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Determining the chemical composition and *in vitro* digestibility of forage species used in small ruminant production systems in the english speaking Caribbean – Part 1

H.A.Jack[1,2*], L.M. Cranston[1], J.L. Burke[1], M. Knights[3], and P.C.H. Morel[1]

[1]*Animal Science Group, School of Agriculture and Environment, Massey University, New Zealand*
[2] *Caribbean Agricultural Research and Development Institute,*
The University of the West Indies Campus, St Augustine, Trinidad and Tobago
[3] *University of Trinidad and Tobago – Biosciences, Agriculture and Food Technologies - Eastern Caribbean Institute for Agriculture and Forestry Campus, Trinidad and Tobago*

**Corresponding author email:* h.jack@massey.ac.nz; jack.heidianna@gmail.com

The nutritional evaluation of tropical forages in the Caribbean is limited. Therefore, this study was done to provide information on the nutritive value of 12 forages used in regional small ruminant production systems in the Caribbean region, utilising three different methods of forage evaluation. Samples of seven grasses *Brachiaria arrecta, Brachiaria ruziziensis, Cynodon dactylon, Cynodon nlemfuensis, Digitaria eriantha, Megathyrsus maximus and Pennisetum purpureum*; two leguminous multipurpose tree species (LMPTs) *Gliricidia sepium* and *Leucaena leucocephala* and three non-leguminous multipurpose tree species (NLMPTs) *Moringa oleifera*, *Morus alba* and *Trichanthera gigantea* were collected from one of two sites located in Trinidad and Jamaica. Analysis of nutritive value was done using proximate analysis, *in vitro* assays and near infrared spectroscopy (NIRS). *Cynodon nlemfuensis* had the highest crude protein (CP, 191.2 g/kg DM) of the grasses and *Leucaena leucocephala* and *Moringa oleifera* the highest CP (263.6 and 232.5 g/kg DM respectively) of the LMPTs and NLMPTs. The NLMPTs, *Moringa oleifera* and *Morus alba* had the highest starch, highest *in vitro* digestible organic matter in dry matter (IVDOMD, 651 and 655 g/kg DM respectively) and metabolisable energy (ME, 10.62 and 10.68 MJ/kg DM respectively) and the lowest neutral detergent fibre (NDF) of the MPTs. There was a strong positive relationship between the IVDOMD and both the CP and starch fractions and a strong negative relationship between the IVDOMD and both the NDF and acid detergent fibre (ADF) fractions. The NIRS predicted values had a strong relationship with the gross chemical CP, NDF, IVDOMD and ME) values of tropical forage species (R^2 = 0.91, 0.86, 0.70 and 0.80 respectively). Overall, the forage species were above the minimum CP, IVDOMD and ME required to be classified as intermediate to good quality forages.

Keywords: Caribbean, forage evaluation, small ruminants, Trinidad and Tobago

There is a growing demand for livestock products within the Caribbean Community (CARICOM). This is thought to be a direct result of rising affluence and urbanisation, as well as a rapidly increasing population which is projected to rise from 18 million to 22 million by 2050 (Asiedu 2001; FAO-UN 2014). Further, there is currently a high regional demand for animal protein from small ruminants and local meat production from the sector currently meets only 20 - 25% of the regional demand (Avril et al. 2011; Lallo et al. 2016). Increasing production may require addressing some of the major constraints particularly the high dependence on costly imported concentrate feed in the region (Singh et al. 2006). One approach to addressing this challenge is increasing the utilization of locally available feeds including forages which may allow for the development of more sustainable feeding systems (Avril et al. 2011).

There is a wide range of tropical forages that are used in small ruminant production systems in the Caribbean (Hernández and Sánchez 2014). These include grasses which are often presented as cut and carry predominantly in intensive production systems. With their efficient C_4 photosynthetic pathway, tropical forages

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undergo faster maturation than their temperate counterparts becoming more fibrous and less digestible over a short period of time (Leng 1990). This reduction in the nutritive value of grasses results in the need for supplementation with commercial feeds, especially during the dry season when the quality of grasses declines severely (Lallo 2015). There is also a wide range of fodder trees or multipurpose tree type species (MPTs) that are known for their higher concentrations of protein, vitamins and minerals when compared to the grasses. The high and more consistent nutritive value of these MPTs make them good supplemental forages that may improve the overall feeding value of the more fibrous tropical grasses (Wilson 1969; Topps 1992).

The nutritive value of forages can vary significantly depending on environmental factors including weather and cultural practices (Sarwar 1999; Hughes et al. 2012). This necessitates the on-going evaluation of these resources to inform their management and optimal use in farming systems. There are several ways to evaluate the quality of forages including *in vivo* studies and laboratory methods. *In vivo* studies are often labour intensive, costly and time consuming and consequently laboratory methods which are faster and more cost effective are commonly used as alternatives to *in vivo* methods (Carro et al. 1994). However, in the Caribbean the application of these laboratory methods to tropical feeds is limited because of a lack of access to well-equipped laboratory facilities as well as their characteristically low analytical capacity (Dardenne and Salgado 2015). This may explain why the analysis of regional forage resources utilising these laboratory methods is not routinely done nor is it extensively reported in the literature. This information can be used to better inform how forages are handled, fed and supplemented for optimal use as feeds for small ruminants (Madsen et al. 1997). Therefore, this study aimed to characterise the nutritive profile of seven grasses, two leguminous multipurpose trees and three non-leguminous multipurpose trees used in small ruminant production systems in the Caribbean utilising proximate analysis, near infrared spectroscopy (NIRS) and *in vitro* assays.

Materials and methods

Site description

Samples for all species were collected from one of two sites (Site 1 and Site 2) (Table 1). Site 1 is the forage bank at the University of Trinidad and Tobago, Valsayn Campus, Trinidad and Tobago (10.63° N, 61.41° W) and Site 2 is the forage bank at New Wales, Manchester, Central Jamaica (17.93 °N, 77.52 °W). The total rainfall for January 2018 at Site 1 when samples were harvested was 60.8 mm; the minimum and maximum temperatures at the time of harvest were 22.2 and 30.1 ^{0}C respectively; the predominant soil type at Site 1 is Piarco fine sand. The total rainfall for January 2018 at Site 2 when samples were harvested was 77.4 mm; the minimum and maximum temperatures at the time of harvest were 24.4 and 30.5^{0}C respectively; and the predominant soil type is St. Ann's clay loam.

Table 1: Forage species used and harvested site

Forage type			
Grasses	**Scientific name**	**Common name**	**Site***
	Brachiaria arrecta	Tanner grass	1
	*Brachiaria ruziziensis***	cv. Mulato II	1
	Cynodon dactylon	Bermuda grass	2
	Cynodon nlemfuensis	African star grass	2
	Digitaria eriantha	Pangola grass	1
	Megathyrsus maximus	Guinea grass	1
	Pennisetum purpureum	Elephant grass	1
LMPTs			
	Gliricidia sepium	Gliricidia	1
	Leucaena leucocephala	Leucaena	1
NLMPTs			
	Moringa oleifera	Moringa	1
	Morus alba	Mulberry	1
	Trichanthera gigantea	Trichanthera	1

LMPTs: Leguminous multipurpose tree species; NLMPTs: Non-leguminous multipurpose tree species
*Site 1: Forage bank at the University of Trinidad and Tobago - Valsayn Campus, Trinidad and Tobago;
Site 2: Forage bank at New Wales, Manchester, Central Jamaica
**Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *Brachiaria brizantha* x *Brachiaria decumbens*)

Selection of forage species

Forage species were selected based on informal consultations with regional stakeholders across the Caribbean Community (CARICOM), including farmers who utilise these in their production systems as well as livestock scientists who have highlighted their potential use in small ruminant production systems in the region. A total of 12 species were selected including seven grass species; two leguminous multipurpose tree species (LMPTs) and three non - leguminous multipurpose tree species (NLMPTs) (Table 1).

Harvesting and preparation of forage samples

On 16 January 2018, samples (n = 3) for ten forage species were harvested from Site 1. These included the leaves and stems of five grasses: *Brachiaria arrecta* (*B. arrecta*), *Brachiaria ruziziensis* (*cv. Mulato II (Brachiaria ruziziensis x Brachiaria brizantha x Brachiaria decumbens, hereafter referred to as B. ruziziensis*)), *Digitaria eriantha* (*D. eriantha*), *Megathyrsus maximus* (*M. maximus*) *and Pennisetum purpureum* (*P. purpureum);* young and mature leaves and stems of two leguminous multipurpose tree species (LMPTs): *Gliricidia sepium* (*G. sepium*) and *Leucaena leucocephala* (*L. leucocephala*); and young and mature leaves and stems of three non-leguminous multipurpose tree species (NLMPTs): *Moringa oleifera (M. oleifera), Morus alba* (*M. alba*) and *Trichanthera gigantea* (*T. gigantea*)). On 23 January 2018 samples (n = 3) of leaves and stem for two grass species (*Cynodon dactylon* (*C. dactylon*) *and Cynodon nlemfuensis* (*C. nlemfuensis*) were harvested from Site 2. For Site 1, the size of plots for each species was 28 m^2 (comprised of two sub plots each 14 m^2) and for Site 2, the size of plots for each species was 291 m^2. At both sites, plots for grass species were cut prior to the harvesting date so that all species had a regrowth of 35 days. Samples (n = 3) for each grass species were randomly harvested (manually chopped with a machete) at approximately 5 - 7 cm above ground level with each of the three replicates comprising cuts from several individual plants in one of three different locations within plots 1 and 2.

Samples (n = 3) for each tree species were randomly harvested (manually chopped with a machete) from all parts of the tree canopy with each of the three replicates comprising cuts from several individual trees within the plots. Immediately after harvesting, all samples collected were dried at 60^0C for 48 hours in a forced-air oven. The dried samples were ground before being packaged (wrapped in triple plastic layers and boxed) and exported to the Food and Nutrition Laboratory, Massey University, New Zealand for analysis. Upon arrival, the samples were ground further with a Thomas hammer mill (screen size:1 mm) and analysed using proximate analysis, *in-vitro* assays and near infrared spectroscopy (NIRS).

Proximate analysis

Samples were analysed for dry matter (DM) by drying at 105 °C in a convection oven (AOAC 930.15). The total nitrogen content was determined by combustion (AOAC 968.06) using a Leco CNS 200 analyser (Leco Corporation, St Joseph, MI, USA) and the crude protein (CP) was computed by multiplying the N values obtained by a factor of 6.25. Starch was determined using an α-amylase Megazyme kit (AOAC 996.11). The neutral detergent fibre (NDF) (with heat stable amylase) and acid detergent fibre (ADF) fractions were determined by the method of Van Soest et al. (1991) as well as the Tecator Fibretec System (AOAC 973.18). The ash content was determined by total combustion at 550 °C (AOAC 942.05) and the organic matter was calculated as the difference between the dry matter content and the ash content. Fat was determined by using the Soxtec method (AOAC 2003.06) and the gross energy (solid) using a bomb calorimeter.

In vitro digestibility

The *in vitro* dry matter digestibility (DMD) and *in vitro* organic matter digestibility (OMD) were measured using the pepsin-cellulase method of Roughan and Holland (1977). The digestible organic matter content in dry matter (IVDOMD) was calculated from the organic matter (percentage) in the diet multiplied by the OMD. The *in vitro* metabolisable energy (ME) of the forages (MJ ME/kg DM) was calculated as DOMD × 0.163 (AFRC 1993).

Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) was used to estimate the chemical components of forage samples including CP, NDF, ADF, lipid, OM, ash, digestible organic matter in dry matter (NIRS DOMD) and the Metabolisable Energy (NIRS ME). The tropical forage samples were scanned using a Bruker MPA NIR spectrophotometer (Ettlingen, Germany). The resulting NIR spectra were then analysed using Optic user software (OPUS) version 5.0. (Ettlingen, Germany). The calibrations for each component were developed using NIRS after scanning finely ground temperate pasture samples in the range of 400 - 2500 nm.

Statistical analysis

Statistical analysis was done in the R environment for statistical computing and visualisation (Team 2013). A linear model with forage as a fixed effect was fitted to the data using both Carr (Fox and Weisberg 2011) and Agricolae (de Mendiburu and de Mendiburu 2019) R packages. Means and superscripts were generated using both Emmeans (Lenth et al. 2019) and Multcomp (Hothorn et al. 2016) R packages which help to separate significantly different means using the Tukey's multiple comparison test. Differences were considered statistically significant if $P \leq 0.05$.

Pearson correlation coefficients between the digestibility data and the proximate chemical components, as well as the Pearson correlation coefficients between proximate chemical components, IVDOMD, *in vitro* ME and their respective NIRS values were generated using the Corrr package version 0.2.1 (Jackson 2016). Simple linear regressions were carried out to investigate the relationships between the chemical composition values generated by the NIRS method and those obtained by proximate analysis using both ggplot2 (Wickham 2016) and ggpmisc (Aphalo 2016) R packages.

Results and discussion

Chemical composition of forages (proximate analysis and NIRS)

Crude protein

In the current study, the average proximate and near infrared CP concentration observed for the MPTs was 213.0 and 219.9 g/kg DM respectively (Tables 2 and 3). MPTs are typically known for their high CP content when compared to tropical grasses which makes them suitable high-protein forage supplements particularly during the dryer parts of the year when both the quality and yield of tropical grasses decline severely (Osuji and Odenyo 1997; Huyen et al. 2012).

There were differences in the concentration of CP among the various grass species. For example, *C. nlemfuensis* had the highest proximate and NIRS CP concentrations for the grass species. The CP concentration reported for the species in the Caribbean range between 98 - 140 g/kg DM (Aumont et al. 1995; Miller et al. 2004). The differences in the concentration of CP between the studies may be because of differences in plant factors (samples comprising leaves or stem; maturity; cultivars) and environmental factors including weather and cultural practices (Sarwar 1999; Hughes et al. 2012). Though values may typically range between 110 - 160 g CP/kg DM for *C. nlemfuensis*, values up to 242 g CP/kg DM have been reported for the species (Caro-Costas et al. 1976). Of all the grasses, *B. ruziziensis* had the lowest proximate CP and NIRS - CP (67.6 and 51.8 g/kg DM respectively) which by both methods were statistically similar (P > 0.05) to those of *D. eriantha* (87.1 and 60.1 g/kg DM respectively) and *M. maximus* (90.3 and 93.6 g/kg DM respectively). The lower values obtained for *M. maximus* and *D. eriantha* despite the early regrowth (35 days) were consistent with the literature for the species at a similar stage of regrowth (5.3 - 12.0 g CP/kg DM for *D. eriantha* (Chaiwang et al. 2011; Fanchone et al. 2012) and between 86 - 140 g/kg DM for *M. maximus* (Lima et al. 2013; Melesse et al. 2017) and may be an indication of the lower quality of these grass species. However, *B. ruziziensis* is one of the improved tropical cultivars and CP concentrations between 110 - 160 g/kg DM and sometimes up to 210 g CP/kg DM have been reported for this forage (Guiot 2005; Argel et al. 2007). Generally, all grass species except *B. ruziziensis* were above the critical 70 g CP/kg DM required to support at least maintenance requirements of animals and for adequate NDF use in fibrous basal diets (Lazzarini et al. 2009).

With respect to the MPTs, *L. leucocephala* had the highest concentration of proximate CP which was statistically similar (P > 0.05) of *M. oleifera*. A similar pattern was observed for the NIRS method where *L. leucocephala* had the highest NIRS CP concentration among the MPTs. Both *L. leucocephala* and *M. oleifera* are rich in protein which may measure up to 260 g/kg DM for *L. leucocephala* and over 300 g CP/kg DM for *M. oleifera* (Gill et al. 2007; Heuze et al. 2019). Values obtained were comparable to values reported for *L. leucocephala* (302 - 318 g/kg DM) and *M. oleifera* (160 - 205 g/kg DM) in the Caribbean (Edwards et al. 2012; López et al. 2017). The proximate CP value for *T. gigantea* was the lowest recorded for the MPTs. However, the NIRS value was high (203.7 g CP/kg DM) and comparable to the NIRS CP values of other protein-rich species as *G. sepium* (215.6 g CP/kg DM), *M. oleifera* (218.1 CP/kg DM) and *M. alba* (190.2 g CP/kg DM). The CP values obtained by both methods for these MPTs were within the range reported in the literature for these MPTs ; (CSIRO 2007). The species were generally above the 160 g/kg DM optimum for supporting growth in growing lambs (Haddad et al. 2001). Further, these species provide more than 11 - 13 % CP which is required for optimal microbial protein (MCP) synthesis in small ruminants, if an adequate supply of energy is provided. After optimal CP levels for microbial synthesis have been attained, further increases in CP may function as bypass protein making MPTs a good source of rumen degradable and bypass protein (MCP and true protein from the diet) (Stern et al. 1994).

Carbohydrates

Both the rapidly degradable non-structural carbohydrates as well as the structural ones are critical precursors to volatile fatty acids (VFAs) which are the main sources of energy for ruminants (Rosales 1997; Faverdin 1999). Tropical grasses, in comparison to other forages, are often higher in the fibrous fractions (Huyen et al. 2012). The proximate NDF concentration for grasses ranged between 699 - 756 g/kgDM (Table 2). Similar results were observed for the NIRS method where the concentration of both NIRS NDF ranged between 696 - 783 g/kg DM (Table 3). The proximate NDF values for the MPTs ranged between 379 - 505 g/kg DM and 308 - 415 g/kg DM for NIRS. Unlike the MPTs, the NDF values obtained for grasses were above 660 g/kg DM which is associated with restricted intake in ruminants (Islam et al. 2003; Bezabih et al. 2014).

For the MPTs, the proximate NDF concentrations for the LMPTs (501 - 505 g/kg DM) and *T. gigantea* (502 g/kg DM) were higher than those typically reported for these species. For instance in studies done by Rosales (1997), *G. sepium*, *L. leucocephala* and *T. gigantea* had the lowest concentration of the cell wall fractions ranging between 294 - 308 g/kg DM for NDF and between 217 - 248 g/kg DM) for ADF when compared to that of other MPTs which ranged between 318 - 613 g/kg DM for NDF and 264 - 620 for ADF. The lower values obtained by Rosales (1997) were more comparable to NIRS NDF and NIRS ADF values in this study (308 - 374 g/kgDM and 162 - 237 g/kg DM respectively). The observed differences between the proximate values of the current study and those of Rosales (1997) may be as a result of differences in samples used which comprised both stems and leaves in the current study and leaves only for studies done by Rosales (1997).

The generally low CP and high NDF concentration in grasses may limit both dry matter and energy intake and subsequently reduce performance (Islam et al. 2003; Arthington and Brown 2005). However, supplementation of tropical grasses with the MPTs may increase the CP concentration; reduce the concentration of the cell wall fractions and therefore improve the overall intake of diets comprising mainly fibrous tropical grasses with low nutritive value (Lazzarini et al. 2009).

Table 2: Proximate chemical composition (g/k DM) (including the crude protein (CP), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, fat, organic matter (OM), ash) and gross energy (GE, MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) (n = 3 per forage species)

	CP	Starch	NDF	ADF	Lignin	Fat	OM	Ash	GE
Grasses									
Brachiaria arrecta	109.5[bc]	3.71[a]	705[cd]	449[def]	77.3[ab]	17.9[ab]	820[b]	122.0[bcd]	17.4[def]
*Brachiaria ruziziensis**	67.6[a]	4.85[a]	715[cd]	414[cdef]	40.5[a]	18.8[abcd]	843[b]	94.1[a]	17.9[f]
Cynodon dactylon	142.8[cd]	12.58[ab]	748[cd]	388[bcdef]	67.0[a]	12.3[a]	685[a]	126.3[cd]	17.2[bcd]
Cynodon nlemfuensis	191.2[ef]	1.06[a]	699[c]	383[bcde]	59.6[a]	18.3[abc]	804[b]	108.4[ab]	17.3[cde]
Digitaria eriantha	87.1[ab]	3.92[a]	727[cd]	497[f]	78.5[ab]	21.8[bcd]	838[b]	95.5[a]	17.9[f]
Megathyrsus maximus	90.3[ab]	1.96[a]	756[d]	472[ef]	57.2[a]	18.6[abcd]	791[b]	137.1[de]	16.9[bc]
Pennisetum pupureum	105.5[b]	1.52[a]	704[cd]	436[cdef]	45.3[a]	26.1[cde]	757[ab]	146.4[e]	16.7[b]
LMPTs									
Gliricidia sepium	192.6[ef]	12.92[ab]	501[b]	335[abc]	188.1[c]	31.3[e]	807[b]	112.0[bc]	19.1[g]
Leucaena leucocephala	263.6[h]	4.59[a]	505[b]	347[abcd]	185.4[c]	26.3[de]	847[b]	92.8[a]	20.1[h]
NLMPTs									
Moringa oleifera	232.5[gh]	28.36[c]	386[a]	284[ab]	99.9[ab]	46.3[f]	836[b]	93.5[a]	19.8[h]
Morus alba	205.3[fg]	25.36[c]	379[a]	250[a]	139.5[bc]	22.1[bcd]	755[ab]	146.8[e]	17.8[ef]
Trichanthera gigantea	171.1[de]	23.68[bc]	502[b]	363[bcde]	196.5[c]	22.4[bcd]	684[a]	225.5[f]	16.0[a]
SEM	6.61	2.37	10.4	21.5	12.8	1.54	18.4	3.09	0.097
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

All means carrying the same superscripts within columns are not significantly different (P >0.05)
*Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *Brachiaria brizantha* x *Brachiaria decumbens*)

Table 3: NIRS predicted values for the chemical composition (g/kg DM) (including the crude protein, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, fat, organic matter (OM), ash) and *in vitro* metabolisable energy (IV-ME, MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non leguminous multipurpose tree species (NLMPTs)

	CP	NDF	ADF	Fat	OM	Ash	DOMD	IV-ME
GRASSES								
Brachiaria arrecta	104.4[bc]	715[cd]	403[ef]	17.80[cd]	889[ef]	76.8[abc]	507[a]	6.99[ab]
*Brachiaria ruziziensis**	51.8[a]	717[cd]	382[e]	27.29[d]	881[def]	70.1[ab]	517[ab]	7.08[ab]
Cynodon dactylon	111.3[c]	699[cd]	394[e]	5.41[a]	894[f]	66.0[a]	595[b]	7.30[abc]
Cynodon nlemfuensis	172.6[d]	696[c]	388[e]	6.85[ab]	881[def]	85.3[cd]	601[b]	8.25[cd]
Digitaria Eriantha	60.1[ab]	709[cd]	373[e]	17.94[cd]	883[def]	72.7[ab]	570[ab]	7.69[bcd]
Megathyrsus maximus	93.6[abc]	783[e]	425[f]	16.52[bc]	881[def]	68.9[ab]	496[a]	6.27[a]
Pennisetum pupureum	120.8[c]	739[d]	372[e]	20.14[cd]	864[cdef]	80.7[bcd]	565[ab]	7.91[bcd]
LMPTs								
Gliricidia sepium	215.6[d]	308[a]	200[bc]	38.84[e]	853[cd]	88.9[d]	728[c]	8.66[de]
Leucaena leucocephala	272.1[e]	318[a]	162[a]	49.14[f]	846[bc]	112.0[e]	770[cd]	9.77[ef]
NLMPTs								
Moringa oleifera	218.1[d]	415[b]	212[cd]	51.15[f]	860[cde]	92.0[d]	759[cd]	9.90[f]
Morus alba	190.2[d]	311[a]	181[ab]	58.97[f]	815[ab]	113.8[e]	839[de]	12.21[g]
Trichanthera gigantea	203.7[d]	374[b]	237[d]	38.55[e]	809[a]	116.8[e]	913[e]	10.25[f]
SEM	8.96	8.1	6.1	1.95	6.35	2.33	17.3	0.227
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

All carrying the same superscripts within columns are not significantly different (P > 0.05)
*Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *Brachiaria brizantha* x *Brachiaria decumbens*)

In vitro digestibility and ME content of forages

The IVDOMD and ME obtained in this study (Table 4) were within the range reported in the literature for tropical grasses and MPTs (Durmic et al. 2017). The MPTs may all be medium to good quality forages for ruminants as the IVDOMD and ME obtained for the MPTs were greater than 500 g/k DM and 7.5 MJ/kg DM respectively (Bediye et al. 2007). Overall the ME values were comparable to those considered to be acceptable for sheep, goats and cattle (9.97 - 10.52 MJ/kg DM) (Marie-Madeleine et al. 2012).

The IVDOMD and ME obtained for the MPTs may be as a result of the characteristically high concentrations of CP and the low concentrations of NDF observed for the MPTs (Arthington and Brown 2005). The positive impact of CP on the IVDOMD may be demonstrated by the strong positive correlation between CP and the IVDOMD (0.82, $P \leq 0.05$) and the negative effect of cell wall fractions on the IVDOMD may be demonstrated by the strong negative correlation between the NDF (- 0.98, $P \leq 0.05$) and ADF (-0.86, $P \leq 0.05$) with IVDOMD (Table 5). Conversely, the low concentrations of CP and high concentrations of NDF in grasses may have resulted in the overall lower digestibility of these species. This negative relationship between both NDF and ADF with the IVDOMD was also reported by (Kamalak et al. 2005).

The correlation of lignin with IVDMD, IVOMD and IVDOMD were 0.72, 0.69 and 0.65 respectively. Throughout the literature there have been conflicting reports on the accuracy of using the concentration of lignin to predict the digestibility of feeds. For instance in some studies lignin was not as strongly correlated with digestibility as the ADF fraction (Moss and Givens 1990) whereas for other authors lignin was the cell wall fraction more strongly correlated with digestibility (Jung and Allen 1995). The contradictions in reports may be because the spatial distribution of lignin in the cell-wall matrix and not the concentration of lignin, impacts more readily on the digestibility of feeds. Therefore feeds with a higher lignin concentrations may not always be the least digestible (Reeves 1987).

The IVDOMD and ME of grasses were comparable across species but varied for the MPTs. However, for the MPTs, *M. oleifera* and *M. alba* had the highest IVDOMD (651 and 655 g/kg respectively) and ME (10.62 and 10.68 MJ/kg DM respectively) which may be related to the overall higher nutritive value of these species in comparison to the other MPTs.

Trichanthera gigantea and *G. sepium* had the lowest IVDOMD and ME which were comparable to the values reported in the literature for these species (Durmic et al. 2017). The low IVDOMD obtained for *T. gigantea* (585 g/kg DM) may be better explained by the lower proximate CP (171.1 g/kg DM); and higher proximate NDF (502 g/kg DM). *Trichanthera gigantea* also had the highest ash content of all the MPTs (225.5 g/kg DM). Greater proximate ash content has also been associated with lower digestibility and energy in forage and may also explain the lower IVDOMD obtained for the species (Negesse et al. 2009; Balraj et al. 2018). For *G. sepium*, the lower proximate CP (192.6 g/kg DM) and higher concentrations of proximate NDF (501 g/kg DM) compared to *M. alba* and *M. oelifera* may be more aligned to the lower IVDOMD (599 g/kg DM) observed for the species. Though the NIRS DOMD and NIRS ME values obtained for the grasses were comparable to their respective *in vitro* values, those for the MPTs were higher and the ranking of the species differed between the methods. For instance, based on the *in vitro* method, both *M. oleifera* and *M. alba* ranked the highest and *T. gigantea* the lowest in terms of their IVDOMD and ME, however for the NIRS method, the DOMD and ME of *T. gigantea* was comparable to that of *M. alba* and higher than that of *M. oleifera*. Overall, species were above the minimum IVDOMD and ME required for tropical forage species to be classified as intermediate to good quality forage (Bediye et al. 2007).

Table 4: Digestibility (*in vitro* dry matter digestibility (IVDMD, g/g DM); *in vitro* organic matter digestibility (IVOMD, g/g DM); *in vitro* digestible organic matter in dry matter (IVDOMD, g/kg DM) and metabolisable energy (ME, MJ/kg DM) of grasses, leguminous multipurpose tree species (LMPTs) and non leguminous multipurpose tree species (NLMPTs) that are used in small ruminant production systems in the Caribbean (n = 3 per forage species)

	IVDMD	IVOMD	IVDOMD	ME*
GRASSES				
Brachiaria arrecta	0.581^{b}	0.574^{ab}	515^{ab}	8.40^{ab}
*Brachiaria ruziziensis***	0.582^{b}	0.582^{b}	526^{b}	8.58^{b}
Cynodon dactylon	0.575^{ab}	0.571^{ab}	511^{ab}	8.32^{ab}
Cynodon nlemfuensis	0.592^{b}	0.591^{b}	530^{b}	8.65^{ab}
Digitaria eriantha	0.572^{ab}	0.572^{ab}	517^{b}	8.42^{b}
Megathyrsus maximus	0.560^{a}	0.553^{a}	496^{a}	8.08^{ab}
Pennisetum pupureum	0.586^{b}	0.580^{b}	517^{b}	8.42^{b}
LMPTs				
Gliricidia Sepium	0.662^{c}	0.671^{c}	599^{cd}	9.76^{cd}
Leucaena leucocephala	0.668^{c}	0.683^{c}	614^{d}	10.01^{d}
NLMPTs				
Moringa oleifera	0.705^{d}	0.726^{d}	651^{e}	10.62^{e}
Morus. Alba	0.729^{e}	0.749^{d}	655^{e}	10.68^{e}
Trichanthera gigantea	0.696^{d}	0.687^{c}	585^{c}	9.53^{c}
SEM	0.004	0.005	4.04	0.066
p-value	<.0001	<.0001	<.0001	<.0001

All means carrying the same superscripts within columns are not significantly different (P > 0.05)
* ME = *in vitro* digestible organic matter (IVDOMD) x 0.163
**Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *Brachiaria brizantha* x *Brachiaria decumbens*)

Table 5: Correlation coefficients between proximate components and *in vitro* digestibility of forages used in small ruminant production systems in the Caribbean

	Chemical composition and the respective correlation coefficients*						
Variable	Ash	CP	Fat	Starch	NDF	ADF	Lignin
IVDMD	0.27	0.78	0.58	0.80	-0.98	-0.84	0.72
IVOMD	0.17	0.80	0.61	0.78	-0.99	-0.85	0.69
IVDOMD	0.02	0.82	0.66	0.74	-0.98	-0.86	0.65

* Correlation coefficients ≥0.310 and ≤-0.310 are significant at P ≤ 0.05)
CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; IVDOMD: *in vitro* digestible organic matter in dry matter

Table 6: Correlation coefficients between gross chemical composition and *in vitro* metabolisable energy values predicted by near infrared spectroscopy (NIRS) for 12 forage species used in small ruminant production systems in the Caribbean

Variable	**Correlation coefficients***
Dry matter	0.50
Ash	0.86
Crude protein	0.94
Fat	0.11
Neutral detergent fibre	0.94
Acid detergent Fibre	0.79
In vitro digestible organic matter in dry matter	0.84
In vitro metabolisable energy	0.89

* Correlation coefficients ≥0.310 and ≤-0.310 are are significant at p ≤0.05

Overall, there was a strong relationship between the proximate CP ($r = 0.94$, $P \leq 0.05$), proximate NDF ($r = 0.94$, $P \leq 0.05$), IVDOMD ($r = 0.84$, $P \leq 0.05$) and ME ($r = 0.89$, $P \leq 0.05$) and their respective NIRS values (Table 6). With respect to CP and NDF, the high coefficient of correlation was expected as the NIRS prediction models for these are known to be precise because of the extensive use of NIRS to measure these components (Dardenne and Salgado 2015). Further, the results suggest that the NIRS model was also effective at predicting the IVDOMD and the *in vitro* ME of tropical forages. Further, the high correlations suggest that even with a NIRS calibration model built primarily on temperate pasture, reasonable predictions can be obtained for tropical forages (Figures 1 and 2). Several efforts have been made by the French Agricultural Research Centre for International Development (CIRAD) and its southern partners to establish NIRS calibration models for tropical forage (Dardenne and Salgado 2015). This is critical as it may improve the reliability and consistency of NIRS tools for generating more accurate data on the nutritive value of tropical forages. This characteristically rapid and cost-effective method of analysis can therefore be used for the routine evaluation of a range of forages in the Caribbean which may indirectly lead to optimised livestock management and productivity.

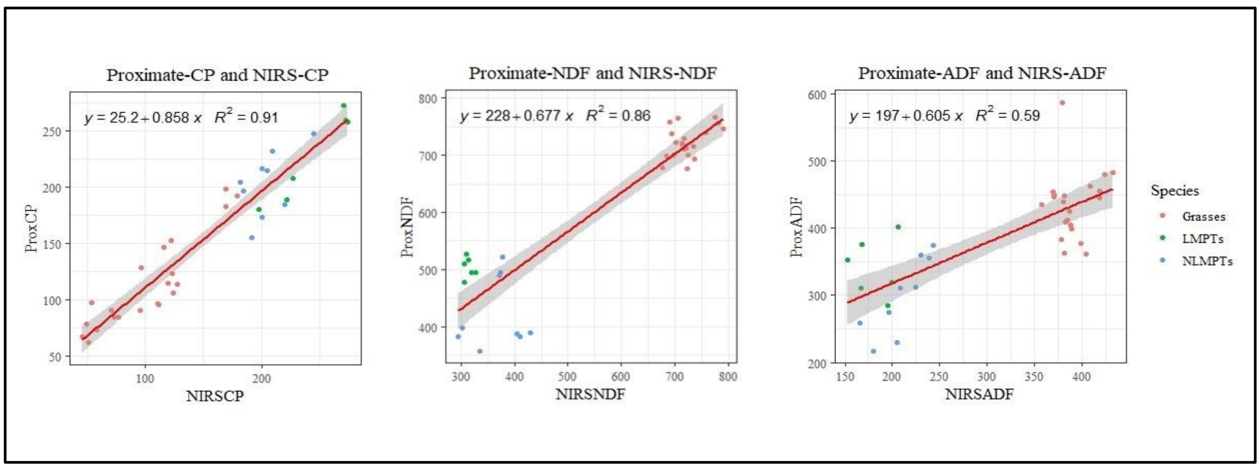

Figure 2: The relationship between gross chemical CP, NDF and NDF with their respective near infrared spectroscopy (NIRS) values

*Terms used: ProxCP: proximate crude protein; NIRSCP: crude protein determined by near infrared spectroscopy; ProxNDF: proximate neutral detergent fibre; NIRSNDF: neutral detergent fibre determined by near infrared spectroscopy; ProxADF; proximate acid detergent fibre; NIRSADF: acid detergent fibre determined by near infrared spectroscopy; LMPTs: leguminous multipurpose tree species; NLMPTs: non-leguminous multipurpose tree species

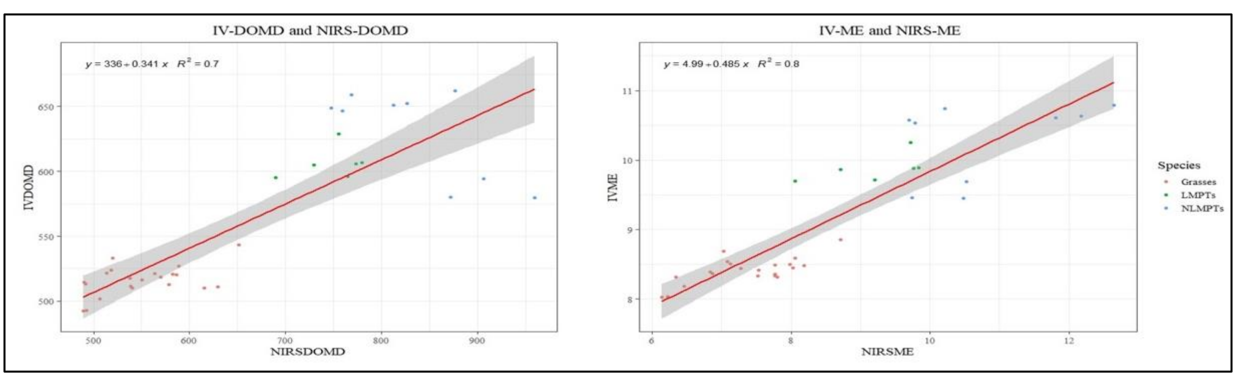

Figure 3: The relationship between the *in vitro* digestible organic matter in dry matter (DOMD) and metabolisable energy (ME) with their respective near infrared spectroscopy (NIRS)-predicted values

Conclusion

The MPTs were of overall high nutritive value, which was as a result of the high concentrations of CP and the low concentration of cell wall fractions including NDF and ADF. This resulted in a high *in-vitro* digestibility (585 - 655 g/kg DM) and metabolisable energy (9.53 - 10.68 MJ/kg DM) observed for the MPTs. The NLMPTs were of higher nutritive value than the LMPTs with *M. alba* and *M. oleifera* leading in terms of most of their chemical components, *in vitro* digestibility and metabolisable energy. *Cynodon nlemfuensis* in particular recorded CP as high as 191.2 g/kg DM. There was a strong relationship between the proximate CP, proximate ADF, proximate NDF, the IVDOMD and *in vitro* ME and their respective NIRS values. We found very close associations between NIRS and proximate values which suggests that the NIRS method can be adopted and will therefore be of benefit because of its accuracy in predicting the nutritive characteristics of tropical forages used in small ruminant production systems in the Caribbean.

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