Fermentation kinetics and *in vitro* digestibility of tropical forages used in sheep and goat production systems in the Caribbean

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The *in vitro* fermentation kinetics and digestibility of six tropical grasses and five multipurpose tree species (MPTs) found in the Caribbean were determined by the current study. The *in vitro* gas production was measured and fitted to a dual and single pool model to determine the fermentation kinetics of these forages. The average rate of the fast pool (C_1) for grasses was over 20% higher than that of the MPTs. The volume of the fast pool by the dual pool model (V_1) for grasses was 40% lower and the slow pool (V_2) 30% greater than that of the MPTs. There was a strong negative relationship between the fast pool of the dual pool model (V_1) and the neutral detergent fibre (NDF) (r = -0.781) and acid detergent fibre (ADF) (r = -0.655) fractions and a positive relationship between the V_1 and the digestible organic matter in dry matter (DOMD) (r = 0.537). *Moringa oleifera* was at the higher end of the range for the total gas production by the dual pool model (Vt_{scho}) (132.1 ml/g DM) and single pool model (Vt_{orsk}) (131.5 ml/g DM) at 48 hours which was followed by that of *Gliricidia sepium* (116.1 and 113.5 ml/g/DM respectively) and *Morus alba* (116.9 and 113.7 g/kg DM respectively). At 48 hours, the Vt_{scho} and Vt_{orsk} for *Trichanthera gigantea* and *Leucaena leucocephala* were at the lower end of the range for the forages (*Trichanthera gigantea*: 70.8 and 65.7 ml/g DM and *Leucaena leucocephala*: 89.1 and 86.2 ml/g DM for the dual and single pool models respectively). *Moringa oleifera* was at the higher end of the range for the total volatile fatty acid (VFA) production whereas *Trichanthera gigantea* was at the lower end of the range. All MPTs except *Trichanthera gigantea* and *Leucaena leucocephala,* were more fermentable than the grasses and based on chemical composition, *in vitro* digestibility, fermentation parameters and end products, *Moringa oleifera* and *Morus alba* demonstrated overall high performance whereas *Trichanthera gigantea* performed poorly.

Keywords: Caribbean, forage, fermentation kinetics, *in vitro* digestibility, small ruminants

The nutritive value of feeds for ruminants is determined by both the concentration of its chemical components as well as the rate and extent to which the feed is digested (Getachew et al. 2004). The *in vitro* gas method is used to determine the rate and extent to which feed is digested and has been based on measured relationships between *in vivo* digestibility of feeds; *in vitro* gas production and; the chemical composition of feeds (Menke 1988).

The *in vitro* gas method involves the incubation of feedstuffs with buffered rumen fluid *in vitro* primarily for measuring the digestion of soluble and insoluble carbohydrates (Menke et al. 1979). When incubated, the carbohydrates in feeds are fermented to produce volatile fatty acids (VFAs) including acetate, propionate and butyrate (Menke et al. 1979). In addition to VFAs, other gases (primarily including CO_2 and CH_4) are produced and the microbial biomass (MBM) is increased through the microbial fermentation of substrate (Getachew et al. 1998). The close association between the cumulative gas production and the fermentation of carbohydrates to VFAs is well known and gases produced are used to reflect the production of these short chain fatty acids (Getachew et al. 2002). Measuring the production of VFAs is critical as these represent a major source of energy for the ruminant providing up to 80% of their energy requirement (Annison 1970). Both protein and fats also produce gases but in small and negligible amounts respectively (Getachew et al. 2004; Wolin 1960).

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The cumulative gas produced *in vitro* can be fitted to mathematical models (France et al. 2000). These models are used to estimate *in vitro* gas production kinetics or the rate and extent a substrate or feed has been fermented and or degraded which can be used to estimate the potential animal performance when that feed is fed (France et al. 2005; Üçkardeş and Efe 2014). Both the dual pool logistic model by Schofield et al. (1994) and single – pool model by Ørskov and McDonald (1979) are commonly used to estimate the kinetics of ruminal fermentation (Peripolli et al. 2014). However, information on the fermentation kinetics, *in vitro* digestibility and how these relate to the nutritive value of forages in the Caribbean, is lacking. Therefore, this study aims to determine and report on the fermentation kinetics of a range of tropical forages including six grasses, two leguminous multipurpose tree species (LMPTs) and three non-leguminous multipurpose species (NLMPTs). This will be done using both the dual-pool logistic model by Schofield et al. (1994) and the single pool model by Ørskov and McDonald (1979). Additionally, the study aims to determine the *in vitro* digestibility and fermentation end products including the VFAs and MBM. The study also aims to determine how the fermentation kinetics of forages relate to their nutritive value.

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; and the predominant soil type at Site 1 was the 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; the predominant soil type is the St. Ann's clay loam.

Table 1: List of species used in the study. Both the scientific and common names of species and the site of harvest are presented

Forage type			
Grasses	**Scientific name**	**Common name**	**Site**
	Brachiaria arrecta	Tanner grass	1
	Brachiaria ruziziensis	Mulato grass (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

Terms used: LMPTs: leguminous multipurpose tree species; NLMPTs: non-leguminous multipurpose tree species
Site 1: Valsayn, Trinidad and Tobago. Site 2: Manchester, Jamaica

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).

Harvesting and preparation of forage samples

On 16 January 2018, samples (n = 3) from ten forage species were harvested from Site 1. These included the leaves and stems of five grasses: *Brachiaria arrecta* (*B. arrecta*), *Brachiaria ruziziensis* (*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 from two grass species (*Cynodon dactylon* (*C. dactylon*) *and Cynodon nlemfuensis* (*C. nlemfuensis*) were harvested from Site 2. All grasses 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 Sites 1 and 2. Samples (n = 3) for each tree 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 at different locations within Site 1. 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 (Export Permit no. 2017065045) 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).

Chemical 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.

Fermentation kinetics parameters and end products

Forage samples (n = 2) were analysed using the Alltech IFM^{TM} system. About 1.4 L of rumen fluid was collected approximately two hours

post-morning feed from a lactating dairy cow fed a typical diet consisting of pasture, grass silage and maize silage, 0.5 kg molasses and 1.5 kg of a pelleted compound feed as part of the regular management of cows through a robotic system (Lely Astronaut). Once collected, the rumen fluid was strained using 2 layers of cheese cloth and was mixed with 250 ml of a reducing agent and 5.6 L of McDougall (1948) buffer solution resulting in a rumen fluid to buffer ratio of 20:80. For each forage species, approximately 0.5 g of dried sample, ground to a size of 2 mm was weighed into 250 ml bottles in duplicates and incubated at 39°C in 100 ml of rumen-buffer inoculum for 48 hours (Mould et al., 2005). During the incubation period, gas production was measured continuously using an automated pressure transducer system by Pell and Schofield (1993). The cumulative gas production (ml/g DM digested) was fitted to a dual pool logistic model by Schofield et al. (1994) to estimate degradation rate constant of the fast pool (fast rate, FR hr^{-1}); the degradation rate constant of the slow pool (slow rate, SR hr^{-1}); and the respective gas production volumes including the fast pool (FP ml/g DM) and slow pool (SP ml/g DM) for each forage species. The total gas production (ml/g DM digested) (GP ml/g DM) was calculated as FP+SP (ml/g DM). The cumulative gas production was also fitted to the single pool model by Ørskov and McDonald (1979) where the gas production from the immediately soluble fraction (a ml/g DM), gas production from the soluble fraction (b ml/g DM), the gas production rate constant (c hr^{-1}) and the potential gas production (a+b ml/g DM) were determined. The apparent dry matter digestibility (aDMD %) or the percent of incubated feed DM left after the 48h incubation (undigested residue that contains microbial biomass (MBM)), was determined by the Tilley and Terry (1963) method. The true dry matter digestibility (tDMD %) was measured after the solubilisation of the MBM in the undigested residue and was estimated using the batch culture *in vitro* digestibility method (Mould et al. 2005; Tilley and Terry 1963) after treating the residue with a neutral detergent solution (Goering and Van Soest 1970). The MBM was estimated as the difference between the aDMD and the tDMD (Goering and Van Soest 1970). The digestible organic matter in dry matter (DOMD) was generated from the tDMD utilising the following formula:

$$DOMD = \frac{[OM\ weight - (NDR\ weight - Ash\ weight)]}{DM\ weight}$$

Where OM weight = % OM in substrate x substrate weight
NDR weight = neutral detergent residues weight (the residues after 48 hr fermentation were treated with NDR solution to remove MBM)
Ash weight = Ash of NDR residue
DM weight = % DM in substrate x substrate weight.

The metabolisable energy (ME) was calculated as the DOMD x 0.163 (AFRC 1993). After 48 hours of incubation of the forage samples, individual and total VFA concentrations were determined by gas chromatography according to Erwin et al. (1961) using an Agilent GC 7890B (FID detector).

Statistical analysis

Statistical analysis was done in the R environment for statistical computing and visualisation (Team 2013). Data on the nutritive value of forages, *in vitro* digestibility, fermentation kinetics and fermentation end products were fitted to a linear model. Anova was used to obtain the least significant differences and P values for the model differences. Pearson's correlation between the digestibility data and the proximate chemical components, as well as the Pearson's

correlation between proximate chemical components and fermentation parameters were generated using the Corrr package (version 0.2.1 (Jackson 2016)). Correlations were considered significant if $P \leq 0.05$.

Cumulative gas production data for both samples were fitted to the dual-pool and single pool models using R environment for statistical computing and visualisation (Team 2013) to determine the fermentation kinetics:

Dual pool logistic model by Schofield et al. (1994):

$$Vt_{scho} = \left[\frac{V_1}{1 + exp^{(2+4 \times C1\ x\ (L-T))}}\right] + \left[\frac{V_2}{1 + exp^{(2+4\ x\ C2\ x\ (L-T))}}\right]$$

where Vt_{scho} = measured gas volume at time *t*; V_1 and C_1, = asymptotic cumulative gas volume and fractional degradation rate for pool 1; V_2 and C_2 = respective parameters for pool 2. *T* is the time and *L* is the lag time for both pools. One value for each parameter V_1, C_1, V_2, and C_2 was obtained for forage samples (n = 2 for each species) and averaged to obtain predicted cumulative gas volumes using the dual-pool logistics model (Schofield et al. 1994). The cumulative gas volumes were illustrated by graphs using ggplot2 (Wickham 2016).

Single pool model by Ørskov and McDonald (1979):

$$Vt_{orsk} = a + b\,(1 - e^{-c(t)})$$

where Vt_{orsk} = the measured gas volume at time *t*, *a* = gas production from the immediately soluble fraction, *b* = gas production from the soluble fraction, $a + b$ = the potential gas production and *c* = gas production rate constant. The above fermentation parameters *a*, *b* and *c* were predicted by fitting original gas volumes to the single pool model of Ørskov and McDonald (1979). The averages of the parameters *a*, *b* and *c* were used to predict cumulative gas fitted to the single-pool model (Ørskov and McDonald 1979) and were illustrated by graphs using ggplot2 (Wickham 2016).

Results

Chemical composition

The chemical composition of the species was described in Jack et al. (2021) and are presented in Table 2. The average CP concentration of the grasses was 113.42 g/kg DM and that of the MPTs was 213.0 g/kg DM. The NDF of the grasses ranged between 699 – 756g/kg DM and ADF between 383 – 497 g/kg DM. The NDF and ADF observed for the MPTs ranged between 379 – 505 gNDF/kg DM and 250 – 363 gADF/kg DM respectively. The NLMPTs had an average starch concentration of 25.80 g/kg DM which was 17.04 g/kg DM higher ($P < 0.0001$) than that of the LMPTs (8.76 g/kg DM). The MPT *Trichanthera gigantea* had the highest ash concentration (225.5 g/kg DM, $P < 0.0001$) measuring up to 114.2 g/kg DM more than the average value observed for the other MPTs species (111.3 g/kg DM).

In vitro digestibility

In the current study the aDMD, tDMD, DOMD and ME were measured for all species (Table 3). The aDMD ranged between 40.4 – 55.1% for grasses and 34.7 – 61.5% for the MPTs. The tDMD, ranged between 55.2 – 64.9% and 59.0 – 77.4% for grasses and MPTs respectively. The DOMD for grasses was 51.2 – 65.1% and for MPTs was 55.3 – 73.9%. The ME was estimated and ranged between 8.18 – 10.42 MJ/kg DM and 8.85 – 11.83 MJ/kg DM for grasses and MPTs respectively.

Table 2: Gross chemical composition (g/k DM) (including the crude protein, 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) used in small ruminant production systems in the Caribbean (n=3 per forage species)*

	CP	Starch	NDF	ADF	Lignin	Fat	OM	Ash	GE
GRASSES									
Brachiaria arrecta	109.5	3.71	705	449	77.3	17.9	878	122.0	17.4
Brachiaria hybrid**	67.6	4.85	715	414	40.5	18.8	906	94.1	17.9
Cynodon dactylon	142.8	12.58	748	388	67.0	12.3	874	126.3	17.2
Cynodon nlemfuensis	191.2	1.06	699	383	59.6	18.3	892	108.4	17.3
Digitaria eriantha	87.1	3.92	727	497	78.5	21.8	905	95.5	17.9
Megathyrsus maximus	90.3	1.96	756	472	57.2	18.6	863	137.1	16.9
LMPTs									
Gliricidia sepium	192.6	12.92	501	335	188.1	31.3	888	112.0	19.1
Leucaena leucocephala	263.6	4.59	505	347	185.4	26.3	907	92.8	20.1
NLMPTs									
Moringa oleifera	232.5	28.36	386	284	99.9	46.3	906	93.5	19.8
Morus alba	205.3	25.36	379	250	139.5	22.1	853	146.8	17.8
Trichanthera gigantea	171.1	23.68	502	363	196.5	22.4	774	225.5	16.0
LSD (0.05)	20.5	7.4	32.3	66.7	39.7	4.8	9.6	9.6	0.3
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0 .0001	<0 .0001	< 0.0001

*Table adapted from Jack et al. (2021)

** *Brachiaria* hybrid *(Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Table 3: The apparent dry matter digestibility (aDMD) %), true dry matter digestibility (tDMD %), digestible organic matter in dry matter (DOMD %) and metabolisable energy (ME MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

Grasses	aDMD	tDMD	DOMD	ME
Brachiaria arrecta	46.8	58.6	54.8	8.8
Brachiaria hybrid *	45.5	57.4	56.6	9.1
Cynodon dactylon	47.8	60.6	65.1	10.4
Cynodon nlemfuensis	55.1	64.9	62.8	10.1
Digitaria eriantha	47.1	58.9	58.3	9.3
Megathyrsus maximus	40.4	55.2	51.2	8.2
LMPTs				
Gliricidia sepium	46.2	65.5	66.1	10.6
Leucaena leucocephala	37.5	59.0	59.4	9.5
NLMPTs				
Moringa oleifera	61.5	73.7	73.9	11.8
Morus alba	56.9	77.4	73.9	11.8
Trichanthera gigantea	34.7	64.2	55.3	8.9
LSD (0.05)	5.96	5.27	11.11	1.77
P value	< 0.0001	< 0.0001	0.011	0.011

* *Brachiaria* hybrid (cv.) Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Fermentation parameters and gas production

The fermentation parameters after fitting the dual pool logistic model of Schofield et al. (1994) and the single pool model of Ørskov and McDonald (1979) to the cumulated gas volumes are presented in Table 4 and Figures 1 and 2. In the dual pool model, the *L* ranged between 1.24 – 1.93 hrs for the grasses and 0.50 – 1.19 hrs for the MPTs. There was no lag time calculated for the single pool model. The fast pool rates (C_1) observed for the dual pool logistic model ranged between 0.22 – 0.37 hr^{-1} for grasses and 0.20 – 0.27 hr^{-1} for the MPTs, and the slow pool rates (C_2) were between 0.03 – 0.04 hr^{-1} for grasses and 0.04 – 0.06 hr^{-1} for the MPTs. The rate of gas production for the single pool Ørskov model (*c*) ranged between 0.02 – 0.06 hr^{-1} for grasses and 0.04 – 0.13 hr^{-1} for the MPTs. The total gas production for grasses determined by the dual pool logistic model (Vt_{scho}) and the total gas production determined by the single pool model (Vt_{orsk}) were 90.3 – 108.1 and 89.8 – 111.5 ml/g DM, respectively, and for the MPTs were 70.8 – 132.1 ml/g DM and 65.7 – 131.5 ml/g DM, respectively.

Table 4: The fermentation parameters of the dual pool logistic model by Schofield et al. (1994) and the single pool model by Ørskov and McDonald (1979) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

	Dual pool logistic model						Single pool model			
	L	V_1	V_2	C_1	C_2	Vt_{scho}	*a*	*b*	*c*	Vt_{orsk}
Grasses										
Brachiaria arrecta	1.93	22	74.3	0.268	0.038	96.6	4.39	103.4	0.047	95.8
Brachiaria hybrid*	1.18	28	75.1	0.220	0.036	102.7	11.66	105.6	0.043	101.8
Cynodon dactylon	1.41	16	91.9	0.235	0.037	108.1	6.78	127.4	0.036	111.5
Cynodon nlemfuensis	1.60	16	90.2	0.261	0.031	105.9	8.7	154.5	0.023	107.7
Digitaria eriantha	1.78	21	81.3	0.369	0.043	102.5	6.54	108.4	0.053	102.8
Megathyrsus maximus	1.24	21	69.2	0.290	0.044	90.3	7.48	88.4	0.058	89.8
LMPTs										
Gliricidia sepium	0.50	40	76.6	0.269	0.058	116.1	5.06	108.7	0.121	113.5
Leucaena leucocephala	1.18	28	61.2	0.257	0.047	89.1	5.33	82.6	0.081	86.2
NLMPTs										
Moringa oleifera	0.51	51	81.3	0.219	0.060	132.1	2.23	129.6	0.126	131.5
Morus alba	1.14	43	73.7	0.2	0.049	116.9	1.44	114.2	0.09	113.7
Trichanthera gigantea	1.19	26	44.8	0.26	0.038	70.8	14.07	59.1	0.043	65.7
LSD (0.05)	0.79	15.6	22.64	0.18	0.01	18.89	6.42	37.53	0.03	21.50
P value	0.037	0.006	0.033	0.797	0.008	0.002	0.031	0.011	< 0.0001	0.003

* *Brachiaria* hybrid (cv.) Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Terms used: *L*: lag time; V_1: fast pool (ml/g DM), V_2: slow pool (ml/g DM), C_1: fast rate (hr^{-1}), C_2: slow rate (hr^{-1}); Vt_{scho}: total gas production by Schofield et al. 1994; *a*: gas production from the immediately soluble fraction (ml/g DM); *b*: gas production from the insoluble or slowly degradable fraction (ml/g DM); *c*: rate of gas production from the slowly degradable fraction (hr^{-1}); Vt_{orsk}: total gas production by Ørskov and McDonald (1979) (ml/g DM)

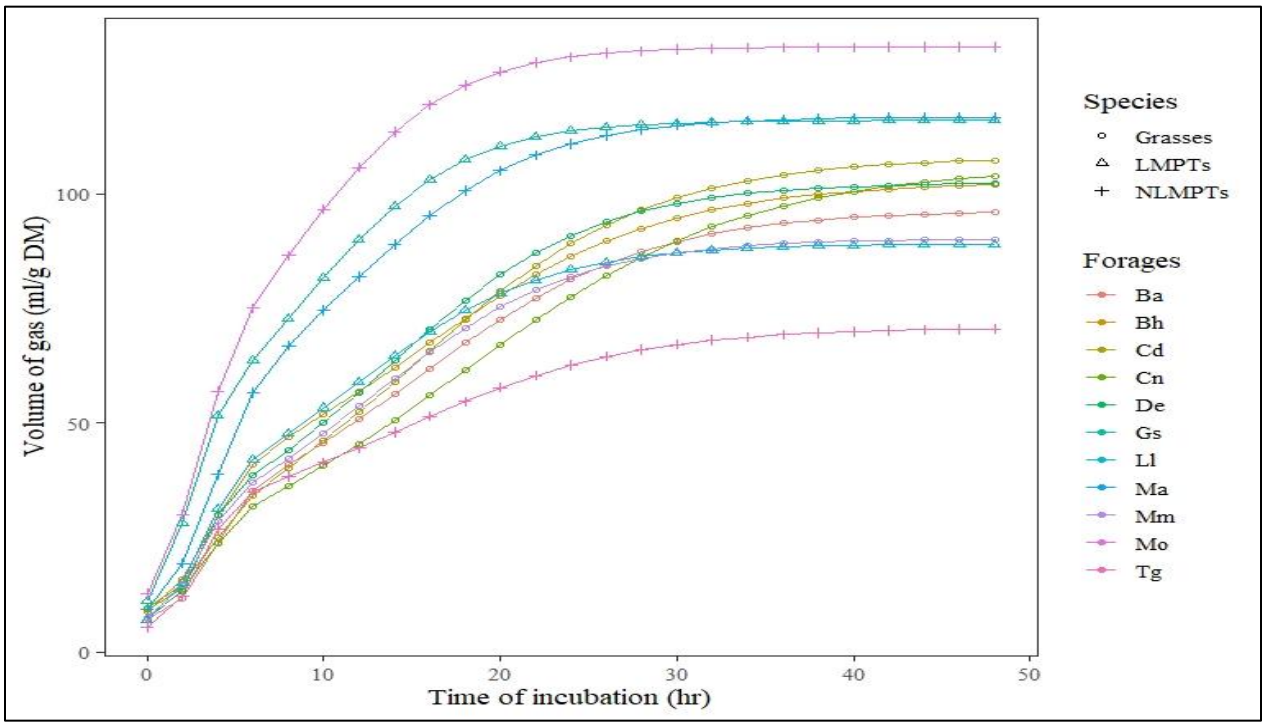

Figure 1: The mean cumulative gas volume for all forage species using the dual pool logistic model by Schofield et al. (1994)

Terms used: Ba: *Brachiaria arrecta*; Bh: *Brachiaria* hybrid (*Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)); Cd: *Cynodon dactylon*; Cn: *Cynodon nlemfuensis*; De: *Digitaria eriantha*; Gs: *Gliricidia sepium*; Ll: *Leucaena leucocephala*; Ma: *Morus alba*; Mm: *Megathyrsus maximus*; Mo: *Moringa oleifera*; and Tg: *Trichanthera gigantean*

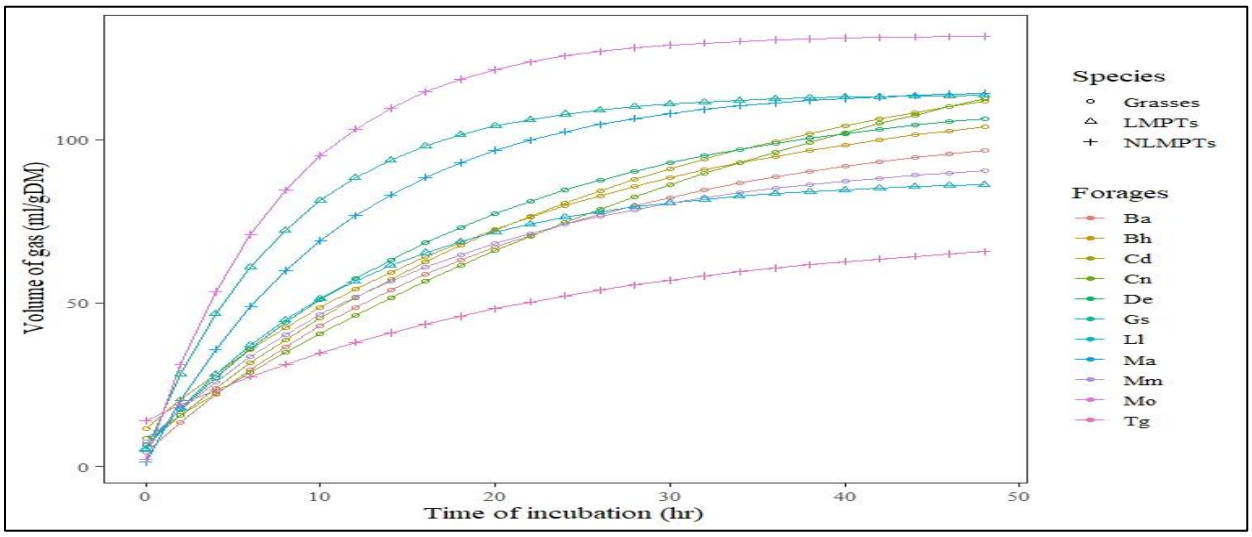

Figure 2: The mean cumulative gas volume for all forage species using the single pool model by Ørskov and McDonald 1979

Terms used: Ba: *Brachiaria arrecta*; Bh: *Brachiaria* hybrid (*Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)); Cd: *Cynodon dactylon*; Cn: *Cynodon nlemfuensis*; De: *Digitaria eriantha*; Gs: *Gliricidia sepium*; Ll: *Leucaena leucocephala*; Ma: *Morus alba*; Mm: *Megathyrsus maximus*; Mo: *Moringa oleifera*; and Tg: *Trichanthera gigantean*

Relationship between chemical composition and fermentation parameters

The relationship between the chemical components of forages and fermentation parameters can be observed in Table 5. There was a strong positive relationship between the fats and the slow pool of the dual-pool model (V_2) (r = 0.725) and the gas pool of the slowly degradable fraction for the single-pool model (*b*) (r = 0.811). The relationship between the NDF and the V_2 and *b* gas pools were negative (r = -0.651 and -0.751 respectively). There was a significant positive relationship between the Vt_{scho} and fat, GE and the DOMD (r = 0.428, 0.473 and 0.773 respectively). The relationship between the ash and the Vt_{scho} and the Vt_{orsk} were negative (r = -0.547 and -0.578 respectively). There was a significant and negative relationship between the Vt_{scho} and the ADF (r = -0.428).

Table 5: The correlation between chemical components (g/k DM) (including crude protein (CP), fat, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, ash), gross energy (GE, MJ/kg DM), digestible organic matter in dry matter (DOMD, %) and the fermentation parameters for the dual and single pool models

	Dual pool Logistic Model (Schofield et al. 1994)					Single pool Model (Ørskov and McDonald 1979)			
	V_1	V_2	C_1	C_2	Vt_{scho}	a	b	c	Vt_{orsk}
CP	0.470*	-0.163	-0.265	0.414	0.195	-0.284	0.046	0.518*	0.135
Starch	0.592*	-0.236	-0.194	0.477*	0.219	-0.151	-0.140	0.500*	0.144
NDF	-0.781*	0.301	0.265	-0.651*	-0.298	0.359	0.122	-0.751*	-0.197
ADF	-0.655*	0.047	0.476*	-0.363	-0.428*	0.335	-0.176	-0.488*	-0.352
Lignin	0.359	-0.539*	0.009	0.476*	-0.210	0.002	-0.481*	0.521*	-0.290
Fat	0.746*	-0.074	-0.136	0.725*	0.469*	-0.341	0.058	0.811*	0.400
Ash	-0.055	-0.585*	-0.050	-0.188	-0.547*	0.431*	-0.517*	-0.216	-0.578*
GE	0.526*	0.111	-0.116	0.615*	0.473*	-0.491*	0.134	0.705*	0.428*
DOMD	0.537*	0.448*	-0.140	0.524*	0.773*	-0.553*	0.476*	0.556*	0.742*

* $P \leq 0.05$ (n = 22); Terms used: V_1: fast pool (ml/g DM), V_2: slow pool (ml/g DM), C_1: fast rate (hr^{-1}), C_2: slow rate (hr^{-1}); Vt_{scho}: total gas production by Schofield et al. 1994; *a*:gas production from the immediately soluble fraction (ml/g DM); *b*: gas production from the insoluble or slowly degradable fraction (ml/g DM); Vt_{orsk}: total gas production by Ørskov and Mcdonald (1979) (ml/g DM); *c*: rate of gas production from the slowly degradable fraction (hr^{-1}); LMPTs: leguminous multipurpose tree species; NLMPTs: non-leguminous multipurpose tree species

Fermentation end products

The total VFAs ranged between 13.09 – 17.09 mmol/l for grasses and 8.92 – 20.81 mmol/l for the MPTs (Table 6). The MBM values obtained for grasses ranged between 112 – 170 mg/g DM and MPTs between 140 – 340 mg/g DM.

Table 6: The volatile fatty acid concentrations (% molar proportions); total volatile fatty acid (TVFA, mmol/l); the acetate to propionate ratio (A:P); and microbial biomass (MBM) yield (mg/g DM) after 48 hour incubation of grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

Grasses	Ac	Pr	Isobut	But	Isoval	Val	TVFA	A:P	MBM
Brachiaria arrecta	58.7	30.6	0.361	8.70	0.563	1.050	15.05	1.92	136
Brachiaria hybrid*	53.2	36.7	0.292	8.33	0.324	1.185	15.00	1.45	137
Cynodon dactylon	60.7	31.8	0.368	5.96	0.522	0.668	13.82	1.93	147
Cynodon nlemfuensis	63.7	31.1	0.383	3.65	0.466	0.707	17.09	2.05	112
Digitaria eriantha	58.4	31.9	0.258	8.28	0.295	0.835	15.68	1.83	135
Megathyrsus maximus	60.4	31.0	0.390	6.63	0.558	1.061	13.16	1.95	170
LMPTs									
Gliricidia sepium	66.1	26.2	0.304	5.77	0.560	1.069	16.01	2.53	222
Leucaena leucocephala	64.9	27.0	0.388	6.07	0.682	0.897	13.09	2.41	247
NLMPTs									
Morus alba	63.0	25.0	0.386	9.48	0.737	1.369	18.32	2.52	235
Moringa oleifera	60.1	26.2	0.469	11.14	0.846	1.242	20.81	2.30	140
Trichanthera gigantea	73.2	24.9	0.126	1.31	0.208	0.253	8.92	2.94	340
LSD (0.05)	3.94	3.02	0.11	1.29	0.19	0.24	2.15	0.34	21.56
P value	< 0.0001	< 0.0001	0.003	< 0.0001	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001

* *Brachiaria* hybrid *(Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*))

Terms used: Ac: acetate; Pr: propionate; Isobut: isobutyrate; But: butyrate; Isoval: isovalerate and Val: valerate

Discussion

Fermentation parameters were derived using the dual pool logistics model of Schofield et al. (1994) and the single pool model of Ørskov and McDonald (1979). The different parameters from each of the models give critical insight into the fermentation kinetics of a range of tropical forages. Tropical grasses are typically high in fibrous fractions which decrease the fermentability of forages and in the current study NDF and ADF were generally high (NDF: > 650 and ADF: > 400 g/kg DM for NDF and ADF respectively) (Gemeda and Hassen 2014; Nussio et al. 1998; Van Soest et al. 1991). From the dual pool model, the size of the slow and fast pools was determined, and the more fibrous grasses had a 40% smaller fast pool (V_1) and 30% larger slow pool (V_2) than the MPTs. Correlation analysis between the fermentation parameters and chemical composition of the forages in this study suggest that the more digestible species (eg. MPTs) with lower ADF and NDF fractions were strongly associated with a larger V_1 pool, than the generally less digestible grasses with higher fibre fractions. The size of the different pools must also be considered in combination with rates of gas production. Surprisingly, the average C_1 for grasses was over 20% higher than that of the MPTs. There is no clear explanation for the higher C_1 observed for the grasses given their less fermentable nature. However, if the average size and rates of the individual gas pools are considered, grasses were overall slower to ferment and produce gas compared to the MPTs.

Further, fitting the single pool model to the accumulated gas production data provided additional information about the fermentation of the forages studied. An overall estimate of the rate of fermentation (c) of the slowly fermentable pool (b) was obtained and the MPTs fermented (0.086 hr^{-1}) twice as fast as the grasses (0.043 hr^{-1}). The lower rate of

degradation estimated by the model for grasses may further indicate the lower fermentability of grasses compared to the MPTs. Additionally, the average *b* pool for the fibrous grasses was approximately 16% higher than that of the MPTs which was expected and consistent with the literature (Bezabih et al. 2014). The increase in the gas volumes for grasses was different to the MPTs where gas production increased but plateaued or was near plateauing before the 48 hours (Figures 1 and 2). The low degradability of grasses over time may indicate that nutrients are not as readily accessed in tropical grasses. This may explain the generally lower performance on these forages and the importance of supplementing with MPTs of higher degradability, to ensure that there is an adequate supply of both bypass and rumen degradable nutrients (Soliva et al. 2008).

The Vt_{scho} and Vt_{orsk} at 48 hours of incubation for the grasses were within the range reported in the literature for tropical species (65.6 to 174.2 ml/g DM) (Gemeda and Hassen 2014; Teguia et al. 1999). Also, based on Table 5 as well as Figures 1 and 2, the Vt_{scho} and Vt_{orsk} observed for the MPTs at 48 hours were wide ranging. This may be linked to differences in the chemical composition of the individual species and the presence or absence of anti-nutritional factors in the MPTs (Apori et al. 1998; Kafilzadeh and Heidary 2013). The Vt_{scho} and Vt_{orsk} at 48 hours for *M. oleifera* was at the higher end of the range for gas production which was followed by *G. sepium* and *Morus alba.* The comparably greater fermentability of these species is not surprising given their higher nutritive value in comparison to other tropical MPTs (Hernández and Sánchez 2014; Valdes et al. 2017). At 48 hours, the Vt_{scho} and Vt_{orsk} for *T. gigantea* and *L. leucocephala* were at the lower end of the range for the MPTs, however, supplementation with more fermentable species may improve the overall digestibility of diets comprising these forage species.

Further, the lower gas volumes observed for the *G. sepium* and *L. leucocephala* at 48 hours may also be explained by the high MBM yield. Digestible substrate is either partitioned towards the synthesis of MBM or fermentation gases and there is often an inverse relationship between gas production (or VFA production) and the synthesis or yield of MBM (Blümmel and Bullerdieck 1997). Microbial biomass represents an important source of amino acids (70 to 80 % of supply (AFRC 1992)) required to support production in ruminants (Nolan 1981). Having the right balance of both protein and energy supports high microbial efficiency (Clark et al. 1992). Therefore, forages must be selected on a combination of both gas production potential and the potential to yield microbial biomass (Hoover and Stokes 1991; Makar 2004).

Volatile fatty acids constitute the major source of energy for the ruminant providing 70 to 80% of its energy requirements (Annison 1970; Bergman et al. 1965; Warner 1964). In the current study, the total VFA production ranged between 13.1 to 17.09 mmol/l for grasses and 8.92 to 20.81 mmol/l for MPTs. The molar proportions of VFAs produced is influenced by the substrate fermented which in turn influences the amount of gas produced (Beuvink and Spoelstra 1992). High concentrations of fermentable substrate yield higher concentrations of propionate resulting in lower A:P ratios compared to less rapidly fermented substrate that yield higher concentrations of acetate and butyrate; and lower propionate leading to higher A:P ratios (Janssen 2010). However, the A:P ratios observed for the more fibrous grasses was at a lower range compared to that of the MPTs in the current study. There is no clear explanation for this as the higher nutritive value and fermentability of the MPTs was expected to result in lower A:P ratios than the tropical grasses of low nutritive value and fermentability. The time at which values were measured may have affected the observed values of the current study. For example, Niderkorn et al. (2011) and Rivero et al. (2020) observed lower A:P ratios at earlier sampling times (3.5 hrs) compared to later sampling

times (24 hours). Therefore, at earlier sampling times, the breakdown of the more fermentable fractions may have led to a greater production of propionate and therefore lower A:P ratios particularly for the more fermentable MPTs.

Conclusion

Overall, the MPTs were more fermentable than the grasses based on the fermentation parameters derived using the dual and single pool models. The correlation analysis between fermentation parameters and chemical composition of the forages observed indicated that the more digestible species (eg. MPTs) with lower ADF and NDF fractions may be a greater source of readily available fermentable nutrients than the more fibrous grasses. The overall higher Vt_{scho} and Vt_{orsk} for the MPTs at 48 hours also suggests the higher fermentability of these species. Further, the comparably higher total gas production observed for *M. alba* and *M. oleifera* at 48 hours suggest that these more fermentable species can be used to supplement and improve the fermentability of diets comprising grasses or MPTs as *T. gigantea* and *L. leucocephala* with a lower total gas production at 48 hours. Based on the overall chemical composition, *in vitro* digestibility and fermentation parameters, *M. oleifera and M. alba* demonstrated high performance whereas *T. gigantea* performed poorly.

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