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In Vitro Gas Production of Common Southeast Asian Grasses in Response to Variable Regrowth Periods in Vietnam

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Abstract: The relationship between DM yield/cutting and the fermentable organic matter (FOM) content of tropical grasses was appropriately investigated to re-assess optimal grass maturity to feed dairy cattle. Nine different grass species belonging to the genera *Brachiaria* spp. (Mulato II, Ruzi), *Panicum* spp. (Guinea, Hamil, Mombasa, TD58), and *Pennisetum* spp. (King, Napier, VA06) were chemically analysed and subjected to an in vitro gas production (IVGP) test. For 72 h, gas production (GP) was continuously recorded with fully automated equipment. A triphasic, nonlinear, regression procedure was applied to analyse GP profiles. Across all the grasses, it was found that the neutral detergent fibre (NDF) contents increased with increasing maturity of the grass while the CP contents decreased with increasing NDF contents. In all nine grasses, digestible organic matter (dOM) was significantly affected by the week of cutting but IVGP was similar between the weeks of cutting in Ruzi, Hamil, Mombasa, and Napier grasses. Except for Guinea grass, the lowest dOM values were found when the grasses were cut after ≥5 weeks of regrowth. Harvesting grass one or two weeks earlier than the normal cutting time is a practically relevant intervention in increasing forage quality and productivity of dOM and fermentation potential.

Keywords: tropical grasses; ruminants; nutritive values; in vitro gas production; methane production; volatile fatty acids

1. Introduction

Dairy cows in Southeast (SE) Asian countries such as Thailand and Vietnam typically produce 4000–4500 kg of milk per lactation cycle with an average fat content below 4% [1–3]. In view of the low level of milk production compared to cows in temperate climates, the observed low milk fat content can be considered unexpected. It is generally accepted that the relatively low milk yield is not caused by genetics, given that the dairy cows are typically crossbreds, predominantly Holstein Friesian (>87.5%) and local breeds [4]. Thus, it is reasonable to infer that environmental factors and management practices, such as nutrition, predominantly influence milk production rather than genetics [2,5]. Acetic acid (Hac) and ß-hydroxybutyric acid (Hbu) are important precursors of fatty acid synthesis in the mammary glands of dairy cows [6]. It can, therefore, be suggested that the supply of Hac and Hbu to the mammary glands of Thai and Vietnamese dairy cows is insufficient. It is well known that the aforementioned precursors of milk fat originate predominantly from organic matter that is fermented in the rumen [7]. It thus would appear that the rations typically fed in Thailand and Vietnam contain insufficient fermentable organic matter (FOM) to yield Hac and Hbu to ensure milk fat synthesis.

Fresh grasses are mainly used to compose dairy rations in SE Asian countries. According to custom, Thai and Vietnamese farmers practice cutting intervals of 6 to 9 weeks, depending on the grass species in question, i.e., typically grasses that belong to the genera *Pennisetum*, *Panicum*, and *Brachiaria*. Cutting intervals of 6 to 9 weeks result in high dry matter (DM) yields per cutting but the harvested grasses are physiologically mature and, therefore, very fibrous and low in crude protein (CP). Furthermore, Huyen et al. [8] recently reported that, across the three aforementioned genera of grasses, in vitro gas production [9,10] was, on average, only ~9% greater compared to that of rice straw, thereby indicating that the FOM content of tropical grasses is relatively low when they are harvested under practical farming conditions. In temperate grasses, such as *Lolium perenne*, it is well established that a prolonged cutting interval is negatively associated with the FOM content of the grass [11]. In tropical grasses, however, the relationship between DM yield/cutting and FOM content is poorly understood due to a dearth of studies addressing this association. As such, whether the relationship between cutting interval/maturity of fresh grass and rumen digestibility, as found in temperate grasses, holds true for tropical grasses is still unknown. Therefore, the objective of the current research was to provide novel information on the relationship between the cutting intervals of common Southeast Asian grasses and rumen degradation. To achieve this, we conducted an in vitro study using cumulative gas production and organic matter (OM) degradability as primary indicators of the FOM content of tropical grasses. We hypothesized that a shorter regrowth period of tropical grasses commonly used for dairy rations in SE Asia would result in increased fermentability.

2. Materials and Methods

2.1. Grass Collection

Nine different grass species (Mulato II (*Brachiaria ruziziensis* × *Brachiaria decumbens* × *Brachiaria brizantha*); Ruzi (*Brachiaria ruziziensis*); Guinea (*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs, formerly named *Panicum maximum* Jacq.); Hamil (*Panicum maximum* cv. Hamill); Mombasa (*Panicum maximum* cv. Mombasa); TD58 (*Panicum maximum* cv.TD58); King (*Pennisetum purpureum* × *Pennisetum glaucum*); Napier (*Pennisetum purpureum* Schumach.); and VA06 (*Pennisetum purpureum* × *Pennisetum americanum*)) were harvested at up to nine different weekly regrowth ages from June to August 2018 at the Animal Husbandry Research and Development Centre for Mountainous Zone (ARDC), Song Cong town, Thai Nguyen province, Vietnam. The centre is located at 21°29′14″ N 105°48′47″ E and experiences an annual rainfall of 2168 mm with an average temperature of 23 °C. The plot area used for each grass variety was 400 m². An initial fertilizer dressing of N:P:K with 160:80:80 kg/ha/yr was applied at sowing, with further annual applications at the same rate. Annually, an amount of 20 tons/ha/yr of cattle manure was applied manually. The cattle manure used was not chemically analysed, but typically manure from Vietnamese dairy cattle contains N:P:K with a ratio of 4.0:1.9:1.6 [12].

Chemical analyses and gas production were carried out on grasses at four cutting time points, excluding Napier and VA06 grasses of *Pennisetum* spp. and Guinea and TD58 grasses of *Panicum* spp. The selection of these time points was based on practical harvest times in Vietnam, including two time points before practical cutting, one during practical cutting, and one for late cutting. As it is unknown whether normal practical cutting provides precise nutritive information for determining the suitable cutting age, two grasses from each of the two most commonly used genera, based on the advice of recognized experts in ruminant nutrition, were selected for additional evaluation. These selections ranged from week 1 to 9 for *Pennisetum* spp. and from week 1 to week 6 for *Panicum* spp.

At each grass plot, a 10 m × 10 m area was marked out for sampling, and by walking in a 'W' pattern, 20 evenly spaced cores were manually collected using a sickle. The grass was harvested, leaving around 10 cm of stubble above ground level. After harvesting, each selected species and harvesting time grass sample was manually cut to 3 cm and mixed thoroughly before collecting a 5 kg representative sample which was divided equally

into two bags (one for analysis and one for reserve) and stored at $-20\ ^\circ$C in Vietnam. Subsequently, all the frozen grass samples were transported to Wageningen University & Research (Wageningen, The Netherlands), maintaining $-20\ ^\circ$C conditions, for analyses.

2.2. Chemical Analyses

Upon arrival at Wageningen, the frozen fresh grass samples were thawed and dried for 16 h at 70 $^\circ$C before being ground (1 mm screen) using a cross beater mill (Peppink 100 AN, Deventer, The Netherlands) and analysed in duplicate for DM, crude ash. The OM content was calculated as the difference between DM and ash contents. Ether extract (EE) was determined by the Soxhlet method with petroleum ether as a solvent following AOAC [13] method no. 963.15. Crude protein was calculated from nitrogen (N $\times$ 6.25) obtained via the Kjeldahl method [14]. The neutral detergent fibre (NDF; with heat stable α-amylase) content was analysed according to Van Soest et al. [15] while acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were determined according to Van Soest [16].

2.3. In Vitro Gas and CH$_4$ Production

Cumulative in vitro gas (IVGP) and methane (CH$_4$) production over 72 h were measured in a fully automated gas production system [9]. Each ground and dried grass (~0.5 g) was accurately weighed in quadruplicate 250 mL fermentation bottles (Schott, Mainz, Germany). Duplicate bottles were randomly distributed across three runs. Blank bottles (rumen fluid without grass) were used in triplicate for each run. The two non-lactating Holstein-Friesian rumen fluid donor cows were fed grass silage (NEL, 4.37 MJ/kg DM; CP, 99 g/kg DM; NDF, 675 g/kg DM) ad libitum and had free access to water. Approximately 350 mL of rumen fluid was collected from each cow using a tube inserted via the oesophagus before the morning feeding at the research farm of Wageningen University, The Netherlands. Subsequently, the rumen fluid was pooled and filtered through cheesecloth and subsequently mixed (1:2 *v/v*) with an anaerobic buffer/mineral solution [15] under continuous flushing with CO$_2$. Prior to inoculation, the fermentation bottles were placed in a shaking water bath kept at 39 $^\circ$C and pre-flushed with CO$_2$. Sixty mL of buffered rumen fluid was added to the bottle before being connected to fully automated gas recording equipment for 72 h. After this time, the bottles were disconnected and placed on ice and 0.6 mL of the solution was pipetted into a 1.5 mL Eppendorf tube, and 0.6 mL of an internal standard solution (isocapronic acid) was added before vigorous mixing. After 5 min of centrifugation at 14,000$\times$ *g*, a 0.75 mL sample of the supernatant was taken and mixed with an equal volume (1:1, *v/v*) of a stock solution composed of 25 mL of 85% (*v/v*) orthophosphoric acid dissolved in 200 mL Millipore water (Merck KGaA, Darmstadt, Germany) and 300 mL of a 4 g/L 4-methylvaleric acid (internal standard) for volatile fatty acids (VFA) analysis. The mixture was then stored at $-20\ ^\circ$C pending analysis. VFA were analysed using a gas chromatograph (Trace GC Ultra, Thermo Scientific, Milan, Italy) equipped with a flame ionization detector and an Agilent HP-FFAP column (Agilent Tech., Santa Clara, CA, USA; 30 m length, 0.53 mm i.d., 1 μm film) using hydrogen as carrier gas (25 kPa, constant pressure). Isocaproic acid was used as an internal standard.

After 72 h of incubation, fermentation fluids from sample bottles were filtered in respective crucibles (P2 standard with pore size 40–100 μm, Foss, Hillerød, Denmark) with a filter plate of sintered glass and 0.5 cm washed and incinerated sea sand (VWR, art. no. 1.07711.5000). Before using the crucibles, they were washed with hot water and dried at 103 $^\circ$C for 1 h, then ashed at 530 $^\circ$C for 1 h and finally placed in a desiccator for 1 h to cool down before weighing with an analytical balance of 0.1 mg precision. The crucibles containing fermentation fluids were then vacuum drained and washed with hot distilled water by a cold extraction unit (FT 121 Fibertec™, Foss, Hillerød, Denmark) to remove microbial matter from the undegraded substrates, and then dried at 103 $^\circ$C for 4 h and ashed at 530 $^\circ$C for 2 h. The difference between these two values was termed residual OM.

The degraded OM (OMd) was calculated as the difference between incubated and residual OM after 72 h of fermentation.

Precisely 10 µL of the headspace gas was collected from each fermentation bottle and directly injected into a gas chromatograph to determine headspace CH_4 production at 0, 3, 6, 9, 12, 24, 30, 36, 48, 60, and 72 h, as described by Pellikaan et al. [10,17]. Briefly, measured CH_4 production in individual bottles was expressed relative to the maximum production in each bottle and was fitted iteratively with a monophasic model. Methane production at each individual valve opening was then calculated, and cumulative CH_4 was determined as the sum of the increase in headspace CH_4 production between two successive valve openings, and the amount of CH_4 vented from the bottle.

2.4. Curve Fitting and Calculations

Gas and CH_4 production from all samples were corrected for the corresponding production by blank bottles at each time point [9,10]. Before curve fitting, the cumulative gas production curves of quadruplicate bottles per sample were visually inspected and coefficients of variation (CV) were determined for values at 8, 12, 24, 48, and 72 h. If the CV > 10%, the gas production curves were evaluated for outlier replicate bottles. The non-linear least squares regression procedure was used [18] and the data were fitted according to the following equation, as outlined by Groot et al. [19]:

$$GP = \sum_i^n \frac{A_i}{1 + \left(C_i / t \right)^{B_i}}$$

where GP (mL/g OM) is the cumulative produced gas or CH_4; n = total number of phases; i = number of phases; Ai (mL/g OM) is estimated asymptotic gas or CH_4 production in phase i; Bi is a constant determining the switching characteristic of the curve in phase i; Ci (h) is the time at which half of the asymptotic gas or CH_4 production was reached in phase i; and t (h) is the time of incubation.

A tri-phasic model (n = 3) was fitted to the cumulative gas production following the procedure as described by Groot et al. [19], where phases 1 and 2 are assumed to relate to the fermentation of the soluble and non-soluble fraction, respectively, and phase 3 is assumed to be related to microbial turnover. The time windows related to the asymptotes of GP for phases 1, 2, and 3 (A1, A2, and A3, respectively) were pre-set from 0 to 3, 3 to 20, and 20 to 72 h after the start of incubation of the substrate, respectively, to enable the estimation of the various parameters (Bi and Ci, respectively). The aforementioned time points were empirically determined by Van Gelder et al. [20] based on the work of Cone et al. [21]. Data on CH_4 production were also fitted according to the above-mentioned model where n = 1.

2.5. Calculations and Statistical Analyses

The total VFA in fermentation fluid at 72 h was calculated as the sum of Hac, propionic acid (Hpr), butyric acid (Hbu), valeric acid (Hva), isobutyric acid (iso-Hbu), and isovaleric acid (iso-Hva). The branched-chain volatile fatty acids (BCVFA) in fermentation fluid were calculated as the sum of iso-Hbu and iso-Hva. The non-glucogenic to glucogenic ratio (NGR) was calculated as described by Ørskov [22]:

[acetate + 2 × (Hbu + isoHbu) + Hva + iso-Hva]/[Hpr + Hva + iso-Hva].

The most commonly used grass in Vietnam for each genus (Mombasa, Mulato II, and King grass) was selected to calculate the estimated yield of FOM indicators as an example. Normal practical cutting was considered as 100% of in vitro digestible OM (dOM) and fermentation potential (GP, A1 + A2) yield, whereafter the percentage of other cutting yields was calculated. For Mombasa and Mulato II grass, biomass yield equations (kg

DM/ha/yr) were derived from data reported by Hare et al. [23,24], respectively, after the conversion of biomass yields per year:

$$Y_{Mo} = 0.1120x^2 + 52.080x \ (0 \leq x \leq 90; R^2 = 0.95)$$

$$Y_{Mu} = 0.7423x^2 + 34.672x \ (0 \leq x \leq 90; R^2 = 0.99)$$

For King grass, biomass yield (kg dry matter/ha/yr) was determined using the equation provided by Sales et al. [25]:

$$Y_{Ki} = -1.2426x^2 + 282.64x \ (0 \leq x \leq 120)$$

where Y_{Mo}, Y_{Mu}, and Y_{Ki} are the estimated yield of Mombasa, Mulato II, and King grass, respectively; x is cutting time in days after regrowth.

Pearson's correlation coefficients between predicted (i.e., GP, TVFA, etc.) and predicting (i.e., cutting ages, chemical components, etc.) variables were determined if data were normally distributed as tested using Kolmogorov–Smirnov. If data were not normally distributed, Spearman's correlation coefficients were used. Effects of regrowth age within each grass were subjected to analysis of variance (ANOVA) using the PROC MIXED procedure [18] using the following model:

$$Y_{ij} = \mu + H_i + R_j + e_{ij}$$

where Y_{ij} = response variable (i.e., GP-72, CH_4-72 production, fermentation kinetics parameters), μ is the overall mean, H_i is the effect of harvest time (i = 1 to 9 regrowth week), R_j is the random effect of run j (j = 1 to 3), and e_{ij} is the residual error term. Differences among harvest times within each grass were determined using the least square means procedure and Tukey's multiple comparisons. Studentized residuals were checked for normal distribution. Residuals were checked per grass species (*n* = 9) with Kolmogorov–Smirnov. All response variables for all grasses were found to be normally distributed except for the BCVFA of Mombasa grass. Therefore, the data of BCVFA were transformed and normality was achieved by applying a square transformation. Throughout, the level of statistical significance was pre-set at *p* < 0.05 while a trend was declared at 0.05 ≤ *p* < 0.10.

3. Results and Discussion

3.1. Chemical Composition of Tropical Grasses at Different Regrowth Ages

The OM content of the grasses and advanced cutting age (Table 1) showed a moderate correlation (*r* = 0.56, *p* < 0.001, *n* = 49). Those belonging to the *Pennisetum* genus (especially King and VA06) generally showed a stronger correlation of OM content with cutting age (*r* = 0.83, *p* < 0.001, *n* = 21). This trend is consistent with the findings of Mutimura et al. [26] who reported that the OM content of Napier grass increased until 90 d after planting and then declined. The increase in OM might be attributed to the fact that grass is still in the development stage, during which OM accumulates relative to the inorganic matter.

The values related to cell wall constituents (NDF, ADF, and ADL) increased with the advancement of grass maturity (*r* = 0.56, 0.60, 0.61, and *p* < 0.001, respectively). The current data were found to be in line with other previous reports [27,28]. Tropical grasses develop thick-walled cells with increased cell wall fractions, including cellulose, hemicellulose, and lignin, as a structural adaptation to minimize photorespiration, enhancing overall resilience to tropical environmental conditions, that helps contribute to the plant's robustness owing to both the thickness and composition of cell walls [29]. Consequently, the NDF content of tropical grasses is higher than temperate grasses (60–75 vs. 35–67% DM) [30–32].

Table 1. Chemical composition (g/kg dry matter) of nine common Southeast Asian grasses at different stages of maturity.

Grass	Week	OM	CP	EE	NDF	ADF	ADL
Mulato II	2	851	226	28.8	519	255	20.3
	4	846	147	25.7	498	234	19.3
	6	858	113	19.2	607	316	24.5
	8	860	118	18.1	656	362	30.9
Ruzi	2	889	179	32.2	536	268	21.5
	4	878	163	25.7	505	234	21.4
	6	915	116	24.5	660	354	26.7
	8	888	162	25.6	622	328	29.8
Guinea	1	864	226	24.4	600	327	21.2
	2	882	218	28.7	599	313	20.2
	3	912	175	26.5	657	349	26.5
	4	877	180	26.3	677	375	31.6
	5	870	143	31.7	659	366	30.6
	6	876	137	28.5	675	373	30.6
Hamil	2	844	254	29.7	572	293	14.9
	4	876	97	23.3	732	413	27.5
	5	845	96	-	-	410	-
	6	840	85	-	-	409	-
Mombasa	2	871	171	25.5	641	350	20.2
	4	860	124	28.6	669	365	20.1
	5	876	114	26.5	696	375	19.0
	6	884	90	23.4	730	406	24.4
TD58	1	827	226	22.3	565	270	19.1
	2	857	107	28.6	673	364	22.2
	3	875	132	28.6	688	372	20.1

Grass	Week	OM	CP	EE	NDF	ADF	ADL
TD58	4	873	138	24.5	644	310	18.1
	5	855	148	27.5	676	362	21.2
	6	863	165	24.3	667	350	16.9
King	3	859	181	24.8	598	336	17.2
	5	869	136	33.4	602	336	19.4
	7	896	87	27.9	646	377	23.6
	9	920	103	23.7	659	409	47.3
Napier	2	881	161	30.9	620	331	21.3
	3	859	165	26.7	581	316	22.5
	4	879	184	25.9	538	270	20.5
	5	871	176	28.9	569	310	23.6
	6	858	167	29.0	594	318	27.9
	7	882	140	28.0	646	353	28.0
	8	890	117	24.7	670	362	23.6
	9	911	132	18.7	696	397	33.1
VA06	1	824	298	28.8	491	276	21.4
	2	811	223	23.6	541	287	30.0
	3	867	256	23.7	560	304	19.4
	4	851	156	25.8	593	324	22.5
	5	872	139	26.8	646	354	25.8
	6	892	102	26.8	682	395	32.1
	7	913	88	24.7	694	395	35.5
	8	902	74	20.4	717	436	51.5
	9	902	89	23.6	706	411	44.0

ADF = acid detergent fibre, ADL = acid detergent lignin, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, OM = organic matter, - = not determined.

In the present study, an increase in the cell wall constituents of tropical grasses (for instance, NDF) was associated with a decrease in CP content with advancing cutting ages, and this decrease was even more pronounced at later stages ($r = -0.75$, $p < 0.001$), which is consistent with observations of others in temperate grasses [33–35]. A notable example is the CP content of VA06, which significantly decreased from approximately 30 to 7.4% in the DM between the first and eighth week. Despite the decline in CP content with advancing grass maturity, the final CP content still exceeded the minimum CP level (7%) required for rumen function [36], although the CP content in rations recommended by the NRC [37] for lactating cows ranges from 14 to 18% DM.

The lipid content (EE) of the selected grasses ranged from 1.9 to 3.2% DM, which is comparable to the values reported by Melesse et al. [38] for tropical grasses (1.1–3.1% DM). The EE of the *Brachiaria* genus in the current study correlated well with increasing maturity age ($r = -0.77$, $p = 0.02$, $n = 8$). The other grasses showed a trend of an increase in EE content from the early to the middle stage and then a decline from the middle to the late stage of maturity.

In general, the reduction in cell contents, in particular CP content, was countered by the accumulation of structural carbohydrates as the grass matured.

3.2. In Vitro Gas and CH$_4$ Production Parameters of Grasses Belonging to the Brachiaria Genus

As shown in Table 2, the in vitro dOM, cumulative CH$_4$ production measured after 72 h of incubation, and branched-chain volatile fatty acids (BCVFA) of Mulato II grass

were significantly influenced by harvesting time. The highest numerical values of this grass were found at week 4 in all parameters (except for BCVFA), which is earlier than the commonly used cutting time (week 6) under practical farming conditions in Vietnam. Nevertheless, the highest quantity of cumulative CH_4 production measured after 72 h of incubation (CH_4-72) observed at week 4 may raise environmental concerns, whereas the CH_4 percentage (CH_4:GP-72) was not different between cutting weeks. This is unexpected due to the low content of fibre at week 4 compared to the other weeks. These findings contrast with Neto et al. [39] and Ruggieri et al. [40] who reported that forages rich in structural carbohydrates tend to yield greater amounts of CH_4 and a decreased digestibility compared to forages higher in non-structural carbohydrates. It is well known that high levels of non-fibre carbohydrates in the diet stimulate rumen Hpr production, which subsequently reduces CH_4 synthesis by the methanogens [41,42]. In Ruzi grass, total volatile fatty acid (TVFA) was highest when cut at week 4, whilst other parameters were not affected by grass maturity. Cutting grass at a later stage (i.e., after week 6) should not be beneficial in terms of fermentable organic matter (FOM) content.

Table 2. In vitro 72 h organic matter digestibility (dOM), gas (GP-72) and methane production (CH_4-72) parameters and volatile fatty acids related values of two grasses (Mulato II and Ruzi) belonging to the *Brachiaria* genus grown between 2 and 8 weeks.

Grass	Week	dOM	GP-72	A1 + A2	CH4-72	CH4:GP-72	TVFA	BCVFA	NGR	A:P
		g/kg OM		mL/g OM		% of GP-72	mM	% of TVFA	mol/mol	
Mulato II	2	781 [ab]	259	199	44.5 [b]	17.2	75.4	3.15 [a]	3.33	2.80
Mulato II	4	791 [a]	275	218	49.5 [a]	18.1	77.7	2.86 [ab]	3.50	3.04
Mulato II	6 *	724 [b]	246	183	41.4 [c]	17.0	71.4	2.58 [b]	3.38	2.76
Mulato II	8	726 [b]	234	180	39.7 [d]	17.1	75.9	2.60 [b]	3.40	2.81
Pooled SE		6.36	9.70	10.8	1.84	1.00	1.89	0.09	0.13	0.19
p value		0.031	0.100	0.091	<0.001	0.510	0.065	0.035	0.811	0.150
Ruzi	2	775	267	222 [x]	46.1	17.4	79.7 [ab]	3.08	3.45	3.01
Ruzi	4	794	272	216 [x]	47.8	17.8	80.5 [a]	2.68	3.60	3.01
Ruzi	6 *	710	247	193 [y]	42.6	17.3	75.3 [ab]	2.53	3.32	2.88
Ruzi	8	735	249	187 [y]	38.4	15.6	74.4 [b]	2.80	3.33	2.84
Pooled SE		8.90	12.5	8.97	3.02	1.46	2.01	0.07	0.16	0.17
p value		0.052	0.215	0.047	0.150	0.233	0.030	0.093	0.227	0.358

[a,b,c,d] Values within column and within grass with different superscripts differ ($p < 0.05$); [x,y] Values within column and within grass with different superscripts show a trend to be different ($0.05 \leq p < 0.10$). * Normal cutting age in Vietnam. A1 + A2 = in vitro fermentation potential of the soluble in insoluble carbohydrates; A:P = acetic to propionic acid ratio; BCVFA = branched-chain volatile fatty acids; NGR = non-glucogenic to glucogenic ratio; OM = organic matter; TVFA = total volatile fatty acid.

Overall, due to the high fermentation potential values (i.e., dOM, A1 + A2 and TVFA) and high values of volatile fatty acids used for milk fat synthesis (i.e., BCVFA), the recommended harvest age for grasses belonging to the *Brachiaria* genus appears to be at week 4 after the previous cut.

3.3. In Vitro Gas and CH_4 Production Parameters of Grasses Belonging to the Panicum Genus

The variation in in vitro gas and CH_4 production parameters of grasses belonging to the *Panicum* genus was found to be large (Table 3). In Guinea grass, cutting at the first three weeks of regrowth had more advantages (except for CH_4 production) than late cuttings. Normal practical cutting (week 5) resulted in higher values of in vitro dOM, GP-72, and A1 + A2 production compared to cutting one week earlier. Week 4 had the lowest values over almost all parameters. These discrepancies are not easy to explain but it can be speculated that the variation in those parameters does not properly reflect the FOM content. Cumulative CH_4-72, expressed in both terms (g/kg OM and proportion) was not systematically affected by harvest time, with week 4 having the least amount of CH_4 being different to the other weeks. This is due to the lowest value of fibre content at

week 4 compared to other weeks. The relationship between structural carbohydrates and CH_4 production was mentioned in the previous section.

Table 3. In vitro 72 h organic matter digestibility, gas (GP-72) and methane production (CH_4-72) parameters and volatile fatty acids related values of four grasses (Guinea, Hamil, Mombasa, and TD58) belonging to the *Panicum* genus grown between 1 and 6 weeks.

Grass	Week	dOM	GP-72	A1 + A2	CH4-72	CH4:GP-72	TVFA	BCVFA	NGR	A:P
		g/kg OM		mL/g OM		% of GP-72	mM	% of TVFA	mol/mol	
Guinea	1	739 ab	251 ab	182 ab	46.1 a	18.5	74.9	3.69 a	3.68	3.24
Guinea	2	762 a	251 a	190 a	48.0 a	19.4	78.0	3.74 a	3.72	3.31
Guinea	3	713 c	248 a	180 ab	47.1 a	19.2	77.6	3.44 ab	3.77	3.34
Guinea	4	625 e	189 c	116 c	27.2 b	14.4	71.3	3.01 c	3.68	3.20
Guinea	5 *	678 d	249 ab	182 ab	46.4 a	18.5	73.1	3.35 ab	3.58	3.12
Guinea	6	645 e	220 bc	167 b	43.2 a	19.7	70.2	3.25 ab	3.65	3.17
Pooled SE		4.28	7.59	5.58	2.83	1.74	2.25	0.11	0.14	0.18
p value		<0.001	0.007	<0.001	0.002	0.070	0.079	0.023	0.323	0.264
Hamil	2	768 a	254	193	49.5	19.5	78.0 a	3.86 a	3.56	3.17
Hamil	4	669 b	255	183	49.7	18.3	74.4 ab	2.93 b	3.61	3.11
Hamil	5 *	670 b	234	181	47.8	19.3	73.5 ab	2.90 b	3.60	3.13
Hamil	6	621 b	237	172	42.3	18.9	70.7 b	2.89 b	3.53	3.07
Pooled SE		7.13	15.1	12.3	4.62	1.57	2.48	0.09	0.12	0.16
p value		0.011	0.500	0.299	0.560	0.384	0.016	0.008	0.777	0.320
Mombasa	2	709	252	187	47.5	18.9	73.9	3.25 a	3.59	3.14
Mombasa	4	726	280	210	52.2	18.7	76.3	3.20 a	3.58	3.15
Mombasa	5 *	704	273	211	46.9	17.1	81.7	2.95 b	3.57	3.08
Mombasa	6	672	265	188	45.4	17.3	74.8	2.80 c	3.60	3.10
Pooled SE		11.1	7.36	4.26	3.41	1.73	2.08	0.03	0.10	0.16
p value		0.310	0.378	0.225	0.072	0.575	0.405	0.002	0.727	0.725
TD58	1	797 a	286	197	54.2 ab	19.1	78.0	4.19 a	3.92 a	3.36 a
TD58	2	774 ab	267	203	51.8 ab	19.2	76.3	3.34 b	3.53 b	3.07 b
TD58	3	735 bc	270	201	49.3 ab	18.2	77.4	2.94 c	3.56 b	3.07 b
TD58	4	773 ab	293	207	53.9 a	18.5	79.2	3.19 bc	3.73 ab	3.16 b
TD58	5 *	728 c	271	200	49.2 ab	18.3	73.5	3.16 bc	3.63 b	3.14 b
TD58	6	740 bc	264	195	45.1 b	17.3	75.1	3.24 bc	3.68 ab	3.16 b
Pooled SE		7.08	5.43	6.97	2.61	1.02	1.7	0.08	0.12	0.15
p value		0.009	0.087	0.670	0.009	0.171	0.115	<0.001	0.028	0.016

[a,b,c,d,e] Values within column and within grass with different superscripts differ ($p < 0.05$). * Normal cutting age in Vietnam. A1 + A2 = in vitro fermentation potential of the soluble in insoluble carbohydrates; A:P = Hac to Hpr ratio; BCVFA = branched-chain volatile fatty acids; NGR = non-glucogenic to glucogenic ratio; TVFA = total volatile fatty acid.

For Hamil grass, dOM gradually declined ($p < 0.001$) with grass maturity whilst GP and A1 + A2 did not differ among weeks, although a numerical decrease was observed. A1 + A2 values were different between weeks 2 and 6 with no difference in CH_4-72 or CH_4:GP-72 values. Cutting at the normal practical cutting time (week 5) did not differ from the other weeks, except for dOM and BCVFA. Under the assumption that NGR and A:P are the indicators related to milk fat synthesis, cutting at any given week between 2 and 6 produced similar results. Week 2 had the highest dOM, TVFA, and BCVFA values, making it the most suitable harvest time for Hamil grass without concerns about increased CH_4 production.

For Mombasa grass, except for BCVFA, expressed as a percentage of TVFA, which had high values at either week 2 or week 4, all other parameters did not vary with cutting age.

For TD58 grass, significant effects of cutting time were observed for dOM, CH_4-72, BCVFA, NGR, and A:P. The lowest values were observed in either week 2 or 3 for dOM, BCVFA, NGR, and A:P corresponding to the high NDF content at these two weeks. GP, A1 + A2, CH_4 proportion, and TVFA were similar throughout the harvesting ages. The NGR and A:P ratio had the highest value at week 1 but were similar from weeks 2 to 6. It should be noted that cutting every week would produce the biomass with the highest FOM.

In the present study, the NDF content of these four grasses was found to be negatively correlated with dOM and BCVFA concentrations ($r = -0.66$, -0.89; $p = 0.003$, <0.001, respectively). In general, it appears that harvesting grasses belonging to the *Panicum* genus before two weeks of regrowth provides the highest concentration of FOM biomass and, therefore, can be expected to yield the greatest milk fat content by dairy cows in Vietnam.

3.4. In Vitro Gas and CH$_4$ Production Parameters of Grasses Belonging to the Pennisetum Genus

As seen in Table 4, grasses belonging to the *Pennisetum* genus generally displayed a wide variation in their in vitro GP and CH$_4$ emission potentials.

Table 4. In vitro 72 h organic matter digestibility (dOM), gas (GP-72) and methane production (CH$_4$-72) parameters and volatile fatty acids related values of three grasses (King, Napier, and VA06) belonging to the *Pennisetum* genus grown between 1 and 9 weeks.

Grass	Week	dOM	GP-72	A1 + A2	CH4-72	CH4:GP-72	TVFA	BCVFA	NGR	A:P
		g/kg OM		ml/g OM		% of GP-72	mM	% of TVFA	mol/mol	
King	3	731 [a]	241 [b]	179 [c]	42.2 [b]	17.6	73.7	3.27 [a]	3.82	3.37 [a]
King	5	751 [a]	271 [a]	211 [a]	50.9 [a]	18.9	77.9	3.09 [a]	3.68	3.13 [b]
King	7 *	703 [a]	262 [a]	204 [b]	48.6 [a]	18.6	76.7	2.62 [b]	3.65	3.03 [b]
King	9	623 [b]	240 [b]	183 [c]	43.0 [b]	17.9	71.7	2.62 [b]	3.65	3.10 [b]
Pooled SE		8.08	9.66	9.23	2.54	1.40	1.17	0.10	0.13	0.17
p value		0.009	0.006	0.002	0.003	0.051	0.149	0.043	0.091	0.026
Napier	2	758	269	210	50.8 [ab]	18.9 [ab]	76.8	3.20 [ab]	3.73 [a]	3.33 [a]
Napier	3	757	265	195	52.7 [a]	20.0 [a]	76.9	3.25 [a]	3.78 [a]	3.34 [a]
Napier	4	762	285	206	53.2 [a]	18.7 [ab]	78.5	3.16 [ab]	3.79 [a]	3.24 [ab]
Napier	5	760	284	218	50.8 [ab]	18.0 [ab]	77.3	3.20 [ab]	3.74 [a]	3.23 [ab]
Napier	6	751	285	213	51.9 [a]	18.3 [ab]	76.3	3.20 [abc]	3.76 [a]	3.26 [ab]
Napier	7 *	770	278	217	52.3 [a]	18.9 [ab]	79.8	2.85 [abc]	3.65 [a]	3.15 [b]
Napier	8	740	287	211	52.2 [a]	18.2 [ab]	77.3	2.89 [bc]	3.67 [a]	3.18 [b]
Napier	9	728	271	207	46.2 [b]	17.1 [b]	77.8	2.67 [c]	3.53 [b]	2.98 [b]
Pooled SE		8.56	11.3	11.2	2.56	1.26	1.97	0.08	0.12	0.17
p value		0.087	0.160	0.120	0.013	0.029	0.804	0.009	0.003	0.012
VA06	1	764 [a]	216 [c]	152 [b]	39.6 [c]	18.1	69.9 [c]	4.16 [a]	3.77 [ab]	3.41 [a]
VA06	2	737 [ab]	245 [ab]	184 [ab]	46.0 [b]	18.8	73.8 [bc]	3.46 [bc]	3.84 [a]	3.40 [a]
VA06	3	756 [a]	274 [a]	204 [a]	51.8 [a]	19.0	77.1 [ab]	3.49 [b]	3.72 [ab]	3.31 [ab]
VA06	4	766 [a]	276 [a]	214 [a]	53.7 [a]	19.3	77.6 [ab]	3.23 [c]	3.81 [ab]	3.32 [ab]
VA06	5	758 [a]	289 [a]	217 [a]	54.1 [a]	18.9	78.4 [a]	2.91 [d]	3.71 [ab]	3.20 [bc]
VA06	6	702 [b]	261 [ab]	185 [ab]	50.6 [a]	19.5	74.3 [abc]	3.38 [bc]	3.71 [ab]	3.22 [bc]
VA06	7 *	706 [b]	287 [a]	212 [a]	51.8 [a]	18.1	78.5 [a]	2.69 [d]	3.75 [ab]	3.21 [bc]
VA06	8	627 [c]	253 [ab]	184 [ab]	45.6 [b]	18.1	71.0 [c]	2.78 [d]	3.66 [b]	3.09 [c]
VA06	9	643 [bc]	254 [ab]	187 [ab]	46.7 [b]	18.5	75.6 [ab]	2.68 [d]	3.76 [ab]	3.22 [bc]
Pooled SE		10.2	11.6	12.3	2.73	1.25	1.77	0.11	0.12	0.18
p value		<0.001	0.01	0.008	<0.001	0.442	<0.001	<0.001	0.048	<0.001

[a,b,c,d] Values within column and within grass with different superscripts differ ($p < 0.05$). * Normal cutting age in Vietnam. A1 + A2 = in vitro fermentation potential of the soluble in insoluble carbohydrates; A:P = Hac to Hpr ratio; BCVFA = branched-chain volatile fatty acids; NGR = non-glucogenic to glucogenic ratio; TVFA = total volatile fatty acid.

King grass exhibited a gradual decrease ($p < 0.001$) in dOM with advancing grass maturity similar to the other grasses. This decrease is due to plant growth and development, where over time grasses contain more fibrous materials such as cellulose and lignin, which are more challenging to digest and require more fermentation for breakdown. It is worth noting that frequent cuttings at 5 weeks of regrowth are the most suitable in view of King grass's chemical composition. Both normal practical and late cutting resulted in a reduction in NGR and A:P compared to very early cutting (week 3) which had relatively low GP and A1 + A2 values. Generally, forages rich in structural carbohydrates tend to result in greater CH$_4$ emissions; however, the result of King grass exhibited the opposite trend.

For Napier grass, it is noteworthy that no significant differences were found in fermentation potential values (i.e., dOM, GP-72, A1 + A2, and TVFA) across cutting weeks. Increased grass maturity led to a decline in BCVFA, NGR, and A:P ratios. Cutting Napier

grass before 6 weeks of regrowth appears optimal in terms of fermentability and generation of precursors for milk fat synthesis.

Cutting VA06 grass at a very early stage (i.e., in the first week) resulted in the lowest values of GP, A1 + A2 and TVFA, and produced the highest values of BCVFA, NGR, and A:P ratios. The highest values for degradable organic matter and fermentability were observed for normal practical cutting of this grass (week 5), but precursors for milk fat synthesis were less favourable. Overall, considering all parameters, week 4 appears to be the most suitable cutting time for VA06.

Meanwhile, the CP content of those grasses belonging to the *Pennisetum* genus was found to be positively correlated with BCVFA ($r = 0.81$ and $p < 0.001$). This finding aligns with the studies by Bowen et al. [43] and Musco et al. [44], who reported that grasses with lower protein levels (compared to other feedstuffs) led to lower ammonia-N and branched-chain fatty acid concentrations because these acids are derived from the degradation of some amino acids (i.e., valine, proline, isoleucine, and leucine).

Overall, the data of grasses belonging to the *Pennisetum* genus indicate that they are best harvested at either week 4 or 5 in terms of digestibility and fermentability.

3.5. Relative Yield (%) of FOM Indices of Three Grasses

To affect milk fat content, the FOM content of the grasses is important but cutting earlier or later than the common practice will affect biomass yield and, as a result, the total amount of FOM produced. Data on biomass yield in relation to the cutting time of Mombasa [23], Mulato II [24], and King grass [25] were used to calculate the relative yields of DM, dOM, GP, and A1 + A2, and the results were compared with those calculated for the current practical cutting time. As can be seen in Table 5, the yield of DM biomass of Mombasa increased with cutting age, while the total amount of dOM was lower for both early and late cutting compared to the normal cutting time at week 5. However, cutting at 4 weeks of regrowth produced on average 20% additional relative yield of fermentation potential (GP and A1 + A2) than week 5. The relative biomass yields of Mulato II gradually increased with increasing maturity across all parameters. Mulato II cut at 8-week intervals compared to the normal cutting interval of 6 weeks showed an average increase of ~13.3% in relative yields of in vitro dOM and fermentation potential (GP and A1 + A2). The decrease in the DM biomass of King grass when cutting age advances might be attributed to the fact that this grass is still in the developmental stage, during which OM accumulates relative to the inorganic matter (Table 1). The effect of King grass maturity on all parameters was more pronounced in week 5 than in week 7 with, on average, around a 9% increase. The total biomass yield of dOM of King grass showed a gradual decline with delayed harvesting times. However, cutting grass at week 3 might not be a good harvesting strategy due to lower values of relative yields of GP and A1 + A2 compared with cutting at week 5.

Table 5. Relative yields (%) of dry matter content, in vitro digestible organic matter (dOM) and in vitro fermentation potential (GP, A1 + A2) of three grasses for early and late cutting [1] compared to practical harvest time (100%) in Vietnam.

Grass	Mombasa				Mulato II				King			
Regrowth Week	2	4	5	6	2	4	6	8	3	5	7	9
DM	95.8	98.6	100	101	68.4	84.2	100	116	116	108	100	92.2
dOM	95.1	99.0	100	96.8	73.8	92.0	100	116	115	112	100	83.9
GP	112	127	100	126	71.5	92.8	100	110	102	108	100	86.7
A1 + A2	101	114	100	107	73.8	98.9	100	114	97.3	108	100	84.9

[1] Assuming additivity of harvest time. DM = dry matter content; GP = in vitro cumulative gas production; A1 + A2 = in vitro fermentation potential of the soluble in insoluble carbohydrates. Based on data from Hare et al. [19,20] and Sales et al. [21].

Overall, the implementation of a well-timed grass-cutting strategy depends on selecting the appropriate parameter to enhance milk fat content while also balancing the

demand for a large quantity of low-quality feed against the need for smaller amounts of higher-quality feed.

4. Conclusions

Harvesting tropical grasses one or two weeks earlier than normally practised is a practically relevant intervention for increasing forage quality and productivity of dOM and fermentation potential, thereby proving our hypothesis. The methane proportion was not significantly affected by grass maturity (except for Ruzi and Guinea). Even within the same genus, grasses still exhibit different patterns of in vitro gas and CH_4 production. These results provide important insights into the potential use of fermentable organic matter indicators of tropical grasses in combination with improvements in nutritive value to meet dairy nutrition requirements.

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