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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 fermentable organic matter (FOM) content of tropical grasses was appropriately investigated to re-access 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 analyzed 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 fiber (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 weeks of cutting in Ruzi, Hamil, Mombasa, and Napier grass. 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 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 those of cows in temperate climates, the observed low milk fat content can be considered unexpected. Acetic acid (Hac) and ß-hydroxybutyric acid (Hbu) are important precursors of fatty acid synthesis in the mammary gland of dairy cows [4]. 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 [5]. 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

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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*. [6] recently reported that, across the three aforementioned genera of grasses, *in vitro* gas production 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 Lollium perenne, it is well established that a prolonged cutting interval is negatively associated with the FOM content of the grass [7]. 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. This question prompted us to conduct the current *in vitro* study using cumulative gas production and OM degradability as primary indicators of the FOM content of the tropical grasses. We hypothesized that a shorter regrowth period of tropical grasses commonly used for dairy rations in SE Asia results in an increased fermentability.

2. Materials and Methods

2.1. Grass Collection

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 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. Plot area used for each grass variety was 400 m2. An initial fertiliser 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, the amount of 20 tons/ha/yr of cattle manure was applied manually.

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 × 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. Grass was harvested, leaving around 10 cm 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 °C in Vietnam. Subsequently, all frozen grass samples were transported to Wageningen University & Research (Wageningen, the Netherlands), maintaining -20 °C conditions, for analyses.

2.2. Chemical Analyses

Upon arrival at Wageningen, frozen fresh grass samples were thawed and dried during 16 h at 70 °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. Crude protein was calculated from nitrogen (N × 6.25) obtained via the Kjeldahl method [8]. The neutral detergent fibre (NDF; with heat stable α-amylase) content was analysed according to Van Soest *et al*. [9] while acid detergent fibre (ADF), and acid detergent lignin (ADL) contents were determined according to Van Soest [10].

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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 [11]. Each ground and dried grass (~ 0.5 g) was accurately weighed in quadruplicate 250 ml fermentation bottles (Schott, Mainz, Germany). All samples 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 rumen fluid was collected from each cow using a tube inserted via the esophagus 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 [11] under continuous flushing with CO_2. Prior to inoculation, the fermentation bottles were placed in a shaking water bath kept at 39 °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 which 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 × 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) ortho-phosphoric 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 VFA analysis. The mixture was then stored at -20 °C pending analysis. Volatile fatty acids (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; 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 Manufacturing Co.) 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 °C for 1 h, then ashed at 530 °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™, Denmark) to remove microbial matter from the undegraded substrates, and then dried at 103 °C for 4 h and ashed at 530 °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.* [12,13]. Briefly, measured CH_4 production in individual bottles was expressed relative to the maximum production in each bottle and were 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 [11,12]. The non-linear least squares regression procedure was used [14] and the data were fitted according to the following equation, as outlined by Groot *et al.* [15]:

$$GP = \sum_{i=1}^{n} \frac{Ai}{1 + (Ci/t)^{Bi}}$$

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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.* [15], 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 phase 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.* [16] based on the work of Cone *et al.* [17]. Data on CH_4 production were also fitted according to the above-mentioned model where n = 1.

2.5. Calculations and Statistical Analyses

Total VFA in fermentation fluid at 72 h was calculated as the sum of Hac, propionic acid (Hpr), 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 Ørskov [18]:

$$[\text{acetate} + 2 \times (\text{Hbu} + \text{isoHbu}) + \text{Hva} + \text{iso-Hva}] / [\text{Hpr} + \text{Hva} + \text{iso-Hva}].$$

The most commonly used grass in Vietnam for each genus (Mombasa, Mulato II, 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.* [19,20], respectively, after conversion of biomass yields per year:

$$Y_{Mo} = 0.1120x^2 + 52.080x \ (0 \le x \le 90; R^2 = 0.95)$$
$$Y_{Mu} = 0.7423x^2 + 34.672x \ (0 \le x \le 90; R^2 = 0.99)$$

For King grass, biomass yield (kg dry matter/ha/yr) was determined using the equation provided by Sales *et al.* [21]:

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

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

Effects of regrowth age within each grass were subjected to analysis of variance (ANOVA) using the PROC MIXED procedure [14] 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. Throughout, the level of statistical significance was pre-set at $P < 0.05$ while a trend was declared at $0.05 \le P < 0.10$.

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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 = 0.61, 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 finding of Mutimura *et al.* [22] 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.

Table 1. Chemical composition (g/kg dry matter) of nine grasses at different stages of maturity commonly used in Vietnam.

Grass[1]	Week	OM	CP	EE	NDF	ADF	ADL	Grass[1]	Week	OM	CP	EE	NDF	ADF	ADL
Mulato II	2	851	226	28.8	519	255	20.3	TD58	3	875	132	28.6	688	372	20.1
	4	846	147	25.7	498	234	19.3		4	873	138	24.5	644	310	18.1
	6	858	113	19.2	607	316	24.5		5	855	148	27.5	676	362	21.2
	8	860	118	18.1	656	362	30.9		6	863	165	24.3	667	350	16.9
Ruzi	2	889	179	32.2	536	268	21.5	King	3	859	181	24.8	598	336	17.2
	4	878	163	25.7	505	234	21.4		5	869	136	33.4	602	336	19.4
	6	915	116	24.5	660	354	26.7		7	896	87	27.9	646	377	23.6
	8	888	162	25.6	622	328	29.8		9	920	103	23.7	659	409	47.3
Guinea	1	864	226	24.4	600	327	21.2	Napier	2	881	161	30.9	620	331	21.3
	2	882	218	28.7	599	313	20.2		3	859	165	26.7	581	316	22.5
	3	912	175	26.5	657	349	26.5		4	879	184	25.9	538	270	20.5
	4	877	180	26.3	677	375	31.6		5	871	176	28.9	569	310	23.6
	5	870	143	31.7	659	366	30.6		6	858	167	29.0	594	318	27.9
	6	876	137	28.5	675	373	30.6		7	882	140	28.0	646	353	28.0
									8	890	117	24.7	670	362	23.6
Hamil	2	844	254	29.7	572	293	14.9		9	911	132	18.7	696	397	33.1
	4	876	97	23.3	732	413	27.5								
	5	845	96	-	-	410	-		1	824	298	28.8	491	276	21.4
	6	840	85	-	-	409	-		2	811	223	23.6	541	287	30.0
									3	867	256	23.7	560	304	19.4
Mombasa	2	871	171	25.5	641	350	20.2	VA06	4	851	156	25.8	593	324	22.5
	4	860	124	28.6	669	365	20.1		5	872	139	26.8	646	354	25.8
	5	876	114	26.5	696	375	19.0		6	892	102	26.8	682	395	32.1
	6	884	90	23.4	730	406	24.4		7	913	88	24.7	694	395	35.5
									8	902	74	20.4	717	436	51.5
TD58	1	827	226	22.3	565	270	19.1		9	902	89	23.6	706	411	44.0
	2	857	107	28.6	673	364	22.2								

[1] Mulato II = Brachiaria ruziziensis (B. ruziziensis × B. decumbens × B. brizantha); Ruzi = B. ruziziensis; Guinea = Panicum maximum Jacq.; Hamil = P. maximum cv. Hamill; Mombasa = P. maximum cv. Mombasa; TD58 = P. maximum cv.TD58; King = Pennisetum purpureum × P. glaucum; Napier = P. purpureum Schumach.; VA06 = P. purpureum × P. Americanum. ADF = acid detergent fibre, ADL = acid detergent lignin, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, OM = organic matter, - = not determined.

The values related to cell wall constituents (NDF, ADF and ADL) increased with the advancement of grass maturity (r = 0.55, 0.60, 0.67 and P < 0.001, respectively). The current data were found to be in line with other previous reports [23,24].

Tropical grasses develop thick-walled cells with increased cell wall fractions, including cellulose, hemicellulose, and lignin, as a structural adaptation to minimize photorespiration, enhance overall resilience to tropical environmental conditions, that helps contribute to the plant's robustness owing to both the thickness and composition of cell walls [25]. Consequently, the NDF content of tropical grasses is higher than temperate grasses (60-75 vs 35-67% DM) [26–28].

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In the present study, an increase in cell wall constituents of tropical grasses was associated with a decrease in CP content with cutting ages as cutting age advances, and this decrease was even more pronounced at later stages (r = -0.74, P < 0.001), which is consistent with observations of others in temperate grasses [29–31]. 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 concentration still exceeded the minimum CP level (7%) required for rumen function [32] although the CP content in rations recommended by the NRC [33] for lactating cows ranges from 14 to 18% DM.

The lipid concentration (EE) of the selected grasses ranged from 1.9 to 3.2% DM, which is comparable to the values reported by Melesse *et al.* [34] 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). Other grasses showed a trend of an increase in EE content from early to the middle stage and then a decline from 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, most of the parameters (except for CH_4 expressed as a percentage of total gas production and A:P) of Mulato II grass were significantly influenced by harvesting time. The highest values of *in vitro* dOM, GP after 72 h incubation (GP-72), TVFA and NGR were found at week 4, which is earlier than the commonly used cutting time (week 6) under practical farming conditions in Vietnam. Nevertheless, the growth of Mulato II after week 4 also produced the highest quantity of cumulative CH_4 production measured after 72 h of incubation (CH_4-72), whereas the CH_4 percentage (CH_4:GP-72) was not different between cutting weeks. This can be explained by the low content of fibre at week 4 compared to other weeks. These findings are in line with Neto *et al.* [35] and Ruggieri *et al.* [36] 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. In Ruzi grass, GP-72 was not affected by grass maturity, but most of other parameters indicated that week 4 is the most suitable harvesting age. Cutting grass at late stage (i.e. week 8) should not be beneficial in term of FOM content.

Both Mulato II and Ruzi, belonging to the *Brachiaria* genus, showed that NDF was negatively correlated with dOM (r = -0.96, -0.998 with P = 0.04, 0.002, respectively).

Overall, due to the high fermentation potential values (i.e. dOM, GP-72 and A1+A2) and high values of volatile fatty acids used for milk fat synthesis (i.e., NGR and A:P), the recommended harvest age for grasses belonging to the *Brachiaria* genus appears to be at week 4 after the previous cut.

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	CH_4-72	CH_4:GP-72	TVFA	BCVFA	NGR	A:P
		g/kg OM	ml/g OM			% of GP-72	mM	% of TVFA	mol/mol	
Mulato II	**2**	**783^{a}**	**258^{ab}**	198^{ab}	45.2^{ab}	17.4	75.8^{ab}	3.17^{a}	3.20^{b}	2.80
Mulato II	4	788^{a}	276^{a}	218^{a}	49.5^{a}	18.1	77.8^{a}	2.86^{b}	3.62^{a}	3.04
Mulato II	6*	725^{b}	247^{ab}	185^{b}	42.0^{b}	17.0	71.8^{b}	2.58^{c}	3.25^{b}	2.78
Mulato II	8	726^{b}	233^{b}	179^{b}	40.2^{b}	17.2	76.3^{ab}	2.61^{c}	3.25^{b}	2.81
Pooled SE		5.9	10.3	10.7	1.81	1.00	1.78	0.09	0.17	0.18
P value		<0.001	0.02	0.004	0.004	0.411	0.042	<0.001	0.01	0.05
Ruzi	2	772^{a}	270	222^{a}	46.7^{ab}	17.5^{a}	79.7^{a}	3.07^{a}	3.47^{ab}	3.02^{x}
Ruzi	4	794^{a}	273	216^{a}	48.0^{a}	17.8^{a}	80.5^{a}	2.71^{b}	3.61^{a}	3.01^{x}
Ruzi	6*	710^{b}	246	193^{b}	42.2^{bc}	17.2^{a}	75.3^{b}	2.50^{c}	3.30^{b}	2.87^{xy}
Ruzi	8	731^{b}	251	189^{b}	38.3^{c}	15.4^{b}	74.4^{b}	2.84^{ab}	3.34^{b}	2.84^{y}

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Pooled SE	7.7	12.1	9.2	2.87	1.45	2.14	0.07	0.16	0.17
P value	<0.001	0.086	0.006	0.001	0.006	0.002	<0.001	0.004	0.031

[a,b,c] 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 ≤ 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.

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 were 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, A1+A2, TVFA and BCVFA 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.

For Hamil grass, dOM gradually declined (P < 0.001) with grass maturity whilst GP did not differ among weeks (P = 0.184) although a numerical decrease was observed. A1+A2 values were different between week 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, A1+A2, 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 dOM and BCFA, 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 ages.

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	CH_4-72	CH_4:GP-72	TVFA	BCVFA	NGR	A:P
		g/kg OM	ml/g OM			% of GP-72	mM	% of TVFA	mol/mol	
Guinea	**1**	**741^{ab}**	**248^{ab}**	181^{ab}	45.9^{a}	18.4^{a}	74.7^{abc}	3.66^{ab}	3.65	3.22^{ab}
Guinea	2	760^{a}	255^{a}	191^{a}	48.4^{a}	19.5^{a}	78.6^{a}	3.78^{a}	3.72	3.31^{ab}
Guinea	3	711^{bc}	249^{a}	180^{ab}	47.2^{a}	19.3^{a}	77.6^{ab}	3.46^{abc}	3.77	3.34^{a}
Guinea	4	627^{e}	190^{c}	116^{c}	27.2^{b}	14.4^{b}	71.4^{c}	3.00^{d}	3.68	3.19^{ab}
Guinea	5*	679^{cd}	248^{a}	182^{ab}	46.3^{a}	18.4^{a}	72.9^{abc}	3.34^{bc}	3.57	3.11^{b}
Guinea	6	646de	219^{b}	167^{b}	43.1^{a}	19.6^{a}	70.2^{c}	3.22^{cd}	3.65	3.16^{ab}
Pooled SE		8.9	7.3	6.7	2.94	1.70	2.33	0.10	0.14	0.19
P value		<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	0.051	0.035
Hamil	2	768^{a}	254	193^{a}	49.4	19.5	77.8^{a}	3.86^{a}	3.56	3.17
Hamil	4	669^{b}	255	183^{ab}	46.8	18.3	74.7^{ab}	2.93^{b}	3.60	3.11
Hamil	5*	668^{b}	240	184^{ab}	45.6	19.1	73.8^{ab}	2.92^{b}	3.59	3.13
Hamil	6	621^{c}	237	171^{b}	44.8	19.0	70.4^{b}	2.88^{b}	3.54	3.07
Pooled SE		7.7	12.3	11.1	3.51	1.57	2.34	0.09	0.11	0.16
P value		<0.001	0.184	0.026	0.117	0.254	0.005	<0.001	0.673	0.184
Mombasa	2	708^{a}	252	190	47.6	18.3	74.4	3.25^{a}	3.59	3.13
Mombasa	4	729^{a}	280	210	51.9	18.9	75.7	3.20^{a}	3.57	3.15

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Mombasa	5*	708^{ab}	237	189	45.9	20.2	79.2	2.97^{b}	3.57	3.12
Mombasa	6	672^{b}	265	187	45.1	17.2	75.0	2.80^{b}	3.60	3.12
Pooled SE		8.4	15.5	10.0	4.30	1.71	2.13	0.04	0.11	0.16
P value		0.006	0.364	0.290	0.199	0.518	0.140	<0.001	0.910	0.943
TD58	1	797^{a}	286^{ab}	198	54.1^{ab}	19.2	78.1^{ab}	4.18^{a}	3.92^{a}	3.36^{a}
TD58	2	769^{ab}	269^{ab}	202	51.9^{ab}	19.1	76.0^{ab}	3.34^{b}	3.54^{b}	3.07^{b}
TD58	3	734^{c}	273^{ab}	201	49.3^{ab}	18.0	77.2^{ab}	2.92^{c}	3.56^{b}	3.07^{b}
TD58	4	772^{ab}	295^{a}	210	54.4^{a}	18.7	79.2^{a}	3.19^{b}	3.73^{ab}	3.16^{b}
TD58	5*	728^{c}	271^{ab}	200	49.2^{ab}	18.4	73.6^{b}	3.15^{bc}	3.62^{b}	3.14^{b}
TD58	6	742^{bc}	262^{b}	196	45.1^{b}	17.5	75.9^{ab}	3.22^{b}	3.66^{b}	3.16^{b}
Pooled SE		7.2	6.5	7.2	2.82	0.99	1.78	0.08	0.12	0.15
P value		<0.001	0.040	0.650	0.003	0.074	0.050	<0.001	<0.001	<0.001

^{a,b,c}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 TD58 grass, significant effects of cutting time were observed for dOM, GP-72, CH_4-72, BCVFA, NGR and A:P with trend for CH_4:GP-72 ($P = 0.074$) and TVFA ($P = 0.050$). 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 was found to be significantly different, but A1+A2 was similar throughout the harvesting ages, suggesting that the asymptote GP associated with microbial turnover (A3) contributed to this difference. There was a similar trend for GP-72 and CH_4 proportion which did not significantly differ from week 1 to 5, with week 6 having the lowest values. Inversely, NGR and A:P ratio had the highest value at week 1 but were similar from week 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 ($r = - 0.66, -0.94$; $P = 0.003, <0.001$, respectively). In general, it appears that harvesting grasses belonging to the *Panicum* genus after two weeks of regrowth provides the highest concentration of FOM biomass and, thereby, 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.

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 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 a relative 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.

Normal practical cutting at week 7 of Napier grass yielded the highest values for dOM and A1+A2, but relative low values for BCVFA, NGR and A:P ratios. Although no significant differences were found in GP-72, CH_4 percentage and TVFA across cutting weeks, increased grass maturity led to a decline in BCVFA, NGR and A:P ratios. Cutting at either week 4 or week 5 appears optimal in term of fermentability and generation of precursors for milk fat synthesis.

Cutting VA06 grass at a very early stage (i.e., first two weeks) resulted in the lowest values of GP, A1+A2 and TVFA, however 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 favorable. Overall, considering all parameters, week 4 appears to be the most suitable cutting time for VA06.

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Meanwhile, CP content of King, Napier and VA06 grass was found to be positively correlated with BCFVA (r = 0.98, 0.76, 0.90 and P = 0.02, 0.03, <0.001, respectively). This finding aligns with the studies by Bowen *et al.* [37] and Musco *et al.* [38], which reported that grasses had lower protein level (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, leucine). Methane proportion was not significantly affected by the maturity of all three grasses.

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.

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	CH_4-72	CH_4: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^{b}	42.3^{b}	17.7	73.7^{ab}	3.27^{a}	3.82^{a}	3.37^{a}
King	5	752^{a}	272^{a}	212^{a}	50.9^{a}	18.9	77.9^{a}	3.09^{a}	3.68^{b}	3.13^{b}
King	7*	701^{b}	262^{ab}	203^{ab}	48.5^{a}	18.6	76.3^{ab}	2.63^{b}	3.65^{b}	3.03^{b}
King	9	623^{c}	240^{b}	182^{b}	42.8^{b}	17.9	71.7^{b}	2.61^{b}	3.65^{b}	3.10^{b}
Pooled SE		7.6	11.2	10.0	2.73	1.48	1.08	0.09	0.13	0.17
P value		<0.001	0.009	0.002	<0.001	0.325	0.010	<0.001	<0.001	<0.001
Napier	2	758^{ab}	269	210^{ab}	50.8^{ab}	18.9	77.4	3.17^{a}	3.73^{a}	3.33^{a}
Napier	3	756^{abc}	265	195^{b}	52.8^{a}	20.0	76.7	3.25^{a}	3.78^{a}	3.34^{a}
Napier	4	763^{ab}	284	208^{ab}	53.2^{a}	18.7	78.2	3.17^{a}	3.79^{a}	3.24^{ab}
Napier	5	758^{abc}	284	218^{a}	50.7^{ab}	17.9	77.9	3.23^{a}	3.74^{a}	3.24^{ab}
Napier	6	751^{abc}	282	211^{ab}	52.0^{a}	18.5	76.8	3.21^{a}	3.76^{a}	3.27^{ab}
Napier	7*	771^{a}	276	216^{a}	52.0^{a}	19.0	79.8	2.84^{bc}	3.66^{ab}	3.16^{b}
Napier	8	738^{abc}	285	209^{ab}	52.0^{a}	18.3	77.3	2.88^{b}	3.67^{ab}	3.18^{b}
Napier	9	722^{c}	271	206^{ab}	46.7^{b}	17.2	77.6	2.67^{c}	3.53^{b}	2.96^{c}
Pooled SE		8.1	11.2	10.7	2.60	1.33	1.58	0.07	0.13	0.17
P value		0.007	0.137	0.024	0.007	0.102	0.577	<0.001	0.002	<0.001
VA06	1	769^{a}	212^{d}	148^{d}	39.4^{c}	18.3	70.0^{c}	4.16^{a}	3.79^{ab}	3.41^{a}
VA06	2	736^{ab}	245^{cd}	184^{c}	46.0^{b}	18.7	73.8^{abc}	3.45^{bc}	3.84^{a}	3.41^{a}
VA06	3	756^{a}	274^{abc}	204^{abc}	51.8^{a}	19.0	77.1^{a}	3.49^{b}	3.72^{abc}	3.31^{ab}
VA06	4	766^{a}	277^{ab}	214^{a}	53.7^{a}	19.3	77.6^{a}	3.23^{c}	3.81^{ab}	3.32^{ab}
VA06	5	758^{a}	289^{a}	217^{a}	54.2^{a}	18.9	78.4^{a}	2.91^{d}	3.72^{bc}	3.20^{c}
VA06	6	708^{b}	266^{abc}	190^{bc}	51.0^{a}	19.3	74.6^{abc}	3.31^{bc}	3.71^{bc}	3.22^{bc}
VA06	7*	706^{b}	287^{a}	212^{ab}	51.8^{a}	18.1	78.4^{a}	2.69^{d}	3.75^{abc}	3.21^{c}
VA06	8	627^{c}	253^{bc}	184^{c}	45.7^{b}	18.1	71.0^{bc}	2.78^{d}	3.66^{c}	3.09^{d}
VA06	9	643^{c}	253^{bc}	186^{c}	46.7^{b}	18.5	75.6^{ab}	2.68^{d}	3.77^{abc}	3.23^{bc}
Pooled SE		6.7	9.8	10.6	2.76	1.21	1.77	0.08	0.12	0.18
P value		<0.001	<0.001	<0.001	<0.001	0.126	<0.001	<0.001	0.001	<0.001

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, total amount of FOM produced. Data on biomass yield in relation to cutting time of Mombasa ([19]), Mulato II ([20]) and King grass ([21]) were used to calculate the relative yields of DM, dOM, GP and A1+A2 and results were compared with those calculated for the current practical cutting time. As can be seen in Table 5, yield of DM biomass of Mombasa increased with cutting age, while total amount of dOM was lower for both early and late cutting compared to the normal cutting time at week 5. However, cutting after 4 weeks of regrowth produced on average 12% additional relative yield of fermentation potential (GP, 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 ~12.3% 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

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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 week 7 with, on average, around 10% increase. The total biomass yield of dOM of King grass was shown 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 to 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.

Parameter	Mombasa				Mulato II				King			
	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	95.3	99.7	100	97.1	73.9	91.5	100	116	116	112	100	84.1
GP	101	114	100	114	70.9	92.8	100	109	102	109	100	86.7
A1+A2	95.8	108	100	101	72.6	97.9	100	112	98	109	100	84.8

[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 of 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 practiced 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 the grass maturity (except for Ruzi and Guinea). Even within the same genus, grasses still exhibit different patterns of *in vitro* gas and CH_4 productions. 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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