In vitro Digestibility and Gas Production Characteristics of Four Napier (*Pennisetum purpureum*) Cultivars as Fresh Fodder

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Abstract

Napier grass was first introduced to Malaysia in the 1920's and there were many cultivars introduced in Malaysia since 1950's. However, there is a need to have comparative evaluation of these Napier cultivars so that definite recommendations can be made in the choice and management of the respective cultivars. The experiment was conducted to evaluate the *in vitro* digestibility and gas production characteristic of four Napier (*Pennisetum purpureum*) cultivars, namely Common, Silver, Red and Dwarf Napier. Common, Silver and Red Napier are classified as tall cultivars while Dwarf Napier is a short cultivar. Gas production was determined at 2, 4, 6, 8, 12, 24, 32, 36, 48, 72 and 96 h of incubation period and its kinetics was described using the equation $p = a + b\ (1 - e^{-ct})$. Dwarf Napier had the highest ($P < 0.05$) nutritive quality (16% CP; 75% IVDMD and 73% IVOMD) among the cultivars. Overall, tall cultivars were higher ($P < 0.05$) in NDF, ADF, and ADL content than Dwarf Napier cultivar. The potential gas production (A+B) at 96 h incubation period was higher ($P < 0.05$) in Dwarf Napier cultivar (65 mL/200 mg DM) compared to other tall cultivars (< 57 mL/200 mg DM). There were no significance differences ($P > 0.05$) in the rate of gas production (C) of Napier cultivars which ranged from 0.024 to 0.035 h^{-1}. The metabolisable energy (ME) was significantly higher in Dwarf and Red Napier cultivars (8.7 MJ/kg DM) compared to Silver and Common Napier cultivars. The cumulative gas production within 32 h was highest ($P< 0.05$) in Red and Dwarf Napier cultivars. The total VFA concentration, acetic and butyric acid content of Napier cultivars ($P>0.05$) ranged from 52 to 73 mM, 88 to 70%, 6.2 to 6.8%, respectively.. Dwarf Napier cultivar had superior nutritional quality. Dwarf and Red Napier cultivars could be classified as high quality grasses due to their high digestibility, gas production and degradation rates compared to the other cultivars. The low quality of Common and Silver Napier cultivars is mainly reflected by the extensive lignification of their cell wall structure.

Keywords: Napier cultivars, nutritive quality

Introduction

Napier grass is a C_4 type tropical grass and commonly used in tropical countries as feedstuff for ruminants. It is a tall, perennial grass originated from tropical Africa. Napier grass is adaptable to a wide range of regions with annual rainfalls between 700 to 2500 mm and altitude from sea level up to 2000 m but does not tolerate flooding. The most suited temperature for optimum growth of Napier grass is between 30-35°C and no growth takes place below 10°C. The tall Napier cultivars are susceptible to frost

damage, in contrast to the dwarf type. Napier grass can be propagated vegetatively from stem cutting, withstands several cuttings and rapidly regenerates producing forages.

Many cultivars of Napier grass (*Pennisetum purpureum*) have been introduced since 1950's which include Common, Red, Taiwan, Indian, Uganda, King grass, Zanzibar and Kobe which are classified as tall cultivars whereas Dwarf, Dwarf "Mott" and Australian Dwarf Napiers are short cultivars. Generally, high yielding tall cultivars are normally grown for cut-and-carry system in Malaysia. Common Napier is among the highest yielding crops and has a better nutritive value compared to Uganda Napier (Halim et al., 2013). Red Napier was high in metabolisable energy and this crucial parameter reflected the actual level of energy available for absorption (Haryani et al., 2012). Dwarf Napier is high in leaf to stem ratio and this associates with good forage quality. However, Silver Napier was recently introduced with little information regarding the performance of this cultivar. There is a need to have comparative evaluation of these Napier cultivars so that definite recommendations can be made in the choice and management of the respective cultivars such as Common, Red, Dwarf and Silver Napier Cultivars. *In vitro* gas production technique (IVGPT) was invented to evaluate the nutritional quality of feed by measuring the rate of production of fermentation gases that can be used to predict the rate of feed degradation, assuming that the amount of gas produced reflects the amount of substrate degraded (Lopez et al., 2000). Unlike this technique, an *in vivo* technique is time consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large scale feed evaluation. Due to limitation in the number of animals and constraint of animal welfare, this technique has been practiced worldwide. In IVGPT, the feedstuff is incubated with buffered rumen fluid and the gas produced is measured as indirect indicator of fermentation kinetics. The degraded feed portion will be fermented to short chain fatty acids, gases (mainly CO_2 and CH_4) and microbial biomass. Fermentation of protein generates relatively small gas production (130 ml gas/g substrate), compared to the fermentation of carbohydrate (340-370 ml gas/g substrate) (Cone and Gelder, 1999). The gas production from fat fermentation is negligible (1-2 ml gas/g substrate) (Menke and Steingass, 1988).

Comparative studies of dry matter productivity and nutritional content of Napier cultivars had been conducted in a number of places (Halim et al., 2013; Ansah et al., 2010; Manyawu et al., 2003). Nevertheless, there were limited comparative studies conducted to assess the digestibility and degradability of different Napier cultivars. Therefore, the objective in this study was to evaluate the *in vitro* digestibility and gas production characteristics of four Napier (Common, Red, Dwarf and Silver) cultivars as fresh fodder.

Materials and Methods

Preparation and Management of Experimental Materials

A field experiment was conducted at Field 2, Universiti Putra Malaysia (UPM), Malaysia to carry out the above objective. The soil texture of the experimental area is clay as classified by Soil Taxonomy Classification (USDA) and determined by the Texture AutoLookup (TAL). The details of the soil texture are clay (45.13%), silt (42.14%) and sand (30.53%) (Jusoh, 2005). Basal fertilization was applied during grass establishment at the rate of 60 kg N, 60 kg P and 50 kg K ha^{-1} and 2 tonnes of Ground Magnesium Limestone (GML) ha^{-1}. Stem cuttings of the four Napier cultivars

(Common, Red, Dwarf and Silver) were collected from Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Malaysia. The planting of the Napier grass was done by stem cuttings of about 20 cm in length which were placed 45° from the ground level with half of the nodes buried in the soil and the other nodes left exposed for tiller emergence. The plot size was 5 m x 4 m with the planting distances of 0.5 m between plants and 1 m between rows. All plants were watered twice a day and plant regrowth was harvested after 12th wk of plot establishment period. The selected cultivars of Napier grass (*Pennisetum purpureum)* of Common, Silver, Red and Dwarf were established in three replicates. The pooled samples within 6 to 8 wk harvesting age (1st cutting) for the respective cultivars were used for further analysis.

Chemical Composition Analysis

The fresh grass was harvested for each plot by cutting 20 cm above ground level in the randomly selected 1 m × 1 m quadrat. Grass samples obtained were oven-dried at 65°C for 48 h and calculated as dry matter yield per hectare. The subsamples were analyzed using standard procedures for chemical composition for dry matter (DM), organic matter (OM), crude protein (CP) and Ash content according to AOAC (1990) procedure. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were measured according to Van Soest's procedure (Van Soest et al., 1991).

In vitro Gas Production

The gas production profiles were measured according to the method described by Menke and Steingass (1988). The rumen fluid used as inoculum was collected before the morning feeding from three rumen-fistulated crossbred goats which were fed a mixture of fresh grasses and transferred into pre-warmed thermos bottles. The rumen fluid was composited and strained using two layers of cheese cloth under continuous flushing with CO_2.

Approximately 0.2 g DM of ground samples (1 mm) with a 1:2 (v/v) mixture of rumen fluid and buffer medium was placed in a glass syringe (FORTUNA® Optima glass syringe), incubated at 39°C in a water bath and shaken at regular times. Incubation was performed in triplicates. The blanks without substrate were included and used to evaluate the gas production in the exclusion of substrates. The volume of gas released was recorded at 2, 4, 6, 8, 12, 24, 32, 36, 48, 72, and 96 h of incubation period.

Sample replicates were terminated at 48-incubation period for the determination of *in vitro* dry matter and organic matter digestibility. The residues were filtered using sintered glass crucible (coarse porosity no. 1, pore size 100-160 µm) and oven-dried at 105°C for 48 h to estimate the dry matter disappearance. The OM of dry residue was determined by the incineration of muffled furnace at 550°C for 3 h. The IVOMD was calculated by using the formula below:

$$\text{IVOMD}(\%) = \frac{Initial\ \ organic\ \ matter\ (mg) - Residue\ \ organic\ \ matter\ \ (mg)}{Sample\ \ orgnaic\ \ matter\ (mg)} \times 100$$

The *in vitro* degradability data were fitted into the equation $P = a + b\ (1 - e^{-ct})$ (Orskov and McDonald, 1979), with the NEWAY computer programme.

VFA Determination

Volatile fatty acids (VFA) from each sample were determined as described by Filipek and Dvorak (2009) with modifications. The molar concentration of

acetic, propionic and butyric acids were analyzed by using 6890N Network GC System (Agilent Technologies Inc., USA) fitted with DB-FFAP, 122-3232 (Nitroterephthalic acid modified polyethylene glycol) polyethylene glycol capillary column (30m x 0.25mm internal diameter x 0.25µm) (Agilent Technologies Inc., USA) and Flame Ionization Detector (FID) . One µl sample was injected into GC for analysis. The oven temperature was 90-180 °C and Nitrogen gas was used as a carrier gas.

Determination of Gross Energy and Metabolisable Energy Content

Gross energy was determined using Automatic IKA® Adiabatic Bomb Calorimeter C2000, Germany whereas the metabolisable energy (ME) was estimated based on a formula derived by Menke and Steingass (1988):

$$ME\ (MJ/kg) = 2.20+0.1357\ GP+0.0057\ CP+0.0002859\ CP^2$$

where,
GP=Gas produced after 24 h for each treatment
CP=Crude protein content of each treatment

Statistical Analysis

The statistical analysis was subjected to analysis of variance, using completely randomized design (CRD) with the general linear model (GLM) procedure of SAS 9.3 (Statistical Analytical System Institute Inc. Cary, North Carolina, USA). The difference between treatment means was compared by least significant difference (LSD) test. The level of significance used to determine the differences between treatments is $P < 0.05$.

Results and Discussion

Based on a study conducted by Halim et al. (2013), the Napier cultivars could be classified into two distinct groups. Common, Silver and Dwarf Napier cultivars are classified as tall types since the grass height exceeds 130 cm whilst Dwarf Napier cultivar as a short type with the height of less than 100 cm.

The highest calculated dry matter yield of pooled samples (6 to 8 wk of age) was observed in Common (44 t//ha/yr) followed by Red (31 t/ha/yr), Silver (27 t/ha/yr) and Dwarf Napier (25 t/ha/yr). A comparative study of 4 Napier cultivars was conducted in Ghana by Ansah et al. (2010) and the dry matter yield reported differed significantly between cultivars ranging from 25 to 45 t/ha/yr. There were significant differences ($P < 0.05$) among the four Napier cultivars in term of nutritional composition (Table 1). The CP content of Napier cultivars ranged from 8.71 to 15.90%. Dwarf Napier recorded the highest ($P < 0.05$) CP content of 15.90% while Common Napier recorded the lowest CP content of 8.71%. The CP contents of Napier cultivars are consistent with the findings of Lounglawan et al. (2014) and Halim et al. (2013). Nevertheless, the CP content recorded was above the minimum requirement (7%) for sustainability of rumen microbes (Lazzarini et al., 2009). The CP content of Dwarf Napier was close to 16% CP required for optimal milk output (Imaizumi et al., 2010). In Australia, 5 species of temperate grasses known as Perennial ryegrass, Cocksfoot, Fescue, Phalaris, Prairie grass and Short rotation ryegrass contained high protein (> 20% CP) and non-structural (< 50% NDF) carbohydrates (Fulkerson et al., 2007) compared to tropical grasses. Thus, these pastures can provide protein level in excess to a cow requirement. The differences in quality between temperate and tropical

grasses especially Napier grass could have resulted in the proportion of structural carbohydrate that might suppress the cell content especially CP content. The hot and humid characteristics of tropical region are believed to have influenced the quality of the grass due to high temperature, which normally enhances the rate of plant development and reduces digestibility (Buxton, 1996).

Table 1. Mean chemical composition of fresh Napier cultivars within 6 to 8 wk old at first cutting

Napier cultivar	CP %	NDF %	ADF %	ADL %	Hemi-cellulose %	Cellulose %	GE (MJ/kg DM)
Common	8.71^{c}	72.77^{a}	44.99^{a}	11.15^{a}	27.78^{b}	31.84^{a}	16.93^{b}
Silver	10.83^{b}	71.87^{a}	39.63^{b}	8.74^{ab}	32.24^{a}	30.89^{a}	17.08^{b}
Red	10.44^{b}	67.76^{b}	40.70^{b}	6.63^{bc}	27.06^{b}	34.07^{a}	17.09^{ab}
Dwarf	15.90^{a}	64.29^{c}	31.33^{c}	5.32^{c}	32.96^{a}	24.59^{b}	17.31^{a}
SEM[1]	0.56	0.70	1.00	1.00	0.92	1.22	0.08

^{abc} Means with different superscripts in the same column differ significantly ($P < 0.05$)
[1]SEM: Standard error of the mean

There were significant ($P < 0.05$) differences in cell wall content among the Napier cultivars. The NDF, ADF and ADL content among cultivars ranged from 64 to 73, 31 to 45 and 5 to 11% DM, respectively. Dwarf Napier had the lowest ($P < 0.05$) cell wall composition (64% NDF; 31% ADF and 5% ADL) than those observed in the tall cultivars. The NDF content of Napier cultivars was above the minimum level of dietary NDF (> 25% NDF) as recommended by NRC. Increase in the proportion of roughage NDF in a diet would reduce energy density, intake and productivity of dairy cows (Kanjanapruthipong et al., 2001; Mertens, 1997), whereas low fiber content in a diet can alter rumen fermentation resulting in severe acidosis. Tall cultivars yielded high lignin and cellulose (>30%) content compared to the Dwarf Napier. Common Napier was the most lignified cultivar (11% ADL) and the least was shown by the Dwarf Napier. Lignin structure is beneficiary to the plants themselves as it gives support to the plant structure, limits water loss by reducing the permeability of the cell wall and impedes pathogens. Consequently, it is well established that lignin content in forages is negatively correlated with digestibility. In nutritional perspectives, the main anti-quality factor of lignin in forages limits the digestion of the structural carbohydrates, mainly cellulose and hemicellulose.

The IVDMD and IVOMD were significantly ($P < 0.05$) affected by the selection of cultivars (Table 2). Based on the results obtained, the IVDMD of Napier cultivars varied from 55 to 75%. A wide range of Napier dry matter digestibility was reported in several studies, ranging from 53 to 80% (Budiman et al., 2012; Wijitphan et al., 2009). Both Red and Dwarf Napiers had high digestibility above 65% IVDMD. According to Mugeriwa et al. (1973), the IVDMD values were greater than 65% indicating good feeding value and values below this threshold level would result in reduced dry matter intake due to lowered digestibility. The IVOMD of Napier cultivars ranged from 51 to 73%. These values were

relatively higher compared to the study by Evitayani et al. (2004) who obtained the organic matter digestibility of tropical grasses ranging from 51.% (*P. purpuphoides*) to 64.4% (*P. purpureum*). In general, the digestibility of Napier grass can be reflected by several factors, for instances the cultivar selection and management practices such as harvesting age, cutting interval and cutting height (Zailan et al., 2015; Lounglawan et al., 2014). Furthermore, high ambient temperature might inhibit the digestibility due to the increase in lignification of plant cell wall whilst the increases of structural carbohydrates (cell wall) suppress the pool size in the cellular content, such as crude protein and water soluble carbohydrate (Van Soest, 1982). Common Napier had the highest yield and fiber content. In contrast, Dwarf Napier had the lowest yield but superior in nutritive value. It is recommended for farmers to balance between quantity and quality in selecting the cultivars such as Red Napier. Red Napier has medium dry matter yield, crude protein and high in digestibility. The quality could be improved by the inclusion of supplementation such as hay and concentrates. The recommended proportion of forage-to-concentrate in dietary NDF should be between 60 to 40 and 40 to 60. In comparison the average digestibility of temperate grasses in Canada ranged between 89 to 92% *in vitro* true digestibility (Thorvaldsson et al., 2007). In Netherlands, a study conducted by Bruinenberg et al. (2002) showed that the digestibility of 4 temperate grasses ranged from 54 to 84% dry matter digestibility. Therefore, this information indicates that the digestibility of Napier cultivars (55 to 75% IVDMD) is within the range reported for several temperate grasses.

Table 2. *In vitro* digestibility (%) of fresh Napier cultivars at 48-h incubation time

Napier cultivar	IVDMD	IVOMD
Common	54.62[d]	50.78[d]
Silver	59.58[c]	54.68[c]
Red	66.34[b]	61.80[b]
Dwarf	75.49[a]	73.07[a]
SEM[1]	0.64	0.53

[abcd] Means with different superscripts in the same column differ significantly ($P < 0.05$)
[1]SEM: Standard error of the mean

The cumulative gas production rapidly increased with increasing incubation time from 2 to 48 h, and remained relatively constant from 48 to 96 h (Table 3). Red Napier cultivar had the highest gas production within 24-h incubation period ranging from 6 to 47 ml/ 200mg DM. Nevertheless, gas production of after 24 h was higher in Dwarf versus Red, Common and Silver Napiers. The asymptotic gas production (B) ranged from 44 to 62 ml, potential gas production (A+B) from 43 to 66 ml and the rate of gas production (C) from 0.024 to 0.035 h^{-1} (Table 4). Highest value ($P < 0.05$) of asymptotic gas production and potential gas production were observed in Dwarf Napier with 65 and 66 ml / 200 mg DM, respectively. Low gas production in tall cultivars (43 to 56 ml of potential gas production) was expected because of high fibrous fraction. The result is in agreement with Kamalak et al. (2005). Nevertheless, there were no significant differences between cultivars in term of degradation rates. Both

Common and Red Napiers reached their asymptotes faster than others since the gas produced by both cultivars within 24 h incubation period were more than 80% of potential gas production. This indicated that most of fermentable carbohydrates fraction available in Common and Red Napiers were fermented into volatile fatty acids (acetic, butyric and propionic acids) within 24 h. Gas production from fermentation of protein is relatively small as compared to carbohydrates fermentation, whereas the fermentation of fat to gas production is negligible (Wolin, 1960). However, Menke and Steingass (1988) suggested that the incubation time chosen was less dependent on the time needed to achieve maximum gas production, but more on the expected rumen retention time. Furthermore, it could be expected that Napier grass would remain in the rumen for at least 48 h (Ansah et al., 2013).

Table 3. Gas production (ml/ 200 mg DM) of fresh Napier cultivars at 96-h incubation period

Napier cultivar	Incubation time (h)											
	2	4	6	8	10	12	24	32	36	48	72	96
Common	6.28^{a}	11.95^{a}	16.75^{b}	20.80^{c}	24.22^{c}	27.12^{c}	37.20^{c}	40.07^{c}	40.94^{d}	42.34^{d}	43.07^{c}	43.19^{c}
Silver	6.45^{a}	12.04^{a}	17.00^{b}	21.39^{bc}	25.28^{bc}	28.72^{bc}	42.57^{b}	47.59^{b}	49.34^{c}	52.67^{c}	55.16^{b}	55.79^{b}
Red	6.41^{a}	13.84^{a}	20.15^{a}	25.50^{a}	30.05^{a}	33.92^{a}	47.62^{a}	51.64^{a}	52.88^{b}	54.93^{b}	56.07^{b}	56.26^{b}
Dwarf	7.35^{a}	13.55^{a}	19.02^{ab}	23.84^{ab}	28.11^{ab}	31.90^{ab}	47.34^{a}	53.21^{a}	55.35^{a}	59.69^{a}	63.51^{a}	64.80^{a}
SEM[1]	0.50	0.70	0.89	1.03	1.11	1.15	0.94	0.72	0.66	0.70	1.04	1.22

^{abc} Means with different superscripts in the same column differ significantly ($P < 0.05$)

[1]SEM: Standard error of the mean

Table 4. *In vitro* gas production characteristics and estimated metabolisable energy content of fresh Napier cultivars

Napier cultivar	B[1]	A+B[2]	C[3]	ME (MJ/kg DM)
Common	43.65^{c}	43.21^{b}	0.030^{a}	7.28^{c}
Silver	55.90^{b}	56.02^{b}	0.024^{a}	8.07^{b}
Red	58.64^{b}	56.30^{b}	0.028^{a}	8.75^{a}
Dwarf	65.21^{a}	65.52^{a}	0.035^{a}	8.76^{a}
SEM[1]	1.24	1.34	0.003	0.13

^{abc} Means with different superscripts in the same column differ significantly ($P < 0.05$)
[1]SEM: Standard error of the mean
[2]Asymptotic gas production (ml/200 mg DM)
[3]Potential gas production (ml/200 mg DM)
[4]Production rate of constant (ml/h)

The metabolisable energy (ME) is crucial as it reflects the actual energy level available for utilization. The values for estimated ME ranged from 7.28 in Common Napier to 8.76 MJ/kg DM in Dwarf Napier. According to Haryani et al. (2012), the ME content of Napier cultivars varied from 8.60 to 9.60 MJ/kg DM. The ME obtained in this study is

within the range reported by Evitayani et al. (2004) of between 6.4 to 9.3 MJ/kg DM and Mlay et al. (2006) of between 6.1 to 9.2 MJ/kg DM for tropical grasses. Menke and Steingass (1988) suggested a strong correlation between ME value measured *in vivo* and predicted ME from 24 h *in vitro* gas production and crude protein content of the forages. In the present study, both Red and Dwarf Napiers had the highest ($P < 0.05$) ME content. This showed that the ME content was very consistent with nutrient content, digestibility and gas production of these cultivars.

There was no significant difference ($P > 0.05$) of total VFA, acetic and butyric acids between cultivars (Table 5). The VFA concentration, acetic and butyric acids of Napier cultivars ranged between 52 to 73 mM, 68 to 70% and 5 to 7%, respectively. As predicted, the tall cultivars rich in cell wall content tended to yield more acetic ($P > 0.05$) than Dwarf Napier. The higher cell wall content of Napier grass as expressed in NDF and ADF would explain the difference in proportion of acetic, propionic and butyric acids. The fermentation of major cell wall component, particularly cellulose resulted in the formation of acetic acid and CO_2. This is in agreement with Murphy (1982), where the fermentation of structural carbohydrate yielded high amount of acetic acids and low amount of propionic acids. Consequently, high ($P < 0.05$) propionic acid was observed in Dwarf Napier than tall cultivars. Rapidly fermentable carbohydrate produces relatively higher propionate as compared to acetate and the reverse takes place when slowly fermentable carbohydrates are fermented (Getachew et al., 1998). Based on the results, the acetic to propionic ratio was higher in tall cultivars ($P < 0.05$) compared to Dwarf Napier. This was due to the least NDF fraction and high in cell contents present in Dwarf Napier compared to the taller cultivars. According to Seymour et al. (2005), milk production was mainly derived by the butyric and propionic as dry matter energy intake increased in proportion to milk production. Besides, they also found that the milk fat was positively correlated with acetic to propionic ratio.

Table 5. Volatile fatty acids composition of fresh Napier cultivars

Napier cultivar	Total VFA (Mm)	Acetic (%)	Propionic (%)	Butyric (%)	A:P
Common	52.15[a]	70.41[a]	22.78[c]	6.80[a]	3.10[a]
Silver	72.97[a]	69.88[a]	24.45[ab]	5.57[a]	2.87[ab]
Red	70.06[a]	70.48[a]	23.31[bc]	6.21[a]	3.03[ab]
Dwarf	52.65[a]	68.39[a]	24.88[a]	6.74[a]	2.77[b]
SEM[1]	7.78	0.89	0.51	0.54	0.92

[abc] Means with different superscripts in the same column differ significantly (P<0.05)

[1]SEM: Standard error of the mean

Conclusion

Red and Dwarf Napier had the highest *in vitro* digestibility (IVDMD and IVOMD), total gas production and degradation rates among the cultivars. This indicates that both cultivars are easily degraded, fermented and absorbed for utilization for body maintenance and production.

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