Research Article

Nutrient Contents and *In vitro* Ruminal Fermentation of Tropical Grasses Harvested in Wet Season in the Philippines

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Abstract | In tropical countries, perennial grasses are the key feed resources for ruminants; grasses having high nutritional characteristics are important for maintaining the productivity and health of animals. In this study, the nutrient composition and *in vitro* ruminal degradation characteristics as well as the short-chain fatty acid (SCFA) production of 7 grasses grown over short cutting intervals were compared with those of corn and rice straw. Grasses were harvested at 35 – 39 days after regrowth in the wet season in Laguna, Philippines. Crude protein (CP) contents were not remarkably different among the grasses (from 9 to 11% in a dry matter (DM) basis, $p>0.05$). Neutral detergent fiber (NDF) contents varied from 63% to 73% DM ($p<0.05$); the lowest was found in Napier grass (*Pennisetum purpureum*) and the highest in Para grass (*Brachiaria mutica*). Non-fiber carbohydrate (NFC) content was the highest in corn, followed by Jarra grass (*Digitaria milanjiana*) and Gamba grass (*Andropogon gayanus*) ($p<0.05$). The *in vitro* NDF degradability was also the highest in corn, followed by that in Jarra grass and Guinea grass (*Panicum maximum*) ($p<0.05$). SCFA production and gas production was the highest in corn, followed by Jarra grass, Guinea grass, and Signal grass (*Brachiaria decumbens*), and the lowest in rice straw ($p<0.05$). These findings suggested that the nutritional characteristics, particularly NFC and NDF contents, and rumen degradation varied across grasses harvested at short cutting intervals. Among the grasses investigated in this study, Jarra grass and Guinea grass contained potentially high nutritive value due to the highest rumen fermentation characteristics.

Keywords | Gas production, *In vitro* rumen fermentation, Nutrient composition, Short-chain fatty acids, Tropical forages

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INTRODUCTION

The population of the Philippines is close to 100 million and is expected to reach over 140 million by the year 2040 (Philippines Statistics Authority, 2006). With the increase in population, the consumption of meat and milk will increase, which would also lead to an increased demand for animal feed. Forage production is very vital in raising ruminants such as cow, buffalo, goat, and sheep. In general, grasses and rice straw are used as basic feeds for ruminants. Rice straw is an abundant feed resource, but its protein content is considerably lower and goes not meet the requirement of animals (Bakrie et al., 1996). Grasses such as Napier grass (*Pennisetum purpureum*) and Para grass (*Brachiaria mutica*) have often been used for ruminant feeding in cut-and-carry system and grazing (Moog, 2006). In the Philippines, grasses grow perennially and can be harvested 5 to 7 times a year when soil and water conditions are well managed (Moran, 2005; Orden et al., 2015). Although plant yield increases with age, the digestibility of grasses decreases (Moran, 2005). Thus, cutting intervals of grasses are recommended to be around 40 days (Moran, 2005; Manyawu et al., 2003; Lounglawan et al., 2014). Whole-crop corn, which includes the ear, is also valuable forage because it contains high nutrients suitable for animals which require high energy. However, corn cultivation needs to sow the seeds with each time, and requires more fertilizer and water than those required for grasses. Moreover, forage corn production directly competes with corn for human consumption. Thus, a perennial grass production system with short cutting interval and multiple harvesting would be valuable for meat and milk production in the tropical environment.

Several domestic and foreign grasses grow in the Philippines, although plant production is affected by climatic conditions (mainly wet and dry seasons). Supporting livestock production necessitates the accumulation of nutritional data of grasses under different area, climate, and soil conditions (Nasrullah et al., 2004). The *in vivo* digestibility is an important parameter of feed evaluation; however, *in vitro* ruminal degradation characteristics are also useful because they allow the rapid evaluation of many samples (Makkar, 2004). Further, the *in vitro* gas production technique is one of the useful tools for forage evaluation since production of gas after incubation of rumen microorganisms with grasses as substrates has been known to be highly correlated with metabolisable energy content and feed intake by ruminants (Menke and Steingass, 1988; Blümmel and Ørskov, 1993). Short-chain fatty acids (SCFAs) produced from feed degradation in rumen are recognized to be a better parameter than digestibility for forage evaluation because they are the primary energy sources for ruminants (Blummel et al., 1999).

This study aimed to determine the nutritive values in terms of nutrient composition and *in vitro* rumen degradability as well as SCFA production from 7 kinds of perennial grass grown for short cutting intervals in the wet season. Rice straw and corn were used as maker samples for low and high nutritional forage, respectively. The second object was to determine the alternative parameters of SCFAs in the *in vitro* rumen by correlating their concentrations with nutritional composition and other parameters from *in vitro* test.

MATERIALS AND METHODS

Forages

The 7 introduced grasses species and corn were harvested at the field of University of the Philippines, Los Banos in August, 2010. Samples of Gamba grass (*Andropogon gayanus*), Signal grass (*Brachiaria decumbens*), Para grass (*Brachiaria mutica*), Jarra grass (*Digitaria milanjiana*), Guinea grass (*Panicum maximum*), Napier grass (*Pennisetum purpureum*), and Splendida (*Setaria sphacelata* var. *splendida*) were randomly collected from 3 quadrants (0.5 m x 0.5 m) at 35 – 39 days of age following a cutting height of around 15 cm from the ground. On the other hand, corn was harvested at dough stage, 65 days of age. Rice straw was obtained from a local farm. All forages were chopped to 2 – 3 cm, and then dried at 60°C for 48 – 72 hours to determine the dry matter (DM) content and used for the following *in vitro* experiment and chemical analysis.

In vitro Rumen Degradation of Forages

In vitro rumen degradability test was performed by the method of Uddin et al., (2010) with some modifications. Dried forages as above were ground to pass through 1 mm screen. Samples (0.5 g) collected from three different plots were individually weighed into 100 mL capacity glass vial in duplicate. Rumen fluid was collected before morning feeding from 2 crossbred cattle cows with rumen cannula fed grass hay and concentrates (1:1) at maintenance level. Rumen fluid was filtered through four layers of cheesecloth and mixed with McDougal buffer at 1:2 ratio, then flushed by CO_2 at 39°C for 10 min. Fifty mL of this medium were dispensed into each vial and these vials were covered with rubber cap and aluminum ring, and then incubated in a water bath at 39°C. To eliminate the gas from buffer solution and excess flushed CO_2, the gas volume produced after 10 min of incubation was released. Gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h. In another incubation run, vials were opened at 48 h and collected the undegradable feed particles by centrifuge at 1,000 × *g* for 10 min. The residues were freeze-dried and subjected to NDF analysis to determine NDF degradability. Supernatant was obtained for the analysis of SCFA.

Chemical Analyses

Organic matter (OM), CP, ether extract (EE) contents were analysed as described in AOAC International (2002). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed as outlined by Van Soest et al. (1991) and both fiber contents were expressed as ash free forms. Non-fiber carbohydrate (NFC) content was calculated as OM – CP – NDF – EE. SCFA concentration in the *in vitro* rumen culture was determined by HPLC equipped with ion exclusion chromatography and a post-column pH-buffered electroconductivity detection method (Shimadzu, Kyoto, Japan).

Statistical Analyses

Data of nutrient composition and *in vitro* rumen fermentation were analysed using a one-way analysis of variance (ANOVA) and tested by Tukey's test, performed using SAS 9.3 (SAS Institute, Carry, USA). Moreover, data of SCFA and other parameters from 27 samples were analysed by pairwise correlations of variables using STATA 13.1 (Statacorp, College Station, Texas, USA).

RESULTS AND DISCUSSION

Plant heights of grasses were higher in Napier grass and

Table 1: Plant height, nutrient composition of 7 grasses, corn and rice straw

	Plant height	DM	OM	CP	EE	NFC	NDF	ADF	ADL
	cm	%	% DM						
Gamba grass	151 ab	22.9 b	92.2 b	10.8 a	2.84 a	8.69 bc	69.9 bcd	39.7 ab	4.94 a
Signal grass	95.9 c	20.2 bc	90.3 c	9.59 a	2.00 bcd	8.10 bcd	70.6 abc	40.2 ab	4.12 ab
Para grass	104 c	18.2 cd	88.5 d	9.58 a	1.82 bcd	4.08 ef	73.0 a	38.8 b	3.50 bcd
Jarra grass	93.2 c	17.1 cde	88.2 d	8.91 a	2.19 abc	9.51 b	67.6 de	41.0 ab	3.76 bc
Guinea grass	138 b	19.7 bc	88.2 d	9.19 a	2.19 abc	5.17 de	71.6 ab	43.6 a	4.33 ab
Napier grass	165 a	14.3 de	82.4 e	10.8 a	2.74 a	5.60 cde	63.2 f	39.2 ab	3.92 bc
Splendida	–	13.1 e	81.8 e	9.40a	2.50 ab	1.87 f	68.0 cde	38.5 b	3.24 cd
Corn	–	19.7 bc	94.2 a	9.81 a	1.59 cd	15.3 a	67.5 de	33.4 c	2.81 d
Rice straw	–	89.2 a	76.0 f	5.16 b	1.29 d	3.66 ef	65.9 ef	39.6 ab	3.86 bc
sem	4.23	0.86	0.32	0.52	0.15	0.63	0.56	0.94	0.17

NFC: non-fiber carbohydrate; **ADL:** acid detergent lignin; –: not determined; **sem:** standard error of the means; Means with different superscripts within a column are significantly different (p<0.05)

Gamba grass, moderate in Guinea grass, and lower in Signal grass, Para grass and Jarrah grass (Table 1). The DM contents of forage except for rice straw were 13%–23% as shown in Table 1. These low DM values are not specific because these forages were grown in wet season when the soil at the experimental site had water, and fresh forage could absorb sufficient amount of water from the soil. CP contents ranged from 9%–11 % DM except for rice straw (5% DM). Protein requirement in feed for producing milk needs to be higher than 11% DM in tropical countries (Moran, 2005). Thus, rice straw did not meet the protein requirement for productive animals, and the protein contents of grasses were found to be at the minimum level. Unlike stems, leaves contain more proteins owing to the activity of photosynthetic enzymes such as ribulose bisphosphate carboxylase/oxygenase (rubisco) (des Francs et al., 1985). The proportion of leaves and stems influenced the CP content of the forage (Moran, 2005). In this study, forages were harvested around 40 days after regrowth, and leaf proportion would be higher than that after later cutting. Although the CP contents in grasses were almost two times as high as rice straw, their level was insufficient for productive animals. Moran (2005) described that high-yielding dairy cows required Napier grass harvested every 30 days to maintain the high protein level. Sufficient protein from grasses can be obtained by using shorter cutting intervals of less than 40 days. Alternatively, feeding legume and tree leaves as protein supplement to animals is thought to be effective (Aban and Bestil, 2013; Basitan and Jarcia 2013; Sultana et al., 2015).

The 7 grasses investigated showed NFC contents of 6% DM on an average; this value was lower than reported for other tropical grasses (approx. 10%–15% DM) (Kozloski et al., 2007; Magalhães et al., 2010). Among the grasses investigated, Gamba grass, Signal grass, and Jarra grass contained relatively higher NFC. Corn contained the highest NFC among the forages, which could be due to the accumulation of starch in the ear part. Several papers showed higher NFC contents in whole crop corn (18%–40%DM) than that found in the present study (Johnson et al., 2003; Magalhães et al., 2010; Samiei e al., 2015). This difference could have been caused by the amounts of grains and their maturity. In this study, the ear of corn was not completely filled with grains and had not matured well. Increasing NFC content in corn could be possible with more precise management. A fraction of NFC included rapidly fermentable carbohydrates, including soluble sugars and starch, in the rumen, and these compounds supply energy (i.e., adenosine triphosphate; ATP) for microbial growth (Kingston-Smith and Theodorou, 2000). In particular, ATP from carbohydrates fermentation in the rumen is important to convert NH_3 into microbial proteins (Bakrie et al., 1996). Although ATP can be supplied mainly from fiber degradation, these structural carbohydrates in fiber are degraded slowly; thus rapidly fermentable carbohydrates such as NFC would be more beneficial to increase microbial protein synthesis (Kingston-Smith and Theodorou, 2000). Little information is available on the effects of dietary NFC on milk production in the tropics; however, in the temperate regions, optimal NFC concentration in a diet of high-yielding dairy cow was 30% DM (Huhtanen and Nousiainen, 2012). In this study, grasses were found to contain considerably less NFC than the requirement for dairy cows. Grains contain high amounts of NFC, but their high price and competition with human food has popularised NFC supply from grasses in recent years. In temperate climate, a novel variety of perennial ryegrass that contains increased concentration of sugars was bred. Miller et al. (2001) reported that cows fed high-sugar ryegrass variety showed increased milk and milk protein production and decreased urea N excretion than those fed normal variety. Thus, collecting more data on NFC content of grasses and selecting and/or breeding high-NFC varieties of tropical

Table 2: *In vitro* ruminal NDF degradability, degradable NDF content and short-chain fatty acids (SCFAs) of 7 grasses, corn and rice straw

	Gas production	NDF degradability	Degradable NDF content	Total SCFAs	Acetic acid	Propionic acid	Butyric acid	A/P ratio
	mL	%	% DM	---------------- m mol/L	---------------------			
Gamba grass	73.2 cd	53.9 cd	37.6 bc	49.7 cd	33.8 bc	11.7 de	4.07 bc	2.88 a
Signal grass	76.0 c	59.1 abc	41.8 ab	49.8 cd	32.8 cd	13.2 bc	3.78 c	2.49 e
Para grass	69.7 de	50.7 d	37.0 c	47.7 de	31.4 d	12.2 cd	4.08 bc	2.58 cde
Jarra grass	84.0 b	63.7 ab	43.1 a	53.3 b	35.4 b	13.6 b	4.31 bc	2.60 bcde
Guinea grass	74.7 c	59.3 abc	42.5 a	51.7 bc	34.0 bc	13.5 b	4.22 bc	2.53 ed
Napier grass	72.8 cde	58.6 bc	37.0 c	49.7 cd	33.0 cd	12.1 cd	4.60 b	2.72 bc
Splendida	68.8 e	54.5 cd	37.0 c	48.3 cde	32.5 cd	11.9 d	3.88 c	2.73 ab
Corn	99.5 a	65.1 a	43.9 a	61.4 a	40.2 a	15.5 a	5.73 a	2.59 bcd
Rice straw	63.1 f	56.8 cd	37.4 bc	45.9 e	31.2 d	10.8 e	3.84 c	2.88 a
sem	0.84	1.25	0.91	0.68	0.43	0.21	0.12	0.027

A/P ratio: the ratio of acetic acid and propionic acid; **sem:** standard error of the means; Means with different superscripts within a column are significantly different ($p<0.05$).

grasses are necessary. This might reduce the requirement of concentrate for the cut-and carry and grazing systems.

In forages investigated here, the dominant nutrient was NDF, which varied from 63% to 73% DM. NDF contents in grasses were similar to rice straw. Napier grass, one of the major grasses in the Philippines, contained the lowest NDF despite the higher plant height. Grant et al. (1974) reported similar results where NDF contents in Napier grass grown at Los Banos, Philippines, were 62% and 66% DM at 45 and 60 days cutting age, respectively. However, in Kenya, Napier grass harvested at 70–90 days after cutting had 71%–78% DM of NDF (Gwayumba et al., 2002), indicating that cutting age greatly affected NDF contents in Napier grass. Para grass showed the highest NDF contents among the collected plants despite moderate plant height. The contents of ADF, the less digestible fiber, were the lowest in corn, whereas those in grasses were similar to those in rice straw. These values were similar to those reported by Feedipedia (2015) except for Jarra grass, which was not listed. The ADL content was the lowest in corn and highest in Gamba grass. Fukushima and Dehority (2000) reported that ADL contents in Napier grass and Guinea grass increased from 4% DM at the start of florescence to 7%–11% DM at complete seed formation with less than half of green leaves remaining. Grasses cut within 40 days after regrowth remained in the vegetative and beginning florescence stage, and hence, lignin was not likely accumulated in the plant. Lignin was shown to depress the digestibility of fibers owing to strong binding between lignin and cellulose (Moran, 2005). Among the grasses, corn, and rice straw, the correlation between NDF degradability and ADL content was low ($r = -0.26$, $p>0.05$, data not shown). Lignin in the form of acetyl bromide-soluble lignin (ABSL) showed higher correlation ($r = -0.97$) with digestibility (Fukushima and Dehority 2000; Fukushima and Kerley, 2011). Therefore, evaluation of fiber characteristics in grasses is needed to determine the ABSL content as lignin.

Corn showed the highest NDF degradability, gas production and SCFA concentration among the forages studied (Figure 1, Table 2), indicating that it had highly degradable fibers and high NFC contents. The *in vitro* ruminal gas production was the highest in corn, lowest in rice straw, and intermediate in the 7 grasses. Unlike gas production, NDF degradability was lowest in Para grass, high in Jarra grass followed by Guinea grass, and Signal grass. Rumen degradable NDF contents were approximately 42% DM in Jarra grass, Guinea grass and Signal grass, and these were significantly higher than those in other grasses ($p<0.05$).

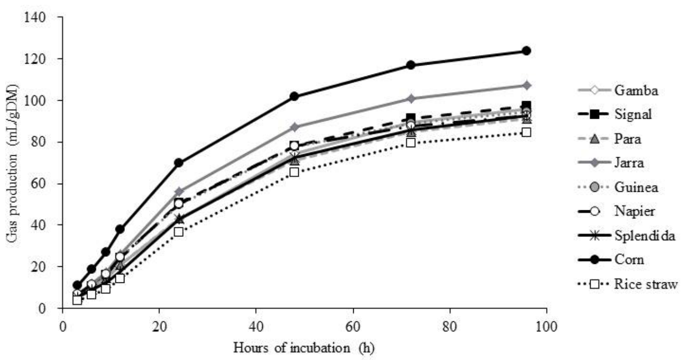

Figure 1: *In vitro* rumen gas production from 7 grasses, corn and rice straw

SCFA are the primary source of metabolisable energy in ruminants; thus their production in the rumen would be important parameters for feed evaluation (Moran, 2005). SCFA concentration in the *in vitro* rumen after 48-h in

Table 3: Coefficient of correlation for *in vitro* rumen short-chain fatty acids (SCFA) concentration and nutrient composition, gas production, degradability

	OM	CP	NDF	ADF	ADL	NFC	Gas production	DM degradability	NDF degradability	Degradable NDF content
SCFA	0.65 ***	0.29 ns	−0.07 ns	−0.47 *	−0.37 ns	0.84 ***	0.96 ***	0.66 ***	0.71 ***	0.70 ***

*p<0.05; *** p<0.001; **ns:** not significant.

cubation was the highest in corn, and lowest in rice straw, and intermediate in the 7 grasses (Table 2). Jarra grass produced relatively higher SCFA than the other grasses. The ratio of acetic and propionic acid (A/P ratio) in the rumen culture from Gamba grass and rice straw were higher than the others, but there was no marked difference in this ratio among these grasses. The A/P ratio is related to the milk fat and lactose content, since acetic acid is metabolised to fatty acids, whereas propionic acid undergoes gluconeogenesis (Moran, 2005). According to the present *in vitro* ruminal experiments, milk component from cows fed the 7 grasses does not seem to be largely different, but *in vivo* experiment will be required to evaluate the effect of grass feeding to milk composition.

Our results showed that SCFA concentration was highly correlated to NFC but not to NDF contents (Table 3). SCFAs are produced from not only fiber degradation but also soluble sugars and starch. These findings indicate that the amount of soluble sugars and storage carbohydrates are directly correlated to SCFA production since most of these carbohydrates can be fermented well in the rumen. However, the amounts of structural carbohydrates were not correlated to SCFA production. Degradabilities varied across different grasses; thus, degradable NDF content was more correlated to SCFA than to NDF content itself. Although NDF degradability is one of the important parameters for the evaluation of fiber quality, monitoring SCFA production based on the utilization of storage and structural carbohydrates in the rumen is better. Measurement of SCFA is laborious and costly. Our findings suggested a high correlation between the *in vitro* ruminal gas production and SCFA production from tropical grasses. Similar findings were reported in temperate grasses (Blümmel and Ørskov, 1993; Blümmel et al., 1999). In the present study, SCFA concentration was estimated from gas production by using the following equation:

$$y = 0.4155x + 19.354 \ (n = 27, r = 0.96)$$

where y indicates SCFA concentration (mmol/L at 48 h) and x indicates gas production (mL/0.5g for 48 h). Measurement of gas production is simple and does not involve any cost and could be useful for the estimation of SCFA production from grasses. Further studies are needed to identify the relation between *in vitro* gas production and metabolisable energy content of grasses and feed intake by ruminant using the grasses grown in the Philippines. These data will be helpful for feed evaluation of grasses by using *in vitro* ruminal gas production systems.

CONCLUSION

The CP content in seven kinds of tropical grasses investigated in this study was comparable, but the NDF content was highly varied across the grasses even though they were harvested at the similar regrowth periods. The *in vitro* ruminal SCFA production of the grasses was higher than that of rice straw and lower than that of corn. Jarra grass and Guinea grass showed the highest SCFA production among the grasses, indicating that these two grasses could supply more metabolisable energy to ruminants than the others. The results also showed SCFA production was better correlated with *in vitro* ruminal gas production than with NDF degradability. Thus, *in vitro* ruminal gas production would be a convenient parameter to assess SCFA production from tropical grasses and would be useful for forage evaluation in future researches. Further, research should also be conducted in the dry season to determine the nutritional characteristics and plant production of these grasses throughout the year.

CONFLICT OF INTEREST

No competing financial or other interest exists.

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AUTHORS' CONTRIBUTION

All the authors contributed equally.

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