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YIELD AND NUTRITIVE QUALITY OF NAPIER (*PENNISETUM PURPUREUM*) CULTIVARS AS FRESH AND ENSILED FODDER

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ABSTRACT

Napier grass is a C4 type tropical grass and commonly used as ruminant feedstuffs due to its promising yield. The preservation of Napier grass is to ensure continuous feed supply for the animals during shortage of forages as well as preserving the quality of the grasses. The study on nutritive quality of fresh and ensiled Napier cultivars was conducted at March 2014 at Universiti Putra Malaysia, Malaysia. Four Napier cultivars (Common Napier, Silver Napier, Red Napier and Dwarf Napier) were sown in a randomized complete block design with three replications. The Common, Silver and Red Napier were classified as tall types and Dwarf Napier as short cultivar. Results revealed that Common Napier and Red Napier had significantly higher ($P \leq 0.05$) dry matter yield (DMY). Both Silver and Dwarf Napier have the lowest DMY ($P \leq 0.05$) regardless of the grass height. Generally, Dwarf Napier had the highest leaf to stem ratio (3.18) and nutritive value (10% crude protein (CP); 66% *in-vitro* dry matter digestibility (IVDMD); 56% *in-vitro* organic matter digestibility (IVOMD)) than tall cultivars. The process of fermentation clearly decreased the nutritive value of Napier cultivars. The ensiling process decreased ($P \leq 0.05$) neutral detergent fibre (NDF) content (72 to 67%), IVDMD (69 to 61%)and increased ($P \leq 0.05$) the IVOMD (52 to 58%) and gross energy (16 to 17 MJ/kg).Regardless of the feed types, the CP content of Napier cultivars surpassed 7% of the minimum requirement for rumen microbial sustainability. There were interaction ($P \leq 0.05$) between cultivars and feed types on CP, potential gas production (A+B), metabolisable energy (ME) and degradation rates (C). The CP content of Common, Red and Dwarf Napier increased numerically due to ensiling process. Nevertheless, the ME content and C value declined gradually after the ensiling process. The compaction during ensiling process could affect the quality of Napier cultivars in particular the IVDMD and the degradation rates due to leaching of nutrient. It is concluded that the nutritive value of Napier cultivars were generally decreased due to preservation mechanism. Despite, the CP content and total gas production of Common Napier increased significantly after ensiled.

Keywords: ensiled, napier cultivars, yield.

INTRODUCTION

Forages are essential in animal production systems hence the most important feed as a substitute for concentrates normally used for feeding livestock. In fact, forages were certainly known as a cost effective feed rather than commercial concentrate. The substitution of forage to concentrate from 30 to 70% in dairy cattle diet could reduce up to 30% cost of production (Sanh *et al*., 2002).

Guinea and Napier are the common cultivated grasses species in Malaysia. Guinea grass yielded from 9 to 12 tonnes dry matter yield (DMY) ha^{-1} cut^{-1} with cumulative mean more than 20 tonnes ha^{-1} yr^{-1}(Ahmed *et al*., 2012; Munyasi *et al*., 2015). Besides, the Napier grass tends to produce higher dry matter yield and surpass most other tropical grasses with yield of 70 tonnes ha^{-1} yr^{-1} (Wijitphan *et al*., 2009). Napier grass was first introduced to Malaysia in the 1920's, since then many cultivars had been introduced in Malaysia, namely as Common Napier, Red Napier, Taiwan Napier, Dwarf Napier, Dwarf "Mott", Australian Dwarf, Indian Napier, Uganda Napier, Zanzibar Napier, Kobe Napier and King grass (Halim *et al*., 2013;Haryani *et al*., 2012). Several comparative studies on the effect of harvesting age showed that the optimal harvesting ages of Napier grass were within 5 to 9 weeks old (Zailan *et al*., 2016; Lounglawan *et al*., 2014). Repeated cuttings at shorter interval will reduced the cumulative DMY whereas prolonged harvesting age will reduced the quality of the grasses. The tropical grasses tend to reach its maturity faster, become fibrous and lignified compared to temperate grass. Moreover, leaf fraction which is more digestible and nutritious compared to stem declined as the species advanced towards maturity (Mustaque *et al*. 2010). Therefore, silage is most suitable method in preserving the nutritional quality of Napier grass within the optimum harvesting ages.

Napier grass grows best in high-rainfall areas not less than 1200mm rainfall yr^{-1} but it does not tolerate flooding (Legel, 1990). The estimated area prone to flood disaster is 9% of total area in Malaysia (D/iya *et al*., 2014). In spite of flood, tropical countries are more vulnerable to drought compared to temperate countries and therefore, the conservation of feed is crucial to preserve the quality and supply adequate feed to livestock. Silage was found to be more suitable than hay making process because of high relative humidity, more than 90%, will easily spoil the hay. In an attempt to preserve the nutritional quality, especially protein content, the changes in nutritive value of grasses during ensiling process need to be assessed.

The conservation of Napier grass among the plentiful tropical forages is preferable due to its high forage yield and favorable concentration of soluble carbohydrates in providing good fermentation condition. However, at early harvesting age, when it presents a promising nutritive value, the high moisture, high buffer power and the low sugar concentration can result in low quality silage. The presence of topsoil layer during fodder harvesting might introduce the undesirable microorganism during ensiling, mainly clostridia. Proliferation of clostridia may appear under specific circumstances of low water soluble carbohydrates in conjunction with high moisture and temperature. The presence of clostridia in silage has reduced digestibility and intake characteristics and increases the incidence of metabolic disturbances in animals to which they fed (Yeruham, *et al*. 2003). In an attempt to produce good quality silage, 35 to 40% dry matter content and at least 2% of sugar content in feed materials were required as recommended by Ohmomo *et al*. (2000). Increase in dry matter content of silage, particularly by wilting technique benefits both environmental and nutritional value of silage. Moreover, the breakdown of protein content by plant protease cease by increasing the dry matter content (Soderlund, 1995). The optimum pH for protease activity is between pH 4 to 8. Proteolysis and growth of spoilage microorganism can be inhibited by reducing the pH value. Thus, the quality of silage depends on the harvesting age, nutritional composition before ensiled and the method of ensiling.

Hence, the general objective of this study was to evaluate the yield and nutritive value of four Napier (*Pennisetum purpureum*) cultivars as fresh and ensiled fodder.

MATERIALS AND METHODS

Preparation and management of experimental materials: Field experiment was conducted at Field 2, Universiti Putra Malaysia (UPM), Malaysia. The experimental plot is located in a Tropical humid zone, 3°00'24.3 North latitude and 101°42'10.3 East longitude, with an average rainfall of 2507 mm per annum. The average minimum and maximum temperature were 23.6°C and 32.9°C, respectively and average relative humidity was 74%. The soil texture 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 clay (30.53%) (Jusoh, 2005).

The stem cutting of four cultivars (Common Napier, Silver Napier, Red Napier and Dwarf Napier) were collected from Malaysian Agriculture Research and Development Institute (MARDI). The Napier grasses were planted in March 2014 on a seedbed that had been prepared by ploughing and disc harrowing. Planting grass by stem cutting about 20 cm length and placed 45° from the ground level with half of the nodes buried in the soil and other nodes left exposed for tiller emergence. The Napier grass cultivars were planted in plot size of 5 m x 4 m using a randomized complete-block design (RCBD) with three blocks of the four cultivars in each block. Grasses were planted in rows with five rows with the planting distances of 0.5 m between plant and 1 m between rows. Basal fertilization applied during grass establishment were 60 kg N, 60 kg P and 50 kg K ha^{-1} using urea (46% N), triple superphosphate, TSP (20% P) and muriate of potash, MOP (50% K), respectively and 2 tonnes of Ground Magnesium Limestone (GML) ha^{-1}. All plants were watered twice a day and plant re-growth was harvested at 12th weeks after sowing. Subsequent harvesting was done at 6-8 weeks of harvesting ages for further analysis.

Dry matter production and chemical analysis: The 3rdcutting Napier grasses were used as experimental materials by cutting 20 cm above ground level from randomly selected 1 m × 1 m quadrate. The grass yield obtained from quadrate sampling was oven-dried at 65°C for 48h and later calculated as dry matter per hectare. The grass samples 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).The gross energy (GE) content of the grasses was measured using IKA® Adiabatic Bomb Calorimeter C2000, Germany whereas the metabolisable energy (ME) content was estimated based on 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

***In-vitro* studies:** Animals and rumen fluid extraction. The gas production profiles were measured using method described by Menke and Steingass (1988). The rumen fluid used as inoculum was collected before morning feeding from three rumen-fistulated crossbred goats which fed on mixture of fresh grasses and transferred into pre-warmed thermos bottles. The rumen fluid was composited and strained using two layers of cheesecloth under continuously flushing with CO_2.

In-vitro gas production: Approximately 0.2 g DM of grounded samples (1 mm) with a 1:2 (v/v) mixture of rumen fluid and buffer medium was placed in a glass syringes (FORTUNA®Optima glass syringe), incubated at 39°C of water bath and being shaken at regular times. Incubation was performed in triplicates. The blanks without substrate was 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.

In-vitro digestibility and degradability characteristics: Sample replicates were terminated at 48 h incubation period for determination of *in-vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD). 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:

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

Where, OM = Organic matter

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

Silage making: The entire plot was then harvested and wilted on the field for 5 h. Later, the chopped grass was compacted using the auto-silager machine and kept in anaerobic condition of sealed container. After 28 days of ensiling, the samples were taken at 15, 30 and 45 cm from the top of the container and pooled as a composite sample. The samples were oven-dried at 65°C, ground through 1.5 mm sieve for chemical analysis and *in-vitro* studies as described previously.

Statistical Analysis: A one-way analysis of variance (ANOVA) was used to determine the dry matter production and leaf to stem ratio of fresh Napier cultivars with general linear model procedures of SAS 9.3 (Statistical analytical Institute Inc. Cary, North Carolina, USA). Each cultivars were pooled for silage fermentation and the comparative data on nutritional quality and kinetic fermentation of fresh and ensiled Napier were assigned via completely randomized design with 4 x 2 factorial experiments (4 cultivars x 2 feed types). The difference between treatment means was measured by the Least Square Means. The level of significance used to determine the differences between treatments is $P \leq 0.05$.

RESULTS AND DISCUSSION

Dry matter production and leaf to stem ratio of fresh Napier cultivars: According to the result obtained, the Silver Napier had a similar DM yield ($P>0.05$) as Dwarf Napier (≤ 4 tonnes ha^{-1} cut^{-1}) regardless of the grass height. Common Napier had the highest DM yield ($P \leq 0.05$) of 5.8 tonnes ha^{-1} cut^{-1} followed by Red Napier with 4.7 tonnes ha^{-1} cut^{-1} (Table 1). The cultivars were classified into tall cultivars (Common, Silver and Red Napier) and short cultivars (Dwarf Napier). One of a few local reports comparing different varieties of Napier grass showed that the height of tall types exceeds 130 cm while the short types were shorter than 90 cm (Halim *et al*, 2013). Numerically, the mean of DM yield for tall cultivar (Common, Silver and Red Napier) were higher than Dwarf Napier and this result is in agreement with Obok *et al*. (2012) where the plant height influenced the dry matter production. These differences of height are due to the length of internodes and the pattern of internodes influenced by the differentiation of cells of apical meristems (Rodrigues *et al*., 1986). The leaf to stem ratio (LSR) is one of the criterion in evaluating the quality of the pasture grass because the higher proportion of leaves compared to stem indicate a better nutritive value. As expected, the short internodes of Dwarf Napier recorded the highest LSR (3.18) compared to the tall cultivars which ranged from 0.74 to 1.84. The result showed that Common Napier had a higher proportion of stem compared to leaves with the LSR of 0.74. Therefore, Common Napier can be classified as high yielding and stemmy cultivars than others. Red Napier was the leafier (1.84 LSR) cultivar among the tall types and this has a significant implication in nutritive quality as the leaves contain higher level of nutrient and less fibrous compared to stems fraction.

The nutritional quality of Napier cultivars: The NDF, ADL, cellulose and gross energy (GE) content were significantly affected ($P \leq 0.05$) by cultivars (Table 2). Dwarf Napier had the lowest ($P \leq 0.05$) NDF and ADL content as it was reflected by the LSR as described previously. Among the tall cultivars, Red Napier had the lowest NDF and ADL content. Moreover, the GE content of Red Napier was the highest (17.25 MJ/kg) among the others. As calculated, the mean of CP content was highest in Dwarf (10.42 %), followed by Silver (9.61%) and least in both Red (8.80%) and Common (8.67%) Napier. This was reflected by the composition of Napier leaf fraction where twice CP content found than in stem fraction (Ansah *et al*., 2010). The CP content in present study surpassed the minimum CP level of 7% required for rumen microbial sustainability (Lazzarini *et al*., 2009). There was no cultivar that was entirely high in DM yield and nutritive quality. For instances, Common Napier had the highest yield and lowest CP content. In contrast, Dwarf Napier was low yielding and it had the highest CP contents. An increase in the structural carbohydrates (cell wall) had reduced the digestibility as well as suppressing the cellular content including the portion of CP content as indicated by da Silva *et al*. (2011). The finding was in agreement with Khan and Chaudhry (2011), where the IVDMD and IVOMD were higher in feed contained higher nutrient and lower fiber content. The cell wall fraction is the major factor influencing the voluntary feed intake and digestibility of forages as described by Harper and McNeill (2015). Fortunately, there was no significance difference ($P>0.05$) in digestibility (63 to 66% IVDMD; 53 to 56% IVOMD) between the cultivars. The dry matter digestibility of Napier cultivars were slightly lower compared to results obtained by Manyawu*et al.* (2003) ranging from 68 to 71% IVDMD.

Changes in nutritional quality of ensiling Napier cultivars: All grasses were well ensiled based by pH value (3 to 4) and a lack of visible mold or spoilage observed. It is generally accepted that pH values of silage less than 4 (McDonald *et al*., 1991). Based on the interpretation of silage analyses by Kung and Shaver (2002), pH for good quality grass and legume silage in the tropics ranges between 4.3 and 4.7. The pH value obtained in this study was in agreement with McDonald *et al*. (1991) but lower than 4.3-4.7 reported by Kung and Shaver (2002). There was interaction ($P\leq0.05$) between cultivars and feed types on CP content (Figure 1). The CP content of Common Napier increased significantly ($P\leq0.05$) from 8.13 to 9.26% after ensiling process. Increase of CP content ($P>0.05$) in Napier cultivars after ensiled could be accompanied by the populated anaerobic microorganism during ensiling process (Zakaria, 2011). In spite of that, a reduction ($P\leq0.05$) of CP content in Silver Napier after ensiling resulted by proteolysis occurring during wilting and ensiling by plant enzymatic activity and microbial fermentation (Duniere *et al*., 2013).The NDF content of ensiled Napier grass was clearly affected ($P\leq0.05$) by the process of fermentation. The reduction of NDF content from 72 to 67% can be explained by the hydrolysis of NDF content during fermentation process (Huisden *et al*., 2009). The IVDMD were markedly decreased ($P\leq0.05$) after ensiling process. This can be explained by the losses of digestible portion which reflected primarily by the losses of soluble in effluent as well as associated with the extensive fermentation. Interestingly, the IVOMD of Napier grass increased significantly ($P\leq0.05$) from 52 to 58% after ensiling. Thus, it seems that the improvement of IVOMD was contributed by microorganisms involved during fermentation of ensiled grass. The GE content of Napier grass increased significantly ($P\leq0.05$) after ensiling process and the increased in silage energy concentration could be attributed by the formation of fermentation products particularly organic acids. The Red Napier had the highest ($P\leq0.05$) metabolisable energy (ME) among the fresh cultivars (Figure 4). Nevertheless, the ME of Napier cultivars decreased significantly ($P\leq0.05$) due to ensiling process. The ranged of ME content in tropical grasses varies from 6.10 to 9.23 MJ/kg (Mlay *et al*., 2006; Evitayani *et al*., 2004). Regardless of the feed types, the ME content for both fresh (8.14 to 8.64 MJ/kg) and ensiled (6.83 to 7.38 MJ/kg) Napier cultivars were within the range of ME content in tropical grasses as reported by Mlay *et al*. (2006) and Evitayani *et al*. (2004).

Kinetics fermentation of fresh and ensiled Napier cultivars: The cumulative gas production increased with increasing time of incubation. In spite of that, the plateau of fresh and ensiled Napier gas production reached at 32 hours and 72 hours incubation period, respectively. The cumulative gas produced by fresh Napier was 3 to 6 ml higher ($P\leq0.05$) than ensiled over 48 hours incubation period(Figure 2).The end-products of carbohydrates fermentation were primarily short chain fatty acids (acetate, butyrate and propionate acids) and gases (carbon dioxide, methane and hydrogen gases). The incubated feed was prolonged to 96 hours due to the undefined asymptotic gas production (B). The fresh and ensiled Napier cultivars showed a negative value on gas production of immediately soluble fraction (A). The negative value obtained indicated that there has to be an initiation period for degradation to start microbial colonization known as a lag phase (Blummel and Becker, 1997). None of the results showed a positive result and this positive result assuming that there is a component that degraded rapidly or a presence of soluble component (Orskov *et al*., 1980).

There was interaction ($P\leq0.05$) between cultivars and feed types on potential gas production (A+B) (Figure 3). The A + B value was highest ($P\leq0.05$) recorded in fresh Napier cultivars but not in Common Napier. This might be reflected by the significant increase in CP content of ensiled Common Napier that contributed to the total gas production. As calculated, the gas produced by fresh and ensiled Napier at 24 hours incubation was up to 79 and 75% of A + B, respectively .The degradation rates (C) of Napier cultivars were significantly affected by the interaction between cultivars and feed types (Figure 5). The degradation rates of fresh Napier cultivars (0.045 to 0.050 h^{-1}) were slightly higher than reported by Kariuki *et al*. (2001) (0.044 h^{-1}) and the decreased in degradation rates was in agreement with Kis *et al*. (2005) due to ensiling process. Presumably this reflected by the low fraction of easy fermentable carbohydrates that loss during ensiling period. This is because the gas yielded predominately by fermentation of carbohydrates (340-370 ml gas/g substrate) followed by protein (130 ml gas/g substrate) and negligible in fat fermentation (1-2 ml gas/g substrate) (Cone and Gelder, 1999; Menke and Steingass, 1988).

Table 1. Dry matter yield and leaf-to-stem ratio of fresh Napier cultivars

Cultivars	Dry matter yield (kg/ha/cut)	Leaf-to-stem ratio
Common	5823.33^{a}	0.74^{c}
Silver	3577.83^{b}	1.06^{c}
Red	4374.00^{ab}	1.84^{b}
Dwarf	3358.67^{b}	3.18^{a}
SEM	486.03	0.17
	Treatment comparison	
Treatment	P-value	
Cultivar	*	*

* Refer to significant differences at $P \leq 0.05$
$^{a, b, c}$ Within a row, means without a common superscript differ ($P \leq 0.05$)

Table 2. Nutritional quality of fresh and ensiled Napier cultivars.

Treatment	Nutritional composition (%)					Energy (MJ/kg)
	NDF	ADF	ADL	Hemicellulose	Cellulose	Gross energy
Cultivar						
Common	71.59^{a}	41.22	6.17^{a}	30.37	35.05^{a}	16.90^{c}
Silver	70.82^{a}	42.15	5.26^{b}	28.67	36.89^{a}	17.05^{b}
Red	68.74^{b}	40.29	4.80^{b}	28.45	35.49^{a}	17.25^{a}
Dwarf	66.11^{c}	36.00	4.50^{b}	30.16	31.50^{b}	16.84^{c}
SEM	0.74	0.40	0.32	0.94	0.51	0.05
Feed types						
Fresh	71.68^{a}	41.51^{a}	5.92^{a}	30.17	35.59^{a}	16.88^{b}
Ensiled	66.95^{b}	38.31^{b}	4.44^{b}	28.66	33.87^{b}	17.14^{a}
SEM	0.51	0.29	0.22	0.65	0.36	0.03
			Treatment comparison			
Treatment			P-value			
Cultivar	*	ns	*	ns	*	*
Feed types	*	*	*	ns	*	*
Interaction	ns	ns	ns	ns	ns	ns

NDF: Neutral detergent fiber, ADF: Acid detergent fibre, ADL: Acid detergent lignin; * Refer to significant differences at $P \leq 0.05$; $^{a, b, c}$ Within a row, means without a common superscript differ ($P \leq 0.05$); ns refers to non-significant difference

Table 3. *In-vitro* gas production and digestibility of fresh and ensiled Napier cultivars

Treatment	Gas production (mL)		Digestibility (%)	
	A	B	IVDMD	IVOMD
Cultivar				
Common	-2.59	50.94	65.62	56.11
Silver	-2.02	50.10	63.11	53.01
Red	-2.74	51.46	63.74	53.67
Dwarf	-2.41	48.24	65.81	56.04
SEM	0.23	0.70	2.06	2.14
Feed types				
Fresh	-3.46^{a}	52.12^{a}	68.75^{a}	51.63^{b}
Ensiled	-1.42^{b}	48.26^{b}	60.38^{b}	57.78^{a}
SEM	0.51	0.48	1.46	1.58
		Treatment comparison		
Treatment		P-value		
Cultivar	ns	Ns	ns	ns
Feed types	*	*	*	*
Interaction	ns	Ns	ns	ns

A: Gas production of immediately soluble fraction, B: Gas production of insoluble fraction, IVDMD: In-vitro dry matter digestibility, IVOMD: In-vitro organic matter digestibility; * Refer to significant differences at $P \leq 0.05$; $^{a, b}$Within a row, means without a common superscript differ ($P \leq 0.05$); ns refers to non-significant difference

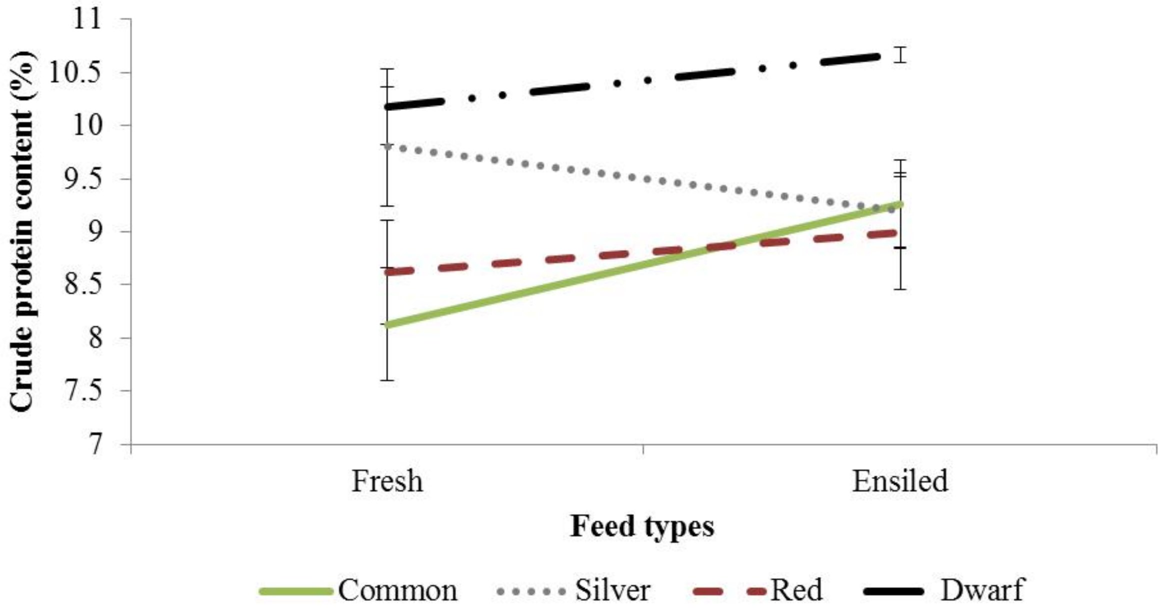

Figure 1. The interaction of crude protein (±SE) content between fresh and ensiled Napier cultivars

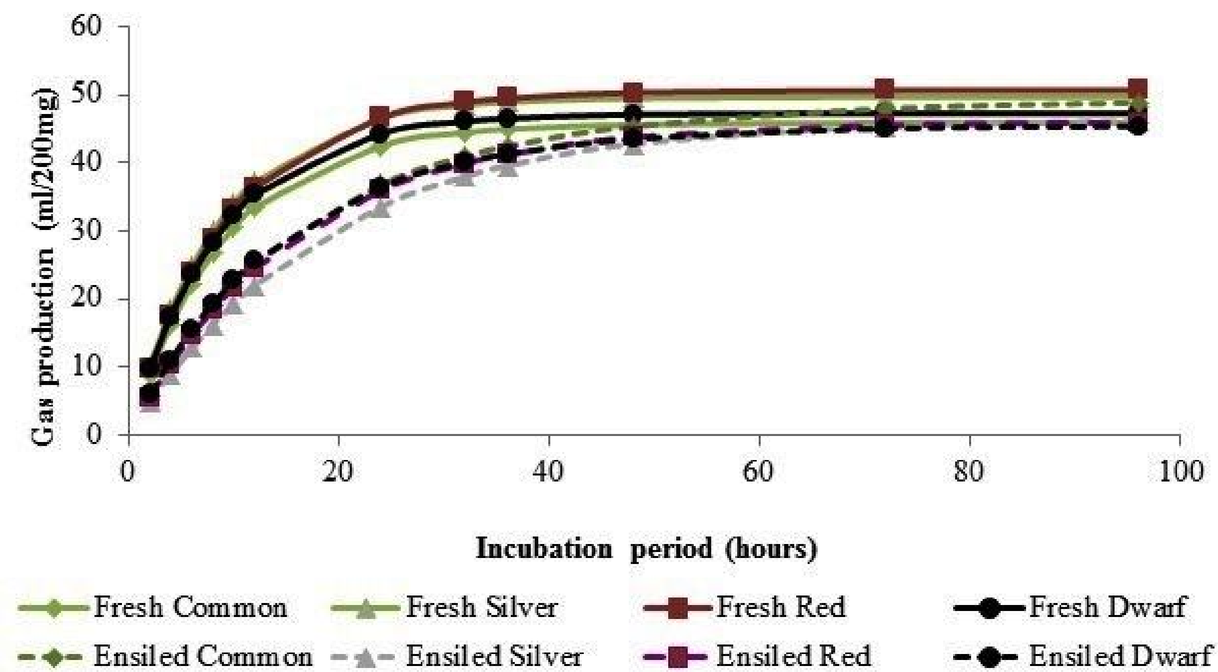

Figure 2. Kinetic fermentation of fresh and ensiled Napier cultivars over 96 hour incubation period

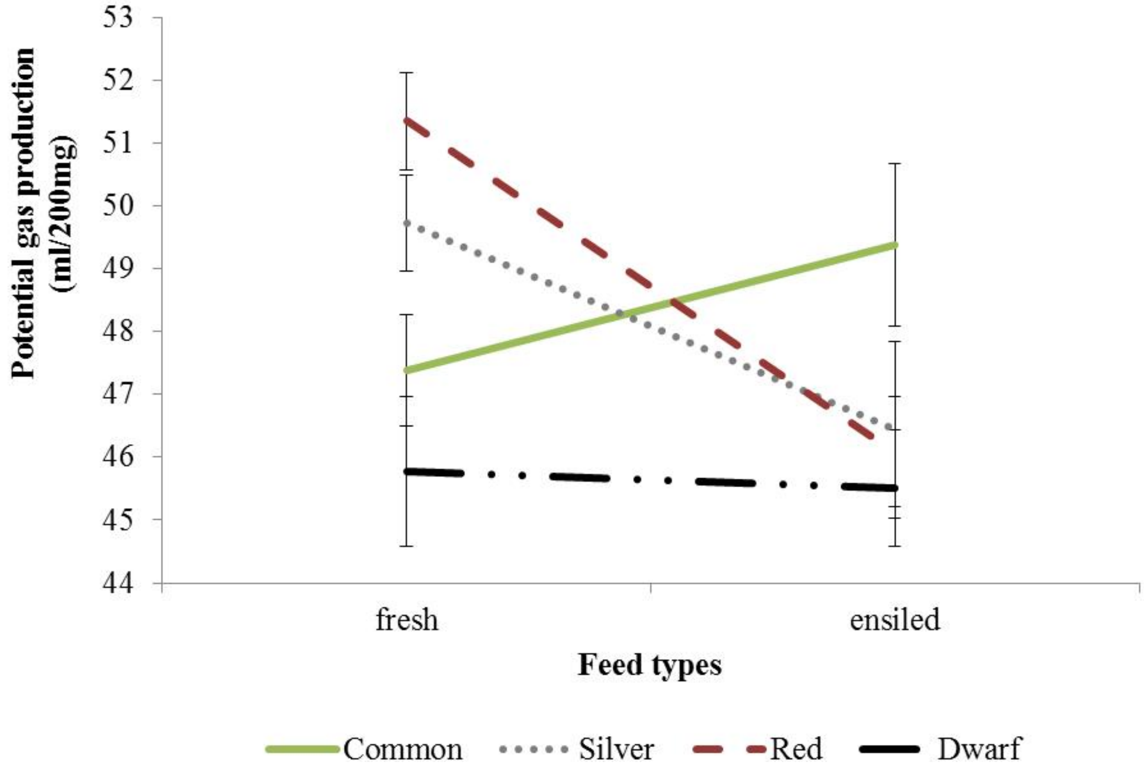

Figure 3 The interaction of potential gas production (A + B) (±SE) of fresh and ensiled Napier cultivars

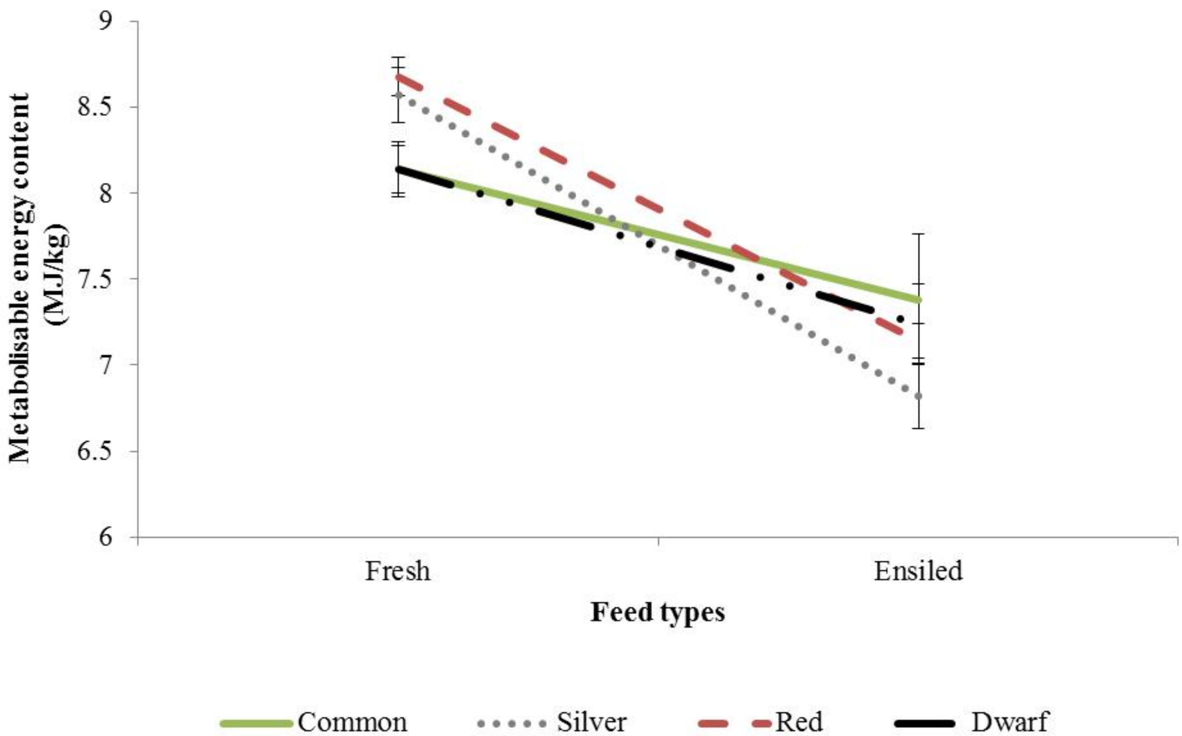

Figure 4. The interaction of metabolisable energy content of fresh and ensiled Napier cultivars

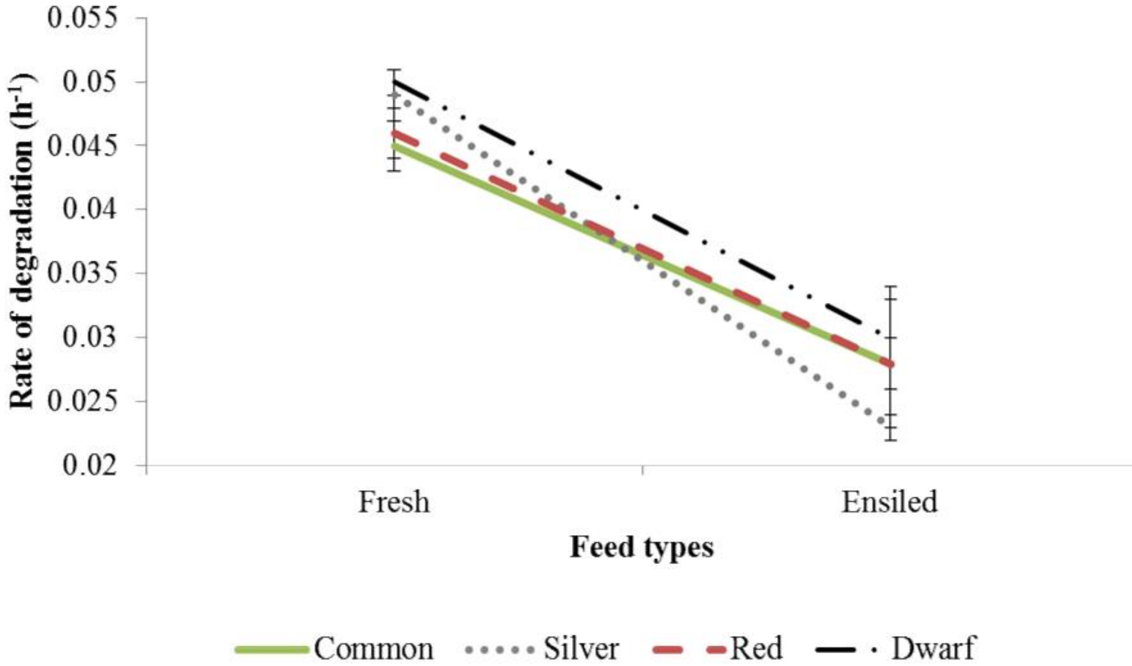

Figure 5. The interaction of degradation rates (C) (±SE) of fresh and ensiled Napier cultivars

Conclusions: Common and Red Napier were high yielding grasses. Ensiling of Napier cultivars declined the digestibility (IVDMD and IVOMD) and degradability rates. Fermentation of available substrates contributed to the formation of organic during ensiling process increased the energy content of Napier cultivars. The CP content of Common, Red and Dwarf Napier increased and loss of CP content observed in Silver Napier after ensiling process.

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