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Assessment of Nutritional Potential and Methane Mitigation Efficacy of Napier Grass Cultivars

Yakup BILAL [1]* | **Bilal SELCUK [1]** | **Tuğba BAKIR [1]** | **Emrah KAYA[2]** | **Adem KAMALAK[1]**

[1] Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Animal Science, Kahramanmaraş, Turkey

[2] Iğdır University, Faculty of Agriculture, Department of Animal Science, Iğdır, Turkey

Correspondence
[1]Iğdır University, Faculty of Agriculture, Department of Plant Protection, 76000, Iğdır, Turkey
Email: emrah.kaya@igdir.edu.tr

Abstract

Napier grass is cultivated as a high-yielding forage crop and widely utilized in the nutrition of grazing animals such as cattle and goats due to its rapid growth rate, expansive leaf surface, and ability to thrive even in low-quality soils, making it a valuable animal feed option. In this study, the chemical compositions, in vitro gas (GP) and methane (CH4) production, metabolizable energy (ME), organic matter digestibility (OMD), true digestible dry matter (TDDM), microbial protein (MP), partitioning factor (PF), microbial protein synthesis efficiency (MPSE, and relative feed values (RFV) of the grasses Packhong1 (Penisetum purpureum x Penisetum americanum), Chinese Pennisetum (Penisetum purpureum x Penisetum alopecurides), and Dwarf Elephant (Pennisetum purpureum cv. Mott) were investigated. Significant differences were found in the chemical contents of napier varieties used in the study (p<0.05). The crude protein (CP) values of the Napier grass varieties were 12.21, 13.58, and 12.04%, while the acid detergent fibre (ADF) values were 51.91, 50.58, and 47.09%, respectively. Following a 24-hour incubation period, GP values were 70.16, 73.42, and 85.36 ml, with corresponding CH4 of 13.08, 16.68, and 16.08%. The TDDM contents were measured at 231.96, 253.82, and 239.61, and the PF values were found to be 3.58, 3.75, and 3.04. The findings highlight variations in chemical composition and GP characteristics among the varieties, offering insights for more efficient feed utilization and environmental sustainability in livestock management. In conclusion, additional in vivo studies are warranted to assess the impact of Napier grass varieties on feed intake.

Keywords: Digestion, Feed value, Gas production, Napier grass

INTRODUCTION

The escalation of greenhouse gases has emerged as one of the most critical global concerns in recent years, prominently contributing to climate change (Seyedin et al., 2022). The primary greenhouse gases generated by human activities are carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). The escalating production of these greenhouse gases worldwide poses a threat to life, while it has been determined that the absence of these gases could reduce the Earth's average surface temperature by approximately -18°C (Ma, 1998). This situation underscores the vital role these gases play in the survival of life on Earth. However, a rapid increase in the concentration of these gases leads to climate change and global warming. According to a statement by the United Nations Food and Agriculture Organization (FAO), the agricultural sector is responsible for 18% of greenhouse gas emissions worldwide, with approximately 80% of these emissions attributed to livestock (Swain et al., 2016). It is anticipated that global temperatures will rise slightly above 1°C per year, and global warming is expected to decrease feed quality by reducing digestibility and crude protein (CP) content in feeds (Polley et al., 2013; Rojas-Downing et al., 2017). Tropical grasses typically utilize C4 metabolic pathways for photorespiration, while most temperate grasses employ C3 carbon fixation pathways. Often, C4 metabolic pathways lead to faster lignin accumulation in plant tissues, which can be a factor altering dry matter intake (DMI) and digestibility in ruminants (Wilson, 1994). Napier grasses (*Pennisetum purpureum*) are a type of C4 carbon-fixing monocot perennial grass known for their natural presence in tropical regions across Africa,

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especially in East Africa (Khan et al., 2007). They are recognized for their high productivity capacity and use as livestock feed, being long, robust, and deep-rooted perennial grasses (Woodard and Prine, 1991). Napier grasses come in various varieties and new Napier grass varieties emerge through interbreeding (Dowling, 2012). The aim of this study is to determine the potential nutritional values, relative feed values (RFV), and anti-methanogenic properties of Packhong1 (*Pennisetum purpureum* X *Pennisetum americanum*), Chinese Pennisetum (*Pennisetum purpureum* X *Pennisetum alopecurides*), and Dwarf Elephant Grass (*Pennisetum purpureum* cv. Mott).

MATERIALS and METHODS

Napier grasses used in this study were harvested from a private greenhouse in Antalya in the first year of cultivation and in the pre-flowering period in 2023. These greenhouse-harvested Napier grasses were dried in the shade for 7 days and subsequently ground to prepare them for the milling process. The grasses used in the study were then individually ground using a 1 mm feed mill. The chemical analysis of the ground feeds, including dry matter (DM), crude ash (CA), ether extract (EE), and CP content, was conducted in triplicate following the method outlined by AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to the procedure described by Van Soest et al. (1991). For the determination of fermentation parameters, the ground Napier grass varieties were weighed at approximately 200 mg ± on a sensitive balance and placed into 100 ml special glass syringes. Subsequently, 30 ml of a previously prepared artificial saliva solution and rumen fluid obtained from three Merino sheep were added to the glass syringes at a 1:2 ratio. The glass syringes were then subjected to a 24-hour incubation in a water bath set at 39°C, in triplicate, following the method of Menke et al. (1979). After the 24-hour incubation, the gas measurements within the glass syringes were determined using a methane analyzer (Sensor Europe GmbH, Ekrath, Germany). The measurement values obtained were calculated according to the formula as reported by Goel et al. (2008).

$$CH_4 \text{ (ml)} = GP \text{ (ml)} \times CH_4 \text{ (\%)} \quad (1)$$

In this formula:

GP represents the gas production after 24 hours of incubation (200 mg DM).

The metabolic energy (ME) and organic matter digestibility (OMD) of the grasses used in the study were determined according to the formula provided by Menke and Steingass (1988).

$$ME \text{ (Mj kg}^{-1} \text{ DM)} = 2.2 + (0.136 \times GP) + (0.057 \times CP) + (0.002859 \times EE \times EE) \quad (2)$$

$$OMD \text{ (\%)} = 14.88 + 0.8893 \times GP + 0.448 \times CP + 0.651 \times CA \quad (3)$$

ME: Metabolic Energy (Mj kg^{-1}),

GP: Gas Production (ml),

CP: Crude Protein (%),

EE: Ether Extract (%),

CA: Crude Ash (%)

OMD: Organic matter digestibility (%)

Neutral detergent acid (NDF) and acid detergent fibre (ADF) values of Napier grass varieties were determined according to the method reported by Van Soest et al. (1991). The relative feed values of these grasses were calculated according to the following formula (Van Dyke and Anderson, 2000).

$$DMD \text{ (\%)} = (88.9 - (0.779 \times \%ADF)) \quad (4)$$

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DMI (%) = (120 / %NDF) (5)

RFV = (%DMD X %DMI) / 1.29 (6)

In this formula;

DMD: Dry Matter Digestion

DMI: Dry Matter Intake

RFV: Relative Feed Value

After measuring the gases and methane produced in special glass syringes following a 24-hour incubation period, the inoculants were transferred into 100 ml beakers. Subsequently, 70 ml of NDF solution was added, and the mixture was boiled on a hot plate device for 60 minutes. Upon completion of the boiling process, the substrates in the beakers were filtered through glass filters with the aid of suction. After filtration, the glass filters were placed in an oven and kept at 70°C for 120 minutes. The glass filters were then removed from the oven, cooled in a desiccator, and weighed using a sensitive balance. The weights obtained were calculated using the formula described below (Blümmel et al., 1997a).

TDDM (%): (Amount of incubated substrate - Amount of substrate after filtration) X 100 (7)

PF = (TDDM / GP) (8)

MP (mg) = (TDDM - (2.2 X GP)) (9)

MPSE = ((TDDM - (2.2 X GP)) / TDDM) X 100 (10)

TDD (%) = (TDDM / Amount of incubated substrate) X 100 (11)

In these formulas:

TDDM: True Digestible Dry Matter

PF: Partitioning Factor

MP: Microbial Protein

EMP: Microbial Protein Synthesis Efficiency

TDD: True Digestibility Degree

The study's data were subjected to ANOVA and Duncan multiple range tests. Principal component analysis was conducted using the chemical composition and *in vitro* gas production parameters as variables, with Napier grass varieties used as the classification criterion.

RESULTS

The chemical compositions of Napier grass varieties are provided in Table 1. Significant differences in the chemical content of the Napier grass varieties used in the study were observed ($p < 0.05$). The ADF content, which represents the proportion of more resistant fibrous components in the feeds, differed significantly among the varieties, with Packhong1 grass having higher ADF values compared to other Napier varieties. This indicates that Packhong1 grass contains a higher amount of resistant fibers compared to other varieties. The EE content of Napier varieties did not show a significant difference among all varieties ($p > 0.05$). The NDF represents the fiber content of plant cell wall components in the feeds, and statistically, there was no significant difference in NDF values among the varieties ($p > 0.05$). The CA value, which indicates the mineral content of the feed, was 14.10% for

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Dwarf Elephant Grass, which is 10.95% and 9.67% higher compared to the other two varieties. This difference suggests that Dwarf Elephant grass contains more mineral content than the others. The CP content represents the protein content of the feeds. Dwarf Elephant grass had the highest CP value at 13.58%, while the other varieties had lower CP values at 12.21% and 12.04%. The varieties did not show a significant difference in their ME values ($p > 0.05$). The OMD represents the proportion of digestible organic matter in the feeds. Dwarf Elephant and Chinese Pennisetum varieties had higher OMD ratios compared to the Packhong1 variety. These findings indicate that Dwarf Elephant and Chinese Pennisetum varieties are superior in terms of digestible organic matter. The GP in the feeds represents the amount of gas produced during feed fermentation. In this study, GP was measured for 200 mg of dry matter. Chinese Pennisetum grass had a higher GP value (36.07 ml) compared to the other varieties, indicating a higher fermentation potential and more GP. The other varieties, Packhong1 and Dwarf Elephant grasses, had lower GP values compared to the Chinese Pennisetum variety, suggesting lower fermentation potential and less GP.

Table 1. *Chemical compositions of the varieties*

Varieties	**DDM**	**CA**	**CP**	**EE**	**NDF**	**ADF**	**GP**	**ME**	**OMD**
Packhong1	92.69[a]	10.95[b]	12.21[a]	4.51	64.57	51.91[b]	30.51[a]	7.57	54.61[a]
Dwarf E.	94.57[b]	14.10[c]	13.58[b]	5.24	65.84	50.58[b]	31.24[a]	7.84	57.93[b]
Chinese P.	95.19[c]	9.67[a]	12.04[a]	4.46	64.04	47.09[a]	36.07[b]	7.72	58.65[b]
SEM	0.12	0.31	0.25	0.33	2.29	0.71	0.98	0.27	0.97
Sig.	.000	.000	.002	.103	.734	.001	.003	.633	.013

[abc] Differences between means with different letters within the same row are significant ($P < 0.05$). DDM: Dried Dry Matter, CA: Crude Ash, CP: Crude Protein, EE: Ether Extract, GP: Gas Production (200 Mg DM), OMD: Organic Matter Digestibility, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ME: Metabolic Energy (Mj Kg^{-1}), SEM: Standard Error Of The Mean. Sig: Significance Level.

The RFV, DMD, and DMI values of Napier grass varieties are provided in Table 2. The DMD of Napier grass varieties was found to be statistically significant ($p<0.05$). In the study, the highest RFV among the Napier grass varieties was determined to be 70.22 in Dwarf Elephant grass. The DMD and DMI values were found to be the highest in Chinese Pennisetum grass, with values of 52.20% and 1.87%, respectively.

Table 2. *Digestion values of the varieties*

Varieties	**RFV**	**DMD (%)**	**DMI (%)**
Packhong1	69.79	48.45[a]	1.85
Dwarf E.	70.22	49.49[a]	1.82
Chinese P.	52.20	52.20[b]	1.87
SEM	0.55	0.06	3.16
Sig.	.001	.800	.187

RFV: Relative Feed Value, DMD: Dry Matter Digestibility, DMI: Dry Matter Intake. SEM: Standard Error of the Mean. Sig: Significance Level.

The *in vitro* GP and digestion parameters of Napier grass varieties in Table 3 showed statistically significant differences ($p<0.05$). The *in vitro* GP and CH_4 production amounts of Napier grass varieties were observed to be the highest in Chinese Pennisetum grass, with values of 85.36 ml and 13.73 ml,

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respectively. The TDDM amount, PF, MP, MPSE, and TDD were found to be the highest in Dwarf Elephant grass, with values of 253.82 mg, 3.75, 104.96 mg, 41.33%, and 52.97%, respectively.

Table 3. Gas production and digestion parameters of the varieties

Varieties	GP	CH_4(ml)	CH_4(%)	TDDM	PF	MP	EMP(%)	TDD (%)
Packhong1	70.16^a	9.13^a	13.08^a	231.96^a	3.58^b	89.69^b	38.69^b	49.61^a
Dwarf E.	73.42^a	12.26^b	16.68^b	253.82^b	3.75^b	104.96^c	41.33^b	52.97^b
Chinese P.	85.36^b	13.73^b	16.08^b	239.61^a	3.04^a	66.55^a	27.73^a	50.05^a
SEM	2.17	0.64	0.46	4.59	0.09	5.24	1.87	0.82
Sig.	.001	.001	.000	.000	.009	.001	.001	.013

[abc] The difference between means with different letters in the same column is significant ($P<0.05$). GP: Gas Production (500 mg DM), TDDM: True Digestible Dry Matter, PF: Partitioning Factor, MP: Microbial Protein, MPSE: Microbial Protein Synthesis Efficiency, TDD: True Digestibility Degree, SEM: Standard Error of the Mean, Sig: Significance Level.

DISCUSSION

The nutrient content of feed ingredients may vary depending on factors such as the climate and soil conditions in the cultivation area, harvesting stage, and storage methods of feed ingredients (Akyıldız, 1986; Kutlu, 2001; Şehu, 2002). The CP content in feeds has been reported to be an important component for producing high-quality feeds (Mohamad et al., 2022). It has been reported that when the CP content of feed ingredients is below 8%, the enzymatic activities of microorganisms in the rumen may be limited, leading to an inadequate supply of required ammonia in the rumen (Norton, 2003; Cappellozza et al., 2013). Since the CP content of the Napier grass varieties used in the study is above 8%, it can be said that the enzymatic activities of microorganisms in the rumen are at an adequate level. In this study, the CP value of the Packhong1 variety used was determined as 12.21%. In a study conducted by Ahamed et al. (2021), the CP values of Packhong1 grasses were found to be between 9.49% and 11.23%. In another study, CP values of Packhong1 grasses were observed to be between 8.64% and 12.64% (Lounglawan et al., 2014). The CP values of Dwarf Elephant grass were determined as 13.94% in the study conducted by Sirait (2018). The CA content of feeds, when exceeding 14%, has been reported to lead to contamination with foreign substances (Nauman and Bassler, 1993). In this study, the CA values of the Napier grass varieties met the desired values for Chinese Pennisetum and Packhong1 grasses. Plant fibers play an essential role in ruminant animals. Ruminant animals can digest plant substrates with the help of enzymes not present in other mammals, thereby contributing to the life cycle of rumen microorganisms (Van Soest, 1994). Sirait (2018) found NDF-ADF contents of Dwarf Elephant grass to be 54.02%, 73.88%, and 34.02%, 44.11%, respectively, in his research. The NDF content of Dwarf Elephant grass in this study is similar, but the ADF content is slightly higher. Lounglawan et al. (2014) determined the NDF-ADF contents of Packhong1 grasses as 59.63-76.49 and 37.86-46.99, respectively. The NDF findings in this study are similar to the findings in the study, while the ADF findings are slightly higher. The GP of feeds is related to the amount of fermentable carbohydrates (Sampath et al., 1995). Volatile fatty acids produced during fermentation react with buffer solution, leading to indirect GP (Wolin, 1960). Therefore, it is believed that the evaluation of feeds should consider not only GP but also digestible nutrient content and MP production (Blümmel et al., 1997b). Studies have reported that higher PF values in feeds increase MPSE, feed intake, and digestibility (Blümmel et al., 1997b; Blümmel and Lebzien, 2001). The most suitable value of PF, an important part of MPSE, is reported to be between 2.75 and 4.41 (Blümmel et al., 1997b; Blümmel and Lebzien, 2001). In this study, the PF values of Napier grass varieties were found to be within the values reported by animal nutrition experts. In some studies, feeds have been classified based on their anti-methanogenic properties. According to this classification, those with values between 11-14% are

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considered to have low anti-methanogenic properties, those with values between 6-11% are considered to have medium anti-methanogenic properties, and those with values between 0-6% are considered to have high anti-methanogenic properties (Lopez et al., 2010). In this study, the Packhong1 grass among the Napier grass varieties used had low anti-methanogenic properties, while Dwarf Elephant and Chinese Pennisetum grasses were found to have no anti-methanogenic effect.

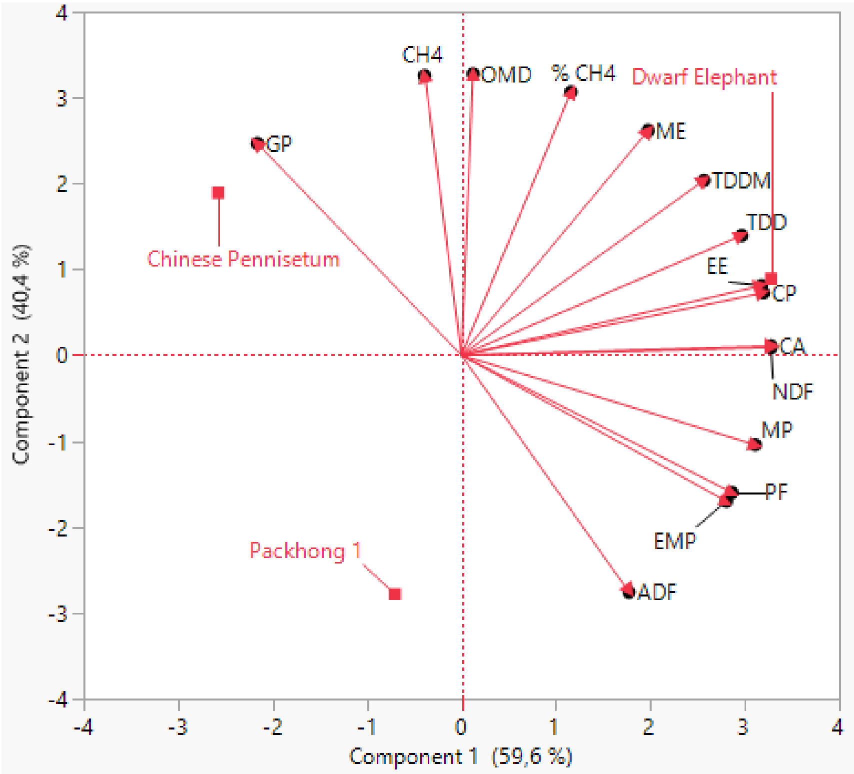

Figure 1. *Principal component analysis of varieties using chemical composition and GP parameters*

Figure 1 shows the principal component analysis of napier grass varieties. The first two principal components of Napier grass varieties accounted for 100% of the total. The variables GP, TDDM, PF, MP, EMP, TDD, NDF, CP, CA, and EE exhibited the highest factor loadings, with -0.22, 0.26, 0.29, 0.31, 0.28, 0.30, 0.33, 0.32, 0.33, and 0.32, respectively, explaining 59.6% of the variability between chemical composition and *in vitro* GP parameters through the first component (PC 1). The variables CH_4, %CH_4, ME, ADF, and OMD exhibited the highest factor loadings, with 0.40, 0.38, 0.32, -0.34, and 0.40, respectively, explaining 40.4% of the variability between chemical composition and *in vitro* GP parameters through the second component (PC 2). Dwarf Elephant grass was characterized by high %CH_4, ME, TDDM, TDD, EE, CP, CA, NDF, MP, PF, and EMP (Figure 1). Chinese Pennisetum grass was characterized by high GP, CH_4, and OMD variables, while Packhong1 grass was characterized by high ADF content.

CONCLUSION

According to the chemical composition of the Napier grass varieties used in the study, Packhong1 has a higher ADF content compared to the other two varieties. In terms of CP content, Dwarf Elephant variety is significantly different from the other varieties ($p<0.005$). Regarding *in vitro* GP and digestion parameters, Packhong1 variety exhibits lower gas and CH_4 production compared to the other varieties. However, it has the lowest observed value for TDDM. Chinese Pennisetum grass has the lowest PF value among the varieties while having the highest OMD value. Based on these findings, it is evident that there are differences among the varieties in terms of chemical composition and GP characteristics. These findings provide valuable information for more efficient feed utilization and environmental

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sustainability in the livestock industry. However, further *in vivo* studies are needed to examine the effects of these results on animal performance in more detail.

CONFLICTS of INTEREST

The authors declare there is no conflict of interest.

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