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Modulatory effects of *Macaranga tanarius* leaves on rumen fermentation and fatty acid biohydrogenation in sheep: an *in vitro* study

S. Jotham[1], A. I. Muhammad[1,3,] and A. A. Samsudin[1,2*]
[1] *Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.*
[2] *Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, Serdang 43400, Malaysia.*
[3] *Department of Animal Science, Faculty of Agriculture, Federal University Dutse. P.M.B. 7156, Jigawa State, Nigeria.*
Corresponding Email: anjas@upm.edu.my

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ABSTRACT

This study investigated the *in vitro* rumen fermentation characteristics, metabolism, and apparent biohydrogenation of fatty acids in *Macaranga tanarius* leaves (MTL) as a potential feed for sheep, compared to Napier grass (NG). Rumen fluid from four cannulated Dorper sheep was used to assess the *in vitro* fermentation kinetics of 200 mg of each forage. The analysis included gas production, *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD), volatile fatty acid (VFA) production, metabolizable energy (ME), and fatty acid profiles, including biohydrogenation. Metabolizable energy (ME), gas production, and the *in vitro* digestibility of dry matter (IVDMD) and organic matter (IVOMD) were significantly higher ($P < 0.05$) for the MTL group than for the NG group. This was accompanied by higher crude protein (CP), ether extract (EE) and non-fibre carbohydrates (NFC) in the MTL group. Conversely, neutral detergent fibre (NDF) and acid detergent lignin (ADL) were negatively correlated with CP, IVDMD, and IVOMD ($P < 0.001$), while strong positive correlations were observed among CO, IVDMD, and IVOMD ($P < 0.001$). Overall, *Macaranga tanarius* leaves demonstrated superior *in vitro* rumen fermentation efficiency, evidenced by enhanced digestibility, energy yield, and biohydrogenation capacity relative to Napier grass. These findings suggest that *M. tanarius* leaves hold significant promise as a sustainable and nutritious feed resource for sheep, with implications for optimizing ruminant nutrition and productivity.

Keywords: Biohydrogenation, Digestibility, Macaranga tanarius, Rumen fermentation, Sheep feed

INTRODUCTION

Forages are the cornerstone of ruminant nutrition, particularly in the tropics, offering the most economical feed source (Jiwuba *et al.,* 2021). Trees and shrubs represent a readily available and often cost-effective feed resource for livestock, supporting rural livelihoods. Beyond their nutritional value, these plants offer diverse benefits, including medicinal properties, soil enrichment, and land demarcation (Mercy *et al*., 2017). Ensiling or drying tree leaves for inclusion in ruminant feed formulations alongside grains is a common practice.

Macaranga tanarius, a medium-sized evergreen dioecious tree native to Malaysia, is widely

distributed throughout tropical regions (Fiala *et al*., 2011). This pioneer species thrives in cleared areas, along roadsides, and in secondary forests of West Malaysia and exhibits an ant-plant symbiotic relationship for herbivore defence. Known as "Mahang" in Malaysia, *Macaranga* species, including *M. tanarius*, have a history of traditional medicinal use due to their bioactive compounds (Mazlan *et al.*, 2013). Beyond medicinal applications, *M. tanarius* offers economic (timber), social (glue, firewood), and environmental (shade, shelter, soil improvement) benefits. Notably, the protein, lipid, carbohydrate, and amino acid-rich food bodies produced on its young stems, leaf blades, and stalks suggest potential nutritional value for livestock.

Accurately predicting the feeding value of potential feedstuffs through cost-effective and efficient *in vitro* methods is crucial for animal nutrition (Md *et al*., 2013). The *in vitro* gas production technique, a common method for estimating feed digestibility, utilises rumen fluid collected from cannulated animals (Foster et al., 2023; Pashaei et al., 2010; Widiawati & Thalib, 2007). The rumen's unique enzymatic capacity facilitates the digestion of soluble and insoluble carbohydrates, with the resulting gas production correlating with volatile fatty acid (VFA) production, a primary energy source for ruminants (Wang *et al.*, 2020). The profile of major VFA (acetate, propionate, butyrate) is largely influenced by the composition of the consumed feed, with higher cell wall content generally leading to increased acetate and decreased propionate production, and vice versa (Lu *et al*., 2020). The objective of this study was to determine the effects of *Macaranga tanarius* leaves, as a potential basal diet for Dorper sheep, on rumen fermentation profiles, metabolism, and the apparent biohydrogenation of fatty acids. This was achieved using an *in vitro* fermentation approach.

MATERIALS AND METHODS

Forage Sample Collection and Preparation

Macaranga tanarius foliage, comprising a blend of young and mature leaves, was randomly harvested from multiple trees within the Universiti Putra Malaysia (UPM) campus, Seri Kembangan, Selangor, Malaysia. This collection strategy ensured a representative sample of varying leaf maturity stages. Napier grass (*Pennisetum purpureum*) was cut at the vegetative growth stage, approximately 8–9 weeks after emergence, as determined by visible stem elongation and leaf development. Both types of forage were shade-dried with frequent manual turning until the weight stabilised, indicating a final moisture content of less than 10%. The dried materials were ground using a hammer mill and sieved into two distinct particle sizes: 1.0 mm for proximate and fibre composition analysis, and 2.0 mm for *in vitro* gas production assays.

Chemical Analysis

Proximate and fibre composition of the forage samples was determined using standard procedures. Dry matter (DM) was quantified by drying samples to a constant weight in a forced-air oven at 105 °C (AOAC, 2016; Method 934.01). Organic matter (OM) was estimated by subtracting the ash content from dry matter (DM). Ash content was determined by incineration at 550 °C for 6 hours (AOAC 942.05). Crude protein (CP) was analysed via the Kjeldahl (AOAC 990.03), and values were converted using a factor of 6.25. Ether extract (EE) was measured using Soxhlet extraction technique with petroleum ether (AOAC 920.39). Crude fibre (CF) was quantified by sequential digestion with dilute sulphuric acid and sodium hydroxide (AOAC 978.10). Fibre fractions, including neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL), were determined by a sequential fibre analysis method adapted from Adeyemi *et al*. (2015). Non-fibre carbohydrates (NFC) were calculated by differences:

$$NFC\ (\%) = 100 - (CP + EE + Ash + NDF)$$

Sample Preparation and Tannin Extraction

Tannin extraction was performed using a modified method based on Khatun *et al*. (2014). Briefly, 400 mg of finely milled *Macaranga tanarius* leaf sample was accurately weighed into a test tube. Pigments and lipophilic impurities were removed by adding 40 mL of diethyl ether containing 1% (v/v) acetic acid, incubating for 5 minutes, and carefully decanting the ether layer. The residue was then extracted with 20 mL of 70% (v/v) aqueous acetone in a sealed tube

(cotton wool and aluminium foil to limit light exposure). The sample underwent continuous agitation on an orbital shaker for 2 hours at ambient temperature to facilitate phenolic compound extraction. The mixture was filtered through Whatman No. 1 filter paper after shaking. The resulting tannin-rich filtrate was stored at 4 °C for subsequent quantitative analysis.

Estimation of Anti-Nutritional Metabolites

Total phenol and tannin concentrations in the plant samples were determined spectrophotometrically using the Folin-Ciocalteu reagent in combination with sodium carbonate (Na_2CO_3), following the method by Horvat *et al*. (2022). Absorbance was read at 725 nm, and total phenolic content was quantified against a tannic acid standard curve, with results expressed in tannic acid equivalents (TAE). To differentiate non-tannin phenolics, polyvinylpolypyrrolidone (PVPP) was used to selectively precipitate tannins from the extract.

Total phenolic content was determined, followed by the separate measurement of non-tannin phenols. The total tannin content was then calculated as the difference between these two values. Condensed tannins were quantified using a colourimetric method with a butanol-HCl reagent mixture (95:5 v/v) and a 2% ammonium iron (III) sulfate solution in 2N HCl). Absorbance was recorded at 550 nm, and the condensed tannin percentage was computed using the following formula:

Condensed tannin (%) = (A_{550} × 78.26 × dilution factor) / Dry Matter (%)

Hydrolysable tannins were estimated indirectly by subtracting the condensed tannin content from the total phenolic content.

***In vitro* Digestibility (72 h)**

The *in vitro* gas production technique was performed as described by Navarro Ortiz & Roa Vega (2020), using rumen fluid collected from four cannulated Dorper sheep. These animals were maintained on a consistent diet comprising rice straw, Napier grass, *Macaranga tanarius* leaves, and a commercial concentrate in a 60:40 forage-to-concentrate ratio, administered twice daily. Rumen fluid was collected before the morning feeding, strained through four layers of cheesecloth, and immediately transported to the laboratory in pre-warmed thermos flasks to maintained anaerobic conditions. Upon arrival, equal volumes of rumen liquor from each donor were pooled and combined with a pre-prepared buffer solution in a 1:2 (v/v) ratio while continuously purging with carbon dioxide (CO_2). The buffer medium was composed of the following per litre of distilled water: A bicarbonate-ammonium buffer was prepared by combining a macromineral solutions (5.7 g Na_2HPO_4, 0.6 g $MgSO_4 \cdot 7H_2O$, and 6.2 g KH_2PO_4), a micromineral mixture (8 g $FeCl_3 \cdot 6H_2O$, 1 g $CoCl_2 \cdot 6H_2O$, 13.2 g $CaCl_2 \cdot 2H_2O$, and 10 g $MnCl_2 \cdot 4H_2O$), and a bicarbonate-ammonium solution (4 g $(NH_4)HCO_3$ and 35 g $NaHCO_3$). This was supplemented with reducing solution (625 mg $Na_2S \cdot 9H_2O$ in 4 mL of 1 M NaOH) and resazurin indicator (100 mg resazurin/L)

The buffered rumen inoculum was maintained in a shaking water bath at 39 °C. For fermentation assay, 200 mg of oven-dried, finely ground forage sample (*Macaranga tanarius* or Napier grass) was weighed into calibrated 100 mL gas-tight glass syringes. Each syringe was then filled with 30 mL of the buffered rumen fluid under anaerobic conditions. Pistons were lubricated and inserted to expel residual air, and gas collection tubes were sealed with clips. The baseline piston position was recorded immediately. Control treatments included: (i) blanks (rumen-buffer only), (ii) standard hay, and (iii) standard concentrate. Each treatment and control were run in triplicate within a batch. Measurements of cumulative gas production were taken at intervals of 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours. To ensure reproducibility, the entire fermentation procedure was replicated in three separate incubation runs using freshly collected rumen inoculum each time.

pH Determination

The pH of the rumen inoculum was measured at the beginning (0 h) and end (72 h) of incubation period using a calibrated Mettler-Toledo digital pH meter (Mettler-Toledo Ltd., Leicester, UK).

Kinetic Fermentation

Cumulative gas production data were fitted

to the exponential model of Navarro Ortiz & Roa Vega (2020) using NEWAY software:

$$V = a + b\,(1 - e^{-ct})$$

Where V is the cumulative gas volume produced at incubation time t (ml), a is the gas volume from the rapidly fermentable fraction (mL), b is the gas volume from the slowly fermentable fraction (mL), and c is the fractional rate constant of gas production (h^{-1}).

Estimation of Metabolizable Energy

Metabolizable energy (ME) and *in vitro* organic matter digestibility (IVOMD) for *M. tanarius* leaves (MTL) and Napier grass (NG) were estimated using equations from Rojas-González *et al*. (2024). The equation are as follows:

$$ME\ (MJ/kg\ DM) = 0.057 \times CP + 0.136 \times GP_{24} + 2.20 + 0.0029 \times CF$$

$$IVOMD\ (\%) = 0.45 \times CP + 0.651 \times Ash + 0.0889 \times GP_{24} + 14.88$$

Where ME is metabolizable energy (MJ/kg dry matter), IVOMD is *in vitro* organic matter digestibility (%), CP = crude protein content (%), GP_{24} is the volume of gas production at 24 hours (ml/200 mg dry matter), CF is crude fibre content (%), and Ash is ash content (%).

***In Vitro* Dry Matter and Organic Matter Digestibility**

After 72 hours of incubation, the fermentation residues from each syringe were emptied into pre-weighed, oven-dried beakers. To determine the undigested dry matter, these beakers and their contents were then dried in a forced-air oven at 105 °C for 24 hours. The *in vitro* dry matter digestibility (IVDMD) was calculated as the difference between the initial sample dry matter and the residual dry matter. For *in vitro* organic matter digestibility (IVOMD), the dried residues were incinerated at 550 °C for 6 hours in a muffle furnace to determine ash content. The organic matter content of the residue was calculated by subtracting the ash weight from the residual dry matter. The IVOMD was then determined as the difference between the initial organic matter and the residue of organic matter.

IVDMD or IVOMD [%] = {(initial DM or OM [g] – undigested DM or OM [g])} / (initial DM or OM [g]) × 100

Methane Produced Estimation

Methane production (CH_4) during the *in vitro* fermentation was estimated based on the volatile fatty acid (VFA) proportions using equation from Behan *et al.* (2024):

$CH_4 = 0.5 \times [Acetate] + 0.5 \times [Butyrate] - 0.25 \times [Propionate]$

where CH_4 is the amount of methane produced (mmol) and [Acetate], [Butyrate], [Propionate] are the respective VFA concentrations (mmol/L)

***In Vitro* Dry Matter Digestibility (IVDMD%)**

After the 72-hour incubation, the contents of each syringe, including blanks, were transferred into pre-weighed, sintered glass crucibles. To ensure complete transfer of fermentation residues, each syringe was rinsed multiple times with distilled water, and the rinsate was added to the corresponding crucibles. Residual moisture removed via vacuum suction. The crucibles and their contents were then dried in a convection oven at 105 °C for 24 hours to determine the final residue weight, which was used to calculate the in vitro dry matter digestibility (IVDMD).

Volatile Fatty Acid (VFA) Analysis

Volatile fatty acid concentrations were determined using a modified procedure based on Behan *et al.* (2024). Briefly, frozen rumen fluid samples were thawed at room temperature for approximately 2 hours. A 1.0 mL aliquot from each sample was mixed with 200 µL of a 25% (w/v) metaphosphoric acid solution to precipitate proteins. After 30 minutes at room temperature, the mixture was centrifuged at 3000 × *g* for 10 minutes at 24 °C. Following centrifugation, 500 µL of the clear supernatant was transferred to a gas chromatography (GC) vial and mixed with an equal volume (500 µL) of a 20 mM 4-methyl-n-valeric acid solution, which served as an internal standard. VFA components were separated and quantified using a Hewlett-Packard 6890 gas chromatograph (Agilent 6890, Mississauga, ON, Canada) equipped with a fused silica capillary column (15 m × 0.32 mm i.d., 0.25 µm film thickness) and a flame ionisation detector (FID).

Ammonia-N (NH_3-N) Analysis

Ammonia nitrogen (NH_3–N) levels were quantified using a colorimetric method adapted from Silva *et al.* (2023). A standard curve was prepared using varying concentrations of ammonium sulfate standards. The absorbance of the test samples was then measured at 420 nm using a spectrophotometer (Secomam, Domont, France), with readings taken within 5–10 minutes after zeroing the instrument with a blank. Sample concentrations were then determined from the standard curve.

Fatty Acid Analysis

The fatty acid composition of the rumen substrate was analysed at 0 and 24 hours of incubation. Lipid extraction was performed using a modified procedure described by Troegeler-Meynadier *et al.* (2014). Briefly, thawed rumen fluid was subjected to lipid extraction with a 2:1 (v/v) chloroform-methanol mixture. The extracted lipids were subsequently converted into fatty acid methyl esters (FAME) for gas chromatographic analysis.

Biohydrogenation of Fatty Acids

The apparent biohydrogenation (BH) of key unsaturated fatty acids oleic (C18:1n-9c), linoleic (C18:2n-6c), and linolenic (C18:3n-3) was calculated based on the difference in their relative concentrations between the start (0 h) and end (24 h) of the incubation period, as described by Adeyemi *et al*. (2015). The following equation was used:

Apparent biohydrogenation (%) =

$$100 \times \frac{(CFA)i - (CFA)f}{(CFA)i}$$

where $(CFA)_i$ and $(CFA)_f$ represents the percentages of each specific unsaturated fatty acid at 0 h and 24 h, respectively.

Statistical Analysis

All data were analysed using a one-way analysis of variance (ANOVA) with the general linear model (GLM) procedure in SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Tukey's Honestly Significant Difference (HSD) test was used to compare means, with a significance level set at $P < 0.05$.

RESULTS

Forage Chemical Composition, Anti-Nutritional Constituents and *In Vitro* Digestibility

The chemical composition of the experimental forages is presented in Table 1. On a dry

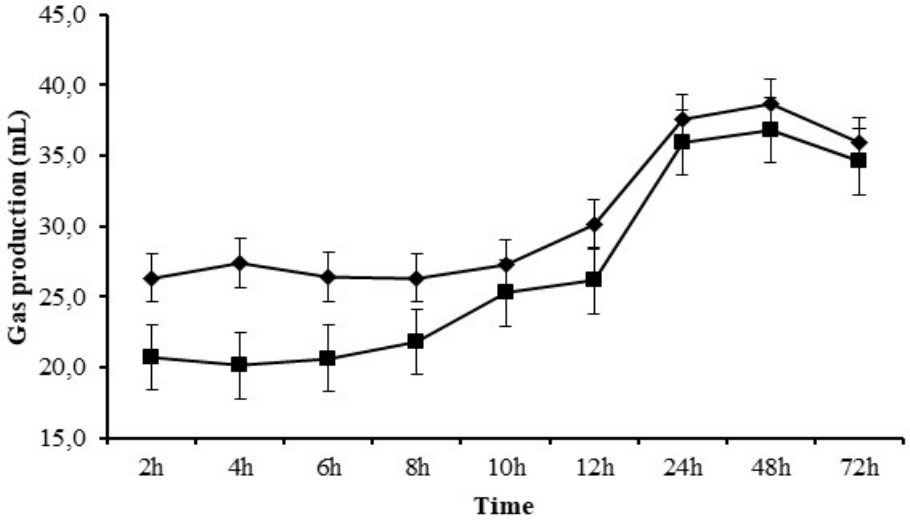

Figure 1. A gas production profile for MTL and NG during *in vitro* incubation. MTL: *Macaranga tanarius* leaves, NG: Napier grass. The symbols represent MTL (◆) and NG (■). Data points are mean values based on triplicate determination. Error bars represent standard error of the mean.

Table 1. Nutrient Composition of Forages (*Macaranga tanarius* Leaves and Napier Grass)

Parameter (%)	Treatments (DM basis)	
	MTL (n:6)	NG (n:6)
Dry matter	42.77	23.26
Organic matter	93.12	92.30
Ash	6.89	7.70
Crude protein	18.17	9.85
Crude fibre	16.31	31.75
Either extraction	4.67	1.56
Non-fiber carbohydrate	35.28	12.30
Neutral detergent fibre	35.00	68.59
Acid detergent fibre	24.67	50.67
Acid detergent lignin	9.73	25.09
Hemicelluloses	22.40	55.63
Energy (Cal/g)	4261.00	3761.67

MTL: *Macaranga tanarius* leaves, NG: Napier grass

Table 2. Anti-Nutritional Constituents of *Macaranga tanarius* Leaves

Parameter (%)	Sample (DM)
	MTL
Total polyphenol	2.13
Non-tannin	0.63
Tannins	1.49
Condensed tannin	0.002
Hydrolysable tannin	1.49

MTL: *Macaranga tanarius* leaves

matter (DM) basis, *Macaranga tanarius* leaves (MTL) had higher concentrations of dry matter, organic matter, crude protein, ether extract, and energy content compared to Napier grass (NG). The secondary metabolic compounds found in MTL are detailed in Table 2. *In vitro* cumulative gas production profiles for both forages are illustrated in Figure 1. Both forages showed an exponentially increasing trend in gas production, peaking at 24 hours of incubation, with MTL consistently yielding higher gas volumes. Following the 24-hour peak, gas production gradually declined for both forages with extended incubation up to 72 hours.

No significant differences ($P > 0.05$) were observed between the forages in terms of *in vitro* fermentation kinetics, net gas production (NP), metabolizable energy (ME), and partitioning factor (PF). In contrast, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) differed significantly between treatments (Table 3). Further correlation analysis revealed a significant negative relationship ($P < 0.001$) between the content of neutral detergent fibre (NDF) and acid detergent lignin (ADL) content with crude protein (CP), IVDMD, and IVOMD, indicating that higher protein content is associated with improved digestibility (Table 4).

Fermentation Products

The concentrations of individual volatile fatty acids (VFAs), acetate, propionate, butyrate, isobutyrate, and valerate, and ammonia-nitrogen (ammonia-N) in the rumen fluid after 72 hours of *in vitro* incubation are presented in Table 5. No significant differences ($P > 0.05$) in total VFA concentrations were observed between *Macaranga tanarius* leaves (MTL) and Napier grass (NG) across the 24, 48, and 72 hours, significant differences ($P < 0.05$) were detected in their proportions at the 24-hour time point. Similarly, no significant differences ($P > 0.05$) were found between MTL and NG in ammonia-nitrogen con-

centrations, methane (CH_4) production, and microbial biomass throughout the incubation (Table 5).

Relationship Between Chemical Constituents and Fermentation Production

As detailed in Table 6, crude protein (CP) and non-fibre carbohydrate (NFC) content exhibited a strong positive correlation with the concentrations of all individual fermentation products (acetate, propionate, butyrate, isobutyrate, and valerate). Conversely, gas production at 24 hours of incubation was negatively correlated with these fermentation products. A strong positive relationship was also observed between neutral detergent fibre (NDF), acid detergent fibre (ADF), and ammonia-nitrogen (NH_3-N) concentrations and fermentation parameters.

Analysis of fermentation kinetics (Table 7) revealed a weak positive correlation between CP and the potential fermentation fraction (*A*) and the potential fermentation rate (*B*). Conversely, NDF and ADF were negatively correlated with the potential fermentation fraction (A).

Fatty Acid Profile and Apparent Biohydrogenation

Table 8 presents the fatty acid profile of the rumen inoculum at 24 hours. No significant differences (P > 0.05) were observed between forages for many individual fatty acids, including lauric acid (C12:0) and myristic acid (C14:0). However, concentration of linolenic acid (C18 : 3*n* – 3), eicosapentaenoic acid (C20 : 5*n* – 3), docasahexaenoic acid (C22 : 6*n* – 3), total saturated fatty acids and total unsaturated fatty acids differed significantly (P < 0.05). The final biohydrogenation product, stearic acid (C18:0), was significantly lower (P < 0.05) in the *Macaranga tanarius* leaves (MTL) treatment, suggesting a less complete biohydrogenation process. While the biohydrogenation rates of linolenic acid and oleic acid were similar between forages (P > 0.05), the extent of linoleic acid biohydrogenation was significantly different (P 0.05).

DISCUSSION

The elevated crude protein (CP) content observed in Macaranga tanarius leaves (MTL) underscores its potential as a valuable, cost-effective protein supplement for ruminants, with CP levels surpassing those reported for several wild leafy vegetables (Hassan and Umar 2006). The CP concentration in Napier grass (NG) obtained in this study aligns with previously documented ranges (Kondo *et al*., 2015), confirming its nutritional consistency. Importantly, the CP content in MTL is likely sufficient to supply adequate nitrogen for rumen microbial activity, thereby enhancing fibre degradation and volatile fatty acid (VFA) production (Lamidi 2010).

Table 3. *In Vitro* Fermentation Kinetics, Net Gas Production and Digestibility of Forages (*Macaranga tanarius* Leaves and Napier Grass) after 72 Hours of Incubation

	Treatments (DM basis)			
Fermentation parameters	MTL (n:9)	NG (n:9)	SEM	*P-value*
NGP (ml)	50.33	47.79	2.75	0.5528
IVDMD (%)	88.74[a]	61.55[b]	2.34	<.0001
IVOMD (%)	79.41[a]	57.78[b]	3.60	0.0013
ME (mj / kg DM)	9.15	8.04	0.13	0.2274
PF24 (mg DMD/ML gas)	1.50	2.08	0.05	0.0994
A (ml)	34.91	33.34	0.93	0.2511
B (ml)	46.05	45.46	2.73	0.8899
C (ml/h)	0.05	0.05	0.01	1.00
A+B (ml)	80.96	78.79	2.88	0.6324

[a, b] Means with different superscripts on the same row significantly differ (P < 0.05). A: volume of gas produced from soluble fraction; B: volume of gas produced from insoluble fraction; C: rate of degradability; A+B: potential degradability; NGP: Net gas production; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; ME: metabolizable energy; PF24: partitioning factor, MTL: *Macaranga tanarius* leaves; NG: Napier grass

Table 4. Pearson Correlation (R) of Chemical Composition, *In Vitro* Dry Matter and *In Vitro* Organic Matter Digestibility of Forage

Parameters	Chemical Components									
	IVDMD	IVOMD	DM	ASH	OM	CP	NDF	ADF	ADL	HEM
IVDMD	1									
IVOMD	0.98***	1								
DM	-0.99***	-0.96**	1							
ASH	-0.89*	-0.86*	0.88*	1						
OM	0.89*	0.86*	-0.88*	-1***	1					
CP	0.99***	0.96**	0.98***	-0.9*	0.9*	1				
NDF	-0.99***	-0.96**	0.96**	0.87*	-0.87*	-0.99***	1			
ADF	-1.00***	-0.97**	0.99***	0.88*	-0.88*	-1***	1***	1		
ADL	-0.66	-0.56	0.62	0.46	-0.46	-0.72	0.75	0.71	1	
HEM	-0.96**	-0.98***	0.95**	0.92**	-0.92**	-0.93**	0.92*	0.94**	0.42	1

CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; ADL: acid detergent lignin, ash: ash, OM: organic matter, DM: dry matter, HEM: hemicelluloses, IVOMD: *in vitro* organic matter digestibility, IVDMD: *in vitro* dry matter digestibility *P < 0.05, **P < 0.01, ***P < 0.001; MTL = *Macaranga tanarius* leaves and NG = Napier grass

These findings support the observations of Hariadi and Santosa (2010), who reported that microbial activity can be hampered by CP levels below 70 g kg^{-1} DM due to nitrogen deficiency. Conversely, the threshold identified in this study appears to promote microbial proliferation and optimizes rumen fermentation efficiency (Njidda & Nasiru, 2010), highlighting the potential of MTL as a strategic dietary intervention for improving ruminant productivity.

The non-fibre carbohydrate (NFC) content of MTL (35.28% DM) was notably higher than that of NG (12.3% DM) and generally exceeds values reported for typical tropical forages, while NG's NFC content falls within the expected range for tropical grasses (Magalhães *et al.*, 2010). NFC, comprising readily digestible components like starch, sugars, pectin, and fermentation acids, serves as a crucial energy source (ATP) for rumen microbial growth, although ATP is primarily derived from fibre degradation.

The relatively low condensed tannin content observed in both forages in this study, particularly in Macaranga tanarius leaves (MTL), may be partly attributed to the oven-drying method employed. Previous studies have shown that oven and sun drying often reduce polyphenol and tannin concentrations when compared to shade drying, potentially due to the inactivation of tannins by moisture loss or the formation of protein-tannin complexes during the drying process (Vitti et al., 2005). From a nutritional perspective, the low tannin content in Macaranga tanarius leaves (MTL) confers significant advantages (Mueller-Harvey, 2006). While moderate tannin levels can enhance nitrogen utilization and aid in controlling gastrointestinal parasites (Makkar, 2003), excessive tannins are well-documented to impair digestibility, suppress feed intake, and hinder nutrient absorption (Frutos et al., 2004). The minimal tannin levels observed in this study suggest that MTL can provide valuable bioactive compounds without eliciting the detrimental effects associated with high tannin concentrations. Consequently, MTL emerges as a promising forage resource capable of enhancing rumen fermentation efficiency and nutrient utilization in ruminants, all while safeguarding animal health and productivity (Makkar, 2003).

The cumulative gas production profiles indicated that the highest forage degradation occurred at 24 hours of incubation, likely due to the higher NFC content, particularly in MTL. Gas production, a recognised indicator of carbohydrate degradation, corroborates finding from Flint *et al.* (2012) on microbial degradation of complex carbohydrates. The significant differences observed in *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were expected, as higher fibre content typically correlates with lower digestibility. The significant negative correlations between neutral detergent fibre (NDF) and acid detergent lignin (ADL) with CP, IVDMD, and IVOMD further support this, as increased cell wall components, especially lignin, hinder microbial enzyme access to structural polysaccharides, potentially reducing microbial activity and even increasing methane (CH_4) production.

The lack of significant differences in *in vitro* fermentation kinetics between *Macaranga tanarius* leaves (MTL) and Napier grass (NG)

Table 5. Volatile Fatty Acids Concentrations In Rumen Fluid at 24, 48 and 72 Hours of *In vitro* Fermentation of Napier Grass and *Macaranga* Leafmeal

Parameter	Treatment (DM basis)			
	MTL (n:18)	NG (n:18)	SEM	*P-value*
pH	6.78	6.83	0.004	0.15
0 h incubation				
Total VFA (Mmol/L)	28.54	28.72	0.24	0.92
Acetic	11.88	12.00	0.16	0.91
Propionic	8.49	8.53	0.08	0.10
Butyric	5.77	5.76	0.11	0.99
Isopropionic	0.75	0.76	0.01	0.96
Isobutyric	1.64	1.68	0.05	0.93
A:P	1.39	1.40	0.01	0.85
CH_4 (Mmol/L of Gas)	6.70	6.75	0.04	0.87
NH_3 (Mg/100ml)	35.72	30.00	0.30	0.06
24 h incubation				
Total VFA (Mmol/L)	25.93^a	22.27^b	0.12	0.01
Acetic	11.56^a	10.12^b	0.06	0.01
Propionic	7.94^a	6.57^b	0.04	0.002
Butyric	3.40^a	3.34^b	0.02	0.03
Isopropionic	0.74^a	0.63^b	0.01	0.0002
Isobutyric	2.00^a	1.61^b	0.01	<.0001
A:P	1.46^b	1.54^a	0.01	0.02
CH_4 (Mmol/L of Gas)	5.64^a	5.09^b	0.03	0.03
NH_3 (Mg/100ml)	44.68	41.89	0.25	0.27
48 h Incubation				
Total VFA (Mmol/L)	25.15	24.14	0.13	0.84
Acetic	11.28	11.06	0.19	0.31
Propionic	6.62	6.33	0.08	0.69
Butyric	4.23	3.75	0.07	0.47
Isopropionic	0.84	0.72	0.01	0.13
Isobutyric	2.04	2.06	0.02	0.12
A:P	1.70	1.78	0.01	0.26
CH_4 (Mmol/L of Gas)	5.99	5.94	0.07	0.93
NH_3 (Mg/100ml)	24.03	23.53	0.22	0.80
72 h incubation				
Total VFA (Mm/L)	19.94	17.59	0.44	0.46
Acetic	9.00	8.30	0.14	0.50
Propionic	6.24	5.13	0.14	0.29
Butyric	3.19	2.98	0.13	0.82
Isoproionic	0.50	0.36	0.02	0.27
Isobutyric	1.02	0.82	0.03	0.33
A:P	1.50	1.66	0.02	0.28
CH_4 (Mmol/L of Gas)	4.53	4.36	0.09	0.79
NH_3 (Mg/100ml)	24.03	23.53	0.39	0.09

[a,b] Means with different superscripts on the same row significantly differ ($P < 0.05$). NG: Napier grass, MTL: *Macaranga tanarius* leaves, SEM: Standard error of means.

suggests similar rates of nutrient breakdown and microbial activity. However, it is important to note that fermentation kinetics do not always directly reflect the extent of substrate degradation and utilization by rumen microbes (Pashaei *et al*., 2010). The observed weak positive correlation between CP content and potential gas production (fraction A, soluble) at 72 hours suggests that adequate protein levels may support increased microbial growth and subsequent fermentation over longer incubation periods. Conversely, the negative correlation observed between NDF and ADF content with potential gas production (A) and the rate constant (B, C) aligns with previous research by Kuliv and Kafilzadeh (2015b), who also found that higher fibre content negatively impacts fermentation kinetics in *in vitro* incubated pasture grasses. This indicates that the structural components of these forages limit the readily available substrate for rapid fermentation.

The observed trend of decreasing acetate, propionate, and butyrate production after 24 hours aligns with typical *in vitro* fermentation patterns as substrate availability changes over time. The highest ammonia-N (NH_3-N) concentration at 24 hours, followed by a decrease at 48 and 72 hours, could indicate peak protein degradation and subsequent utilization by rumen microbes for growth, consistent with (Jin *et al*., 2013) findings in purple prairie clover fermentation, although their temporal pattern showed an initial decrease followed by an increase. The high ammonia-N at 24 hours in the current study may indeed suggest enhanced microbial protein synthesis during this active fermentation phase.

The higher acetate-to-propionate (A:P) ratio observed in Napier grass (NG) compared to *M. tanarius* leaves (MTL) is likely a consequence of its greater proportion of structural carbohydrates (cellulose and hemicellulose). This aligns with the findings of Md et al. (2013), who reported

Table 6. Correlation (R) Between Chemical Composition (DM) and Volatile Fatty Acid (Mm /L) Production for Feeds Incubated in Buffered Rumen Fluid For 24 Hours

Parameters	Individual VFA Products					
	Acet	Pro	But	Isop	Isob	A:P
CP	-0.84*	-0.93***	-0.62	-0.79	-0.78	0.52
NFC	-0.84*	-0.94***	-0.66	-0.81	-0.8	0.56
NDF	0.85*	0.94***	0.66	0.82*	0.81	0.55
ADF	0.85*	0.95***	0.66	0.82*	0.79	0.3
24H	-0.21	-0.43	-0.23	-0.23	-0.27	0.46
NH_3-N	0.69	0.66	0.77	0.59	0.72	0.37

CP: crude protein (%DM basis); NFC: non-fibre carbohydrate (%DM basis); NDF: neutral detergent fibre (%DM basis); ADF: acid detergent fibre (%DM basis); gas 24h: gas 24 h: ml/200 mg DM. NH_3-N: ammonia nitrogen: Acet: acetic acid, Pro: propionic acid, But: buturic acid, Isop: Isopropionic acid, 1001 Isob: Isobutyric acid, A:P acetate to propionate ratio. $*P < 0.05$ and $*** P < 0.001$.

Table 7. Pearson Correlation (R) Between Chemical Composition (G Kg-1 DM) and *In Vitro* Gas Production Characteristics

Parameter	Gas production constants of kinetic fermentation		
	A (ml gas 0.2g-1DM)	B (h-1)	C (h-1)
CP	0.247	0.025	0.002
NDF	-0.244	-0.041	-0.020
ADF	-0.239	-0.025	-0.007

CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; A: potential gas production (ml 0.2 g-1 DM); A volume of gas produced from insoluble fraction; B, volume of gas produced from soluble fraction; C, gas production rate constant from the insoluble fraction.

Table 8. Fatty Acid Composition of Rumen Fluid (% of Total FA) and Rate of Biohydrogenation at 24 Hours of Incubation

Parameter	Treatments (DM basis)			
	MT (n:18)	NG (n:18)	SEM	*P-values*
C12:0, lauric	1.27	1.21	0.2	0.64
C14:0, myristic	2.14	2.32	0.02	0.31
C14:1, myristoleic acid	1.10	1.20	0.01	0.30
C15:0, pentadecanoic	1.73	1.84	0.01	0.07
C16:0, palmitic	19.22	20.17	0.12	0.30
C16:1n-7, palmitoleic acid	1.81	1.61	0.05	0.62
C16:1n-9, hexadecenoic acid	1.59	1.52	0.03	0.79
C18:0, stearic	48.49	53.57	0.37	0.09
C18:1n-9, oleic	2.72	2.71	0.03	0.98
Trans-11 C18:1	2.72	2.71	0.06	0.99
CLAc9t11	0.90	0.99	0.01	0.09
CLAc12t10, linolelaidic acid	0.78	1.24	0.03	0.15
C18:2n-6, linoleic	1.42	1.07	0.05	0.36
C18:3n-3, linolenic	1.12[a]	0.65[b]	0.02	0.02
C20:4n-6, arachidonic acid	0.43	0.47	0.01	0.17
C20:5n-3, eicosapentaenoic	1.12[a]	0.34[b]	0.03	0.01
C22:5n-3, docosapentaenoic	0.41[a]	0.34[b]	0.01	0.02
C22:6n-3, docosahexaenoic	2.00[b]	3.33[a]	0.06	0.01
Total saturated FA (SFA)	72.00[b]	78.00[a]	0.12	<.0001
Total unsaturated FA (UFA)	27.15[a]	20.88[b]	0.11	<.0001
Total monounsaturated FA	12.18	11.09	0.09	0.14
Total PUFA n-3	4.59	5.44	0.08	0.17
Total PUFA n-6	8.57	6.55	0.02	0.06
Total PUFA	14.01[a]	8.13[b]	0.12	<.0001
Total Trans FA	2.72	2.71	0.06	0.99
Total CLA	1.82	2.23	0.05	0.26
n-6: n-3	0.45	0.28	0.01	0.06
Unsaturated FA: Saturated FA	0.39	0.27	0.01	0.09
Polyunsaturated FA: Saturated FA	0.19	0.10	0.01	0.11
Δ9desaturase index	0.04	0.03	0.01	0.33
Apparent Biohydrogenation (%)				
C18:1n9	88.70	87.03	0.22	0.33
C18:2n-6c	90.87[a]	80.60[b]	0.30	0.001
C18:3n-3	81.70	79.67	0.21	0.23

[a, b] Means with different superscripts on the same row significantly differ ($P < 0.05$). MTL: *Macaranga tanarius* leaves, NG: Napier grass, SEM: Standard error of means.

that fibrous carbohydrates in forage cell walls favour acetate production over propionate. Furthermore, the lower methane (CH_4) concentration in MTL, particularly when compared to NG at all time points, can be attributed to the presence of tannins. Secondary metabolites, such as tannins found in tropical legumes, are known to reduce methane production by inhibiting fibre digestion, which could explain the observed differences between the forages. The higher total VFA concentration in MTL at 24 hours, coinciding with peak gas production, likely indicates a greater extent of initial substrate degradation. While the *in vitro* VFA production levels were relatively low overall, (Pashaei *et al*., 2010) suggested that high ammonia-N concentrations in vitro can sometimes hinder gas release due to its basic nature, potentially leading to an underestimation of VFA production based on gas volume, especially with rapidly degradable non-fibre carbohydrates (NFC) and crude protein (CP) sources, possibly due to shifts in VFA molar proportions and ammonia-N accumulation.

The observed strong positive correlation between crude protein (CP), non-fibre carbohydrates (NFC), and the concentrations of key fermentation products (acetate, propionate, butyrate, isobutyrate, and valerate) highlights the role of these readily fermentable fractions in rumen metabolism. Conversely, neutral detergent fibre (NDF), acid detergent fibre (ADF) exhibited a strong negative correlation with these products, suggesting that fibrous components inhibit the production of these specific metabolites. These findings are consistent with the general understanding that nutrient fractions play differential roles in modulating rumen fermentation dynamics (Pashaei *et al*., 2010). Also, they demonstrated that readily digestible crude protein (CP) and high degradability may negatively influence gas production, offering a potential explanation for the observed results. This suggests a complex interplay between the specific chemical composition of the feed and the dynamics of rumen fermentation, where nutrient fractions like CP and non-fibre carbohydrates can have multifaceted effects on microbial activity and gas output.

The inherently low fatty acid content of the forages used in this study is less than 5% of their dry matter, with α-linolenic acid (*C*18 : 3n - 3) as the dominant component (Palmquist *et al*., 2005) likely contributed to the lack of significant effects on the overall fatty acid profile of the rumen fluid. The method of forage preservation may also have been a contributing factor. Studies have shown that fresh and ensiled grasses promote greater biohydrogenation (BH) of linoleic (*C*18:2*n*6*c*) and α-linolenic (*C*18:3*n*-3) acids compared to hay (He *et al*., 2012). Thus, the exclusive use of dried forages in the current study may have limited their impact on lipid metabolism. This is supported by our finding that the concentrations of oleic acid (*C*18 : 1*n*9*c*), linoleic acid, and α-linolenic acid did not differ significantly between treatments, suggesting that lipolysis and the early stages of BH were not substantially affected by forage type. Furthermore, a stable rumen fluid pH, maintained within the optimal physiological range across all treatments, was unlikely to have limited fatty acid transformation, as low ruminal pH is known to impair microbial activity and hinder BH (Fuentes *et al.*, 2011).

CONCLUSION

This *in vitro* study reveals *Macaranga tanarius* leaves (MTL) significantly boost rumen fermentation efficiency, showing higher gas production and organic matter degradation, comparable to Napier grass (NG) in VFA and ammonia -N output. MTL also offers superior digestibility, lower tannins, and higher metabolizable energy, IVDMD, and IVOMD than NG. These findings suggest MTL can provide crucial nutrients for ruminant maintenance and productivity. Incorporating MTL into ruminant diets presents a promising, accessible feed for smallholder farmers, potentially enhancing livestock performance and reducing reliance on conventional feeds. Further *in vivo* research is needed to confirm these benefits in real-world conditions.

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