



Vegetative growth response and in vitro evaluation of three multipurpose fodder tree leaves and shrubs as potential feeds for farm animals

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Abstract In a completely randomized design, the vegetative growth and nutritive value of three tree leaves *Tectona grandis*, *Gmelina arborea*, and *Acacia mangium* compared to berseem (*Trifolium alexandrinum*) hay as a standard high-quality roughage for ruminants were studied. Growth, chemical composition, in vitro gas production (GP), and fermentation characteristics for 48 h of incubation were measured. Results of measured growth parameters showed that the highest mean value of total height, stem base diameter, crown width, shoot fresh weight, and shoot dry weight were observed in *Gmelina arborea*, *Tectona grandis*, and *Acacia mangium*, respectively.

Gmelina arborea had higher ($P < 0.05$) concentrations (%) crude protein (CP) and lower acid detergent fiber (ADF) compared to berseem hay. The lowest ($P < 0.05$) CP was observed with *Tectona grandis*; however, it had more ($P < 0.01$) neutral detergent fiber (NDF) compared to the berseem hay. All tree leaves had higher ($P < 0.05$) concentrations (%) of total phenolics, and total tannins compared to the berseem hay. Furthermore, *Acacia mangium* had the lowest GP ($P < 0.01$) GP (mL/g DM) and the rate (h) of GP ($P < 0.01$), while had the highest ($P < 0.05$) lag of GP (h) compared to berseem hay. *Acacia mangium* showed the lowest methane production (mL/g

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DM) and the highest methane production expressed as mL/g degraded DM (*dDM*), with no significant differences between *Gmelina arborea*, *Tectona grandis*, and berseem hay. At the same time, *Tectona grandis* showed the highest ($P < 0.05$) total (mM) and individual (%) short chain fatty acids concentrations compared to berseem hay. *Acacia mangium* showed the lowest ($P < 0.01$) truly degraded DM and organic matter (mg/g incubated) and protozoal count ($P < 0.05$) ($10^5/\text{mL}$). Also, *Acacia mangium* had lower ($P < 0.05$) ammonia-N concentration (mg/dL), metabolizable energy (MJ/kg DM), and microbial protein production (mg/g DM). Overall, *Tectona grandis* and *Gmelina arborea* are more recommended than *Acacia mangium* due to their higher rumen fermentation characteristics and nutrient degradability; however, *Acacia mangium* has an importance from an environmental view as it had lower methane production compared to other plants.

Keywords Agroforestry · Degradability · Fermentation · Methane · Sustainability · Tree leaves

Introduction

In arid and semi-arid areas, multipurpose trees and shrubs can be used to feed ruminants during all year seasons (Sallam et al. 2019; Ebeid et al. 2020; Kholif et al. 2022a; Morsy et al. 2022). Although multipurpose trees are used as pastures, information on the nutritive value of tree leaves *Tectona grandis*, *Gmelina arborea*, and *Acacia mangium* is scarce, especially as ruminant feeds.

Generally, plant multipurpose trees are rich in secondary metabolites that can modify and alter ruminal fermentation profiles at appropriate doses (Salem et al. 2014). However, the effectivity of secondary metabolites on ruminal fermentation depends mainly on the dose, bioactivity, and concentration (Salem et al. 2016). Rumen microorganisms can degrade alkaloids, saponins, tannins, and phenolics and use them as an energy source, without negative effects on rumen fermentation (Kholif and Olafadehan 2021; Kholif 2023).

Tectona grandis Linn. f is a tropical multipurpose tree belonging to the family of Lamiaceae that is originally distributed in tropical countries (Chávez-Salgado et al. 2022; Daramola 2022). The leaves contain

considerable concentrations of bioactive constituents such as phenols (~70 gallic acid equivalents per g), flavonoids (~667 mg per 100 g), tannins (~1583 mg per 100 g) and alkaloids (~1778 mg per 100 g) (Ogunmefun et al. 2017; Asdaq et al. 2022; Daramola 2022) with antioxidant and antibacterial effects (Shofa et al. 2021). Dégbéet al. (2018) showed that the extract of *Tectona grandis* leaves is a rich source of phenols (141–206 mg/mL), tannins (93–122 mg/mL), and flavonoids (124–183 mg/mL). In Nigeria, Daramola (2022) screened the chemical composition of *Tectona grandis* and showed that the leaves contained 13.28% protein, 28% fiber, 2% fat, 11% ash, 55 mg saponin/g, 44 mg phenol/g and 10 mg tannin/g. While, in Indonesia, Shofa et al. (2021) observed that *Tectona grandis* contained 9% crude protein (CP), 29% fiber, 6.24 µg flavonoid/mL, and 11.64 µg antioxidant IC₅₀/mL. Datt et al. (2008) determined the nutritive value of 12 species including *Tectona grandis* using the in vitro gas production (GP) technique and observed that the in vitro dry matter (DM) and organic matter (OM) degradability were 37 and 39%. Moreover, they noted that the metabolizable energy (ME) concentration was 6.76 MJ/kg.

Gmelina arborea is a multipurpose leguminous tree commonly present in the tropics (Binuomote et al. 2022). Leaves are rich in nutrients with low levels of antinutritional compounds (Binuomote et al. 2022). The leaves contain about 13% CP, 1.2% tannins, 1.80% oxalates, 3.10% flavonoids, 0.85% phytates, and 0.72% saponins. Mixing *Gmelina arborea* with other feedstuffs is recommended compared to feeding them solely, where its feeding showed poor nutrient utilization (Bandawa et al. 2020; Binuomote et al. 2022). Binuomote et al. (2022) fed West African Dwarf sheep *Gmelina arborea* to replace *Panicum maximum* and observed an increase in feed intake and the concentrations of total volatile fatty acids, lactic acid, and ruminal ammonia-N as well as ruminal pH.

Acacia mangium Willd is a leguminous plant found in many tropical and non-tropical areas. The plant contains a high concentration of CP ranging from 13 to 32% (Kusuma et al. 2022). The leaves contain considerable concentrations of secondary metabolites, including flavonoids, saponins, alkaloids, tannins, and phenolics in addition to 29% DM, 8% CP, 54% neutral detergent fiber (NDF), 29% acid detergent fiber (ADF), and 24% hemicellulose. In an in vitro experiment, Maulana et al. (2022) evaluated concentrate

feed pellets containing different levels of tannins from *Acacia mangium* and observed unaffected ruminal DM, OM, and fibers digestibility; however, decreased CP digestibility.

We hypothesized that the three multipurpose tree leaves in arid and semi-arid regions with high availability and low cost can be used as alternative feeds and their different concentrations of nutrients and secondary metabolites will positively affect the rumen fermentation profiles differently to alter ruminal fermentation and lower methane (CH₄). This study has a novelty in the field of agroforestry and animal feeding since in the Middle East no scientific reports are available on this topic, and today sustainable farming systems need to find alternatives that boost yields rationally and those field practices include planting such multipurpose trees. Thus, the objective of this study was to assess the nutritive value of three tree leaves including *Tectona grandis*, *Gmelina arborea*, and *Acacia mangium* compared to berseem hay as a standard ruminant feed on in vitro GP, CH₄ production, degradability, and fermentation parameters.

Materials and methods

Plant materials and vegetative growth

The experiment was carried out on three highly rapid growth multipurpose tree species *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* (aged 8 years) were planted at Ismailia governorate (30°29'15.55" N, and 32°14'25.43" E). The three species were planted under experimental replication in a sandy soil at (2.5×2) space with 840 trees/fed-dan in by the end of 2013 and irrigated using a dripping irrigation system with treated sewage water. Tree's total height, stem base diameter, crown width, shoot fresh weigh and shoot dry weight, were measured at the time of collecting samples, to evaluate the three tree species growth response under Egyptian weather conditions.

Samples of leaves and shoots were collected from three different sites with 10 trees for each tree species, for each site at the same plantation for fresh, dry weight, and chemical analysis. The leaves of each plant from the same site were mixed into one sample

and considered as a replicate. After drying for 7 days at room temperature, samples were ground and sieved through a 0.5 mm mesh screen before storing in a dark room until analysis.

Chemical analysis

Feed samples were analyzed for ash after burning the samples in a muffle furnace at 550 °C for 12 h (method ID 942.05), CP using the Kjeldahl method (method ID 954.01), and ether extract (EE) using diethyl ether in Soxhlet extractors (method ID 920.39) according to the official methods of AOAC (2006). Fiber fractions (i.e., ADF and acid detergent lignin (ADL)) were determined according to the official methods of AOAC (2006). Neutral detergent fiber content was determined without alpha amylase but with sodium sulfite following the procedure of Van Soest et al. (1991) and expressed exclusive of residual ash. Non-structural carbohydrates, cellulose, hemicelluloses, and OM were calculated.

Total phenolic contents of the ethanolic extract were determined by the Folin–Ciocalteu method (Singleton et al. 1999). The vanillin-HCl method of Broadhurst and Jones (1978) was used to measure total condensed tannins, which are expressed as milligrams of Catechin equivalents per g dry weight of the extract (mg EC/g DM).

In vitro gas production

This study was conducted at the Advanced Laboratory of Animal Nutrition, Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Egypt. All procedures followed protocols approved and authorized by the Institutional Animal Care and Use Committee of Alexandria University (ALEXU-IACUC/08–24-04–05-32–4).

The in vitro study was carried out using a semi-automatic system of GP using 100 mL serum bottles (Cat# 223747; Wheaton Science Products, Millville, NJ, USA). Prior to the study, bottles were washed thoroughly and allowed to dry before being used. The system was equipped with a pressure transducer and a data logger (Bueno et al. 2005). Rumen contents were collected freshly from three adult-fasted Egyptian cow steers slaughtered at the slaughterhouse of Alexandria University for each experimental run. Before

slaughtering, cows were ad libitum fed a diet containing concentrates, berseem hay, and rice straw at 5:4:1 (DM basis), with free access to water. The rumen contents were collected considering the standardized procedure for sampling, storage, and use of ruminal contents recommended by Fortina et al. (2022). At the slaughterhouse, the interval between the animal death and the rumen fluid collection was less than 10 min. Before the collection of ruminal inoculums, hot water was collected inside a plastic container. The collected materials were kept separately under anaerobic conditions in pre-warmed thermos containers at 39 °C. The collected rumen contents were blended separately for 10 s before squeezing through 3 layers of cheesecloth at 39 °C under a continuous flushing with CO₂. Artificial saliva was prepared according to MB9 buffer (Onodera and Henderson 1980) recipe containing (per L): 9.83 g NaHCO₃, 3.69 g Na₂HPO₄, 0.60 g KCl, 0.47 g NaCl, 0.30 g (NH₄)₂SO₄, 0.061 g MgC₁₂·6H₂O, 0.0293 g CaC₁₂·2H₂O. The buffer was maintained in a water bath at 39 °C. The buffer and ruminal fluid were mixed at 3:1 (v/v) and the pH were measured using a benchtop pH meter (ADWA model, Romania). Then, 60 mL of the artificial saliva and ruminal liquor were dispensed into the serum bottles containing the substrate.

In this study a completely randomized design with four treatments: 1) berseem hay, (2) *Tectona grandis* leaves, (3) *Gmelina arborea* leaves, and (4) *Acacia mangium* leaves. Samples of berseem hay and tree plants (0.500 g ± 0.02) were weighed and immediately inserted individually into the previously labeled 100 mL serum bottles. Six bottles were used for each plant. Three bottles were used for the fermentation parameters and protozoal count, and the other three were used to measure truly degraded DM (*d*DM) and truly degraded OM (*d*OM). Additional 3 bottles containing only rumen fluid and buffer solution without any plant leaves were used as blanks. Another 3 bottles containing rumen inoculum, buffer solution, and clover hay were used as internal standard bottles. Before incubation, bottles were sealed, manually checked, and incubated in a forced-air oven at 39 °C for 48 h. The bottles were shaken manually after recording gas pressure bi-hourly. The amount of the GP in all bottles at each measuring time was estimated according to the regression equation predicated between gas volume versus pressure relationship $V = 4.974 \times p + 0.171$ ($n = 500$; $R^2 = 0.98$; where: V is

a gas volume (mL); p is the measured pressured (psi). Individual bottles were considered technical replicates. Incubation was repeated in 3 successive weeks, where rumen contents were collected from 3 cows for each run. The run was considered as the experimental unit.

The NLIN procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC) was used to estimate the kinetic of GP according to France et al. (2000) as: $y = A \times [1 - e^{-c(t-Lag)}]$ The equation represents the relationship between the volume of total GP production at time t (h), denoted as y , and the variables A , c , and Lag (h). A is the asymptotic GP (mL/g DM), c represents the fractional rate of GP (/h), and Lag (h) indicates the discrete lag time before any GP. At the end of incubation after 48 h, 1.2 mL of gases in the bottle's headspace was collected in a 5 mL vacutainer tube (BD Vacutainer® Tubes, Franklin Lakes, NJ, USA) using a syringe for the chromatograph measurement of CH₄ concentration (Model 7890, Agilent Technologies, Inc., CO 80537, Santa Clara, CA, USA). Calculations were made based on the amounts of CH₄ produced (Longo et al. 2006). The matching blank values were subtracted from the net values of GP and CH₄.

Nutrient degradability and fermentation profile

At the end of incubation at 48 h, bottles were placed in ice to stop the microbial fermentation process. Degradability of DM (i.e., *d*DM) and *d*OM were measured according to Blümmel et al. (1997). The *d*DM and *d*OM values were calculated from the difference between the amounts of the incubated DM and OM and those remaining non-degraded. The partitioning factor at 24 h of incubation (PF₂₄; mg *d*DM: mL gas) was estimated (Blümmel et al. 1997). The volume of gas produced (mL/200 mg DM) at 24 h incubation (GY₂₄) was calculated. Metabolizable energy was calculated according to Menke et al. (1979). Microbial crude protein (MCP) production was calculated according to Blümmel et al. (1997).

At the end of incubation, the fermentation was stopped by placing the bottles on ice for 5 min, and then rumen pH was measured directly within 3 min of sampling using a portable pH meter (ADWA model, Romania) in the fermentation bottles. Samples (5 mL) of the supernatant fermented fluid from each bottle were taken in glass tubes for determination

of ammonia–N ($\text{NH}_3\text{-N}$) and total and individual SCFA concentrations. A subsample of 3 mL was preserved with 3 mL of 0.2 M hydrochloric acid solution for measurement of $\text{NH}_3\text{-N}$ concentration. Four mL of supernatant fermented fluid from each bottle were mixed with 1 mL of 10% formaldehyde, gently shaken, and placed in a refrigerator at 4 °C until protozoal count according to Dehority (1993) where 1 mL of 10% formaldehyde fixed sample was diluted with 1 mL of distilled water, and then 0.5-mL sample was taken with a Pasteur pipette (BRAND, 7712, Wertheim, Germany) and put into a Neubauer chamber (BRAND, 7178-10, Wertheim, Germany), and observed on a contrast microscope at a 400× magnification. Individual SCFA concentrations were determined according to Palmquist and Conrad (1971) using gas chromatography (Thermo Fisher Scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 autosampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 μm i.d., and 25 m length; J&W Agilent Technologies Inc., Palo Alto, CA). Hydrogen at 1.35 mL/min was used as a carrier gas. Air, hydrogen, and nitrogen fluxes (make-up gas) were kept at 450, 40, and 35 mL/min, respectively. A 0.1 μL aliquot was injected in the spitless mode for the entire run with 31.35 mL/min of H_2 flux (63.432 Pa). Injector and flame ionization detector (FID) temperatures were held isothermally at 250 °C. The oven heating slope was 80 °C (1 min), 120 °C (20 °C/min for 3 min), and 205 °C (10 °C/min for 2 min), with 9 min overall analytical time. A mixture of known concentrations of SCFAs was used as an external standard (Sigma Chemie GmbH, Steinheim, Germany) to calibrate the integrator. Ruminal $\text{NH}_3\text{-N}$ concentration was measured calorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, California) using a commercial kit (Preston 1995).

Statistical analysis

The vegetation parameters data were analyzed using the PROC GLM procedure in a one-way analysis of variance (ANOVA) using SAS Studio (SAS Institute, Cary, NC, USA) according to the following statistical model: $Y_{ij} = \mu + P_i + e_{ij}$; where Y_{ij} is the observed response variable, μ is the overall mean, P_i is the plant species effect, and e_{ij} represents the residual error. Then, parameters were tabulated as mean values $\pm$ standard deviation (SD), following Snedecor and Cochran (1989) method.

In vitro fermentation data were analyzed as a randomized design using the PROC GLM procedure in a one-way ANOVA of SAS. Data from each of the three runs of the same sample of the substrate were averaged before statistical analysis. Mean values of each run (three runs) were used as the experimental unit. The statistical model was: $Y_{ij} = \mu + P_i + \varepsilon_{ij}$; where: Y_{ij} represents every observation of the i th plant, P_i is the plant species effect, and ε_{ij} is the experimental error. Tukey's test was used for the multiple comparisons among mean values.

Results

Vegetation parameters

Total height (m), stem base diameter (cm), crown width (m), and shoot fresh and dry weight (kg), are detailed in Table 1. *Gmelina arborea* followed by *Tectona grandis*, and *Acacia mangium* had the highest total height, stem base diameter, and shoot fresh and dry weights. Moreover, the highest shoot fresh and dry weights (ton/hectare) were found in *Gmelina arborea* followed by *Tectona grandis*, and *Acacia mangium* (Table 2).

Table 1 Growth parameters (mean $\pm$ standard deviation) for the three species

	Total height (m)	Stem base diameter (cm)	Crown width (m)	Shoots fresh weight (kg)	Shoots dry weight (kg)
<i>Tectona grandis</i>	4.86 $\pm$ 0.34	20.92 $\pm$ 4.12	5.68 $\pm$ 2.73	16.54 $\pm$ 1.60	11.43 $\pm$ 1.28
<i>Gmelina arborea</i>	5.72 $\pm$ 0.66	32.10 $\pm$ 5.10	3.82 $\pm$ 0.36	20.99 $\pm$ 2.58	13.20 $\pm$ 2.57
<i>Acacia mangium</i>	3.83 $\pm$ 0.35	16.30 $\pm$ 3.85	2.91 $\pm$ 0.50	14.56 $\pm$ 2.68	9.90 $\pm$ 1.97

Table 2 Fresh and dry weight for each tree species/hectare (means $\pm$ standard deviation)

	Age (years)	Trees (num/ hectare)	Shoots fresh weight (ton/hectare)	Shoots dry weight (ton/hectare)
<i>Tectona grandis</i>	8	2000	33.08 $\pm$ 3.20	22.85 $\pm$ 2.56
<i>Gmelina arborea</i>	8	2000	41.99 $\pm$ 5.15	26.40 $\pm$ 5.15
<i>Acacia mangium</i>	8	2000	29.12 $\pm$ 5.36	19.80 $\pm$ 3.94

Table 3 Chemical composition (mean $\pm$ SD) of berseem hay, *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* tree leaves (% DM basis)

	Berseem hay	<i>Tectona grandis</i>	<i>Gmelina arborea</i>	<i>Acacia mangium</i>	<i>P</i> value
OM	88.5 $\pm$ 2.41	82.8 $\pm$ 2.22	91.6 $\pm$ 2.93	92.9 $\pm$ 2.85	0.376
CP	13.5 ^b $\pm$ 0.94	7.62 ^c $\pm$ 0.55	19.7 ^a $\pm$ 0.27	14.8 ^b $\pm$ 0.33	0.028
EE	2.7 ^c $\pm$ 0.12	3.04 ^c $\pm$ 0.09	5.58 ^a $\pm$ 0.11	4.14 ^b $\pm$ 0.09	0.016
NDF	46.8 ^b $\pm$ 1.49	71.3 ^a $\pm$ 1.04	56.4 ^b $\pm$ 2.13	55.4 ^b $\pm$ 1.96	0.008
ADF	32.9 ^b $\pm$ 2.98	51.4 ^a $\pm$ 1.44	28.8 ^b $\pm$ 0.95	33.6 ^b $\pm$ 0.98	0.024
ADL	5.3 ^b $\pm$ 0.32	13.78 ^a $\pm$ 0.88	13.81 ^a $\pm$ 0.75	14.24 ^a $\pm$ 0.89	0.010
Hemicellulose	13.9 ^c $\pm$ 0.74	19.90 ^b $\pm$ 1.00	27.63 ^a $\pm$ 1.56	21.78 ^b $\pm$ 1.03	0.031
Cellulose	32.6 ^a $\pm$ 2.22	37.63 ^a $\pm$ 2.04	14.99 ^b $\pm$ 1.00	19.35 ^b $\pm$ 1.18	0.005
TP	2.20 ^b $\pm$ 0.010	2.76 ^a $\pm$ 0.212	2.75 ^a $\pm$ 1.004	2.66 ^a $\pm$ 0.117	0.002
TT	0.60 ^b $\pm$ 0.022	1.14 ^a $\pm$ 0.073	1.17 ^a $\pm$ 0.053	1.14 ^a $\pm$ 0.075	0.001

ADF acid detergent fiber; ADL acid detergent lignin; CP crude protein; EE ether extract; NDF neutral detergent fiber; OM organic matter; TP total phenolics; TT total tannins

Means in the same row with different superscripts differ, $P < 0.05$. *P* value is the observed significance level of the *F*-test for treatment

Chemical composition

As shown in Table 3, nutrient concentrations differed between the plant trees and compared with berseem hay. *Tectona grandis* showed the highest ($P = 0.028$) CP content (19.7%) compared to other trees, while *Gmelina arborea* showed the lowest content (7.62%). The highest NDF concentration ($P = 0.008$) was observed in *Tectona grandis* leaves (71.3%), while the lowest concentration (55.4%) was observed in the leaves of *Acacia mangium*. Compared to berseem hay, *Gmelina arborea* leaves had higher hemicellulose ($P = 0.031$) and EE ($P = 0.016$) and lower ADF ($P = 0.024$) and cellulose ($P = 0.005$) contents (Table 3). *Tectona grandis* leaves had more NDF (71.3%) ($P = 0.008$) and ADF (51.4%) ($P = 0.024$) concentrations. *Acacia mangium* leaves showed minimal differences in the

concentrations of CP, NDF, and ADF compared to berseem hay. All tree leaves had the greatest concentrations of total phenolics ($P = 0.022$) and total tannins ($P = 0.001$) compared to berseem hay.

In vitro gas production

Figure 1 shows the in vitro ruminal GP of berseem hay, *Tectona grandis*, *Gmelina arborea*, and *Acacia mangium* tree leaves incubated for 48 h. *Acacia mangium* showed the lowest asymptotic GP ($P = 0.016$) and the rate of GP ($P = 0.002$) and had the highest GP lag ($P = 0.023$) compared to berseem hay (Table 4). No significant differences were observed in the rate of GP and the lag of GP between *Gmelina arborea* and *Tectona grandis*. Compared to berseem hay the lag phase of those foliages was comparable, however, *Tectona grandis* and *Gmelina arborea* showed low lag of GP ($P = 0.032$) compared to berseem hay.

Fig. 1 In vitro ruminal gas production (mL/g incubated DM) of berseem hay, *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* tree leaves incubated for 48 h

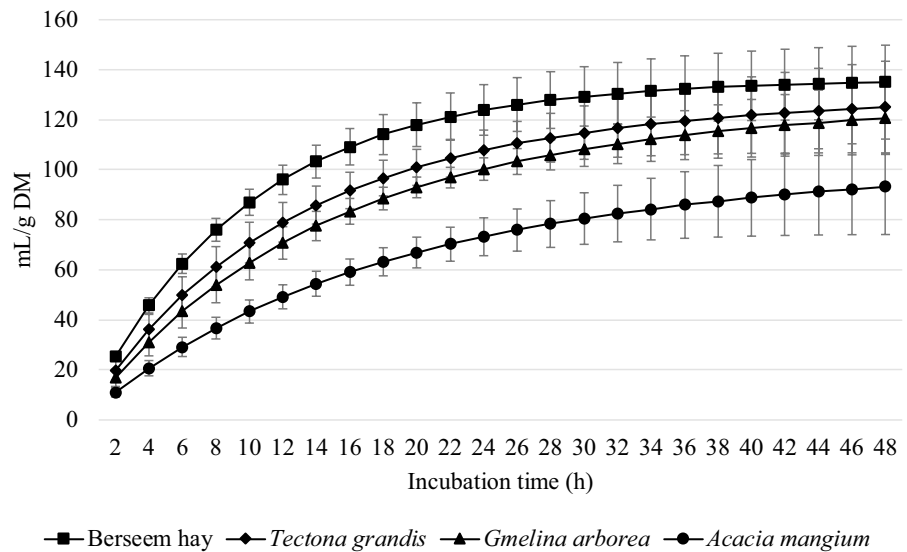


Table 4 In vitro rumen gas kinetics and methane production of berseem hay, *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* tree leaves incubated for 48 h

	Berseem hay	<i>Tectona grandis</i>	<i>Gmelina arborea</i>	<i>Acacia mangium</i>	SEM	<i>P</i> value
<i>Gas production kinetics</i> ¹						
A	136.3 ^a	131.6 ^{ab}	128.5 ^{ab}	108.3 ^b	1.38	0.016
c	0.104 ^a	0.087 ^{ab}	0.075 ^{bc}	0.062 ^c	0.0072	0.002
Lag	2.58 ^b	2.57 ^b	2.67 ^b	2.97 ^a	0.105	0.032
<i>Methane production</i> ²						
mL/g DM	8.63 ^a	7.61 ^{ab}	7.8 ^{ab}	6.96 ^b	0.518	0.023
mL/g dDM	13.3 ^b	13.7 ^b	13.2 ^b	16.4 ^a	1.10	0.014

Means in the same column with different superscripts differ, $P < 0.05$. *P* value is the observed significance level of the *F*-test for treatment; SEM = standard error of the mean

¹A is the asymptotic GP (mL/g DM), *c* is the rate of gas production (/h), *Lag* is the initial delay before gas production begin (h)

²Methane was measured at the end of incubation at 48 h

Acacia mangium had the lower CH₄ production as mL/g DM ($P = 0.023$) and mL/g dDM ($P = 0.014$) compared to berseem hay. No significant differences among *Gmelina arborea*, *Tectona grandis*, and berseem hay in CH₄ production as mL/g DM ($P = 0.023$) and mL/g dDM ($P = 0.014$).

Fermentation profile

The higher ($P = 0.001$) total and individual (i.e., acetate, propionate, butyrate, valerate, iso-valerate and iso-butyrate) SCFA concentrations (mM) were

found in *Tectona grandis* compared to berseem hay (Table 5). However, compared to berseem hay, *Acacia mangium* and *Gmelina arborea* were comparable in total SCFA, acetate, butyrate, and iso-butyrate concentration. The tree leaves of the three plants had less contents of acetate ($P = 0.004$), propionate ($P = 0.005$) and iso-valerate ($P = 0.014$) proportions compared to berseem hay. However, the plants showed higher butyrate ($P = 0.002$) and valerate ($P < 0.001$) proportions. *Acacia mangium* showed the lowest acetate and highest valerate proportions.

Table 5 Short chain fatty acids (SCFA) profile of berseem hay, *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* tree leaves incubated for 48 h

	Berseem hay	<i>Tectona grandis</i>	<i>Gmelina arborea</i>	<i>Acacia mangium</i>	SEM	<i>P</i> value
Total SCFA, mM	59.4 ^b	94.6 ^a	55.6 ^b	50.0 ^b	3.51	0.001
Acetate, mM	37.1 ^b	58.4 ^a	34.3 ^b	30.4 ^b	2.12	0.001
Acetate, %	62.4 ^a	61.7 ^b	61.7 ^b	60.8 ^c	0.20	0.004
Propionate, mM	9.80 ^b	14.31 ^a	8.24 ^{bc}	7.83 ^c	0.503	0.001
Propionate, %	16.5 ^a	15.1 ^b	14.8 ^b	15.6 ^b	0.24	0.005
Butyrate, mM	6.59 ^b	11.73 ^a	7.52 ^b	6.88 ^b	0.505	0.003
Butyrate, %	11.1 ^c	12.4 ^b	13.5 ^a	13.8 ^a	0.25	0.002
Valerate, mM	1.06 ^b	2.16 ^a	1.26 ^b	1.19 ^b	0.075	0.001
Valerate, %	1.78 ^c	2.29 ^b	2.27 ^b	2.38 ^a	0.028	<0.001
Iso-valerate, mM	3.83 ^b	5.99 ^a	3.23 ^{bc}	2.78 ^c	0.255	0.001
Iso-valerate, %	6.47 ^a	6.34 ^a	5.77 ^b	5.57 ^b	0.168	0.014
Iso-butyrate, mM	1.03 ^b	2.00 ^a	1.05 ^b	0.89 ^b	0.124	0.008
Iso-butyrate, %	1.71	2.11	1.88	1.78	0.131	0.227

Means in the same row with different superscripts differ, $P < 0.05$. P value is the observed significance level of the F -test for treatment; SEM = standard error of the mean

No differences were observed between berseem hay and *Tectona grandis* (Table 6). *Acacia mangium* had the lowest ($P = 0.001$) dDM and dOM compared to berseem hay and the other plants. The highest pH ($P = 0.024$) was observed with *Acacia mangium*,

which showed the lowest NH_3-N ($P = 0.003$), ME ($P < 0.001$) and MCP ($P < 0.001$). Lower NH_3-N ($P = 0.003$), ME ($P < 0.001$) and GY_{24} ($P = 0.038$) were observed with the tree leaves compared to

Table 6 Degradability and fermentation profile of berseem hay, *Tectona grandis*, *Gmelina arborea* and *Acacia mangium* tree leaves incubated for 48 h

	Berseem hay	<i>Tectona grandis</i>	<i>Gmelina arborea</i>	<i>Acacia mangium</i>	SEM	<i>P</i> value
<i>Degradability</i> ¹						
DM	677 ^a	648 ^a	605 ^b	435 ^c	14.1	0.001
OM	634 ^a	596 ^a	592 ^a	416 ^b	15.8	0.001
<i>Fermentation</i> ^{2,3}						
pH	5.88 ^b	5.86 ^b	6.02 ^{ab}	6.23 ^a	0.086	0.024
NH_3-N	19.0 ^b	14.6 ^c	24.6 ^a	14.5 ^c	0.99	0.003
ME	6.34 ^a	5.57 ^c	6.05 ^b	5.03 ^d	0.062	<0.001
PF ₂₄	5.49 ^b	6.05 ^a	6.04 ^a	5.99 ^a	0.152	0.033
MCP	404.3 ^a	411.2 ^a	384.0 ^a	274.0 ^b	10.55	<0.001
GY_{24}	183.2 ^a	167.4 ^b	165.9 ^b	168.5 ^b	4.59	0.038
Protozoa ($10^5/mL$)	5.15 ^a	4.15 ^b	4.35 ^b	3.75 ^c	0.293	0.022

Means in the same column with different superscripts differ, $P < 0.05$. P value is the observed significance level of the F -test for treatment; SEM = standard error of the mean

¹DM is dry matter degradability (dDM ; mg/g incubated), OM is organic matter degradability (dOM ; mg/g incubated), NDF is neutral detergent fiber degradability (mg/g incubated), ADF is acid detergent fiber degradability (mg/g incubated)

² NH_3-N is ammonia-N (mg/dL), ME is metabolizable energy (MJ/kg DM), PF₂₄ is the partitioning factor at 24 h of incubation (mg dDM : mL gas), MCP is microbial CP production (mg/g DM), GY_{24} is the volume of gas produced (mL/200 mg DM) at 24 h incubation

³Calculated according to Blümmel et al. (1997): as: ME (MJ/Kg DM) = $2.20 + 0.136 GP + 0.057 CP$; PF₂₄ = dDM (mg/g DM)/total gas production at 24 h of incubation; GY_{24} = mL gas per g DM/g DMD; MCP (mg/g DM) = mg DMD - (mL gas $\times$ 2.2 mg/mL)

berseem hay. All the tree leaves had lower ($P=0.022$) protozoal count compared to berseem hay.

Discussion

Vegetation parameters

Our results on vegetation parameters (total height, stem base diameter, crown width, shoot fresh weight, shoot dry weight) agree and are within that reported by others (Adekunle et al. 2011; Mohamed et al. 2017). They mentioned that both *Gmelina arborea* and *Tectona grandis* are suitable for the rapid production of great volumes of timber, although *Tectona grandis* grows slower than *Gmelina arborea*, when *Gmelina arborea* achieves high biomass at an early age during the first 5–6 years (Onyekwelu and Stimm 2002). The same trend was shown by Krisnawati et al. (2011) and Hegde et al. (2013) regarding the *Acacia mangium* growth and productivity. According to Hlaing (2022) the high growth rate parameters of *Tectona grandis* (collar diameter, height, number of leaves, leaf fresh weight, root fresh weight, shoot fresh weight, leaf dry weight, root dry weight, shoot dry weight, total biomass and leaf area) might be due to the fast-growing nature of the species, and their sturdiness quotient (SQ), generally plants with high SQ value, will be less sturdy and intolerant to strong wind, drought, and frost resulting in substantial losses counter to seedlings with lower SQ values. Moreover, the differences in dry matter accumulation return to variations in nutrient uptake between species (Baligar et al. 2008; Rotowa and Adeagbo 2019). When Venn and Harrison (2000, 2001) mentioned that the growth rates of *A. mangium* in plantations established in the Philippines, surpassed all other species of *Eucalyptus* as well as *Gmelina arborea*. *A. mangium*, along with the species just mentioned, has been considered the third highest growth rate forest tree species in the world nevertheless in poor sites. *A. mangium* has a great potential to grow rapidly even in extremely hostile soil conditions (Garay et al. 2004; Maiti 2006; Torres Vélez and Del Valle 2007).

Chemical composition

Crude protein concentrations ranged between 7.62 and 19.7% for *Tectona grandis* and *Gmelina arborea*, respectively compared to 13.5% for berseem

hay. Suitable concentrations of CP ensure the growth and building of tissue in animals (Hristov et al. 2019). The NDF concentrations ranged between 55.4 and 71.3% with *Acacia mangium* and *Tectona grandis*, respectively compared to berseem hay which had 53.8% NDF. High concentrations of fiber in animal feeds can cause intestinal irritation and decrease nutrient availability (Terry et al. 2019; Zhou et al. 2022). Therefore, some treatments are recommended to decrease and reduce the negative effects of the high fiber concentration in *Tectona grandis*. Generally, the concentrations of different nutrients varied between the plants, and this could be due to several factors such as genetic differences that influence their protein content, or environmental factors such as soil quality, climate, temperature and sunlight, and stage of growth of each plant (Fowler et al. 1990; Langyan et al. 2022). In the present study, different nutrient concentrations between the evaluated plants were most likely due to genetic effects since most of other conditions were the same.

All tree leaves showed higher concentrations of total phenolics, and total tannins compared to berseem hay, which may affect feed utilization by ruminal microbes. Phenolic compounds are generally higher in tree leaves compared to berseem hay due to several factors that include evolutionary adaptation as trees have evolved to produce phenolic compounds as a defense mechanism against herbivores and pathogens (Pratyusha 2022). Moreover, phenolic compounds in tree leaves play an important structural role, contributing to the toughness and resilience of leaves, which is less critical for the context of berseem hay (Bhattacharya et al. 2010). Trees accumulate phenolic compounds during their growth period, while berseem hay, is harvested earlier, and thus phenolic contents may not reach peak levels (Brahmi et al. 2022). Additionally, trees have longer growth cycles and diverse ecological roles, which is reflected in higher levels of these compounds compared to fast-growing forage crops like berseem hay (Pratyusha 2022). In our study, the concentrations of both phenolics and total tannins were almost the same. Phenolics and tannins have antimicrobial properties to alter ruminal fermentation, animal health and CH₄ production (Kholif and Olafadehan 2021; Kholif 2023).

At high concentrations, such metabolites are toxic to ruminal microbiota (Cedillo et al. 2014; Morsy et al. 2024).

In vitro gas production

The fermentability of the substrates and the presence of secondary metabolites are the main reasons for the observed changes in gas and CH₄ production. Higher fermentability and suitable concentrations of secondary metabolites may increase GP and reduce CH₄ production (Kholif 2023). In our study, *Acacia mangium* showed the lowest GP and rate of GP and the highest lag of GP indicating less nutritive value and fermentability compared to other evaluated trees. However, the concentrations of CP and fiber fractions (NDF and ADF) did not differ between *Acacia mangium* and berseem hay, which indicates that other factors caused the observed differences. Total phenolics and tannins for *Acacia mangium* and berseem hay, respectively may be the main reason (Kholif and Olafadehan 2021). The lowered *d*OM and protozoal number in *Acacia mangium* reflected the lowered GP as production and rate of production (Kholif et al. 2024) as there is a strong correlation between OM digestibility and total GP (Getachew et al. 2004).

The asymptotic GP and rate of GP did not differ between *Gmelina arborea* and *Tectona grandis* compared to berseem hay indicating a good fermentability with both trees compared to *Acacia mangium* which had the lowest asymptotic GP and rate of GP. However, the concentrations of CP and fiber concentrations differed between all evaluated plants. The higher fiber and the lower CP contents in *Tectona grandis*, and higher CP in *Gmelina arborea* were expected to affect these parameters; however, the expected effects were masked by the effect of total phenols and tannins in *Gmelina arborea* and *Tectona grandis*. Compared to the fermentation of carbohydrates, the fermentation of protein produces relatively smaller amounts of gas (Makkar et al. 1995).

Acacia mangium showed the lowest CH₄ production per g DM, with no differences between *Gmelina arborea*, *Tectona grandis*, and berseem hay. However, when CH₄ production was calculated based on degradable DM, *Acacia mangium* produced the highest amount of CH₄. It was expected that all the tree leaves would lower CH₄ production compared to berseem hay due to their high concentrations of phenolics

and tannins since plant secondary metabolites may suppress CH₄ production. The different responses between the incubated tree leaves may be related to the rate of feed degradation (Setiawan et al. 2022). Moreover, the lowered protozoal count in *Acacia mangium* treatment is possibly the reason for the lowered CH₄ production (Kholif and Olafadehan 2021). The concentrations of secondary metabolites in the tree leaves are another possible reason; however, such effects cannot be confirmed since all the tree leaves contain high concentrations of phenolics and tannins. Type and concentrations of secondary metabolites affect cellulolytic bacteria (Patra and Saxena 2009) and reduce the production of H₂ required for methanogenesis (Goel and Makkar 2012), and inhibit ruminal CH₄-producing bacteria (Goel and Makkar 2012). Moreover, Setiawan et al. (2022) reported that incubating a mixture of three leaves containing *Acacia mangium* did not affect in vitro CH₄.

Fermentation profile

Generally, the tree leaves of *Gmelina arborea* and *Acacia mangium* showed lower proportions (%) of acetate, propionate and iso-valerate compared to berseem hay indicating less fermentability with the tree plants compared to berseem hay. Within the plant trees, *Tectona grandis* showed the highest total and individual SCFA concentrations indicating higher ruminal fermentation and feed degradation, which is associated with its chemical composition and nutrient degradability compared to *Gmelina arborea* and *Acacia mangium*. Ruminal fermentation characteristics (e.g., SCFA) depend on the composition of the plants and the microbial activity against fiber fractions (e.g., cellulose, hemicellulose) and carbohydrates concentrations which later produce SCFA (Cammack et al. 2018). In the present study, *Tectona grandis* had the highest NDF and ADF concentrations, which also explain the higher acetate production with *Tectona grandis* (Elghandour et al. 2014b).

However, the concentrations of phenolic compounds and tannins cannot be ignored as a reason for the differences in total and individual SCFA between berseem hay and *Tectona grandis* since concentrations of secondary metabolites can differ between plants affecting rumen microbial activity and fermentation end products such as SCFA (Kholif et al. 2021). Moreover, we can speculate that the degradability of

fiber fractions differed between the measured samples. Setiawan et al. (2022) observed that incubating a mixture of three leaves containing *Acacia mangium* in pellet feed at tannin levels of 0%, 1%, and 2% DM feed did not affect SCFA production (Table 5). *Acacia mangium* showed the lowest *d*DM and *d*OM. Generally, nutrient degradation in the rumen is greatly influenced by several factors related to the plant material and the rumen environment. Lignified plants are harder to degraded (Khattab et al. 2013); therefore, the high ADL concentration in *Acacia mangium* may be a possible reason (Kholif et al. 2022b) for its low rumen degradability. Additionally, cellulose at different concentrations can cause different rates of nutrient degradation since ruminal microbes have high ability to degrade it. In the present study, both of *Acacia mangium* and *Gmelina arborea* had the lowest cellulase concentrations which may result in lower *d*DM and *d*OM compared to berseem hay and *Tectona grandis*. Moreover, the calculated MCP showed that *Acacia mangium* had the lowest value, which is an indicator of depressed bacterial growth and activity in the incubation medium, especially fibrolytic microorganisms (Morgavi et al. 2000; Singla et al. 2021). Accessibility of nutrients for rumen microorganisms allows for their degradability (Elghandour et al. 2014a). Maulana et al. (2022) observed unaffected ruminal DM, OM, and fibers digestibility and decreased CP digestibility when incubated (in vitro) feed pellets containing *Acacia mangium* as a source of tannins.

All tree leaves showed low $\text{NH}_3\text{-N}$, which cannot be explained based on the concentration of CP from berseem hay and plant leaves, since *Tectona grandis* and *Acacia mangium* had less CP concentration compared to berseem hay. Such results (i.e., $\text{NH}_3\text{-N}$ concentration) confirm the assumption that secondary metabolites in feeds, especially tannins can bind dietary protein and protect them from microbial degradation (Frutos et al. 2004). In the present study, the concentrations of total phenolics and tannins were higher in plant the tree leaves compared to berseem hay and that may explain why all tree leaves produced less $\text{NH}_3\text{-N}$ during the fermentation. Moreover, the decrease in total ruminal protozoal in the incubation media of the tree leaves can also explain the results of $\text{NH}_3\text{-N}$ concentration because ruminal protozoa are related to protein degradation (Jouany 1996).

Acacia mangium showed the lowest protozoal count; however, *Gmelina arborea* and *Tectona grandis* also showed low protozoal counts compared to berseem hay (Table 6), which partially explains the low CH_4 production with *Acacia mangium* (Nowak et al. 2016; Kholif and Olafadehan 2021). The presence of secondary metabolites with antimicrobial properties is a possible reason (Kholif and Olafadehan 2021). *Acacia mangium* showed the lowest $\text{NH}_3\text{-N}$, ME and MCP; however, all the tree leaves showed less ME and GY_{24} compared to berseem hay indicating low activity and growth of ruminal microbes in the rumen. The key factors in such effects are the concentrations of nutrients and secondary metabolites in the tree leaves. As mentioned before, inappropriate levels of secondary metabolites depress ruminal fermentation, decrease nutrient utilization, and depress the growth and activity of ruminal microbes by impairing the balance between energy and protein release in the rumen.

The PF_{24} differed between the fermented substrates due to variations in plant nutrient composition, fiber content, presence of secondary metabolites, microbial community structure, and overall fermentation dynamics within the rumen (Blümmel et al. 1997). The high PF_{24} observed with the tree leaves compared to berseem hay indicates their potential for high conversion of degraded substrate into microbial biomass (Blümmel et al. 1997). Higher nutrient contents provide the ruminal microbes with their needs for growth and activity to degrade nutrient and produce gases. In the present study, the tree leaves produced less gases per each degraded unit of DM.

Conclusions

The studied plant trees influenced rumen fermentation compared to conventional forages (i.e., berseem hay) in ruminant diets. From ruminants nutritional view, *Tectona grandis* and *Gmelina arborea* are more recommended than *Acacia mangium* due to their higher rumen fermentation characteristics and nutrient degradability; however, *Acacia mangium* had the lowest methane production (per g DM), but the highest methane production per g degradable DM compared to other plants. More studies are recommended to measure the concentrations of all secondary

metabolites in the tree plants and study the adaptation of rumen microbes to the antimicrobial effects of secondary metabolites in the evaluated plants in in vivo studies to find out the suitable levels of their inclusion in the ruminant diets.

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Data availability The original contributions presented in this study are included in the article, and further inquiries can be directed to the corresponding authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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