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Nutrient composition, *in vitro* true digestibility (IVTD) and methane production potential of fodder tree leaves

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ABSTRACT

This work was carried out to estimate the nutrient composition and methane production potential of various tree leaves to develop a database on methane production. Highest organic matter (OM) content was recorded in cashew leaves (96.87%) and lowest value was recorded in mulberry leaves (86.03%). Ether extract (EE) content of various tree leaves ranged from 0.54 to 8.74%. Neutral detergent fibre (NDF) was highest in *velvel* leaves (65.03%) and lowest in *Murungai* leaves (19.48%). Lowest *in vitro* true digestibility was observed in *velvel* leaves (41.59%) and highest value was observed in *maramalli* leaves (75.59%). Mulberry leaves had lowest methane production potential at half life ($t_{1/2}$) (0.21 ml/100 mg truly digested substrate) and highest methane production potential at half life ($t_{1/2}$) was recorded in *velvel* leaves (1.98 ml/100 mg truly digested substrate). *Agathi* leaves had highest ($P<0.05$) methane production potential at 24 h (2.72 ml/100 mg truly digested substrate) compared to all other tree leaves. It can be concluded that average OM, EE and NDF content of various tree leaves was 90.83, 4.67 and 40.53%, respectively. Average methane production potential of different tree leaves at half life and 24 h was 0.93 and 1.55 ml /100 mg truly digested substrate, respectively.

Key words: *In vitro* true digestibility, Methane production potential, Nutrient composition

Methane is emitted both from natural as well as anthropogenic sources. Natural sources include wetlands, wild ruminants, ocean, lakes and termites. Global methane production ranged between 350–820 Tg/year. Owing to its high global warming potential (25 times CO_2) and its short atmospheric lifetime (12 years) reducing methane emission is a good option for reducing global warming. Methane emitted through enteric fermentation by ruminants (30%) is not only an important greenhouse gas associated with global warming, approximately 6–8% of dietary gross intake energy is lost to the atmosphere as methane. This research work was carried out to estimate the nutrient composition and methane production potential of various fodder tree leaves to develop a database on methane production and to develop suitable strategies to mitigate methane emission from ruminants.

MATERIALS AND METHODS

Analysis of tree leaves for nutrient composition: Air-dried fodder tree leaves were further oven dried at 50–60°C overnight and ground through 1 mm sieve. These samples were subjected for the estimation of total ash (TA), ether extracts (EE) (AOAC 2012) and neutral detergent fibre (NDF) (Goering and Van Soest 1970). Based on the total ash content, organic matter (OM) was calculated for all the fodder tree leaves. The OM, EE and NDF contents of different fodder tree leaves are presented in Table 1.

Collection of rumen inoculum for in vitro *experiments:* Rumen contents were collected from male calves fed on paddy straw based ration using stomach tube and filtered using 4 layered muslin cloth.

In vitro *gas production test IVGPT:* About 200 mg of ground samples (1mm) were transferred into the 100 ml calibrated glass syringes and exactly 30 ml of rumen inoculum (Menke and Steingass 1988) was dispensed into the syringes. After removing the gas bubbles the nozzle was closed using a rubber cork.

The syringes were incubated in a shaking water bath at 39°C in triplicates. Gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h interval and the data were analysed using Graph Pad Prism (version 5.0) to estimate half life ($t_{1/2}$).

Estimation of in vitro *true digestibility (IVTD):* About 500 mg of ground samples (1 mm) were transferred into the 100 ml calibrated glass syringes and exactly 40 ml of rumen inoculum (Makkar *et al.* 1995) was dispensed into the syringes. After removing the gas bubbles the nozzle was closed using a rubber cork. The syringes were incubated

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Table 1. Organic matter (OM), ether extract (EE) and neutral detergent fibre (NDF) content of fodder tree leaves (% dry matter basis) (mean ±SE)

Name of the tree leaves	Scientific name	OM (%)	EE (%)	NDF (%)
Vagai	*Albizia lebbeck*	92.23±0.18	3.60±0.17	40.55±0.55
Glyricidia	*Glyricidia glabra*	89.54±0.09	3.36±0.13	37.40±0.47
Agathi	*Sesbania grandiflora*	91.82±0.21	4.54±0.14	21.63±0.07
Neem	*Azadirachta indica*	92.38±0.00	2.90±0.07	34.91±0.20
Mulberry	*Morus alba*	86.03±0.06	3.59±0.35	28.55±0.06
Subabul	*Leucaena leucocephala*	92.38±0.15	5.52±0.21	33.80±0.09
Kodukapuli	*Inga dulce*	91.90±0.01	6.56±0.05	50.24±0.35
Kalyana Murungai	*Erythrina indica*	91.24±0.06	6.45±0.52	48.01±0.04
Maramalli	*Millingtonia hortensis*	93.72±0.01	4.12±0.13	26.97±0.12
Velvel	*Acacia leucophloea*	94.09±0.02	4.84±0.04	65.03±0.02
Baniyan tree	*Ficus bengalensis*	86.54±0.39	3.45±0.12	61.45±0.01
Arasan	*Ficus religiosa*	86.06±0.06	3.47±0.04	40.63±0.23
Luna (noni)	*Morinda citrifolia*	93.85±0.04	3.87±0.04	42.76±0.30
Poovarasan	*Thespesia populnea*	88.62±0.03	5.13±0.02	42.78±0.03
Karuvel	*Acacia nilotica*	93.84±0.03	6.37±0.14	42.17±0.52
Grape	*Mahonia aquifolium*	89.51±0.07	4.92±0.49	46.61±0.71
Cashew	*Anacardium occidentale*	96.87±0.02	1.82±0.12	51.88±0.01
Mango	*Mangifera indica*	88.67±0.03	0.54±0.06	47.14±0.05
Murungai	*Moringa oleifera*	89.32±0.08	8.74±0.05	19.48±0.12
Tapioca	*Manihot esculenta*	88.10±0.06	8.32±0.02	27.95±0.08
Udaiyan	*Lannea coromendnicaea*	90.81±0.10	5.90±0.03	41.10±0.14
	Mean ±SE	90.83±0.64	4.67±0.43	40.53±2.57

in a shaking water bath maintained at 39°C in triplicates.

After the end of 24 h of incubation, contents in the syringes were transferred into spoutless beakers by repeated washing with neutral detergent solution (NDS). The contents were refluxed for 1 h using Fibertec equipment and filtered through pre-weighed Gooch crucible (Grade I). The residues were dried in the hot air oven and weighed.

Estimation of methane production potential: Ground

$$\text{True digestibility (\%)} = \frac{\text{weight of the dry matter taken for incubation- NDF residue}}{\text{weight of the dry matter taken for incubation}} \times 100$$

samples (1mm) of about 200 mg were weighed and transferred into the 100 ml calibrated glass syringes and exactly 30 ml of rumen inoculum (Menke and Steingass 1988) was dispensed into the syringes. The syringes were incubated in a shaking water bath maintained at 39°C in triplicates. Total gas production was recorded both at half life ($t_{1/2}$) and 24 h for tree leaves with half life less than 16 h. For other tree leaves gas samples were collected at half life after recording the total gas production. Gas samples were collected in vacuum container to estimate the concentration of methane in gas chromatograph (GC) using a calibration gas consisting of 22.53% methane, 1.05% hydrogen, 33.30% carbon dioxide and 43.12% nitrogen. Helium gas was used as carrier gas with oven temperature at 60°C, injector temperature at 100°C and detector temperature at 200°C.

Based on the true digestibility, methane production potential per 100 mg truly digested substrate was calculated for all the tree leaves.

Statistical analysis: Completely randomized design (CRD) was the experimental design followed for all the *in vitro* experiments. The *in vitro* true digestibility and methane production potential were statistically analyzed using one-way analysis of variance (One Way-ANOVA) to compare the means (SAS/ SPSS version 15.0 for windows). When significant difference was detected the multiple range tests was used to separate the mean value.

RESULTS AND DISCUSSION

Results of nutrient composition indicated that OM content of various tree leaves ranged from 86.03 to 96.87% with an average value of 90.83% (Table 1). Highest OM content was recorded in cashew (*Anacardium occidentale*) leaves and lowest value was recorded in mulberry (*Morus alba*) leaves. Ether extract content of tree leaves varied from 0.54 to 8.74% with an average value of 4.67%. NDF content was highest in *velvel* (*Acacia leucophloea*) leaves and lowest value was observed in *murungai* (*Moringa oleifera)* leaves. Average NDF content of different tree leaves was 40.53%. Results of OM, EE and NDF content of cashew (*Anacardium occidentale)*, glyricidia (*Glyricidia glabra)*, *agathi* (*Sesbania grandiflora)* and subabul (*Leucaena leucocephala)* were similar to the findings of Reddy and Elanchezhian (2008).

Results of *in vitro* experiments revealed that *in vitro* true digestibility (IVTD) of various fodder tree leaves ranged from 41.59 to 75.59% with an average value of 61.82% and the difference was statistically significant ($P<0.05$) (Table 2). *Vagai* (*Albizia lebbeck*), neem (*Azadirachta indica*), baniyan (*Ficus bengalensis*) and *luna* (*Morinda citrifolia*) tree leaves had similar IVTD values. The variation among *agathi* (*Sesbania grandiflora*), *kalya murungai* (*Erythrina indica*), *poovarasan* (*Thespesia populnea*), cashew (*Anacardium occidentale*), mango (*Mangifera indica*), *murungai* (*Moringa oleifera*), tapioca (*Manihot esculenta*) and *udaiyan* (*Lannea coromendnicaea*) tree leaves with respect to IVTD was statistically nonsignificant. Lowest IVTD was observed in *velvel* (*Acacia leucophloea*) leaves and highest value was recorded in *maramalli* (*Millingtonia hortensis*) leaves. However, Kamalak *et al*. (2004) reported that *in vitro* digestibility for tannin rich tree leaves varied from 41.70 to 52.80%. Similar *in vitro* digestibility values for *vagai* (*Albizia lebbeck*) and *Agathi* (*Sesbania grandiflora*) leaves were reported by Sanchez *et al*. (2000). *In vitro* digestibility of *vagai* (*Albizia lebbeck*), glyricidia (*Glyricidia glabra*), neem (*Azadirachta indica*) and *kalyana murungai* (*Erythrina indica*) tree leaves were in accordance with Mohamed Amanullah *et al*. (2006).

Methane production potential of various tree leaves at half life ($t_{1/2}$) ranged from 0.21 to 1.98 ml/100 mg truly digested substrate with an average value of 0.93 ml/100 mg truly digested substrate and significant difference ($P<0.05$) was found among the tree leaves. *Vagai* (*Albizia lebbeck*), glyricidia (*Glyricidia glabra*), neem (*Azadirachta indica*), subabul (*Leucaena leucocephala*), mulberry (*Morus alba*), *kodukkapuli* (*Inga dulce*), *kalyana murungai* (*Erythrina indica*), baniyan (*Ficus bengalensis*), *karuvel* (*Acacia nilotica*), grape (*Mahonia aquifolium*), cashew (*Anacardium occidentale*), mango (*Mangifera indica*) and *udaiyan* (*Lannea coromendnicaea*) tree leaves had significantly lower ($P<0.05$) methane production potential at half life ($t_{1/2}$) compared to other fodder tree leaves. *Maramalli* (*Millingtonia hortensis*), *velvel* (*Acacia leucophloea*), *arasan* (*Ficus religiosa*), *luna* (*Morinda citrifolia*), *poovarasan* (*Thespesia populnea*), *Murungai* (*Moringa oleifera*) and tapioca (*Manihot esculenta*) tree leaves had significantly ($P<0.05$) higher methane production potential than other tree leaves at half life ($t_{1/2}$).

Methane production potential of various tree leaves at 24 h ranged from 0.92 to 2.72 ml/100 mg truly digested substrate with an average value of 1.55 ml/100 mg truly digested substrate and the difference was significant ($P<0.05$). *Agathi* (*Sesbania grandiflora*) leaves had significantly ($P<0.05$) higher methane production potential at 24 h (2.72 ml/100 mg truly digested substrate) compared to all other tree leaves. In general tree leaves produces less

Table 2. *In vitro* true digestibility (%), half life (h) and methane production potential (ml) of fodder tree leaves

Name of the tree leaves	Scientific name	*In vitro* true digestibility (IVTD) (%)	Half life ($t_{1/2}$)(h)	Methane production potential (ml/100 mg truly digested substrate)	
				Half life ($t_{1/2}$)	24 h
Vagai	*Albizia lebbeck*	$51.17±0.09^{b}$	9.15	$0.63±0.07^{abc}$	$1.76±0.18^{a}$
Glyricidia	*Glyricidia glabra*	$56.40±0.24^{cde}$	10.96	$0.65±0.09^{abc}$	$1.75±0.10^{a}$
Agathi	*Sesbania grandiflora*	$65.56±0.22^{h}$	11.13	$1.14±0.13^{bcd}$	$2.72±0.21^{b}$
Neem	*Azadirachta indica*	$54.46±0.40^{bcd}$	10.81	$0.30±0.06^{a}$	$0.92±0.13^{a}$
Mulberry	*Morus alba*	$59.25±0.18^{ef}$	7.80	$0.21±0.04^{a}$	$1.07±0.06^{a}$
Subabul	*Leucaena leucocephala*	$73.83±0.34^{i}$	20.21	$0.98±0.03^{abcd}$	-
Kodukapuli	*Inga dulce*	$60.27±0.15^{efg}$	13.68	$0.67±0.09^{abc}$	$1.02±0.15^{a}$
Kalyana murungai	*Erythrina indica*	$62.85±0.91^{fgh}$	13.18	$0.88±0.33^{abcd}$	$1.43±0.33^{a}$
Maramalli	*Millingtonia hortensis*	$75.59±0.69^{i}$	33.47	$1.18±0.07^{bcde}$	-
Velvel	*Acacia leucophloea*	$41.59±0.51^{a}$	36.50	$1.98±0.06^{e}$	-
Baniyan	*Ficus bengalensis*	$52.34±0.53^{bc}$	47.91	$0.86±0.09^{abcd}$	-
Arasan	*Ficus religiosa*	$72.52±0.25^{i}$	16.98	$1.66±0.11^{de}$	-
Luna (noni)	*Morinda citrifolia*	$53.60±0.79^{bcd}$	30.67	$1.32±0.14^{cde}$	-
Poovarasan	*Thespesia populnea*	$65.52±0.77^{h}$	22.92	$1.33±0.07^{cde}$	-
Karuvel	*Acacia nilotica*	$72.51±0.07^{i}$	14.38	$0.89±0.06^{abcd}$	$1.71±0.07^{a}$
Grape	*Mahonia aquifolium*	$56.90±0.65^{de}$	19.87	$0.70±0.08^{abc}$	-
Cashew	*Anacardium occidentale*	$65.24±0.58^{h}$	22.40	$0.22±0.02^{a}$	-
Mango	*Mangifera indica*	$65.48±0.57^{h}$	19.10	$0.47±0.14^{ab}$	-
Murungai	*Moringa oleifera*	$64.60±0.52^{h}$	18.40	$1.22±0.25^{bcde}$	-
Tapioca	*Manihot esculenta*	$64.17±0.99^{gh}$	25.60	$1.33±0.42^{cde}$	-
Udaiyan	*Lannea coromendnicaea*	$64.41±0.58^{gh}$	21.40	$0.84±0.21^{abcd}$	-
	Mean ±SE	61.82±1.85	20.31	0.93±0.10	1.55±0.21

[abcdefghi] Means with different superscripts in a column differs significantly ($P<0.05$).

methane than other feedstuffs which might be attributed to the presence of plant secondary metabolites such as tannin, saponin and phenolic compounds (Kamra *et al*. 2012).

It can be concluded that average OM, EE and NDF contents of various tree leaves were 90.83, 4.67 and 40.53%, respectively. Average methane production potential of different tree leaves at half life and 24 h was 0.93 and 1.55 ml /100 mg truly digested substrate, respectively.

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