Nutritional potentialities of some tree leaves based on polyphenols and rumen in vitro gas production

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Abstract

Aim: The study was conducted to evaluate eight tree leaves based on polyphenolic content and rumen in vitro incubation and gas production technique (RIVIGPT) for their nutritive potentiality.

Materials and Methods: Eight selected tree leaves, namely Sesbania grandiflora, Melia dubia, Dillenia spp., Artocarpus heterophyllus, Commiphora caudata, Moringa oleifera, Leucaena leucocephala, and Acacia auriculiformis, were selected for proximate composition, forage fiber fractions, total phenolics (TPs), non-tannin phenols (NTPs), total tannins (TTs), condensed tannins (CTs), and hydrolysable tannins (HTs); RIVIGP with and without polyethylene glycol (PEG); and in vitro dry matter digestibility (IVDMD) (modified in vitro two stage) analysis was conducted. On the basis of RIVIGPT, the in vitro digestible organic matter (IVDOM) and dry matter intake (DMI) was calculated.

Results: Crude protein (CP) content of tree leaves ranged from 9.59 to 25.81%, neutral detergent fiber (NDF) 28.16 to 53.33%, acid detergent fiber (ADF) 21.26 to 41.7%, acid detergent lignin (ADL) 3.62 to 21.98%, TP 1.83 to 17.35%, TT 0.40 to 15.47%, and CTs 0.02 to 15.26%. IVDMD (%) was ranged from 64.95 to 88.12. The mean metabolizable energy (ME) (MJ/Kg) of tree leaves estimated with and without PEG was 7.75±0.56 and 8.75±0.39, and 37.09±2.64, initial gas production (a) 9.81±2.41 and 7.42±0.80, in vitro gas production at 24 h (IVGP24) (ml) 31.06±4.14 and 39.50±4.430, the predicted IVDOM (%) 55.44±4.15 and 61.98±3.03, and DMI (g/Kg W0.75) (r=0.838, p<0.05) and negatively correlated with CP, ADF, ADL, TP, CT, IVGP24, IVGP96, a+b, k (r=0.731, p<0.05), IVDMD, IVDOM, and DMI (r=0.838, p<0.05) and negatively correlated with a and t1/2. ME, ADF, and ADL contents were negatively correlated with ME (r=−0.867, p<0.05), IVGP24 (r=−0.858, p<0.05), IVGP96 (r=−0.858, p<0.05), a+b (p<0.05), k (r=0.828, p<0.05), IVDOM (r=−0.853, p<0.05), and DMI and positively correlated with a and t1/2, CP, IVGP24, IVGP96, a+b, k, and DMI were negatively correlated with ME, IVGP24, IVGP96, a+b, k, and DMI were positively correlated with a and t1/2.

Conclusion: In the present study, the potentiality of tree leaves was assessed based on CP, ADF, ADL, TP, CT, IVGP, ME, IVDOM, predicted IVDOM, and predicted DMI. Based on this, it can be concluded that S. grandiflora, M. dubia, M. Oleifera, and L. leucocephala were graded as best; A. heterophyllus and C. caudata as moderate; and Dillenia spp. and A. auriculiformis as lowest potential ruminant feed.

Keywords: chemical composition, in vitro, in vitro dry matter digestibility, in vitro digestible organic matter, metabolizable energy, polyethylene glycol, rumen in vitro incubation and gas production, ruminants, tannins, tree leaves.

Introduction

The inadequacy of nutrients is a major limitation for livestock as a deficit of 728 mT (64%) of green fodder and 157 mT (25%) of dry fodder with 27% of crude protein (CP) and 24% of total digestible nutrients in India which is expected by 2020 [1]. This can partly be overcome by feeding tree leaves, as huge quantity of biomass is available from fodder trees, which provide nitrogen, energy, minerals and vitamins and additionally have laxative effect and reduce cost of feeding [2].

Suitability of inclusion in the feeding can be assessed either by in situ or rumen in vitro incubation and gas production techniques (RIVIGPT), which would be complementary to traditional chemical measurements [3]. In comparison to in vivo feeding trial,
the RIVIGPT is simple, less expensive, less time consuming and allow more control over experiment. As being more efficient than other in vitro techniques, it is suggested for determining the nutritive value of feeds containing anti-nutritive factors and for evaluating the microbial fermentation of ruminant feeds and its impact on fermentation products [4,5].

Therefore, the present study was conducted to evaluate nutritive potentialities of eight tree leaves such as Sesbania grandiflora, Melia dubia, Dillenia spp., Artocarpus heterophyllus, Commiphora caudata, Moringa oleifera, Leucaena leucocephala, and Acacia auriculiformis of different parts of Karnataka state based on polyphenolic content and RIVIGPT. The selected tree leaves are grown in this region and used in animal feeding. Except S. grandiflora, A. heterophyllus, M. Oleifera, and L. leucocephala, other tree leaves are new to investigation and there is no literature with regard to nutritive value, and hence, this study will be useful in comparing among the tree leaves.

Materials and Methods

Ethical approval

The Institutional Animal Ethics Committee approved to collect rumen fluid from the cannulated cow.

Collection of tree leaves and processing

Tree leaves of S. grandiflora (Agase, Kan.), M. dubia (Hebbevu, Kan.), Dillenia spp. (Kaadu kanganu, Kan.), A. heterophyllus (Halasu, Kan.), C. caudata (Konda Maavu, Kan.), M. oleifera (Nugge, Kan.), L. leucocephala (Subabul), and A. auriculiformis (Acacia) were chosen from different parts of Karnataka state. About 2-3 Kg of tree leaves from the single source were hand plucked, oven dried at 55°C for 48 h, and grounded to pass through size of 1 mm sieve for further analysis (Kan. regional language, Kannada).

Determination of nutritive components and polyphenolic fractions

These selected tree leaves were analyzed in triplicates for proximate composition, forage fiber fractions [5], total phenolics (TPs), non-tannin phenols (NTPs), total tannin phenols (TTPs), condensed tannins (CTs), and hydrolysable tannins (HTs) [4]; RIVIGP with and without polyethylene glycol (PEG) [6]; in vitro dry matter digestibility (IVDMD) (modified in vitro two stage) [7]; and prediction of in vitro digestible organic matter (IVDOM) and dry matter intake (DMI) [8].

RIVIGP

The selected tree leaves were subjected to RIVIGP (with and without PEG) for estimating metabolizable energy (ME) and rate of gas production. A lactating dairy cow producing 3 Kg of milk per day, fitted with a flexible rumen cannula of large diameter (Bar Diamond Inc., USA), receiving a basal diet consisting of finger millet straw and CFM (maize 60%, WB 35%, mineral mixture 2%, urea 2%, and salt 1%) was used as donor cow for rumen fluid. For RIVIGP, rumen fluid was collected before offering CFM.

Feed samples (200±10 mg) were incubated with and without PEG in 100 ml calibrated glass syringes in triplicate with 30 ml mixed rumen suspension with three blank incubations and standards [6]. For PEG treatment, PEG is added twice the amount of feed sample, and blank samples with PEG were also incubated for estimating corrected gas production. Cumulative gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 h of incubation.

Data on gas production were fitted to the exponential equation \( Y=a+b \left(1-e^{-ct}\right) \), where \( Y \) (mL) was defined as gas production at time \( t \), \( a \) (mL) was the initial gas production, \( b \) (mL) was the gas production during incubation, \( a+b \) or \( D \) (mL) was the potential gas production, and \( c \) (mL/h) was the fractional gas production.

The equations used to estimate the IVDOM and ME [5] are as follows:

\[
\text{ME (MJ/Kg)} = 2.20 + 0.1357 \text{ GP} + 0.0057 \text{ CP} + 0.0002859 \text{ EE}^2
\]

IVDOM (%) = 14.88 + 0.8893 GP + 0.0448 CP + 0.0651 TA

Where

- \( GP \) = Corrected net gas production, ml/200 mg DM.
- \( CP \) = Crude protein, g/Kg DM.
- \( EE \) = Ether extract g/Kg DM.
- \( TA \) = Total ash, g/Kg DM.

Predicted daily intake (DMI) (g DM/Kg W0.75) [8] was estimated using the following equation:

\[
\text{DMI}=18.9-0.23(a+b)+687(c)+0.11\text{CP}(g/\text{Kg DM})
\]

Where \( a \) (mL) was the initial gas production, \( b \) (mL) was the gas production during incubation, and \( c \) (mL/h) was the fractional gas production.

IVDMD

The IVDMD was carried out using Ankom<sup>®</sup> Fiber Analyzer where F57 Ankom Filter Bag (porosity: 25 µ) was used for extraction. Bags were made up of N-free Monofilament Polyester Screen Printing Fabrics.

About 400mg of dry forage samples (2 mm) were weighed into F57 Ankom Filter Bags and subjected for 48 h incubation in Mold’s buffer/rumen fluid mixture in sealed Erlenmeyer flasks followed by treatment with NDS. The dry residues were weighed, and digestibility was calculated using the following equation [7,9].

\[
\text{IVDMD} = \left(\frac{\text{Initial wt. of sample (DM)} - \text{Final wt. of sample- Blank}}{\text{Initial wt. of sample (DM)}}\right) \times 100
\]

Polyphenolic fractions

TPs were estimated by Folin–Ciocalteu reaction [4] using gallic acid as a standard. For the CT fraction, the extract was treated with butanol–HCl in the presence of ferric ammonium sulfate, and CT expressed as leucocyanidin equivalent as A550 nm×782.6 weight.
of sample DM, where 550 nm is absorbance at 550 nm assuming that the effective E1%, 1 cm, 550 nm of leucocyanidin is 460. The phenolic content of the supernatant after precipitating with polyvinylpolypyrrolidone (100 mg) was measured by the Folin–Ciocalteu reaction and this was regarded as the NTP. TTPs were calculated as the difference between TP and NTP. HTs were calculated as the difference between TTP and CT.

**Statistical analysis**

The data on RIVIGP were subjected to nonlinear regression using GraphPad Prism software to assess gas kinetics (exponential decay equation model). Pearson correlation analysis was used to assess the relationship between chemical composition and ME, IVGP\textsuperscript{(in vitro gas production at 24 h), IVDMD, IVDOM, and DMI of the tree leaves.}

**Results**

**Chemical composition**

The nutrient composition of the tree leaf samples is presented in Table-1. The DM content of fresh tree leaves was low in *S. grandiflora* (20.31%) and high in *A. heterophyllus* (37.45%). The OM content was low in *C. caudata* (87.97%) and high in *A. auriculiformis* (93.95%). The CP content ranged from 9.59% (*Dillenia* spp.) to 25.81% (*L. leucocephala*). The level of NDF ranged from 28.16% (*L. leucocephala*) to 53.33% (*Dillenia* spp.). The ADF ranged from 21.26% (*S. grandiflora*) to 41.7% (*Dillenia* spp.). The level of ADL ranged from 3.62% (*S. grandiflora*) to 21.98% (*A. auriculiformis*).

**Polyphenolic fractions**

Polyphenolic fractions (on DM basis) of the eight tree leaves are presented in Table-2 [4]. The TP content of tree leaves was high in *A. heterophyllus* (17.35%) and low in *S. grandiflora* (1.77%). The NTP was rich in *Dillenia* spp. (5.7%) and low in *S. grandiflora* (1.00%). TTP content was more in *A. heterophyllus* (15.47%) and low in *M. oleifera* (0.40%). The potent source of CT and HT was *A. heterophyllus* (15.26%) and *Dillenia* spp. (4.72%), respectively.

**Gas production kinetics**

RIVIGP values of tree leaves with or without PEG are shown in Table-3. The IVGP\textsuperscript{24} (ml) was more in *M. oleifera* (42.63) and less in *A. auriculiformis* (8.68). The IVGP\textsuperscript{1} (ml) was higher for *S. grandiflora* (50.54) and less for *A. auriculiformis* (10.27). The DM (ml) value was more in *S. grandiflora* (47.48) and lower for *A. auriculiformis* (9.64). k was higher for *M. dubia* and lower for *Dillenia* spp., and 1/2 (h) was highest for *Dillenia* spp. and least for *M. dubia*. Among the evaluated tree leaves, energy content (ME, MJ/Kg) was higher in *S. grandiflora* (9.87) and lower in *A. auriculiformis* (5.03).

**IVDMD**

IVDMD of tree leaves was analyzed using Ankom\textsuperscript{20} Fiber analyzer based on modified in vitro two-stage method which is shown in Table-4. IVDMD of tree leaves ranged from 64.95% (*A. auriculiformis*) to 88.12% (*M. dubia*) and an average of 76.02%.
Table-2: Polyphenolic fractions (%) of tree leaves on DMB.

| Tree leaves                        | TP1   | NTP2   | TTP3   | CT4   | HT5   |
|-----------------------------------|-------|--------|--------|-------|-------|
| Sesbania grandiflora              | 1.77±0.06 | 1.00±0.03 | 0.77±0.02 | 0.02±0.01 | 0.75±0.05 |
| Melia dubia                       | 1.89±0.11 | 1.11±0.02 | 0.77±0.06 | 0.19±0.02 | 0.58±0.03 |
| Dillenia spp.                     | 11.67±0.56 | 5.70±0.40 | 5.97±0.43 | 1.25±0.01 | 4.72±0.35 |
| Artocarpus heterophyllus          | 17.35±0.14 | 1.88±0.01 | 15.47±0.19 | 15.26±0.01 | 0.21±0.03 |
| Acacia auriculiformis             | 9.04±0.66 | 4.95±0.14 | 4.09±0.08 | 2.37±0.05 | 1.72±0.01 |
| Moringa oleifera                 | 1.83±0.19 | 1.44±0.03 | 0.40±0.08 | 0.19±0.01 | 0.20±0.07 |
| Leucaena leucocephala            | 4.76±0.12 | 2.68±0.15 | 2.07±0.20 | 1.99±0.01 | 0.09±0.08 |
| Commiphora caudata               | 4.98±0.22 | 3.35±0.32 | 1.63±0.26 | 1.25±0.01 | 0.38±0.11 |

All values were mean of triplicates. TP by Folin–Ciocalteu reaction [4], NTP by Folin–Ciocalteu reaction using PVPP [4].

The PEG inclusion during incubation resulted in increased ME, a, D, IVGP, ME, IVDOM, and DMI and of tree leaves and decreased t_{1/2}. In Dillenia spp., A. heterophyllus, and A. auriculiformis tree leaves, it was more pronounced.

Correlation analysis (r)

Pearson correlation analysis was made to test the relationship between chemical composition and ME, IVGP, IVDOM, and DMI values and is presented in Table-5. CP was positively correlated with ME, IVGP, IVGP, a+b, k (r=0.749, p<0.05), IVDOM, and DMI (r=0.838, p<0.05) and negatively correlated with a (r=−0.451) and t_{1/2} (r=−0.575). NDF, ADF, and ADL contents are negatively correlated with ME (r=−0.899, p<0.05), IVGP (r=−0.867, p<0.05), IVGP (r=−0.858, p<0.05), a+b (r=−0.828, p<0.05), k (r=−0.877, p<0.05), IVDOM (r=−0.674), IVDOM (r=−0.853, p<0.05), and DMI (r=−0.538) and positively correlated with a (r=0.424) and t_{1/2} (r=0.683). TP, TTP, and CT are negative correlation of non-significant difference with ME, IVGP, IVGP, a+b, k, IVDOM, IVDOM, and DMI and positively correlated with a (r=0.808, p<0.05) and t_{1/2}. The initial gas production was more in tannin-containing feeds which may due to easily degradable OM. At 96 h incubation, tannins inhibited the gas production (negative correlation) which may be due to fiber bound tannins in tree leaves. The negative relationship between chemical composition and phenolic compounds, with in vitro degradability of legumes at 24 h of in vitro incubation may be due to presence of ADF and CT in them, but on addition of PEG reversed that situation [3, 10, 11]. ME (MJ/Kg) was positively correlated with IVGP (r=0.938, p<0.05), IVGP (r=0.875, P=0.05), (a+b) (r=0.813, p<0.05), k (r=0.731, p<0.05), IVDOM, IVDOM (r=0.985, p<0.05), and DMI (r=0.727, p<0.05) and negatively correlated with a and t_{1/2}.

Discussion

Chemical composition

In the present study, CP varied from 9.59 to 25.81%, and it was in similar range for tree leaves as in published reports [12-17]. Similarly, the NDF, ADF, and ADL contents of analyzed tree leaves were in a instead of the similar range [13,18,19].

In the present study, there were considerable variations in chemical compositions between the tree leaves. This was due to differences in genotype, environment, stage of maturity, and harvesting [20-22]. The multipurpose tree leaves contained moderate levels of CP, minerals, and vitamins that are deficient in many low-quality roughages and it was found that CP level above the threshold level (11-12%) is required for the moderate level of ruminant production [23].

Polyphenolic fractions

The literature on the polyphenolic content of tree leaves depicts that the concentration remarkably varies from source to source. The polyphenolic content estimated was lower [17,24,25] than the present values, whereas a similar range of values was also observed in the past [15,16,26-28].

Tannin composition in plants depends on type of plant, photosynthetic capacity, soil fertility, environmental conditions, maturity of the leaves, and processing and analytical method employed in analysis [4]. In general, the intake of CT below 5% improves the utilization of feed by ruminants, mainly because of a reduction in ruminal protein degradation and, as a consequence, greater availability of mainly essential amino acids for absorption in the small intestine. Values of CTs exceeding 5% on dry matter basis could inhibit microbial activity, depress dry matter digestibility, and reduce voluntary intake [29].

Gas production kinetics

In the present study, the lower level of ME and RIVIGP parameters is closely related to its CP, fiber fractions, and tannins as reported by previous studies [24,30,31,32,33].
Table 3: Rumen in vitro gas production values of tree leaves with and without PEG 6000.

| Tree leaves          | Me (MJ/Kg) | PEG 6000 | Mean of triplicates | Potential cumulative gas production (ml) | Rapidly produced gas (ml) | Half-life (t₁/2) | Rate of gas production (h⁻¹) | Corrected net gas production (ml/96 h) |
|----------------------|------------|----------|---------------------|-----------------------------------------|--------------------------|-----------------|-------------------------------|----------------------------------------|
| Sesbania grandiflora | 9.87±0.18  | 10.01±1.20 | 4.23±0.75 | 23.05±3.2 ±0.81 | 2.63±1.12 | 2.74±0.87 | 9.03±0.71 | 51.33±0.97 |
| Artocarpus heterophyllus | 5.03±0.87 | 6.70±0.89 | 6.89±0.89 | 6.21±0.87 | 4.05±0.77 | 4.11±0.42 | 4.11±0.42 | 4.11±0.42 |
| S. grandiflora        | 8.36±0.11  | 9.35±0.75 | 12.03±3.2 | 3.69±0.54 | 2.16±0.19 | 2.16±0.19 | 2.16±0.19 | 2.16±0.19 |
| Moringa               | 5.05±0.55  | 5.95±0.71 | 7.05±0.71 | 5.05±0.54 | 4.05±0.26 | 4.05±0.26 | 4.05±0.26 | 4.05±0.26 |
| Commiphora caudata    | 7.96±0.54  | 8.76±0.62 | 9.33±0.62 | 8.05±0.54 | 4.14±0.31 | 4.14±0.31 | 4.14±0.31 | 4.14±0.31 |
| Dillenia spp.         | 38.01±1.66 | 8.85±0.27 | 1.34±0.31 | 0.98±0.16 | 0.98±0.16 | 0.98±0.16 | 0.98±0.16 | 0.98±0.16 |

Effect of PEG on kinetics of gas production

The improvement in gas production in Dillenia spp., A. heterophyllus, and A. auriculiformis tree leaves was due to affinity of PEG to tannins[32-35]. It was also found that there was only minor improvement in IVGP² and IVGPₘ in L. leucocephala and C. caudata due to the low level of CT in them. Addition of PEG also increased the ME, IVDOM % content, and DMI (g/Kg W₀⁷⁵) in these tree leaves.

In general, the improvement in fermentation in each species by adding PEG almost certainly reflects its deactivation of secondary compounds [11,35,36]. High CT (proanthocyanidins) and fiber (NDF and ADL) contents reduce digestibility [31,32] while low CP affects the acceptability of browse [25]. Similarly, in the present study, lower level of IVGP was observed in those tree leaves which are containing more CT, NDF, ADL, and ADL.

Based on ME, ADL, and predicted DMI of tree leaves, it can be categorized as best, moderate and poor potential source of fodders for ruminants [26]. S. grandiflora, M. dubia, M. oleifera, and L. leucocephala were best potential fodders due to higher ME (>8.08 MJ) and predicted DMI (≥111 g) and lower ADL (<8.62%); A. heterophyllus and C. caudata were moderate potential due to moderate in ME (7.96 MJ) and predicted DMI (48.7-82.4 g) and slightly higher ADL (9-15.3%), whereas, Dillenia spp. and A. auriculiformis were lowest potentiality due to lower ME (5-6.2 MJ) and predicted DMI (41-48.1 g) and very higher ADL (15.25-21.98%) in them.

Conclusion

In the present study, the nutritive potentiality of tree leaves was assessed based on CP, ADF, ADL, TP, CT, IVGP, ME, IVDMD, IVDOM, and DMI. It can be concluded that S. grandiflora, M. dubia, M. oleifera, and L. leucocephala were graded as the best due to higher CP, ME, IVGP, IVDMD, IVDOM and DMI and lower polyphenols and fiber fractions; A. heterophyllus and C. caudata were graded as the moderate potential and Dillenia spp. and A. auriculiformis were graded as the lowest potential as a ruminant feed. While the addition of PEG in Dillenia spp. and A. auriculiformis improved the RIVIGP but it did not impact their overall nutritive ranking. However, more studies are required to characterize these feeds better.
### Table 4: IVDM and predicted IVDOM and DMI (g/Kg W^{0.75}) of tree leaves.

| Tree leaves          | IVDM^1 | IVDOM^2 (%) | DMI^2 (g/Kg W^{0.75}) |
|----------------------|--------|-------------|-----------------------|
| Sesbania grandiflora | 78.93±1.11 | 69.05±0.41 | 90.84±0.42 | 110.2±0.21 | 116.3±0.39 |
| Melia dubia          | 88.12±2.43 | 66.61±0.17 | 69.01±0.31 | 136.8±0.42 | 125.0±0.51 |
| Dillenia spp.        | 75.92±1.43 | 47.27±0.42 | 57.44±0.18 | 41.9±0.17  | 64.6±0.28  |
| Artocarpus heterophyllus | 71.43±1.78 | 50.94±0.32 | 63.19±0.42 | 48.7±0.53  | 94.7±0.61  |
| Acacia auriculiformis | 64.95±1.86 | 32.52±0.19 | 43.50±0.38 | 48.1±0.42  | 72.1±0.51  |
| Moringa oleifera     | 86.26±1.32 | 60.10±0.22 | 77.77±0.32 | 111.4±0.38 | 104.9±0.43 |
| Leucaena leucocephala| 70.62±0.64 | 57.87±1.21 | 60.88±0.19 | 134.0±0.52 | 153.7±0.58 |
| Commiphora caudata   | 71.96±0.71 | 59.12±0.82 | 64.17±0.91 | 82.4±0.72  | 103.9±0.75 |

All values were mean of triplicates. *Indicates with PEG and -indicates without PEG. 1IVDM (%) - As per modified two-stage fermentation using Ankom^TM Fiber Bag Analyzer. 2IVDM (%)=14.88+0.8893 CP+0.0448 CP+0.0651 TA. 3DMI (g DM/Kg W^{0.75})=18.9-0.23 (a+b)+687(c)+0.11CP (g/Kg DM). IVDMD=In vitro dry matter digestibility, IVDOM=In vitro digestible organic matter, PEG=Polyethylene glycol, DMI=Dry matter intake.

### Table 5: Correlation coefficients (r) between in vitro fermentation parameters, IVDM, IVDOM, and DMI with chemical composition and total polysaccharides and tannins.

| Parameter      | PEG | CP | NDF | ADF | ADL | TP | TTP | CT | ME (MJ/Kg) |
|----------------|-----|----|-----|-----|-----|----|-----|----|------------|
| ME (MJ/Kg)     |     |    |     |     |     |    |     |    |            |
| PEG            |     |    |     |     |     |    |     |    |            |
| CP             |     |    |     |     |     |    |     |    |            |
| NDF            |     |    |     |     |     |    |     |    |            |
| ADF            |     |    |     |     |     |    |     |    |            |
| ADL            |     |    |     |     |     |    |     |    |            |
| TP             |     |    |     |     |     |    |     |    |            |
| TTP            |     |    |     |     |     |    |     |    |            |
| CT             |     |    |     |     |     |    |     |    |            |
| ME (MJ/Kg)     |     |    |     |     |     |    |     |    |            |

*p<0.05; *indicates with PEG and -indicates without PEG. CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, ADL=Acid detergent lignin, TP=Total phenolic, TTPs=Total tannin phenols, CT=Condensed tannins, ME=Metabolizable energy (MJ/Kg DM), IVGP=Gas production volume (ml/0.2 g DM) after 24 h of incubation, IVDMD (%)=14.88+0.8893 CP+0.0448 CP+0.0651 TA. DMI (g DM/Kg W^{0.75})=18.9-0.23 (a+b)+687(c)+0.11CP (g/Kg DM). IVDMD=In vitro dry matter digestibility, IVDOM=In vitro digestible organic matter, PEG=Polyethylene glycol, DMI=Dry matter intake.

Competing Interests

The authors declare that they have no competing interests.

References

1. GOI. (2012) Report of the Working Group on Animal Husbandry and Dairying. 12th Five Year Plan, 2012-2017. Planning Commission, New Delhi.
2. Devendra, C. (1992) Nutritional potential of fodder trees and shrubs as protein sources in ruminant nutrition. Legume trees and other fodder trees as protein sources in ruminant nutrition. FAO Anim. Prod. Health Pap, 102: 50-62.
3. Carlos, A.S.C., Henry, L.L.S. and Francisco, J.S.S. (2005) Chemical composition, in vitro gas production and in situ degradability. Anim. Feed Sci. Technol., 123-124(12): 1277-289.
4. Makkar, H.P.S. (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to through in vivo feeding trials with respect to palatability and intake.

Authors’ Contributions

TMP and KCS planned and supervised the entire research work. KSG carried out the experimental work and laboratory analysis. VN, TT, YBR, and BCU prepared the manuscript along with data analysis. All authors read and approved the final manuscript.

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overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res., 49(3): 241-256.
5. Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for dietary, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74(10): 3583-3589.
6. Menke, K.H. and Steingass, H. (1988) Estimation of the energetic feed value from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev., 28: 47-55.
7. Goering, H.K. and Van Soest, P.J. (1970) Forage Fibre Analysis. Agricultural Handbook. Agricultural Research Services, US. Department of Agriculture, Washington, D.C.
8. Khazaal, K., Dentinho, J.M., Ribeiro, J.M. and Ørskov, E.R. (1995) Prediction of apparent digestibility and voluntary intake of hays fed to sheep: Comparison between using fibre components, in vitro digestibility or characteristics of gas production or nylon bag degradation. Anim. Sci., 61(3): 527-538.
9. Mould, F.L., Morgan, R., Morgan, R.E., Kliem, K.E. and Krystallidou, E. (2005) A review and simplification of the in vitro incubation method. Anim. Feed Sci. Technol., 123: 155-172.
10. Yissehak, K. and Janssens, G.P.J. (2013) Evaluation of nutritive value of leaves of tropical tanniferous trees and shrubs. Livest. Res. Rural Dev., 25(12): 14-21.
11. Salem, A.Z.M., Salem, M.Z.M., El-Adawy, M.M. and Robinson, P.H. (2006) Nutritive evaluations of some browse tree foliage during the dry season: Secondary compounds, feed intake and in vivo digestibility in sheep and goats. Anim. Feed Sci. Technol., 127(3-4): 251-267.
12. Patel, V.R., Choubey, M., Raval, A.P. and Desai, M.C. (2018) Influence of feeding Albizia lebbeck and Terminalia arjuna leaves on growth performance and nutrient utilization in Surti kids. Indian J. Anim. Nutr., 35(1): 76-81.
13. Raja, K.K. and Parthasarathy, M. (2009) In vitro nitrogen degradability of some forages, top feeds and fibrous crop residues. Anim. Feed Sci. Technol., 9: 97-101.
14. Sultana, N., Alimon, A.R., Huque, K.S., Sazili, A.Q., Yaakub, H., Hossain, J. and Baba, M. (2015) The feeding value of Moringa (Moringa oleifera) foliage as replacement to conventional concentrate diet in Bengal goats. Adv. Anim. Vet. Sci., 3(3): 164-173.
15. Jayanegara, A., Wina, E., Soliva, C.R., Marquardt, S., Kreuzer, M. and Leiber, F. (2011) Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Anim. Feed Sci. Technol., 163(2-4): 231-243.
16. Reddy, D.V. and Elanchezian, N. (2008) Evaluation of tropical tree leaves as ruminant feedstuff based on cell contents, cell wall fractions and polyphenolic compounds. Anim. Nutr. Rural Dev., 20(5): 35-55.
17. Pal, L., Patra, A.K., Sahoo, A. and Kumawat, P.K. (2015) Evaluation of several tropical tree leaves for methane production potential, degradability and rumen fermentation in vitro. Livest. Sci., 180: 98-105.
18. Mishra, A.S., Tripathi, M.K., Vaidhyananthan, S. and Jakkomla, R.C. (2013) Nutritional evaluation of fallen tree leaves as source of roughage in complete feed blocks for sheep. Anim. Nutr. Feed Technol., 13(2): 223-234.
19. Girma, M., Getachew, A. and Getinet, A. (2015) Chemical composition and in vitro organic matter digestibility of major indigenous fodder tree leaves and shrubs in Northeastern Drylands of Ethiopia. Livest. Res. Rural Dev., 27(2): 26.
20. Arhab, R., Machebeuf, D., Aggoun, M., Bousslehoua, H., Viala, D. and Besle, J.M. (2009) Effect of polyethylene glycol on in vitro gas production and digestibility of tannin-containing feedstuffs from North African arid zone. Trop. Subtrop. Agron., 10(3): 475-486.
21. Elghandour, M.M.Y., Va’quez-Chagoya’n, J.C., Salem, A.Z.M., Kholif, A.E., Martínez Castañeda, J.S., Camacho, L.M. and Cerrillo-Soto, M.A. (2014) Effects of Saccharomyces cerevisiae at direct addition or pre-incubation on in vitro gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci., 13(2): 295-301.
22. Ramachandran, M., Bharathidhasan, A. and Balakrishnan, V. (2015) Nutrient composition, in vitro true digestibility (IVTD) and methane production potential of fodder tree leaves. Ind. J. Anim. Sci., 85(5): 494-497.
23. ARC. (1984) The Nutritive Requirements of Ruminant Livestock. Agricultural Research Council, Commonwealth Agricultural Bureau, Farnham, Royal, UK.
24. Singh, S., Pathak, A.K., Sharma, R.K. and Khan, M. (2015) Effect of tanniferous leaf meal based multi-nutrient blocks on feed intake, haematological profile, immune response and body weight changes in Haemonchus contortus infected goats. Vet. World, 8(5): 572-579.
25. Dey, A., Dutta, N., Sharma, K. and Pattanaik, A.K. (2008) Effect of dietary inclusion of Ficus indica leaves as a precoatent of proteins on the performance of lambs. Small Rum. Res., 75(2-3): 105-114.
26. Chander, D., Datta, M. and Singh, N.P. (2008) Assessment of fodder quality of leaves of multipurpose trees in subtropical humid climate of India. J. For. Res., 19(3): 209-214.
27. Baruah, L., Malik, P.K., Kolte, A.P., Dhali, A. and Bhatta, R. (2018) Methane mitigation potential of phyto-sources from Northeast India and their effect on rumen fermentation characteristics and protozoa in vitro. Vet. World, 11(6): 809-818.
28. Khan, N., Ankur, R., Keshab, B., Sharma, R.K. and Vasi, B. (2014) Effect of feeding mixed silage of oat fodder and jamun leaves on nutrient utilization in goats. Indian J. Anim. Sci., 84(1): 85-87.
29. Min, B.R., Barry, T.N., Attwood, G.T. and McNabb, W.C. (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Anim. Feed Sci. Technol., 106(1-4): 3-19.
30. Kannalak, A., Canbolut, O., Gurbuz, Y., Ozay, O. and Orkose, E. (2005) Chemical composition and its relationship to in vitro gas production of several tannin containing tree and shrubs leaves. Asian Aust. J. Anim. Sci., 18(2): 203-208.
31. Gemedes, B.S. and Hassen, A. (2015) Effect of tannin and species variation on in vitro digestibility, gas, and methane production of tropical browse plants. Asian Australas. J. Anim. Sci., 28(2): 188-199.
32. Huyen, N.T., Fryganas, C., Uittenbogaard, G., Mueller-Harvey, I., Verstegen, M.W.A., Hendriks, W.H. and Pellikaan, W.F. (2016) Structural features of condensed tannins affect in vitro ruminal methane production and fermentation characteristics. J. Agric. Sci., 154(8): 1474-1487.
33. Hatw, B., Stringano, E., Mueller-Harvey, I., Hendriks, W.H., Carbonero, C.H., Smith, L.M.J. and Pellikaan, W.F. (2016) Impact of variation in structure of condensed tannins from safinon (Onobrychis vicifolia) on in vitro ruminal methane production and fermentation characteristics. J. Anim. Physiol. Anim. Nutr., 100(2): 348-360.
34. Makkah, H.P.S., Blummel, M. and Becker, K. (1995) Formation of complexes between polyvinyl-polypropyrolidone or polyethylene glycol and tannins and their implications in gas production and true digestibility in in vitro techniques. Br. J. Nutr., 73(6): 897.
35. Salem, A.Z.M., Robinson, P.H., El-Adawy, M.M. and Hassan, A.A. (2007) In vitro fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. Anim. Feed Sci. Tech., 138(3): 316-330.
36. Rajkumar, K., Bhar, R., Kannan, A., JadHAV, R.V., Singh, B. and Mal, G. (2015) Effect of replacing oat fodder with fresh and chopped oak leaves on in vitro rumen fermentation, digestibility and metabolizable energy. Vet. World, 8(8): 1021-1026.