Indian Journal of Animal Sciences **88** (9): 1085–1089, September 2018/Article

In vitro digestibility and fermentation kinetics of some north eastern Himalayan tree leaves using cattle rumen fluid as inoculum

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Received: 18 September 2017; Accepted: 30 May 2018

ABSTRACT

A study was undertaken to evaluate few north eastern Himalayan forest tree leaves [Blemkar (*Buddleja asiatica*), Phrengpa (*Quercus walliasehiana),* Maar (*Castanopsis indica*)*,* Maarma (*Spiraea canescens*), Domkar (*Symplocus racemosa*), Matekpa (*Quercus fenestrate*), Zimbu *(Lingustrm myrsinites*), Baggar (*Berberis aristata)* and Ngek sing (*Symplocos crataegoides*)] for their potential to manipulate rumen fermentation. An *in vitro* gas production study was conducted for 96 h to obtain the t½ (h) of various leaves. A time dependent increase in gas production was observed for all tree leaves during 96 h incubation period. The rate constant of gas production (c) was highest for Phrengpa and Ngek sing leaves. IVTDMD (%) and TVFA (mM/dl) at t½ (h) was highest for Zimbu, Maarma and Ngek sing tree leaves. Moreover, microbial biomass production (MBP) per unit digested dry matter was highest for Maar followed by Phrengpa and Maarma leaves at t½ (h). Therefore, from the present study, it can be inferred that these tree leaves can influence *in vitro* rumen fermentation positively and might serve as newer feed resource for livestock.

Key words: Cattle rumen fluid, Himalayan tree leaves, *In vitro* digestibility, Fermentation kinetics, t½

High cost of conventional feeds and its shortage are major constraints limiting livestock productivity among marginal farmers. To meet the present demands of livestock population, tropical trees and shrubs emerged as alternative, since they are rich in protein, minerals, vitamins and soluble carbohydrates. Gupta *et al.* (2016) reported that some tree leaves in Ayodhya hills of Purulia district (West Bengal) have a great potential to serve as local livestock fodder due to high content of protein and metabolizable energy. Furthermore, supplementation of low quality roughage with tree foliages either fresh or as leaf meal has been found to be effective in improving the animal performance during the dry season while lowering the cost of production at the same time (Pandey *et al.* 2017). Therefore, as there is inadequate data regarding the nutritive value of various tree fodders and shrubs, the present study was designed to exploit few tree leaves found in north eastern Himalayan regions by *in vitro* gas production technique for ascertaining their role as livestock feed.

MATERIALS AND METHODS

Collection, preparation and chemical analysis of tree leaves: Tree leaves of Blemkar, Phrengpa, Maar, Maarma, Domkar, Matekpa, Zimbu, Baggar and Ngek sing were collected from sub-tropical to temperate region (Arunachal Pradesh), located between 26.28° to 29.30° N latitudes and 91.30° to 97.30° E longitudes, at 3000–13000 ft. above sea level. All leaves harvested from three different trees at the height of 6–12 ft. After mixing properly, leaves were dried at 50°C for 72 h in a forced hot air oven. Dried tree leaves were grinded in a hammer mill and passed through 1 mm and stored in an air tight container for further studies. Samples were analyzed for proximate principles (OM, CP, EE) as per the AOAC (2005) procedures. NDF (estimated without amylase), ADF (both expressed as inclusive of ash) and ADL were analysed according to the methods described by Van Soest *et al*. (1991). The total phenols, total tannins (PVPP-tannins) were determined as per the procedure given by Makkar (2003).

Preparation of inoculums: Rumen liquor (RL) was collected from two growing Jersey crossbred calves, maintained on a diet of paddy straw and concentrates mixture (38% maize grain, 24% mustard cake, 35% wheat bran, 2% mineral mixture and 1% common salt) @ 60:40 ratio. Concentrate mixture was offered to the animal twice a day at 9:00 AM and 4:30 PM to maintain uniformity in rumen fermentation. RL was collected just before morning feeding from the donor animal by stomach tube, filtered through 3 layers of muslin cloth and transported in insulated thermo flask under anaerobic conditions to the laboratory. The inoculums/incubation medium was prepared by mixing

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Table 1. Chemical composition (on % DM basis) and phenolic constituents of different tree leaves

Tree leaf	Chemical composition and phenolic constituents									
	OM	CP	EE	T-CHO	NDF	ADF	Cellulose	ADL	Total tannin phenols (%)	Total phenolics (%)
Blemkar	95.9	18.9	1.5	75.5	54.6	43.2	23.5	19.7	1.23	2.48
Phrengpa	95.9	14.7	3.3	77.9	58.6	39.2	20.5	18.7	6.05	8.53
Maar	95.5	12.9	2.5	80.1	52.2	44.7	23.8	20.9	4.57	5.75
Maarma	96.4	7.4	2.8	86.2	42.2	26.1	14.9	11.1	10.40	11.21
Domkar	93.9	12.0	2.5	79.4	47.6	29.1	16.9	12.2	1.01	2.40
Matekpa	96.9	14.0	2.2	80.7	57.4	41.8	22.2	19.6	5.64	6.87
Zimbu	94.3	12.4	1.2	80.7	41.7	25.0	14.6	10.4	5.93	8.04
Baggar	95.3	9.9	3.4	82.0	56.5	38.3	23.5	14.8	3.56	6.18
Ngek sing	92.2	20.6	6.4	65.2	51.2	21.4	11.9	9.5	1.54	3.66

rumen liquor with buffer in the ratio of 1:2 (Menke and Steingass 1988). Incubation medium (30 ml) was dispensed anaerobically in each 100 ml syringe for *in vitro* studies.

Gas production kinetics: To determine the rate of degradation of tree leaves, 200±10 mg of each tested tree leaves were incubated in triplicate with 30 ml buffered rumen inoculum (Menke and Steingass 1988) in 100 ml calibrated glass syringes for 96 h, with recording of the gas production (GP) after 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h of incubation. Reference standard feedstuffs (dried maize fodder) as per Menke and Steingass (1988) was used. The data generated was processed as per Sigma Software (version 3.5) for calculating $t_{½}$ (h), potential GP and rate of constant. The GP kinetic parameters were calculated from the time dependent (0 to 96 h) *in vitro* cumulative GP data by applying single pool logistic model as depicted below. The assumption were made that the rate of GP is proportional to both the accumulated microbial mass and to the amount of digestible substrate remaining (Schofield *et al*. 1994).

$$Y(t) = b/[(1+ \exp \{(2 + 4C (Lt)\}]$$

where, Y (t), GP (ml) after time t; b, asymptotic value of the component (total potential GP, ml); c, specific rate of fermentation; µm, maximum rate of GP (b×c); L, lag time (the time axis intercept). At the incubation point (Y, b/2), i.e. inflection occurs half way to the maximum gas volume and thus, $t_{½} = L + (\mu m \times b/2)$.

Total gas, IVTDMD, total volatile fatty acids and microbial biomass at t½ (h): After t½ h of incubation, total gas production was recorded by visual assessment of the calibrated scale on the syringe. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing only the inoculum). Total volatile fatty acids (TVFA) were determined as described by Barnett and Reid (1957). For estimation of *in vitro* true dry matter digestibility (IVTDMD), the pellets obtained after centrifugation were refluxed with neutral detergent solution, filtered through sintered glass crucible sand the residues were dried in hot air oven. The loss in weight was considered as IVTDMD. Microbial biomass estimation was calculated as per Blummel *et al*. (1997).

Statistical analysis: The experimental obtained analyzed using simple one way ANOVA. The significant mean differences were compared by Duncan's Multiple Range Test (Kramer *et al.* 1957).

RESULTS AND DISCUSSION

Chemical composition of tree leaves: Chemical composition of the tree leaves are represented in Table 1. Highest CP content (20.6%) was found in Ngek sing. Minimum ADL content (9.5%) was observed in Ngek sing tree leaves. In general, most of the tree leaves were rich in CP content, except Maarma tree leaves (CP content was 7.4%). Total tannin phenolics varied from 1.0% to 10.4% in all the 9 leaves.

In vitro *gas production upto 96 (h): In vitro* gas production up to 96 hours revealed a time dependent increase ($P<0.01$) in gas production for all leaves (Table 2). On an average, overall gas production of different tree leaves sharply increased up to 24 h of incubation, thereafter the rate of gas production was lowered and it was negligible in between 48 to 96 h. Gas produced at different incubation period by Maar, Matekpa and Phrengpa leaves was lower ($P<0.01$) than others.

Rumen fermentation kinetics at t½ (h): Potential gas production was comparatively higher for incubation of Baggar, Ngek sing, Domkar and Zimbu leaves (Table 3). This could be due to compositional variation in fibre and other constituents that determine substrate degradability, ensuing gas production in *in vitro* ruminal fermentation studies. Variation in CP and NDF concentrations among the tree leaves are responsible for variations in amount of substrate OM fermented and short chain fatty acids formed (Njidda and Nasiru 2010). Khazaal *et al.* (1995) indicated that the potential gas production is associated with the degradability of the feed. Thus, higher values obtained for potential gas production on incubation of tree leaves, may indicate enhanced fermentability and better nutrient availability for rumen micro-organisms (Elghandour *et al*. 2016). On an average, potential gas production was higher for three tree leaves, i.e. Baggar, Ngek sing, and Zimbu tree leaves, with an average value of 31.1 ml. Average CP content of the above tree leaves was 14.3%. The reasonable CP and soluble carbohydrate content seems to be major

Table 2. *In vitro* gas production (ml/200 mg substrate) by tree leaves

Tree leaf	Hours post-incubation										
	2	4	6	8	10	12	24	36	48	72	96
Blemkar	4.2^{BC}	6.1^{C}	8.5^{B}	10.7^{BC}	12.5^{CD}	14.5^{CD}	21.1^{C}	24.2^{C}	25.0^{C}	25.3^{C}	25.7^{C}
Phrengpa	3.6^{B}	5.5^{C}	6.9^{B}	7.9^{B}	8.4^{B}	8.8^{B}	10.2^{B}	10.9^{B}	11.5^{B}	12.5^{B}	13.3^{B}
Maar	1.4^{A}	1.8^{A}	2.4^{A}	3.0^{A}	3.3^{A}	3.6^{A}	4.8^{A}	5.7^{A}	5.9---^{A}	6.2^{A}	6.5^{A}
Maarma	3.4^{AB}	5.3^{C}	7.8^{B}	9.3^{B}	11.0^{C}	12.5^{C}	19.6^{C}	24.1^{C}	25.6^{CD}--	26.6^{CD}	27.3^{CD}
Domkar	6.6^{D}	10.5^{D}	13.7^{C}	16.1^{D}	18.2^{F}	20.1^{E}	28.9^{E}	35.7^{E}	38.5-^{F}	40.4^{F}	41.1^{F}
Matekpa	2.2^{AB}	3.1^{AB}	4.2^{A}	5.0^{A}	5.5^{A}	6.0^{A}	7.6^{AB}	8.8^{AB}	9.5^{B}	10.2^{B}	10.6^{B}
Zimbu	6.5^{D}	9.25^{D}	11.5^{C}	13.3^{CD}	14.6^{DE}	16.0^{D}	22.3^{CD}	26.3^{CD}	28.0^{CDE}	29.0^{CDE}	29.3^{CDE}
Baggar	2.8^{AB}	4.6^{BC}	7.4^{B}	10.4^{B}	13.3^{CD}	16.0^{D}	25.3^{DE}	28.5^{D}	29.9^{E}	31.4^{E}	32.1^{E}
Ngek sing	5.6^{CD}	8.6^{D}	11.4^{C}	14.4^{D}	16.9^{EF}	18.7^{E}	24.9^{D}	27.8^{CD}	28.9^{DE}	29.6^{DE}	29.9^{DE}
SEM	0.240	0.314	0.373	0.454	0.512	0.529	0.837	0.994	1.05	1.09	1.10
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCDEF}Values with different superscripts in a column differ significantly (P<0.01).

Table 3. Fermentation constant and t½ (h) of tree leaves

Tree leaf	Potential gas production (ml/200 mg DM)	Rate constant (c)	Half time (t½, h)	Lag phase (h)	R Square
Blemkar	25.9^{C}	0.07^{C}	10.4^{AB}	1.3^{AB}	0.89
Phrengpa	12.2^{B}	0.08^{D}	8.3^{A}	2.8^{CD}	0.85
Maar	6.4^{A}	0.06^{B}	12.5^{CD}	2.9^{CD}	0.88
Maarma	27.6^{CD}	0.05^{A}	13.5^{CD}	2.4^{C}	0.95
Domkar	29.7^{DE}	0.05^{A}	14.2^{D}	3.5^{DE}	0.91
Matekpa	10.3^{B}	0.05^{A}	12.9^{CD}	1.6^{B}	0.82
Zimbu	29.6^{DE}	0.05^{A}	12.8^{CD}	4.1^{E}	0.92
Baggar	32.1^{F}	0.06^{B}	11.2^{BC}	0.69^{A}	0.93
Ngek sing	31.7^{EF}	0.08^{D}	9.1^{AB}	0.83^{A}	0.86
SEM	0.91	0.004	1.04	0.31	–
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01	–

^{ABCDEF}Values with different superscripts in a column differ significantly (P<0.01).

Table 4. Effect of tree leaves on ruminal gas and methane production (ml/200 mg) at t½ (h)

Tree leaf	Gas production (ml)	Gas production (ml/g DM)	Gas production (ml/g DDM)
Blemkar	14.7^{D}	83.1^{EF}	227.4^{E}
Phrengpa	6.7^{B}	37.6^{B}	109.2^{A}
Maar	4.1^{A}	22.4^{A}	101.3^{A}
Maarma	11.1^{C}	62.4^{C}	116.3^{AB}
Domkar	17.3^{E}	92.4^{F}	181.5^{D}
Matekpa	6.3^{B}	35.9^{B}	190.3^{D}
Zimbu	14.3^{D}	74.8^{DE}	130.5^{AB}
Baggar	12.3^{C}	67.4^{CD}	147.5^{BC}
Ngek sing	14.7^{D}	83.4^{EF}	167.7^{CD}
SEM	0.862	4.64	8.25
Level of significance	P<0.01	P<0.01	P<0.01

^{ABCDEF}Values with different superscripts in a column differ significantly (P<0.01).

determinant in inflicting substrate degradation and ensuring potential gas production. However, it was observed that Maar leaves contain highest amount of lignin (20.9%) hence, its potential gas production was also lowest. Likewise finding was also reported by Bhatt *et al*. (2014) with crop residue having varying content of CP and soluble carbohydrate. The half time i.e. t½ (h) was lowest for Phrengpa (8.3 h) and highest for Domkar leaves. Maximum lag phase was observed in case of Zimbu tree leaves (4.1 h). Furthermore, difference in half time and lag phase of the tested tree leaves might be due to presence of variable amount of anti-nutritional factors like saponins, tannins (Goel *et al.* 2008, Benchaar *et al*. 2008). In the present study, highest t½ (h) in Domkar leaves is a major factor for its slow degradability in comparison to other tree leaves.

In vitro *total gas, microbial biomass production and IVTDMD at t½ (h):* Total gas production (ml/200 mg) at t½ (h) showed significant (P<0.01) difference among the tree leaves (Table 4). Highest gas production was seen on supplementation of Blemkar (227.4 ml/g DMD) followed by Matekpa (190.3 ml/g DMD) and Domkar (181.5 ml/g DMD) leaves. However, lower gas production was recorded in case of Maar and Phrengpa leaves. Reduced gas volume might be due to higher ADF as well as lignin content of these leaves which in turn leads to decreased dry matter degradation resulting in lower gas production. Furthermore, lower gas production might also be due to high concentration of tannic acid and other polyphenolic compounds present in the tree leaves. Condensed tannins (CT) have been widely reported to decrease both rate and extent of gas production (Frutos *et al.* 2002). This suppressive effect probably being a result of reduction in attachment of microbes to feed particles (McAllister *et al*. 1994) or inhibition of ruminal microbial enzymes activity (Waghorn 1996, McSweeney *et al*. 2001).

Microbial biomass production at t½ (h) per unit digested dry matter was improved due to inclusion of Phrengpa, Maar and Maarma leaves (Table 5). IVTDMD was highest for Zimbu, Domkar, Maarma as their t½ (h) was lower, while

Table 5. Effect of tree leaves on microbial biomass production and dry matter degradation at t½ (h)

Tree leaf	Microbial biomass (mg)	Microbial biomass (mg/g DM)	Microbial biomass (mg/g DMD)	IVTDMD (%)	T-VFA (mM/dl)
Blemkar	32.3^{B}	182.7^{B}	499.7^{A}	36.5^{C}	4.5^{C}
Phrengpa	47.0^{C}	265.0^{C}	759.7^{E}	34.8^{C}	3.7^{B}
Maar	31.0^{B}	173.7^{B}	777.1^{E}	22.3^{B}	2.8^{A}
Maarma	70.5^{E}	399.8^{E}	744.2^{DE}	53.7^{F}	5.7^{E}
Domkar	57.5^{D}	306.5^{CD}	600.7^{B}	51.1^{EF}	5.2^{D}
Matekpa	19.4^{A}	109.8^{A}	581.4^{B}	18.9^{A}	3.5^{B}
Zimbu	78.4^{E}	408.8^{E}	713.0^{DE}	57.3^{G}	6.8^{F}
Baggar	56.4^{D}	308.1^{CD}	675.6^{CD}	45.6^{D}	4.7^{C}
Ngek sing	55.2^{D}	314.2^{D}	631.0^{BC}	49.8^{E}	5.1^{D}
SEM	3.62	19.2	18.2	2.6	0.228
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCDEFG}Values with different superscripts in a column differ significantly (P<0.01).

moderate IVTDMD was observed in case of Baggar and Ngek sing tree leaves. Minimum IVTDMD was found as a result of supplementation of Matekpa and Maar leaves as these tree leaves contained relatively high level of ADL resulting in lower *in vitro* degradation. The digestibility is dependent on the cell wall constituent (fibre), especially the NDF and lignin (Bakshi and Wadhwa 2004). Likewise observation was also reported by Camacho *et al.* (2010). Moreover, higher TVFA production was also observed in case of Zimbu, Domkar, Maarma, Baggar and Ngek sing leaves due to higher IVTDMD of these tree leaves. Positive correlation exists between IVTDMD and TVFA production of feeds (Valenciaga *et al*. 2009).

On the basis of chemical composition and *in vitro* rumen fermentation, it can be inferred that, Baggar and Ngek sing leaves had highest IVTDMD, TVFA and microbial biomass production, hence they may be used as potential livestock feed. Although, *in vivo* trial should be performed before further recommendation.

ACKNOWLEDGEMENT

The financial support in form of institutional fellowship to the senior author by NDRI is thankfully acknowledged.

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