new and more sustainable alternative strategy to rehabilitate the plantation workforce is also urgently needed.

*Conflict of interest:* The authors declare no conflict of interest. Views expressed are personal.


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# Potential of native forest leaves to modulate *in vitro* rumen fermentation and mitigate methane emission

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**Tree foliages rich in phytochemicals can be used as sustainable fodder for livestock to modulate rumen fermentation for cleaner and improved production. Samples of nine different forest tree leaves were collected from hilly regions of Arunachal Pradesh, India to study their effect on *in vitro* rumen fermentation and methane production. After 24 h of incubation, highest ($P < 0.05$) gas production (ml/g DM/24 h) was observed in *Symplocos racemosa* among the leaves. Methane production (ml/g DDM/24 h) was lowest ($P < 0.05$) in *Symplocos crataegoides* followed by *Berberis aristata* leaves, while *in vitro* true dry matter digestibility was highest ($P < 0.05$) for *Berberis aristata* leaves. In case of rumen fermentation attributes, *B. aristata* and *S. crataegoides* produced maximum volatile fatty acid and microbial biomass amongst other screened leaves. Therefore, these leaves can be used as a fodder supplement to address feed scarcity and reduce methanogenesis in ruminants of the North East hilly regions of India.**

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INDIAN livestock accounts for 11% of worldwide enteric methane ($CH_4$) emissions[1]. Apart from being a potent environmental pollutant, $CH_4$ production causes a considerable feed energy (2–12% of gross energy) in ruminants, which could otherwise be used for productive purposes[2]. Dietary intervention modestly abates enteric methane emissions in ruminants. However, the availability of poor-quality roughages in tropical countries further adds to increased enteric methane emissions[3]. Scarcity of fodder not only has an adverse effect on livestock productivity, but it becomes challenging for farmers to fulfil the maintenance requirements of livestock. Sustainable feeding practices using agroforestry plants with high nutritional value can replace costlier feed ingredients, reduce food feed competition and lower greenhouse gas emissions in ruminants of the North East hilly regions of India[4]. Tree foliages are a promising source of proteins, minerals and vitamins which can fulfil the nutritional inadequacy of poor-quality feed resources[5,6]. In addition to valuable nutrients, these foliages are endowed with different types of phytochemicals or plant bioactive compounds which can help in combating increased methane emissions produced from feeding of low-quality roughages[7]. Plant bioactives such as essential oils, saponins, tannins, flavonoids, etc. modulate rumen fermentation and the microbial population which lowers enteric $CH_4$ emissions[8]. Choudhary *et al.*[4] observed in an *in vitro* study that leaves of *Spiraea canescens* and *Lingustrum myrsinites* produced a positive effect on nutrient digestibility which can aid in boosting livestock productivity. Supplementing animals with tree foliages either fresh or as leaf meal in low-quality roughage diets has been found to improve nutrient utilization and reduce production costs[9]. Adoption of selected plants species increased ruminant productivity, decreased pasture time and minimized methane emissions[10]. Bhatt *et al.*[3] found that plant secondary metabolites found in *Blepharis scindica* and *Trigonella foenum-graecum* roughages in the form of feed blocks lowered methane production by 49.3% and 26.8% respectively. However, till date scarce literature is available regarding the nutritional worth of forest tree leaves as livestock feed. Holistic screening of these agroforestry plants would be beneficial for deciding their inclusion level in ruminant diets. Therefore, the present study was planned to evaluate *in vitro* methanogenesis and rumen fermentation potential of tree leaves widely distributed in hills of Arunachal Pradesh, India, so as to identify novel phytogenic feed for environment-friendly ruminant production in these regions.

The tree leaves used in this analysis were collected from the study area of Arunachal Pradesh. Each leaf was collected twice from separate trees ranging in height from 6 to 12 ft, mixed properly, and dried in a hot-air oven at 50°C for 72 h. A hammer mill was used to grind the dried tree leaves, which were then sieved at 1 mm, for further laboratory analysis and *in vitro* tests.

Rumen liquor for *in vitro* gas production technique was obtained from Jersey crossbred calf that were stall-fed on a diet of paddy straw and concentrates in a 60 : 40 ratio was fed according to the National Research Council (NRC), USA[11]. To formulate the inoculums, rumen liquor from a donor animal was collected and combined with buffer in a 1 : 2 ratio[12]. In each 100 ml syringe, 30 ml of incubation medium was administered anaerobically for *in vitro* experiments.

In 100 ml calibrated glass syringes, 200 + 10 mg air-equilibrated tree leaves were incubated with 30 ml buffered rumen inoculum (10 ml rumen fluid + 20 ml buffer)[12] and put in a water bath maintained at 39°C for 24 h. Each sample was incubated in triplicate, and each sample was incubated three times.

After 24 h of incubation, the gas produced due to substrate fermentation was determined by subtracting the gas generated in the blank syringe (which had no substrate but only inoculum) from the total gas produced in the syringe containing substrate and inoculum. For methane analysis, 1 ml gas was injected into a gas chromatograph (NetelUltima-2100) with a stainless steel column filled with Porapak-Q from the headspace of a syringe in an airtight syringe (Hamilton). The estimated methane percentage in the gas sample was used to calculate methane production (methane volume (ml) = methane per cent × total gas produced (ml) in 24 h)[13].

Total volatile fatty acid (TVFA) in rumen liquor (incubation medium after 24 h of incubation) was measured according to Barnett and Reid[14]. Volatile fatty acid (VFA) fractionation method of Cottyn and Boucque[15] was used. Samples were processed for rumen enzyme estimation as described in the literature[16,17]. For estimation of carboxymethyl cellulase, xylanase and amylase enzymes, the substrates used were carboxymethyl cellulose, xylan and starch respectively. The reducing sugars thus produced were estimated as monosaccharides by the dinitrosalicylic acid method[18] and $\beta$-glucosidase analysed according to Shewale and Sadana[19]. Microbial biomass was calculated as explained by Blummel *et al.*[20]. The pellets collected after separation were refluxed with neutral detergent solution for a hour, passed through G1 crucibles and the residue was left to dry in oven to calculate the *in vitro* true dry matter digestibility (IVTDMD), and true dry matter digestibility was measured by loss in weight. The total digestible nutrition (TDN) was estimated using the equation proposed by Krishnamoorthy *et al.*[21] and the metabolizable energy (ME) content of tree leaves according to the NRC, USA[22]. Ciliates were identified using the method proposed by Hungate[23], while total and differential protozoal numbers were determined as described by Kamra *et al.*[24].

The experimental data generated by different tree leaves in an *in vitro* gas production test were analysed

**Table 1.** Effect of tree leaves on ruminal gas, methane and total volatile fatty acid (TVFA) production (ml/200 mg) *in vitro*

| Tree | Gas production (ml/g DM/24 h) | Gas production (ml/g DDM/24h) | $CH_4$ production (ml/g DM/ 24 h) | $CH_4$ production (ml/g DDM/24 h) | TVFA (mM/dl) | Acetate (%) | Propionate (%) | Butyrate (%) | Acetate: propionate |
|---|---|---|---|---|---|---|---|---|---|
| *Buddleja asiatica* | $129.8^{B}$ | $234.3^{AB}$ | $25.9^{CD}$ | $46.7^{A}$ | $7.2^{BC}$ | $63.6^{B}$ | $22.0^{C}$ | $14.4^{A}$ | $2.9^{A}$ |
| *Quercus walliasehiana* | $72.1^{A}$ | $301.6^{BCD}$ | $15.1^{B}$ | $63.1^{C}$ | $6.9^{BC}$ | $62.8^{AB}$ | $20.0^{A}$ | $17.2^{E}$ | $3.1^{B}$ |
| *Costanpsis indica* | $39.9^{A}$ | $304.6^{BCD}$ | $8.6^{A}$ | $65.6^{C}$ | $5.4^{A}$ | $63.8^{B}$ | $20.0^{A}$ | $16.2^{CDE}$ | $3.2^{B}$ |
| *Spiraea canescens* | $132.4^{B}$ | $326.1^{D}$ | $25.3^{CD}$ | $62.3^{C}$ | $6.5^{ABC}$ | $63.8^{B}$ | $20.0^{A}$ | $16.2^{CDE}$ | $3.2^{B}$ |
| *Symplocos racemosa* | $186.4^{C}$ | $320.8^{CD}$ | $37.6^{E}$ | $64.5^{C}$ | $7.3^{C}$ | $64.0^{B}$ | $20.2^{A}$ | $15.8^{BCD}$ | $3.2^{B}$ |
| *Quercus fenestrate* | $46.5^{A}$ | $292.4^{BCD}$ | $9.7^{A}$ | $61.3^{BC}$ | $5.8^{AB}$ | $63.2^{B}$ | $20.0^{A}$ | $17.0^{DE}$ | $3.2^{B}$ |
| *Lingustrm myrsinites* | $126.9^{B}$ | $247.1^{ABC}$ | $24.6^{C}$ | $47.9^{AB}$ | $6.7^{ABC}$ | $65.7^{C}$ | $19.5^{A}$ | $14.8^{AB}$ | $3.0^{B}$ |
| *Berberis aristata* | $144.4^{BC}$ | $238.5^{AB}$ | $28.2^{D}$ | $46.6^{A}$ | $7.6^{C}$ | $61.7^{A}$ | $22.7^{C}$ | $15.7^{BC}$ | $2.7^{A}$ |
| *Symplocos cratagoides* | $127.9^{B}$ | $214.0^{A}$ | $27.4^{CD}$ | $45.9^{A}$ | $7.5^{C}$ | $63.8^{B}$ | $21.0^{B}$ | $15.2^{ABC}$ | $3.0^{B}$ |
| SEM | 6.42 | 8.12 | 1.25 | 1.71 | 0.180 | 0.178 | 0.151 | 0.156 | 0.028 |
| Level of significance | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.05$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ |

$^{ABCDE}$Values with different superscripts in a column differ significantly ($P < 0.01$).
DM, Dry matter; DDM, Digested dry matter; SEM, Standard error of means.

using a simple one-way ANOVA employing SPSS 14.0 (2005). Duncan's multiple range test was used to compare the means, and significant values were defined as those with a probability of less than 0.05.

Table 1 presents results of the effect of different tree leaves on *in vitro* gas and methane production for 24 h incubation. Highest ($P < 0.01$) gas production (ml/g DM/24 h) was observed for *Symplocos racemosa*, which was 77% higher compared to *Costanpsis indica* leaves. However, gas production in terms of digested dry matter was highest ($P < 0.01$) for *S. canescens* and *S. racemosa*. Methane production (ml/g DM/24 h) was lowest ($P < 0.01$) for *C. indica* followed by *Quercus fenestrate* leaves. *In vitro* methane per unit of digested dry matter was lowest ($P < 0.01$) for *Symplocos crataegoides*, *Berberis aristata* and *Buddleja asiatica* leaves.

TVFA production was higher ($P < 0.05$) for *B. aristata*, *S. crataegoides*, *S. racemosa* and *B. asiatica* leaves; it varied from 5.4 to 7.6 mM/dl (Table 1). Propionate production was 14.1% higher ($P < 0.01$) due to incubation of *B. aristata* leaves in comparison to *L. myrsinites* leaves. Acetate to propionate ratio ($P < 0.01$) was lowest for *B. aristata*, followed by *B. asiatica* and *S. crataegoides* leaves.

Cellulase enzyme activity (μmol/ml/h) was highest ($P < 0.01$) for *B. aristata* leaves, followed by *Q. fenestrate*, *S. canescens* and *S. cratagoides* among the screened tree leaves (Table 2). Similarly, activities of xylanase and $\beta$-glucosidase enzymes were higher ($P < 0.01$) for *B. aristata* leaves compared to the others. However, no significant effect was observed on amylase activity among the tree leaves.

Table 3 presents results of the effect of different tree leaves on rumen protozoal count ($\times 10^4$/ml). Lowest ($P < 0.01$) population of holotrich, spirotrich and total protozoa was observed for *B. aristata* leaves. Total rumen protozoal count was 48.6% lower for *B. aristata* than the other tree leaves. Highest number of holotrichs was observed for *S. racemosa* followed by *L. myrsinites* leaves.

Microbial biomass production (mg/g DM) was highest with the incubation of *S. crataegoides* and *B. aristata* leaves, and it was lowest for *C. indica* among the other screened leaves (Table 3). Microbial biomass production (mg/g DM) was 82.8% higher with the addition of *S. crataegoides* leaves. IVTDMD was highest ($P < 0.01$) for *B. aristata* (60.6%) followed by *S. cratagoides* (59.7%) leaves (Table 3). Lowest IVTDMD (%) was observed for *C. indica* followed by *Q. fenestrate* and *Q. walliasehiana* leaves. TDN content of tree leaves varied from 40.4% to 63.7% on DM basis, while ME content varied from 1.3 to 2.4 Mcal/kg DM (Table 3). The ME and TDN values were highest for *B. aristata* and *S. cratagoides* leaves.

The chemical composition of the screened leaves is discussed in the companion study[4]. The increase in gas production (mg/g DM) in *S. racemosa*, *B. aristata* and *S. canescens* leaves might be partly due to more availability of organic matter for fermentation or presence of soluble sugars[25,26]. Lower gas production in case of *C. indica* and *Q. fenestrate* leaves might be due to higher acid detergent fibre and lignin content, which result in lower dry matter degradation. Interestingly, there was low methane production by tree leaves having low tannin content, which might be due to the presence of other plant secondary metabolites such as saponins, flavanoids, and essential oils that play a synergistic role in decreasing methane production[27–30]. Furthermore, samples containing both hydrolysable and condensed tannins are more successful in reducing total gas and methane production than those containing only hydrolysable tannins[31]. *S. crataegoides* and *B. aristata* leaves contained high ether extract content, i.e. 6.4% and 3.4% respectively[4]. Therefore, low methane production in these leaves may be related to their fat content. Lee *et al.*[32] reported that methane production from feedstuff is negatively correlated with ether

**Table 2.** Effect of tree leaves on rumen enzyme activity (μmol/ml/h) *in vitro*

| Tree | Enzyme activity | | | |
|---|---|---|---|---|
| | Carboxymethyl-cellulase | Xylanase | Amylase | β-glucosidase |
| *B. asiatica* | $2.88^{AB}$ | $6.28^{B}$ | 13.68 | $0.332^{ABC}$ |
| *Q. walliasehiana* | $3.39^{BCD}$ | $7.32^{B}$ | 17.55 | $0.316^{ABC}$ |
| *C. indica* | $3.03^{BC}$ | $5.94^{B}$ | 17.28 | $0.363^{ABC}$ |
| *S. canescens* | $3.85^{DE}$ | $6.49^{B}$ | 17.98 | $0.463^{C}$ |
| *S. racemosa* | $2.25^{A}$ | $4.04^{A}$ | 13.18 | $0.308^{ABC}$ |
| *Q. fenestrate* | $3.88^{DE}$ | $6.58^{B}$ | 15.63 | $0.294^{AB}$ |
| *L. myrsinites* | $2.79^{AB}$ | $6.32^{B}$ | 12.10 | $0.211^{A}$ |
| *B. aristata* | $4.44^{E}$ | $7.22^{B}$ | 19.60 | $0.448^{BC}$ |
| *S. crataegoides* | $3.64^{CD}$ | $6.18^{B}$ | 16.78 | $0.350^{ABC}$ |
| SEM | 0.105 | 0.173 | 0.682 | 0.016 |
| Level of significance | $P < 0.01$ | $P < 0.01$ | NS | $P < 0.01$ |

$^{ABCDE}$Values with different superscripts in a column differ significantly ($P < 0.01$); NS, Non significant.

**Table 3.** Effect of tree leaves on microbial biomass (MB) production, dry matter degradation and rumen protozoal number ($\times 10^4$/ml) *in vitro*

| Tree | MB (mg/g DM) | MB (mg/g DDM) | IVTDMD% | TDN | ME (Mcal/kg DM) | Holotrich | Spirotrich | Total protozoa |
|---|---|---|---|---|---|---|---|---|
| *B. asiatica* | $120.7^{C}$ | $217.8^{A}$ | $55.4^{E}$ | $50.1^{C}$ | $1.8^{C}$ | $0.35^{BC}$ | $11.6^{C}$ | $11.95^{B}$ |
| *Q. walliasehiana* | $93.8^{BC}$ | $386.8^{B}$ | $24.1^{B}$ | $53.6^{D}$ | $1.9^{D}$ | $0.31^{BC}$ | $10.1^{BC}$ | $10.41^{B}$ |
| *C. indica* | $54.2^{A}$ | $413.2^{BC}$ | $13.1^{A}$ | $41.3^{A}$ | $1.4^{A}$ | $0.29^{BC}$ | $10.8^{BC}$ | $11.09^{B}$ |
| *S. canescens* | $262.5^{EF}$ | $646.5^{E}$ | $40.6^{C}$ | $55.5^{D}$ | $2.0^{D}$ | $0.28^{BC}$ | $11.7^{C}$ | $11.98^{B}$ |
| *S. racemosa* | $173.6^{D}$ | $297.7^{A}$ | $58.3^{EF}$ | $62.3^{E}$ | $2.3^{E}$ | $0.63^{D}$ | $9.6^{BC}$ | $10.23^{B}$ |
| *Q. fenestrate* | $68.4^{AB}$ | $430.1^{BC}$ | $15.9^{A}$ | $40.4^{A}$ | $1.3^{A}$ | $0.33^{BC}$ | $10.0^{BC}$ | $10.33^{B}$ |
| *L. myrsinites* | $234.5^{E}$ | $456.4^{BCD}$ | $51.4^{D}$ | $46.4^{B}$ | $1.6^{B}$ | $0.53^{CD}$ | $8.8^{B}$ | $9.33^{B}$ |
| *B. aristata* | $287.9^{FG}$ | $475.3^{CD}$ | $60.6^{F}$ | $63.7^{E}$ | $2.4^{E}$ | $0.06^{A}$ | $6.1^{A}$ | $6.16^{A}$ |
| *S. crataegoides* | $315.8^{G}$ | $529.2^{D}$ | $59.7^{F}$ | $54.2^{D}$ | $1.9^{D}$ | $0.47^{BCD}$ | $10.2^{BC}$ | $10.67^{B}$ |
| SEM | 13.2 | 12.6 | 2.54 | 1.09 | 0.048 | 0.028 | 0.31 | 0.317 |
| Level of significance | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ | $P < 0.01$ |

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

IVTDMD, *In vitro* true dry matter digestibility; TDN, Total digestible nutrients; ME, Metabolizable energy.

extract content, because ether extract fractions are mostly not fermented by rumen microbes and unsaturated fatty acids are toxic to methanogenic bacteria.

In the present study, *B. aristata*, *S. crataegoides*, *S. racemosa* and *B. asiatica* leaves had higher TVFA production. VFA production has a strong positive relationship with dry matter digestibility[33], and it is also in accordance with the present findings. Increased propionic acid production with the incubation of *B. aristata*, *B. asiatica* and *S. crataegoides* leaves was due to shift in rumen fermentation towards propionate at the expense of acetate production[34]. Moreover, reduced protozoal population with incubation of *B. aristata* is also associated with the increase in the proportion of propionic acid[35].

The lowest total protozoal count was observed with addition of *B. aristata* and *L. myrsinites* tree leaves, which indicates that methane gas production per unit digested dry matter was low for the leaves for these two trees. A reduction in protozoa count may be attributed to the presence of essential oils or saponins in these tree leaves, as these plant secondary metabolites show antiprotozoal activity[36,37]. However, the difference in response of rumen protozoa to saponins or essential oils might be due to different chemical and physical structure of saponins or essential oils in different leaves[38]. The population of holotrichs was increased in case of *S. racemosa* leaves, followed by *L. myrsinites.* Increased holotrich population might be due to higher content of soluble sugars in these leaves due to lower lag phase during degradation[4].

The activity of fibre-degrading enzymes, e.g. carboxymethyl cellulase, xylanase and β-glucosidase was highest in case of *B. aristata* leaves. Incubation of *B. aristata* leaves decreased the total protozoal population. Defaunated animals have higher bacterial and fungal populations, which are the main sources of fibrolytic enzymes in rumen[39]. Therefore, alteration in the rumen microbiota might have resulted in increased fibrolytic enzymes activity in case of *B. aristata* leaves.

Microbial biomass (mg/g DM) production was maximum with incubation of *B. aristata* and *S. crataegoides* leaves. In the present study, lower methane production in *B. aristata* and *S. crataegoides* leaves promoted microbial anabolism and synthesis of microbial biomass due to increased availablity of $H^+$ ions from reduced methanogenesis[40].

Highest *in vitro* dry matter digestibility was found with *B. aristata* and *S. cratagoides* leaves. Both the leaves increased activity of fibre degrading enzymes such as cellulase, $\beta$-glucosidase and xylanase, which resulted in higher digestibility. *C. indica*, *Q. fenestrate* and *Q. walliasehiana* leaves had higher content of lignin and tannin[4] which consequently lowered dry matter digestibility. In the present study, the dry matter digestibility range of different tree leaves is comparable to that reported by Datt *et al.*[41]. Likewise, the ME value was also highest for *B. aristata* followed by *S. racemosa* leaves, depicting a positive correlation between ME value and dry matter digestibility. In the present study, *Q. fenestrate* and *C. indica* leaves had lower ME value due to high lignin content in these leaves. Lignin causes decrease in digestibility, which reduces gas production and results in low ME value of the feeds[42]. TDN and ME content of these leaves were similar to those reported by Gupta *et al.*[5].

Based on the present findings, it can be concluded that among the screened tree leaves, *B. aristata* and *S. cratagoides* showed higher fibrolytic enzymatic activity, energy content, dry matter digestibility and volatile fatty acid production along with reduced methane production. Hence, these two tree foliages can be included in the feeding module of livestock reared in the North East hilly regions of Arunachal Pradesh, to mitigate methane emission and address feed scarcity. However, *in vivo* studies should be carried out to determine the effect of the selected tree leaves on animal performance and its association with the rumen environment before practical recommendations.


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# Relationship between Cerambyciid borer (Insecta: Coleoptera) infestation and human-induced biotic interferences causing mortality of kharsu (*Quercus semecarpifolia* Smith in Rees) oak trees in Garhwal, Western Himalaya, India

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**Stem and wood boring beetles significantly damage kharsu oak trees leading to their mortality and decline in the Garhwal region of Western Himalaya, India. The relationship established between the prevalent biotic factors (extensive lopping and grazing) and the degree of borer infestation in Chakrata hills, Uttarakhand, revealed a strong correlation between the two. Density–girth class relationship in borer-infested oak stands revealed a higher degree of past disturbance compared to uninfested oak stands, with maximum infestation in girth class 61–80 cm and between 2601 and 2700 msl.**

**Keywords:** Biotic interference, oak, stand composition, stem and wood borer, tree density.

OAKS are the dominant climax tree species of the moist temperate forests ecosystem in the Indian Himalayan region[1], where over 35 species of oak are reported spread along an elevation gradient of 800–3800 m amsl (ref. 2). Five species of evergreen oak, namely *Quercus leucotrichophora* (banj), *Quercus floribunda* (moru), *Quercus semecarpifolia* (kharsu), *Quercus glauca* (phaliyant/harimj) and *Quercus lanuginosa* (riyanj) grow naturally in the Western Himalaya[1], of which *Q. semecarpifolia*

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