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

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1. Chakraborthy, K., Sudhakar, S., Sarma, K. K., Raja, P. L. N. and Das, A. K., Recognizing the rapid expansion of rubber plantation—a threat to native forests in parts of northeast India. Curr. Sci., 2018, 114(1), 207–213.
2. Reddy, C. S., Jha, C. S. and Dadhwal, V. K., Assessment and monitoring of long-term forest cover changes (1920–2013) in Western Ghats biodiversity hotspot. J. Earth Syst. Sci., 2016, 125, 103–114.
3. Thomas, S. and Jacob, J., The Gadgil–Kasturirangan reports on Western Ghats and concerns of the plantation sector. Rubber Sci., 2013, 26, 167–174.
4. Jha, C. S., Dutt, C. B. S. and Bawa, K. S., Deforestation and land use changes in Western Ghats, India. Curr. Sci., 2000, 79(2), 231–238.
5. Satheesan, V., Jayakumar, M., Varadan, M. and Unni, N., Effect of conversion of natural forests into plantation crops and water use in the Western Ghats. In Proceedings of the Fifth Kerala Science Congress, Kottayam, 28–30 January 1993, pp. 71–73.
6. George, B., Varkey, J. K., Thomas, K. T., Jacob, J. and Mathew, N. M., Carbon dioxide emission reduction through substituting synthetic elastomers with natural rubber. In Kyoto Protocol and the Rubber Industry (eds Jacob, J. and Mathew, N. M.), Rubber Research Institute of India, Kottayam, 2006, pp. 133–146.
7. Jacob, J., Rubber tree, man and environment. In Natural Rubber: Agromanagement and Crop Processing (eds George, P. J. and Jacob, C. K.), Rubber Research Institute of Kottayam, India, 2000, pp. 599–610.
8. Jacob, J., Forestry and plantations: opportunities under the Kyoto Protocol. Econ. Polit. Wkly, 2005, 60, 2043–2045.
9. Raj, S. and Jacob, J., Identification of agro-climatically suitable areas for natural rubber cultivation in left wing extremism affected states. Rubber Sci., 2018, 31(2), 152–163.
10. Kumar, B. M., Land use in Kerala: changing scenarios and shifting paradigms. J. Trop. Agric., 2005, 42(1–2), 1–12.
11. Joseph, J. and Jacob, J., Over-dependence of Indian rubber industry on imported natural rubber of long-term sustainability. Rubber Sci., 2018, 31(1), 1–9.
12. Narasimhan, T. E., Natural rubber production declines, demand-supply gap rises to 45%. Business Standard; https://www.business-standard.com/article/economy-police/natural-rubber-production-declines-demand-supply-gap-rises-to-45-119031300213_1.html (accessed on 26 November 2021).
13. Ahrends, A., Hollingsworth, P. M., Ziegler, A. D., Fox, J. M., Chen, H., Su, Y. and Xu, J., Current trends of rubber plantation expansion may threaten biodiversity and livelihoods. Global Environ. Change, 2015, 34, 48–58.
14. Chen, H., Yi, Z.-F., Schmidt-Vogt, D., Ahrends, A., Beckschäfer, P. and Klein, C., Pushing the limits: the pattern and dynamics of rubber monoculture expansion in Xishuangbanna, SW China. PLoS ONE, 2016, 11(2), 1–15.
15. Fox, J. M., Castella, J. C., Ziegler, A. D. and Westlay, S. B., Rubber plantation expands in mountainous southeast Asia: what are the consequences for the environment? Asia Pacific Issues, East-West Centre, 2014, vol. 114, pp. 1–8.
16. Roy, M., Saha, A. and Roy, M., Ecological impact of rubber plantations: Tripura perspective. Int. J. Curr. Res., 2014, 2(11), 10334–10340.
17. Mazumdar, A., Datta, S., Choudhary, B. K. and Mazumdar, K., Do extensive rubber plantation influence local environment? a case study from Tripura, Northeast India. Curr. World Environ., 2014, 9(3), 768–779.
18. Gupta, N., Rajvanshi, A. and Badola, R., Climate change and human–wildlife conflicts in the Indian Himalayan biodiversity hotspot. Curr. Sci., 2017, 113(5), 846–847.
19. Kale, M. P., Tahukdar, G., Panigrahy, R. K. and Singh, S., Patterns of fragmentation and identification of possible corridors in northern Western Ghats. J. Indian Soc. Remote Sensing, 2010, 38, 401–413.
20. Tijskens, S., Weed management on a rubber plantation with special reference to minimum tillage cultivation. In JIRCAS International Symposium, Japan, Series No. 4, 1996, pp. 65–75.

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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 (CH₄) emissions⁴. Apart from being a potent environmental pollutant, CH₄ production causes a considerable feed energy (2–12% of gross energy) in ruminants, which could otherwise be used for productive purposes⁵. 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 emissions⁶. Scarcity of fodder not only has an adverse effect on livestock productivity, but it becomes challenging for farmers to fulfill 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 India⁷. Tree foliages are a promising source of proteins, minerals and vitamins which can fulfill the nutritional inadequacy of poor-quality feed resources⁸,⁹. 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 roughages⁸. Plant bioactives such as essential oils, saponins, tannins, flavonoids, etc. modulate rumen fermentation and the microbial population which lowers enteric CH₄ emissions⁶. 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 costs⁹. Adoption of selected plant species increased ruminant productivity, decreased pasture time and minimized methane emissions⁹,¹⁰. Bhattacharyya et al.¹⁰ 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 and was fed according to the National Research Council (NRC), USA¹¹. To formulate the inoculums, rumen liquor from a donor animal was collected and combined with buffer in a 1:2 ratio¹². 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 air-tight 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 Reid¹⁴. Volatile fatty acid (VFA) fractionation method of Cottyn and Bouquè⁵ was used. Samples were processed for rumen enzyme estimation as described in the literature¹⁶,¹⁷. 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 method¹⁸ and β-glucosidase analysed according to Shewale and Sadana¹⁹. 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, USA²². Ciliates were identified using the method proposed by Hungate²³, 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.
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 Symlocos 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 Symlocos 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. fenestrare, S. canescens and S. crataegoides among the screened tree leaves (Table 2). Similarly, activities of xylanase and β-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 ($\times 10^7$/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. crataegoides (59.7%) leaves (Table 3). Lowest IVTDMD (%) was observed for C. indica followed by Q. fenestrare and Q. wallisiahenea 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. crataegoides leaves.

The chemical composition of the screened leaves is discussed in the companion study. 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. Lower gas production in case of C. indica and Q. fenestrare 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. Furthermore, samples containing both hydrolysable and condensed tannins are more successful in reducing total gas and methane production than those containing only hydrolysable tannins. S. crataegoides and B. aristata leaves contained high ether extract content, i.e. 6.4% and 3.4% respectively. Therefore, low methane production in these leaves may be related to their fat content. Lee et al. reported that methane production from feedstuff is negatively correlated with ether

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/24 h) | CH₄ production (ml/g DM/24 h) | CH₄ production (ml/g DDM/24 h) | TVFA (mM/dl) | Acetate (%) | Propionate (%) | Butyrate (%) | Acetate:propionate |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------|-------------|----------------|--------------|------------------|
| Buddleja asiatica          | 129.8A                        | 234.3AB                       | 25.9CDB                      | 46.7A                         | 7.2B         | 63.6B       | 22.0E           | 14.4A        | 2.9A              |
| Quercus wallisiahenea       | 72.1A                         | 301.6BCD                      | 15.1B                        | 63.1C                         | 6.0B         | 62.8AB      | 20.0E           | 17.2E        | 3.1B              |
| Costanpsis indica           | 39.9A                         | 304.6BCD                      | 8.6A                         | 65.6B                         | 5.4A         | 63.8B       | 20.0E           | 16.2D        | 3.2B              |
| Spiraee caneascens          | 132.4B                        | 326.1D                        | 25.3BCD                      | 62.3B                         | 6.5B         | 63.8AB      | 20.0E           | 16.2D        | 3.2B              |
| Symlocos racemosa           | 186.4C                        | 320.8CD                       | 37.6BCD                      | 64.5C                         | 7.3F         | 64.0B       | 20.2E           | 15.8B        | 3.2B              |
| Quercus fenestrate          | 46.5A                         | 292.4BCD                      | 9.7A                         | 61.3BC                         | 5.8AB        | 63.2B       | 20.0E           | 17.0B        | 3.2B              |
| Lingustrum myrsinites       | 126.9B                        | 247.1ABC                      | 24.6C                        | 47.9AB                         | 6.7B         | 65.7C       | 19.5A           | 14.8B        | 3.0B              |
| Berberis aristata           | 144.4BC                       | 238.5AB                       | 28.2D                        | 46.6A                         | 7.6C         | 61.7A       | 22.7E           | 15.2B        | 2.7A              |
| Symlocos crataegoides       | 127.9B                        | 214.0A                        | 27.4CD                       | 45.9A                         | 7.5C         | 63.8B       | 21.0B           | 15.2AB       | 3.0B              |
| SEM                         | 6.42                          | 8.12                          | 1.25                         | 1.71                          | 0.180        | 0.178       | 0.151           | 0.156        | 0.028             |

**Level of significance**: $P < 0.01$ for $P < 0.01$ for $P < 0.01$ for $P < 0.01$ for $P < 0.01$; $P < 0.05$ for $P < 0.05$ for $P < 0.05$ for $P < 0.05$ for $P < 0.05$; SEM, Standard error of means.
In the present study, acids are toxic to methanogenic bacteria. Not fermented by rumen microbes and unsaturated fatty extract content, because ether extract fractions are mostly

| Tree                  | Carboxymethyl-cellulase | Xylanase | Amylase | β-glucosidase |
|-----------------------|-------------------------|----------|---------|--------------|
| B. asiatica           | 2.88<sup>AB</sup>       | 6.28<sup>B</sup> | 13.68   | 0.332<sup>ABC</sup> |
| Q. wallisaeathiana    | 3.39<sup>BCD</sup>      | 7.32<sup>B</sup> | 17.55   | 0.316<sup>B</sup>  |
| C. indica             | 3.03<sup>BC</sup>       | 5.94<sup>B</sup> | 17.28   | 0.363<sup>ABC</sup>|
| S. canescens          | 3.85<sup>BE</sup>       | 6.49<sup>B</sup> | 17.98   | 0.463<sup>D</sup>  |
| S. racemosa           | 2.25<sup>B</sup>        | 4.04<sup>B</sup> | 13.18   | 0.308<sup>ABC</sup>|
| Q. fenestrate         | 3.88<sup>BE</sup>       | 6.58<sup>B</sup> | 15.63   | 0.294<sup>AB</sup> |
| L. myrionsites        | 2.79<sup>AB</sup>       | 6.32<sup>B</sup> | 12.10   | 0.211<sup>A</sup>  |
| B. aristata           | 4.44<sup>E</sup>        | 7.22<sup>B</sup> | 19.60   | 0.448<sup>BC</sup> |
| S. crataegoides       | 3.64<sup>CD</sup>       | 6.18<sup>B</sup> | 16.78   | 0.350<sup>ABC</sup>|
| SEM                   | 0.105                   | 0.173     | 0.682   | 0.016         |

Level of significance: P < 0.01

Table 3. Effect of tree leaves on rumen enzyme activity (μmol/ml/h) in vitro

| Tree                  | MB (mg/g DM) | MB (mg/g DDM) | IVTDMD% | TTN | ME (Mcal/kg DM) | Holotrich | Spirotrich | Total protozoa |
|-----------------------|--------------|---------------|---------|-----|----------------|-----------|------------|----------------|
| B. asiatica           | 120.7<sup>C</sup> | 217.8<sup>B</sup> | 55.4<sup>B</sup> | 50.1<sup>C</sup> | 1.8<sup>F</sup> | 0.35<sup>B</sup> | 11.6<sup>C</sup> | 11.95<sup>B</sup> |
| Q. wallisaeathiana    | 93.8<sup>BC</sup>  | 386.8<sup>B</sup> | 24.1<sup>B</sup> | 53.6<sup>B</sup> | 1.9<sup>D</sup>  | 0.31<sup>B</sup> | 10.1<sup>B</sup> | 10.41<sup>B</sup> |
| C. indica             | 54.2<sup>A</sup>   | 413.2<sup>BE</sup> | 13.1<sup>F</sup> | 41.3<sup>A</sup> | 1.4<sup>A</sup>  | 0.29<sup>B</sup> | 10.8<sup>B</sup> | 10.11<sup>B</sup> |
| S. canescens          | 262.5<sup>EF</sup> | 646.5<sup>F</sup> | 40.6<sup>F</sup> | 55.5<sup>D</sup> | 2.0<sup>D</sup>  | 0.28<sup>B</sup> | 11.7<sup>B</sup> | 11.98<sup>B</sup> |
| S. racemosa           | 173.6<sup>D</sup>  | 297.7<sup>B</sup> | 58.3<sup>EF</sup> | 62.3<sup>E</sup> | 2.3<sup>E</sup>  | 0.63<sup>B</sup> | 9.6<sup>BC</sup> | 10.23<sup>B</sup> |
| Q. fenestrate         | 68.4<sup>AB</sup>  | 430.1<sup>BC</sup> | 15.9<sup>A</sup> | 40.4<sup>A</sup> | 1.3<sup>A</sup>  | 0.33<sup>B</sup> | 10.0<sup>B</sup> | 10.33<sup>B</sup> |
| L. myrionsites        | 234.5<sup>B</sup>  | 456.4<sup>CD</sup> | 51.4<sup>D</sup> | 46.4<sup>B</sup> | 1.6<sup>B</sup>  | 0.53<sup>CD</sup> | 8.8<sup>B</sup>  | 9.33<sup>B</sup>  |
| B. aristata           | 287.9<sup>D</sup>  | 475.3<sup>EF</sup> | 60.6<sup>B</sup> | 63.7<sup>EF</sup> | 2.4<sup>F</sup>  | 0.06<sup>A</sup> | 6.1<sup>B</sup>  | 6.16<sup>A</sup>  |
| S. crataegoides       | 315.8<sup>B</sup>  | 529.2<sup>CD</sup> | 59.7<sup>B</sup> | 54.2<sup>B</sup> | 1.9<sup>D</sup>  | 0.47<sup>CD</sup> | 10.2<sup>C</sup> | 10.67<sup>B</sup> |
| SEM                   | 13.2          | 12.6         | 2.54         | 1.09         | 0.048        | 0.028         | 0.31         | 0.317       |

Level of significance: P < 0.01

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 digestibility31, 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 production34. Moreover, reduced protozoal population with incubation of B. aristata is also associated with the increase in the proportion of propionic acid35.

The lowest total protozoal count was observed with addition of B. aristata and L. myrionsites 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 protozoal count may be attributed to the presence of essential oils or saponins in these tree leaves, as these plant secondary metabolites show anti-protozoal activity36,37. 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 leaves38. The population of holotrichs was increased in case of S. racemosa leaves, followed by L. myrionsites. Increased holotrich population might be due to higher content of soluble sugars in these leaves due to lower lag phase during degradation4.

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. Defecated animals have higher bacterial and fungal populations, which are the main sources of fibrolytic enzymes in rumen39. 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 availability of H+ ions from reduced methanogenesis40.
 Highest in vitro dry matter digestibility was found with *B. aristata* and *S. crataegoides* leaves. Both the leaves increased activity of fibre degrading enzymes such as cellulase, β-glucosidase and xylanase, which resulted in higher digestibility. *C. indica*, *Q. fenestrate* and *Q. wallisii* leaves had higher content of lignin and tannin 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. 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. TDN and ME content of these leaves were similar to those reported by Gupta et al.

Based on the present findings, it can be concluded that among the screened tree leaves, *B. aristata* and *S. crataegoides* 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.

1. Malik, P. K., Kolte, A. P., Dhali, A., Sejain, V., Thirumalaisamy, G., Gupta, R. and Bhatt, R., GHG emissions from livestock: challenges and ameliorative measures to counter adversity. In Greenhouse Gases: Selected Case Studies (ed. Manning, A. J.), 2021, Rijeka, Croatia, 2016, pp. 1–16.
2. Patra, A. K., Min, B. R. and Saxena, J., Dietary tannins on microbial ecology of the gastrointestinal tract in ruminants. In Dietary Phytochemicals and Microbes, Vol. 237 (ed. Patra, A. K.), Springer, Dordrecht, The Netherlands, 2012, p. 62.
3. Bhatt, R. S., Sahoo, A., Sarkar, S., Saxena, V. K., Soni, L., Sharma, P. and Gadekar, Y. P., Dietary inclusion of nonconventional roughages for lowering enteric methane production and augmenting nutrient-valorization of meat in cull sheep. *Anim. Feed Sci. Technol.*, 2021, 273, 114832.
4. Choudhary, S., Santra, A., Sarkar, S. and Das, S. K., In vitro digestibility and fermentation kinetics of some north eastern Himalayan tree leaves using cattle rumen fluid as inoculum. *Indian J. Anim. Sci.*, 2018, 88, 1085–1089.
5. Gupta, R., Dutta, T. K., Kundu, S. S., Chatterjee, A., Gautam, M. and Sarkar, S., Nutritional evaluation of tree leaves of Ayodhya hills of Purulia district, West Bengal. *Indian J. Anim. Nutr.*, 2016, 33, 404–410.
6. Sarkar, S., Mohini, M., Mondal, G., Pandita, S., Nampoothiri, V. M. and Gautam, M., Effect of supplementing *Aegle marmelos* leaves on in vitro rumen fermentation and methanogenesis of diets varying in roughage to concentrate ratio. *Indian J. Anim. Res.*, 2018, 52, 1180–1184.
7. Sarkar, S., Mohini, M., Sharma, A., Tariq, H. and Pal, R. P., Effect of supplementing *Leucaena leucocephala* leaves alone or in conjunction with malic acid on nutrient utilization, performance traits, and enteric methane emission in crossbred calves under tropical conditions. *Trop. Anim. Health Prod.*, 2021, 53, 1–10.
8. Arango, J. et al., Ambition meets reality: achieving GHG emission reduction targets in the livestock sector of Latin America. *Front. Sustain Food Syst.*, 2020, 4, 1–9; https://doi.org/10.3389/fsufs.2020.00065.
9. García-González, R., López, S., Fernández, M. and González-J., S., Effects of the addition of some medicinal plants on methane production in a rumen simulating fermenter (RUSITEC). In International Congress Series, Elsevier, 2006, vol. 1293, pp. 172–175.
10. Yusuf, A. O., Egbinnola, O. O., Ekunseitan, D. A. and Saleem, A. Z. M., Chemical characterization and in vitro methane production of selected agroforestry plants as dry season feeding of ruminants livestock. *Agrofor. Syst.*, 2020, 94, 1481–1489.
11. National Research Council, Nutrient Requirements of Dairy Cattle, National Academy Press, Washington, DC, USA, 2001, 7th rev. edn.
12. Menke, K. H. and Steingass, H., Estimation of the energetic feed value obtained by chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.*, 1988, 28, 7–55.
13. Agarwal, N., Kamra, D. N., Chatterjee, P. N., Kumar, and Chaudhary, L. C., In vitro methanogenesis, microbial profile and fermentation of green forages with buffalo rumen liquoras influenced by 2-bromoethanesulfonic acid. *Asian–Aust. J. Anim. Sci.*, 2008, 21, 818–823.
14. Barnett, A. J. G. and Reid, R. L., Studies on the production of volatile fatty acids from grass by rumen liquor in artificial rumen. The volatile fatty acid production from fresh grasses. *J. Agric. Sci.*, 1957, 48, 315–321.
15. Cottyn, B. G. and Bouquec, C. V., Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.*, 1968, 16, 105–107.
16. Hristov, A. N., McAllister, T. A. and Cheng, K. J., Effect of diet, digesta processing, freezing and extraction procedure on some polysaccharide degrading activities of ruminal contents. *Can. J. Anim. Sci.*, 1999, 79, 73–81.
17. Agarwal, N., Agarwal, I., Kamra, D. N. and Chaudhary, L. C., Diurnal variation in the activities of hydrolytic enzymes in different fractions of rumen contents of Murrah buffaloes. *J. Appl. Anim. Res.*, 2000, 18, 73–80.
18. Miller, G. L., Use of dinitrosalicylic acid reagent for determining reducing sugar. *Anal. Chem.*, 1959, 31, 426–428.
19. Shewale, J. G. and Sadana, J. C., Cellulase and β-glucosidase production by a basidiomyocyte species. *Can. J. Microbiol.*, 1978, 24, 1204–1216.
20. Blummel, M., Makkar, H. P. S. and Becker, K., In vitro gas production: a technique revisited. *J. Anim. Physiol. Anim. Nutr.*, 1997, 77, 24–34.
21. Krishnamoothy, U., Soller, H., Steingass, H. and Menke, K. H., Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies in vitro. *Anim. Feed Sci. Technol.*, 1995, 52, 177–188.
22. National Research Council, Nutrient Requirements of Dairy Cattle, National Academy Press, Washington, DC, USA, 1989, 6th rev. edn.
23. Hungate, R. E., The rumen protozoa. In *The Rumen and its Microbes*, Academic Press, New York, USA, 1966, pp. 92–147.
24. Kamra, D. N., Sawal, R. K., Pathak, N. K., Kewalramani, N. and Agarwal, N., Diurnal variation in ciliate protozoa in the rumen of black buck (*Antilope cervicapra*) fed green forage. *Lett. Appl. Microbiol.*, 1991, 13, 165–167.
25. Molina-Bobero, I. C. et al., Effect of the addition of *Endoreolobium cyclocarpum* pods and *Glicidica sepium* forage on dry matter degradation, volatile fatty acid concentration, and in vitro methane
production. *Trop. Anim. Health Prod.*, 2020; https://doi.org/10.1007/s11250-020-02324-4

26. Sahoo, A., Sarkar, S., Lal, B., Kumawat, P., Sharma, S. and De, K., Utilization of fruit and vegetable waste as an alternative feed resource for sustainable and eco-friendly sheep farming. *Waste Manage.*, 2021, 128, 232–242.

27. Kamra, D. N., Pawar, M. and Singh, B., Effect of plant secondary metabolites on rumen methanogens and methane emission by ruminants. In *Dietary Phytochemicals and Microbes* (ed. Patra, A. K.), Springer, Dordrecht, The Netherlands, 2012, pp. 351–370.

28. Hundal, J. S., Wadhwa, M. and Bakshi, M. P. S., Effect of supplementing essential oils on the *in vitro* methane production and digestibility of wheat straw. *J. Anim. Nutr.*, 2016, 1, 14; doi:10.21767/2572-5459.100014.

29. Sarkar, S., Mohini, M., Nampoothiri, V. M., Mondal, G. and Pandita, S., Effect of tree leaves and malic acid supplementation to wheat straw based substrates on *in vitro* rumen fermentation parameters. *Indian J. Anim. Nutr.*, 2016, 33, 421–426.

30. Valencia-Salazar, S. S., Jiménez-Ferrer, G., Arango, J., Molina-Botero, I., Chirinda, N., Piñeiro-Vázquez, A. and Kú-Vera, J., Enteric methane mitigation and fermentation kinetics of forage species from southern Mexico: *in vitro* screening. *Agrofor. Syst.*, 2021, 95, 293–305.

31. Bhatta, R., Saravanan, M., Baruah, L. and Prasad, C. S., Effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. *J. Appl. Microbiol.*, 2015, 118, 557–564.

32. Lee, H. J., Lee, S. C., Kim, J. D., Oh, Y. G., Kim, B. K., Kim, C. W. and Kim, K. J., Methane production potential of feed ingredients measured by *in vitro* gas test. *Asian–Aust. J. Anim. Sci.*, 2003, 16, 1143–1150.

33. Valenciaga, D., Chongo, B., Herrera, R. S., Torres, V., Oramas, A. and Herrera, M., Effect of regrowth age on *in vitro* dry matter digestibility of *Pennisetum purpureum* cv. CUBACT-115. *Cuban J. Agric. Sci.*, 2009, 43, 79–82.

34. Shingfield, K. J., Ahvenjarvi, S., Toivonen, V., Vanhatalo, A., Hultenan, P. and Grinari, J. M., Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. *Br. J. Nutr.*, 2008, 99, 971–983.

35. Hess, H. D., Monosale, L. M., Lascano, C. E., Carulla, J. E., Díaz, T. E. and Kreuzer, M., Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effect on *in vitro* ruminal lipid turnover and methanogenesis. *Aust. J. Agric. Res.*, 2003, 54, 703–713.

36. Patra, A. K. and Saxena, J., Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Antone Van Leeuwenhoek*, 2009, 96, 363–375.

37. Bhatta, R., Baruah, L., Saravanan, M., Suresh, K. P. and Sampath, K. T., Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis in *in vitro*. *J. Anim. Physiol. Anim. Nutr.*, 2013, 97, 446–456.

38. Waghorn, G. C. and McNabb, W. C., Consequences of plant phe nolic compounds for productivity and health of ruminants. *Proc. Nutr. Soc.*, 2003, 62, 383–392.

39. Chaudhary, L. C., Srivastava, A. and Singh, K. K., Rumen fermentation pattern and digestion of structural carbohydrate in buffalo (*Bubalus bubalis*) calves as affected by ciliate protozoa. *Anim. Feed Sci. Technol.*, 1995, 56, 111–117.

40. Ungerfeld, E. M., Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. *Front. Microbiol.*, 2015, 6, 1–7.

41. Datt, C., Niranj, M., Chhabra, A., Chatopadhayay, K. and Dhim an, T. R., Forage yield, chemical composition and *in vitro* digestibility of different cultivars of maize (*Zea mays L.*). *Indian J. Dairy Sci.*, 2006, 59, 177–180.

42. Makkar, H. P. S., Sen, S., Blummel, M. and Becker, K., Effect of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Acacia auriculiformis* on rumen fermentation. *J. Agric. Food Chem.*, 1998, 46, 4324–4328.

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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 region1, 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* (phalijant/harimj) and *Quercus lanuginosa* (riyani) grow naturally in the Western Himalaya1, of which *Q. semecarpifolia*

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