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## Evaluation of Moringa enriched multinutrient block on *in vitro* methanogenesis and fermentation in rumen liquor of buffalo

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### Abstract

The aim of the study was to evaluate *Moringa oleifera* enriched multi-nutrient blocks (mMNB) using *in-vitro* gas production, in view of the need for nutritionally safe and environmentally sustainable feeding strategies in ruminants. Levels of 2.5%, 5% and 10% *Moringa oleifera* enriched multi-nutrient blocks were prepared by the cold process method and subjected to *in-vitro* evaluation. The *In-vitro* true digestibility was significantly ( $p < 0.01$ ) higher in 10% mMNB, followed by 5% mMNB, compared with the control MNB. Total gas production was significantly ( $p < 0.01$ ) lower in 2.5%, 5% and 10% mMNB compared with the control. Methane production, a key contributor to enteric greenhouse gas emissions, was significantly ( $p < 0.01$ ) lower in 10% mMNB (19 ml/g DM), followed by 5% mMNB (22 ml/g DM) and 2.5% mMNB (23 ml/g DM), compared with the control MNB (30 ml/g DM). Thus, it was concluded that incorporation of 10% *Moringa oleifera* in multi-nutrient blocks can serve as an effective dietary strategy for methane mitigation without adversely affecting digestibility compared with other MNBs.

**Keywords:** *In vitro*, Moringa, methane, multinutrient block

### Introduction

Livestock rearing plays a pivotal role in the development of the rural economy by providing livelihood security, nutritional support, and employment opportunities. Animal husbandry contributes substantially through the supply of milk, meat, wool, manure, urine, and energy, while also generating self-employment for rural youth. At present, nearly 60-65% of the rural population depends primarily on cattle rearing. Despite its importance, the productivity of dairy animals remains relatively low due to limited genetic potential, inadequate nutrition, and suboptimal management practices (BHAS, 2019; FAO, 2011) [15, 12]. Among these factors, improving nutritional status is considered a key strategy for enhancing productive performance in dairy animals.

India faces a chronic shortage of livestock feed resources, with deficits of approximately 64% in concentrate feed and 63.5% in green fodder. According to the Government of India and IGFRI reports (GOI report, 2012; IGFRI Vision 2025 Document, 2013) [16], this situation poses a serious challenge for small and marginal dairy farmers. Garg *et al.* (2013) [13, 14] and Singh *et al.* (2007) [39] reported that farmers often rely on limited quantities of costly concentrates supplemented with natural grasses, tree leaves, and crop residues such as rice straw, wheat straw, and maize stalks. These low-quality roughages are deficient in essential nutrients, thereby limiting animal growth and production. Previous studies have demonstrated that treatment of such roughages with urea and other non-protein nitrogen (NPN) sources can improve nutrient intake and milk yield by enhancing rumen microbial activity (Wadhwa *et al.*, 2012) [45].

Urea molasses mineral blocks (UMMB) have been developed as an effective nutritional supplement to address deficiencies in low-quality diets. According to Leng and Preston (1984) [22] and Sansoucy *et al.* (1988) [38], UMMB typically consist of urea, molasses, protein sources, minerals, and binding agents, and serve as a continuous source of soluble nitrogen, fermentable energy, and minerals for rumen microorganisms. Supplementation with UMMB helps maintain an optimal ammonia concentration in the rumen, thereby improving rumen

fermentation and digestive efficiency (Leng and Preston, 1984) [22]. Garg *et al.* (2002) [13, 14] and Makkar (2012) [27] reported that UMMB supplementation enhances the utilization of dry fodder and reduces feed wastage. Consequently, improvements in milk yield and fat content, body growth, and reproductive efficiency have been widely reported in ruminants (Sansoucy *et al.*, 1988; Garg *et al.*, 2002) [38, 13, 14].

*Moringa oleifera*, a fast-growing tree widely distributed in tropical and subtropical regions, has recently attracted considerable attention in animal nutrition due to its rich nutrient profile and functional properties. Recent analyses indicate that *M. oleifera* leaves are an excellent source of crude protein (22.99-29.36%), as well as vitamins (A, C, and several B vitamins) and essential minerals such as calcium, phosphorus, potassium, and iron, making them highly nutritive for animal diets (Sultan, 2020) [42]. In addition to macronutrients, the leaves contain bioactive secondary metabolites, including polyphenols and flavonoids that exert antioxidant and other functional effects beneficial for animal health (Islam, 2025) [18]. The crude protein content of moringa leaves ranges from 23 to 30.3% on a dry matter basis, with nearly 47% present as rumen bypass protein (Becker, 1995) [5], along with a favourable amino acid composition and polyphenolic antioxidants (Pakade *et al.*, 2013) [33]. Compared to conventional protein supplements such as soybean meal and sesame cake, moringa leaves represent a cost-effective and sustainable protein source (Kholif *et al.*, 2015) [20].

In addition to their nutritional advantages, moringa leaves contain low crude fibre (approximately 5.9%), comparable to soybean meal, and can be harvested multiple times within a season. Dried moringa leaves can be stored for extended periods without significant nutrient loss (Mendieta-Araica *et al.*, 2011) [30]. The presence of natural antioxidants, including polyphenols, flavonoids, carotenoids, polysaccharides, alkaloids, and vitamins, further enhances their potential as a functional feed ingredient. Several studies have reported beneficial effects of moringa leaf supplementation on production and economic traits in cattle and crossbred animals, along with possible modulation of rumen fermentation.

Incorporation of *Moringa oleifera* into urea molasses mineral blocks (UMMB) has emerged as a promising nutritional strategy for mitigating enteric methane emission in ruminants. *Moringa oleifera* leaves are rich in bioactive secondary metabolites such as tannins, saponins, flavonoids, and phenolic compounds, which are known to modulate rumen microbial ecology and suppress methanogenic archaea (Makkar *et al.*, 2007; Soliva *et al.*, 2004) [26, 41]. When included in UMMB, *Moringa* provides a sustained supply of fermentable nitrogen and energy along with these phytochemicals, thereby improving rumen fermentation efficiency while reducing hydrogen availability for methane synthesis (Patra and Saxena, 2011) [35]. Several in-vitro and in-vivo studies have demonstrated that supplementation of *Moringa* leaf meal significantly reduces methane production without adversely affecting nutrient digestibility or animal performance (Adejoro *et al.*, 2019; Jayanegara *et al.*, 2012) [2, 19]. Thus, enrichment of UMMB with *Moringa oleifera* represents an environmentally sustainable feeding approach that combines improved utilization of low-quality roughages with effective methane mitigation. Incorporation of *Moringa oleifera* into UMMB may therefore not only improve

nutrient utilization but also influence rumen fermentation patterns and methane production. In this context, the present study was undertaken to prepare and evaluate a moringa-enriched multi-nutrient block and to assess its effects on *in vitro* methanogenesis and rumen fermentation characteristics using buffalo rumen liquor.

## Materials and Methods

### Preparation of Moringa enriched multinutrient block

Multinutrient blocks were prepared by using cold process method. Firstly, mature moringa leaves were plucked from the tree after 9.00 am and kept it for drying under the direct sunlight continuously for a week. Once it got dried, the twigs and other impurities were removed from the dried leaves manually. Then the leaves were grinded into a coarse powder and stored in dry place. After that, urea was added to molasses, stirred and left standing overnight. Next morning, the rest of the ingredients were mixed together on a polythene sheet. To obtain a uniform distribution in the whole premix, common salt, moringa powder, being the smaller quantity, was mixed with the cement, before mixing with the other dry ingredients. The urea-molasses mixture was poured into this premix and mixed thoroughly by hand. A known amount of this semi-solid mixture (2.0 kg) was put in the frame of manual block making machine and pressed for 30-60 seconds. Then blocks were removed kept on the polythene sheet. The blocks were left at room temperature to air-dry so as to be hard enough for handling. Composition of Moringa enriched multinutrient blocks (mMNBs) were given in Table 1.

### Preparation of substrate and MNBs

Individual raw materials of concentrate and forage mixture and mMNBs were dried in hot air oven at 100±2 °C overnight, ground to pass through to 1 mm sieve separately and stored in airtight container.

### Preparation of inoculum

Rumen liquor was collected from two fistulated buffaloes fed on a diet of wheat straw and concentrate mixture in 1:1 ratio. The animals were fed at the rate of 2.5 kg dry matter/100 kg of body weight. The rumen liquor was sampled just before feeding (0 h) from two animals and transported in insulated flasks under anaerobic conditions to the laboratory, pooled in equal proportions and used as a source of inoculum.

### In vitro gas production test

The substrate (wheat straw, concentrate mixture and mMNB) was milled to pass through 1mm sieve and 200±10 mg was weighed in glass syringes of 100 ml capacity. The incubation medium was prepared as described by Menke and Steingass (1988) [31] and 30 ml was dispensed anaerobically in each syringe. Syringes were incubated at 39 °C for 24h.

### Estimation of gas and methane production

After 24h incubation gas production was estimated by the displacement of piston during incubation. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing no substrate, but only the inoculum and buffer) from total gas produced in the syringe containing substrate and inoculum. The gas produced in standard syringe (containing standard

wheat straw and concentrate mixture) was used to check day to day variation in the quality of inoculum. *in vitro* methane production was estimated by taking the sample gas after 24h incubation from the head space in the air tight syringe and injected to the gas chromatogram fitted with flame ionization detector and stainless-steel column packed with Porapak-Q (1.5m x 3.2mm x 2mm). The temperature for injector column and detector was adjusted as 40 °C, 50 °C and 100 °C and flow rate for nitrogen gas through column was 30, 300 and 30 mL/min. Gas sample (2 mL) from syringe was injected into GC through injection port. The standard gas used for methane estimation composed of methane (CH<sub>4</sub>) and 50% Corban di oxide (CO<sub>2</sub>).

### ***In vitro* true digestibility (IVTD)**

The IVTD of feed was determined after termination of incubation. The contents of syringes were transferred quantitatively, into spoutless beakers by repeated washings with 100 ml neutral detergent solution. The flask contents were refluxed for 1 h and filtered through pre-weighed Gooch crucibles (Grade G1). The dry matter content of the residue was weighed and *in vitro* true digestibility of feed was calculated as follows by Van Soest *et al.* (1991) [44].

### **Statistical Analysis**

The experimental data generated were analyzed using MS Excel. The parameters were analyzed using one way ANOVA with Dunken's post hoc testing to compare experimental groups. For all statistical analyses, probability values less than 0.05 were considered as significant.

## **Results and Discussion**

### ***In vitro* gas production and methane emission**

The results of *in vitro* fermentation of moringa-enriched multi-nutrient blocks (MNBs) are presented in Table 2. Total gas production was recorded as 127, 122, 120, and 121 ml/g DM in the control, 2.5%, 5%, and 10% moringa-enriched MNBs, respectively. The control MNB exhibited significantly higher ( $p < 0.01$ ) total gas production compared to all moringa-enriched treatments, indicating a moderating effect of moringa inclusion on rumen fermentation.

Methane production decreased progressively with increasing levels of moringa incorporation and was observed to be 30, 24, 22, and 19 ml/g DM in the control, 2.5%, 5%, and 10% moringa-enriched MNBs, respectively (Table 2). Methane production was significantly ( $p < 0.01$ ) lower in the 10% moringa-enriched MNB compared to the other treatments, demonstrating a clear dose-dependent inhibitory effect of moringa inclusion on methanogenesis. Results indicating that 2.5, 5 and 10% mMNBS reducing methane emission by 21.68%, 25.9% and 37%, respectively compared to control (Figure 1).

The present findings are in agreement with earlier reports by Leitanthem *et al.* (2022) [21], Dey *et al.* (2014) [9], and Soliva *et al.* (2005) [40], who documented reduced methane production following the inclusion of moringa leaves in ruminant diets. Leitanthem *et al.* (2022) [21] reported a significant ( $p < 0.05$ ) increase in total gas production along with a reduction in methane emission in cattle diets supplemented with moringa leaves. Similarly, Dey *et al.* (2014) [9] observed reduced methane production coupled with increased total gas production when wheat straw was supplemented with moringa leaves under *in vitro* conditions using buffalo rumen liquor. Parra-García *et al.* (2019) [34]

also reported a significant reduction in methane production in the presence of *Moringa oleifera* leaf extract. Soliva *et al.* (2005) [40] observed a 17% reduction in methane production in diets containing moringa leaves compared with those containing rapeseed meal or soybean meal, without any adverse effects on rumen fermentation or nutrient digestibility.

Several mechanisms have been proposed to explain the reduction in methane production, including direct inhibition of methanogenic archaea (Carulla *et al.*, 2005) [6], reduced fibre degradation (Tiemann *et al.*, 2008) [43], and decreased ruminal protein degradation (Salem *et al.*, 2013) [37]. Moringa leaves are rich in secondary metabolites such as tannins and saponins, which are known to suppress methanogens and protozoa, thereby altering rumen microbial activity and fermentation pathways. In the present study, the progressive reduction in methane production with increasing levels of moringa-enriched MNBs may be attributed to the presence of these bioactive compounds (Machmüller *et al.*, 2020) [23]. Total gas production during *in vitro* fermentation primarily reflects the formation of volatile fatty acids, including acetate, propionate, and butyrate, resulting from carbohydrate fermentation. The relatively stable gas production observed up to 10% moringa inclusion suggests that rumen fermentation efficiency was not adversely affected, despite the significant reduction in methane emission. This indicates that moringa-enriched MNBs can effectively mitigate methane production while maintaining normal rumen fermentation, supporting their potential as a sustainable dietary strategy for ruminants.

### ***In vitro* true digestibility**

*In vitro* true digestibility (IVTD, %) was recorded as 62, 61, 62, and 64 in the control, 2.5%, 5%, and 10% moringa-enriched multinutrient blocks, respectively (Table 2). The IVTD was significantly higher ( $p < 0.01$ ) in the 10% moringa-enriched MNB compared to the other treatments.

These results are consistent with the findings of Leitanthem *et al.* (2022), who reported improved ruminal digestibility in moringa leaf-supplemented diets. Similarly, Dey *et al.* (2014) [9] observed increased true dry matter digestibility (TDMD) and true organic matter digestibility (TOMD) when moringa leaves were supplemented to straw-based diets. Supplementation with moringa leaves has been reported to improve digestibility and overall feeding value due to their high-quality protein and balanced nutrient composition (Cohen-Zinder *et al.*, 2016) [8].

Soliva *et al.* (2005) [40] reported that *in vitro* organic matter digestibility of diets containing moringa leaves or extracts was comparable to or higher than that of diets containing conventional protein supplements. Malik *et al.* (2021) [28] observed that a 15% moringa-incorporated urea molasses block exhibited approximately 60% DM digestibility compared to normal UMMB, which is in agreement with the present findings. Previous studies have reported a wide range of *in vitro* DM digestibility values (64.8-79.0%) for moringa-based feeds (Makkar and Becker, 1997; Foidl *et al.*, 1999; Aregheore, 2002; Al-Masri, 2003; Manh and Verstegen, 2003) [25, 11, 4, 3, 29]. Mendieta-Araica *et al.* (2011) [30] reported average DM and OM digestibility values of 74% and 77%, respectively.

Abdel-Raheem and Hassan (2021) [11] also observed a significant ( $p < 0.05$ ) increase in DM and OM digestibility in buffalo calves when moringa leaf meal was included at 15%

in the concentrate mixture. The improvement in digestibility may be attributed to the presence of readily fermentable nutrients in moringa, which positively influence rumen microbial population and activity. It is well established that increased microbial efficiency in the rumen enhances nutrient utilization. Furthermore, supplementation with UMMB has been reported to improve intake and digestibility of fibrous feeds (Yami, 2007) [46]. Studies by Mubi *et al.* (2013) [32], Mahesh and Mohini (2014) [24], and Choubey *et al.* (2016) [7] also support the role of UMMB-based feed technologies in improving nutritional efficiency, though some reported differences were statistically non-significant. Firsoni *et al.* (2017) [10] reported significantly higher DM digestibility and total gas production in animals fed Gliricidia-enriched multinutrient blocks compared to conventional UMMB.

**Table 1:** Formulation used for preparing MNB and Moringa enriched MNBs

| Ingredients<br>(on a percentage basis) | Formulation (%) |           |         |          |
|----------------------------------------|-----------------|-----------|---------|----------|
|                                        | MNB (control)   | 2.5% mMNB | 5% mMNB | 10% mMNB |
| Moringa                                | -               | 2.5       | 5       | 10       |
| Wheat bran                             | 33              | 30.5      | 28      | 23       |
| Mustard oilcake                        | 15              | 15        | 15      | 15       |
| Molasses                               | 35              | 35        | 35      | 35       |
| Urea                                   | 5               | 5         | 5       | 5        |
| Mineral Mixture                        | 3               | 3         | 3       | 3        |
| Cement                                 | 7               | 7         | 7       | 7        |
| Salt                                   | 2               | 2         | 2       | 2        |
| Total                                  | 100             | 100       | 100     | 100      |

**Table 2:** Effect of Moringa enriched MNB on *in vitro* fermentation

| MNBs        | Gas (ml/g DM)             | Methane (ml/g DM)        | IVTD (%)                 |
|-------------|---------------------------|--------------------------|--------------------------|
| Control MNB | 126.63 <sup>a</sup> ±4.98 | 30.12 <sup>a</sup> ±0.16 | 62.19 <sup>b</sup> ±0.19 |
| 2.5 % mMNB  | 121.65 <sup>b</sup> ±5.77 | 23.59 <sup>b</sup> ±0.26 | 61.28 <sup>c</sup> ±0.33 |
| 5% mMNB     | 119.98 <sup>b</sup> ±6.67 | 22.32 <sup>b</sup> ±0.35 | 62.03 <sup>b</sup> ±0.67 |
| 10% % mMNB  | 121.23 <sup>b</sup> ±4.41 | 18.98 <sup>c</sup> ±0.19 | 64.17 <sup>a</sup> ±0.17 |
| SEM         | 4.34                      | 0.24                     | 0.34                     |
| P-Value     | <0.01                     | <0.01                    | <0.01                    |

a,b,c Superscript bearing means values within row differ significantly

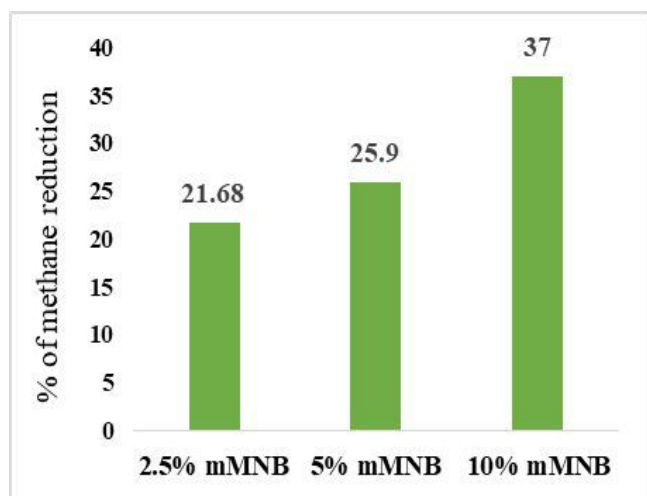


**Fig 1:** Percentage inhibition in *in vitro* methane production by Moringa enriched MNBs

## Conclusion

It can be concluded that supplementation with moringa-enriched multi-nutrient blocks effectively reduced *in vitro* methane production while improving dry matter and organic matter digestibility. Among the treatments evaluated, inclusion of 10% *Moringa oleifera* in the multi-nutrient block yielded the most desirable response, highlighting its potential as an eco-friendly and nutritionally efficient feed supplement for improving rumen fermentation and mitigating methane emission in buffalo. However, further *in vivo* validation under field conditions is required to confirm these benefits, with emphasis on long-term animal performance, feed intake, milk yield and composition, rumen microbial dynamics, economic feasibility, and practical adoption before large-scale recommendation.

## Declaration of competing interest

Authors do not have any conflict of interest.

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