Niger seed (*Guizotia abyssinica*) alters *in vitro* rumen fermentation and reduces methane emission

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Oilseeds can be used to manipulate ruminal fermentation for attaining cleaner and better production. The effect of feed-grade niger seeds was studied on *in vitro* rumen fermentation parameters in comparison to sunflower seeds. Methane production decreased (*P* ≤ 0.01) with incorporation of both seeds. *In vitro* digestibility of dry matter and organic matter reduced at higher incorporation levels (above 5%, DM basis). Fermentation pattern of niger and sunflower seed-based total mixed rations was highly comparable. The relatively higher anti-methanogenic potential of niger seeds could make it a better choice for alleviation of enteric methane and formulation of environment-friendly rations.

**Keywords:** Digestibility, methane emission, niger and sunflower seeds, rumen fermentation, total mixed rations.

Reducing the formation of enteric methane (CH$_4$) and minimizing the environmental impact of ruminant production is currently an important goal of dairy nutritionists. Being a potent greenhouse gas (GHG), CH$_4$ is a major concern regarding climate change globally. Enteric CH$_4$ emission from ruminants accounts for about 3.3% and 17% of global GHGs and CH$_4$ emissions respectively. Moreover, CH$_4$ emission represents up to 15% loss of gross energy intake in ruminant livestock. In this context, feeding of oilseeds has received much attention as a mechanism to mitigate methane emission from ruminants. However, for obtaining optimum benefits from the supplemental oilseeds; they must not be detrimental to ruminal fermentation and nutrient digestibility. Niger seeds (NS) are a natural source of fat rich in polyunsaturated fatty acids (PUFA), particularly linoleic acid. PUFA are more toxic to ruminal microbes than saturated fatty acids (SFA), and can disturb the function of ruminal microbial cell membranes. Additionally, PUFA may act as a sink for hydrogen produced by rumen microbiota on account of bio-hydrogenation. Therefore, incorporation of niger seeds as a lipid source in ruminant rations (as an alternative to conventional lipid sources) may alter ruminal fermentation and diminish CH$_4$ production effectively.

Indian niger seeds contain about 55% linoleic acid and 25% oleic acid. They are free from toxic constituents and apparently well suited for all classes of livestock that can digest fibrous feeds. However, there is lack of information in the available literature regarding their influence on *in vitro* as well as *in vivo* rumen fermentation and methane production. In contrast, sunflower seeds (SS) have been commonly used as a lipid source for formulating energy-dense transition diets and as a means of mitigating methane emissions in ruminants, and hence are broadly described in the literature. They contain over 40% oil, which consists of a high proportion of linoleic acid (62–75%). These seeds are considered safe, and have high palatability and nutritive value for ruminants. Hence, it seems logical to evaluate the *in vitro* pattern of niger seeds in comparison to sunflower seeds. Therefore, the present study was undertaken with the objective to evaluate the effect of graded levels (3%, 5%, 7% and 9%) of niger seeds in total mixed rations (TMRs) on *in vitro* rumen fermentation, *in vitro* digestibility and CH$_4$ production in comparison to the similar incremental levels of sunflower seeds.

**Materials and methods**

**Animals and feeding**

Three rumen cannulated adult male Murrah buffaloes fed on a maintenance ration consisting of wheat straw, available green forage and concentrate mixture were used as rumen liquor donors for *in vitro* incubations. Animals were individually housed in a well-ventilated stall, having provision for adequate sunlight and amenities for individual feeding. *Ad libitum* clean and fresh drinking water was made available to the animals thrice daily. Rumen contents were collected from the animals immediately before the morning feeding. All the experimental procedures were put into practice after due approval by the Institutional Animal Ethics Committee.
**TMR preparation and in vitro incubations**

Animal feed-grade samples of niger and sunflower seeds were dried at 65°C for 48 h, ground in the laboratory Wiley mill and stored in ziplock bags prior to the preparation of experimental rations. Roughages (maize fodder and wheat straw) and concentrate mixture were mixed to obtain TMRs with 60%-40% portions for forage and concentrate. Ground niger and sunflower seeds, both at incremental levels of 7.5%, 12.5%, 17.5% and 22.5% in the concentrate mixture (corresponding to 3%, 5%, 7% and 9% of total dry matter (DM)) were used to formulate experimental TMRs (n = 8). The control TMR (TMRc) was devoid of niger and sunflower seeds, and had the same forage to concentrate ratio (60 : 40). TMRs with similar incremental levels of niger and sunflower seeds were balanced for ether extract (EE) and crude protein (CP).

The rumen contents collected in plastic thermos (pre-heated at 39°C) were immediately transported to the laboratory, where they were flushed continuously with carbon dioxide and strained through four layers of cheese cloth. Prior to incubation, 100 ml calibrated glass syringes (Fortuna Optima, Germany) were placed in an incubator (39°C) were immediately transported to the laboratory, where they were flushed continuously with carbon dioxide and strained through four layers of cheese cloth. Prior to incubation, 100 ml calibrated glass syringes (Fortuna Optima, Germany) were placed in an incubator (39°C ± 1) for 72 h. About 200 mg sample of each air-equilibrated TMR was incubated with 30 ml of buffered rumen inoculum in the glass syringes for 24 h. Incubation runs were performed thrice in triplicates.

**In vitro methane and digestibility determination**

In vitro gas production technique was used for the evaluation of different TMRs. After 24 h, total gas production was measured and representative gas samples (10 ml) were collected from the headspace of the glass syringes in airtight syringes for CH₄ analysis. These gas samples were injected into the gas chromatograph (Nucon 5700, India) fitted with stainless-steel column and flame ionization detector (FID). The temperature of the injector, column and detector was 40°C, 50°C and 100°C respectively.

Pistons of syringes were removed and pH was measured immediately using a pH meter. Thereafter, the contents of each glass syringe were centrifuged at 3000 rpm for 15 min to separate the supernatant (used for NH₃-N and VFA estimation). Pellets obtained after centrifugation were refluxed with neutral detergent solution, filtered through sintered glass (G-1) crucibles, and the residues were dried in a hot-air oven at 100°C for estimation of in vitro DM digestibility (IVDMD). This is the difference between the weights of DM incubated and DM of the residue left behind. In vitro true organic matter digestibility (IVOMD) was estimated by ashing the residue at 550°C for 2 h. The net gas production along with in vitro total DM digestibility (IVTDMD) and in vitro total organic matter digestibility (IVTOMD) was used for the calculation of partitioning factor (PF) and microbial biomass production (MBP).

**Chemical analyses**

Standard methods of AOAC were followed for determining DM, CP (N × 6.25), EE and total ash. Fibre fractions like neutral detergent fibre (NDF) and acid detergent fibre (ADF) were assayed according to Van Soest et al. NH₃–N was estimated from supernatant samples using Kjeldahl method. For VFA analysis 0.8 ml of the supernatant sample mixed with 0.2 ml of metaphosphoric acid solution (250 g/l) was injected into the gas chromatograph (fitted with FID and stainless steel column packed with Chromosorb-101), following the protocol described by Erwin et al. All the analyses were done in triplicate.

**Statistical analysis**

The data were analysed by one-way analysis of variance using SAS software. Means were separated for statistical significance at 1% level (P ≤ 0.01) using post-hoc comparison by Tukey’s Studentized Range Test.

**Results and discussion**

**Chemical composition of samples and total mixed rations**

Table 1 presents the chemical composition of feed samples and TMRs used for the in vitro study. NS and SS contained approximately similar levels of organic matter (OM), EE and CP. However, NDF content (DM basis) of NS (254 g/kg) was slightly lower than that of SS (285 g/kg), whereas ADF content of NS (212 g/kg) was slightly higher than that of SS (191 g/kg).

All presented values for samples are within the normal range as reported in the literature. Incorporation of sunflower and niger seeds at various levels changed the chemical composition of the treatment TMRs in comparison to TMRc (negative control). However, TMRs containing similar incremental levels of niger and sunflower seeds possessed almost similar chemical composition.

**Effect on in vitro gas production and methane emission**

As shown in Table 2, all NS- and SS-based rations decreased (P ≤ 0.01) net gas production (GV₂₄h) and CH₄ emission almost in a linear fashion in comparison to control. Reduced rate of GV₂₄h by treatments compared to the control indicates diminished ruminal degradability. Relatively comparable GV₂₄h up to 5% incorporation level of
Table 1. Chemical composition (g/kg –1 dry matter (DM)) of samples and total mixed rations containing graded levels of niger and sunflower seeds

| Ingredient          | SS   | NS   | TMRc  | TMRs-3 | TMRs-5 | TMRs-7 | TMRs-9 | TMRn-3 | TMRn-5 | TMRn-7 | TMRn-9 |
|---------------------|------|------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| Organic matter      | 965  | 948  | 896   | 901    | 898    | 901    | 900    | 898    | 899    | 898    | 897    |
| Crude protein       | 198  | 212  | 131   | 133    | 134    | 136    | 137    | 133    | 134    | 136    | 137    |
| Ether extract       | 402  | 395  | 27    | 38     | 45     | 51     | 58     | 38     | 45     | 51     | 57     |
| Neutral detergent fibre | 285 | 254  | 485   | 492    | 497    | 508    | 517    | 491    | 496    | 503    | 513    |
| Acid detergent fibre | 191  | 212  | 300   | 301    | 303    | 305    | 308    | 302    | 304    | 307    | 310    |
| ASH                 | 35   | 52   | 104   | 99     | 102    | 99     | 100    | 102    | 101    | 102    | 103    |

SS, Sunflower seeds; NS, Niger seed; TMRc, Control total mixed rations; TMRs (3, 5, 7, 9), TMR containing SS at 3%, 5%, 7% and 9% respectively; TMRn (3, 5, 7, 9), TMR containing NS at 3%, 5%, 7% and 9% respectively.

Table 2. In vitro gas production and associated parameters of total mixed rations containing graded levels of sunflower and niger seeds

| Parameter | TMRc | TMRs-3 | TMRs-5 | TMRs-7 | TMRs-9 | TMRn-3 | TMRn-5 | TMRn-7 | TMRn-9 | SEM    |
|-----------|------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Total gas, GV 24h (ml/g) | 208.75d | 198.33c | 195.83abc | 193.45abc | 190.66ab | 196.25bc | 195.00abc | 192.91abc | 189.50a | 0.66   |
| Methane (%) | 32.07d | 27.79d | 26.94cd | 25.86bc | 25.12bc | 27.02cd | 26.58cd | 25.81bc | 24.14a | 0.23   |
| Methane (ml/g) | 66.63e | 54.88d | 52.55ed | 54.81de | 52.79ed | 51.58de | 49.51de | 45.52a | 0.61   |
| IVDMD (%) | 67.18d | 67.031c | 66.22c | 64.56b | 63.19a | 66.69c | 66.15c | 64.35b | 63.09a | 0.17   |
| IVOMD (%) | 67.18c | 67.031c | 66.22c | 64.56b | 63.19a | 66.69c | 66.15c | 64.35b | 63.09a | 0.17   |
| PF | 3.22a | 3.41b | 3.39b | 3.33b | 3.29b | 3.37b | 3.36b | 3.32b | 3.30b | 0.01   |
| MBP (mg) | 254.30 | 277.86d | 272.28ed | 252.91 | 249.81 | 270.23d | 269.88d | 256.62 | 249.57a | 1.48   |

IVDMD, In vitro dry matter digestibility; IVOMD, In vitro true organic matter digestibility; PF, Partitioning factor; MBP, Microbial biomass production. a–eMeans in the same row with different superscripts differ (P < 0.01).

Table 3 presents the in vitro fermentation pattern of treatment rations. The pH values for all treatments were within the range (5.4 and 6.7) acceptable for optimal rumen fermentation. Lack of effect on pH may be due the inability of these seeds to elicit any effect on the concentration of total volatile fatty acids (tVFA). Our results are in agreement with those of Beauchemin et al. who reported that feeding sunflower seeds does not affect the rumen pH or tVFA concentration.

NH3–N concentration decreased (P ≤ 0.01) linearly in response to the graded levels of NS and SS compared to control (Table 3). Reduction in NH3–N with the addition of NS and SS in TMR at 5% (DM-basis) would be optimal for ruminal ecosystem, microbial activity and thus nutrient digestibility. Significant reduction in in vitro digestibility may be attributed to anti-microbial activity of PUFA, and thus slower rate of substrate degradation at higher incorporation levels. This indicates that degradation rate may also affect ruminal fermentation of unsaturated fat sources like niger and sunflower seeds. Partitioning factor (PF), which is the measure of efficiency of microbial protein synthesis in vitro25, remained within the normal range (2.74–4.41). Higher values of PF for TMRs-3, TMRs-5, TMRn-3 and TMRn-5 imply better utilization of the oilseed proteins by rumen microbes at 3% and 5% inclusion levels of both niger and sunflower seeds. This may be attributed to higher OM digestibility at these levels.

Effect on in vitro rumen fermentation

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of sunflower and niger seeds indicates the antimicrobial activity of these seeds on ruminal microbiota. Reduced levels of \( \text{NH}_3-N \) might have occurred due to suppression of the population and activity of ruminal protozoa\(^{27}\), which are the key players for ruminal protein degradation\(^{28}\), or reduced peptidolytic activity of ruminal bacteria\(^{29}\). The decreasing trend observed in the concentration of ammonia-N is consistent with previous findings\(^{30,31}\). Incorporation of NS and SS at various incremental levels did not alter tVFA concentration in contrast to TMRs. However, the molar proportion of acetate increased at 9% inclusion levels, whereas propionate concentration showed a linearly increasing (\( P \leq 0.01 \)) trend with the incorporation of treatments. Furthermore, treatments did not affect the molar proportions of butyrate, isobutyrate, isovalerate and valerate. Increased acetate concentration at 9% inclusion level may be probably related to high fibre content of these rations; whereas the observed increase in propionate is justified by the reduction in methane production, as the generation of propionate incorporates reducing equivalents\(^{32}\). Increased molar proportion of propionate in this study is in line with earlier findings\(^{33,34}\). Despite the increase in propionate, no change was observed in acetate propionate ratio. Our results are inconsistent with those of other researchers, who did not observe any effect on the molar proportions of VFA upon sunflower seed supplementation\(^{35,36}\). Increased molar concentration of propionate indicates that NS- and SS-based TMRs may have a positive consequence on the productive performance of animals.

**Conclusion**

Fermentation pattern of niger seeds was highly comparable to sunflower seeds. NS depicted higher potential of reducing \( \text{CH}_4 \) relative to SS. Reduction in *in vitro* digestibility at higher levels may limit the inclusion level of the seeds to 5%. However, the outcome of this study needs an *in vivo* validation in various classes of ruminants.

**Conflict of interest:** The authors declare no conflict of interest.

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