Effect of feeding pellet containing *Leucaena leucocephala* and *Ficus infectoria* leaves on *in vivo* methane emission in Barbari goats

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ABSTRACT

Effect of feeding complete pellet feed containing tree leaves on methane emission was studied in male Barbari goats. Three types of complete pellet feed was formulated, viz. control pellet (C) having gram straw (60%) and concentrate mixture (40%), treatment 1 (T1) and treatment 2 (T2) containing dried *Leucaena leucocephala* and *Ficus infectoria* leaves respectively. All types of pellets were isonitrogenous. Growing male Barbari goats (12; 3–4 month-old; average body weight 11.01±0.49 kg) were divided into 3 groups (Gr 1, Gr 2 and Gr 3) as per completely randomized design. Gr 1 was fed with control pellet while Gr 2 and Gr 3 was fed with T1 and T2 pellet respectively. After 6 weeks of experimental feeding, a digestion trial of 6-day duration was conducted. There was no difference in the DMI (g)/day between the 3 groups. Dry matter digestibility and digestibility of other nutrients were comparable among all groups of goats. Rumen liquor was collected from each animal to study rumen fermentation metabolites. Rumen pH, ammonia-N and nitrogenous fractions (total nitrogen, TCA-ppt N, NPN) were statistically similar in all the groups. Total volatile fatty acids (mmol/dl) was significantly higher in Gr 3 (10.57) and Gr 2 (9.52) as compared to control group (8.67). Fractions (%) of volatile fatty acids (acetic acid, propionic acid and butyric acid) were similar in different groups. *In vivo* methane emission in different groups of goats was estimated by SF₆ technique as per procedure standardized in our lab. Methane production (g/day) was 8.29 in Gr 1, 7.47 in Gr 2 and 6.72 in Gr 3. There was 9.89 and 18.93% lower methane production in Gr 2 and Gr 3 as compared to control group of goats fed with complete pellet feed. From the present study, it can be concluded that incorporation of dried *L. leucocephala* and *F. infectoria* leaves in the complete pellet feed can reduce the methane production in goats.

Key words: Barbari goats, *Ficus infectoria*, *In vivo* methane, *Leucaena leucocephala* Nutrient digestibility, Rumen metabolites

Tree leaves are a natural component of goat feed and goat relishes to feed on leaves of the trees. Plant secondary metabolites like tannins and saponins manipulate the rumen fermentation pattern and reduce the methane emission from the animals (Kamra 2008). Tree leaves are the source of secondary metabolites like tannins, condensed tannin etc. Incorporation of extracts of tree leaves in *in vitro* gas production test reportedly reduced the methane production (Kumar et al. 2011). Incorporation of tree leaves in the complete pellet feed can reduce the methane production and improve the feed fermentation. Several studies have been conducted to replace a part of concentrate mixture with different leaf meal mixture as a strategic supplement in the diet of goat (Anbarasu et al. 2004, Patra et al. 2006, Pal et al. 2010). Pal et al. (2010) reported that leaf meal mixture can replace the concentrate mixture without any adverse effect on growth, nutrient utilization and rumen fermentation parameters. Feed fermentation by ruminants is producing around 85 million tonnes methane per year and 82% of total livestock emission. Measuring of methane by SF₆ tracer technique is widely used in many countries for CH₄ emission measurements on grazing and pen fed animals. Methane production from the feed varied depending upon the composition of feed (Kumar et al. 2007). With this background, the present experiment was conducted to study the effect of incorporation of *L. leucocephala* and *F. infectoria* leaves in the complete pellet on nutrient digestibility, rumen fermentation metabolites and *in vivo* methane production in goats.

MATERIALS AND METHODS

Animal management and rations: Growing male Barbari goats (12, 3–4 month-old age, average body weight 11.01±0.49 kg) were divided into 3 groups (Gr 1, Gr 2 and Gr 3) as per completely randomized design (CRD). All the goats were kept under uniform management conditions by housing them in well ventilated sheds. Three types of complete pellet feed, viz. control pellet having gram straw (60%) and concentrate mixture (40%), treatment 1 (T1),
and treatment 2 (T2) containing dried *L. leucocephala* and *Ficus infectoria* leaves were formulated. All the goats were fed with complete pellet feed having concentrate mixture and Bengal gram straw in 40:60 ratio. Composition of control pellet was 60% gram straw, 22.8% barley, 12% groundnut cake, 4% wheat bran, 0.8% mineral mixture and 0.4% salt. The composition of T1 pellet was 60% gram straw, 20% barley, 7% groundnut cake, 1.8% wheat bran, 10% dried *L. leucocephala* leaves, 0.8% mineral mixture and 0.4% salt while T2 pellet was 60% gram straw, 17% barley, 10% groundnut cake, 1.8% wheat bran, 10% dried *Ficus infectoria* leaves, 0.8% mineral mixture and 0.4% salt. Gr 1 was fed with control pellet, Gr 2 and Gr 3 were fed with T1 and T2 pellet, respectively. All types of pellets were iso-nitrogenous.

**Experimental procedure:** An experimental feeding on 3 groups of goats was carried out for 90 days. Weighed quantities of respective pellet were offered to different group of goats at 08:00 AM and 2:00 PM daily in a completely randomized design (CRD). *Ad lib.* water was provided and was changed twice daily throughout the experimental period. Pellet offered and residues were sampled weekly for subsequent analysis of DM to determine DM intake.

After feeding for 6 weeks, 6-day duration digestion trial was conducted. During digestion trial mean body weight of group of goats was 12.10±0.69 kg. Digestion trial was conducted in metabolic cages having facility for individual feeding and separate collection of feces and urine. Representative samples of pellet offered and remnants were taken daily in previously tared trays and kept in a hot air oven at 100±2°C overnight for DM estimation. The dried material obtained during the trial period was pooled, ground and stored for proximate and fibre analysis. The faeces voided in 24 h by the individual animal were collected in a previously weighed container. After weighing the fecal container, the feces were thoroughly mixed and a representative sample from each animal was taken in a previously labeled polythene bags. From the sample, a representative sample from each animal was taken in a container, the feces were thoroughly mixed and a previously weighed container. After weighing the fecal container, the feces were thoroughly mixed and a representative sample from each animal was taken in a previously labeled polythene bags. From the sample, a suitable aliquot (10%) of faeces was kept for drying at 100±2°C in a hot air oven for dry matter determination.

In vivo methane emission in different groups of goats was estimated by sulfur hexafluoride (SF6) technique (Johnson *et al*., 1994) as per standardized procedure for goats in our lab. Animals were dosed orally with permeation tube filled with pure sulfur hexafluoride (SF6) gas and standardized for constant release rate of tracer SF6 gas. A halter fitted with a capillary tube is placed on the animals head and connected to an evacuated sampling canister. All the animals were accustomed to carry canister fitted with halter assembly for two weeks prior to sampling. Sampling of expired and eructated air containing mixture of gases like methane, SF6 was conducted in the evacuated canister for each animal. One background canister was also placed in the shed area for estimation of methane and SF6 concentration in the surroundings. Methane and SF6 concentration of sampled canister was analysed and methane emission was calculated in excess to background level.

**Laboratory and statistical analysis:** Nutrient composition of feed, residue and faeces were analysed as per AOAC (1995). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose (HC) were determined as per Robertson and Van Soest (1981). The CT content of leaves was estimated by Butanol-HCl method (Makkar 2000).

The pH of rumen liquor was determined immediately after collection, with an electronic pH meter (Eutech pH tester 30, Thermo Fischer Sci. Inc., USA) calibrated against standard buffer solution. The ammonia nitrogen (NH3-N) in rumen liquor was estimated by distillation method. Total nitrogen and trichloro acetic acid (TCA) precipitated nitrogen was estimated by micro Kjeldahl’s method (AOAC 1995). Non protein nitrogen of rumen liquor was calculated by subtracting the TCA precipitated nitrogen from total nitrogen content of rumen liquor. Rumen liquor samples were prepared by adding 0.2 ml of 25% metaphosphoric acid/ml of rumen liquor, allowing it to stand for 2 h followed by centrifugation at 5,000 rpm for 20 min. Supernatant was used for estimation of volatile fatty acids (VFAs). Estimation of volatile fatty acids was done using Clarus-580 gas chromatograph (Perkin Elmer, Singapore) equipped with flame ionization detector and capillary column as per Cotton and Boucque (1968).

The data collected during study were statistically analyzed using generalized linear model (GLM) procedures of analysis of variance (ANOVA). The means of the treatments were compared using Duncan’s Multiple Range Test (DMRT) as per Snedecor and Cochran (1989). All the analysis was performed using SPSS v 7.5 software package.

**RESULTS AND DISCUSSION**

**Chemical composition of pellets:** The chemical composition of different types of complete pellet on dry matter basis fed during experimental period is presented in Table 1. All type of pellets were iso-nitrogenous having crude

| Attribute | Control pellet | T1 pellet | T2 pellet |
|-----------|---------------|----------|----------|
| OM        | 88.99         | 82.13    | 84.67    |
| CP        | 11.50         | 11.15    | 11.19    |
| EE        | 1.44          | 1.49     | 1.46     |
| NDF       | 49.50         | 40.84    | 45.20    |
| ADF       | 34.93         | 25.92    | 30.50    |
| Cellulose | 27.58         | 19.39    | 22.32    |
| Hemicellulose | 14.57   | 14.92    | 14.70    |
| TCHO      | 76.04         | 69.48    | 73.01    |
| ADL       | 5.99          | 4.33     | 5.78     |

OM, Organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; TCHO, total carbohydrate.
Protein (CP) content around 11%. The ratio of gram straw and concentrate mixture was maintained 60:40 in the pellet feed. The dried leaves powder was mixed in the pellet at the rate of 10% of total dry matter. The different nutrient was similar in the pellet feed. NDF and ADF were 49.50 and 34.93% for control; 40.84 and 25.92% for T1; 45.20% and 30.50% for T2 pellet respectively. The condensed tannin (% DM basis) of dried *L. leucocephala* and *Ficus infectoria* leaves was 2.90 and 10.80, respectively.

**Intake and nutrient digestibility:** There was no difference in the DMI (g)/day between different groups. There was no significant difference (P>0.05) in the dry matter intake (g/kg W₀.₇₅/day) of different group of goats (Table 2). The voluntary feed intake expressed as a percentage of live weight of goats in the tropical environment ranged from 1.1 to 4.1% (NRC 1981, Kearl 1982).

The DMI/day on per cent body weight basis varied from 5.10 to 5.55% in different groups of goats. Higher intake of dry matter may be attributed to the form of feed which is pellet type. Generally pellet form of feed has more intake as compared to normal feed. Dry matter and organic matter digestibility among the different group of goats was comparable. Digestibility of crude protein and ether extract were also similar among different group of goats. Pal et al. (2010) reported significant reduction in digestibility of DM, OM, CP and EE on replacement of 50% (on weight basis) of concentrate mixture with *L. leucocephala*, *M. azedarach* and *M. alba* leaf meal mixture. Srivastava and Sharma (1998) and Mahanta et al. (1999) also reported a lower CP digestibility as a substitute to conventional protein supplements. No significant effect on digestibility of nutrients might be due to lower level (10%) of incorporation of dried leaves as compared to previous study. In present experiment, tree leaves were incorporated in the diet not as a part of ration but a source of plant secondary metabolites that can affect the methane production with no adverse effect on other nutritional parameters. The digestibility of fibre fractions (NDF, ADF, cellulose and hemicellulose) did not differ significantly (P>0.05) between dietary treatments. The findings are in agreement with previous workers (Mahanta et al. 1999, Ondiek et al. 2000). The digestibility of total carbohydrate was also similar among all the groups.

**Rumen fermentation metabolites:** Rumen pH was statistically similar in all the groups (Table 3). The values were within normal range of 6–7 indicating no adverse effect on ruminal environment and microbes. No significant difference was reported in various nitrogenous fractions (total nitrogen, TCA-ppt N, NPN) of different group of goats. Pal et al. (2010) and Anbarasu et al. (2002) also reported no difference in nitrogenous fractions of rumen liquor on addition of leaf meal mixture in the ration. Total volatile fatty acids (mmol/dl) was significantly (P<0.05) higher in Gr 3 and Gr 2 as compared to Gr 1 fed with control pellet. Fractions (%) of volatile fatty acids (acetic acid, propionic acid and butyric acid) were similar in different group of goats (Table 3).

### Table 2. Dry matter intake and nutrient digestibility in different group of goats

| Attribute                  | Gr 1   | Gr 2   | Gr 3   | Mean   | SEM    | P-Value |
|----------------------------|--------|--------|--------|--------|--------|---------|
| B. Wt. (kg)                | 13.22  | 12.25  | 10.85  | 12.10  | 0.69   | 0.406   |
| W₀.₇₅ (kg)                 | 6.91   | 6.53   | 5.96   | 6.47   | 0.27   | 0.403   |
| DMI (g/d)                  | 673.79 | 669.25 | 564.31 | 635.78 | 35.48  | 0.399   |
| DMI (% b.wt.)              | 5.10   | 5.55   | 5.28   | 5.31   | 0.22   | 0.074   |
| DMI (g/kg W₀.₇₅/day)       | 97.02  | 103.30 | 95.23  | 98.52  | 3.78   | 0.702   |

**Nutrient digestibility (%):**

| Nutrient | DM | OM | CP | EE | NDF | ADF | Cellulose | Hemicellulose | TCHO |
|----------|----|----|----|----|-----|-----|-----------|--------------|------|
|          | 54.17 | 58.33 | 59.66 | 75.92 | 37.37 | 29.15 | 52.49 | 57.09 | 57.80 |
|          | 56.40 | 59.56 | 58.86 | 61.65 | 43.96 | 38.01 | 57.05 | 58.26 | 66.72 |
|          | 55.20 | 61.13 | 55.55 | 51.31 | 44.95 | 44.04 | 56.68 | 47.18 | 57.27 |
|          | 55.26 | 59.67 | 58.02 | 6.96  | 42.09 | 37.07 | 55.07 | 54.18 | 60.60 |
|          | 0.58  | 0.99  | 1.92  | 5.52  | 1.68  | 3.04  | 2.30   | 2.74  | 1.94  |

DM, Dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; TCHO, total carbohydrates.

### Table 3. Rumen fermentation pattern in different group of goats

| Attribute                  | Gr 1       | Gr 2       | Gr 3       | Mean      | SEM      | P-value |
|----------------------------|------------|------------|------------|-----------|----------|---------|
| pH                        | 6.57       | 6.67       | 6.52       | 6.59      | 0.077    | 0.760   |
| TVFA (mmol/dl)             | 8.67       | 9.52       | 10.57      | 9.59      | 0.29     | 0.011   |
| Total-N (mg/dl)            | 81.20      | 81.20      | 86.80      | 83.06     | 5.19     | 0.899   |
| NH₃-N (mg/dl)              | 18.90      | 16.80      | 21.70      | 19.13     | 0.91     | 0.076   |
| NPN (mg/dl)                | 35.00      | 32.20      | 35.70      | 34.30     | 6.10     | 0.732   |
| TCA ppt-N (mg/dl)          | 46.20      | 49.00      | 51.10      | 48.76     | 4.11     | 0.906   |
| VFA proportion (%)         | 65.05      | 54.94      | 54.89      | 54.96     | 0.77     | 0.997   |
| Acetic acid                | 28.54      | 27.40      | 27.20      | 27.71     | 0.70     | 0.749   |
| Propionic acid             | 16.40      | 17.64      | 17.90      | 17.31     | 0.95     | 0.823   |

a,b Means with different superscript differ significantly at P<0.05.
**METHANE PRODUCTION ON TREE LEAVES CONTAINING PELLET FEED**

In vivo methane emission: In vivo methane emission in different groups of goats was estimated by SF$_6$ technique as per standardized procedure. Methane production (g/day) was 8.29 in Gr 1, 7.47 in Gr 2 and 6.72 in Gr 3. There was 9.89 and 18.93% lower methane production in Gr 2 and Gr 3 fed with tree leaves incorporated pellet feed as compared to Gr 1 of goats fed with complete pellet feed. Methane production (g/kg DMI) was 13.34 in Gr 1, 11.52 in Gr 2 and 12.00 in Gr 3. There was lower methane production in treatment groups of goat fed with pellet containing dried L. leucocephala and F. infectoria leaves. Tree leaves, a source of secondary metabolites like tannins and their extracts, have been found to reduce methane production in many in vitro studies (Kumar et al., 2011, Kamra et al., 2006). Plants rich in condensed tannins have been shown to reduce rumen methanogenesis probably due to both directly through an inhibition of the growth of methanogens and indirectly through a reduction in fibre digestion, which decreases H$_2$ production. They are low molecular weight compounds that do not play any role in primary plant metabolism like growth and reproduction. The function of these secondary metabolites in plants is to protect the plants against predation, infection or by restricting grazing by herbivores. Kumar et al. (2011) studied the effect of extracts (water, ethanol and methanol) of leaves of Mangifera indica, Eugenia jambolana, Aegle marmelos, Ziziphus spina-christi, Azadirachta indica and Ficus religiosa on methane production and other rumen fermentation characteristics in an in vitro gas production test. They reported that methanol and ethanol extract of Mangifera indica inhibits methane production by 35.7 and 23.2% respectively. Methanol extract of E. jambolana also inhibited methane emission by 24.1%. Puchala et al. (2005) fed condensed tannin containing forage Lespedeza cuneata to the goats and found that methane emission per day and per kg DMI was lower for L. cuneata than for crabgrass/tall fescue (7.4 vs 10.6 g/d and 6.9 vs 16.2 g/kg DMI). Tannins suppressed daily methane release by 7% on average, reduced ruminal ammonia concentration and urinary nitrogen excretion in sheep fed ryegrass silage (Carulla et al., 2005). For practical feeding purpose, it is quite difficult to use extracts of plant leaves. So in present study, dried plant leaves were incorporated in the pellet feed to reduce the methane production. There was a reduction in NDF and ADF content of pellet (T1 and T2) after incorporation of tree leaves. Composition of ration has a significant effect on the methane generation of feed (Kumar et al., 2007), which might have contributed in reducing the methane production from the T1 and T2 pellet in comparison to control pellet.

From the present study, it can be concluded that incorporation of dried L. leucocephala and F. infectoria leaves in the complete pellet feed can reduce the methane production without adverse effect on fermentation and digestibility of feeds.

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