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Effect of graded levels of condensed tannin (CT) from *Mimosa pudica* on *in-vitro* methane production

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Introduction

Livestock in the country are primarily being fed on fibrous feed resulted in high enteric methane (CH_4) emission along with low nutrients availability to host animal. Rumen methano genesis is necessary for the host system as this process ensure the removal of fermentative H_2 through the reduction of CO_2 into CH_4. At the same time this process is wasteful because the emission also represents a loss of dietary energy (6-12% of gross energy intake) apart from contributing to global warming.

Worldwide livestock contribute around 90-95 Tg methane to the pool with a contribution of 12-13% from the Indian livestock. Various nutritional and other approaches have been attempted with highly variable success rate in the country and elsewhere for the enteric methane amelioration. The cost of the item used for the mitigation purpose, adaptation of ruminal microbes and toxicity to either host animal or inhabiting microbes are few important criteria for an economic, sustainable and effective amelioration approach (Malik *et al.* 2015).

Herbal materials are being used by the peoples since ages; however, their anti-methanogenic effect is recently established. The anti-methanogenic effect of different herbal materials mainly lies in their secondary metabolites which are highly effective even at very low concentration (Bhatta *et al.*, 2014). Keeping these facts in view, a study was carried to ascertain the effect of varying levels of CT on *in vitro* total gas and methane production.

Materials and Methods

A series of *in vitro* studies was conducted to investigate the effect of variable levels of condensed tannin (CT) from mimosa (*Mimosa pudica*) on total gas and methane production. For conducting *in vitro* studies, rumen liquor was collected from cannulated Holstein Friesian male cattle and immediately filtered through four layers of cheese cloth into a pre-warmed beaker flushed with CO_2 to maintain the temperature and anaerobic conditions. Basal diet comprising ragi straw and concentrate mixture was prepared by mixing these two ingredients in the ratio of 70:30. 0.2 g oven dried sample was incubated in 100 ml capacity glass syringe with 10 ml rumen liquor and 20 ml buffer flushed with CO_2. Six replicates of each sample were incubated at 39^0 C for 24 hrs in a continuous flow water bath. In first set, the low levels viz. 0 (T_0), 8 (T_1), 16 (T_2) and 24 (T_3) mg/kg of condensed tannin (CT) were studied for their effect on *in vitro* methane production. Due to the non responsive effect of these dosages, the levels of CT were revised as 0 (T_0), 70 (T_1), 140 (T_2) and 210 (T_3) mg/kg of basal diet in the second set of study. However, in next study the moderate levels of CT viz. 0, 20, 30 and 40 mg/kg basal diet were used for the evaluation. The methane in total gas was analyzed by gas chromatograph. Ammonical nitrogen and TVFA was estimated as per the standard procedure. Protozoa were enumerated using methodology of Kamra *et al.* (1991).

Results and Discussion

Data from the first experiment (Table 1) in the series did not reveal any significant change in total gas production as well as *in vitro* methane on the addition of graded levels of CT. The total gas production however was numerical higher than that recorded in control treatment T_0. Similarly, the *in vitro* methane production was also little higher than the control group. The study clearly indicated that the supplementation of CT at such low graded levels do not have any impact on methane production.

Table 1: Effect of low levels of CT on *in vitro* total gas (ml/g DM) and methane production (ml/g DM)

Levels	**Total gas***	**CH_4 ***
T_0	122	20.7

T_1	155	24.9
T_2	160	25.4
T_3	148	24.4

* Each figure is based on six replicate values

The revised levels with higher dosages envisaged significant changes in both *in vitro* total gas and methane production on the supplementation of graded levels of CT from 0 to 210 mg/kg DM (Table 2). The total gas production decrease significantly ($p<0.05$) with increasing levels of CT and lowest total gas production was recorded in the treatment T_3 where the basal diet was supplemented with 210 mg CT/kg of basal diet. Similarly, the *in vitro* methane production also decreased with graded levels of CT. The highest methane production was recorded in control treatment (T_0), while lowest in treatment T_3. Data indicated 26, 51 and 65% reduction in *in vitro* methane production as compared to control. The *in vitro* dry matter digestibility also decreased significantly ($p<0.05$) as compared to control. Thus, it can be inferred from the study that the reduction in methane production was accompanied with reduction in DMD and thereby overall fermentation of the basal diet. The total protozoal counts (x 10^5/ml) was also significantly ($p<0.05$) lower in treatments group than the control (Table 2). The variation in protozoal numbers in treatment T3 was also significantly ($p<0.05$) lower than the treatment T_1 and T_2. The significant variation in protozoal numbers along with lower DMD among the different treatments were accountable for the lower methane production as compared to control.

Table 2: Effect of graded levels of HT on *in-vitro* total gas, methane and other fermentation parameters

Particulars	**CT (mg/kg DM)**				**SE**	**Sig**
	T_0	T_1	T_2	T_3		
Total gas (ml/g DM)	157.2^d	111.3^c	83.2^b	53.0^a	10.1	0.001
CH_4 (ml/g DM)	30.1^d	22.0^c	14.6^b	10.7^a	1.9	0.001
IVDMD (%)	61.2^c	53.2^b	54.8^{ab}	53.5^a	0.8	0.001
Total protozoa (x 10^5/ml)	7.3^c	6.7^b	6.3^b	5.6^a	0.1	0.001

Mean values in a row bearing different superscripts differ significantly ($p<0.05$)

As *in vitro* methane production with higher dosages of CT (Table 2) was accompanied with decrease in DMD, therefore, another experiment with moderate levels of CT was conducted and data is presented in Table 3. Results from the study indicated that *in vitro* methane production decreased with increasing levels without affecting the DMD (Table 3) as compared to control treatment. Likewise, no substantial change was recorded in TVFA production among the different treatment in the study. The supplementation at 20mg/kg of DM did not affect the methane production as compared to control; however, the supplementation at 30 and 40 mg levels (T_2 and T_3) decreased the methane production by 19% as compared to control treatment.

Table 3: Effect of moderate levels of CT on *in-vitro* total gas and methane production

Particulars	**Treatment**			
	T_0	T_1	T_2	T_3
Total gas (ml/g DM)	183	166	151	152
CH_4 (ml/g DM)	31.0	31.0	24.9	25.1
IVDMD (%)	52.3	53.3	53.8	54.2
TVFA (mM/100ml)	9.30	8.05	7.80	7.45

Conclusion

In vitro studies revealed that the CT from *Mimosa pudica* at lower graded levels did not have any impact on methane production, while at higher dosages the methane production decreased significantly with simultaneous decrease in DMD.

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