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In Vitro Evaluation of Total Mixed Rations containing Graded Levels of *Tinospora cordifolia*

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ABSTRACT

The present study was conducted to assess the chemical composition and *in vitro* nutritional worth of total mixed rations (with R:C ratio of 50:50) containing graded levels of *Tinospora cordifolia* stem powder. *T. cordifolia was* added to the total mixed ration (TMR) at the levels of 0%, 1%, 2%, 3%, 4% and 5% (on DM basis) to formulate six TMRs designated as TMR1, TMR2, TMR 3, TMR 4, TMR 5 and TMR 6, respectively. No significant difference was observed in net gas production, truly degraded substrate, partitioning factor, OM digestibility, NDF digestibility, microbial mass production, efficiency of microbial mass production, short chain fatty acids and metabolizable energy among the total mixed rations tested. The ammoniacal nitrogen (NH_3-N, mg/dl) was higher ($P<0.05$) in TMR 1 (26.50) and lower ($P<0.05$) in TMR 6 (20.60) than other TMRs evaluated. The *in vitro* methane production was significantly ($P<0.05$) lower in TMR 3 containing 2% *T. cordifolia* on DM basis than control TMR (TMR 1). Thus, *T. cordifolia* stem powder incorporated at the rate of 2% of DM in the diet of ruminants has the potential to reduce methane production.

HIGHLIGHTS

- There was no effect on nutrient digestibility and microbial mass production of TMRs containing graded levels of *Tinospora cordifolia*.
- Ammonia-N showed a declining trend with increasing level of *Tinospora cordifolia* in total mixed ration.
- The *in vitro* methane production was reduced ($P<0.05$) in TMR containing 2% *T. cordifolia* on DM basis.

Keywords: *In vitro*, *Tinospora cordifolia*, Methane

India is a country where livestock is extremely important to the economy. India ranks 1st with livestock population of about 535.78 million (National Livestock Census, 2019). *Tinospora cordifolia* (Giloy), also known as "Guduchi" in Sanskrit, belongs to the Menispermaceae family, it is considered to be one of the big, diverse and deciduous climbing shrub having flowers of greenish-yellow colour that bloom at higher elevations in various Asian countries including India, Sri Lanka and Myanmar. In Hindi, this plant is also known as 'giloya' that refers to the holy nectar that has kept celestial creatures perpetually young and saved them from old age according to Hindu mythological terms.

T. cordifolia contains important minerals and nutrients that are beneficial to both animal as well as human health (Rahal *et al.,* 2014). Since the discovery of a wide range of pharmacological properties, including immunomodulation, anticancer, hepatoprotective, and hypoglycemic effects, as well as the isolation of various compounds, including alkaloids, sesquiterpenoids, diterpenoids, phenolics, steroids, aliphatic compounds, and polysaccharides, *T. cordifolia* has attracted significant research interest (Singh and Chouhdary, 2017).

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Widespread resistance to synthetic feed additives has sparked interest in alternate use of these herbal plants. These herbal plants have various advantages over synthetic products, including the fact that they are inexpensive and so easily accessible to farmers. The synthetic medicine albendazole did not induce as much mortality as the aqueous and alcoholic fruit extracts of *T. cordifolia*, however, greater concentrations are more efficient than lower concentrations (Jogpal *et al.,* 2021). In addition, the herbal plants are biodegradable and are environmental friendly. These also have the advantage of avoiding toxic residues in milk and meat and are least likely to bio-accumulate in animal tissues. Thus, the present study was planned to determine the effect of graded levels of *T. cordifolia* stem powder on the *in vitro* nutrient utilization of total mixed ration and methane production in buffalo inoculum.

MATERIALS AND METHODS

Chemical analysis

An experiment was conducted at Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Total mixed ration (TMR) was formulated with roughage to concentrate ratio of 50:50 on DM basis. Table 1 provides the ingredient composition of the TMR used in this study. The sample of TMR was dried in hot air oven (60ºC, 24 h) and then grounded to pass through 1.0 mm sieve and stored in plastic containers for chemical estimation. The finely ground sample was analyzed for proximate (AOAC, 2005) and cell wall constituents (Van Soest *et al*., 1991). ADICP and NDICP was estimated and expressed as % of DM (Licitra *et al.,* 1996).

Table 1: Ingredient composition of TMR for *in vitro* study

Ingredient	Composition (% DM basis)
Maize fodder	50.0
Maize	21.0
Soybean meal	10.5
Wheat bran	7.5
Deoiled rice bran	6.5
Rice polish	3.0
Mineral mixture	1.0
Salt	0.5

In vitro evaluation

T. cordifolia stem powder *was* added to the total mixed ration (TMR) at the levels of 0%, 1%, 2%, 3%, 4% and 5% (on DM basis) to formulate six TMRs designated as TMR1, TMR2, TMR 3, TMR 4, TMR 5 and TMR 6, respectively which were used as substrates. Rumen fluid collected from male buffaloes fitted with rumen fistulae maintained on 2 kg conventional concentrate mixture (maize-38, mustard cake-15, SBM- 15, deoiled rice bran-12, wheat bran-10, rice polish- 7, mineral mixture-2, common salt- 1 part), 17 kg green fodder, 3 kg wheat straw and *ad lib* urea molasses mineral block (UMMB) were used as a donor for rumen liquor. The rumen contents were collected and then strained through 4 layered muslin cloth. The strained rumen liquor (SRL) was added to the buffer media (containing macro, micro mineral solutions, resazurin and a bicarbonate buffer solution prepared as per (Menke *et al*., 1979; Menke and Steingass, 1988) in 1:2 ratio. The medium was kept at 39ºC in a water bath and flushed with CO_2. Thirty ml of buffered rumen fluid was dispensed into 100 ml calibrated glass syringes containing 375 mg test feed under the anaerobic conditions. Syringes were sealed with rubber tube and plastic clip and placed in a water bath at 39ºC for 24 h. blank was also run in triplicate with each set which only contained buffered rumen liquor. After 24 h, the volume produced in each syringe was recorded and the contents of syringes were transferred to spoutless beaker, boiled with neutral detergent solution for estimating the OM and NDF digestibility (Van Soest and Robertson, 1988). The amount of gas produced was used to calculate ME. The partitioning factor (PF) was calculated as per the method described by France *et al*. (1993).

Determination of ME availability

The ME value of the substrate was calculated by using the following equation developed by Menke *et al*. (1979).

$$ME\ (kg) = 1.24 + 0.146\ G\ (ml/200\ mg\ DM) + 0.007\ CP + 0.0244\ EE$$

where,

ME = Metabolizable energy, MJ/kg DM; G = Net gas production, ml/200mg DM; CP = Crude protein, g/ kg; EE = Ether extract, g/kg.

STATISTICAL ANALYSIS

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Tukey's b.

RESULTS AND DISCUSSION

Chemical composition of TMR

The chemical composition of TMR used during *in vitro* analysis is given in Table 2. The TMR used for *in vitro* study contained concentrate mixture and maize green fodder in 50:50 ratio on w/w basis. The organic matter (OM) of TMR was 91.05%, crude protein 13.02% and ether extract 3.25%. The total ash percentage in concentrate was 8.95%, whereas the NDF and ADF were 51.50% and 32.15%. The hemicellulose was 19.35% and ADL was 5.15%. The total carbohydrate content was 74.78%, ADICP was 5.94% and NDICP was 8.81%.

In vitro evaluation

The net gas production (NGP, ml/g DM/24h) was 160.00, 156.41, 163.09, 156.86, 159.62 and 165.69 in TMRs 1, 2, 3, 4, 5 and 6, respectively (Table 3). There was no significant difference in NGP among the TMRs. The truly degraded substrate (TDS) showed no significant difference in TMR 1, 2, 3, 4, 5 and 6 with the numerical value of 313.69, 316.87, 323.14, 319.96, 326.24 and 329.41 mg. The partitioning factor (PF, mg/ml) in TMR 1, 2, 3, 4, 5 and 6 was 3.01, 3.00, 3.05, 3.07, 2.98 and 2.92, respectively. No significant difference was observed in PF in the present study. The OM digestibility (%) in the TMRs containing graded levels of *T. cordifolia* was similar to that of control TMR (TMR 1) with numerically highest value in TMR 3 (containing 2% *T. cordifolia* on DM basis).

The NDF digestibility (NDFD) and DM digestibility (DFD) varied non significantly among the TMRs with numerically highest value in TMR 3 (containing 2% *T. cordifolia* on DM basis) (Table 3). The microbial mass production (MMP) also followed the similar trend with numerically highest value in TMR 3 (53.54 mg). The short chain fatty acids varied non significantly (P>0.05) among the TMRs. The metabolizable energy (ME) availability ranged from 7.51 to 7.78 MJ/kg DM among the TMRs tested and varied non significantly (P>0.05).

Table 2: Chemical composition of TMR used for *in vitro* study

Item	OM	CP	EE	Total ash	NDF	ADF	Hemi-cellulose	ADL	ADICP	NDICP	TCHO
TMR	91.05	13.02	3.25	8.95	51.50	32.15	19.35	5.15	5.94	8.81	74.78

Table 3: Effect of level of *T. cordifolia* on the *in vitro gas* production and digestibility of nutrients in the TMRs (24 h)

Parameter	Level of *T. cordifolia* (%DM basis)						SEM
	0	1	2	3	4	5	
	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5	TMR 6	
NGP, ml/gDM/24h	160.00	156.41	163.09	156.86	159.62	165.69	1.54
TDS, mg	313.69	316.87	323.14	319.96	326.24	329.41	1.62
PF, mg/ml	3.01	3.00	3.05	3.07	2.98	2.92	0.03
OMD, %	57.60	55.98	59.46	57.49	56.93	57.80	0.37
NDFD, %	31.13	28.49	34.15	30.96	30.05	31.46	0.61
MMP, mg	48.69	47.02	53.54	51.96	48.79	46.86	1.26
EMMP, %	26.95	26.55	27.87	28.25	26.21	24.61	0.64
DMD, %	64.00	62.65	65.57	63.92	63.46	64.20	0.31
SCFA, mmole	0.71	0.69	0.72	0.69	0.70	0.73	0.01
ME, MJ/kg DM	7.61	7.51	7.70	7.52	7.60	7.78	0.04
NH_3-N, mg/dl	26.50^{c}	24.80^{bc}	23.30^{b}	23.80^{bc}	24.20^{bc}	20.60^{a}	0.56

Table 4: Methane production from fermentation of total mixed rations containing graded levels of *T. cordifolia* (24 h)

Parameter	Level of *T. cordifolia* (%DM basis)						SEM
	0	1	2	3	4	5	
	TMR1	TMR2	TMR3	TMR4	TMR5	TMR6	
CH_4, ml	14.57[b]	14.29[b]	10.33[a]	11.21[a]	11.70[a]	11.88[a]	0.48
CH_4, ml/100mg DM	7.25[b]	7.04[b]	5.04[a]	5.44[a]	5.59[a]	5.60[a]	0.26
CH_4, ml/100mg DMD	11.33[b]	11.24[b]	7.88[a]	8.30[a]	8.80[a]	8.73[a]	9.38
CH_4, ml/100mg OMD	15.05[b]	15.03[b]	10.46[a]	10.92[a]	11.70[a]	11.54[a]	0.58

Means bearing different superscripts in a row differ significantly (P<0.05).

The ammonia nitrogen in TMR 1, TMR 2, TMR 3, TMR 4, TMR 5 and TMR 6 was 26.50, 24.80, 23.30, 23.80, 24.20 and 20.60 mg/dl, respectively. The ammonia nitrogen level showed a declining trend from TMR 1 to TMR 6 with increasing level of *T. cordifolia* in the concentrate. The results are in tune with those of Dorantes-Iturbide *et al.* (2022) reported that lambs supplemented with polyherbal additive (based on *Tinospora cordifolia*, *Ocimum sanctum*, *Whitania somnifera, Andrographis paniculata* and *Azadirachta indica*) had lower ruminal ammonia nitrogen concentration, perhaps due to lower ruminal degradability of the protein consumed.

Methane production from fermentation of TMRs containing graded levels of *T. cordifolia* is given in Table 4. CH_4 (ml), CH_4 (ml/100mg DM), CH_4 (ml/100mg DMD) and CH_4 (ml/100mg OMD) varied significantly among the TMRs containing graded levels of *T. cordifolia*. The methane production (ml) was significantly (P<0.05) reduced in TMR 3 (containing 2% *T. cordifolia* on DM basis). Beyond that level, the methane production was similar in TMR 3, TMR 4 and TMR 5. Similar trend was observed in methane production (expressed as ml/100 mg DM, ml/100 mg DMD, ml/ 100 mg OMD) among the TMRs with significant decline in TMR 3.

CONCLUSION

In vitro screening of TMRs containing *T. cordifolia* stem powder at graded levels revealed that net gas production, nutrient digestibility (OM, NDF, DM) and microbial mass production were higher (P>0.05) in TMR 3 (containing 2% *T. cordifolia* on DM basis) whereas *in vitro* methane production was significantly (P<0.05) reduced at this level. Thus, *T. cordifolia* stem powder incorporated at the rate of 2% of DM in the diet of ruminants has the potential to reduce methane production.

NGP- Net gas production, TDS- Truly degraded substrate, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, NH_3-N-Ammoniacal nitrogen, R:C ratio was 60:40 on dry matter basis, all TMRs contained maize fodder, Means bearing different superscripts in a row differ significantly (P<0.05)

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