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## Effect of inclusion of *Tinospora cordifolia* stem powder on *in vitro* feed fermentation

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### Abstract

*Tinospora cordifolia* stands as a testament to the rich botanical heritage of traditional medicine. Its diverse array of bioactive compounds contributes to a myriad of health benefits, ranging from immune system support to anti-inflammatory and antioxidant effects. In the direction of recognize new ways to mitigate methane emission from ruminants. *T. cordifolia* was evaluated using *In vitro* gas production technique. The dry powder of *T. cordifolia* stem was included at the level of 0, 10, and 15% of substrate. The addition of *T. cordifolia* stem powder under *in vitro*-gas production system has not shown any effect on total gas and methane production, Dm degradability, NH<sub>3</sub>-N, VFAs concentration. However, protozoa count was significantly reduced.

**Keywords:** Methane, *Tinospora cordifolia*, feed fermentation, cattle rumen liquor

### Introduction

*Tinospora cordifolia*, commonly known as Giloy or Guduchi, is a versatile and potent medicinal plant deeply rooted in Ayurveda, the ancient Indian system of medicine. This herbaceous vine has been a staple in traditional medicine for centuries, celebrated for its wide range of therapeutic properties. It is a large deciduous climbing shrub found belongs to the Menispermaceae family and is native to the tropical regions of India, Myanmar, and Sri Lanka. The plant is characterized by its heart-shaped leaves and is known for its climbing nature. It thrives in a variety of soil types and is often found in the wild, clinging to trees and other supporting structures. Though every part of the plant has therapeutic value, the stem is the most frequently used part for the medicinal preparations. The medicinal properties of *T. cordifolia* can be attributed to its rich composition of bioactive compounds (Anjum *et al.*, 2023) [1]. Some of the key constituents include alkaloids, diterpenoid lactones, glycosides, steroids, and polysaccharides. These compounds work synergistically to impart the plant's therapeutic effects. Methane (CH<sub>4</sub>) production from ruminant animals, such as cattle, sheep, and goats, is a significant concern due to its environmental impact. Ruminants have a unique digestive system that involves fermentation in the stomach, particularly in the rumen, where microbes break down complex carbohydrates. During this process, methane is produced as a byproduct and released through belching. Efforts to address methane emissions from ruminant animals are important for sustainable agriculture and climate change mitigation. *T. cordifolia* as sole substrate was reported to reduce the methane production and protozoa count (Bhatta *et al.*, 2013; Wang *et al.*, 2017) [2, 5]. Therefore, the present study was aimed to evaluate the stem of *T. cordifolia* supplemented to improve the feed fermentation.

### Materials and Methods

In the present study, plants were sun-dried in order to reflect practical harvest condition and grounded. The effects of *T. cordifolia* stem powder on rumen fermentation were investigated by *in vitro* gas production method (Menke *et al.*, 1979) [3]. Cattle rumen liquor was used as inoculums. The substrate (200mg, concentrate mixture and wheat straw in 40:60 ratio) along with 30 ml buffered rumen liquor was incubated in syringes at 39 C for 24 h. the *T. cordifolia* stem powder was added @ 0, 10 and 15% of the substrate. After 24 h, total gas production VFAs. Another set was run for *in vitro* digestibility, where 400 mg substrate was taken with 40 ml of buffered rumen liquor.

### Results

Chemical composition of concentrate mixture, grass hay and TC used in *in vitro* studies  
The values of different proximate principles, cell wall and mineral constituents of concentrate

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mixture, local grass hay and *T. cordifolia* (TC) are presented in the Table 1. The values of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), total ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicelluloses (% dry matter basis), for concentrate were, 97.85, 93.93, 17.90, 3.63, 6.07, 65.20, 10.68, 2.92, 54.52, for

grass hay, 92.56, 93.62, 5.77, 2.44, 6.38, 40.37, 68.63, 43.94, 36.00, 24.69 and For TC, moisture (%), OM, CP, EE, TA, NDF, ADF, cellulose, hemicelluloses (% dry matter basis), 73.75, 92.97, 7.24, 1.18, 7.03, 54.35, 33.53, 22.67, 6.83, 1.13 respectively.

**Table 1:** Composition of concentrate, grass hay and *T. cordifolia* used *in vitro* studies

Attributes	Concentrate	Hay	<i>T. cordifolia</i>
DM	97.85±1.82	92.56±0.05	73.75±0.88
OM	93.93±0.61	93.62±0.38	92.97±0.53
CP	17.90±0.24	5.77±0.27	7.24±0.40
EE	3.63±0.15	2.44±0.33	1.18±0.19
TA	6.07±0.61	6.38±0.38	7.03±0.53
NFE	61.19 ±0.18	40.37±2.06	54.35±1.80
NDF	65.20±3.32	68.63±1.25	33.53±0.85
ADF	10.68 ±0.08	43.94±3.61	22.67±2.77
Cellulose	2.92±0.06	36.00±3.19	6.83±0.42
Hemicellulose	54.52±3.40	24.69±2.37	1.13±0.17
Ca	0.20±0.04	0.45±0.10	0.99±0.34
P	0.51±0.05	0.41±0.05	1.75±0.88

The *in vitro* gas (ml/g DM) and methane (ml/g DDM) production was similar in all the groups. The gas production ranged from 151 to 156 ml/g DM and methane production was 59.50, 58.36 and 56.78 in 0, 10 and 15% inclusion of *T. cordifolia* stem powder, respectively. The ammonia nitrogen (mmol/L), total volatile fatty acids and proportion of acetate, propionate and butyrate produced were similar with all the three inclusion levels of *T. cordifolia* medium indicating that the *in vitro* feed fermentation was not affected with any level

of *T. cordifolia* powder. There were gradual decreases in protozoa population in the fermented medium with the increasing level of *T. cordifolia*. At 15% inclusion level the protozoa count was significantly lower compared to control. Dry matter digestibility was 56.60, 55.35 and 55.82% at 0, 10 and 15% inclusion levels respectively, indicating that *T. cordifolia* stem powder did not hamper *in vitro* feed digestibility.

**Table 2:** Effect of inclusion of *T. cordifolia* on *in vitro* feed fermentation

Inclusion level	Gas production (ml/g DM)	Methane (ml/g DDM)	NH <sub>3</sub> -N (mmol/L)	Protozoa (10 <sup>4</sup> /ml)				IVTDMD (%)
					Acetate	Propionate	Butyrate	
0	152.43	59.50	13.0	2.4	59.32	31.92	12.46	56.6
10	154.58	58.36	12.0	2.2	59.35	30.9	12.95	55.35
15	151.57	56.78	2.04	2.04	60.36	29.7	13.88	55.82
SEM	1.17	0.11	0.09	0.09	0.50	0.44	0.40	0.79
P-value	0.994	0.630	0.041	0.041	0.348	0.077	0.208	0.798

Means bearing different superscript (a, b) in a column differ significantly ( $p < 0.05$ )

## Discussion

Chemical composition of concentrate mixture, grass hay and *T. cordifolia* the values of proximate principles and fibre fractions of concentrate mixture, grass hay was in normal range. The CP concentration of concentrate mixture and grass hay was 17.90 and 5.77 which was sufficient to meet the protein requirement of the animal. The CP content of *T. cordifolia* was 7.24, it contains sufficient amount of calcium (0.99%) and phosphorous (1.75%) The chemical composition of *T. cordifolia* stem powder was comparable with the values reported by Rahel *et al* (2014). The inclusion of *T. cordifolia* stems powder in the incubation medium at the levels of 0, 10 and 15% of the substrate could not influence *in vitro* feed fermentation including *in vitro* gas and methane production, concentration of metabolites like ammonia, VFA in the fermented medium. Though there was significant reduction in the protozoa count at 15% inclusion level but this reduction did not reflect in terms of methane production. The reason might be that though a portion of protozoa population is associated with the methanogens in the rumen but it is not always necessary that protozoa population reduction should be accompanied with methane inhibition. Low inclusion levels might be the reason for no effect of *T. cordifolia* on

feed fermentation.

## Conclusion

Inclusion of *T. cordifolia* stem powder @ 0, 10 and 15% of the substrate under *in vitro* gas production system had not exerted any effect on feed fermentation, however protozoa count was significantly reduced revealing antiprotozoal activity of *T. cordifolia* stem powder hence can be further evaluated for methane inhibition.

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