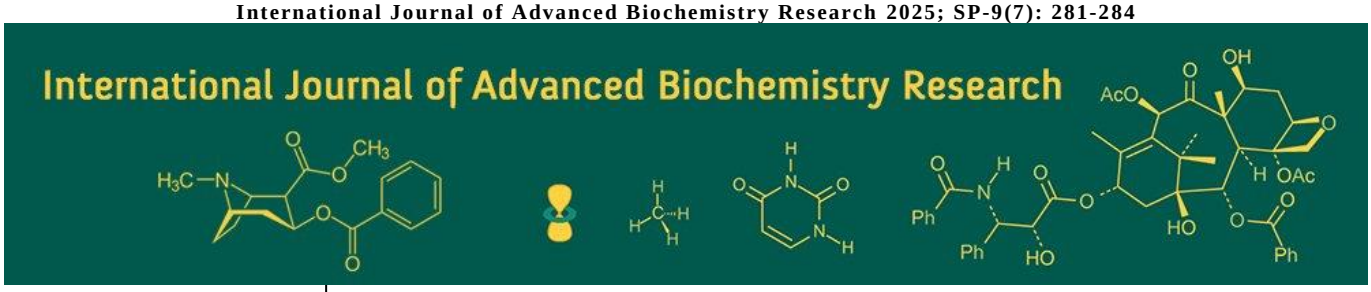

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(7): 281-284
www.biochemjournal.com
Received: 08-05-2025
Accepted: 12-06-2025

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Effect of dry leaves of *Senna gardneri* on *in vitro* dry digestibity and methane emission in cattle

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i7Sd.4820

Abstract
Methane emissions from ruminants, especially cattle, constitute a significant source of anthropogenic greenhouse gases creating considerable obstacles for achieving sustainable livestock farming. This investigation aims to assess the effectiveness of *Senna gardneri*, a tannin-containing plant, in reducing *in vitro* methane emissions and dry matter digestibility (DMD) in cattle. Dry *Senna gardneri* leaves were incorporated into total mixed rations (TMR) in varying concentrations from 0% to 8%, with a corresponding decrease in wheat straw content. Rumen fluid was collected from experimental cattle, processed and analyzed for methane production and DMD using gas chromatography. The results showed no significant variation in *in vitro* dry matter digestibility (IVDMD) compared to the control TMR. Methane emissions were notably reduced with Senna gardneri supplementation, with mean observed values of total gas production, CH4 (ml/100 mg of DM) and CH4 (ml/100 mg of DDM) recorded at 9.00, 1.17 and 2.39, respectively for 2% supplementation. These findings indicate that *Senna gardneri* shows promise as a promising anti-methanogenic feed additive to curb methane emissions without negatively impacting feed digestibility, thereby supporting more sustainable ruminant livestock production practices.

Keywords: *In vitro* digestibility, methane emission, SF_6 tracer technique & gas chromatography

Introduction
The emission of methane from ruminants, particularly cattle, represents a significant source of anthropogenic greenhouse gases, thereby posing a critical challenge for sustainable livestock production. Cattle are the highest daily emitters of methane among ruminants, contributing significantly to global greenhouse gas emissions. Methane emissions from livestock, particularly cattle, account for approximately 14-16% of global methane emissions.

In vitro methodologies provide a controlled experimental framework to investigate the efficacy of various feed additives in mitigating methane production within the rumen. *Senna gardneri*, a plant recognized for its bioactive compound known as a tannin, emerges as a potential candidate for such interventions. Condensed tannins (CTs) and hydrolysable tannins (HTs) have been identified as effective agents in reducing enteric methane (CH4) emissions in ruminants. These tannins interact with proteins and carbohydrates, thereby disrupting the microbial fermentation processes in the rumen responsible for methane production. Incorporating these tannins into ruminant diets presents a promising strategy for mitigating greenhouse gas emissions from livestock. The primary objective of this study is to evaluate the impact of Senna gardneri on *in vitro* methane emissions and dry matter digestibility when incorporated into cattle rumen fluid. Through this investigation, the study seeks to elucidate the potential of *Senna gardneri* in reducing methane emissions while maintaining or enhancing feed digestibility in ruminant livestock.

Materials and Methods
The present study was carried out at Animal nutrition research station, College of Veterinary Science and Animal Husbandry, Anand from mid of April to June. Dry leaves of *Senna gardneri* were added to the TMRs at level varying from 0 to 8% by deducting the level of wheat straw simultaneously.

Table 1: *In-vitro* study of total mixed ration with different levels of *Senna gardneri* (%)

Total mixed ration with different levels of *Senna gardneri*	Ingredients (%)		
	Wheat Straw	Concentrate Mixture	*Senna gardneri*
SG0 (40:60)	60	40	0
SG1 (40:59:1)	59	40	1
SG2 (40:58:2)	58	40	2
SG3 (40:57:3)	57	40	3
SG4 (40:56:4)	56	40	4
SG5 (40:55:5)	55	40	5
SG6 (40:54:6)	54	40	6
SG7 (40:53:7)	53	40	7
SG8 (40:52:8)	52	40	8

Rumen liquor was collected from an experimental cattle two hours prior feeding without watering in the morning using a stomach tube & strained through four layers of muslin cloth, resulting in Strained Rumen Liquor (SRL).
The SRL was kept at 39±1 °C in a thermos flask and flushed with CO_2 gas for further analysis. For *in-vitro* studies artificial saliva (McDougall buffer) was freshly prepared.

Determination of *in-vitro* digestibility and methane production
Feed samples were grinded using 1.0 mm screen. 500 mg & 200 mg samples were placed in 100 mL glass syringes for estimation of dry matter digestibility & methane production, respectively. Three syringes were kept as a blank. Fresh macro and micromineral solutions, buffer solutions & resazurin were combined in a flask and incubated at 39 °C with continually flushed CO_2 in the medium. Total 35 syringes were filled and put in a shaker water bath at 39 °C.
For *in-vitro* digestibility 48 hr incubation, each syringe's contents were filtered through a pre-weighed Gooch crucible, dried and weighed. Undigested leftovers in Gooch crucibles were oven dried at 70 °C for 24 hours, then cooled in desiccators and weighed to determine dry matter and *in-vitro* DMD (Menke *et al.*, 1979) [4].
After 24 hours of incubation, gas samples were directly injected into Gas Chromatography (GC) from each syringe and methane concentrations were compared to a reference methane sample. The column temperature was kept at 50ºC and nitrogen was employed as a carrier gas with a flow rate of 30 ml/min. Calibration was done with standard methane gas. The peak area was integrated using a Thermo Fisher integrator (Johnson *et al.* 1994) [3].

Statistical Analysis
The experimental data collected during the trial period was analyzed using the two-way analysis of variance (ANOVA) with method in SPSS16 through computer software as prescribed by Snedecor and Cochran (1994) [7].

Results and Discussion
The proximate composition and fiber fraction data of the prepared TMRs are detailed in the table 2 showcasing their nutritional content.
Table 3 illustrates the impact of *Senna gardneri* on *in vitro* dry matter digestibility. The parameters include total gas production (in ml), CH4 percentage, CH4 per 100 mg of digestible dry matter (DDM) and CH4 volume per 100 mg of dry matter (DDM) in table.4

Table 2: Chemical Composition and Fiber Fraction of the Total Mixed Ration

Parameter	T1
Crude protein	13.11
Ether extract	1.74
Crude fibre	26.35
Nitrogen-free extract	44.48
Total ash	14.31
Organic matter	85.69
Neutral detergent fibre	62.54
Acid detergent fibre	41.10
Cellulose	27.11
Hemicellulose	21.44
Calcium	1.27

***In vitro* dry matter digestibity**
The assessment of *in vitro* dry matter digestibility (IVDMD) revealed no significant differences (p<0.05) in comparison to the control total mixed ration (TMR). The inclusion of *Senna gardneri* at 5% and 6% levels in TMR exhibited IVDMD values of 50.35% and 50.65%, respectively. However, the minimum digestibility was recorded at a 7% inclusion level in TMR.
These results diverge from the findings of Patra *et al.* (2006) [5], Yogianto *et al.* (2014) [10], and Yuliana *et al.* (2014) [11], who reported significant reductions in IVDMD with the incremental inclusion of tannin-containing ingredients. Conversely, Chaudhari (2018) and Gosvami (2019) [18] documented significantly higher (p<0.05) *in vitro* DMD values compared to control (66.19%).

Table 3: ***in vitro*** Dry Matter Digestibility (DMD) of TMRs with different levels of *Senna gardneri*

Groups	*In vitro* DMD (%)
SG0 (40:60)	47.7±1.81
SG1 (40:59:1)	45.7±0.87
SG2 (40:58:2)	48.85±1.49
SG3(40:57:3)	47.75±1.99
SG4(40:56:4)	44.75±0.68
SG5(40:55:5)	50.35±2.23
SG6(40:54:6)	50.65±0.54
SG7(40:53:7)	44.05±3.53
SG8(40:52:8)	48.3±3.53

Means with different superscripts within a column differ significantly (p<0.05).

***In vitro* methane gas production**
The mean observed values of total gas production (ml), CH4 (ml/100 mg of DM) & CH4 (ml/100 mg of DDM) were 9.00, 1.17 & 2.39 for 2% *Senna gardneri* supplementation,

respectively. These values were significantly differed (p<0.05) as compared to control.

Our finding was in accordance with Pellikaan *et al.* (2011) [6], Soltan *et al.* (2012) [8], Yogianto *et al.* (2014) [10] & Yuliana *et al.* (2014) [11], who observed significantly (p<0.05) reduced CH4 emissions compared to control. while on contrary, Williams *et al.* (2011) [9] & Gosvami (2018) [18] indicated not significant alter *in vitro* methane production.

Table 4: *In-vitro* methane gas production (Total gas production (ml), CH4%, CH4 ml/100 mg DDM & CH4 ml/100 mg DDDM)

Groups	Total gas Production (ml)	CH4 (%)	CH4 ml/100 mg DDM	CH4 ml/100 mg DM
SG0	12.25^{abcd}±0.85	23.53^{a}±0.67	4.02^{de}±0.58	1.96^{abc}±0.28
SG1	10.50^{cd}±0.65	22.35^{ab}±0.59	3.55^{de}±0.69	1.62^{ab}±0.32
SG2	9.00^{d}±0.71	21.52^{b}±0.23	2.39^{e}±0.48	1.17^{c}±0.24
SG3	11.50^{bcd}±1.66	22.45^{ab}±0.81	5.27^{bcd}±0.94	2.52^{abc}±0.45
SG4	10.25^{d}±0.63	21.50^{b}±0.67	4.74^{cd}±0.14	3.16^{a}±1.10
SG5	14.00^{abc}±0.71	18.90^{c}±0.68	5.23^{bcd}±0.44	2.63^{abc}±0.22
SG6	15.75^{a}±1.80	19.80^{c}±0.60	6.09^{abc}±0.48	3.09^{bc}±0.24
SG7	14.50^{ab}±1.19	22.08^{ab}±0.10	6.78^{ab}±0.56	2.99^{bc}±0.35
SG8	15.75^{a}±1.93	21.87^{ab}±0.41	7.10^{a}±0.77	3.43^{a}±0.37
SE.m.	0.45	0.28	0.30	0.18
CD (0.05)	3.58	0.105	1.75	0.105
CV%	19.54	7.79	24.04	36.23

Means with different superscripts within a column differ significantly (p<0.05).

Conclusion

This study evaluated the effects of *Senna gardneri* on *in vitro* methane emissions and dry matter digestibility (DMD) in cattle. It was found that the inclusion of Senna gardneri with various level found non significant alter. The assessment of methane gas production for 2% *Senna gardneri* supplementation showed significant reductions in total gas production by 26% and methane emissions by 40% compared to the control, aligning with studies that observed reduced methane emissions. Overall, *Senna gardneri* demonstrated potential in reducing methane emissions while maintaining digestibility, warranting further research to optimize its use.

Declarations

The permission for animal experiments was granted by the Institutional Animal Ethics Committee (IAEC: 402/AN/2023).

Funding

The research grant was received from the project of Kamdhenu University, Gandhinagar.

Data Availability

The authors acknowledge that the data presented in this study will be available upon reasonable request.

Conflict of Interest

All the authors affirm that they have no conflicts of interest

Author Contributions

All the contributors contributed to the conception and planning of the study. Material preparation, data collection and analysis were performed by Dr. Jignesh H. Vansola, Dr. Paresh R. Pandya, Dr. Kalpesh K. Sorathiya & Dr. Minnat M. Patel. The first draft of the manuscript was written by Dr. Jignesh H. Vansola, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript

Acknowledgment

The authors are grateful to the Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Anand. I am thankful for providing the necessary facilities and financial support for undertaking this study.

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