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Effect of dry leaves of *Senna gardneri* on feed intake, body weight, nutrient digestibility, rumen parameters & methane emission in cattle

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Abstract
This study investigated the effects of *Senna gardneri* supplementation on methane emissions, nutrient intake and rumen fermentation in cattle. Fourteen adult cattle were randomly assigned to control (T_1) and treatment (T_2) groups with 7 in each, with the latter receiving 2% *Senna gardneri* in their total mixed ration (TMR) over a 70-day period. The results indicated no adverse effects on body weight, with similar weight gain in both groups. However, the T_2 group exhibited significantly lower dry matter intake (DMI) and crude protein intake (CPI). Despite these reductions, the digestibility coefficients of various nutrients remained unaffected. Notably, *Senna gardneri* supplementation enhanced rumen microbial activity, as evidenced by increased total volatile fatty acids (TVFAs) and ammoniacal nitrogen (NH_3-N) levels. Methane emissions were significantly reduced by 11% and dietary energy loss through methane was decreased by 19.28% in treatment (T_2) group. Furthermore, Feed costs were significantly lower in the T_2 group. These findings suggest that *Senna gardneri* can be effectively incorporated into cattle diets to mitigate methane emissions and reduce feed costs without compromising animal health, warranting further research on its impact on growth and production performance.

Keywords: *Senna gardneri*, methane emission, rumen fermentation, nutrient digestibility

Introduction

India had the largest livestock population globally with 535.78 million, reflecting a 4.6% increase from the 2012 census. Cattle make up 35.94% of this population, numbering around 192.49 million, with a growth rate of 0.83% (DADH, 2019) [6]. Methane, a major greenhouse gas, is 25 times more potent than carbon dioxide and contributes about 14% to global greenhouse gas emissions, with livestock, particularly cattle, responsible for 51% of methane emissions, exacerbating global warming (Kumari, 2019) [13].

Volatile fatty acids (VFAs) such as acetate, propionate and butyrate, produced during anaerobic digestion in the rumen, are crucial energy sources for animals but also generate methane (CH_4) and carbon dioxide (CO_2), released through eructation. Research on rumen microbial fermentation focuses on improving feed utilization, animal productivity, health and food safety, with methane production influenced by species, age, size, feed intake, type, quality and environmental conditions (Shibata, 2010) [28].

Plants containing secondary metabolites, especially tannins, exhibit significant antibacterial properties and can be used to suppress undesired bacteria in the rumen, thereby modifying the microbial ecosystem (Agarwal *et al.*, 2006) [1]. Tannins, found in hydrolysable and condensed forms, are increasingly being incorporated into ruminant diets to enhance the quality of consumable products and reduce methane and nitrogen emissions (Jeronimo, 2016; Carulla, 2005) [11, 3].

Senna gardneri, a species within the genus *Senna* (Leguminosae), is distributed across Brazil, India, Australia and Africa. It possesses numerous beneficial properties, including anti-allergic, anti-inflammatory, antioxidant, antibacterial, antimicrobial, analgesic, antiparasitic, insecticidal, antitumor, hepatoprotective and antifungal effects (Ricarte *et al.*, 2017) [27]. The leaf extracts of *Senna gardneri* have demonstrated significant anticancer activity, particularly against human colon and glioblastoma cancer cell lines (Silva *et al.*,

2016) [29]. This plant is rich in polyphenolic compounds, containing approximately 425g/kg on a dry matter basis, with tannins constituting 80% of the total polyphenolic content (Ricarte *et al.*, 2017) [27].

Tannins can bind with proteins, minerals and polysaccharides, forming complexes that affect nutrition and reduce feed intake by creating an unpleasant mouthfeel (Makkar, 2003; Lesschaeve & Noble, 2005) [17, 15]. Condensed tannins can enhance dietary protein absorption by binding proteins in the rumen and releasing them in the acidic abomasum and alkaline small intestine (Mueller-Harvey, 2006) [18]. Tannins also inhibit protozoa, methanogens and other hydrogen-producing bacteria, thereby reducing methane production (Patra and Saxena, 2010) [23]. Methanogens in the rumen primarily use hydrogen and CO_2 as substrates, so introducing compounds that inhibit their activity can reduce methane synthesis (Tavendale *et al.*, 2005) [31].

Materials and Methods

The present research was carried out on 14 cattle for 70 days at the Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Anand from mid of April to June with average body weight of 287.57±16.21 kg of both groups. The experimental animals were randomly assigned to two treatment groups, each with seven animals.

Treatment 1 consist of TMR feed with 60% roughage and 40% concentrate, which replace with 2% of *senna gardneri* by deducting deoiled rice bran form TMR in T_2. All experimental animals were provided with total mixed ration (TMR) to meet their nutritional requirements (ICAR, 2013) [10].

Observations Recorded

Feeding and Maintenance of Animals All experimental animals were fed TMR to meet their nutrient needs, as per ICAR (2013) [10]. Animals in the T_2 groups were fed TMR as per T_1 (Roughage: Concentrate; 60:40) but with additional supplement of 2% *Senna gardneri* in TMR. The animals were fed separately twice a day, in the morning and evening. Controlled exercise sessions were conducted for a duration of two hours in the morning and one hour in the afternoon, during which the animals had unrestricted access to clean and fresh drinking water. Prior to the commencement of the trial, all animals were dewormed using a broad spectrum anthelmintic.

Dry leaves of *Senna gardneri* were obtained from the Department of Medicinal and Aromatic Plants at Anand Agricultural University, Anand, Gujarat. *Senna gardneri* Contain 12% Tannin on dry matter basis.

Digestion Trial

A 6-day digestion trial was conducted to assess nutrient digestibility in experimental animals. Leftover feed from each experimental animal was collected, weighed and representative samples were stored for analysis. Similarly, labelled samples of TMR (Total Mixed Ration) were also kept to determine dry matter content. These dried samples were pooled over 6 days, ground through a 1.0 mm sieve and stored in airtight containers for further proximate and fibre analysis.

Faeces were collected and weighed daily, with samples taken for nitrogen and dry matter content estimation. Dried, pooled and ground samples were stored for further analysis. Records of TMR intake, leftovers and faeces output were maintained daily. The samples of different TMR offered, leftover and faeces of each animal were analyzed for proximate principles as per AOAC (2005) [2] and for fibre fractions as per Van Soest (1991) [32].

Rumen Parameters

Rumen liquor (150 ml) was collected at 0, 3 and 6 h of post feeding through a stomach tube against negative pressure created by a suction pump from each experimental animal (Lane *et al.*, 1968) [14]. The collected rumen liquor was strained through four layered muslin cloth and referred as Strained Rumen Liquor (SRL). The SRL was brought to the laboratory in a pre-warmed (39±1 °C) thermos flask for further analysis, Samples were estimated by using various analysis methos viz. Rumen pH analyzed by using pH meter, TVFAs estimated using steam distillation method using Markham micro-distillation assembly. The samples of SRL were analyzed for ammonia-N by Pearson and Smith, 1943 and total-N by Kjeldahl's method. After centrifugation of SRL, Soluble-N in supernatant of SRL after was estimated by Kjeldahl's method, while similarly non-protein-nitrogen estimated except Trichloroacetic acid added of SRL.

Methane Production Estimation Using Sulphur Hexafluoride (Sf6) Tracer Technique

Permeation tubes were filled with SF6 gas and kept at 39 °C for 4 weeks. The release rates were monitored and standardized. Afterward, 14 tubes were inserted into the rumen of each cattle. Breath samples were collected daily for three consecutive days using PVC canisters from cattle fed control and *senna gardneri* supplemented TMR.

Breath samples were analyzed for CH4 and SF6 using a Gas Chromatograph. The emission rate of CH4 was calculated using the permeation tube emission rate and the CH4/SF6 concentration ratio. Energy loss as CH4 was calculated as a percentage of gross, digestible and metabolizable energy intake. (Johnson *et al.*, 1994) [12]

Cost of Feeding

The cost of feeding for cattle was calculated from the records of daily feed consumption and by considering the procurement price of feed ingredients, green fodder and *Senna gardneri* in cattle.

Statistical Analysis

The experimental data collected during the trial was analyzed using the independent t-test and two-way ANOVA in SPSS16 software, following the guidelines by Snedecor and Cochran (1994) [30]. Treatment and period means were compared using the Duncan Multiple Range Test, considering a p-value of less than 0.05 as statistically significant.

Results And Discussion

Proximate composition of different feed ingradients

The proximate composition and fibre fractions (NDF and ADF) of the TMR and green grass offered to experimental cattle are presented in Table 1. TMR-1 (T_1; control), TMR-2 (2% dry leaves of *senna gardneri* in T_1) and green which contained 13.11, 12.77 and 4.06% crude protein, 1.74, 1.73 and 2.43% ether extract, 26.35, 26.08 and 31.16%

crude fibre, 44.48, 44.74 & 48.76% NFE and 14.31%, 14.67% & 13.59% total ash, respectively.

Table 1: Proximate composition (%) and fibre fractions of TMR & Green (on DMB)

Parameter	T_1	T_2	Green
Crude protein	13.11	12.77	4.06
Ether extract	1.74	1.73	2.43
Crude fibre	26.35	26.08	31.16
Nitrogen-free extract	44.48	44.74	48.76
Total ash	14.31	14.67	13.59
Organic matter	85.69	85.33	86.41
Neutral detergent fibre	62.54	63.52	67.54
Acid detergent fibre	41.10	41.18	46.14
Cellulose	27.11	26.83	31.63
Hemicellulose	21.44	22.34	21.40
Calcium	1.27	1.24	0.90

Body Weight

The initial average body weights of the animals in both the T_1 and T_2 treatment groups were identical at 287.57 kg. By the end of the experiment, the final body weights were maintained at 317.87 kg for the treatment group and 311.55 kg for the control group**.** Similar findings have been noted in previous research studies by (Okoruwa and Omoragbon (2017) [21]; Pathak *et al.* (2017) [22]; Ningrat *et al.* (2018) [19]; Chaudhari (2018) [4]; Ibrahim *et al.* (2024) [9].

Nutrients Intake

There was significant (p>0.05) reduction in average DMI, CPI, DCPI & TDNI in *Senna gardneri* supplemented group as it depicted in Table 2.

These findings were accompanied by researcher Ojha *et al.* (2010) [20]; Magnani *et al.* (2023) [16] with related to DMI, while it was higher in tannin supplemented group in Poornachandra *et al.* (2019) [25] experiment.

CPI & DCPI were notably higher in previous experiment conducted by (Zhang *et al.* (2019)) [34]. Poornachandra *et al.* (2019) [25] observed no effect of tannin supplementation in CPI.

On contrary to our findings, Prajapati (2016) [26], Chaudhari (2018) [4] & Gosvami (2018) [8] observed non significant difference in TDNI.

Table 2: Body weight, Feed intake & Nutrient intake

Parameters	T_1	T_2
Body weight (Kg)	302.51±6.45	300.04±5.93
DMI (Kg/d)	7.02b±0.09	6.14a±0.14
DMI (kg/100 kg BW)	2.35b±0.05	2.04a±0.04
CPI (g/day)	754.83b±9.22	619.30a±20.91
CPI (g/100 kg BW)	252.96b±5.86	204.65a `±7.19
DCPI	539.84b±6.59	454.75a±15.35
DCPI (g/100 kg BW)	180.92b±4.19	150.28a±5.28
TDNI (Kg/day)	3.57b±0.05	3.18a±0.06
TDNI (Kg/100 kg BW)	1.20b±0.03	1.05a±0.03

* a, b, superscripts in a column differ significantly (P<0.05)

Digestibility of Nutrients

The mean digestibility of nutrient remained unaltered no significant change was noted, it showed numerically higher crude protein & crude fibre digestibility. Similar to our finding, Dey and De (2014) [7] noted non significant alter in nutrient digestibility.

Table 3: Average values of digestibility coefficient (%) of nutrients

Attributes (%)	T_1	T_2	SE.m	CV%
DMD	54.03±1.03	53.97±0.54	0.56	3.86
OMD	59.22±0.92	58.17±0.51	0.52	3.22
CPD	71.52±0.67	73.43±0.57	0.50	2.57
CFD	45.23±1.67	47.08±1.23	1.03	8.04
EED	62.71±3.97	60.54±2.81	2.36	13.78
NFED	61.24±1.08	61.47±0.68	0.59	3.62
NDFD	45.20±1.17	46.59±0.79	0.71	5.55
ADFD	36.52±1.50	37.20±0.06	0.79	7.98
HCD	62.21±2.23	64.32±1.40	1.30	7.68
CD	56.62±0.98	56.07±0.68	0.59	5.42

* a, b, superscripts in a column differ significantly (P<0.05)

Rumen Parameters

Rumen pH remained unaltered during 3h & 6h in treatment group while it was found non significant differ between treatment and control. Average TVFAs values were significantly (p>0.05) higher in *Senna* supplemented group, Poornachandra *et al.* (2019) [25] & Chaudhari (2018) [4] found significant effect on TVFAs production.

There was significantly (p<0.05) higher concentration of ammonia-N in treatment groups than control and it was higher at 3 h than 0 h and 6 h in both control & treatment groups with 30.6% increase in NH3-N concentration in *Senna gardneri* supplemented group. Chaudhari (2018) [4] obtained similar result for NH3-N. Similar result obtained for total N as it was numerically higher in treatment group as compared of control one and it was higher at 3 h than 0 h and 6 h in both control & treatment groups. Chaudhari (2018) [4] & Gosvami (2019) [8] obtained similar result. Both NPN & TCA precipitable N were found non significant while soluble N was statistically higher in T_2 as compare to control.

Table 4: Periodical Mean values of Ruminal pH, TVFA, Ammonical-N, total-N, TCA precipitable nitrogen, NPN and Soluble-N concentration in different treatment groups

	Hours of sampling			
Treatments	P1 (0 hr)	P2 (3 hr)	P3 (6 hr)	Mean±S.E.
Rumen pH				
T_1	7.37±0.04	7.14±0.06	7.24±0.06	7.25±0.04
T_2	7.23±0.07	7.12±0.07	7.12±0.1	7.16±0.05
TVFAs				
T_1	9.71±0.29	10.12±0.17	9.60±0.22	9.81a±0.14
T_2	10.07±0.32	11.54±0.39	12.54±0.38	11.38b±0.26
Ammonical N				
T_1	10.90±0.55	14.20±0.60	9.60±0.44	11.57a±0.43
T_2	16.4±0.66	18.4±1.27	15.2±1.34	16.67b±0.67
Total N				
T_1	70.4±4.40	108.60±12.24	72±3.50	83.67±5.17
T_2	71±3.11	117±9.47	85.8±5.42	91.27±4.75
TCA Precipitable Nitrogen				
T_1	47.6±2.33	80.4±10.22	47.2±2.19	58.4±4.32
T_2	43.8±2.15	66.44±6.10	54.60±4.5	54.93±2.94
NPN				
T_1	22.80±1.09	29.60±0.91	25.20±1.28	25.87±0.76
T_2	21.60±1.15	30.80±1.83	25.60±2.17	26.00±1.16
Soluble N				
T_1	22.8±2.87	28.2±3.35	24.8±2.66	25.27a±1.71
T_2	27.2±1.57	50.6±4.88	31.2±4.07	36.33b±2.66

* a, b, superscripts in a column differ significantly (P<0.05)

Enteric Methane Emission from Experimental Cattle & Feed Cost

Methane Emissions

The average methane emission in T_1 & T_2 groups as g/day was 195.86 & 174.20, while as g/kg DMI it was 45.30 & 37.26, respectively. Daily Methane emission in T_2 group reduced significantly ($p<0.05$) by 11% compared to T_1 group. Similarly, when expressed as g/kg DMI methane emission reduced significantly ($p<0.05$) by 17.74 % cattle in T_2 group.

Methane emission in terms of DDMI g/kg was 84.37 in control & 68.82 in *Senna gardneri* supplemented group which is non significantly differed from control but, it was 18.43% less as compared to control.

These results are consistent with those of previous research by (Woodward *et al.* (2004); Cieslak *et al.* (2012); Prajapati (2016); Malik *et al.* (2017); Chaudhari (2018; Gosvami (2019); Magnani *et al.* (2023)) [33, 5, 26, 25, 4, 8, 16].

Table 5: Methane emission, Energy loss through emission & Feed cost

Parameters	T_1	T_2
CH4 (g/day)	195.86^b±6.89	174.20^a±7.04
CH4 g/kg DDMI	84.37±3.40	68.82±3.08
CH4 g/kg DMI	45.30^a±1.58	37.26^b±1.78
Energy loss as CH4 (% GEI)	22.50^b±0.79	18.16^a±0.87
Energy loss as CH4 (% DEI)	26.88^b±0.94	21.69^a±1.04
Energy loss as CH4 (% MEI)	33.19^b±1.16	26.66^a±1.27
Total Feed cost	5455.94^b±51.100	5110.50^a±104.62
Daily Feed cost	77.94^b±0.73	73.01^a±1.49

* a, b, superscripts in a column differ significantly ($P<0.05$)

The daily energy loss in the form of CH4 as % of GEI was statistically different in T_1 & T_2 group was 22.50 & 18.16, respectively. It was 19.28 % of less GEI loss compared to control TMR. Similar outcomes have been observed in both Energy loss as CH4 (% DEI) & (% MEI).

Average total feed cost was Rs.5455.94 & 5110.50, while average daily feed cost was Rs.77.94 & 73.01 in T_1 & T_2 group, respectively. There was significant difference observed in both total & daily feed cost among the groups as due to lower intake in treatment group. These observations mirror those reported in earlier research by Dey and De (2014) [7]; Chaudhari (2018) [4]; Gosvami (2019) [8].

Conclusions

Senna gardneri supplementation had no negative impact on animal body weight and significantly decreased dry matter and nutrient intake. Digestibility of nutrient remained unchanged. Furthermore, *Senna gardneri* significantly increased total volatile fatty acids (TVFA), ammoniacal nitrogen (N) and soluble nitrogen (N), indicating improved rumen microbial activity, although the increase in average total nitrogen concentration in strained rumen liquor (SRL) was not significant. Additionally, *Senna gardneri* supplementation led to a significant reduction in daily methane emissions by 11% and dietary energy loss through methane by 19.30%. The daily feed cost and total feed cost over 70 days were also significantly lower in the *Senna gardneri* supplemented group. Therefore, it can be concluded that incorporating dry *Senna gardneri* leaves at a 2% inclusion rate in the total mixed ration (TMR) of cattle is beneficial, resulting in a significant reduction in methane emissions without any adverse effects on the animals.

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. Minnat M. Patel, Dr. Paresh R. Pandya & Dr. Kalpesh K. Sorathiya & Dr. J. H. 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

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