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Dr. Nayan H Raval
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Paresh R Pandya
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Kalpesh K Sorathiya
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Ashish N Patel
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Safi G Vahora
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Corresponding Author:
Dr. Nayan H Raval
Animal Nutrition Department, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Effect of replacing wheat straw with urad straw in TMR on rumen fermentation and enteric methane emission in adult cattle

Nayan H Raval, Paresh R Pandya, Kalpesh K Sorathiya, Ashish N Patel and Safi G Vahora

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Abstract
The present study was carried out to evaluate the effect of replacing wheat straw with urad straw in total mix ration on rumen fermentation & methane emission in adult cattle. The study was conducted on 15 adult cattle for 70 days by splitting them into three groups of five each. In control group (T_1) 70% wheat straw and 30% concentrate mixture were fed to experimental cattle. Animals in group T_2 were fed 35% wheat straw, 35% urad straw and 30% concentrate mixture and in group T_3 animals were fed 70% urad straw and 30% concentrate mixture. The cattle were fed total mix ration as per ICAR (2024) feeding standard to fulfill their nutrient requirement. Rumen fermentation parameters were also statistically ($p>0.05$) similar among the treatment groups except that total volatile fatty acids (TVFA) and total nitrogen concentration were significantly ($p<0.05$) higher in group T_3. Daily average enteric methane emission was analyzed by using sulphur hexafluoride tracer technique. Methane emission significantly ($p<0.05$) reduced in T_3 and T_2 by 13.58% and 8.71%, as compared to T_1. Similarly, methane emission as g/kg DMI & DDMI was significantly ($p<0.05$) lower in T_3 and T_2 by 14.93 & 21.23% and 11.92% & 14.42%, respectively, as compared to T_1. The daily energy loss in the form of CH_4 as % of GEI was significantly ($p<0.05$) lower in T_3 & T_2 by 22.77 & 17.61 as compared to T_1. Similarly, energy loss in the form of CH_4 as % of DEI & MEI was also significantly ($p<0.05$) decreased by in 22.71 & 23.94% in T_3 and 17.54 & 18.95% T_2, respectively, as compared to T_1. These findings indicate that replacing wheat straw with urad straw improves rumen fermentation and mitigate methane emissions due to its anti-methanogenic property, thereby supporting more sustainable ruminant livestock production practices.

Keywords: Methane emission, energy, SF_6 tracer technique

Introduction

Global warming, intensified by rapid urbanization and industrialization, is significantly driven by methane emissions, particularly from ruminant livestock through enteric fermentation (Yohannes, 2015) [18]. Methane is 28 times more potent than carbon dioxide in terms of global warming potential (Panchauri *et al.*, 2014), with India's cattle alone contributing around 4.92 Tg of CH_4 annually (ICAR-NIANP, 2018) [6]. The widespread use of low-quality cereal straws like wheat and paddy, necessitated by climatic challenges and inconsistent feed availability, results in poor nutrient intake and reduced productivity in livestock. As a solution, integrating legume straws such as urad (black gram), moong, and moth into animal feed offers superior nutritional value and better palatability (Jadhav, 2000), while effectively utilizing readily available agricultural byproducts from pulse cultivation (Win *et al.*, 2021) [17].

Legume straws not only enhance feed efficiency but also contain tannins known for their anti-methanogenic properties, aiding in the reduction of enteric methane emissions and promoting environmentally sustainable livestock farming. Studies have shown improved feed value when legume straws are combined with rice straw (Soe *et al.* 2007) [15] or incorporated into complete feed blocks (Venkateswarlu *et al.* 2013) [16], supporting their potential as viable cereal straw alternatives. Recognizing this, the current study aims to assess the impact of replacing wheat straw with urad straw in total mixed rations under *in vivo* conditions, emphasizing the need for continued research in optimizing legume-based feeding strategies to improve both animal productivity and climate resilience.

Materials and Methods

Evaluation of methane emission using Sulphur hexafluoride (SF_6) Tracer Technique

Standardizing SF_6 release rate

About 20 permeation tubes were filled with pure (99.9%) sulphur hexafluoride (SF_6) gas under liquid nitrogen. Permeation tubes containing SF_6 gas were kept at 39 °C in water bath for 4 weeks period. The release rate of SF_6 from each permeation tubes was monitored weekly. Release rate of SF_6 from each permeation tube during 4 weeks period are given in Table 3.4. After standardizing the release rate, 15 permeation tubes containing known release rate of SF_6 were inserted in the rumen of each experimental cattle through mouth.

Canisters and halters

Breath samples were collected from each experimented cattle using evacuated PVC canisters with 2-2.5" ID and 200 psi pressure, PVC end caps (10 kg/cm3 pressure), and a 90° elbow. A short (4") piece of 14" teflon tubing was connected to the valve, with a female 14" fast connect on the upstream end to allow attachment to the halter.

Measurement of enteric methane emission

Breath samples from all experimented animals fed with the above T_1, T_2, T_3 treatments were collected in canisters daily for four days and analysed for CH_4 and SF_6 gases using a Gas Chromatograph instrument equipped with a Porapack N column for CH_4 and a molecular sieve 5A for SF_6 analysis (Johnson *et al.*, 1994) [7]. The column temperature was kept constant at 50 °C, and nitrogen was employed as a carrier gas at a flow rate of 30 ml/min. The CH_4 emission rate was estimated as the product of the permeation tube emission rate and the sample concentration ratio of CH_4 to SF_6.The energy content of CH_4 was calculated to be 13.34 Kcal/g. The National Research Council (1989) [5] was used to compute the amount of energy lost as CH_4 as a percentage of gross energy intake, digestible energy intake, and metabolizable energy intake. The rate of methane emission will be computed as follows:

$$Q\ CH_4 = Q\ SF_6 \times (CH_4)/(SF_6)$$

Where,

CH_4 = Methane emission rate (g/min)

Q SF_6 = Known release rate of SF6 from permeation tube (g/min)

CH_4 = Methane concentration of collected sample in canister (µg/m^3)

SF_6 = SF_6 concentration of collected sample in canister (µg/m^3)

Rumen liquor 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) [9]. The collected rumen liquor was strained through four layered muslin cloth and referred as Strained Rumen Liquor (SRL). The pH of SRL was determined immediately after collection using portable digital pH meter. The concentration of TVFA was determined in SRL by the steam distillation method, using Markham micro-distillation apparatus. The samples of SRL were analysed 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 was estimated by Kjeldahl's method, while similarly non-protein-nitrogen estimated after Trichloroacetic acid added to SRL.

The data generated during the experiment were analyzed by two & one-way analysis of variance (ANOVA) using OPSTAT method as prescribed by Snedecor and Cochran, (2014) [14].

Results and Discussion

Enteric Methane Emission

The average enteric methane emission in T_1, T_2 and T_3 groups as g/day was 187.89, 171.53 and 162.37, respectively. The results showed that, in comparison to the T_1 group, the methane emission in groups T_3 and T_2 was significantly ($p<0.05$) lower by 13.58% and 8.71%, respectively. Lower methane emission in T_2 and T_3 is due to improved nutrient digestibility and rumen fermentation. It is also due to the tannins found in urad straw, which have antimethanogenic characteristics.

Similar pattern of methane reduction was recorded when the results were compared on g/kg of DM and DDM intake basis. The average enteric methane emission in T_1, T_2 and T_3 groups as g/kg DMI & DDMI was 25.25 & 49.58, 22.24 & 42.43 and 21.48 & 39.05, respectively. Statistically enteric methane emission as g/kg DMI & DDMI was significantly ($p<0.05$) reduced by 14.93% & 21.23% in T_3 and 11.92% & 14.42% in T_2 groups, respectively, as compared to T_1 group.

Consistent with our findings, Malik and Singhal (2016) [10] revealed that when buffalo calves were given lucerne fodder-based TMR instead of wheat straw-based TMR, the amount of methane emission was significantly ($P<0.05$) decreased by 21.38% (g/day). Similarly, Gosvami (2019) [4] discovered that the group of bullocks fed lucerne straw containing TMR had 17.71% lower enteric methane emission (g/day) than the group fed wheat straw containing TMR, which was considerably ($p<0.05$) lower. Chaudhari (2018) [2] also discovered that feeding crossbred calves TMR based on pigeon pea straw significantly ($p<0.05$) decreased methane emission by 10.53% (g/day) as compared to wheat straw containing TMR. Contradictory to our findings, Bhavsar (2023) [1] found lower ($p>0.05$) methane emission (g/day) in lactating crossbred cows fed with wheat & moth straw based TMR as compared to only wheat straw based TMR. Joshi (2023) [8] revealed lower ($p>0.05$) methane emission (g/day) in lactating crossbred cows fed with chickpea & wheat straw based TMR and wheat straw based TMR.

Table 1: Enteric Methane Emission from experimental cattle

Attributes	Groups		
	T_1	T_2	T_3
Methane Emission (g/day)	$187.89^a \pm 5.07$	$171.53^b \pm 5.82$	$162.37^b \pm 3.87$
Methane Emission (g/kg DMI)	$25.25^a \pm 0.61$	$22.24^b \pm 0.73$	$21.48^b \pm 0.53$
Methane Emission (g/kg DDMI)	$49.58^a \pm 1.57$	$42.43^b \pm 1.40$	$39.05^b \pm 0.9$

a, b Mean having different superscript in a row differ significantly ($p<0.05$)

Energy Intake and Loss as CH_4

The daily average value of gross energy intake (GEI), energy loss as CH_4 and loss of methane as % of GEI in T_1, T_2 and T_3 groups are presented in table 2.

The daily average of gross energy intake was significantly (p<0.05) higher in group T_3 (21.67 Mcal/day) and T_2 (21.46 Mcal/day) by 11.76% and 10.68%, respectively, as compared to T_1 (19.39 Mcal/day) group. The average of digestible energy intake was significantly (p<0.05) higher in group T_3 (18.14 Mcal/day) and T_2 (17.97 Mcal/day) by 11.77% and 10.72%, respectively, as compared to T_1 (16.23 Mcal/day). The daily average of metabolizable energy intake was significantly (p<0.05) higher in group T_3 (14.91 Mcal/day) and T_2 (14.68 Mcal/day) by 14.25% and 12.49%, respectively, as compared to T_1 (13.05 Mcal/day).

Daily energy loss as CH_4 (Mcal/day) was 2.17, 2.29 and 2.51 in T_3, T_2 and T_1, respectively, which was significantly (p<0.05) lower in T_3 and T_2 by 13.55% and 8.76%, respectively, as compared to T_1. Therefore, the daily energy loss in the form of CH_4 as % of GEI was also significantly lower (p<0.05) in T_3 (10) and T_2 (10.67) as compared to T_1 (12.95). Daily energy loss in the form of CH_4 as % of DEI was also significantly (p<0.05) lower in T_3 (11.94) and T_2 (12.74) as compared to T_1 (15.45). The daily energy loss in the form of CH_4 as % of MEI was also significantly (p<0.05) lower in T_3 (14.53) and T_2 (15.61) as compared to T_1 (19.26).

Table 2: Energy intake and loss as CH_4

Attributes	Groups		
	T_1	**T_2**	**T_3**
Gross Energy intake (Mcal)	19.39^a±0.15	21.46^b±0.1	21.67^b±0.14
Digestible energy intake (Mcal)	16.23^a±0.13	17.97^b±0.08	18.14^{b}±0.12
Metabolizable energy intake (Mcal)	13.05^b±0.12	14.68^a±0.08	14.91^a±0.09
Energy loss as methane	2.51^a±0.07	2.29^b±0.08	2.17^b±0.05
As % of GEI	12.95^a±0.38	10.67^b±0.37	10.00^b±0.23
As % of DEI	15.45^a±0.45	12.74^b±0.44	11.94^b±0.28
As % of MEI	19.26^a±0.58	15.61^b±0.55	14.53^b±0.34

a, b Mean having different superscript in a row differ significantly (p<0.05)

Consistent to our findings, Chaudhari (2018) [2] observed significantly reduction (p<0.05) in energy loss in the form of CH_4 as % of GEI by 15.95% in crossbred calves fed pigeon pea straw based TMR, as compared to wheat straw based TMR. Also, Gosvami (2019) [4] reported significantly (p<0.05) lower energy loss in the methane as % of GEI by 15.66% in bullock fed lucerne straw containing TMR as compared to wheat straw based TMR. In contrast, Gosvami (2019) [4] reported non-significant (p>0.05) lower energy loss in the methane as % of DEI & MEI in bullock fed lucerne straw containing TMR by 15.66% & 14.66%, as compared to wheat straw containing TMR. Chaudhari (2018) [2] also discovered significant (p<0.05) reduction in energy loss in the form of CH_4 as % of MEI in crossbred calves fed pigeon pea containing TMR by 16.4%, as compared to wheat straw containing TMR.

Rumen Parameters

The average values of rumen pH, nitrogen fractions and total volatile fatty acids (TVFA) are depicted in Table 3. Total nitrogen and Total volatile fatty acid concentration of rumen liquor was significantly (p<0.05) higher in T_3 and T_2 group respectively, as compared to T_1. All other parameters were at par among the groups within normal range. Similarly, significantly (p<0.05) higher TVFA concentration observed in gram straw (Chauhan, 2016) and pigeon pea (Chaudhary, 2018) supplemented groups. Contradict to our results, some studies reported non-significant (p>0.05) difference in TVFA concentration (Bhavsar, 2023; Gosvami, 2019; Prajapati, 2016) [1, 4]. Joshi (2023) [8] revealed significantly (p<0.05) higher total N and ammonia N concentration in the lactating cattle group fed with chickpea straw based TMR. Whereas, Gosvami (2019) [4] & Prajapati (2016) [13] reported non-significant (p>0.05) difference in total N and ammonia N in a group fed with legume straw based TMR.

Table 3: Rumen fermentation parameters in crossbred cows

Attributes	T_1	T_2	T_3	SEM	P value
pH	7.41±0.02	7.45±0.04	7.49±0.05	0.035	0.238
Total N	76.35^a±2.04	79.15^{ab}±2.11	80.55^b±2.5	1.18	0.042
NH_3 N	10.50±0.69	11.11±0.53	11.06±0.54	0.39	0.481
TCA-N	45.27±1.54	46.29±1.66	46.67±1.54	0.91	0.53
Soluble N	31.08±1.23	32.85±1.31	33.88±1.92	1.30	0.312
NPN	29.31±1.33	27.07±1.01	26.70±1.54	0.89	0.087
TVFA	9.82^a±0.2	9.99^a±0.38	11.48^b±0.56	0.232	<0.001

a, b Mean having different superscript in a row differ significantly (p<0.05); NPN, non-protein nitrogen; TVFA, total volatile fatty acids

Conclusions

Inclusion of urad straw @ 35% and 70% in TMR (70:30) by replacing wheat straw significantly (p<0.05) reduced enteric methane emission by 8.71% & 13.58% and energy loss as CH_4 8.76% & 13.54%, respectively. It significantly (p<0.05) improved Total N and TVFA concentration in strained rumen liquor. Overall results of the study suggested that replacing wheat straw with urad straw in TMR significantly (p<0.05) reduced methane emission and improves rumen fermentation in adult cattle.

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