

Original Research Article

METHANE MITIGATION IN CROSSBRED BULLOCKS BY UREA TREATED WHEAT STRAW & LUCERNE STRAW BASED TMR

ABSTRACT

Present experiment was conducted to study the effect of feeding lucerne straw and urea treated wheat straw based TMR on nutrients intake, digestibility of nutrients, rumen parameters, rumen microbial protein synthesis and enteric methane emissions in crossbred bullocks. Experiment was conducted on 15 crossbred bullocks for 50 days using *Completely Randomized Design*. The animals in control group (T₁) were fed TMR with 70% wheat straw and 30% concentrate. The animals in experimental group T₂ were fed TMR with 35% urea treated wheat straw, 35% wheat straw and 30% concentrate whereas, animals in T₃ group were fed TMR with 35% lucerne straw, 35% wheat straw and 30% concentrate. The intakes of nutrients and rumen parameters were not affected by the treatments. Digestibility coefficients of CP, EE & HC did not differ significantly among the groups. Similarly, digestibility coefficients of DM & NFE did not differ significantly between T₁ & T₂ group but significant reduction reported in T₃ group. CF, NDF, ADF & Cellulose digestibility was significantly higher in T₂ group, whereas significant reduction noted in NDF, ADF & Cellulose digestibility in T₃ group, as compared to T₁ group. Rumen microbial protein synthesis improved by 17.22% ($p>0.05$) in T₂ group but almost similar value reported in T₃ group, as compared to control group. Average daily methane emission in T₂ and T₃ group reduced significantly by 16.20% and 17.71%, as compared to T₁ group. The energy loss in the form of CH₄ as % of GEI in T₂ and T₃ groups reduced numerically by 11.10% and 15.66% ($p>0.05$), as compared to T₁ group. Hence, inclusion of urea treated wheat straw and

lucerne straw in wheat straw based TMR helped in reducing enteric methane emissions in crossbred bullocks.

Key words: Lucerne straw, Urea treatment, Methane emission, Ruminants, Greenhouse gas.

INTRODUCTION

Livestock sector in India contributes about 50% of the total methane (CH₄) emissions which is a potent greenhouse gas, of which enteric fermentation contributes >49% [11]. The per capita methane production is relatively higher in India than the values reported from developed countries [3,14] and this can be attributed to the poor quality roughages and feeds available to the animals. Over a wide range of diets, enteric CH₄ emissions from ruminant livestock account for 2 to 12% of gross energy intake [12]. Methane emission from enteric fermentation, apart from its association with environmental problem, is also representing a certain amount of energy loss from the animals [6]. It is therefore essential to develop various feeding strategies that simultaneously mitigate methane emission and increase the efficiency of energy utilization. Legume forages have been shown to decrease CH₄ production in ruminants, which is often explained by the low fiber content, high dry matter (DM) intake, faster rate of passage from the rumen and in some cases, the presence of condensed tannins (CT) [3]. Some legumes also contain saponins which has anti-protozoal effect. Lucerne (*Medicago sativa*) fodder a fair source of saponins¹⁵ and grown traditionally on wide scale in India, might be used for mitigation of methane in ruminants fed without any additional input [17]. Urea treatment of cereal straws enhances the quality of straw in terms of increased nitrogen content, improved palatability and digestibility of straw. Thus, the present study was undertaken to evaluate the effect of partial replacement of wheat straw based TMR with lucerne straw and urea treated wheat straw on nutrient intake, digestibility, rumen parameters, microbial protein synthesis and CH₄ emissions in crossbred bullocks.

MATERIALS AND METHODS

On farm study was conducted at Anand Agricultural University, Anand on 15 crossbred bullocks for 50 days using *Completely Randomized Design*. Animals in control group (T₁) were fed total mixed ration (TMR) containing 30% concentrates and 70% wheat straw. The animals in experimental group T₂ were fed TMR with 35% urea treated wheat straw, 35% wheat straw and 30% concentrate whereas, animals in T₃ group were fed TMR with 35% lucerne straw, 35% wheat straw and 30% concentrate. The quantity of TMR offered twice daily i.e. morning and evening to meet the nutrient requirements [10] (Table 1).

Table 1: Ingredients & Chemical composition of total mixed rations

Parameters	Groups		
	T ₁	T ₂	T ₃
Ingredients composition (%)			
Wheat straw	70	35	35
Urea treated wheat straw	0	35	0
Lucerne straw	0	0	35
Maize	5	7	10
Soyabean meal	10	7	0
De-oiled rice bran	5	6	9
Molasses	9	9	10
Mineral mixture	1	1	1
Chemical composition (% on DM basis)			
DM	93.37	93.79	91.34
Crude protein (CP)	9.79	10.56	9.56
Ether extract (EE)	2.54	2.44	2.27
Crude fibre (CF)	26.57	29.86	33.00
Total ash (TA)	13.57	13.66	10.60
Nitrogen free extract (NFE)	47.53	43.48	44.58
Acid insoluble ash (AIA)	7.31	5.15	3.37
Neutral detergent fibre (NDF)	55.92	64.36	62.17
Acid detergent fibre (ADF)	34.65	39.92	39.25
Cellulose (C)	27.88	30.52	30.55
Hemicellulose (HC)	25.26	24.44	22.92

Acid detergent lignin (ADL)	4.35	5.50	6.88
Calcium (Ca)	1.15	1.04	1.35
Phosphorus (P)	0.50	0.50	0.48

Individual feeding of all the bullocks was followed. The bullocks were housed in sheds with proper ventilation, flooring and tying arrangements with facility of individual feeding. They were let loose daily (except during the period of digestion trial) in an open paddock, for two hours in the morning and two hour in the afternoon under controlled conditions for exercise and to access fresh wholesome drinking water. Deworming of all the crossbred bullocks was carried out using broad spectrum anthelmintic before initiation of the experiment.

The daily feed intake was recorded for each experimental animals during the entire feeding trial. A digestion trial was conducted at the end of experimental period to determine digestibility of the nutrients. The arrangement for quantitative collection of faeces was made during trial period of 7 days. A proper record of feed consumed, refusal and faeces voided by each crossbred bullocks was maintained during the entire trial period. Representative samples of feed consumed, refusal & faeces were taken for proximate² and fibre fractions [24] analyses.

Samples of rumen liquor (150 ml) were collected from individual bullock at 0 (before feeding), 2, 4 & 6 h post feeding through a stomach tube against negative pressure created by a suction pump. The pH of strained rumen liquor (SRL) was determined immediately after collection using portable digital pH meter. After pH determination, 1.0 mL of saturated HgCl₂ solution was added to each sample to stop microbial activity. The samples of SRL were analyzed for ammonia-N and total-N by Kjeldahl's method. Soluble-N in supernatant of SRL after centrifuging was estimated by Kjeldahl's method and non-protein nitrogen estimated by Trichloro-acetic acid precipitation of SRL and estimating the N content of

supernatant by Kjeldahl's method. Total volatile fatty acids (TVFAs) concentration was determined by the steam distillation method using Markham micro-distillation apparatus.

Urine samples (100 mL) were collected from individual bullocks for three consecutive days and assayed for allantoin, uric acid and creatinine [25]. Urine samples preserved with sufficient quantity of 1.87 mol/L H₂SO₄ to maintain pH<3.0. Purine derivatives (PD) were measured using Spectrophotometer based on the fact that excretion of creatinine is constant throughout a day, therefore, creatinine was used as an internal marker for estimation of PD [5]. Daily excretion of creatinine was considered as 0.98 mmol/kg W^{0.75} and microbial N supply was calculated from the daily urinary PD excreted⁹.

Breath samples from each experimental bullock were collected daily for three consecutive days in canisters to estimate enteric CH₄ emissions using a sulphur hexafluoride (SF₆) tracer technique [13]. A small permeation tube containing a known release rate of SF₆ gas was inserted into each experimental bullock through mouth. All breath samples were analysed in triplicate using a Gas Chromatograph instrument fitted with a Porapack N column for CH₄ and molecular sieve 5A for SF₆ analysis. Column temperature was maintained at 50°C and nitrogen was used as a carrier gas with flow rate of 30 mL/min. The methane emission rate was calculated as: $Q_{CH_4} = Q_{SF_6} \times (CH_4)/(SF_6)$, where Q_{CH_4} ; methane emissions rate (g/min), Q_{SF_6} = known release rate of SF₆ from permeation tube (g/min), CH_4 =methane concentration of the collected sample in a canister (μg/m³) and SF_6 =SF₆ concentration of collected sample in a canister (μg/m³). Energy content of CH₄ was considered as 13.34 Kcal/g. Loss of energy in the form of CH₄ as % of gross energy intake, digestible energy intake and metabolizable energy intake was calculated.

The cost of feeding for bullocks was calculated from the records of daily feed consumption and by considering the procurement price of feeds and fodder used for feeding of bullocks.

Data were analysed by the analysis of variance as per the methods of Snedecor and Cochran (1994), with the help of SPSS and WASP software programme.

RESULTS AND DISCUSSION

Ingredient and chemical composition of TMRs used for is presented in Table 1. Intake of DM, CP, DCP & TDN in all three groups was statistically similar (Table 2). Results indicate that lucerne straw and urea treated wheat straw based TMRs have non-significant effect on DMI. The results are in agreement with earlier findings [21]. Similarly, non-significant intake of DM, CP & TDN reported although DCP intake was significantly higher in earlier study [4]. However, significant improvement has been reported in intake of DM, CP, DCP & TDN in crossbred heifers fed urea treated wheat straw containing diet, as compared to untreated counterpart [8].

Digestibility coefficients of DM, CP, EE, NFE & Hemicellulose (HC) did not differ significantly among the groups. But, CF, NDF, ADF & Cellulose digestibility was significantly higher in T₂ group which may be due to better microbial activity on account of readily available source of nitrogen (Table 2). This is further supported by higher microbial protein synthesis in T₂ compared to T₁. However, significant reduction noted in NDF, ADF & Cellulose digestibility in T₃ group as compared to T₁ group. Though the results are unexpected, but may be due to higher passage rate from the rumen giving less time for digestibility of fiber fractions. Similar to our findings, non-significant effect of legume straw based TMR on digestibility of CP, EE, CF & HC have been reported in cattle [21,4]. Similarly, non-significant DMD reported in earlier study supplemented urea treated rice straw compared untreated group although CPD, NDFD & ADFD was significantly higher [7].

The rumen parameters also revealed non-significant difference among the treatments (Table 2). Similar results obtained when compared legume straw with cereal straw in earlier studies [19,21]. However in contrast with this study, significantly higher TVFA & NH₃-N

concentration was noted in crossbred dairy cows fed urea treated rice straw as compared to untreated rice straw [7].

Table 2: Effect of Urea treated wheat straw & Lucerne straw based TMR on Nutrient intake, Digestibility & Rumens parameters

Parameters	Groups		
	T ₁	T ₂	T ₃
Nutrient Intake			
DMI (kg/day)	8.21 ± 0.40	8.41 ± 0.63	8.45 ± 0.11
CPI (kg/day)	0.75 ± 0.04	0.83 ± 0.07	0.76 ± 0.01
DCPI (kg/day)	0.50 ± 0.04	0.57 ± 0.05	0.51 ± 0.05
TDNI (kg/day)	4.07 ± 0.21	4.27 ± 0.38	4.01 ± 0.05
Digestibility coefficients (%)			
DM (%)	57.16 ^a ± 1.16	56.89 ^a ± 0.93	52.66 ^b ± 0.34
CP (%)	66.93 ± 2.06	69.52 ± 3.24	68.55 ± 1.03
EE (%)	64.85 ± 4.82	70.14 ± 5.42	62.87 ± 2.46
CF (%)	55.27 ^b ± 1.21	61.70 ^a ± 1.06	53.18 ^b ± 1.46
NFE (%)	63.67 ^a ± 1.41	60.22 ^{ab} ± 1.63	56.46 ^b ± 0.94
NDF (%)	54.30 ^b ± 1.69	59.37 ^a ± 1.32	49.45 ^c ± 0.44
ADF (%)	47.83 ^b ± 1.89	57.18 ^a ± 2.08	42.60 ^c ± 0.59
Hemicellulose (%)	63.24 ^b ± 2.10	62.88 ^b ± 3.25	60.91 ^b ± 0.78
Cellulose (%)	60.23 ^b ± 1.68	66.53 ^a ± 1.51	53.37 ^c ± 0.22
Rumens Parameters			
pH	6.80 ± 0.04	6.82 ± 0.06	6.77 ± 0.01
TVFA (mmol/dl)	10.90 ± 0.20	10.36 ± 0.46	10.38 ± 0.18
Total Nitrogen (mg/dl)	57.12 ± 1.54	62.44 ± 4.29	62.58 ± 3.42
Ammonia – N (mg/dl)	13.37 ± 1.00	12.95 ± 0.91	11.41 ± 0.76
Non Protein Nitrogen (mg/dl)	17.36 ± 0.81	16.38 ± 1.00	16.52 ± 1.30

Soluble Nitrogen (mg/dl)	15.75 ± 0.60	15.61 ± 0.75	14.21 ± 0.72
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^{abc}Means with different superscripts in row for a parameter differ significantly (P<0.05)

Microbial protein synthesis:

The data of various urine parameters and rumen microbial protein synthesis are presented in Table 3. The average rumen microbial protein synthesis in T₁, T₂ and T₃ groups was 83.31, 97.66 and 81.65 g/day, respectively. The results were at par however, rumen microbial protein synthesis increased by 17.22% in T₂ group as compared to control group which may be due to urea treated straw and better digestibility of nutrients in T₂. Significantly higher microbial protein synthesis (8.16% & 37.44%) was reported in buffalo & crossbred calves fed legume straw based TMR, as compared to wheat straw based TMR, respectively [19,4]. In crossbred dairy cows also higher microbial protein synthesis (P<0.05) has been reported on urea treated rice straw, as compared to untreated rice straw [7].

The results of methane emission are delineated in Table 3. Average daily methane emission in T₂ and T₃ group reduced significantly by 16.20% and 17.71%, respectively, as compared to T₁ group. Similarly when expressed as g/kg DMI, CH₄ emission reduced significantly by 19.75% in T₃ group, while non-significantly in T₂ group by 10.32%, as compared to T₁ group. Methane emission (g/kg DDMI) in T₂ & T₃ groups also reduced numerically by 9.93% & 12.92%, respectively as compared to T₁ group. The results revealed that inclusion of urea treated straw and legume straws in ration of crossbred bullocks helps in methane mitigation successfully. This is due to the better digestibility of nutrients and more microbial protein synthesis in rumen as methane emission and rumen microbial protein synthesis are inversely related [21]. In lucerne straw containing group, the methane mitigation may be due to higher tannins and saponin content which is anti-methanogenic. Similar findings have been reported by various researchers. Similarly, Lucerne fodder based TMR feeding significantly reduced methane emission by 12% (49.55 vs. 44.29 g/day) and

30% when expressed in g/kg DMI (17.38 vs.12.20 g/kg DMI) in crossbred calves, as compared to wheat straw based TMR [16]. Significant reduction in enteric methane emission by 6.39% (g/day) and 6.65% (g/kg DMI) also reported in buffalo and 7.79% (g/day) and 9.04% (g/kg DMI) in crossbred cattle, on legume straw based TMR, as compared to wheat straw based TMR [19, 21].

Table 3: Effect of Urea treated wheat straw & Lucerne straw based TMR on Rumen microbial protein synthesis, Methane emission & Economics of feeding

Parameters	Groups		
	T ₁	T ₂	T ₃
Rumen microbial protein synthesis			
Allantoin (mmol/l)	0.267 ± 0.03	0.287 ± 0.01	0.310 ± 0.14
Uric acid (mmol/l)	1.70 ± 0.27	1.93 ± 0.13	2.13 ± 0.40
Creatinine (mmol/l)	1.37 ± 0.17	1.38 ± 0.08	1.69 ± 0.05
PDC index	111.13 ± 7.29	128.40 ± 8.17	108.73 ± 14.08
Total PD excreted (mmol/day)	108.91 ± 7.14	125.83 ± 8.01	106.56 ± 13.80
Absorbed purine (mmol/day)	114.6 ± 8.26	134.33 ± 9.23	112.3 ± 16.09
Intestinal flow of microbial nitrogen supply (g/d)	83.31 ± 6.01	97.66 ± 6.71	81.65 ± 11.70
Enteric Methane emission			
CH ₄ emission (g/day)	347.81 ^a ± 13.34	291.45 ^b ± 11.96	286.20 ^b ± 20.10
CH ₄ emission (g/kg DMI)	42.94 ^a ± 1.43	38.51 ^{ab} ± 2.62	34.46 ^b ± 2.03
CH ₄ emission (g/kg DDMI)	75.23 ± 2.74	67.76 ± 4.65	65.51 ± 4.13
GE intake (Mcal/day)	23.63 ± 1.15	22.82 ± 2.21	22.89 ± 0.26
Energy loss as CH ₄ (Mcal/day)	4.64 ^a ± 0.18	3.89 ^b ± 0.16	3.82 ^b ± 0.27
Energy loss as CH ₄ (% of GEI)	19.73 ± 0.78	17.54 ± 1.36	16.64 ± 1.02
Energy loss as CH ₄ (% of DEI)	23.57 ± 0.93	20.96 ± 1.62	19.88 ± 1.22
Energy loss as CH ₄ (% of MEI)	28.58 ± 1.19	25.37 ± 2.03	24.39 ± 1.51
Economics of feeding			
Daily Feed Cost (Rs.)	58.84 ± 3.15	58.10 ± 4.64	50.14 ± 0.73

^{abc}Means with different superscripts in row for a parameter differ significantly (P<0.05)

Daily gross energy (GE) intake was similar in all three groups (Table 3). However, daily energy loss through CH₄ (Mcal/day) was 4.64, 3.89 and 3.82 in T₁, T₂ and T₃, respectively where significant reduction noted by 16.16% and 17.67 % in T₂ and T₃ than T₁.

The energy loss through methane when calculated as % of GE intake was found less by 11.10% and 15.66%. Similar trend was observed for energy loss through methane as % of DE and ME intake. The results are in agreement with many findings. Similar to this finding, reduction of 7.15%, 9.57% & 15.95% in energy loss through methane as % of GE intake has been reported ($p < 0.05$) in buffalo, crossbred cattle & crossbred calves, respectively, fed legume straw based TMR as compared to wheat straw based TMR [19,21,4]. Energy loss through methane reduced by 20.34% as percent of gross energy intake in sheep fed ammonia treated wheat straw, as compared to untreated counterpart, which supports the present findings [18].

The economics of feeding was calculated based on the records of TMR consumption and considering the actual price of feeds and fodder used to prepare TMR during entire experimental period. Average daily feed cost was Rs.58.84, 58.10 and 50.14 in T₁, T₂ and T₃ group, respectively indicating non-significant differences among the groups (Table 3). Here we can see that average daily feed cost was almost similar in T₁ & T₂ group while 14.78% reduction observed in T₃ group as compared to T₁ group. Also we can decrease the ration of the animal equivalent to energy saved through methane mitigation & thus cost of feed will decrease.

CONCLUSIONS

The results suggested that incorporation of 35% urea treated wheat straw or lucerne straw replacing wheat straw in TMR with ratio of 70:30 for roughage: concentrate reduces daily methane emission as well as energy loss through methane and by 16.20 & 17.71 %, respectively in crossbred bullocks. The dietary energy loss saved can be used by the animals for physiological functions like health, production and reproduction etc.

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