Original Research Article

METHANE MITIGATION IN CROSSBRED BULLOCKS BY UREA TREATED WHEAT STRAW & LUCERNE STRAW BASED Total Mixed Ration

ABSTRACT

Present experiment was conducted to study the effect of feeding lucerne straw and urea treated wheat straw based TMR (Total Mixed Ration) 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 (T_1) were fed TMR with 70% wheat straw and 30% concentrate. The animals in experimental group T_2 were fed TMR with 35% urea treated wheat straw, 35% wheat straw and 30% concentrate whereas, animals in T_3 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 crude protein (CP), ether extract (EE) and hemicellulose (HC) did not differ significantly among the groups. Similarly, digestibility coefficients of dry matter (DM) and nitrogen-free extract (NFE) did not differ significantly between T_1 & T_2 group, and significant reduction was reported in T_3 group. Crude fibre (CF), NDF (neutral detergent fibre), ADF (acid detergent fibre) and cellulose digestibility was significantly higher in T_2 group, whereas significant reduction noted in NDF, ADF and cellulose digestibility in T_3 group, as compared to T_1 group. Rumen microbial protein synthesis improved by 17.22% ($p>0.05$) in T_2 group, but almost similar value was reported in T_3 group, as compared to control group. Average daily methane emission in T_2 and T_3 group reduced significantly by 16.20% and 17.71%, as compared to T_1 group. The energy loss in the form of methane (CH_4) as % of gross energy intake (GEI) in T_2 and T_3 groups reduced numerically by 11.10% and 15.66% ($p>0.05$), as compared to T_1 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 (CH_4) 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 CH_4 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 CH_4 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 saponins[15] 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 CH_4 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 (T_1) were fed total mixed ration (TMR) containing 30% concentrates and 70% wheat straw. The animals in experimental group T_2 were fed TMR with 35% urea treated wheat straw, 35% wheat straw and 30% concentrate whereas, animals in T_3 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 of anthelmintics before initiation of the experiment.

The daily feed intake was recorded for each experimental animal 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 bullock was maintained during the entire trial period. Representative samples of feed consumed, refusal and faeces were taken for proximate [2] and fibre fractions [24] analyses.

Samples of rumen liquor (150 ml) were collected from individual bullock at 0 (before feeding), 2, 4 and 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 $HgCl_2$ 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 H_2SO_4 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 excreted [9].

Breath samples from each experimental bullock were collected daily for three consecutive days in canisters to estimate enteric CH_4 emissions using a sulphur hexafluoride (SF_6) tracer technique [13]**.** A small permeation tube containing a known release rate of SF_6 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 CH_4 and molecular sieve 5A for SF_6 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: $QCH_4 = QSF_6 \times (CH_4)/(SF_6)$, where QCH_4; methane emissions rate (g/min), QSF_6= known release rate of SF_6 from permeation tube (g/min), CH_4=methane concentration of the collected sample in a canister (µg/m^3) and SF_6=SF_6 concentration of collected sample in a canister (µg/m^3). Energy content of CH_4 was considered as 13.34 Kcal/g. Loss of energy in the form of CH_4 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. However, CF, NDF, ADF and cellulose digestibility was significantly higher in T_2 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 T_2 compared to T_1. However, significant reduction noted in NDF, ADF and cellulose digestibility in T_3 group as compared to T_1 group. Though the results are unexpected, but may be due to higher passage rate from the rumen giving less time for digestibility of fibre 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 and NH_3-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 & Rumen 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
Rumen 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

^{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 T_1, T_2 and T_3 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 T_2 group as compared to control group which may be due to urea treated straw and better digestibility of nutrients in T_2. Significantly higher microbial protein synthesis (8.16% and 37.44%) was reported in buffalo and 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 T_2 and T_3 group reduced significantly by 16.20% and 17.71%, respectively, as compared to T_1 group. Similarly when expressed as g/kg DMI, CH_4 emission reduced significantly by 19.75% in T_3 group, while non-significantly in T_2 group by 10.32%, as compared to T_1 group. Methane emission (g/kg DDMI) in T_2 and T_3 groups also reduced numerically by 9.93% and 12.92%, respectively as compared to T_1 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 [insert citations]. 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$^{b\,\pm}$ 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 CH_4 (Mcal/day) was 4.64, 3.89 and 3.82 in T_1, T_2 and T_3, respectively where significant reduction noted by 16.16% and 17.67 % in T_2 and T_3 than T_1. The energy loss though 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% and 15.95% in energy loss thorough 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 T_1, T_2 and T_3 group, respectively indicating non-significant differences among the groups (Table 3). Here we can see that average daily feed cost was almost similar in T_1 and T_2 group while 14.78% reduction observed in T_3 group as compared to T_1 group. Furthermore, we can decrease the ration of the animal equivalent to energy saved through methane mitigation and 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 and 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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