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RESEARCH ARTICLE

Antimethanogenic effects of soybean straw and seaweed (*Sargassum johnstonii*) based total mixed ration in crossbred cows

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Abstract: Ruminants are responsible for 50 % of anthropogenic methane production. Methane has higher global warming potential which is 28 times more than CO_2. The present investigation was conducted to evaluate effect of supplementation of soybean straw and seaweed (*Sargassum johnstoni*) in the ration of dairy cows on methane mitigation and production performance of animals. Six crossbred lactating cows received three treatments in Switch Over Design for 135 days in 3 periods of 45 days each. The treatments consist of T1: TMR with compound concentrate mixture and wheat straw (60:40); T2: TMR with 20 % each of wheat straw and soyabean straw and 60 % compound concentrate mixture and T3: TMR (60:40) with seaweed (*Sargassum johnstonii*). Methane emission was estimated by SF_6 tracer technique. The DM and nutrient intake were at par in T1 and T2, but was significantly low in T3. The milk yield observed was also less in T3 than T1 and T2 which were at par. The methane production was significantly reduced by 20.79 % and 16.53% ($P<0.05$) in soybean (T2) and seaweed (T3) supplemented group as compared to control group. The loss of dietary energy through methane also significantly decreased in T2 and T3 than T1. The results indicated that supplementation of soybean straw and seaweed in TMR has tremendous potential for methane mitigation in crossbred cows. However, further research is needed to ameliorate the feed and nutrient intake by increasing palatability of seaweeds based TMR.

Keywords: Feed efficiency, Methane, Milk yield, Seaweeds, Soybean straw, TMR

Introduction

Methane (CH_4) is a principal source of greenhouse gas emission from enteric fermentation in ruminants (Opio, et al. 2013). In India, per capita methane emission is higher as compared to developed countries (Johnson et al. 2002) due to poor quality roughages and less productivity of animals. Indian livestock emits 9.253 Tg enteric methane annually. Cattle is the largest producer with 4.92 Tg, contributing to 56% of total emission in the country (ICAR-NIANP, 2018). In addition, ruminants loose between 2 to 12 % of the dietary gross energy in the form of CH_4 (Johnson and Johnson, 1995). Different strategies are used to mitigate methane emission of which dietary manipulation is easy and economical. It involves a selection of feeds with secondary metabolites like tannins and saponin (tree leaves, legume straws, babul pods, lucerne etc.), feeds rich in halogenated compounds like seaweeds and processing of feeds and fodder into total mixed ration etc. for abatement in rumen methane emission (Beauchemin et al. 2020).

Seaweeds are macroalgae which are classified as brown algae (*Phaeophyceae*), red algae (*Rhodophyceae*) and green algae (*Chlorophyceae*) on colour basis (Makkar et al. 2016). More than 7500 km of Indian coastline is potential environmental province for growth of seaweeds in Tamil Nadu, Gujarat coast, Lakshadweep and Andaman Nicobar Islands. Recent studies have revealed high antimethanogenic potency of seaweeds in *in vitro* (Maia et al. 2016; Molina-Alcaide et al. 2017; De la Moneda et al. 2019) and *in vivo* with different species (Li et al. 2018; Roque et al. 2019; Kinley et al. 2020). Seaweed supplementation also has positive results on performance of lactating animals with increasing milk yield (Bendary et al. 2013; Singh et al. 2015). Legume forages also help in methane mitigation in ruminants, which is often explained by the presence of condensed tannins, low fibre content, high dry matter intake, and quicker rate of

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passage from the rumen (Beauchemin et al. 2008). Legume straw supplementation significantly reduces production of methane in *in vitro* and *in vivo* compared to cereal straws (Prajapati, 2016; Sherasia et al. 2018; Jasvantgiri, 2019). Milk yield also increases when 50 % of cereal straw is replaced by legume straw in dairy animals (Islam et al. 2020). Present study aimed to investigate effect of soybean straw and seaweed (*Sargassum johnstonii*) based Total Mixed Ration (TMR) on methane mitigation and performance of crossbred dairy cows.

Materials and Methods

Experimental design and animals

The experiment was conducted on 6 HF × Kankrej (50:50) crossbred dairy cows randomly allotted to three treatments in a 3 × 3 Switch Over Design. There were three periods each of 45 days duration. During the experiment, one animal suffered from mastitis and hence removed from experiment. So, results were compiled from the data of 5 animals in each period. Permission was granted by Institutional Animal Ethics Committee (IAEC/310/ANRS/2019) to conduct the experiment. *Sargassum johnstonii* dried biomass was procured from local vender in Veraval coast in Gujarat. It was included @ 8% inclusion level based upon *in vitro* studies (Katwal et al. 2020). The animals received three treatments viz; T1: TMR with compound concentrate mixture and wheat straw (60:40); T2: TMR with 20% each of wheat straw and soyabean straw and 60% compound concentrate mixture and T3: TMR (60:40) as T1 but DORB is replaced with 8 % seaweeds (*Sargassum johnstonii*). Molasses is added at different level to make TMR isocaloric and iso nitrogenous. TMR was prepared in TMR machine with grinding and mixing the different ingredients represented in Table 1.

DM and nutrient intake

The nutrient requirement of animals was met as per ICAR (2013) feeding standards. Daily feed intake was recorded by weighing the feed offered and the leftover of each animal. The DM and nutrient intake were calculated from feed intake. The nutrient composition of TMR is given in Table 2.

Milk yield and composition

Daily milk yield of cows was recorded during morning and evening at time of milking. 4% Fat corrected milk (FCM) and fat protein corrected milk (FPCM) yield were calculated as per NRC (2001):

$$\text{FCM (kg)} = 0.4\,(\text{Milk Yield}) + 15\,(\text{Fat Yield})$$

$$\text{FPCM (kg)} = \text{Milk production (kg)} \times (0.337 + 0.116 \times \text{fat\%}) + 0.06 \times \text{protein \%}$$

Table 1 Ingredient composition (%) of total mixed rations

Ingredient	T1	T2	T3
Wheat straw	40.00	20.00	40.00
Soyabean straw	0.00	20.00	0.00
Compound Con. Mixture	46.0	40.0	46.0
DORB	8.00	10.0	0.00
Seaweed	0.00	0.00	8.00
Molasses	5.00	9.00	5.00
Mineral mixture with salt	1.00	1.00	1.00
Feed cost (₹)	1467.44	1468.6	1613.04

Table 2 Chemical composition of total mixed rations (% on DM Basis)

Parameters	T1	T2	T3
Dry matter	91.98	92.00	91.90
Organic matter	85.74	85.72	81.98
Crude Protein	12.46	12.51	12.80
Ether Extract	3.78	3.91	3.70
Crude Fibre	25.70	25.54	24.82
Total Ash	14.26	14.29	17.94
Acid Insoluble Ash	8.69	5.58	8.34
Nitrogen Free Extract	43.8	43.7	40.7
NDF	66.28	64.55	63.24
ADF	43.04	41.41	42.45
Hemicellulose	23.24	23.12	20.78
Cellulose	23.07	23.54	27.48
Lignin	7.13	7.90	7.39

About 100 ml of milk samples were collected in clean plastic bottles in the morning and evening at fortnightly intervals and were mixed. Analysis of milk composition was done using Lactoscan milk analyser. Milk samples were analysed for Total Solid (%), Fat (%), Solid Not Fat (%), Protein (%), Lactose (%) and Salt (%).

Methane emission and energy balance

Methane emission was measured by collecting breath samples for three consecutive days using sulphur hexafluoride (SF_6) tracer technique. Small permeation tubes (PT) were filled with pure (99.9%) SF_6 gas under liquid nitrogen. After standardizing the SF_6 release rate, PT was inserted into the rumen of experimental animals through mouth. The breath samples of all crossbred cows were analysed for CH_4 and SF_6 gases, using Thermo Fisher ceres 800 Gas Chromatography fitted with Porapack N column for CH_4 and molecular sieve 5A for SF_6 analysis (Johnson et al. 1994). The column temperature was maintained at 50 ºC and nitrogen was used as a carrier gas, with flow rate of 30 ml/min. Energy content of CH_4 was considered as 13.34 Kcal/g. All the samples were analysed in triplicate and the CH_4 emission rate was calculated as:

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

Where, $Q\,CH_4$ = Methane emission rate (g/min), $Q\,SF_6$ = Known release rate of SF_6 from permeation tube (g/min), CH_4 = Methane concentration of collected sample in canister (µg/m^3) and SF_6 = SF_6 concentration of collected sample in canister (µg/m^3).

Energy intake was calculated from TDN intake obtained by conducting digestibility trial on each animal in each period.

Feed efficiency and economics

Feed efficiency was determined as the amounts of DM, DCP and TDN intake per kg milk yield and amount of DMI per kg 4% FCM and FPCM. The feeding cost was calculated from records of daily feed consumption and procurement price of feeds and fodder used in the experiment, based on that economics of milk production was calculated.

Statistical analysis

The experimental data were analysed by analysis of variance using General Linear Model procedure as per the methods of Snedecor and Cochran (1994), with the help of SAS software programme.

Results and Discussion

DM and nutrient intake

The average daily DMI of crossbred cows in T1, T2 and T3 groups was 12.56, 12.25 and 11.26 kg, respectively which was significantly (P=0.0004) low in T3 (seaweed group). The DMI declined by 10.35% in cows fed seaweed as compared to control. The higher reduction in DMI of T3 cows may be due to less palatability of seaweed containing TMR which was less consumed by animals during last 2 weeks of each period. Less DMI resulted in significant low intake of CP, DCP and TDN in T3 than T1 and T2. Roque et al. (2019) observed significant ($P < 0.001$) decrease in dry matter intake of Holstein cows by 10.8 and 38.0% at low (0.5%) and high (1%) level of *Asparagopsis armata* inclusion, respectively compared to control group. However, Singh et al. (2015) incorporated brown seaweed (*Sargassum wightii*) in the diet of lactating Sahiwal cows to the extent of 20% in concentrate mixture without significant difference in DMI among treatment groups. Similar to present findings, no significant difference on DMI of crossbred cows was observed when soybean straw replaced wheat straw up to 50 and 75% level in diet of crossbred cows (Mudgal et al. 2010). No adverse effect of replacing 50% of wheat straw by groundnut straw in TMR was observed in cattle (Sherasia et al. 2018; Jasvantgiri, 2019) and buffalo (Prajapati, 2016).

Milk yield and composition

The data of milk yield and composition are given in Table 3. Daily milk yield was 23.79% lower in T3 than T1 group, which was due to less DM and nutrient intake in T3 group. The average FCM and FPCM yield (kg/d) also reduced by 21.22% and 20.65% in T3 as compared to other groups due to lower milk yield. There was

Table 3 Milk production and composition

Parameter	T1	T2	T3	SEM	P value
Milk yield (kg/d)	8.88	9.07	7.17	0.50	0.0725
FCM yield (kg/d)	8.51	8.50	7.02	0.45	0.1020
FPCM yield (kg/d)	7.01	7.01	5.81	0.36	0.0982
Fat (%)	4.01	3.75	4.03	0.13	0.7804
SNF (%)	8.59	8.54	8.50	0.05	0.5958
Protein (%)	3.07	3.05	3.02	0.01	0.1819
Lactose (%)	4.70	4.67	4.62	0.01	0.0768
Salts (%)	0.70	0.69	0.69	0.004	0.6594
Total solids (%)	12.60	12.30	12.53	0.16	0.8113

no significant difference among all parameters of milk composition in all three treatments. The present findings are in agreement with Mudgal et al. (2010) who reported no significant effect of soybean straw on milk yield in crossbred cows when wheat straw was replaced at 50 and 75% level. Khare et al. (2018) reported that soybean straw could be supplemented upto 20% level without any adverse effect on milk production. Similarly, for seaweeds also Roque et al. (2019) reported 11.6 % reduction in milk yield in cows fed concentrate mixture with 1% *A. armata* compared to control (P<0.001). However, Hong et al. (2015) reported no significant difference in milk yield of Holstein cows with different levels (0%, 2%, and 4%) of Brown Seaweed By-Products (BSB). Singh et al. (2015) reported significant increase in milk yield with incorporation of 20% *Sargassum wightii* in concentrate mixture of lactating Sahiwal cows. The decrease in milk yield in present study was due to low DM and nutrient intake in seaweed group which can be taken care by improving DM and nutrient intake by addressing palatability of seaweed.

Methane emissions

The data pertaining to methane emission is depicted in Table 4. The daily CH_4 emission (g/d) was 236.89, 187.64 and 197.73 g/day. The daily CH_4 emission (g) significantly reduced by 20.79% and 16.53% (P<0.05) in soybean and seaweed supplemented group respectively, as compared to control. Methane emission (g/kg milk) significantly reduced by 22.42% (P<0.05) in soybean supplemented group as compared to control, however, in T3 due to less milk yield, it was at par with T1 in spite of low daily methane emission. The results indicated that the feeding soybean straw based TMR as well as TMR with supplementation of seaweed has remarkable potential for methane mitigation in crossbred cows. Sherasia et al. (2018) reported significant reduction in enteric methane emission by 7.79% (g/day) and 15.13% (g/kg DDMI) in Kankrej cows fed groundnut straw based TMR as compared to only wheat straw based TMR. Chaudhari (2018) also observed 10.5% reduction (P<0.001) in methane emission in crossbred calves offered TMR (50:50) with pigeon pea straw replacing 50% of wheat straw in TMR. Similarly, Roque et al. (2019) observed that methane production (g/d) and g/kg DMI decreased significantly (P<0.0001) by 26.4 and 20.3% at the low (0.5%) level of *A. armata* inclusion and by 67.2 and 42.7% at the high (1%) level of inclusion. Methane intensity (g/kg milk yield) significantly decreased by 26.8 and 60% from cows fed at 0.5% and 1.0% of *A. armata* inclusion level. Our results also revealed reduction in methane due to seaweed; however, due to decrease in milk yield, methane (g/kg milk) was higher.

Energy intake and loss of energy as methane

The energy intake in form of GE, DE, ME and NE was calculated from TDN intake and was found lower in T3 due less DMI. Energy loss (Mcal/d) in form of CH_4 was lower in T2 (20.63%) and T3 (16.50%) compared to T1. The energy loss in form of CH_4 as % of NE intake was 16.73% less in T2 than T1 (Table 4). Hence, in spite of less NE intake in T2, milk yield was at par with T1 as the dietary

Table 4 Methane emission and energy loss in crossbred cows

Parameter	T1	T2	T3	SEM	P value
Methane emission					
CH_4 (g/d)	236.89[a]	187.64[b]	197.73[b]	10.70	0.04
CH_4 (g/DMI)	18.64	15.51	18.93	0.95	0.08
CH_4 (g/DDMI)	36.34	31.80	35.62	1.89	0.28
CH_4 (g/kg milk)	26.71[a]	22.52[b]	29.03[c]	2.35	0.04
Energy Intake (Mcal/d)					
GE	32.59[a]	31.97[a]	27.08[b]	0.83	0.01
NE	13.63[a]	12.98[a]	11.05[b]	0.34	0.01
Energy loss					
Through CH_4 (Mcal/d)	3.15[a]	2.50[b]	2.63[b]	0.14	0.04
Through CH_4 as % of GE intake	9.67	8.14	9.87	0.50	0.22
Through CH_4 as % of NE intake	23.12	19.25	23.51	1.21	0.25

Table 5 Feed efficiency and economics

Parameter	T1	T2	T3	SEM	P value
Milk yield (kg/kg DMI)	0.71	0.74	0.63	0.03	0.2275
FCM yield (kg/kg DMI)	0.68	0.69	0.62	0.03	0.3452
FPCM (kg/kg DMI)	0.56	0.57	0.51	0.02	0.3524
Economics					
Feed cost (₹ /d)	203.90	195.71	195.36	2.83	0.138
Feed cost (₹ /kg milk)	22.93	22.50	28.85	1.67	0.069
Income from sale of Milk (₹ /d)	252.13	264.73	210.43	16.04	0.072
Return over feed cost (₹ /d)	51.91	64.72	15.68	14.21	0.060

energy saved through methane mitigation supported the milk production. In seaweed group (T3), though dietary energy loss through methane was saved by methane mitigation, it could not support the milk yield because, NE intake was significantly less due to less DM and nutrient intake. Sherasia et al. (2018) in Kankrej cows, Prajapati (2016) in Surti buffalo and Chaudhari (2018) in crossbred calves reported significant reduction in energy loss through methane when 50% of wheat straw in TMR was replaced by legume straws.

Feed efficiency and economics

The feed efficiency and economics are given in Table 5. There was no significant difference among treatment groups, however feed efficiency was higher in T1 and T2 group as compared to T3. Feed cost per kg milk production was lower in T2 and T1 as compared to T3 due to less daily milk yield in T3. The sale price of milk was calculated on the basis of minimum and maximum fat and SNF observed during the experiment with Rs. 29.38, 28.32 and 29.26 in T1, T2 and T3, respectively. The return over feed cost was higher in T2 (24.67%) and lower in T3 (69.79%) compared to T1 due to difference in milk production among the groups. Similar to our findings, less feed cost and higher net profit was observed in crossbred dairy cows offered pigeon pea straw (Chetan et al. 2017) or groundnut straw (Islam et al. 2020) replacing cereal straw. Bendary et al. (2013) reported non-significant difference in feed efficiency and economics of feeding in cows fed seaweed @ 50g/head/day as compared to control group. Sharma and Datt (2020) also observed non-significant difference in feed efficiency for milk yield in dairy cows supplemented with red seaweed based powder (*K. alvarezii*: *G. salicornia*: *K. alvarezii* in 1: 1: 1 ratio) @ 1.5 and 3% of ration.

Conclusions

Incorporation of soybean straw @ 20% level in TMR had no adverse effect on animals' performance, helped in reducing methane emission and saving loss of dietary energy through methane. Inclusion of *S. johnstonii* @ 8% in the ration reduced DM and nutrient intake, milk yield but helped in methane mitigation. Hence, further research is required to decide the optimum inclusion level of *S. johnstonii* and measures to ameliorate its palatability to maintain DM and nutrient intake so as to exploit the antimethanogenic potential.

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