Journal of Animal Research: v.7 n.6, p. 1009-1017. December 2017

DOI: 10.5958/2277-940X.2017.00152.8

Effect of Probiotic Supplementation on Nutrient Digestibilities, Growth Performance and Enteric Methane Emissions in Deccani Ram Lambs

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Received: 08 April, 2017 **Revised:** 12 Dec., 2017 **Accepted:** 13 Dec., 2017

ABSTRACT

The present study was designed to investigate the effect of probiotic supplementation on nutrient digestibility, growth performance and enteric methane emissions in Deccani ram lambs. *In vitro* studies were conducted to select the best combination of probiotics based on *in vitro* dry matter digestibilities (IVDMD) and mean methane emissions for inclusion in *in vivo* studies. Increased *in -vitro* dry matter digestibilities and reduced total gas and methane emissions were observed with T4 probiotics (*S. cerevisiae*47@300 × 10^6 CFU /gm+ *S. boulardii*@50 × 10^6 CFU/gm + *L. acidophilus*@45 × 10^6 CFU + *P. freudenreichii*@50 × 10^6 CFU/gm). During second phase, 12 Deccani ram lambs of uniform body weight (16.5±0.64 kg with 130.11±3.00 days of age) were randomly allotted to 2 treatments in a completely randomized design. The nutrient digestibilities ($P<0.05$) increased with probiotic supplementation. The increase in nutrient digestibilities was reflected by higher live weight($P<0.05$) and average daily weight gain ($P<0.05$). Feed efficiency of the animals improved as the feed conversion ratio (kg feed/kg gain) ($P<0.01$), decreased. Higher ($P < 0.05$) Nitrogen, Ca and P balance was observed with probiotic supplementation. Mean enteric methane emissions (l/day) were significantly ($P<0.01$) lower in Group II (10.05± 0.39) than Group I (11.59± 0.70) and the reduction is 21.9 percent as compared to control group. It may be concluded that supplementation of probiotic increased nutrient digestibilities, growth performance and decreased enteric methane emissions, suggesting that the energy loss for ruminants in the form of methane emissions can be reduced efficiently.

Keywords: Probiotic, Methane emissions, Nutrient digestilibities, sheep

Methane (CH_4), together with carbon dioxide (CO_2) and nitrous oxide (N_2O) constitute the three major greenhouse gases (GHG). The emission of GHG from livestock and their impact on climate changes are a major concern worldwide (Steinfeld *et al*., 2006). It has been reported that enteric CH_4 is the most important GHG emitted (50% to 60%), at the farm scale, in ruminant production systems (Ogino *et al*., 2007). Methane emissions from livestock are estimated at about 2.2 billion tons of carbon dioxide equivalent, accounting to 35 percent of the total anthropogenic methane emissions (FAO, 2013). Apart from its potentiality in terms of global warming, methane also accounts for significant energy loss to the animal ranging from 2% to 12% of gross energy (GE) intake (Johnson and Johnson, 1995). Therefore, reducing the production of enteric CH_4 from ruminants without altering animal production is desirable both as a strategy to reduce global GHG emissions and as a means of improving feed conversion efficiency.

There is a growing interest in the use of supplements and in the identification of efficient feed additives, particularly probiotics in livestock production systems. Live yeast, the most commonly used probiotic in ruminant production, has not been extensively tested in mitigation of CH_4 production (Chaucheyras-Durand *et al*., 2008). However, yeasts are capable to show great functional and metabolic

diversity and some strains have been reported to decrease CH_4 production *in vitro* and *in vivo* studies (Newbold and Rode, 2006; Jeyanathan *et al*., 2014; Elanthamil *et al.,* 2017). The mechanisms by which yeasts decrease methanogenesis has been proposed to be by increasing microbial synthesis (Newbold and Rode, 2006) and by stimulating reductive acetogenesis (Chaucheyras *et al*., 1995).

One major goal in increasing the efficiency of nutrient utilization is to alter molar proportions of ruminal volatile fatty acids, increase nutrient digestibility and reduce emission losses. Ideally such production strategies aim at limiting environmentally harmful enteric emissions in addition to the optimization of production potential in sustainable manner. Therefore the present study is designed to identify the best probiotic on the basis of methane reducing capacity in *in vitro* and to evaluate the effect of same in *in vivo* on methane production, nutrient utilization and growth performance in Deccani ram lambs.

MATERIALS AND METHODS

In vitro studies

In vitro studies were conducted to identify the best probiotic supplement for further *in vivo* experimentation in Deccani ram lambs. The techniques used for the experiment were *in vitro* dry matter degradability (IVDMD) and *in vitro* gas production.

Suitable aliquot of gas collected from Gas-tight culture bottles (100ml capacity) consisting rumen contents and feed samples, was withdrawn from the tip of the incubation bottles using gas tight syringe and composition of gas in the headspace of bottles determined using gas chromatograph (450-GC, BRUKER Daltonics, Bremen, Germany).

In vivo studies

Animal, experimental design and management

Eighteen growing Deccani ram lambs aged 130±3.0 d with average body weight of 16.5±0.64 kg were selected for conducting a growth trial for a period of 90 days at Central Research Institute for Dry land Agriculture (CRIDA) Livestock farm, Hyderabad. These animals were randomly divided in to two groups of six animals in each in a completely randomized design.

All the experimental animals were housed in a well-ventilated animal shed with the provision for feeding and watering. The lambs were weighed individually at fortnightly intervals before feeding and watering to observe the body weight changes for an experimental period of 90 days. After 60 days of growth trial, a seven days metabolic trial was carried out on lambs to study the digestibility of nutrients in experimental diets.

Experimental diets

The dietary groups were *viz*., G I: Basal diet (chopped sorghum stover as roughage source) (BD) + group 1 concentrate+ chopped green fodder (4kg), G II: Basal diet + group 1 concentrate supplemented with probiotics (*S. cerevisiae*47@300 × 10^6 CFU /gm+ *S. boulardii*@50 × 10^6 CFU/gm + *L. acidophilus*@45 × 10^6 CFU + *P. freudenreichii*@50 × 10^6 CFU/gm) selected from *in vitro* studies @ 4kg per 100kg concentrate + chopped green fodder (4kg).

Table 1: Chemical composition of experimental feeds (%DM) offered to Deccani ram lambs

Nutrient	Basal diet		Concentrate mixture	
	Green fodder (HN-CO_4)	Dry fodder (Sorghum straw)	Group 1 (control)	Group 2 (Probiotic supplement)
Proximate principles				
Dry matter	20.38	98.59	98.03	97.74
Organic matter	87.22	92.29	91.05	91.07
Crude protein	11.75	3.02	17.96	17.96
Crude fibre	35.86	40.45	14.96	14.96
Ether extract	2.64	2.49	6.34	6.36
NFE	36.97	46.34	49.61	49.59
Total ash	12.78	7.71	8.95	8.93
Cell wall constituents				
NDF	71.34	83.27	48.12	48.17
ADF	41.58	52.34	19.44	19.48
Hemicellulose	29.76	30.93	28.67	28.66
Cellulose	33.78	42.93	11.71	11.74
Minerals				
Ca	0.40	0.34	1.12	1.12
P	0.16	0.24	0.82	0.82

Concentrate mixture used in the experiment consisted of Maize (42), Rice bran (32), Soya meal (25) Mineral mixture (02) and Common Salt (01) per 100 parts. Deccani ram lambs were fed the respective diets *ad-lib.* to meet the nutrient requirements (NRC, 2001) throughout 90 days of feeding trial. The chemical composition of the experimental feeds is summarized in Table 1.

Respiratory chamber

Enteric emissions from the animals were measured using closed respiratory chamber method. The respiration chamber was designed to enable accurate measurements of gaseous exchanges and provide a comfortable and safe environment for the animals.

Respiratory chamber was made of 10 mm transparent acryl panels (0.602 m wide × 1.307 m length × 1.306 m tall, 1.028 m^3 volume) fixed to an iron angle frame. Three air pumps with 13 l/min capacity (AS16-1 Mini Air compressor piston type) each was equipped to draw air from chamber through the pipe and supply air to inside the chamber so that the rate of approximately 39 litres per minute flow was maintained. Based on our previous test, at this air flow rate, the carbon dioxide concentration in the chamber with a 25-kg goat did not exceed 0.5%, a suggested maximum concentration (Klein and Wright, 2006). Air samples from the chamber were collected from various heights at regular interval of 60 min in 24h duration in gas syringes, closed with airtight caps and sealed with parafilm. Composition of gas determined using gas chromatograph (450-GC, BRUKER Daltonics, Bremen, Germany) with three detectors Thermal Conductivity Detector (TCD), Electron Capture Detector (ECD) and Flame Ionization Detector (FID) with a 1041 PWOC Packed/Wide bore On-Column. Carrier gases were nitrogen (N_2), helium (He), hydrogen (H_2) and methane (CH_4) at 500 kPa (max 1000kPa), H2 and air are detector fuel gases.

Statistical analysis

The results obtained were subjected to analysis through software (version 17.0: SPSS, 2005) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test (Duncan, 1955) with a test of significance at $P<0.05$. All the statistical procedures were done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

***In-vitro* studies**

Seven Probiotic treatments viz., *Saccharomyces boulardii* (T1), *Lactobacillus strain* 16 (T2), *Lactobacillus strain* 15 (T3), *S cerevisiae*47 + *S. boulardii* + *L. acidophilus* + *Pediococcusfreudenreichii* (T4), *P. acidilactici* strain U56 (T5), Yea-sac (T6) and *Saccharomyces cerevisiae* (T7) were used to study the effect of probiotic supplementation on IVDMD and *in vitro* methane emissions with sorghum stover as basal diet and the results are presented in Table 2.

It was observed that the *in-vitro* dry matter degradability (%) was 3.5% higher ($P<0.05$) with T4 Probiotic over

Table 2: *In vitro* methane emissions (g/kg IVDMD) and *in vitro* dry matter degradability (%) from *in vitro* coarse crop residues fermentation with different probiotics supplementation

Sl. No.	Experimental diet	Treatment	CH_4 g/kg IVDMD	IVDMD (%)	Methane (ml)*	Total gas (ml)*
1	*Saccharomyces boulardii*	T1	16.16 ± 0.52^a	64.48 ± 0.28^b	11.4± 0.54^a	41.1± 1.06^a
2	*Lactobacillus* strain 16	T2	16.02 ± 0.46^a	64.68 ± 0.46^b	11.3± 0.32^a	40.8± 0.53^a
3	*Lactobacillus* strain 15	T3	16.08 ± 0.22^a	64.54 ± 0.30^b	11.3± 0.20^a	40.9± 0.28^a
4	*S cerevisiae* 47, *S. boulardii* + *L. acidophilus* + *P. freudenreichii*	T4	15.46 ± 0.88^b	66.7 ± 0.18^a	10.9± 0.21^b	39.3± 0.15^b
5	*Pediococcus acidilactici* strain U56	T5	16.58 ± 0.72^a	63.26 ± 0.20^b	11.7± 0.41^a	42.2± 0.32^a
6	Yea-sac (*Saccharomyces cerevisiae* 1026*)*	T6	16.18 ± 1.02^a	64.52 ± 0.36^b	11.4± 0.32^a	41.2± 0.23^a
7	*Saccharomyces cerevisiae*	T7	16.24 ± 0.36^a	63.14 ± 0.28^b	11.4± 0.29^a	41.3± 0.05^a

*****_In vitro_ methane emissions (ml/0.5g) and total gas (ml/0.5g) from *in vitro* coarse crop residues fermentation with different probiotics

group averages. Probiotics promote growth of cellulolytic and lactate-utilizing bacteria (Chaucheyras-Durand *et al*., 2008) and thereby increase degradability of coarse cereal crop residues. Similar increase in IVDMD has been reported by Lila *et al*. (2004) with supplementation of twin-strains of *Saccharomyces cerevisiae* live cells to a basal diet of corn starch, soluble potato starch and sudan grass hay (60.5%, DM basis) plus concentrate mixture (39.5%, DM basis). Malik *et al*. (2010) reported higher IVDMD (P<0.001) with *L. acidophilus* than *S. cerevisiae* at $1 \times 10^{(9)}$ and $3 \times 10^{(9)}$cfu/flask, respectively.

In-vitro gas and methane production

Typical relationship between head-space gas pressure and gas volume from 20 bottles read on 8 occasions during a 24h incubation period for sorghum stover are presented in Fig. 1.

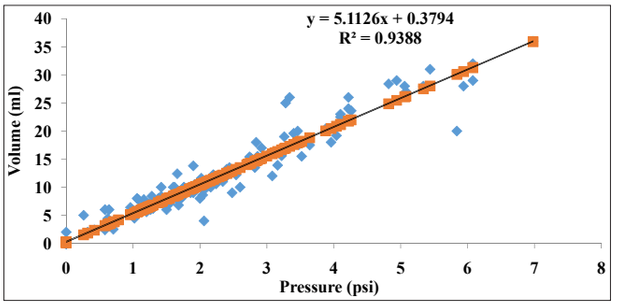

Fig. 1: Typical relationship between head-space gas pressure and gas volume from 20 bottles read on 8 occasions during a 24h incubation period

DM: Dry matter; OM: Organic matter: NDF: neutral detergent fibre; Means with the different superscripts along the row are significantly different; SEM, standard error of the mean

Total gas and mean methane emissions (g/kg IVDMD) were 4.0% lower (P<0.01) with T4 probiotic over group averages (Table 2). Supplementation of probiotics T1, T2, T3 & T4 at 15 mg/0.5 g of sorghum stover had significant influence on the total gas production among the probiotics, whereas the other treatments T5,T6 and T7 did not have a large influence on the *in-vitro* gas production. The results of the present investigation corroborated the findings of Chaucheyras *et al*. (1995), Mwenya *et al*. (2004) who concluded that Probiotic culture might stimulate the acetogens to compete or to co-metabolize hydrogen with methanogens thereby, reducing CH_4 emissions. Similar results were also reported by Galindo *et al*. (2010), Chung *et al.* (2011). Contradictory results were reported by Salem *et al*. (2015) who observed that the addition of *S. Cerevisiae* linearly increased the gas production during the first 12 hour of incubation.

Harikrishna *et al*. (2012) reported total gas was increased with the yeast supplementation. However, Sullivan *et al*. (2012) observed no impact on H_2 and CH_4 production with fermentation of ground corn supplemented with *S. cerevisiae* at either of the concentrations (0.35 or 0.73 g/L). Similarly Elghandour *et al*. (2014) and Yang *et al*. (2015) reported no effect on methane production, when fibrous feeds were incubated with *S. cerevisiae* at various doses.

Feed intake

The present study revealed that the supplementation of probiotic had no effect on Dry matter intakes (DMI) in ram lambs. The results are in agreement with results of Hernandez *et al*. (2009) who noticed no change in DM intake of lambs fed grass diets which contain probiotics. Similar results were also reported by Titi *et al.* (2008), Singh *et al.* (2016).

Nutrient digestibility

In the present study supplementation of probiotic to the ration significantly enhanced digestibilities of major nutrients and the results are presented in (Table 3).

The crude fibre digestibility co-efficient in control and experimental diet were 65.33 ± 0.85 and 71.99 ± 0.98 respectively. Supplementation of probiotic significantly increased (P< 0.01) crude fibre digestibility. The increase in fibre degradability could be due to enhanced cellulolytic activity in the rumen in addition to improved enzymatic activity (El-Waziry and Ibrahim, 2007) and could also be due to selective stimulatory effect of Yeast culture on microbes responsible for fibre degradation. Similar results were also reported by Chaucheyras-Durand *et al*. (2008) who concluded that probiotics deliver many lactic acid bacteria into the rumen and gastrointestinal tract of the animal and promote growth of cellulolytic and lactate-utilizing bacteria, increase pH in the rumen (Mohamed *et*

al., 2009; Paryad and Rashidi, 2009) and increase fibre degradability.

Table 3: Effect on Intake and digestibilities of DM, OM and NDF of experimental rations fed to Deccani ram lambs

Indicators	Group I	Group II	SEM	P
Intake				
DMI kg/day	1.049 ± 0.01	1.029 ± 0.01	0.01	0.01
DMI % LW	4.80 ± 0.32	4.47 ± 0.25		
OMI kg/day	0.961 ± 0.01	0.938 ± 0.01	0.01	0.02
Digestibilities				
DM %	64.32^{b} ± 0.08	70.65^{a} ± 1.28	0.93	0.01
OM %	67.95^{b} ± 0.67	73.37^{a} ± 1.17	0.81	0.02
CP %	66.94^{b} ± 0.68	73.53^{a} ± 1.33	0.93	0.05
CF %	65.33^{b} ± 0.85	71.99^{a} ± 0.98	0.89	0.02
NDF %	68.19^{b} ± 0.71	74.62^{a} ± 0.82	0.84	0.001
ADF %	62.16^{b} ± 0.83	69.82^{a} ± 1.03	1.00	0.001
Cellulose %	70.96^{b} ± 0.74	77.29^{a} ± 0.84	0.83	0.001

DM: Dry matter; OM: Organic matter: EE: Ether extract; NFE: Nitrogen free extract; NDF: neutral detergent fibre; ADF: Acid detergent fibre; Means with the different superscripts along the row are significantly different; SEM, standard error of the mean

Significantly higher (P < 0.01), DM digestibilites were observed in experimental diet (70.65 ± 1.28) as compared to control (64.32 ± 0.08). The increase in DM digestibility could be due to the ability of these microorganisms to modify the ruminal and intestinal milieu and to deliver enzymes and other beneficial substances (Dierck N.A, 1989).

The present study revealed higher digestibility (P<0.05) crude protein digestibility (73.53 ± 1.33) with supplementation of probiotic as compared to control (66.94 ± 0.68). Increase in CP digestibility could be due to increased fibre degradation resulting in release of protein bound to structural carbohydrates in addition to enhanced cellulolytic activity in the rumen.

The neutral detergent fibre and acid detergent fibre coefficients were significantly (P<0.01) increased with probiotic supplementation and could be due to increased total viable bacteria and cellulolytic bacteria (Wallace and Newbold, 1991), which adhere to plant cell wall components and degrade by producing enzymes (Chaucheyras-Durand *et al.*, 2008).

The results of the present study are in agreement with the findings of Singh *et al.* (2016) who reported higher digestibility coefficient for DM, CP, EE, CF and NFE with probiotic supplementation in Barbari kids.

Nitrogen, Ca and P Balance

All the experimental ram lambs were on positive nitrogen balance. Significantly lower (P<0.001) faecal nitrogen loss and higher (P<0.01) N balance were observed with probiotic supplementation than control (Table 4).

Higher (P<0.01) N balance in the present study might be due to increased ruminal N pool , N-retention and post-ruminal amino acid flow by enhancing microbial peptidolytic and proteolytic activities in the rumen with the addition of probiotic supplement (Erasmus *et al*., 1992; Cole *et al*.,

Table 4: Nitrogen, Calcium and Phoshorus balance (g/day) in Deccani ram lambs

Ration	Average daily N intake		Total N intake	Outgo		Total outgo	Balance
	Fodder (green + dry)	Conc.		Faeces	Urine		
Nitrogen Balance							
G 1	10.46± 0.09	8.48 ±0.00	$18.94±0.09^{b}$	$8.78±0.11^{a}$	2.69±0.13	11.47±0.07	$7.47±0.09^{b}$
G 2	11.42±0.10	8.48±0.00	$19.90±0.10^{a}$	$7.74±0.26^{b}$	2.71±0.29	10.45±0.10	$9.45±0.10^{a}$
Calcium Balance							
G 1	3.68±0.03	3.29±0.00	6.98±0.03	1.97±0.16	1.41±0.11	$3.38±0.15^{a}$	$3.59±0.15^{b}$
G 2	3.63±0.04	3.29±0.00	6.92±0.04	1.57±0.17	1.03±0.09	$2.61±0.17^{b}$	$4.32±0.16^{a}$
Phosphorus Balance							
G 1	1.58±0.02	2.41±0.00	$3.99±0.02^{a}$	$1.34±0.04^{a}$	0.98±0.05	$2.32±0.07^{a}$	$1.67±0.08^{b}$
G 2	1.54±0.03	2.41±0.00	$3.95±0.02^{a}$	$1.08±0.04^{b}$	0.69±0.16	$1.76±0.14^{b}$	$2.19±0.14^{a}$

Means with different superscripts in a column differ significantly (P<0.01)

1992; Jouany *et al*., 1998; Enjalbert *et al*., 1999; Paryad and Rashidi 2009). Contrary to the results of the present study, Hernandez *et al*. (2009) reported supplementation of probiotic (yeast culture @5 g/d per sheep) had no effect on N-retention, N-intake and fecal and urinary N in lambs fed with orchard grass hay as basal diet.

Higher Ca balance (P<0.05), and lower Ca excretion lower (P<0.05) were observed with probiotic supplementation. Similarly lower faecal P excretion (P<0.001), total P excretion decreased (P<0.05) and P balance (P<0.01) increased with probiotic supplementation. This may be attributed to improved nutrient digestibility (Abd El-Ghani, 2004) and ruminal digestion (Kamel *et al*., 2004) more likely fiber degradation (Dawson and Tricarico, 2002) and lower excretion of the minerals.

Plane of nutrition

Higher DCP and TDN in ration (P<0.05), and TDN intake (P<0.05) was observed with probiotic supplementation due to increase in nutrient digestibilities (Table 5). Probiotics improve nutrient digestibility (Abd El-Ghani, 2004), and ruminal digestion (Kamel *et al*., 2004) more likely fiber degradation (Dawson and Tricarico, 2002) and this could be reason for higher TDN, DE and ME intake in Group II.

Table 5: Plane of nutrition of Deccani ram lambs as affected by experimental rations

Parameter	Experimental groups		ICAR
	T1	T3	(1998)
Body weight (kg)	22.32±1.48	23.36±1.23	25.0
Dry matter intake/day (kg)	1.05±0.01	1.03±0.01	0.68
Dry mater intake/100 kg b.wt	4.80±0.32	4.47±0.25	2.7
DCP in ration (%) **	7.3^{b} ±0.08	8.1^{a} ±0.15	—
DCP intake (g)	77.0 ±1.32	83.1 ±1.49	33
TDN in ration (%) **	64.1^{b} ±0.78	72.0^{a} ±0.84	—
TDN intake (kg)*	0.67^{b} ±0.01	0.74^{a} ±0.01	0.31
DE intake (M.cal)	3.0 ±0.06	3.3 ±0.06	—
ME intake (M.cal) *	2.4^{b} ±0.04	2.6^{a} ±0.05	1.10

DCP: Digestible crude protein; TDN: Total digestible nutrients; DE: Digestible energy; ME: metabolizable energy.

Body weight changes and growth performance

The increase in the digestibilities of various nutrients reflected in the growth performance of Deccani ram lambs as reflected by higher live weight (P<0.05), average daily gain (P<0.05) with probiotic supplementation. Feed efficiency of the animals reflected in high feed conversion ratio (kg feed/kg gain) (P<0.01), low cost of feed/kg gain (P<0.01) with probiotic supplementation (Table 6). This could be due to better digestibility of nutrients with efficient utilization of absorbed nitrogen. Similar findings were observed by Mutassim, (2008) in lambs and Singh *et al*. (2016) in Barbari kidswith probiotic supplementation. Contrary to this no effect on animal performance was reported by Mikulec *et al*. (2010).

Enteric methane emissions

Enteric methane emissions for 24 h sampling plotted standard curve showing linearity are presented in Fig. 2.

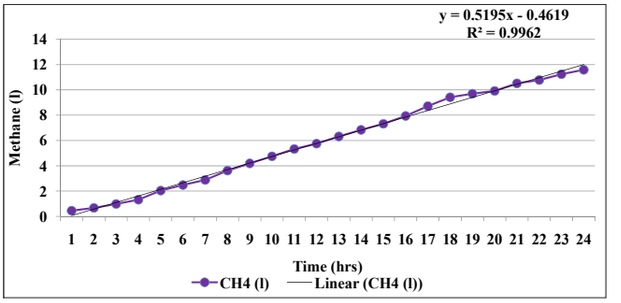

Fig. 2: Enteric methane emissions curve for 24 hrs sampling in Deccani ram lambs

A significantly (P<0.01) lower methane emissions were observed from probiotic supplemented lambs than control group lambs in terms of lower methane in (g)/day (9.05± 0.47 Vs 11.59± 0.70 g/day), methane emissions l/day (6.46 ± 0.33Vs8.28 ± 0.50l/day) and methane emission in l/DMI/day (0.59 ± 0.01 Vs 0.79 ± 0.01l/DMI/day) (Table 7). Over all it was observed that the supplementation of Probiotics reduced (P<0.01) daily methane emissions by 21.9 per cent in growing Deccani ram lambs over control group.

Similar results were also reported by Mwenya *et al.* (2004) who reported that a yeast *Trichosporon sericeum* (4 g/ day) decreased methane by 10% in sheep fed on

Table 6: Average daily gain (g), DMI and cost (Rs.) of feed per kg weight gain in Deccani ram lambs as affected by feeding experimental rations

Group	Initial weight (kg)	Final weight (kg)	Live weight gain (kg)	Average daily gain (g)	DMI/kg weight gain (kg)	Cost of feed per kg gain (₹)
T1	16.3 ± 1.36	22.73± 1.32	6.43^{b} ± 0.41	71.44^{b}± 4.57	14.68^{a}± 0.88	130.05^{a}± 7.55
T2	16.47± 1.21	24.14± 1.20	7.68^{a}± 0.12	85.29^{a} ± 1.34	12.11^{b}± 0.28	127.21^{a}± 2.33
SEM	0.64	0.65	0.20	2.28	0.47	5.47
P	0.97	0.60	0.02	0.02	0.00	0.00

Means with the different superscripts along the row are significantly different; SEM, standard error of the mean

Table 7: Methane (CH_4) emissions in Deccani ram lambs

Indicators	Group I	Group III	SEM	P
B.Wt	22.73 ± 1.32	24.06 ± 1.20	0.82	0.83
M.bwt	10.39 ± 0.45	10.85 ± 0.41	0.28	0.81
DMI/day	1.05^{a} ± 0.01	1.03^{b} ± 0.01	0.01	0.11
CH_4 gm/Day	11.59^{a} ± 0.70	9.05^{b} ± 0.47	0.38	0.01
CH_4 gm/L.WT	11.07^{a} ± 0.72	8.79^{b} ± 0.43	0.38	0.02
CH_4 gm/M.BWT	0.51^{a} ± 0.00	0.38^{b} ± 0.00	0.02	0.00
CH_4 gm/Kg DMI	1.11^{a} ± 0.02	0.83^{b} ± 0.01	0.03	0.00
CH_4 L/Day	8.28^{a} ± 0.50	6.46^{b} ± 0.33	0.27	0.01
CH_4 L/L.WT	7.91^{a} ± 0.51	6.28^{b} ± 0.30	0.27	0.02
CH_4 L/M.BWT	0.35^{a} ± 0.00	0.26^{b} ± 0.00	0.01	0.00
CH_4 L/Kg DMI	0.79^{a} ± 0.01	0.59^{b} ± 0.01	0.02	0.00

DMI: Dry matter intake; CH_4: Methane; Means with the different superscripts along the row are significantly different; SEM, standard error of the mean

a roughage-based diet and also reported that sheep produced significantly less methane when 0.4% yeasts were included in a basal hayand 30% concentrate diet. The effect of probiotics in reducing methane emission could be through the stimulation of acetogens to compete or to co-metabolize hydrogen with methanogens there by, reducing methane emissions (Chaucheyras *et al*., 1995; Mwenya *et al*., 2004; Klieve, 2007).

Contrary to the results of the present study Martin and Nisbet, (1990) reported that *A. oryzae* and *S. cerevisiae* increased methane while Mathieu *et al.* (1996) reported that *S. cerevisiae* could not affect methane release *in-vivo*. These contradictory results on methane might be due to the strain differences between yeasts and the type of diets (Newbold and Rode, 2006).

CONCLUSION

Based on the above findings, it may be concluded that, probiotics can be supplemented to increase the nutrient digestibilities, growth performance and to reduce methane emissions in sheep. Probiotic supplementation in diet has the potential to increase the nutrient utilization and to mitigate the effect of methane production in sheep fed coarse roughage based diets.

ACKNOWLEDGEMENTS

The author is grateful to Sri Vekateswara Veterinary University for providing the financial help and to CRIDA, Hyderabad for providing the facilities for the research work.

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