

Evaluation of methane reduction potential of sheanut cake based concentrate rations by *in vitro* gas production studies

S.Venkateswarlu¹, L.Radhakrishnan^{2*}, R.Karunakaran³, M.Parthiban⁴
and S.T.Selvan⁵

Tamil Nadu Veterinary and Animal Sciences University,
Department of Animal Nutrition,
Madras Veterinary College, Chennai.

ABSTRACT

A study was conducted to evaluate the methane reduction potential of sheanut cake in ruminants. The nutrient composition *viz.*, CP, NDF and ADF contents (%) were 15.2, 42.24 and 30.85 respectively. The total tannin phenol content was 7.08 % and predominantly in the form of hydrolysable tannins (5.85 %). Six isonitrogenous concentrate rations were prepared by including sheanut cake at levels of 0 (control), 15 % (T₁), 17.5 % (T₂), 20 % (T₃), 22.5 % (T₄) and 25 % (T₅) and were evaluated by *in vitro* gas production technique (IVGPT). *In vitro* gas production profiles indicated that there was significant (P<0.01) reduction in total gas production, methane, methane for 100 mg of truly digested substrate and NH₃ - N in sheanut cake based rations compared to control. Methane for 100 mg of truly digested substrate was significantly (P<0.01) reduced by 15.39 %, 20.37 %, 28.23 %, 27.28 % and 28.21 % in T₁, T₂, T₃, T₄ and T₅ respectively. The maximum methane reduction was observed at 20 % level thereafter no further reduction even after increasing sheanut cake. It can be concluded that sheanut cake could be used medium energy and protein source and also a potential feed ingredient in suppressing the methane production in ruminants.

Key words: Methane, sheanut cake, tannins, protozoa

INTRODUCTION

Greenhouse gas emission from ruminant production system is of particular importance because of their consequences

on global climate. Enteric methane (CH₄) is one of the most potent greenhouse gas emitted by ruminants and accounts for 2 to 12 % loss of gross energy of feeds depending on the diet (Johnson and Johnson, 1995). Globally about 80 million tonnes of CH₄ is produced annually, agriculture contributing 47 % of these emissions with ruminants accounting for 39% (Gerber *et al.*, 2013). During past decade, intensive research has been conducted to develop effective and practical means to decrease the CH₄ emission from ruminants (Hristov *et al.*, 2013). Among them, use of unconventional

* Corresponding author email: radhakrish67@yahoo.com

¹ PhD Scholar, ³ Professor and Head, Department of Animal Nutrition, MVC, Chennai

² Professor and Head, Central Feed Technology Unit, Kattupakkam

⁴ Professor, Department of Animal Biotechnology, Madras Veterinary College, Chennai

⁵ Professor and Head, Post Graduate Research Institute in Animal Sciences, TANUVAS

feeds rich in tannins is one of the promising approach (Bhatta *et al.*, 2012). Sheanut cake (obtained from processing of the sheanut for shea butter) is one such potential alternative feed resource in ruminant diets (Kumar *et al.*, 2015). However, use of sheanut cake, level of inclusion and its antimethanogenic properties were not fully explored. Hence, the present *in vitro* study had been undertaken to evaluate the sheanut cake based concentrate ration and its level of inclusion on methane reduction potential and rumen fermentation characteristics.

MATERIALS AND METHODS

Sample Collection and Preparation

Sheanut cake was procured from the industry at Tadepalligudem, West Godavari District of Andhra Pradesh while other ingredients (maize, deoiled rice bran and soybean meal) were procured locally and were ground in a Willey mill to pass through 1 mm sieve and used for further analysis. Six isonitrogenous concentrate rations were formulated by including sheanut cake @ 0 % (control), 15 % (T₁), 17.5 % (T₂), 20 % (T₃), 22.5 % (T₄) and 25 % (T₅) in the ration. Ingredient composition is presented in table 1. Sheanut cake and concentrate rations were analyzed for proximate principles (AOAC, 2007), fibre fractions (Vansoest *et al.*, 1991) and tannins (Makkar, 2003).

In vitro gas production studies

The *in vitro* gas production studies were carried out using Hohenheim gas production technique (Menke and Steingass, 1988). Rumen liquor samples were collected from three crossbred cattle maintained on paddy straw, Napier hybrid grass (Co4) and

concentrate, under anaerobic conditions. About 200 mg of samples incubated with 30 ml of buffered rumen inoculum in 100 ml calibrated glass syringes and were kept in water bath shaking incubator set at 39°C. At the end of incubation period (24 h), the total gas produced was measured and the gas samples were collected in vaccutainer for estimation of methane using gas chromatography (Perkin Elmer, Clarus 500 model) equipped with Flame Ionization Detector (FID). Samples of fermented fluid were collected for estimation of ammonia nitrogen (micro-diffusion method of Conway, 1957), total volatile fatty acids (Barnett and Reid, 1957) and total protozoa (Kamra *et al.* 1991). The pH of the rumen liquor was measured immediately after the end of incubation using digital pH meter. *In vitro* true dry matter digestibility was estimated as per the procedures of Van Soest and Robertson (1988). Metabolisable energy (MJ / kg DM) and Microbial biomass production (MBP) were calculated as per equations suggested by Menke *et al.* (1979) and Blummel *et al.* (1997) respectively.

$$\text{ME (MJ / kg DM)} = 1.06 + 0.157 \times \text{gas (ml / 200 mg DM)} + 0.0084 \times \text{CP (g/kg DM)} + 0.022 \times \text{EE (g/kg DM)} - 0.0081 \times \text{Ash (g/kg DM)}$$

$$\text{MBP (mg)} = [\text{TD (mg)}] - [(2.20 \times \text{net gas volume in ml})]$$

Where, TD = True digestible dry matter (Substrate incubated – Neutral detergent fibre)

Statistical analysis of the data was carried out by analysis of variance (ANOVA) as per the procedures suggested by Snedecor and Cochran (1994) using SPSS version 20.0. Treatment means were compared by using Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical composition of sheanut cake and sheanut cake based concentrate rations were presented in Table 1. Chemical composition revealed that sheanut cake contains 15.2 % crude protein, 8.26 % crude fibre, 7.29 % total ash and 64.47 % nitrogen free extract. The nutrient composition was comparable to that of palm kernel meal (Bhatta *et al.*, 2012). NDF and ADF contents were 42.24 % and 30.85 %, respectively and these values were corroborated with earlier findings (Kumar *et al.*, 2007 and oddoye *et al.*, 2012). However, higher NDF and ADF content were observed by Pousga *et al.* (2007) and Pullaiah (2013) than that reported in the present study.

The total tannin content was 7.08 % and most of them are hydrolysable tannins (5.85%) while condensed tannins were only 1.23 %. Tannins content was similar to that reported by Bhatta *et al.* (2012) in various sheanut by-products. Variation in the chemical composition might be attributed to the method of extraction of shea butter, maturity of nuts, handling of the nuts prior to processing, cultivation practices and agronomic factors (Oddoye *et al.*, 2012).

Chemical composition of concentrates indicates that all the rations were isonitrogenous (20 % crude protein). NDF and ADF content of concentrate rations increased gradually as level of sheanut cake increased in rations due to presence of higher NDF and ADF content in sheanut cake compared to other conventional ingredients used in the ration.

In vitro gas production studies:

Effect of sheanut cake based concentrate rations on total gas (ml), methane (ml), *in vitro* true dry matter digestibility and methane (ml) per 100 mg of truly digested substrate was presented in Table 2. The total gas production had significantly ($P < 0.01$) decreased as the level of sheanut cake increased in concentrate rations. The total gas was lowered by 11.82 %, 13.12 %, 15.48 %, 16.99 % and 19.14% in T₁, T₂, T₃, T₄ and T₅, respectively than control. Similar trend in gas production was observed by Kumar *et al.* (2015) by replacing wheat bran and deoiled rice bran with sheanut cake in concentrate mixture at 0 to 9 % of the ration.

As the level of sheanut cake increased in rations, methane (ml) production was reduced significantly ($P < 0.01$). The minimum level of sheanut cake that reduced the maximum methane was 20%. Similar trend was observed in methane (%) on total gas production. Reduction in the methane emission observed in the present study is in conformity with that of Bhatta *et al.* (2012) and who concluded that reduction of CH₄ was due to presence of active tannins. Kumar *et al.* (2015) also reported significant reduction of methane in feeds containing sheanut cake.

The methane (ml) per 100mg of truly digested substrate was also reduced significantly ($P < 0.01$) with increasing levels of sheanut cake in concentrate rations. The reduction was 15.39, 20.37, 28.33, 27.28 and 28.21 per cent in T₁, T₂, T₃, T₄ and T₅, respectively compared with control.

Highest reduction was observed in T₄ but there was no significant difference between T₃, T₄ and T₅ therefore the minimum level of sheanut cake in rations with maximum methane reduction is 20% inclusion level. Reduction in total gas production and methane production with increasing sheanut cake level could be linked to role of sheanut tannins in reducing protozoa and/or methanogenic archaea population (Bhatta *et al.*, 2012), reducing the digestibility of dry matter and organic matter and the release of H₂ (Jayanegara *et al.*, 2010; Patra and Yu, 2014). Jayanegara *et al.* (2010) reported that there is significant negative relation between total tannins and methane production.

In vitro true dry matter digestibility ranges from 82.78 (control) to 77.50 (T₅) and decreased significantly (P < 0.01) with increasing level of sheanut cake in the concentrates rations compared to control. Decline in digestibility might be attributed to tannins present in sheanut cake forming complexes with proteins and carbohydrates under ruminal pH conditions (McSweeney *et al.*, 2001). Pullaiah (2013) reported that *in vitro* dry matter digestibility (IVDMD) was reduced significantly when sheanut cake is included at 10, 20 % level in complete rations. However Kumar *et al.* (2007) reported that when sheanut cake extract is included at 20 % and 40 % levels there was no significant difference in IVDMD using buffalo rumen liquor. The possible reason for difference in IVDMD may be due to inclusion of different ingredients used in the experimental rations and tannins content in sheanut cake.

***In vitro* fermentation characteristics:**

Effect of sheanut cake and sheanut cake based concentrate rations on pH, ammonia nitrogen, total volatile fatty acids (TVFA) concentration, microbial biomass, ME and protozoal count were presented in table 3. There is no significant change in pH among the treatments. Similar to these results, Travendale *et al.* (2005) also reported no change in pH by addition of tannin rich feeds like *Lotus pendunculatus* and *Medicago sativa*. Ammonia nitrogen content in treatment groups were significantly (P<0.01) reduced compared to control. Results in the present study were in agreement with the findings of Bhatta *et al.* (2012) who reported that when sheanut cake is incubated *in vitro* with and without poly ethylene glycol (PEG), there was significant reduction of ammonia nitrogen in the absence of PEG confirming that tannins present in sheanut cake binds with proteins and reduces their degradation. Proteins that escape being degraded in the rumen would enhance flow of proteins to intestines (Tan *et al.*, 2011), Hence sheanut cake can be incorporated in ruminant diets to protect degradable proteins.

The TVFA concentration decreased numerically when sheanut cake was included in concentrate rations, but there is no significant (P>0.05) difference among the treatment groups. Slight decrease in TVFA in sheanut based rations might be due to presence of tannins. This finding was in consistence with the findings of Getachew *et al.* (2008) with tannic acid and quebracho tannin. Bhatta *et al.* (2012) reported that incubation of sheanut cake with PEG resulted in substantial increase in TVFA.

Microbial biomass (mg/200 mg DM) was significantly ($P < 0.01$) higher in all sheanut cake based rations (86.07 to 87.55) compared to control ration (80.31). However, there is no significant difference among the sheanut cake based rations. Banakar *et al.* (2017) reported similar microbial biomass in different unconventional feeds under *in vitro* conditions. Higher microbial biomass in treatment groups indicate that substrate incubated *in vitro* was well utilized by microbes for their growth and development.

Metabolisable energy (MJ/kg DM) was significantly ($P < 0.01$) higher in control (8.45) and lower in T₅ (7.62). As the level of sheanut cake increased in rations, ME values decreased gradually. ME values reported in the present study was within range (7.39 to 12.02) of values reported by Kumar *et al.* (2015) for different concentrate feed ingredients. The difference in the ME values of feeds is reflective of difference in available carbohydrate, nitrogen content and fibre content of feeds.

There was significant ($P < 0.05$) reduction in the total protozoa count in sheanut cake based ration compared to control. This finding was consistent with the findings of other researchers (Bhatta *et al.*, 2012 and Bharathidasan, 2018). Galindo *et al.* (2008) found that 39.42% reduction in total protozoa population when tannin containing feed *L.leucocephala* was included at 30 % level under *in vitro* conditions. Bhatta *et al.* (2012) also observed higher susceptibility of Entodinia to phenolics present in the sheanut byproducts resulting in decreased methane production by removing the methanogen populations attached to protozoa.

CONCLUSION

It could be concluded that sheanut cake is a potential unconventional feed resource with medium protein and energy content which can be used to replace conventional feed ingredients in concentrate rations for ruminants. At 20% level of inclusion, sheanut cake is having maximum methane reduction potential without much effect on rumen fermentation. Hence, sheanut cake could be used as energy and protein source as well as to reduce enteric methane emission. However, further biological trials need to be carried out to assess the effect of sheanut cake in ruminant rations.

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Table 1 Ingredient and chemical composition of sheanut cake and sheanut cake based concentrate rations

Ingredient	Control	T ₁	T ₂	T ₃	T ₄	T ₅	Sheanut Cake
Maize	27	26	25	25	25	24.5	-
DORB	42	29.5	28.5	26	24	22	-
Soybean meal	28	26.5	26	26	25.5	25.5	-
Sheanut Cake	0	15	17.5	20	22.5	25	-
Mineral Mixture	2	2	2	2	2	2	-
Salt	1	1	1	1	1	1	-
Chemical composition of feed stuffs (% DM basis)							
OM	89.08	90.45	90.52	90.80	91.03	91.49	92.71
CP	20.07	20.16	19.92	20.23	19.98	20.18	15.20
EE	2.22	2.70	2.76	2.85	2.93	2.91	4.78
CF	10.37	9.20	9.17	8.92	8.73	8.30	8.26
NFE	56.42	58.39	58.67	58.80	59.39	60.10	64.47
NDF	25.46	26.59	27.02	27.12	27.32	27.53	42.24
ADF	8.41	11.38	11.95	12.43	12.92	13.43	30.85
TDN*	73.29	71.24	70.78	70.55	70.27	69.96	58.01

*Calculated values

Table 2 Effect of sheanut cake based concentrate rations on total gas (ml), methane (ml), *in vitro* true dry matter digestibility and methane (ml) per 100 mg of truly digested substrate.

Parameter	Control	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	P Value
Total gas production (ml)	38.75 ^a	34.17 ^b	33.67 ^{bc}	32.75 ^{bcd}	32.17 ^{cd}	31.33 ^d	0.47	0.01
Methane (ml)	8.01 ^a	6.60 ^b	6.18 ^b	5.54 ^c	5.53 ^c	5.38 ^c	0.17	0.01
% Methane on total gas production	20.67 ^a	19.32 ^b	18.38 ^{bc}	16.90 ^d	17.15 ^{cd}	17.17 ^{cd}	0.28	0.01
Methane (ml) for 100 mg of truly digested substrate	4.84 ^a	4.09 ^b	3.86 ^{bc}	3.46 ^d	3.52 ^{cd}	3.47 ^d	0.09	0.01
<i>In vitro</i> true dry matter digestibility (%)	82.78 ^a	80.67 ^b	80.22 ^b	79.80 ^b	78.53 ^c	77.50 ^d	0.42	0.01
Methane reduction (%)	-	15.39 ^a	20.37 ^a	28.23 ^b	27.28 ^b	28.21 ^b	1.31	0.01

Each value is mean of six observations

Means bearing different superscripts in the same row differ significantly (P < 0.01)

Table 3 Effect of sheanut cake based concentrate rations on pH, Ammonia Nitrogen, total volatile fatty acids (TVFA) concentration, microbial biomass, ME and protozoal count

Parameter	Control	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	P Value
pH	6.67	6.73	6.77	6.75	6.75	6.78	0.12	0.08
Ammonia -N (mg/dl)**	16.68 ^a	15.05 ^b	14.82 ^b	14.58 ^b	14.35 ^b	14.47 ^b	0.18	0.01
Total Volatile fatty acids (mM/l)	86.37	82.90	80.97	79.40	82.55	80.63	1.01	0.46
Microbial bio mass (mg)**	80.31 ^b	86.17 ^a	86.37 ^a	87.55 ^a	86.29 ^a	86.07 ^a	0.64	0.01
Metabolisable energy (MJ/kg DM)**	8.43 ^a	7.95 ^b	7.86 ^{bc}	7.79 ^{bc}	7.71 ^{bc}	7.63 ^c	0.06	0.01
Total protozoa (× 10 ⁵ /ml)*	5.08 ^a	4.25 ^b	4.17 ^b	4.08 ^b	3.83 ^b	3.67 ^b	0.13	0.02

Each value is mean of six observations

Means bearing different superscripts in the same row differ significantly * P < 0.05 **P < 0.01