DR-1703
[1-6]

RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume Issue : ()

Impact of Supplementing Essential Oils on Reduction of Enteric Methane Emission in Indigenous Dairy Cattle

K. Rajkumar, R. Karunakaran, A. Bharathidhasan, P.T. Gnanaraj, P. Vijayarani

10.18805/ajdfr.DR-1703

ABSTRACT

Background: Globally enteric methane emission is the largest contributor of anthropogenic global warming and methane is the second largest driver of global radiative forcing after carbon dioxide. The present study was conducted to evaluate the effect of supplementing essential oils (EOs) in combination of garlic oil and peppermint oil on enteric methane emission in indigenous dairy cattle.

Methods: Eight indigenous dairy cattle were divided into two groups supplemented with essential oils in combination of garlic oil and peppermint oil (2.4 mL/animal) as treatment group and without supplementation with paddy straw and concentrate feed (60:40) based diet. A digestibility trial was conducted during the last six days of 30 days feeding trial to evaluate the effect of essential oils in combination on nutrients digestibility. The total methane emission was estimated by sulfur hexa fluoride tracer gas technique (SF_6).

Result: No significant difference in DMI, TDN and DCP intake could be noted with supplementation of essential oils in indigenous dairy cattle. However, cows fed with combination of garlic oil (GO) and peppermint oil (PO) significantly ($P<0.05$) reduced the methane emission by 10.60% in comparison to control group. Hence, the feasibility of feeding essential oils can be considered at farm level as a methane mitigation strategy.

Key words: Essential oil, Garlic oil, Methane mitigation, Peppermint oil.

INTRODUCTION

Climate change and livestock are reticular processes, both of which synchronize on a universal scale and their relationship is portentous as the imbalance between world population and world food production. Also challenges regarding the sustainable dairy production with respect to the ever-present environmental problems, especially facing the climate change emphasize the importance of nutritional expertise and optimum feeding management. Enteric methane emission is considered to be the largest potential contributor to the global warming (Caro *et al*. 2016) and worldwide, the livestock contributes up to 40% of the global anthropogenic methane emissions (Key and Tallard, 2012). Methane is most crucial GHG as it has soaring global warming potential, short half-life and loss of gross energy which otherwise can be diverted for animal production. Various attempts have been made for mitigation of methane emission from livestock using halogenated methane analogues, antibiotics, fats and oils, organic acids, but the response and the adoption rate of these compounds was highly variable. The most promising dietary strategies are those which employ feedstuffs that meet another need in the diet (*i.e*., provide a source of energy or nitrogen) or are readily available ingredients that are already being utilized by producers. Plant essential oils are volatile, aromatic compounds with an oily appearance, which are wringed from plants, famous as antimicrobial agent and categorized to zoo-technical additives group such as digestion enhancer, gut flora stabilisers, substance which favorably affects the environment. Essential oils have shown their ability to improve the performance and contribute to a reduction of emission losses in animal husbandry. So far, many studies have been conducted to evaluate the effect of essential oils on methane reduction by *in vitro* (Patra *et al*. 2010 and Jayanegara *et al*. 2010) and *in vivo* (Beauchemin and McGinn, 2006) methods. The data on supplementation of essential oils to indigenous cattle is very limited. Paddy straw is a major agro-residue fed to ruminants in the tropical countries like India. Feeding only rice straw does not provide enough nutrients to the ruminants even for maintenance due to the low nutritive value, also it is claimed that feeding low nutritive value feed leads to increased enteric methane emission in ruminants. Keeping these facts in view, the current study was under taken to assess the effect of

Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India.
[1]Livestock Farm Complex, Chennai-600 051, Tamil Nadu, India.
[2]Department of Animal Biotechnology, Madras Veterinary College, Chennai-600 007, Tamil Nadu, India.

Corresponding Author: K. Rajkumar, Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India. Email: rajkumar.nutrition@gmail.com

How to cite this article: Rajkumar, K., Karunakaran, R., Bharathidhasan, A., Gnanaraj, P.T. and Vijayarani, P. (2022). Impact of Supplementing Essential Oils on Reduction of Enteric Methane Emission in Indigenous Dairy Cattle. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1703.

Submitted: 01-05-2021 **Accepted:** 03-02-2022 **Online:** 04-05-2022

essential oils (combination of garlic oil and peppermint oil) on mitigation of enteric methane emission in indigenous cattle fed with paddy straw-based diet.

MATERIALS AND METHODS

All experiment protocols were approved by the Tamil Nadu Veterinary and Animal Sciences University.

Animal and feeding

Eight indigenous female dairy cattle (304.5±8.66 kg) were randomly selected and divided into two groups with (treatment) and without (control) supplementation of essential oils for a period of thirty days. The cows were maintained at well ventilated animal shed with individual feeding and 24 hours drinking water facilities. All the cattle were fed with paddy straw and concentrate mixture (60:40) based diet. They were fed weighed quantity of paddy straw (3.5 kg/animal/d) and concentrate feed (2.75 kg/animal/d) separately. The concentrate mixture was prepared by mixing of maize (33.5%), soya bean meal (19.5%), de-oiled rice bran (44.0%), mineral mixture (2.0%) and salt (1.0%). The essential oil combination consisting of 1.7 mL of garlic oil and 0.7 mL of peppermint oil were supplemented along with the basal feed to treatment group, *i.e*., 2.4 ml of essential oil combination in total per cow. The dose rate for *in vivo* experiment was calculated based on the assumption of rumen capacity on body weight basis as prescribed by Owens and Goetsch (1986).

Digestibility trial

A digestibility trial was conducted during the last six days of feeding trial to evaluate the effect of essential oil combination on digestibility of feed for indigenous dairy cattle.The representative samples of paddy straw, concentrate feed and dung voided were collected and subjected for proximate analysis (AOAC, 2000). The left-over feed was also collected on the next day and subject to moisture estimation.

Estimation of methane emission by sulfur hexafluoride (SF_6) tracer gas technique

Methane (CH_4) emission from cows in control and treatment groups was estimated using sulfur hexafluoride (SF_6) tracer gas technique (Johnson *et al*. 1994). Brass permeation tubes (12.5 mm × 40 mm) containing SF_6 gas were pre-determined for the release rates and were placed in the rumen (one per animal) 14 days prior to the start of the experiment to allow for the tracer gas to reach steady state in the rumen. Release rates of the permeation tubes used in this study ranged from 3 to 4 mg per day. Gases expired by animals were sampled using a gas collection apparatus (canister) fitted around the neck and attached to a halter (Chaves *et al*. 2006). The PVC canister was placed around the neck of each cow just behind the ears and attached to a halter fitted with an air-tight connection to a 90 cm length of restriction tubing (1.58 mm internal diameter) with an in-line 15 mm filter and flexible nose piece. Samples of expired air were accumulated over five consecutive 24 hours periods in evacuated collection canisters strapped to the cows' shoulders (Fig 1). Also, a canister was hanged in animal shed to record the background concentration of CH_4 and SF_6 gases and they were subtracted from the CH_4 and SF_6 gases collected from the indigenous dairy cattle. The concentration of CH_4 and SF_6 gases in collected gas samples were determined using gas chromatography fitted with Flame Ionization Detector (FID) and Electron Capture Detector (ECD) for methane and SF_6 respectively. The methane emission was determined from CH_4 to SF_6 ratio using the release rate of SF_6 as given in the following formula (Williams *et al*. 2011).

$$RCH_4(g/cow/d) = RSF_6 \times \frac{[(CH_4\ canister) - (CH_4\ background)]}{[(SF_6\ canister) - (SF_6\ background)]} \times \frac{MWCH_4}{MWSF_6} \times 1000$$

Where,

The values in square brackets are concentrations and R- represents rates of CH_4 emission and SF_6 release. Methane emissions have been expressed as absolute values for individual cows (CH_4 g/cow/d).

Statistical analysis

All data collected during the feeding trial were statistically analysed using ANOVA and t test (Snedecor and Cochran, 1980). Pair wise post-hoc comparisons were done using Tukey test. All analyses were carried out using SPSS version 25.0 for windows.

RESULTS AND DISCUSSION

Composition of feeds

Paddy straw is characterized by low digestible nutrients, high amount of silica, deficient in major nutrients and strong ligno-cellulose bonds (Table 1). Cellulose is the main fraction of rice straw which can be hydrolyzed into glucose. However, the presence of lignin and hemicellulose makes the enzymatic hydrolysis of cellulose very difficult, thus preventing the utilization of rice straw (Mussatto *et al*., 2008). Therefore, paddy straws are to be supplemented with concentrate mixtures, which will provide adequate nutrients. The basal diet used for this study met the nutrient requirement for Indian cattle in tropical condition as prescribed by Paul *et al*. (2004). Inadequate production of green fodder and unavailability of quality dry fodder in the country compelled farmers to utilize straws as an important diet for ruminants.

Digestibility trial

The palatability of the feed was unaffected with supplementation of essential oils in indigenous dairy cattle. There was no significant difference in average dry matter intake between control and treatment groups (Table 2). Similar results were also observed by Benchaar *et al*. (2007), who reported that addition of essential oil in alfalfa silage-based feed did not alter the palatability and digestibility of feed in dairy cattle. The unaltered nutrient digestibility between the treatment groups in the present study was due

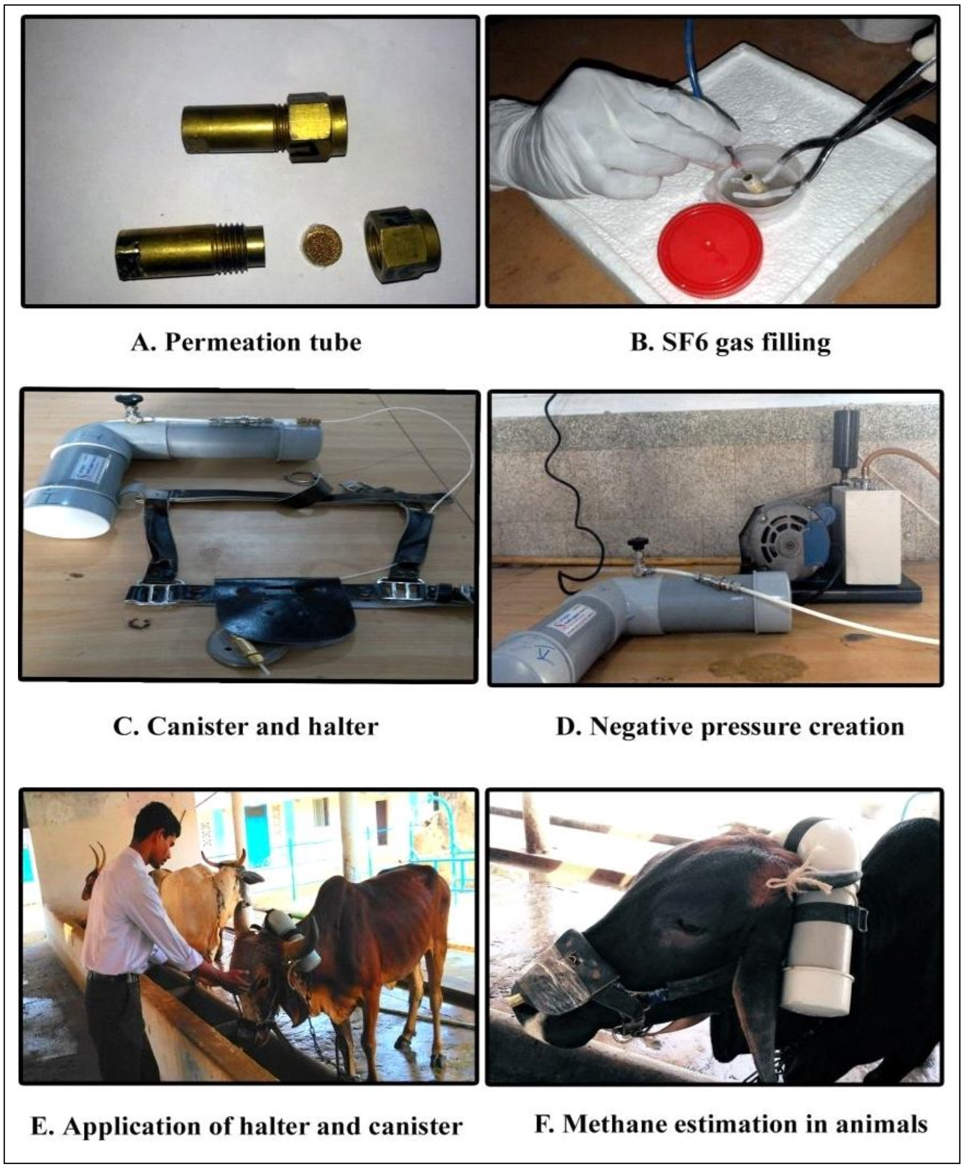

Fig 1: Estimation of methane emission by SF_6 tracer gas technique in indigenous dairy cattle.

Table 1: Composition of compound feed fed to the animals during the trial period**.**

Parameters	Paddy straw (%)	Concentrate feed (%)
Organic matter	82.33	87.90
Crude protein	5.93	21.19
Ether extract	0.83	1.76
Crude fibre	33.16	10.96
Total ash	17.67	12.10
Nitrogen free extract	42.41	53.99
Acid insoluble ash	16.71	4.22
Neutral detergent fibre	70.76	39.40
Acid detergent fibre	45.03	13.76
Acid detergent lignin	3.77	3.50

to the minimum supplementation of EOs (Table 2). Patra *et al*. (2010) also reported that there was no effect on DMI but the digestibility was significantly improved by feeding garlic at 1% of DMI. Ando *et al*. (2003) also reported that when peppermint (200 g/d) was fed to steers, the digestibility of all nutrients tended to be higher than that of the control. The activity of the plant secondary metabolites is also associated with pH, type of diet of the animal; therefore, the dose of the feed additive has to be standardized with the specific feeding regimen of the animal (Calsamiglia *et al*. 2007) for unaltered intake and digestibility. The dose at which the feeding trial was conducted might be minimum and hence no significant improvement in the nutrient digestibility was observed. Also, there was no significant difference in TDN and DCP in control and treatment (Table 2).

Further the basal diet used for the indigenous dairy cattle in the current study for both groups met the recommended nutritive values as per Ranjhan (1991), who reported 2.4 kg TDN and 0.2 kg DCP were required for 300 kg mature Indian cattle. The addition of essential oils with paddy straw and concentrate feed did not affect the nutritive value of feeds.

Estimation of methane emission using sulfur hexafluoride (SF_6) tracer gas technique

Methane emission in the control group was found to be 101.73 g/d whereas the methane emission in garlic oil and peppermint oil supplemented group was 90.95 g/d (Table 3). The supplementation of essential oils in combination of GO and PO at 2.4 mL/d significantly (P<0.01) decreased the CH_4 emission by 10.60% when compared to control group. Similarly, the earlier studies also reported that a reduction of methane emission by supplementation of essential oils in dairy cattle (Yatoo *et al*. 2018). However, the higher methane reduction potential (64%) was observed by the supplementation of methanol extract of garlic without affecting fibre degrading enzymes and in vitro dry matter degradability of feeds (Kamra *et al*. 2006). The addition of EOs to basal diet *in vitro* modulate the rumen fermentation and decreases the methane production effectively (Agarwal *et al*. 2009). However, very few such studies have been done, *in vivo* and a study on dairy cattle reported lesser efficiency with lower magnitude of reduction in methane emission compared to *in vitro* experiments (Beauchemin and Mcginn, 2006).

Other studies showed varying results on methane emission in dairy cattle. Klevenhusen *et al*. (2011) reported that there was no effect of garlic oil on *in vivo* methane reduction. However, 10.06% decrease in methane emission observed in the current study is less compared with earlier studies (Jordan *et al*. 2006; Odongo *et al*. 2007; Martin *et al*. 2008; Beauchemin *et al*. 2009). Further, a decrease in methane emission in earlier studies was, at least in part, attributed to a decrease in DMI (Beauchemin *et al*. 2009). In the current study, the effect of Eos on methane emission was not accompanied by change in DMI. This difference in responses among the authors could be attributed not only to the difference in type and combination of essential oils used and stability of their active principles but also diet provided in these studies which determined the emission

Table 2: Effect of supplementation of essential oils in combination of garlic oil and peppermint oil on nutrient utilization indigenous dairy cattle.

Parameters	Control Basal feed	Treatment Basal feed + Garlic oil 1.7 mL + Peppermint oil 0.7 mL	P-value
Dry matter intake [NS]			
DMI (kg/d)	5.73±0.03	5.48±0.05	0.14
DMI (g/kg$^{0.75}$ / d)	79.27±3.85	75.33±2.78	0.442
Digestibility (%) [NS]			
Dry matter	61.12±1.53	58.95±1.62	0.873
Organic matter	65.97±1.18	64.21±1.65	0.586
Crude protein	68.37±1.45	67.67±1.30	0.971
Ether extract	42.44±3.09	48.17±2.50	0.798
Crude fibre	63.58±1.67	61.50±1.57	0.554
NDF	50.99±1.99	49.35±2.77	0.431
ADF	42.33±2.92	39.08±3.63	0.925
NFE	66.88±1.04	64.79±2.03	0.185
Nutritive value [NS]			
TDN %	58.21±1.09	56.89±1.53	0.598
TDN intake (g/d/ kg MBW)	46.18±2.79	42.75±1.29	0.480
DCP %	6.96±0.18	7.09±0.17	0.839
DCP intake (g/d/ kg MBW)	5.44±0.10	5.29±0.12	0.769

[#]Mean of four observations; [NS] Non significant.

Table 3: Effect of supplementation of garlic oil and peppermint oil in combination on reduction of methane emission in indigenous dairy cattle.

Parameters	Control Basal feed	Treatment Basal feed + Garlic oil 1.7 mL + Peppermint oil 0.7 mL	P-value
Methane emission			
(g/cow/ day)	101.73^{a}±1.92	90.95^{b}±1.46	0.003
(g/kg BW/d)	0.33^{a}±0.01	0.30^{b}±0.01	0.002
(g/kg DMI/d)	17.80^{a}±0.45	15.90^{b}±0.36	0.012
(g/kg digestible DMI/d)**	30.13^{a}±0.73	26.86^{b}±0.49	0.007

Means bearing different superscript in the same row differ significantly.

Different roughage to concentrate ratio affects the methane emission to the maximum extent (Hristov *et al*. 2013). In our study the concentrate feed formulated was based on the paddy straw diet in which ad libitum feeding of paddy straw leads to an elevated ratio of roughage to concentrate. The reduction of the methane emission in the current study was mainly due to the active principles, diallyl disulfide (DADS) (24%) and menthol (26%) present in the GO and PO –respectively.

CONCLUSION

This feeding trial clearly demonstrated that EOs in combination of garlic oil and peppermint oil reduced methane emission without affecting the digestibility of the feed. It was concluded that the methane emission was significantly decreased by 10.06% in EO supplemented treatment group when compared to EO un-supplemented control group for indigenous dairy cattle fed on paddy straw-based diet. Hence essential oil could be thought of an effective and a natural ingredient with properties of reduction in methane emission when fed with concentrate feed.

ACKNOWLEDGEMENT

The authors acknowledged the assistance of Livestock farm complex staff at Madhavaram Milk Colony, Chennai. Thanks also given to the staffs of the Department of Animal Nutrition, Madras Veterinary College, Chennai for providing infrastructure facilities for analysing the methane emission and proximate principles of samples. The funding support of Indian Council of Agriculture Research is also acknowledged.

Conflict of interest: None.

REFERENCES

Agarwal, N., Shekhar, C., Kumar, R., Chaudhary, L.C and Kamra, D.N. (2009). Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Animal Feed Science and Technology. 148(2-4): 321-7.

Ando, S., Nishida, T., Ishida, M., Hosoda, K and Bayaru, E. (2003). Effect of peppermint feeding on the digestibility, ruminal fermentation and protozoa. Livestock Production Science. 82: 245-248.

A.O.A.C. (2000). Official Methods of Analysis. 17th edn, Association of Official Analytical Chemists International, Washington, D.C.

Beauchemin, K.A., Mcginn, S.M., Benchaar. C and Holtshausen, C. (2009). Crushed sunflower, flax or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. Journal of dairy science. 92(5): 2118-2127.

Beauchemin, K.A and McGinn, S.M. (2006). Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. Journal of Animal Science. 84(6):1489-96.

Benchaar, C., Petit, H.V., Berthiaume, R., Ouellet, D.R., Chiquette, J. and Chouinard, P.Y. (2007). Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage. Journal of Dairy Science. 90: 886-897.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P.W and Kamel, C. (2005). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. Journal of Dairy Science. 88(7): 2508-16

Calsamiglia, S., Busquet, M., Cardozo, P., Castillejos, L and Ferret A. (2007). Essential oils as modifiers of rumen microbial fermentation. A review. Journal of dairy science. 90: 2580-2595.

Caro, D., Kebreab, E and Mitloehner, F.M. (2016). Mitigation of enteric methane emissions from global livestock systems through nutrition strategies. Climatic Change. 137: 467-480.

Chaves, A.V., Thompson, L.C., Iwaasa, A.D, Scott, S.L., Olson, M.E., Benchaar, C., Veira, D.M and McAllister, T.A. (2006). Effect of pasture type (alfalfa *vs*. grass) on methane and carbon dioxide production by yearling beef heifers. *Canadian* Journal of Animal Science. 86: 409-418.

Hosoda, K., Nishida, T., Park, W.Y and Eruden, B. (2005). Influence of *menthe × piperita* L. (peppermint) supplementation on nutrient digestibility and energy metabolism in lactating dairy cows. Asian-Australasian Journal of Animal Sciences. 18: 1721-1726.

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P., Adesogan, H.P.S., Yang, A.T., Lee, C., Gerber, P.J., Henderson, B and Tricarico, J.M. (2013). Special topics-Mitigation of methane and nitrous oxide emissions from animal operations: A review of enteric methane mitigation options. Journal of animal science. 91: 5045-5069.

Imai, H., Osawa, K., Yasuda, H., Hamashima, H., Arai, T. and Sasatsu, M. (2001). Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. Microbios. 106:31-39.

Jayanegara, A., Goel, G., Makkar, H.P.S and Becker, K. (2010). Reduction in Methane Emissions from ruminants by plant secondary metabolites: Effects of Polyphenols and Saponins. Sustainable Improvement of Animal Production and Health'. [(Eds) NE Odongo, M Garcia, GJ Viljoen] pp: 151-157.

Johnson, K., Huyler, M., Westberg, H.L and Zimmerman, P.B. (1994). Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. Environmental Science and Technology. 28: 359-362.

Jordan, E., Lovett, D.K., Hawkins, M., Callan, J.J. and O'mara, F.P. (2006).The effect of varying levels of coconut oil on intake, digestibility and methane output from continental cross beef heifers. Animal Science. 82(6): 859-865.

Kamra, D.N., Agarwal, N and Chaudhary, L.C. (2006). Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. In International Congress Series Elsevier. (Vol. 1293, pp. 156-163).

Key, N and Tallard, G. (2012). Mitigating methane emissions from livestock. A global analysis of sectoral policies. Climatic Change. 12: 387-414.

Klevenhusen, F., Zeitz, J.O., Duval, S., Kreuzer, M and Soliva, C.R. (2011). Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. Animal Feed Science and Technology. 166: 356-363.

Malik, P.K., Singhal, K.K., Soren, N.M., Thulasi, A., Jose, L and Santosh, S.S. (2013). Effect of graded level Egyptian clover (*Trifolium alexandrium*) supplementation on in vitro methane production and fermentation pattern. Indian Journal of Animal Sciences. 83(8): 802- 805.

Martin, C., Rouel, J., Jouany, J.P., Doreau, M. and Chilliard, Y. (2008). Methane output and diet digestibility in response to feeding dairy cows on crude linseed, extruded linseed, or linseed oil. Journal of Animal Science. 86(10): 2642-2650.

Mussatto, S.I., Fernandes, M. Milagres, M. and Roberto, I.C. (2008). Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. Enzyme and Microbial Technology. 43(2): 124-129.

Odongo, N.E., Rashi, M.M., Kebreab, E., France, J. and Mcbride, B.W. (2007). Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. Journal of Dairy Science. 90(4): 1851-1858

Owens, F.N. (1986). Digesta passage and microbial protein synthesis. Control of digestion and metabolism in ruminants. 196-226.

Patra, A.K., Kamra, D.N. and Agarwal, N. (2010). Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds *in vitro*. Journal of the Science of Food and Agriculture. 90: 511-520.

Paul, S.S., Mandal, A.B., Mandal, G.P., Kannan A. and Pathak, N.N. (2004). Deriving nutrient requirements of lactating Indian cattle under tropical condition using performance and intake data emanated from feeding trials conducted in different research institutes. Asian Australasian Journal of Animal Sciences. 17: 769-776.

Ranjhan, S.K. (1991). Chemical Composition and Nutritive Value of Indian Feeds and Feeding of Farm Animals. New Delhi: I.C.A.R. Publication.

Snedecor, G.W and Cochran W.G. (1980). Statistical Methods Iowa State University Press, Ames. Statistical methods. 7th ed. The Iowa State University Press, Ames.

Williams, S.R.O., Moate, P.J., Hannah, M.C., Ribaux, B.E., Wales, W.J and Eckard, R.J. (2011). Background matters with the SF_6 tracer method for estimating enteric methane emissions from dairy cows: A critical evaluation of the SF6 procedure. Animal Feed Science and Technology. 170: 265-276.

Yatoo, M.A., Chaudhary, L.C., Agarwal, N., Chaturvedi, V.B and Kamra, D.N. (2018). Effect of feeding of blend of essential oils on methane production, growth and nutrient utilization in growing buffaloes. Asian-Australasian Journal of Animal Sciences. 31(5):672.