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Effect of Feeding of Unconventional Oil Cake Combination on Methane Emission Through Respiration Chamber Study

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ABSTRACT

Background: In India, there are a variety of unconventional feed resources that can be used in ration of ruminant animals. Among all, the most promising are castor bean, mahua, neem and karanj oil cakes which contains quality nutrients like that of conventional cakes. These unconventional oilcakes (UOC) are rich source of phytochemicals such as glycosides, saponins, tannins, essential oils and other which will help in methane reduction. This study was planned to investigate the effect of feeding unconventional oil cakes combination (Karanj: mahua: 75:25) on methane production through respiration chamber.

Methods: Nine cattle calves aged one and half year old were distributed into three groups (Control, T1 and T2), three in each as per completely randomized block design where one group acted as control and two groups (T1 and T2) were experimental. The control animals were fed with basal diet (concentrate+roughage) and T1 and T2 were fed with basal diet and UOC combination at the rate of 7.5% and 10% of concentrate mixture, respectively. The calves were fed for 120 days and methane was estimated through respiration chamber.

Result: Methane production was significantly ($P<0.01$) reduced in experimental group, compared to control. Methane production (L/day) measured in control, T1 and T2 was 75.24±2.02, 65.90±0.97 and 59.85±1.55, respectively. Based on the result we concluded that feeding of UOC combination significantly reduced the methane production.

Key words: Calf, methane, Phytochemicals, Respiration chamber, UOC combination.

INTRODUCTION

It was estimated that 0.63% of methane (CH_4) produced at global level was from India and form that 75% of total methane production was from domesticated cattle (Crutzen *et al.,* 1996). The methane emission in ruminant animals due to anaerobic fermentation is a serious concern, having 23 times more global warming potential than that of carbon dioxide. Apart from that, 2 to 12% of gross energy loss was measured in high-fibre, lignocelluloses compounds in ruminant animals due to anaerobic fermentation (Johnson *et al.,* 1991; Moss *et al.,* 2000). Rumen microbes *viz.,* protozoa, fungi, bacteria and archea play a pivotal role in fiber digestion. The phylum Euryarcheota contains a type of Archaea known as methanogens, which are primarily responsible for the generation of methane in the rumen and hindgut of mammals.

A previous report by Kurihara *et al.* (1999) indicated that the loss energy through methane in cattle fed tropical forage which having a relatively high level of fibre and lignin and low level of non-fibre carbohydrate was higher as compared to cattle fed temperate forages. Furthermore, in developing countries the livestock animals are primarily fed a high-roughage diet with little or no concentrate, which results in increased ruminal methanogenesis. All these aspects of enteric CH_4 production have encouraged the scientific community to find alternatives to mitigate GHG emissions. Although a variety of chemical additives and antibiotics have been studied and utilized to reduce enteric methane emission, but today's customer expectations orient toward the use of "natural source" to alter rumen fermentation pattern.

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Plant bioactive compound with antimicrobial properties such as essential oils, saponin and tannin (Wallace *et al.,* 2002), could be used to reduce methane emissions. These compounds, called 'phytochemicals' or plant secondary metabolites (PSM), describe non-nutritive plant metabolites which are essential for plant survival (*i.e.*, protection against herbivores, pests, microorganisms) and proper growth and reproduction (Patra and Saxena, 2009). Some phytochemicals have a direct toxic effect on methanogens (*e.g.*, condensed tannins) or protozoa (*e.g.*, saponins). These effects, in combination with lower voluntary DM intake and decreased nutrient digestibility from high doses of condensed tannin, have led to their classification as "anti-nutritional factors".

There are many unconventional feed resources available in India which can be included in the ration of ruminant animals (Gowda *et al.,* 2019). Among all, the most promising are castor bean, mahua, neem and karanj oil cakes which contains quality nutrients like that of conventional cakes. Karanj is a medium sized glabrous tree and the seeds are reported to contain on an average about 28-34% oil with high percentage of PUFAs. The Karanj cake contains 30-35% crude protein (CP), total digestible nutrients (TDN) present is 60-65% and 4-7% crude fibre. Karanj cake also contain karanjin and pongamol are the most important toxic factors and responsible for bitter taste (Rao *et al.,* 2013). Mahua is an important economic plant growing throughout the subtropical region of the Indo-Pakistan subcontinent. The mahua seed cake contains CP content of 19-26% and TDN content of 50-55%. Mahua cake contains toxic and bitter mowrin compound. These unconventional oil cakes (UOC) are rich sources of phytochemicals such as glycoside, saponins, tannins, essential oils and others which will help in methane reduction (Rao *et al.,* 2013). Also, it has been reported that these cakes having various functions and beneficial effects for livestock production other than feed value. In addition to feed value, it has been indicated that these cakes have a variety of functions and favorable impacts for animal production. These unconventional oil cakes in lower level would be useful for the preparation of concentrate mixture of the ruminant animal as it is having high protein content and nutrients with a cheaper price (Salem *et al.,* 2004). It is reported that inclusion of this type of feed ingredients with very low level is effective to manipulate the rumen fermentation towards the beneficial mode. With this brief background the present study was undertaken.

MATERIALS AND METHODS

Nine cattle calves aged one and half years old were randomly distributed into three groups, with three calves in each group following completely randomized block design, where one group was control and other two groups were experimental (T1 and T2). All the experimental diets were prepared to meet the NRC (2001) nutrient recommendations. The physical composition of concentrate mixture for different groups were presented in Table 1. The calves in control group were fed concentrate mixture and wheat straw at 50:50 ratio. The animals in T1 group was fed concentrate mixture containing 7.5% of UOC (Karanj+mahua at 75:25 ratio) and wheat straw at 50:50 ratio. The animals in T2 group was fed concentrate mixture containing 10% of UOC (Karanj+Mahua at 75:25 ratio) and wheat straw at 50:50 ratio. One kg green fodder was provided twice in a week to the experimental calves to meet the vitamin A requirement The experimental period was for 120 days and standard housing management were followed in all group animals.

Estimation of methane emission by open circuit respiration chamber

The open circuit respiration calorimeter study involved the measurement of methane concentration in the air going in and coming out from the respiration chamber. Measuring flow rate, temperature of dry and wet bulb and atmospheric pressure were recorded. Methane measured by an infrared gas analyzer, while flow rate and the total volume of air coming out to the chamber were measured by using a flow meter attached with the respiration calorimeter. The dry and wet bulb temperature of air coming out of chamber recorded by dry and wet bulb thermometer, atmospheric pressure was recorded by barometer.

Adaptation of animals in respiration chamber

Calves were weighed in the morning prior to feeding and watering and kept in respiration chamber for 2-3 days for acclimatization and then recording of the respiration data were done at hourly interval for two consecutive days. Calves were provided sufficient amount of clean drinking water, feeding manger was attached to metabolic crate, kept inside the respiration chamber, feeding of the calves was as per protocol given above.

Measurement of respiratory exchange

After offering the feed, the chamber was air tighten by closing the door. Blower was run along with ventilation system of chamber. Recording of temperature of dry and wet bulb, flow rate, air volume, atmospheric pressure was done manually at hourly interval. Methane content of sample of the outgoing and incoming air from the respiratory chamber was determined by the Infrared analyzer attached with the chamber. The chamber was opened after 24 hours. The feed residues were collected and measured and daily dry matter intake (kg/d) were estimated. As the collection of urine and faeces separately was not possible (because of mixing), the calculated digestibility of the animal in the consecutive metabolism trial was used for the calculations.

Calibration of respiration apparatus

The calibration of respiration calorimeter was done following standard procedure describe below:

Calibration of flow meter

The flow meter was frequently calibrated against a standard gas meter. The standard dry gas meter was attached between animal chamber and flow meter to be calibrated. The respiration chamber was run for four hours and volume of outgoing air was measured in both the equipment's and the factor was derived with four such observations for difference in the readings of both the equipment's which is being used for necessary correction in total volume of gas passed from respiration chamber.

Calibration of gas analyzer and analysis of methane

Analysis of methane was made by gas analyzer model attached with respiration calorimetry which was calibrated daily with standard gas as per standard protocol. Concentration of methane in incoming and outgoing respiration gas was measured on onscreen display and recorded at hourly interval. Methane production was calculated as per following formulas:

$$V_{STP} = V273/(273+t) \times (P\text{-}VP)/760$$

Where,
V= Volume at room temperature and pressure.
T= Dry bulb temperature.
P= Barometric pressure in mm Hg.
VP= Vapour pressure.
V_{STP}= Volume at standard temperature and pressure.

Methane production

The total volume of methane (L) was calculated by using following equation

$$\text{CH4 recovery (\%)} = \frac{V_{STP}\,(M_f - M_i)}{100}$$

Where,
M_f= Methane present in outgoing air from the chamber.
M_i= Methane present in incoming air into the chamber.
V_{STP}= Volume at standard temperature and pressure.

RESULTS AND DISCUSSION

The chemical composition of unconventional oil cakes (Karanj and Mahua) was presented in Table 1. The organic matter content of karanj and mahua cake was 92.05% and 85.89%, respectively. The crude protein content of Karanj and Mahua cake was 27.71% and 30.02%, respectively. The tannin present in Karanj and Mahua cake was 3.50% and 0.87%, respectively. The saponin content in Mahua cake was 7.80%. The tannin content of experimental concentrate mixture in control, T1 and T2 group was 0, 1.22 and 1.32%, respectively, while saponin content was 0, 0 and 7.80%, respectively. The effect of feeding Karanj and Mahua cake combination to the growing crossbred calves on methane emission was presented in Table 2 and graphically represented in Fig 1 and 2. The dry matter intake measured during experimental period in chamber shown similar ($p>0.05$) in all the three groups. The average daily DMI (kg/day) were 2.65, 2.69 and 2.81 in the control, T1 and T2 groups of calves, respectively. The methane emission (L/day) were 75.24, 65.90 and 59.85 in control, T1 and T2 groups, respectively. The methane emission (L/day) were significantly ($p<0.05$) lower in UOC mixture fed groups as compared to control group. Even though, DMI among the experimental calves were similar, the methane emission L/kg DMI was significantly lesser in T1 and T2 group calves. However, the emission levels were similar in T1 and T2 experimental group calves.

Table 1: Physical composition of concentrate mixture for different groups of experimental calves (%).

Ingredients	Control	T1	T2
Maize	35	33	33
Soyabean meal	26	22	20
Wheat bran	36	34.5	34
Karnj cake	0	5.625	7.5
Mahua cake	0	1.875	2.5
Mineral mixture	2	2	2
Salt	1	1	1
Total	100	100	100

Tannins are polyphenolic substances which bind protein in aqueous solution. Furthermore, tannins are known to decrease protozoal number and hence methane production due to symbiosis protozoa with methanogen in rumen (Makkar *et al.,* 1995). Tavendale *et al*. (2005) suggested two modes of action of tannin on methanogenesis: first, directly affecting activity or population of methanogens, resulting in lower methane emission and second, indirectly by reduced hydrogen production by lowering feed degradation. Effect of tannin on methane production was demonstrated by Pellikaan *et al.* (2011) who mentioned that *in vitro* methane production was reduced by addition of condensed and hydrolysable tannins and methane production was reduced up to 50% (Patra and Saxena, 2010; Goel and Makkar, 2012). Similarly, addition of polyphenol rich bamboo leaf reduced in-vitro methane production (Jafari *et al.,* 2020) Tannins are known for phyto-chemical compound having a strong potential to inhibit CH4 synthesis in the rumen (Jouany and Morgavi, 2007).

Saponins are natural detergents, chemically defined as high molecular weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety. These compound result in cell death by forming complex with sterols in protozoal cell membrane (Cheeke, 2000). Saponins affect methanogenesis in the rumen by limiting hydrogen availability and induce rechanneling of metabolic hydrogen to propionate production from CH_4 (Wina *et al.,* 2006) and other metabolic pathways like acetogenesis (Patra and Saxena, 2009). They modify ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria. Plants rich in saponin have potential for enhancing flow of microbial protein from rumen, increasing efficiency of feed utilization and decreasing methanogenesis.

Table 2: Effect of feeding UOC combination on methane emission in cattle calves.

Attributes	Control	T1	T2
DMI (kg/d)	2.65±1.30	2.79±1.21	2.81±1.51
Methane (L/d)	75.24^b±2.02	65.90^a±0.97	59.85^a±1.55
Methane (L/kg DMI)	28.38^b±0.52	23.61^a±0.25	21.29^a±0.36

$^{a,\,b}$Mean bearing superscripts in rows differ significantly.

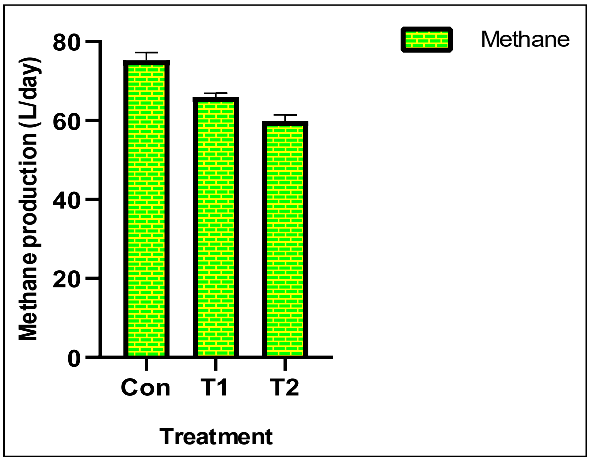

Fig 1: Methane production (L/day).

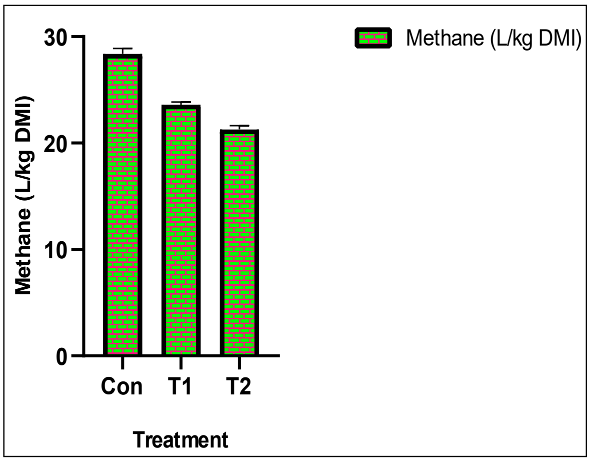

Fig 2: Metha production (L/kg DMI).

It was further reported that methane production was reduced up to 10-25% by addition saponins (Jouany and Morgavi, 2007; Makkar *et al.,* 2007; Patra and Saxena, 2009; Szumacher-Strabel and Cieslak, 2011). Hess *et al.* (2006) reported that *Sapindus saponaria* reduced methane production in Rusitec by 20%, without affecting the methanogens in relation to control and there was a 54% reduction in protozoa population.

Further, Broudiscou *et al.*(2000) reported that, flavonoid-rich extracts of *Equisetum arvense* and *Salvia officinalis* were added to a 50:50 hay+ barley grain diet showed decreased methanogenesis and tannin-rich forages such as sainfoin, sulla, bird's-foot trefoil showed decrease in methane emission in ruminants (Ramirez-Restrepoand Barry, 2005; Waghorn, 2008).

CONCLUSION

It was concluded that, inclusion of unconventional oil cakes like karanj, mahua in ruminant feeding decreased the methane emission, when it was mixed at 75:25 ratio and included at 7.5 and 10.0% of the cattle calves concentrate mixture formulation.

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