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Original Research Article

Efficacy of Reetha (*Sapindus mukorosii*) Fruit extracts in modulating *in vitro* Rumen Fermentation and Methanogenesis in Buffalo

R. K. Singh[1, 2*], Avijit Dey[1], Mala Singh[3], B. S. Punia[1] and S. S. Paul[1]

[1]Division of Animal Nutrition and Feed Technology, ICAR- Central Institute for Research on Buffaloes, Hisar - 125 001, Haryana, India
[2]Division of Animal Nutrition, ICAR- National Dairy Research Institute, Karnal - 132 001, India
[3]Division of Animal Physiology, ICAR- National Dairy Research Institute, Karnal - 132 001, India
**Corresponding author*

A B S T R A C T

Aqueous and ethanolic extract of *Sapindus mukorosii* (SMaq. and $SM_{eth.}$ respectively) were screened for their influences on *in vitro* methane production, feed fermentation and fatty acids composition. *In vitro* fermentation studies were carried out in 125mL serum bottle under anaerobic condition with mixed feed containing oats hay and concentrate mixture as a substrate (60:40) and incubated in 39°C for 24hrs. Methane concentration (%) in the head space gas was reduced ($p<0.001$) linearly with the increasing concentration of both the extracts. However, truly degradable dry matter (%TDDM) was also reduced ($p<0.001$) in treatment groups with higher doses of both the extracts. Increased ($p<0.001$) propionate production with declined ($p<0.001$) acetate production were observed with higher inclusion levels. A: P ratio was declined ($p<0.001$) gradually in higher dose groups. The investigations revealed that, either $SM_{aq.}$ or SM_{eth} extracts supplementation at low dose (0.5mL/40mL of buffered rumen liquor) could be used to reduce methane production without much impeding feed digestibility. However, etanolic extract of reetha (*Sapindus mukorosii*) was found better than aqueous extract in modulating these parameters.

Keywords

Sapindus mukorosii, Methanogenesis, Digestibility

Introduction

India is fortunate with more than 500 million livestock population (DAHDF, 2016). Large population of Indian livestock holding the country at top ranking in milk production (17% of world's) since almost two decades, addressable meat production and other by-products. Currently India is harvesting 155.5MT of milk production and 7.2MT of meat production. Indian milk production scenario is far better than world's production. India is reporting 6.27% growth rate in milk production (DAHDF, 2016). However, world's average growth rate is merely 3.0% (FAO, 2009). Per capita milk availability of India (327g/head/ day) is also far better than world (299g/head/day) (FAO, 2009). Indian agriculture sector is also a main pillar of national GDP (4th largest contributor) with around 1/3rd participation. However, world's average agriculture contribution in GDP is around 6% (FAO, 2009). Indian agriculture GDP is mainly contributed through livestock rearing (around 29% of total agriculture). Currently

dairy sector earned more than 4 lac crore Rs. and 15691 crore Rs. of foreign exchange through meat sector (DAHDF, 2016). With excellent advantages and life sustainable benefits of livestock commodities, such sector is also bound to harm the environment by producing greenhouse gases (GHG) which leads to global warming. In India, ruminants are generally reared on lingo-cellulosic rich feed residue (fibrous diet) resulting in poor feed utilization and more methane emission. Indian livestock produce about 14.3Tg/year methane by enteric fermentation Patra *et al.,* (2008).

Greenhouse gas production from livestock rearing drawn much attention because of its consequences in changing the global climate, which is a global burning issue. Methane comprises up to 16% of global greenhouse gas production Scheehle *et al.,* (2006) and is much deleterious as its warming potential is nearly 25 times greater than that of carbon dioxide. Methane production from agriculture sector represents more than $1/3^{rd}$ of total anthropogenic production while enteric fermentation in ruminant makes the largest single (25%) contribution. The production of methane from ruminants also varies based on geographical location feed composition and quality, feed intake, processing of feed and breed of the animals (FAO, 2009).

The effects of greenhouse gases production on the ecological and social impact have been already addressed and will continue to grow very drastically in future (IPCC, 2007). Carbon dioxide (CO_2), methane (CH_4), nitrous oxide, and sulfur hexafluoride are the important greenhouse gases that are monitored by the United Nations Framework Convention on Climate Change. Methane is the second largest anthropogenic greenhouse gas. It contributed about 14.3% of total anthropogenic greenhouse gas production estimated in 2004 (IPCC, 2007). Atmospheric CH_4 was 1803.2 ppb in 2011. This is many more times greater than in 1750 (IPCC, 2013) and really a serious health and climatic concern.

Many plants secondary metabolites such as saponins, tannins, essential oils and other unknown metabolites from many plant sources were reported to have potential for modulating rumen fermentation with CH_4 suppression Kamra *et al.,* (2008). However, the nature of extracts and their doses, and the composition of diets determine the effectiveness of the additive to modulate the rumen fermentation in positive direction. Saponins or saponins containing plants have been reported to suppress protozoal populations, increased bacterial and fungal populations, propionate production, microbial yield, and decreased methanogenesis in ruminants Hess *et al.,* (2003). However, the efficacy of saponins varies with the source and their doses as well as the nature of substrate used.

Therefore, the goal of the present study was to investigate the graded levels of aqueous and ethanolic extracts of reetha (*Sapindus mukorosii)* on *in vitro* methanogenesis and rumen fermentation pattern on incubation of a mixed feed substrate with rumen liquor of buffaloes.

Materials and Methods

Oats fodder was harvested at pre-bloom stage from ICAR- CIRB, Hisar-India buffalo farm and subjected to drying for overnight in hot air oven at 100^{o}C. After grinded to 1 mm size it was used as substrate for *in vitro* fermentation studies. Hay was also investigated (Table: 1) for proximate analysis (AOAC, 1995) and fibre analysis Van Soest *et al.,* (1991). A standard concentrate mixture was prepared by mixing

ingredients viz. maize grains (37%), groundnut cake (35%), deoiled rice bran (25%), mineral mixture (2%) and common salt (1%) and was also investigated for proximate analysis (Table: 1). A mixed feed was prepared by mixing oats hay and concentrate mixture (60:40) and used as a substrate for incubation during *in vitro* fermentation studies.

For preparation of aqueous extract ($SM_{aq)}$ of reetha (*Sapindus mukorosii*), 10g fruits was finely ground and mixed with 100ml of distilled water in a conical flask. The flask was then kept in an incubator cum shaker at 40°C for 48hrs and the extract was filtered by Whatman filter paper No-I stored at refrigerator (4^oC) for further studies. However, ethanol extract ($SM_{eth)}$ was prepared by adding 10g finely ground sample in 100 ml of 70% aqueous ethanol (ethanol: water, 70:30) in a conical flask and placed in thermo scientific incubator cum shaker for 48hrs, and after incubation the extract was filtered by Whatman filter paper No-1. The filtrate was kept at refrigerator (4^oC) for further study.

Individual *in vitro* fermentation studies were carried out with aqueous ($SM_{aq)}$ and ethanol (SM_{eth}) extracts in 125mL serum bottles with mixed feed containing oat hay and concentrate mixture (60:40) with buffered rumen fluid (2:1) at 39°C under anaerobic condition Menke *et al.,* (1979). Both $SM_{aq.}$ and SM_{eth} were investigated at graded concentrations (@ 0, 0.5, 1.0, 2.0 and 4.0 mL/40mL buffered rumen fluid) in three replicates for each treatment. After 24hrs of incubation, methane concentration in the gas phase and volatile fatty acids in liquid were analyzed by GC (NUCON 5700) using Porapak Q stainless steel and Chromosorb 101 glass column, respectively. *In vitro* true digestible dry matter (TDDM) was estimated by refluxing fermentation residue for 1hrs with neutral detergent solution Van Soest *et al.,* (1991). Data obtained were analyzed using standard statistical procedures (SPSS 16 version, One way ANOVA).

Results and Discussion

Inclusion of SM_{aq} revealed significant ($p<0.001$) reduction (Table 2 and Fig 1) in methane concentration (%) in all treatment groups, in comparison to control group. However, TDDM (%) was significantly ($p<0.001$) reduced in higher doses (0.5mL/40mL onwards). Thus, lower incubated dose of $SM_{aq.}$ was found to be better for methane inhibition with minimal affecting fiber digestibility. NH_3-N concentration was reduced ($p<0.001$) in cumulative higher doses, however, it remained unchanged at lower dose. Acetate concentration (mM/100ml) was significantly ($p<0.001$) reduced at higher concentrations of extract. But, it remained similar ($p>0.05$) in lower doses. Propionate concentration (mM/100ml) was significantly ($p<0.001$) improved at increasing doses of aqueous extract of reetha. However, butyrate concentration was comparable in all incubated dose levels. The A: P ratio was found to be decreased ($p<0.001$) in higher dose levels, when compared to control.

The inclusion of ethanolic extract (SMeth) of reetha revealed significant ($p<0.001$) reduction of methane (%) concentration in all treatment groups, as compared with control group. However, TDMD (%) was comparable at lowest incubated dose level of SMeth only, as compared to control group. NH_3-N concentration was significantly ($p<0.001$) reduced in all treatment groups. All VFA fractions were found to be linearly reduced ($p<0.001$) in treatment groups with higher doses of supplementation, in comparison to control.

Table.1 Proximate composition of substrates (Oats hay and concentrate feed) used during *in vitro* studies

Attributes	Values (%) on DM basis	
	Oats hay	Mixed feed
DM	88.7	91.2
OM	93.3	94.8
CP	7.8	20.7
CF	31.4	13.5
EE	2.07	3.47
Total ash	6.7	5.2
NDF	58.4	39.8
ADF	43.7	26.32

Table.2 Effects of aqueous extract of *Sapindus mukorosii* ($SM_{aq.}$) on *in vitro* rumen fermentation with mixed feed as substrate

Attributes	Control	T_1	T_2	T_3	T_4	SEM	P_{Value}
TDMD%	64.56^{d}	64.21^{d}	62.27^{c}	54.94^{b}	52.22^{a}	0.759	<0.001**
Methane conc. (%)	5.64^{d}	4.78^{c}	2.84^{b}	2.30^{a}	2.68^{b}	0.391	<0.001**
NH_3-N (mg/dl)	18.73^{b}	16.33^{b}	12.27^{a}	10.27^{a}	9.33^{a}	0.738	<0.001**
Acetate (mM/100ml)	4.76^{c}	4.67^{c}	4.61^{c}	4.31^{b}	4.09^{a}	0.036	<0.001**
Propionate (mM/100ml)	1.35^{c}	1.38^{c}	1.43^{c}	1.84^{b}	2.13^{a}	0.018	<0.001**
Butyrate (mM/100ml)	0.60^{c}	0.60^{c}	0.47^{b}	0.31^{a}	0.26^{a}	0.018	<0.001**
A:P	3.49^{c}	3.36^{c}	3.21^{c}	2.38^{b}	1.95^{a}	0.073	<0.001**

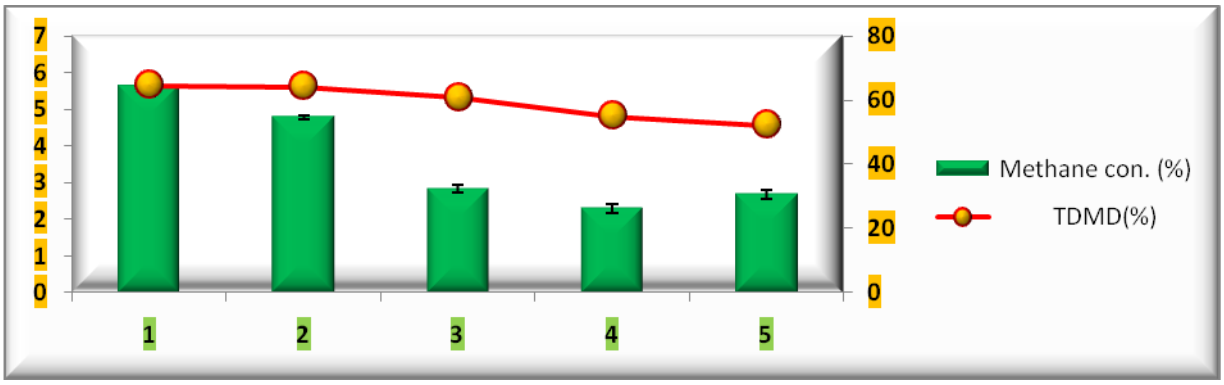
^{abcd} means with different superscripts in the same row differ significantly, **means($p<0.001$).
Control, T_1, T_2, T_3, and T_4 are treatment groups @ 0.0, 0.5, 1.0, 2.0 and 4.0ml of $SM_{aq.}$/40ml of buffered rumen fluid.

Table.3 Effects of ethanol extract of *Sapindus mukorosii* ($SM_{eth.}$) on *in vitro* rumen fermentation with mixed feed as substrate

Attributes	Control	T_1	T_2	T_3	T_4	SEM	P_{Value}
TDMD%	63.95^{c}	64.91^{c}	62.99^{c}	59.20^{b}	54.77^{a}	0.694	<0.001**
Methane conc.%	6.47^{d}	5.19^{c}	5.13^{c}	3.09^{b}	2.24^{a}	0.421	<0.001**
NH_3-N(mg/dl)	19.13^{b}	17.40^{b}	14.99^{ab}	11.63^{a}	10.73^{a}	1.012	<0.001**
Acetate(mM/100ml)	4.46^{cd}	4.61^{cd}	4.17^{c}	3.49^{b}	2.90^{a}	0.063	<0.001**
Propionat(mM/100ml)	1.23^{a}	1.26^{a}	1.33^{a}	2.04^{b}	2.68^{c}	0.018	<0.001**
Butyrate(mM/100ml)	0.46^{c}	0.38^{b}	0.36^{ab}	0.32^{a}	0.37^{b}	0.00	<0.001**
A:P	3.63^{d}	3.66^{d}	3.13^{c}	1.73^{b}	1.13^{a}	0.077	<0.001**

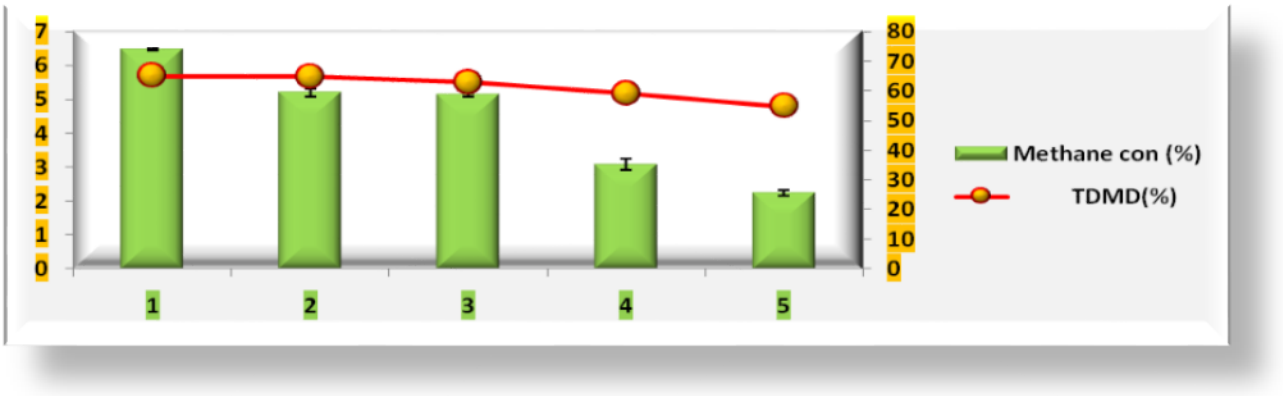
^{abcd} means with different superscripts in the same row differ significantly, **means($p<0.001$).
Control, T_1, T_2, T_3 and T_4 are treatment groups @ 0.0, 0.5, 1.0, 2.0 and 4.0ml of $SM_{eth.}$/40ml of buffered rumen fluid.

Fig.1 Dry matter digestibility vs methane concentration *in vitro* on $_{SMaq.}$ with mixed feed as substrate



1= control. 2, 3, 4 and 5 are incubated groups of $SM_{aq.}$ @ 0.5, 1.0, 2.0, 4.0ml of $SM_{aq.}$/40ml of buffered rumen liquor, respectively.

Fig.2 Dry matter digestibility vs methane concentration *in vitro* on *Sapindus mukorosii* ($SM_{eth.}$) with mixed feed as substrate



1=Control. 2, 3, 4 and 5 are treatment group @ 0.5, 1.0, 2.0 and 4.0ml of $SM_{eth.}$/40ml of buffered rumen liquor, respectively.

However, Propionate concentration was found to be increased ($p<0.001$) linearly on graded doses of incubation. The A: P ratio was found to be significantly ($p<0.001$) decreased in treatment groups with higher doses of inclusion.

Saponins (active phytochemical) investigated for methanogenesis and other rumen fermentation characteristics found in many plant genera, and traditionally saponins containing plants have been used as soap substitutes. Chemically, saponins are high molecular weight glycosides in which a triterpene or steroidal aglycone moiety is linked to one or more sugar chains. The number of sugars, the type of sugars and the stereochemistry of aglycone moieties may vary producing a diverse array of metabolites in this compound class, which might explain the variable potency of biological effects Newbold *et al.*, (2004). Reetha (*Sapindus mukorosii*) is a rich source of saponins and *in vitro* incubation of its aqueous and ethanolic extracts (@ 0.5ml/ 40ml of buffered rumen liquor) resulted 15.75% and 19.78% inhibition of methane reduction, with minimal affecting fibre

digestibilities. However, ethanolic extract of *Sapindus mukorosii* found more promising results (20.71% methane inhibition) in methane inhibition at 1ml of SM_{eth}/40ml of buffered rumen liquor. *In vitro* methanogenesis investigations with aqueous extract of *Spindus mukorosii* fruits were also conducted by Aggarwal *et al.,* (2013), they incubated 0.5ml of extract /30ml of rumen fluid and found 20% reduced methane production, as compared to control group. However, 9.32% TDDM (%) also reduced, as compared to control group. Hess *et al.,* (2004) also conducted similar study on finely ground *Sapindus mukorosii* fruits @1.24g/lt of media, and reported 20% reduced methane concentration, as compared to control group. However, 22.79% reduced TDMD (%) was reported.

Aggarwal *et al.,* (2013) also investigated ethanolic extract of *Sapindus mukorosii* fruits for *in vitro* methanogenesis and reported almost whole methane inhibition as compared to control group. However, they also reported 14.67% reduced TDMD (%). Karma *et al.,* (2008) conducted similar study with ethanolic extract of *Sapindus mukorosii* fruits @ 0.2g/kg DM and reported 1.65ml/g DM methane concentration, as compared with 38.89ml/g DM in control group. They also reported 29.2% reduced TDMD (%), as compared to control group.

In present study, ethanolic extract of *Sapindus mukorosii* was found to be more efficient on methane inhibition (Table 2 and 3) and other fermentation characteristics, as compared to aqueous extract, which may suggest more efficient saponins extraction through ethanol solvent. There is increasing evidence to suggest that addition of saponins in the diets might decrease methane production, which is likely due to a decrease in protozoal numbers and/or methanogenic archaea Hess *et al.,* (2003).

In vitro investigations of ethanolic extract of *Sapindus mukorosii* was found more efficient in methane inhibition with minimal affecting fibre digestibility in lower incubated dose regimen, as compared with aqueous extract on similar dose regimen. However, higher incubated graded doses of either $SM_{aq.}$ or SM_{eth} were found greater extents of methane inhibition with reduced dry matter digestibility.

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