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Effect of *Madhuca longifolia* and *Terminalia chebula* on methane production and nutrient utilization in buffaloes

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ABSTRACT

Deoiled mahua seed cake (*Madhuka longifolia*, M) either alone or in combination with harad seed pulp (*Terminalia chebula*, H) was evaluated for its antimethanogenic activity under *in vitro* as well as *in vivo* conditions. For *in vitro* gas production test, two types of concentrate mixtures were used, one was without M (control) and the other containing M @ 100 g/kg concentrate mixture (M10). The substrate used was concentrate mixture and wheat straw in 50:50 ratios and the rumen liquor collected from two buffaloes fed control diet, pooled in equal proportion was used as inoculum. The four treatments were, control; M10 without harad seed pulp (M10H0); M + 2 mg harad seed pulp/100 mg substarte (M10H2); and M + 4 mg harad seed pulp/100 mg substarte (M10H4). Total gas production was reduced ($P<0.05$) with M10H4 in comparison to control and other two treatments. The percent reduction ($P<0.05$) in methane production was 18.9, 21.2, and 33.3 with M10H0, M10H2, and M10H4, respectively, as compared to control. The production of fermentation products (total volatile fatty acids, their molar proportions, and ammonia nitrogen) was not affected with any of the treatments. The protozoa number was reduced ($P<0.05$) by inclusion of feed additives. For *in vivo* feeding trial, 16 male buffaloes divided into 4 groups of 4 animals each were assigned to the treatments viz; control, M10 without harad seed pulp (M10H0), M10 + harad seed pulp @ 20 g/kg DMI (M10H2), and M10 + harad seed pulp @ 40 g/kg DMI (M10H4) for 21 days followed by a metabolism trial and chamber studies. The intake and digestibility of nutrients (dry matter, organic matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber) was similar in all the four groups. Methane production (L/d) was tended ($P<0.09$) to decrease by 11.7%, 12.9%, and 17.6% in M10H0, M10H2, and M10H4 groups, respectively vs. control. However, heat production and energy utilization by the animals were similar in all the groups. There was no effect of additives on blood parameters; hemoglobin and packed cell volume, total protein, albumin, globulin, and serum enzymes; alanine transaminase, aspartate transaminase, and lactate dehydrogenase. The cell mediated immunity was also not affected with the additives. The results indicated

Abbreviations: A:G, albumin globulin ratio; ADF, acid detergent fiber; ALT, alanine transaminase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BW, body weight; CMI, cell mediated immunity; CP, crude protein; CT, condensed tannin; DE, digestible energy; DM, dry matter; DMI, dry matter intake; DTH, delayed-type hypersensitivity; EDTA, ethyl diamine tetra acetic acid; EE, ether extract; GE, gross energy; IVTD, *in vitro* true digestibility; LDH, lactate dehydrogenase; M10H0, mahua seed cake (100 g/kg DM of concentrate mixture) without harad seed pulp; M10H2, M10 + 20 g harad seed pulp/kg DM; M10H4, M10 + 40 g harad seed pulp/kg DM; ME, metabolizable energy; NDF, neutral detergent fiber; NDS, neutral detergent solution; OM, organic matter; PCV, packed cell volume; PHAP, phytohemagglutinin-P; SEM, standard error of mean; VFA, volatile fatty acid.

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that under *in vitro* condition the combination of additives decreased methane production significantly but under *in vivo* feeding trial, the level used was not sufficient enough to induce significant inhibition in methane production in buffaloes. Further experiments with higher dose for longer period are needed to verify the antimethanogenic activity of the additives tested.

1. Introduction

The livestock contribute to global warming by producing greenhouse gases like carbon dioxide, methane, and nitrous oxide which are well known for their green house effects. Methanogenesis is an essential metabolic process in the rumen to get rid of excess hydrogen produced in the process of carbohydrate fermentation. Methane production in the rumen results in 2–12% loss of feed gross energy in the form of methane and also contributes to global warming (Johnson et al., 1993). Therefore, by controlling the methane emission from the rumen, the livestock productivity can be improved with reduced environmental pollution (Kreuzer et al., 2009). Efforts have been made, using different additives like halogenated methane analogues, propionate enhancers, fats, reductive acetogens, ionophores, bacteriocins, defaunating agents (Boadi et al., 2004) to mitigate methane emission but non of the technologies could be used practically either due to harmful effects on the animals directly or due to presence of chemical residues in the animal products. The plant secondary metabolites (PSM) have antimicrobial activity and therefore, their role in modulating rumen microbial ecosystem to reduce methanogenesis has been conceptualized (Patra et al., 2006, 2010; Kreuzer et al., 2009). A large number of plant parts like leaves, fruits, bark, and seeds containing secondary metabolites like tannin, saponin, essential oils, flavonoids, etc., have been tested as feed additive to reduce methane production in the rumen, but majority of work has been done in *in vitro* conditions using extracts of the plants. There are only limited numbers of feeding trials using plants containing PSM as feed additives to reduce enteric methane emission by animals (*e.g.*, Beauchemin et al., 2007; Patra et al., 2011; Verma et al., 2012; Puchala et al., 2012; Lin et al., 2013). In the authors' laboratory several plants, rich in PSM have been screened for *in vitro* antimethanogenic activity, out of which two plants (deoiled mahua seed cake and harad seed pulp) exhibiting antimethanogenic activity were selected in the present study to explore their practical use at the farm level.

2. Materials and methods

2.1. In vitro experiment

The *in vitro* gas production test (Menke and Steingass, 1988) was used for *in vitro* tested of deoiled mahua seed cake (*Madhuka latifolia*, M) and harad seed pulp (*Terminalia chebula*, H). The substrate (0.2 g) used, was comprised of concentrate mixture and ground wheat straw in 50:50 ratios on fresh weight basis. The rumen liquor collected just before feeding from two buffaloes and pooled in equal proportion was used as inoculum. Two concentrate mixtures were prepared, one without mahua seed cake (control) and the other with M @ 100 g/kg concentrate mixture (M10) (Table 1). The four treatments were, control; M10 without harad seed pulp (M10H0); M10 + 20 g harad seed pulp/kg DM (M10H2); and M10 + 40 g harad

Table 1
Ingredients and chemical composition (g/kg) of concentrate mixtures and wheat straw.

Ingredient	Concentrate mixture (control)	Concentrate mixture (treatment)	Wheat straw
Physical composition (g/kg fed basis)			
Wheat bran	510	440	–
Maize	270	260	–
Deoiled soyabean meal	190	170	–
Deoiled mahua seed cake	0	100	–
Mineral mixture	20	20	–
Salt	10	10	–
Chemical composition (g/kg DM basis)			
OM	915	925	929
CP	195	188	375
EE	41	43	20
NDFom	298	307	789
ADFom	108	120	647
Total ash	85	75	71

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash.