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Effect of herbal feed additives containing saponins on rumen fermentation pattern

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ABSTRACT

Macrotyloma uniflorum (kulthi) seeds, *Asparagus racemosus* (shatavari) roots or *Acacia concina* (shikakai) pods were supplemented to total mixed rations (TMR) @ 0–3% (on DM basis) to assess the impact of herbal feed additives (HFAs) on the *in vitro* rumen fermentation pattern. The saponin content and 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPHH) antioxidant activity was highest in *A. racemosus* than other HFAs. But total phenols, non tannin phenols, true tannins, condensed tannins, vitamin C and flavanoid contents were highest in *M. uniflorum* and lowest in *A. concina*. The dose/level of supplementation of HFAs, irrespective of their nature did not affect net gas production (NGP) and availability of metabolizable energy (ME) from TMR, but digestibility of nutrients and partitioning factor (PF) decreased in comparison to the unsupplemented group. The total and individual volatile fatty acids (VFAs) production; and acetate to propionate ratio was improved when the TMR was supplemented with HFAs at 1% level. The methane and ammonia-N production was depressed at 2% level as compared to control group. Irrespective of the dose, the total VFAs, acetate, and propionate production was higher while ammonia-N decreased in *M. uniflorum* supplemented TMR than other HFAs supplemented groups. Methane production from the TMR was comparable in the diet supplemented with different HFAs, however, diet supplemented with *M. uniflorum* resulted in lower methane production. Amongst the tested HFAs, *M. uniflorum* was a richer source of most of the bio-active compounds. Based on *in vitro* fermentation parameters, *M. uniflorum* supplemented to TMR @ 2% gave the best results.

Key words: Bio-active components, Fermentation pattern, Herbal feed additives, *In vitro*

The ever increasing human population, urbanization and consumer income has led to increased demand for high quality food/animal products. In comparison to 2010, the requirement of meat and milk will increase by 73 and 58% in world and by 109 and 116% in developing countries, respectively in 2050 (FAO 2011). The expected role of ruminants in meeting this demand is very challenging as besides providing milk and meat for human consumption, ruminants emit greenhouse gases such as methane (CH_4), nitrous oxide and carbon dioxide which affect environment. Over past 250 years, CH_4 emission has increased by 149% which possesses 21 times higher global warming potential than CO_2 (Thorpe 2009). About 90% of CH_4 emitted from enteric fermentation come from ruminants. Domesticated ruminants produce about 80 teragram (Tg) methane/annum. The quantity and quality of feed consumed and fermented in the rumen is one of the major factors influencing enteric methane emission.

Herbs containing saponins [steroid or triterpenoid aglycone (sapogenin) linking to one or more oligosaccharide moieties by glycosidic linkage] possess phytochemical, pharmacological and therapeutic properties, and used for curing many diseases, boosting productive and reproductive performance of livestock. Antiulcer, antioxidant, antidiarrhoeal, immunomodulatory, antibacterial, antihepatotoxic, antineoplastic, antihyperglycemic and antilithiatic activities were observed in *M. uniflorum* (Siddhuraju and Manian 2007, Bigoniya *et al.* 2014), *A. racemosus* (Gautam *et al.* 2004, Christina *et al.* 2005) and *A. cinconisa* (Poomanee *et al.* 2015). Pure bio-active compounds like essential oils, tannins and saponins have shown promising results on enteric methane mitigation, rumen metabolites and nutrient utilization (Patra and Yu 2012, Hundal *et al.* 2016a, b). However, little information is available on using HFAs, containing different bioactive compounds which may have synergistic effect on rumen metabolites, nutrient utilization and performance of calves (Bakshi and Wadhwa 2004, Bakshi *et al.* 2005). Therefore, HFAs were used to assess their effect on *in vitro* rumen fermentation and methane production using TMR as a substrate.

MATERIALS AND METHODS

The HFAs, viz. *M. uniflorum* (kulthi) seed, *A. racemosus* (shatavari) roots and *A. concina* (shikakai) pods containing saponins were supplemented individually at 0, 1, 2 and 3%

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to the TMR containing concentrate mixture (maize 30, mustard cake 15, soybean meal 15, wheat bran 10, rice bran 15, deoiled rice bran 12, mineral mixture 2 and common salt 1% each), green oats and wheat straw in 40:15:45 ratio on DM basis.

In vitro *studies:* Three rumen fistulated bucks (*Beetal*) were fed TMR as per NRC (2007) feeding standard. The rumen contents were collected before feeding at 09:00 h in a thermos flask flushed with CO_2 and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender and strained through four layers of muslin cloth. The solution, containing 960 ml distilled water, 0.16 ml micromineral solution, 660 ml bicarbonate buffer, 330 ml macro mineral solution and 1.6 ml resazurine (0.1%) were mixed in a Woulff flask (3 L) with magnetic stirrer in a water bath at 39°C (Menke *et al.* 1979). The mixture was continuously flushed with CO_2. Then SRC was added to the buffer media in the ratio of 1:2. Glass syringes (100 ml; Haberle Labortechnik, Germany) containing 375±5 mg TMR and buffered rumen liquor were incubated in triplicate in a water bath at 39°C and swirled every 60 min over a 24 h incubation period. If the volume of gas in the syringe exceeded 70 mL after 8 h, the volume was recorded and the gas was expelled. After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spoutless beaker, boiled with neutral detergent solution for assessing the true OM and NDF digestibility. Each *in vitro* gas production set was repeated thrice in order to check any variation in the net gas production and other parameters.

Methane estimation: TMR (200 mg) was incubated for 24 h with buffered rumen liquor and respective herbal feed additive in triplicate. After the stipulated period, total gas production was measured. For CH_4 estimation, representative gas sample was taken from the headspace of syringe using a 100 mL Hamilton syringe and injected into Netchrom 9100 gas chromatograph (Netel, India) equipped with flame ionization detector and stainless steel column packed with Porapak Q. The gas flow rates for N_2, H_2 and air were 15, 30 and 300 mL/min, respectively. Temperature of injector oven, column oven and detector were 70, 50 and 100ºC, respectively. A 50/50 mixture of CH_4 and CO_2 (Spancan; Spantech Products Ltd., England) was used as a standard.

Estimation of volatile fatty acids: After 24 h of incubation, a 5 mL aliquot of fluid from each syringe was mixed with 1 mL of 25% meta-phosphoric acid and kept for 1 h at ambient temperature (Erwin *et al*. 1961). Thereafter, it was centrifuged at 5500 rpm for 10 min and clear supernatant was collected and stored at 20ºC until analyzed. The volatile fatty acids were estimated using Netchrom 9100 gas chromatograph (Netel, India) equipped with glass column (packed with chromosorb 101) and flame ionization detector (Cottyn and Boucque 1968). Temperature of injection port, column and detector was set at 250, 175 and 270ºC, respectively. The flow rate of carrier gas (N) through the column was 15 mL/min while flow rate of H_2 and air through FID was 30 and 300 mL/min, respectively. Sample (2 µL) was injected through the injection port using a 10 µL Hamilton syringe. Individual VFA's of the samples were identified on the basis of their retention time and their concentration (mmol) was calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values. The efficiency of rumen fermentation (E), efficiency of fermented hexose energy to VFA energy (E_1) and methane energy (E_2) were calculated by using the equations of Ørskov *et al*. (1968), Czerkawski (1986) and IAEA (1985) respectively as cited by Baran and Zitnan (2002).

Analytical methods: The dry extracts of herbs were screened for heavy metals like arsenic and lead by inductively coupled plasma-optical emission spectrometry (Perkins Elmer Optima 2100 DV Model) (Yeotikar *et al*. 2018); yeast and moulds, pathogens like *E. coli*, *Salmonella* and total *coliforms* (Anonymous 2012). The HFAs were also analyzed for saponins (Baccou *et al*. 1977), condensed tannins (Porter *et al*. 1986) and for DPPH activity (Kumaran and Karakumaran 2007). The essential oils in HFAs were obtained by hydro-distillation with a Clevenger apparatus using 100 g of sample for 6 h and dried over anhydrous sodium sulphate. The finely ground samples of the substrate were analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and total ash (AOAC 2007) and neutral detergent fiber (Van Soest *et al*. 1991). For ammonia estimation, 5 mL of supernatant was mixed with 1 N NaOH and NH_3 evolved was collected in boric acid solution containing mixed indicator and titrated against 0.01 N H_2SO_4 (AOAC 2007).

Table 1. Bioactive components in herbal feed additives (mg % on DM basis)

Herbal feed additive	Local	Saponins	Phenolics				Antioxidants		
			TP	NTP	TT	CT	DPPH	Vit C	Flavonoids
Macrotyloma uniflorum	Kulthi	7.54^{a}	11.19^{c}	0.78^{b}	10.41^{c}	0.40^{b}	2.00^{a}	1.95^{c}	6.62^{c}
Asparagus racemosus	Shatavari	9.35^{b}	7.47^{b}	0.74^{b}	6.72^{b}	0.02^{a}	2.29^{b}	0.63^{b}	4.17^{b}
Acacia concina	Shikakai	7.90^{a}	3.78^{a}	0.49^{a}	3.30^{a}	0.11^{a}	2.08^{a}	0.47^{a}	1.82^{a}
PSE		0.95	1.36	0.21	1.30	2.10	0.67	0.7	2.05
p-value		0.002	<0.001	0.019	<0.001	0.009	0.002	<0.001	<0.001

TP, Total phenols; NTP, Non-tannin phenols; TT, True tannins; CT, Condensed tannins; DPPH 2, 2-diphenyl-1-picryl-hydrazyl-hydrate antioxidant activity; Vit C, Vitamin C; ^{a,b,c}Means with different superscripts in a column differ significantly; PSE, Pooled standard error.

Statistical analyses: The data of active components in selected HFAs was analyzed by simple ANOVA, while that of other parameters by 4 × 3 factorial design (Snedecor and Cochran 1994), taking different herbal feed additives as one factor and level of herbs as second factor, by using SPSS (2009) version 16.0 and the means were tested for the significant difference by using Duncan's multiple range test.

RESULTS AND DISCUSSION

The TMR used as substrate contained 15.4% CP, 2.98% EE, 57% NDF, 37.8% ADF and 31.8% cellulose.

Bioactive components in HFAs: The saponin content in *A. racemosus* was higher (P<0.01) than *M. uniflorum* and *A. concina* (Table 1). In addition to saponins, these HFAs also contained tannins and antioxidants. The total phenols, non tannin phenols, true tannins and condensed tannins content were highest (P<0.01) in *M. uniflorum* followed by A. *racemosus* and the lowest was in *A. concina,* except condensed tannin content which was lowest (P<0.01) in *A. racemosus*. The DPHH antioxidant activity was highest (P<0.01) in *A. racemosus* and the lowest in *M. uniflorum,* but comparable in *A. concina* and *M. uniflorum.* The vitamin C and flavanoid content in *M. uniflorum* was higher (P<0.01) than *A. racemosus* followed by *A. concina*. Essential oils were not detected in the herbal feed additives. The qualitative/quantitative presence of above phytochemical constituents in *M. uniflorum* (Sreerama *et al.* 2010, Prasad and Singh 2015), *A. racemosus* (Kamat *et al*. 2000, Alok *et al*. 2013) and *A. concina* (Khanpara *et al*. 2012, Anonymous 2018) have also been reported earlier.

The heavy metals (arsenic and lead); yeast and moulds were not detected in the dry extracts of the above herbal feed additives. Pathogens *E. coli*, *Salmonella* and total *coliforms* were absent in all the samples of dry extracts.

Effect of dose and nature of HFAs on digestibility and ME availability from the TMR: The dose of supplementing HFAs, irrespective of their nature, did not affect NGP and ME availability from TMR, however, supplementation at 2% level had an edge over control and other levels (Table 2). The digestibility of NDF, true OM and PF decreased (P<0.05) in the HFAs supplemented TMR in comparison to the unsupplemented TMR. Earlier studies revealed that saponin extracts from either *Sapindus saponaria* (Hess *et al*. 2003a), *Quillaja saponaria* or *Yucca schidigera* (Pen *et al*. 2006) supplemented at different doses to different substrates depressed *in vitro* digestibility. Supplementation of saponin containing HFAs decreased the digestibility of NDF by 5.3% and true OM by 1.9% and PF by 5.8% in comparison to the control TMR.

Irrespective of the dose, NGP, digestibility of NDF and true OM, PF and ME availability from TMR were not affected by the nature of HFAs supplemented diets but supplementation of *A. concina* had an edge over other herbs as far as NGP or ME availability was concerned (Table 2). Earlier reports also indicated that using the extracts of saponin containing plants like *Sapindus saponaria* (Hess

Table 2. Effect of dose and nature of saponin containing herb (% DMB) on *in vitro* gas production, digestibility of nutrients and available ME using total mixed ration as substrate

Parameter	Control	Level of herbal feed additives (L), %			PSE	Herbal feed additive			PSE	P value		
		1	2	3		*Macrotyloma uniflorum* (Kulthi)	*Asparagus racemosus* (Shatavari)	*Acacia concina* (Shikakai)		Level	HFA	L × HFA
NGP	169.33	172.62	177.6	173.60	2.90	172.14	172.27	175.13	3.35	0.443	0.716	0.539
NDFD (%)	49.03^{c}	47.14^{b}	46.19^{b}	45.92^{a}	0.64	47.21	47.19	47.35	0.55	0.032	0.975	0.479
TOMD (%)	68.53^{c}	67.44^{ab}	67.39^{ab}	66.84^{a}	0.37	67.56	67.51	67.59	0.32	0.046	0.985	0.512
PF (mg/mL)	2.17^{c}	2.10^{b}	2.02^{a}	2.01^{a}	0.01	2.08	2.08	2.07	0.009	<0.001	0.725	0.659
ME	8.10	8.21	8.32	8.24	0.086	8.19	8.20	8.26	0.10	0.451	0.768	0.615

NGP, Net gas production, ml/g/24 h; NDFD, Neutral detergent fibre digestibility; TOMD, True OM digestibility; PF, Partitioning Factor, mg/ml; ME, Metabolizable energy, MJ/kg DM; ^{a,b,c}Means with different superscripts for different doses of HFAs with in a row differ significantly; PSE, Pooled standard error.

et al. 2003b), *Camellia sinensis* (Hu *et al*. 2006) or *Sesbania sesban* (Goel *et al*. 2008) using substrates like meadow hay: *Arachis pintoi* hay: barley straw in 56: 22: 11 ratio, grass hay: corn in 50: 50 ratio, hay: concentrate mixture in 32: 68 ratio, respectively did not show any adverse effect on the *in vitro* DM digestibility.

Effect of dose and nature of HFAs on the volatile fatty acid production and fermentation efficiency of TMR: The total VFAs, propionate, butyrate, isovalerate and valerate production improved (P<0.01) when the TMR was supplemented with HFAs @ 1% level on DM basis. Supplementation of HFAs beyond 1% did not show any beneficial effects on these parameters (Table 3). The concentration of total VFAs, propionate, butyrate and that of BCFAs increased by 9.5, 7.9, 15.5 and 29.9%, respectively over that of control group. Lila *et al.* (2003) also reported increase in total VFAs when corn starch was supplemented with saponin extract from *Medicago sativa*. However, supplementing different substrates with different saponin extracts from different plants either did not affect or decreased the total VFAs production (Agarwal *et al*. 2006, Goel *et al*. 2008). The A: P ratio improved (P<0.01) with supplementation of HFAs @ 1% of substrate. Hydrogen accumulation hinders the pathway for C2 synthesis and favours C3 production (Van Nevel and Demeyer 1996) resulting in lower C2:C3 ratios. The shift of VFA products from acetate to propionate could probably be explained by the reduction of protozoa population. Manju (2019) also revealed that herb supplementation to the wheat straw based complete feed improved (P<0.01) the rumen fermentation resulting in increased total VFA production and decreased total protozoal count.

As compared to control diet, the relative proportion of acetate decreased (P<0.01) while that of all other VFAs increased (P<0.01) with increase in level of supplementation of saponin containing HFAs (Table 3). The relative proportion of acetate decreased (P<0.01) by 3.95%, while that of propionate, butyrate, valerate and BCFAs increased (P<0.01) by 5.8, 4.2, 11.6 and 26.8%, respectively in comparison to unsupplemented TMR. The highest (P<0.01) efficiency of rumen fermentation (E) was observed in diet supplemented with HFAs at 1% level and lowest (P<0.01) in un-supplemented control diet. The efficiency of fermenting hexose to methane (E_2) decreased (P<0.01) in diet supplemented with HFAs at 1% level, followed by diet supplemented with HFAs at 2%. The high fermentation efficiency in diet supplemented with HFAs at 1% level was probably due to the lowest methane production.

Irrespective of dose of HFA, the total VFAs, acetate and propionate production from the substrate supplemented with *M. uniflorum* was higher (P<0.01) than that supplemented with *A. racemosus* and lowest in *A. concina* (Table 3) supplemented TMR. Butyrate production from the substrate supplemented with *M. uniflorum* and *A. racemosus* was comparable but higher (P<0.01) than that supplemented with *A. concina.* Lila *et al*. (2003) also reported increase in total VFAs when saponin extract of *M. sativa* was supplemented to corn starch. Similarly, propionate production increased by supplementing the corn grain: Chinese wild rye with saponin extract of *Tribulus terrestris* plant (Feng *et al*. 2012). The isovalerate production from the substrate supplemented with *A. racemosus* was comparable with that supplemented with *A. concina*, but higher (P<0.01) than that of *M. uniflorum.* The valerate production from *M. uniflorum* was higher (P<0.01) than that of *A. concina.* The acetate to propionate ratio varied between 2.97 mM/dL (*A. racemosus*) to 3.06 mM/dL (*A. concina).* Overall, TMR supplemented with *M. uniflorum* had an edge over *A. racemosus* and *A. concina* with regards to total and individual VFAs production.

The relative proportion of acetate was the highest (P<0.01) from the TMR supplemented with *M. uniflorum*, comparable with that of *A. concina*, but higher (P<0.01) than *A. racemosus* (Table 3). The relative proportion of propionate, butyrate and isovalerate was the highest (P<0.01) from substrate supplemented with *A. racemosus*. The efficiency of rumen fermentation (E) was similar (74.6 to 74.8%) in diet supplemented with *A. concina* and *A. racemosus*. The efficiency of fermenting hexose to methane (E_2) was the lowest (P<0.01) in diet supplemented with *A. racemosus* and comparable with the diet supplemented with *M. uniflorum*. This confirmed the positive effect of HFAs on rumen fermentation. The high fermentation efficiency in diet supplemented with *A. racemosus* was probably due to the lowest methane production.

Effect of dose and nature of HFAs on in vitro *methane production from TMR:* The *in vitro* methane production expressed as mL/100 mg DM/24 h or as mL/100 mg DOM/ 24 h decreased (P<0.05) at 2% level as compared to unsupplemented diet (Table 4). Patra *et al*. (2006) observed that by supplementing wheat straw: concentrate (50: 50) diet with saponin extract from *A. concina* the methane production was depressed by 18.6% as compared to control group. Jadhav *et al*. (2018) revealed that protozoal count, methane and ammonia-N production decreased linearly up to the 0.8% of tea (*Camellia sinensis*) seed saponins supplemented to different forage to concentrate ratios. Ammonia nitrogen production decreased (P<0.05) at all doses as compared to unsupplemented diet. The lowest ammonia-N production was observed at 2% level of supplementation. *In vitro* ammonia-N concentration decreased (P<0.002) when saponins from *Camellia sinensis* and *Trigonella foenum-greacum* plants were included with the vetch-oat hay (Arhab *et al*. 2014). At 24 h incubation, protozoal counts were reduced by 81.86% and 83.29% for the high levels of *Camellia sinensis* and *Trigonella foenum-greacum*, respectively, which showed that tea saponins depressed ciliate protozoa population, but little effect on the methanogen population in sheep. Further, there was no significant correlation between the protozoa counts and methanogens, but decreased methanogen activity (Wang *et al*. 2011). Kang *et al*. (2016) demonstrated that a high level of *Momordica charantia* saponin (MCS) quickly inhibited *in vitro* fermentation of maize stover while MCS

Table 3. Effect of dose and nature of saponin containing herb (% DMB) on *in vitro* volatile fatty acid production (mM/dL) from total mixed ration

Parameter	Control	Level of herbal feed additives (L), %			PSE	Herbal feed additive			PSE	P value		
		1	2	3		*Macrotyloma uniflorum* (Kulthi)	*Asparagus racemosus* (Shatavari)	*Acacia concina* (Shikakai)		Level	HFA	L × HFA
Total VFA	6.98^{a}	7.22^{b}	7.25^{b}	7.21^{b}	0.014	7.32^{o}	7.20^{n}	6.97^{m}	0.012	<0.001	<0.001	<0.001
Acetate (A)	4.56^{c}	4.51^{a}	4.56^{c}	4.54^{b}	0.005	4.65^{o}	4.54^{n}	4.43^{m}	0.005	<0.001	<0.001	<0.001
Propionate (P)	1.41^{a}	1.55^{b}	1.54^{b}	1.54^{b}	0.005	1.55^{o}	1.53^{n}	1.45^{m}	0.004	<0.001	<0.001	<0.001
Isobutyrate	0.100^{a}	0.118^{b}	0.120^{b}	0.118^{b}	0.002	0.112	0.115	0.115	0.002	<0.001	0.251	0.860
Butyrate	0.71^{a}	0.77^{b}	0.77^{b}	0.76^{b}	0.003	0.76^{n}	0.77^{n}	0.73^{m}	0.003	<0.001	<0.001	<0.001
Isovalerate	0.13^{a}	0.18^{b}	0.18^{b}	0.18^{b}	0.002	0.167^{m}	0.173^{n}	0.170^{mn}	0.001	<0.001	0.035	0.011
Valerate	0.069^{a}	0.083^{c}	0.082^{c}	0.074^{b}	0.001	0.080	0.077	0.075	0.001	<0.001	0.059	0.075
A:P	3.24^{c}	2.90^{a}	2.96^{b}	2.96^{b}	0.006	3.01^{n}	2.97^{m}	3.06^{o}	0.005	<0.001	<0.001	<0.001
Relative proportion (%)												
Acetate	65.34^{c}	62.48^{a}	62.87^{b}	62.92^{b}	0.061	63.61^{n}	63.05^{m}	63.55^{n}	0.053	<0.001	<0.001	0.001
Propionate	20.19^{a}	21.53^{c}	21.23^{b}	21.29^{b}	0.033	21.14^{n}	21.26^{o}	20.78^{m}	0.028	<0.001	<0.001	<0.001
Isobutyrate	1.44^{a}	1.64^{b}	1.66^{b}	1.64^{b}	0.023	1.53^{m}	1.60^{n}	1.65^{n}	0.020	<0.001	0.003	0.101
Butyrate	10.18^{a}	10.67^{b}	10.57^{b}	10.57^{b}	0.032	10.36^{m}	10.63^{o}	10.51^{n}	0.027	<0.001	<0.001	0.067
Isovalerate	1.86^{a}	2.52^{b}	2.54^{b}	2.55^{b}	0.021	2.27^{m}	2.39^{n}	2.44^{n}	0.18	<0.001	<0.001	0.005
Valerate	0.99^{a}	1.154^{b}	1.13^{b}	1.03^{a}	0.018	1.09	1.07	1.07	0.016	<0.001	0.671	0.147
Fermentation efficiency												
E	74.20^{a}	75.02^{c}	74.86^{b}	74.87^{b}	0.016	74.74^{n}	74.84^{o}	74.64^{m}	0.014	<0.001	<0.001	<0.001
E_1	73.75^{a}	74.42^{c}	74.29^{b}	74.28^{b}	0.017	74.20^{n}	74.30^{o}	74.07^{m}	0.014	<0.001	<0.001	<0.001
E_2	16.40^{c}	15.50^{a}	15.67^{ab}	15.72^{b}	0.021	15.75^{mn}	15.68^{m}	15.89^{o}	0.019	<0.001	<0.001	<0.001
MBM	177.68^{a}	182.46^{b}	183.16^{b}	182.31^{b}	0.324	185.44^{o}	182.49^{n}	176.28^{m}	0.28	<0.001	<0.001	<0.001

VFA, Volatile fatty acid; E, Efficiency of rumen fermentation; E_1, Efficiency of fermented hexose energy to VFA energy; E_2, Efficiency of fermented hexose to methane; MBM, Microbial biomass; Means with different superscripts^{a,b,c} for different doses of HFAs and superscripts^{m,n,o} for nature of HFAs with in a row differ significantly; PSE, Pooled standard error.

Table 4. Effect of dose and nature of saponin containing herbs (% DMB) on the *in vitro* methanogenesis from total mixed ration

Parameter	Control	Level of herbal feed additives (L), %			PSE	Herbal feed additive			PSE	P value		
	0	1	2	3		*Macrotyloma uniflorum* (Kulthi)	*Asparagus racemosus* (Shatavari)	*Acacia concina* (Shikakai)		Level	HFA	L × HFA
Ammonical-N	0.055^{b}	0.047^{a}	0.045^{a}	0.046^{a}	0.02	0.048	0.049	0.048	0.025	<0.001	0.067	0.026
CH_4 (%)	18.17	17.21^{b}	16.68^{a}	17.03^{ab}	0.03	17.26	17.38	16.73	0.04	0.023	0.105	0.052
CH_4 (mL/100 mg DM/24 h)	3.93^{c}	3.41^{b}	2.61^{a}	3.11^{ab}	0.05	3.31	3.35	3.33	0.027	0.036	0.087	0.031
CH_4 (mL/100 mg DOM/24 h)	3.33^{c}	3.01^{b}	2.47^{a}	2.95^{ab}	0.08	2.87	2.94	2.89	0.03	0.019	0.073	0.029

CH_4, Methane; DOM, Digestible organic matter; PSE, Pooled standard error; ^{a,b,c}Means with different superscripts for different doses of HFAs with in a row differ significantly.

at low doses has the ability to modulate the rumen fermentation pattern by regulating the number of functional rumen microbes including cellulolytic bacteria and fungi populations, and may have potential as a feed additive applied in the diets of ruminants.

Irrespective of the dose of HFA, methane production from the substrate was comparable in all the herbal supplemented diets. Hess *et al*. (2003a) reported that diet supplemented with *Sapindus saponaria* resulted in decrease in protozoa counts by 54% and methane production by 20% with no effect on methanogens and suggested that defaunation reduced methane production because of a lower H_2 supply thus reducing activity per methanogen. *M. uniflorum* had edge over other HFAs as far as fermentation of diet and methane production was concerned.

There was no significant interaction for NGP, digestibility of nutrients, partitioning factor and ME availability from the TMR but there was significant ($P<0.01$) interaction for total and individual VFAs production and acetate to propionate ratio except for isobutyrate and the best was observed in *M. uniflorum* supplemented at 2% of TMR. Similar trend was observed for molar proportion of VFAs, except for butyrate, isobutyrate and valerate. There was no interaction between HFAs and their level of incorporation on the methane and ammonia production, but lowest values were observed when *M. uniflorum* supplemented at 2% of substrate. Based on *in vitro* fermentation parameters and methane mitigation, *M. uniflorum* @ 2% of DM was selected for further *in vivo* evaluation.

Based on *in vitro* fermentation parameters and methane mitigation results, *M. uniflorum* @ 2% of TMR on DM basis was considered as the best and selected to assess the impact on *in vivo* nutrient utilization from complete feed.

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REFERENCES

Agarwal N, Kamra D N, Chaudhary L C and Patra A K. 2006. Effect of *Sapindus mukorossi* extracts on *in vitro* methanogenesis and fermentation characteristics in buffalo rumen liquor. *Journal of Applied Animal Research* **30**: 1–4.

Alok S, Jain S K, Verma A, Kumar M, Mahor A and Sabharwal M. 2013. Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): A review**.** *Asian Pacific Journal of Tropical Diseases* **3**: 242–51.

Anonymous. 2012. *FSSAI Manual of Methods of Analysis of Foods, Microbiological Methods.* Lab Manual 14, Food Safety and Standards Authority of India, Ministry of Health and Family Welfare, Government of India.

Anonymous. 2018. *Acacia concinna* (Willd.) DC Monograph, pspuok.com/books/ monograph%20on/1.pdf.

AOAC. 2007. *Official Methods of Analysis.* 18th edn. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

Arhab R, Abla R, Aggoun M and Zitouni H. 2014. Effect of *Camellia sinensis* and *Trigonella foenum-greacum* saponins on *in vitro* rumen fermentation of vetch-oat hay. *Emirates Journal of Food and Agriculture* **26**: 723–29.

Baccou J C, Lambert F and Sanvaire Y. 1977. Spectrophotometric method for the determination of total steroidal sapogenin. *Analyst* **102**: 458–66.

Bakshi M P S and Wadhwa M. 2004. Effect of herbal feed additives on productive performance of buffalo calves. *Bubalus bubalis*. *Journal of Buffalo Science and Technique* **10**: 65–70.

Bakshi M P S, Rani N, Wadhwa M and Kaushal S. 2005. Impact of herbal feed additives on the utilization of nutrients *in vitro*. *Indian Journal of Animal Nutrition* **22**: 147–51.

Baran M and •itnan R. 2002. Effect of monensin sodium on fermentation efficiency in sheep rumen (short communication). *Archiv fürTierzucht*, *Dummerstorf* **45**: 181–85.

Bigoniya P, Bais S and Sirohi B. 2014. The effect of *Macrotylo mauniflorum* seed on bile lithogenicity against diet induced cholelithiasis on mice. *Ancient Science of Life* **33**: 242–51.

Christina A J, Ashok K, Packialakshmi M, Tobin G C, Preethi J and Murugesh N. 2005. Antilithiatic effect of *Asparagus racemosus* Willd on ethylene glycol-induced lithiasis in male albino Wistar rats. *Methods and Findings in Experimental and Clinical Pharmacology* **27**: 633–38.

Cottyn B G and Boucque C V. 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agriculture and Food Chemistry* **16**: 105–07.

Czerkawski J W. 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, pp 221–22.

Erwin E S, Marco G J and Emery E M. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *Journal of Dairy Science* **44**: 1768–71.

FAO. 2011. World Livestock 2011 – Livestock in food security. Rome, FAO.

Feng Z H, Cao Y F, Gao Y X, Li Q F and Li J G. 2012. Effect of gross saponins of *Tribulus terrestris* on ruminal fermentation and methane production *in vitro*. *Journal of Animal and Veterinary Advances* **11**: 2121–25.

Gautam M, Diwanay S, Gairola S, Shinde Y, Patki P and Patwardhan B. 2004. Immunoadjuvant potential of *Asparagus racemosus* aqueous extract in experimental system. *Journal of Ethnopharmacology* **91**: 251–55.

Goel G, Makkar H P S and Becker K. 2008. Effects of *Sesbania sesban* and *Carduuspy cnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage- and concentrate-based feeds to methane. *Animal Feed Science and Technology* **147**: 72–89.

Hess H D, Kreuzer M, Diaz T E, Lascano C E, Carulla J E and Solvia C R. 2003a. Saponin rich tropical pods affect fermentation and methanogenesis in faunated and defaunated fluid. *Animal Feed Science and Technology* **109**: 79–94.

Hess H D, Monsalve L M, Lascano C E, Carulla J E, Diaz T E and Kreuzer M. 2003b. Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* pods: effects on *in vitro* ruminal nitrogen turnover and methanogenesis. *Australian Journal of Agricultural Research* **54**: 703–13.

Hu W, Liu J, Wu Y, Guo Y and Ye J. 2006. Effects of tea saponins on *in vitro* ruminal fermentation and growth performance in growing Boer goat. *Archives of Animal Nutrition* **60**: 89–97.

Hundal J S, Wadhwa M and Bakshi M P S. 2016a. Effect of supplementing essential oils on the *in vitro* methane production and digestibility of TMR. *Journal of Animal Nutrition USA* **1**: 14.

Hundal J S, Wadhwa M and Bakshi M P S. 2016b. Methane mitigation potential of tannins and their impact on digestibility of nutrients *in vitro*. *Animal Nutrition and Feed Technology* **16**: 505–13.

IAEA. 1985. *Laboratory Training Manual on the Use of Nuclear Techniques in Animal Nutrition*. Technical Reports Series No.48, International Atomic Energy Agency, Vienna, 301.

Jadhav R V, Kannan A, Bhar R, Sharma O P, Gulati A, Rajkumar K, Mal G, Singh B and Verma M R. 2018. Effect of tea (*Camellia sinensis*) seed saponins on *in vitro* rumen fermentation, methane production and true digestibility at different forage to concentrate ratios. *Journal of Applied Animal Research* **46**: 118–24.

Kamat J P, Boloor K K, Devasagayam T P A and Venkatachalam S R. 2010. Antioxidant properties of *Asparagus racemosus* against damage induced by α-radiation in rat liver mitochondria. *Journal of Ethnopharmacology* **71**: 425–35.

Kang J, Zeng B, Tang S, Wang M, Han X, Zhou C, Yan Q, He Z, Liu J and Tan Z. 2016. Effects of *Momordica charantia* saponins on *in vitro* ruminal fermentation and microbial population. *Asian Australasian Journal of Animal Science* **29**: 500–08.

Khanpara K, Renuka, Shukla V J and Harisha C R. 2012. A detailed investigation on shikakai (*Acacia concinna*linn.)–fruit. *Journal of Current Pharmaceutical Research* **9**: 6–10.

Kumaran A and Karakumaran J. 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT–Food Science and Technology* **40**: 344–52.

Lila Z A, Mohammed N, Kanda S, Kamada T and Itabashi H. 2003. Effect of sarsaponin on rumen fermentation with particular reference to methane production *in vitro*. *Journal of Dairy Science* **86**: 3330–36.

Manju, Dhuria R K, Khinchi R K, Meel P and Meel M S. 2019. Effect of herbs as feed additive on rumen fermentation patterns and haemato-biochemical parameters in marwari rams fed wheat straw based complete feed. *International Journal of Livestock Research* **9**: 32–40.

Menke K H, Rabb L, Salewski A, Steingass H, Fritz D and Schneider W. 1979. The estimation of the digestibility and ME content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agriculture Science* **93**: 217–22.

NRC. 2007. *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. National Research Council, National Academy of Sciences, Washington, DC.

Ørskov E R, Flatt W P and Moe P W. 1968. Fermentation balance approach to estimate extent of fermentation and efficiency of volatile fatty acids formation in ruminants. *Journal of Dairy Science* **51**: 1429–35.

Patra A K and Yu Z. 2012. Effect of Quillaja and Yucasaponins on communities and select populations of rumen bacteria and archaea, and fermentation *in vitro*. *Applied Environmental Microbiology* **78**: 4271–80.

Patra A K, Kamra D N and Agarwal N. 2006. Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Animal Feed Science and Technology* **128**: 276–91.

Pen B, Sar C, Mwenya B, Kuwaki K, Morikawa R, and Takahashi J. 2006. Effects of *Yucca schidigera* and *Quillaja saponaria* extracts on *in vitro* ruminal fermentation and methane emission. *Animal Feed Science and Technology* **129**: 175–86.

Poomanee W, Chaiyana W, Intasai N and Leelapornpisid P. 2015. Biological activities and characterization of the pod extracts from sompoi (*Acacia concinna* linn) grown in northern Thailand. *International Journal of Pharmacy and Pharmaceutical Science* **7**: 237–41.

Porter L J, Hrstich L N and Chan B G. 1986. The conversion of procyanidins and prodelphinidins to cyanidins and delphinidin. *Phytochemistry* **25**: 223–30.

Prasad S K and Singh M K. 2015. Horse gram—an underutilized nutraceutical pulse crop: a review. *Journal of Food Science and Technology* **52**: 2489–99.

Siddhuraju P and Manian S. 2007. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotylo mauniflorum* (Lam.) Verdc.) seeds. *Food Chemistry* **105**: 950–58.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 7th edn. Oxford and IBH Publications, New Delhi.

SPSS. 2009. *Statistical Packages for Social Sciences*. Ver. 16, SPSS Inc., Linois, USA.

Sreerama Y N, Dennis A, Neelam, Vadakkoot B S and Vishwas M P. 2010. Distribution of nutrients and antinutrients in milled fractions of chickpea and horse gram: Seed coat phenolics and their distinct modes of enzyme inhibition. *Journal of Agriculture and Food Chemistry* **58**: 4322–30.

Thorpe A. 2009. Enteric fermentation and ruminant eructation: the role (and control?) of methane in the climate change debate. *Climatic Change* **93**: 407–31.

Van Nevel C J and Demeyer D I. 1996. Control of rumen methanogenesis. *Environmental Monitoring and Assessment* **42**: 73–97.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.

Wang J K, Ye J A and Liu J X. 2011. Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance—a review. *Tropical Animal Health and Production* **44**: 607–706.

Yeotikar P V, Nayyar S, Singh C, Mukhopadhaya C S, Kakkar S S and Jindal R. 2018. Levels of heavy metals in drinking water, blood and milk of buffaloes during summer and winter seasons in Ludhiana, Punjab (India). *International Journal of Pure and Applied Bioscience* **6**: 1265–74.