



Herb Supplementation on Fermentation and Methane Emission  
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## Impact of Type and Level of Herbs Supplemented to Total Mixed Ration on the Fermentation Pattern and *In vitro* Methane Emission

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### ABSTRACT

This study was taken up to assess the effect of dry extract of six herbal feed additives [HFAs; Kulthi (*Dohichos biflorus*), patha (*Cissampelospareria*), aritha (*Sapindustrifoliatus*), methi (*Trigonella foenum graecum*), shikakai (*Acacia concina*) and shatavari (*Asparagus racemosus*)] rich in saponins supplemented at 1-4% of total mixed ration (TMR) on DM basis on the *in vitro* fermentation pattern and methane emission. A TMR with roughage to concentrate (R:C) ratio of 70:30 on DM basis was formulated. The data was analyzed by 2 x 6 x 5 factorial design. The net gas production (NGP), digestibility of NDF and OM, ME availability, total and individual volatile fatty acids (VFAs) were higher ( $P < 0.01$ ) at 24h incubation in comparison to that observed at t $\frac{1}{2}$ . The NGP and ME in the TMR supplemented with shatavari was higher ( $P < 0.01$ ) than all other HFAs supplemented groups and increased ( $P < 0.01$ ) with the increase in the level of HFAs. The total and individual VFAs were the highest ( $P < 0.01$ ) when the diet was supplemented with HFAs at 3% levels. The methane production was lowest ( $P < 0.01$ ) and the fermentation efficiency was the highest in TMR supplemented with shatavari. The methane emission was depressed at all levels of HFAs as compared to control group. The methane emission at t-half was observed to be the lowest in the TMR supplemented with patha, kulthi or shatavari at 2% on DM basis. It was concluded that best response in above parameters could be ascertained in TMR supplemented with shatavari @ 2-3% on DM basis.

**KEYWORDS:** Fermentation, Herbal additives, Methane emission, Nutrient utilization, Total mixed ration

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### INTRODUCTION

The livestock production in low and middle-income countries will increase by 74% over the next decade, while global animal population will increase by around 50% by 2050 (OECD-FAO, 2021). Despite the fact that livestock systems play key role in addressing global food security and promoting economic growth, but if not managed well, they put the environment at risk. Livestock emissions from manure and enteric fermentation represent roughly 32% of global anthropogenic emissions (UNEP and CCAC, 2021).

Different supplements like antibiotics (Karásková et al., 2015) and phytogetic feed additives (Miguel, 2010) have been used worldwide for the mitigation of enteric methane emission. Number of studies has

been conducted to assess the potential of plant secondary metabolites on rumen fermentation. Saponins are phytochemicals commonly found in plants and are composed of steroids, triterpenoids, and steroid alkaloids. Saponins as feed supplements may have the potential to modulate rumen fermentation and improve nutrient utilization in ruminants (Bakshi and Wadhwa, 2010). This study was therefore taken up to assess the effect of different HFAs containing saponins supplemented at varying levels to TMR on the *in vitro* methane emission and nutrient utilization.

### MATERIALS AND METHODS

#### Procurement of herbs

The dry extracts of HFAs (Kulthi: *Dohichos Biflorus*), patha (*CissampelosPareria*), aritha

(*Sapindus Trifoliatius*), methi (*Trigonella Foenum Graecum*), shikakai (*Acacia Concina*) and shatavari (*Asparagus racemosus*) rich in saponins were procured from Konark Herbals and Healthcare, Mumbai, India.

### Total mixed ration

A TMR containing roughage to concentrate ratio of 70:30 (DM basis) was formulated. The roughage portion was made up of wheat straw and green maize in 70:30 ratio, whereas, the concentrate mixture was made up of maize 15, wheat 15, deoiled mustard cake 15, mustard cake 10, soybean meal 10, rice bran 15, deoiled rice bran 16, urea 1, salt 1 and mineral mixture 2% each. The samples of TMR were analyzed for proximate (AOAC, 2007) and cell wall constituents (Van Soest et al., 1991)

### *In vitro* gas production studies

The nutritional value of TMR was evaluated by *in vitro* gas production technique (IVGPT; Menke et al., 1979; Menke and Steingass, 1988). Two sets of samples were incubated, each in triplicate. Blank and sample of standard hay were run in triplicate with each set. In the 1<sup>st</sup> set the HFAs were supplemented @ 1-4% to 375 mg TMR in 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) with buffered rumen fluid and incubated at 39°C for 24h for assessing the NGP, digestibility of nutrients, VFA production and ME content. In the 2<sup>nd</sup> set 200 mg TMR supplemented @ 1-4% HFAs were incubated in a water bath at 39°C for 96 min. in triplicate to determine the rate of degradation of the substrate. The gas volumes were recorded after 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 hr hand swirled every 60 min. for initial 8 hours. The rate and extent of gas production and  $t^{1/2}$  was calculated by non-linear regression by using software 'Graph Pad Prism' (Miller, 2003).

The incubations were terminated at respective  $t^{1/2}$

and the volume of gas was recorded and true substrate degradability was measured (Blummel and Lebzien, 2001) for assessing the NGP, methane emission, digestibility of nutrients, VFA production and ME at  $t^{1/2}$ .

### Estimation of methane and volatile fatty acids

Methane at  $t^{1/2}$  was estimated by gas chromatograph equipped with stainless steel column packed with porapak-Q and flame ionization detector. VFAs were also estimated by using GLC (Netchrom 9100; Netal, India) equipped with a glass column packed with chromosorb 101 (Cottoyn and Boucque, 1968).

### Other fermentation attributes

The fermentation attributes related to hydrogen recovery, VFA utilization index and microbial biomass synthesis, the efficiency of conversion of fermented hexose energy to VFA and efficiency of conversion of fermented hexose energy to methane energy were calculated adopting procedures described in an earlier publication (Deuri et al., 2019).

### Statistical analyses

The data were analyzed by 2 (Incubation periods) x 6 (HFAs) x 5 (Levels of HFAs) factorial design (Snedecor and Cochran, 1994). The data was analyzed 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 contained 10.9% total ash, 18.5% CP, 2.85% EE, 30.3% cellulose and 60.7% NDF. The effect of incubation period irrespective of type of HFA and level of HFAs on the *in vitro* fermentation pattern revealed that NGP, digestibility of NDF and OM, ME and PF were lower ( $P < 0.01$ ) at  $t$ -half in comparison to that at 24h incubation (Table 1).

Table 1. Effect of incubation period, type and level of herbal feed additive, irrespective of one another on the fermentation parameters of TMR

Parameters	Incubation, h	PSE	P-Value	Herbal feed additives				PSE	P-value	Levels of HFAs, %				PSE	P-value			
				Patha	Kulthi	Shikakai	Methi			Aritha	4	4	4			4	0	1
NGP	166.5 <sup>b</sup>	0.59	<0.001	138.1 <sup>a</sup>	136.3 <sup>a</sup>	137.0 <sup>a</sup>	135.2 <sup>a</sup>	136.2 <sup>a</sup>	142.3 <sup>b</sup>	1.02	0.001	133.06 <sup>a</sup>	135.7 <sup>b</sup>	137.2 <sup>b</sup>	139.10 <sup>c</sup>	142.68 <sup>d</sup>	0.93	<0.001
NDFD, %	49.9 <sup>b</sup>	0.20	<0.001	47.3 <sup>a</sup>	50.7 <sup>b</sup>	51.5 <sup>b</sup>	51.4 <sup>b</sup>	48.6 <sup>a</sup>	50.2 <sup>b</sup>	0.50	<0.001	49.80	49.6	50.8	49.95	49.63	0.45	0.351
TOMD, %	88.1 <sup>b</sup>	0.09	0.003	82.1 <sup>a</sup>	83.1 <sup>b</sup>	83.2 <sup>b</sup>	83.2 <sup>b</sup>	82.3 <sup>a</sup>	82.8 <sup>b</sup>	0.16	0.002	82.81	82.7	83.0	82.80	82.70	0.15	0.391
PF	2.21 <sup>b</sup>	0.02	0.002	1.99 <sup>ab</sup>	2.05 <sup>bc</sup>	2.04 <sup>bc</sup>	2.11 <sup>c</sup>	2.01 <sup>ab</sup>	1.94 <sup>a</sup>	0.03	0.001	2.09 <sup>c</sup>	2.10 <sup>c</sup>	2.02 <sup>bc</sup>	1.97 <sup>ab</sup>	1.94 <sup>a</sup>	0.02	<0.001
ME	7.72 <sup>b</sup>	0.021	0.001	7.02 <sup>a</sup>	6.98 <sup>a</sup>	6.98 <sup>a</sup>	6.97 <sup>a</sup>	6.98 <sup>a</sup>	7.15 <sup>b</sup>	0.036	0.008	6.90 <sup>a</sup>	6.93 <sup>ab</sup>	7.01 <sup>bc</sup>	7.07 <sup>cd</sup>	7.14 <sup>d</sup>	0.033	0.001

NGP-Net gas production ml/gDM/24h, NDFD- Neutral detergent fiber digestibility, TOMD-True organic matter digestibility, PF-Partitioning factor, ME-Metabolizable energy MJ/kg DM, Figures with different superscripts in a row differ significantly

The effect of supplementing different HFAs containing saponins, irrespective of other factors revealed that the NGP and ME availability in the TMR supplemented with shatavari was higher ( $P < 0.01$ ) than all other HFA supplemented groups. The NGP was similar in the remaining HFAs supplemented groups. The digestibility of nutrients (OM and that of NDF) in patha and aritha was statistically comparable, but lower ( $P < 0.01$ ) than rest of HFAs supplemented groups. There was no difference in the digestibility of nutrients amongst these groups. Earlier studies revealed that dry extracts of HFAs rich in saponins like kulthi, shatavari or shikakai, irrespective of their level, did not affect ( $P > 0.05$ ) the *in vitro* NGP, digestibility of nutrients and ME (Hundal et al., 2020). Goel et al. (2008) also indicated that using the extracts of saponin containing plants like *Sesbania sesban* using substrates like hay: concentrate mixture in 32: 68 ratio did not show any adverse effect on the *in vitro* DM digestibility. *In vitro* digestibility of DM and organic matter was not changed by *Sesbania graniflora* pods meal (SES) addition (Unnawong et al., 2021). The highest ( $P < 0.01$ ) PF was observed in TMR supplemented with methi, which was comparable with shikakai and kulthi. The lowest ( $P < 0.01$ ) PF was observed in shatavari which in turn was comparable with aritha and patha.

The level of HFAs had significant impact on fermentation pattern of TMR. The NGP and ME availability increased ( $P < 0.01$ ) with the increase in the level of HFAs from 1-4%. However, reverse trend ( $P < 0.01$ ) was observed in case of PF. The level of HFAs did not show any impact on the digestibility of nutrients. However, Hundal et al. (2020) reported that with the increase in level of HFAs from 1-3%, the *in vitro* digestibility of NDF and true OM was depressed ( $P < 0.05$ ) as compared to un-supplemented group.

### Impact of incubation periods, type and levels of HFAs on the volatile fatty acid production

The effect of incubation period, irrespective of other factors like type and level of HFAs revealed that the total and individual volatile fatty acids were higher ( $P < 0.01$ ) at 24h in comparison to that observed at t-half incubation (Table 2).

Table 2. Effect of incubation period, type and level of herbal feed additive, irrespective of one another on the VFAs production (mM/dl) during fermentation of TMR

Parameters	Incubation, h		PSE	P-Value	Herbal feed additives					PSE	P-Value	Levels of HFAs, %					PSE	P-value
	24	t-half			Patha	Kulthi	Shikakai	Methi	Aritha			Shatavari	0	1	2	3		
TVFAs	7.69 <sup>b</sup>	7.48 <sup>a</sup>	0.01	0.001	8.46 <sup>f</sup>	8.22 <sup>e</sup>	6.60 <sup>b</sup>	7.49 <sup>e</sup>	7.55 <sup>d</sup>	7.22 <sup>b</sup>	7.64 <sup>c</sup>	6.87 <sup>a</sup>	7.41 <sup>b</sup>	7.88 <sup>d</sup>	0.01	0.001		
Acetate	5.36 <sup>b</sup>	5.06 <sup>b</sup>	0.005	<0.001	5.81 <sup>f</sup>	5.54 <sup>e</sup>	4.98 <sup>b</sup>	5.09 <sup>d</sup>	5.01 <sup>c</sup>	4.81 <sup>a</sup>	5.18 <sup>c</sup>	5.05 <sup>b</sup>	4.99 <sup>a</sup>	5.34 <sup>d</sup>	0.009	<0.001		
Propionate	1.80 <sup>b</sup>	1.61 <sup>a</sup>	0.002	<0.001	1.79 <sup>d</sup>	1.78 <sup>d</sup>	1.61 <sup>a</sup>	1.61 <sup>a</sup>	1.76 <sup>c</sup>	1.67 <sup>b</sup>	1.66 <sup>b</sup>	1.67 <sup>b</sup>	1.64 <sup>a</sup>	1.75 <sup>c</sup>	0.004	0.003		
Butyrate	0.637 <sup>b</sup>	0.613 <sup>a</sup>	0.005	0.004	0.672 <sup>c</sup>	0.699 <sup>d</sup>	0.588 <sup>ab</sup>	0.609 <sup>b</sup>	0.603 <sup>b</sup>	0.577 <sup>a</sup>	0.614 <sup>a</sup>	0.611 <sup>a</sup>	0.609 <sup>a</sup>	0.627 <sup>a</sup>	0.007	<0.001		
Isovalerate	0.087 <sup>b</sup>	0.077 <sup>a</sup>	0.00	<0.001	0.086 <sup>c</sup>	0.094 <sup>d</sup>	0.079 <sup>b</sup>	0.080 <sup>b</sup>	0.079 <sup>b</sup>	0.073 <sup>a</sup>	0.087 <sup>c</sup>	0.080 <sup>b</sup>	0.078 <sup>a</sup>	0.079 <sup>ab</sup>	0.001	0.004		
Valerate	0.061 <sup>b</sup>	0.048 <sup>a</sup>	0.001	0.002	0.062 <sup>c</sup>	0.064 <sup>c</sup>	0.050 <sup>ab</sup>	0.051 <sup>ab</sup>	0.053 <sup>b</sup>	0.048 <sup>a</sup>	0.056 <sup>bc</sup>	0.053 <sup>ab</sup>	0.052 <sup>a</sup>	0.054 <sup>ab</sup>	0.001	<0.001		
A:P	2.81 <sup>a</sup>	3.34 <sup>b</sup>	0.006	0.001	3.26 <sup>c</sup>	3.13 <sup>d</sup>	3.09 <sup>c</sup>	3.14 <sup>d</sup>	2.88 <sup>a</sup>	2.92 <sup>b</sup>	3.11 <sup>b</sup>	3.06 <sup>a</sup>	3.07 <sup>a</sup>	3.06 <sup>a</sup>	0.009	0.001		

Figures with different superscripts in a row differs significantly

However, a reverse trend ( $P<0.01$ ) was observed in the acetate to propionate ratio which was higher at t-half than that observed at 24h. Singh (2017) also observed that the total and individual VFAs were higher ( $p<0.01$ ) at 24 in comparison to that observed at t-half incubation, however, reverse trend ( $P<0.01$ ) was observed in the acetate to propionate ratio.

The total VFAs, acetate and propionate production was the highest ( $P<0.01$ ) in patha followed by kulthi and the lowest was observed in shikakai, except acetate production which was the lowest in shatavari (Table 2). Lila et al. (2003) also reported increase in total VFAs when corn starch was supplemented with saponin extract from *Medicago sativa*. Similarly, propionate production increased by supplementing the corn grain: Chinese wild rye with saponin extract of *Tribulus terrestris* plant (Feng et al., 2012). However, Agarwal et al. (2006) supplementing different substrates with different saponin extracts from different plants either did not affect or decreased the total VFAs production. The butyrate, isovalerate and valerate production was the highest ( $P<0.01$ ) in kulthi followed by patha and the lowest was observed in shatavari. The A:P ratio was the highest ( $P<0.01$ ) in patha followed by kulthi and shikakai.

Irrespective of incubation period and type of herb used, the total and individual volatile fatty acids were the highest ( $P<0.01$ ) when the diet was supplemented with HFAs at 3% level on DM basis (Table 2). The lowest ( $P<0.01$ ) production of total VFAs was observed at 1%, while that of individual VFAs ( $P<0.01$ ) was at 2% level of HFAs supplementation. Hundal et al. (2020) reported that the total VFAs, propionate, butyrate, isovalerate and valerate production improved ( $P<0.01$ ) when the TMR was supplemented with HFAs even at 1% level on DM basis. Unnawong et al. (2021) revealed that butyrate and acetate to propionate ratio were decreased, and propionate production was increased ( $P<0.05$ ) with the increase in dose of SES.

Irrespective of type and level of HFAs, the relative proportion of all VFAs at t-half was lower ( $P<0.01$ ) than that at 24h (Table 3).

Table 3. Effect of incubation period, type and level of herbal feed additive, irrespective of one another on the relative proportion of VFAs (%) during fermentation of TMR

Parameters	Incubation, h		PSE	P-Value	Herbal feed additives					PSE	P-value	Levels of HFAs, %					PSE	P-value
	24	t-half			Patha	Kulthi	Shikakai	Methi	Aritha			Shatavari	0	1	2	3		
Acetate	65.8 <sup>a</sup>	69.2 <sup>b</sup>	0.09	<0.001	68.7 <sup>c</sup>	67.4 <sup>b</sup>	67.7 <sup>b</sup>	66.7 <sup>a</sup>	66.4 <sup>a</sup>	67.3	67.4	67.3	67.3	67.57	0.14	0.141		
Propionate	23.4 <sup>b</sup>	20.7 <sup>a</sup>	0.03	<0.001	21.1 <sup>a</sup>	21.6 <sup>b</sup>	22.0 <sup>c</sup>	23.1 <sup>d</sup>	23.3 <sup>c</sup>	22.1 <sup>b</sup>	22.1 <sup>b</sup>	22.1 <sup>b</sup>	22.2 <sup>b</sup>	22.28 <sup>b</sup>	0.05	<0.001		
Isobutyrate	0.51 <sup>b</sup>	0.49 <sup>a</sup>	0.01	0.034	0.49 <sup>b</sup>	0.51 <sup>bc</sup>	0.42 <sup>a</sup>	0.49 <sup>b</sup>	0.52 <sup>bc</sup>	0.017	0.49 <sup>ab</sup>	0.53 <sup>b</sup>	0.51 <sup>b</sup>	0.459 <sup>a</sup>	0.015	0.032		
Butyrate	8.27 <sup>b</sup>	7.89 <sup>a</sup>	0.09	0.003	7.94	8.50	7.94	7.98	7.98	0.159	8.16	8.17	8.18	8.00	0.145	0.530		
Isovalerate	1.14 <sup>b</sup>	0.99 <sup>a</sup>	0.005	<0.001	1.01 <sup>a</sup>	1.14 <sup>d</sup>	1.09 <sup>c</sup>	1.015 <sup>a</sup>	1.06 <sup>b</sup>	0.009	1.084 <sup>bc</sup>	1.07 <sup>b</sup>	1.06 <sup>b</sup>	1.010 <sup>a</sup>	0.008	<0.001		
Valerate	0.79 <sup>b</sup>	0.61 <sup>a</sup>	0.010	0.001	0.72 <sup>bc</sup>	0.77 <sup>c</sup>	0.698 <sup>ab</sup>	0.657 <sup>a</sup>	0.709 <sup>ab</sup>	0.018	0.684 <sup>ab</sup>	0.69	0.72	0.685	0.016	0.148		

Figures with different superscripts in a row differ significantly

However, reverse trend ( $P < 0.01$ ) was observed in case of acetate, similar trend ( $P < 0.01$ ) was observed in an earlier study (Singh, 2017). The relative proportion of acetate was the highest ( $P < 0.01$ ) in patha and lowest ( $P < 0.01$ ) in aritha supplemented TMR. Reverse trend ( $P < 0.01$ ) was observed in case of propionate. The relative proportion of isobutyrate was the highest in TMR supplemented with methi and the lowest was observed in shikakai. The relative proportion of butyrate was not affected by the type of HFAs. The relative proportion of isovalerate and valerate was the highest when the TMR was supplemented with kulthi and the lowest in TMR supplemented with shatavari.

Irrespective of incubation period and type of HFAs used, the level of HFAs did not affect the relative proportion of acetate, butyrate and valerate (Table 3). The relative proportion of propionate was improved ( $P < 0.01$ ) at all levels of HFAs supplementation, but amongst the different levels of HFAs the differences were nonsignificant. The relative proportion of isobutyrate was comparable up to 3% level, but depressed ( $P < 0.05$ ) at 4% level. But that of isovalerate was depressed ( $P < 0.01$ ) at all levels of HFA supplementation.

### Impact of incubation periods, type and levels of HFAs on the hydrogen balance and methane emission at t-half

The effect of incubation period, irrespective of other factors revealed that the methane production, hydrogen recovery, hydrogen consumption and VFA utilization index were higher ( $P < 0.01$ ) at t-half as compared to 24h incubation (Table 4). However, reverse trend ( $P < 0.01$ ) was observed in case of fermentation efficiency.

Table 4. Effect of incubation period, type and level of herbal feed additive, irrespective of one another on the hydrogen balance and *in-vitro* methane production at t<sub>1/2</sub> during fermentation of TMR

Parameters	Incubation, h		PSE	P-Value	Herbal feed additives						PSE	P-value	Levels of HFAs, %					PSE	P-value
	24	t-half			Patha	Kulthi	Shikakai	Methi	Aritha	Shatavari			0	1	2	3	4		
Methane, mM	31.1 <sup>a</sup>	33.3 <sup>b</sup>	0.022	0.001	31.4 <sup>a</sup>	31.5 <sup>a</sup>	32.0 <sup>b</sup>	32.3 <sup>c</sup>	32.5 <sup>d</sup>	31.3 <sup>a</sup>	32.3 <sup>b</sup>	32.2 <sup>ab</sup>	32.22 <sup>a</sup>	32.19 <sup>a</sup>	32.22 <sup>a</sup>	0.035	0.002		
HR, %	73.3 <sup>a</sup>	72.3 <sup>b</sup>	0.077	<0.001	68.1 <sup>a</sup>	70.3 <sup>b</sup>	75.1 <sup>c</sup>	74.5 <sup>d</sup>	73.0 <sup>e</sup>	76.2 <sup>f</sup>	76.4 <sup>e</sup>	73.2 <sup>c</sup>	70.83 <sup>a</sup>	70.41 <sup>a</sup>	70.13 <sup>a</sup>	0.119	<0.001		
HC, %	1.17 <sup>a</sup>	1.40 <sup>b</sup>	0.009	<0.001	1.23 <sup>a</sup>	1.21 <sup>a</sup>	1.38 <sup>c</sup>	1.35 <sup>bc</sup>	1.19 <sup>a</sup>	1.31 <sup>b</sup>	1.40 <sup>c</sup>	1.29 <sup>b</sup>	1.315 <sup>b</sup>	1.197 <sup>a</sup>	1.223 <sup>a</sup>	0.014	0.001		
FE, %	75.10 <sup>b</sup>	73.8 <sup>a</sup>	0.014	0.003	74.0 <sup>a</sup>	74.3 <sup>b</sup>	74.4 <sup>c</sup>	74.3 <sup>b</sup>	74.8 <sup>d</sup>	74.9 <sup>e</sup>	74.7 <sup>b</sup>	74.4 <sup>a</sup>	74.46 <sup>a</sup>	74.51 <sup>a</sup>	74.48 <sup>a</sup>	0.021	0.030		
VFA+UI	3.44 <sup>a</sup>	4.01 <sup>b</sup>	0.006	0.002	3.89 <sup>c</sup>	3.79 <sup>d</sup>	3.73 <sup>c</sup>	3.78 <sup>d</sup>	3.50 <sup>a</sup>	3.55 <sup>b</sup>	3.60 <sup>b</sup>	3.72 <sup>a</sup>	3.73 <sup>a</sup>	3.71 <sup>a</sup>	3.70 <sup>b</sup>	0.009	<0.001		
<i>In-vitro</i> methane production at t <sub>1/2</sub>																			
CH <sub>4</sub> , %					32.05 <sup>a</sup>	32.03 <sup>a</sup>	39.64 <sup>c</sup>	35.38 <sup>b</sup>	32.81 <sup>ab</sup>	32.34 <sup>a</sup>	37.86 <sup>b</sup>	37.89 <sup>b</sup>	32.68 <sup>a</sup>	32.19 <sup>a</sup>	31.08 <sup>a</sup>	0.78	<0.001		
CH <sub>4</sub> , ml/100mg DM					3.21 <sup>a</sup>	3.89 <sup>a</sup>	4.52 <sup>b</sup>	3.95 <sup>a</sup>	4.07 <sup>a</sup>	3.85 <sup>a</sup>	4.40 <sup>bc</sup>	4.05 <sup>b</sup>	3.94 <sup>a</sup>	3.59 <sup>a</sup>	3.27 <sup>a</sup>	0.126	0.007		

HR- Hydrogen recovery; HC- Hydrogen consumption, FE-Fermentation efficiency; VFA+UI- volatile fatty acid utilization index; Figures with different superscripts in a row differ significantly.

Irrespective of the incubation period and level of HFAs supplementation, the methane production was lowest ( $P < 0.01$ ) in TMR supplemented with shatavari, comparable with patha and kulthi, while it was highest ( $P < 0.01$ ) in the TMR supplemented with aritha (Table 4). The dry extract of shatavari is rich in saponins, which inhibit rumen ciliate protozoa by binding with sterols present on the protozoal surface (Hristov et al., 1999). Defaunation often lowers rumen methanogenesis because about 25% of ruminal methanogens live in association with protozoa. The elimination of ciliate protozoa, from the rumen, reduced methane emission by 30 to 45% (Lila et al., 2005). The hydrogen recovery was the highest ( $P < 0.01$ ) in TMR supplemented with shatavari and the lowest was observed in patha. The hydrogen consumption was the highest in TMR supplemented with shikakai and the lowest was observed in kulthi. The fermentation efficiency was the highest in TMR supplemented with shatavari and the lowest in TMR supplemented with patha. VFA+UI was the highest in TMR supplemented with patha and the lowest was observed in aritha.

Irrespective of the incubation period and type of HFAs supplemented to the TMR the methane emission was depressed ( $P < 0.01$ ) at all levels as compared to control group (Table 4). 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. Like methane emission, the hydrogen recovery, hydrogen consumption, fermentation efficiency and VFA+UI were depressed at all the levels of HFA supplementation. Unnawong et al. (2021) revealed that by increasing SES levels decreased ( $P < 0.05$ ) protozoal population. The ruminal CH<sub>4</sub> concentration was reduced by 44.1% when 0.6% SES was added after 8 h of incubation.

#### Impact of type and level of HFAs on methane emission at t-half

Irrespective of level of HFA supplemented to the TMR, the methane emission at t-half expressed either as methane per cent or as methane ml/100mg DM

was observed to be the lowest ( $P < 0.01$ ) in the TMR supplemented with patha, kulthi or shatavari (Table 4). Irrespective of the type of HFAs, the methane emission at t-half was observed to be the lowest ( $P < 0.01$ ) when the diet was supplemented at 2% on DM basis.

## CONCLUSION

The screening of different herbal feed additives supplemented at different levels by *in vitro* gas production technique revealed that the best response with respect to digestibility of nutrients, methane emission and ME availability from TMR was observed in shatavari supplemented at the rate of 2-3% of TMR on DM basis.

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