Effect of supplementation of saponin containing herbs on *in vitro* methane production under different feeding systems

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**ABSTRACT**

This study was taken up to assess the effect of herbal feed additives [HFAs; kulthi (*Dohichos biflorus*), patha (*Cissampelos pareria*), aritha (*Sapindus trifoliatus*)] supplemented at 0–3% on DM basis of total mixed rations (TMR) on the *in vitro* methane production and nutrient fermentation in a 3 × 4 factorial design. TMR with different roughage to concentrate ratio (R:C) of 80:20, 75:25, 70:30 and 65:35 on DM basis were formulated. The roughage portion was made up of wheat straw and maize green fodder in 70:30 ratio. The chemical analysis of HFAs revealed that aritha had the highest concentration of both water and methanol soluble saponins; and condensed tannins (Leucocyanidin). Patha followed by kulthi had the highest concentration of vitamin C, flavonoids, total phenols and true tannins. The digestion kinetic parameters revealed that with the increase in level of concentrate in the diet, irrespective of type and level of supplementation of HFAs, the lag phase for fermentation of diet decreased linearly. The data conclusively revealed that the best response with respect to net gas production (NGP), digestibility of nutrients, methane production, volatile fatty acid (VFA) production, ME availability and other fermentation parameters from TMRs with different R:C ratios was observed in kulthi and patha supplemented at the rate of 2% of TMR with R:C ratio of 65:35 on DM basis.

**Key words:** Herbs containing saponins, *In vitro* methane production, Roughage to concentrate ratio

Increasing attention has been placed on greenhouse gas (GHG) emissions in recent years with deteriorating scenario of global warming (O’Mara 2011). Approximately 7–18% of the global GHG emission originate from livestock sector (Hristov et al. 2013). Globally, about 80–115 Tg methane equivalent to 15–20% of total anthropogenic methane is produced/annum by domestic livestock (Houghton et al. 2001).

Number of studies has been conducted to assess the potential of plant secondary metabolites as natural manipulating agents for ruminal fermentation (Hundal et al. 2016a, b, Wallace et al. 2002). Saponins are phytochemicals commonly found in plants and are composed of steroids, triterpenoids, and steroid alkaloids (Wina et al. 2005a, Bakshi and Wadhwa 2010). Saponins as feed supplements may have the potential to modulate ruminal fermentation and improve nutrient utilization in ruminants (Patra and Saxena 2010, Wallace et al. 1994, Hirstov et al. 1999). Both *in vitro* (Hu et al. 2005, Lila et al. 2003) and *in vivo* (Yuan et al. 2007) studies revealed that saponins might reduce the pH and ammonia–N concentration in the rumen. Saponins have been reported to have mixed effect on feed intake decreasing (Lovett et al. 2006), or increasing (Holshausen et al. 2009) or no effect on feed intake in ruminants (Mao et al. 2010). Supplementing the diet of sheep with saponins increased organic matter and fibre digestibility (Lu and Jorgensen 1987). Moreover, addition of saponin-rich plants, such as *Yucca schidigera* has been found to improve growth, feed efficiency and health in ruminants (Cheeke 1996). According to Wina et al. (2005b), many different factors affect the response of ruminants to saponins including sources, levels of supplementation, and diet composition. The present investigation was planned to assess the effect of herbal feed additives containing saponin as active component on the methane production potential using total mixed rations (TMRs) with different roughage to concentrate ratios, commonly used for different categories of animals.

**MATERIALS AND METHODS**

**Bioactive components of HFAs:** The HFAs [kulthi (*Dohichos biflorus*), patha (*Cissampelos pareria*) and aritha (*Sapindus trifoliatus*)] containing saponins were procured from Konark Herbals, Mumbai. Herbs (100 mg) in duplicate were extracted with 7.5 ml of distilled water. The contents were centrifuged at 3,000 g for 10 min. The extraction was repeated again and the extracts were pooled. The aqueous extract obtained was used for the estimation of phenolics/
tannins (Makkar et al. 1993), condensed tannins (Porter et al. 1986), flavonoids (Balabaa et al. 1974), saponins (Baccou et al. 1977) and DPPH (Kumaran and Karakumaran 2007). Simultaneously 1 ml of 20% TCA was added to 1 ml of aqueous extract and kept overnight at 4°C. It was centrifuged and the supernatants were used for the estimation of vitamin-C (Jagota and Dani 1982). The herbs were extracted with 80% methanol following the same procedure as mentioned above. The saponins were estimated from the methanolic extract as well.

Preparation of total mixed rations: Four TMRs with different R:C ratios (80:20, 75:25, 70:30 and 65:35) were prepared. The roughage portion was made up of wheat straw and maize green fodder in 70:30 ratio, while the concentrate mixture was made up of 15% maize, 15% wheat, 15% deoiled mustard cake, 10% mustard cake, 10% soybean meal, 15% rice bran, 16% deoiled rice bran, 1% urea, 1% salt and 2% mineral mixture.

Chemical analysis: The samples were ground to pass through 1 mm sieve and were analyzed for proximate and cell wall constituents. The protein contents were analyzed using standard method (AOAC 2000). The samples of TMR were analyzed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL; Robertson and Van Soest 1981) and cellulose (Crampton and Maynard 1938).

In vitro studies: Rumen fistulated male buffaloes (maintained on 2 kg conventional concentrate mixture, 2 kg green and ad lib. wheat straw) were used as a donor for rumen liquor. The rumen contents were collected at 0 h in double walled (Thermos) flask flushed with CO2 and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender, maintained at 39°C and then strained through 4 layered muslin cloth. The HFAs containing saponins were supplemented @ 1–3% of TMRs in 100 ml double walled (Thermos) flask flushed with CO2 and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender, maintained at 39°C and then strained through 4 layered muslin cloth. The HFAs containing saponins were supplemented @ 1–3% of TMRs in 100 ml double walled (Thermos) flask flushed with CO2 and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender, maintained at 39°C and then strained through 4 layered muslin cloth.

Methane estimation: Methane was estimated by using GLC (Netchrom 9100) equipped with stainless steel column packed with porapak-Q and flame ionization detector. Standard calibration gas (Sigma Gases, New Delhi) consisted of 50% methane and 50% carbon dioxide. The flow rates for nitrogen, hydrogen and zero air were 30, 30, 320 ml/min, respectively. From the headspace of each syringe, 100 ml gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane.

Hydrogen balance: Hydrogen recovery (%) was estimated as (4M+2P+2B) / (2A+P+4B) ×100, the ratio of hydrogen consumed via CH4/VFA was estimated as 4M/ (2P+2B), where acetate (A), propionate (P), butyrate (B) and methane (M) production was expressed in mmol (Demeyer 1991).

Fermentation efficiency: This was calculated on the basis of the equation worked out by Rskov (1975) and modified by Baran and Zitnan (2002). FE = (0.622a + 1.092p + 1.56b) / (a + p + 2b) where a, p and b express the concentrations (µmol) of acetic, propionic and butyric acids respectively in the total concentration of VFAs produced. The final result of this equation is expressed in percentage and shows an amount of energy stored in VFAs as a percentage participation of the initial energy.

VFAs utilization index: This was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) according to Rskov (1975). NGGR = (A + 2B + V) / (P+V) where A, P, B and V express the concentrations (µmol) of acetic, propionic, butyric and valeric acids respectively. Valeric acid is classified as both glucogenic and non-glucogenic VFA because, its oxidation creates 1 mole of acetic acid and 1 mole of propionic acid. Too high NGGR indicates high loss of energy in the form of gases.

Statistical analysis: Data were analyzed by 3 × 4 factorial
design (Snedecor and Cochran 1994), by using SPSS (2007) version 12 and the differences in means were tested by Duncan’s Multiple Range Test.

RESULTS AND DISCUSSION

Chemical composition of TMRs: The chemical composition of TMRs varying in R:C ratios revealed that with increase in level of concentrate in TMRs, the OM, EE and CP content increased, while cellulose, NDF, ADF and ADL content decreased (Table 1). Screening of herbs for bioactive compounds: The data revealed that water soluble saponin content was highest (P<0.01) in aritha, followed by kulthi and lowest in patha, whereas saponin content in methanol extract was highest (P<0.01) in aritha and lowest in kulthi (Table 2). Beside saponins, these were rich in antioxidant activity due to presence of vitamin C and flavonoids. Patha had the highest (P<0.01) concentration of vitamin C and that of flavonoids and aritha had the lowest concentration of these antioxidants. Among plant secondary metabolites, flavonoids have gained importance because of their wide range of biological activities and in particular antimicrobial properties. These natural compounds are believed to have direct effects against methanogens (Bodas et al. 2012) and to be an alternative agent to suppress methane production and improve animal health and productivity. Addition of flavonoid substances enhances fermentation efficiency by improving propionate in detriment of acetate production and clearly depressed hydrogenotrophic methanogenic archaea communities (Seradj et al. 2014). The data revealed that aritha exhibited the highest (P<0.01) DPPH activity followed by patha.

Total phenols and true tannins were highest (P<0.01) in patha followed by aritha and lowest in kulthi. Phenolic compounds, including tannins have been recognized as modulators of rumen fermentation with potential to inhibit methanogenesis (Bhatta et al. 2014, Mangwe et al. 2016). Phenolic compounds play an important role as antimicrobials by interacting with the extracellular microbial enzymes, as well as depriving microbe substrates required for growth (Patra and Saxana 2011). In addition to direct inhibition on methanogenic archaea and protozoa (Patra et al. 2012), it is possible that phenolic compounds could indirectly affect H₂ production (Patra et al. 2017) or form insoluble complexes with protein resulting in suppression of overall microbial degradation and methane emissions (Patra and Saxana 2011). Feeding plants containing total phenols and tannins has been demonstrated to reduce methane and total gas production (Pal et al. 2015).

Condensed tannins (CTs) were highest (P<0.01) in aritha and lowest in patha (Table 2). The molecular weight and chemical structures are primary factors determining the influence of CTs on CH₄ production in ruminants (Spencer et al. 1988, Osborne and McNeill 2001, Liang and Khamsekhiew 2006). Moderate levels of CT (less than 4.0%) in forage legumes can have beneficial responses in ruminants, resulting in higher CP utilization, growth rates and milk yield, but levels of CT exceeding 6% of the diet resulted in negative effect on growth rate and milk yield (Makkar 2003).

Digestion kinetic parameters of gas production in vitro: The gas production profiles indicated that the diet supplemented with kulthi showed the lowest lag phase, highest y max (maximum potential of gas production), highest rate of degradation (k) and lowest t½ (time taken for reaching half asymptote) as compared to other HFA supplemented groups, irrespective of level of supplementation and type of diet (Table 3). With the increase in level of HFA supplementation, irrespective of type of herb and diet, the lag phase decreased and y max increased (P<0.01) linearly. However, rate of degradation (k) and t½ values were not affected by level of supplementation of herbs. With increase in level of concentrate in the diet, irrespective of type and level of supplementation of herbs, the lag period for fermentation of diet decreased (P<0.01) linearly, degradation rate (k) and y max increased linearly (P<0.01), but y min (minimum potential of gas production) and t½ followed the reverse trend (P<0.01).

In vitro screening of optimum level of HFAs containing saponins using TMRs varying in R:C ratios at t-half:

Table 1. Chemical composition of TMRs

| Parameter               | Roughage: Concentrate ratio |
|-------------------------|-----------------------------|
|                        | 80:20 | 75:25 | 70:30 | 65:35 |
| Total ash               | 12.22 | 11.22 | 10.95 | 10.78 |
| Organic matter          | 87.78 | 88.78 | 89.05 | 89.22 |
| Crude protein           | 14.65 | 17.9  | 18.55 | 21.45 |
| Ether extract           | 2.05  | 2.25  | 2.85  | 3.35  |
| Cellulose               | 37.6  | 32.9  | 30.3  | 26.1  |
| Neutral detergent fibre | 69.2  | 64.9  | 60.7  | 57.4  |
| Acid detergent fibre    | 37.6  | 32.9  | 30.3  | 26.1  |
| Hemicellulose           | 31.6  | 32.0  | 30.4  | 31.3  |
| Acid detergent lignin   | 8.10  | 7.55  | 6.60  | 5.60  |

Table 2. Bio-active components in herbal feed additives (% DWB)

| Parameter             | Herbal feed additive | PSE | P value |
|-----------------------|----------------------|-----|---------|
| Aqueous saponin**     | 5.06a                | 5.55b| 5.81c   | -      | 0.00   |
| Methanol saponin**    | 3.75b                | 2.00a| 6.75c   | 0.75   | 0.00   |
| Antioxidants          |                      |     |         |
| Vitamin C**           | 5.68e                | 1.95b| 0.48c   | -      | 0.00   |
| Flavonoids**          | 11.37e               | 6.62b| 0.02e   | 1.16   | 0.00   |
| DPPH AA%**            | 403.96ab             | 400.14a| 446.02c| 11.80  | 0.00   |
| DPPH mg%**            | 2.02ab               | 2.00a| 2.23c   | 0.10   | 0.00   |
| Tannins               |                      |     |         |
| Total phenolics**     | 37.48c               | 11.19a| 14.41b | 4.18   | 0.00   |
| Non tannin phenols**  | 5.93e                | 0.78a| 1.63b   | 1.35   | 0.00   |
| True tannins**        | 31.56e               | 10.41a| 12.78b | 3.40   | 0.00   |
| CT, Leucocyanidin**   | 0.07a                | 0.40b| 2.51c   | -      | 0.00   |

Figures with different superscripts in a row differ significantly (***P<0.01).
The end-product of anaerobic microbial fermentation of carbohydrates in the rumen of ruminants has been reported to be VFAs, carbon dioxide and methane (Camero and Franco 2001). The total VFAs, propionate, acetate, butyrate and that of isobutyrate concentration was highest (P<0.01) from the diet supplemented with kuthi (Table 5), irrespective of the level of HFA and the R:C ratio of diet. The perusal of data on fermentation pattern revealed that total and individual VFAs were highest (P<0.01) when the diet was supplemented at 2% level, irrespective of the type of herb and the type of diet used. The A:P ratio was also the lowest (P<0.01) in the diet supplemented with HFAs at 1%, comparable with that supplemented at 2% level, but lower (P<0.01) than control and the diet supplemented with HFA at 3% level. Indeed, total and individual VFAs increased with high-concentrate level as compared with high-forage level. Unlike many studies, the total VFAs, acetate, propionate concentration decreased (P<0.01) linearly, while that of butyrate increased (P<0.01) linearly with the increase in concentrate mixture in the TMR, irrespective of type and level of HFA supplemented.

The molar proportions of acetate was higher (P<0.01) in diet supplemented with kuthi (70.1%), while that of propionate butyrate and isobutyrate was higher (P<0.01) in diet supplemented with aritha (Table 6) as compared to other HFA supplemented TMRs, irrespective of the level of supplementation and type of TMR. This agrees with the report of Dung et al. (2011) who reported that, even though volatile fatty acids contribute about 70% of the caloric

### Table 3. Effect of herbs containing saponins on the fermentation kinetics of diet.

| Parameter                        | Kulthi | Patha | Aritha | PSE | Levels of HFA (%) | PSE | Roughage to Concentrate ratio | PSE |
|----------------------------------|--------|-------|--------|-----|------------------|-----|-----------------------------|-----|
| Lag time (h)**                   | -0.54^a| -0.95^c| -0.83^b| 0.11| 1.18^a           | 0.09| 75:25                      | 0.58 |
| Ymax (ml)^2                      | 45.36^b| 45.28^b| 43.56^a| 0.12| 42.46^b          | 0.1  | 70:30                      | 65:35|
| Rate of degradation (k)**        | 0.059^a| 0.058^b| 0.057^c| -   | 0.058            | -   | 65:35                      | -   |
| Ymin (ml)^2                      | 17.00^a| 17.29^a| 17.61^b| 0.08| 17.24            | 0.06| 70:30                      | 65:35|
| t½ (h)^2                         | 11.78^a| 11.99^a| 12.21^b| 0.05| 11.95            | 0.04| 65:35                      | -   |

Figures with different superscripts in a row differ significantly (**P<0.01); 1Irrespective of level of supplementation and type of diet; 2Irrespective of type of herb and diet, of supplementation; 3Irrespective of level of supplementation and type of herb.

Amongst the herbs evaluated, supplementation of patha, irrespective of level of supplementation and type of diet resulted in higher (P<0.01) NDF, digestibility of nutrients and availability of ME as compared to other HFAs supplemented groups (Table 4). The partitioning factor varied (P<0.01) from 1.91 mg/ml (patha supplemented diet) to 2.31 mg/ml (diet supplemented with aritha). However, the PF for a given feedstuff can vary with the incubation time partly because of the dynamics of microbial growth. The differences amongst herbs can be attributed to nature of saponin in different herbs.

With the increase in level of supplementation of herbal feed additive, the NDF and availability of ME as compared to other HFAs supplemented groups (Table 4). The partitioning factor varied (P<0.01) from 1.91 mg/ml (patha supplemented diet) to 2.31 mg/ml (diet supplemented with aritha). However, the PF for a given feedstuff can vary with the incubation time partly because of the dynamics of microbial growth. The differences amongst herbs can be attributed to nature of saponin in different herbs.

The type of feed offered to a ruminant can have a major effect on rumen fermentation. The types of diet are potential modifiers of ruminal fermentation and may offer a strategy to reduce protozoal and methanogen populations, thus improving the efficiency of feed utilization in the ruminants (Anantasook and Wanapat 2012). With the increase in level of concentrate in TMRs, the NDF, digestibility of OM and availability of ME increased linearly (P<0.01). However, the digestibility of NDF decreased (P<0.01) linearly up to 2% level of supplementation, clearly indicating the potential of saponin containing herbs at optimum level in manipulating rumen fermentation.

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### Table 4. Effect of herbs, their level of supplementation and type of TMRs on in vitro gas production and digestibility of nutrients at t½

| Parameter                        | Kulthi | Patha | Aritha | PSE | Levels of HFA (%) | PSE | Roughage to Concentrate ratio | PSE |
|----------------------------------|--------|-------|--------|-----|------------------|-----|-----------------------------|-----|
| NGP (ml/g DM)**                  | 97.7b  | 101.7^a| 81.8a  | 0.56| 90.8a            | 0.64| 75:25                      | 0.64|
| NDFD (%)**                      | 28.1^a | 31.7^a| 28.9^b | 0.08| 27.2^a           | 0.09| 70:30                      | 0.09|
| TOMD (%)**                      | 54.7^a | 56.9^a| 55.3^b | 0.05| 54.1^a           | 0.06| 65:35                      | 0.06|
| PF** (mg/ml)                    | 1.97^a | 1.91^a| 2.31^b | 0.04| 2.11             | 0.04| 65:35                      | 0.03|
| ME** (MJ/kg DM)                 | 6.5^b  | 6.6^c | 6.1^d  | 0.02| 6.4^a            | 0.03| 65:35                      | 0.03|

Figures with different superscripts in row differ significantly (**P<0.01); 1Irrespective of level of supplementation and type of diet; 2Irrespective of type of herb and diet, of supplementation; 3Irrespective of level of supplementation and type of herb.
Table 5. Total volatile fatty acids (TVFAs) production from fermentation of TMRs

| Parameter                        | PSE Levels of HFA (%) | PSE Roughage to Concentrate ratio |
|----------------------------------|-----------------------|-----------------------------------|
|                                  | 0 | 1 | 2 | 3 | 0:20 | 75:25 | 70:30 | 65:35 |
| TVFA**                           | 4.5  | 4.01b  | 3.75a  | 0.01 | 4.13c  | 3.96a  | 4.20b  | 4.08b  | 0.01 | 4.13b  | 4.13b  | 4.05a  | 4.07a  | 0.01 |
| Acetate*                         | 3.2e  | 2.78b  | 2.52a  | 0.003 | 2.85c  | 2.74a  | 2.91a  | 2.80a  | 0.003 | 2.86c  | 2.84b  | 2.80a  | 2.81a  | 0.003 |
| Propionate**                     | 0.91b  | 0.82a  | 0.83b  | 0.001 | 0.85b  | 0.82a  | 0.88a  | 0.87a  | 0.001 | 0.87c  | 0.86b  | 0.85a  | 0.85a  | 0.001 |
| Isobutyrate**                    | 0.022b  | 0.014a  | 0.017a  | 0.001 | 0.016a  | 0.017a  | 0.018 | 0.019 | 0.002 | 0.017 | 0.021 | 0.017 | 0.015 | 0.002 |
| Butyrate**                       | 0.36c  | 0.32a  | 0.32a  | 0.001 | 0.334b  | 0.319a  | 0.342b  | 0.335b  | 0.001 | 0.330b  | 0.332b  | 0.328b  | 0.340b  | 0.001 |
| Isovalerate                      | 0.041 | 0.039 | 0.038 | 0.007 | 0.057a  | 0.032a  | 0.035 | 0.033 | 0.008 | 0.032 | 0.056 | 0.034 | 0.036 | 0.008 |
| Valerate                         | 0.025 | 0.028 | 0.023 | 0.004 | 0.023a  | 0.030a  | 0.027 | 0.023 | 0.004 | 0.025 | 0.030a  | 0.023 | 0.025 | 0.004 |
| A:P**                           | 3.49  | 3.83 | 3.02  | 0.002 | 3.33c  | 3.31b  | 3.31b  | 3.22a  | 0.002 | 3.29a  | 3.31b  | 3.32a  | 3.29a  | 0.002 |

Figures with different superscripts in row differ significantly (**P<0.01), 1Irrespective of level of supplementation and type of diet; 2Irrespective of type of herb and diet, of supplementation; 3Irrespective of level of supplementation and type of herb.

Table 6. Relative proportion of volatile fatty acids as affected by supplementation of saponin containing herbs, level of supplementation and type of diet

| Parameter                        | Herbal feed additives1 | PSE Levels of HFA (%) | PSE Roughage to Concentrate ratio |
|----------------------------------|-----------------------|-----------------------|-----------------------------------|
|                                  | 0 | 1 | 2 | 3 | 0:20 | 75:25 | 70:30 | 65:35 |
| Acetate**                        | 70.07c  | 69.43b  | 67.10a  | 0.14 | 68.89  | 68.91 | 69.05  | 68.61 | 0.16 | 69.12  | 68.61 | 69.00  | 68.72 | 0.16 |
| Propionate**                     | 20.14a  | 20.54b  | 22.26c  | 0.04 | 20.77c  | 20.91a  | 20.91a  | 21.33b  | 0.05 | 21.08b  | 20.84a  | 21.06b  | 20.94ab  | 0.05 |
| Isobutyrate**                    | 0.44b  | 0.31a  | 0.46b  | 0.03 | 0.34a  | 0.43a  | 0.38 | 0.46 | 0.04 | 0.41  | 0.42  | 0.39  | 0.39  | 0.04 |
| Butyrate**                       | 7.88a  | 8.06b  | 8.54c  | 0.02 | 8.10a  | 8.15b  | 8.18b  | 8.22b  | 0.02 | 8.02a  | 8.07b  | 8.12b  | 8.44c  | 0.02 |
| Isovalerate                      | 0.92a  | 0.95  | 1.03a  | 0.16 | 1.36a  | 0.84a  | 0.84  | 0.82  | 0.18 | 0.78  | 1.33  | 0.84  | 0.91  | 0.18 |
| Valerate                         | 0.56  | 0.71a  | 0.62  | 0.09 | 0.55a  | 0.76a  | 0.64  | 0.57  | 0.10 | 0.59  | 0.74  | 0.58  | 0.62  | 0.10 |

Figures with different superscripts in row differ significantly (**P<0.01), 1Irrespective of level of supplementation and type of diet; 2Irrespective of type of herb and diet, of supplementation; 3Irrespective of level of supplementation and type of herb.

requirement of ruminants, the nutrients in the diets (supplemented or un-supplemented) undoubtedly affects the amount of volatile fatty production in the rumen. The relative proportion of acetate, valerate and that of BCFAs was not affected by level of supplementation of HFAs (Table 6). However, the proportion of propionate and that of butyrate increased, with increase in level of supplementation HFAs, irrespective of type of herb and type of TMR.

Hydrogen recovery was highest (P<0.01) when diet was supplemented with aritha, but hydrogen consumed via methane/VFA was highest when diet was supplemented with patha (Table 7). In this study, supplementation of diet with different saponin containing HFAs, irrespective of the nature of diet and level of supplementation of herbs, the fermentation efficiency was highest (P<0.01) in diet supplemented with aritha and lowest from diet supplemented with kulthi. VFA utilization index (NGGR) varied (P<0.01) from 3.71 (diet supplemented with aritha) to 4.18 (diet supplemented with kulthi). The non-glucogenic to glucogenic VFA ratio is associated with effects on methane production, milk composition, and energy balance (Morvay et al. 2011). Glucogenic propionate contributes to energy deposition in body tissues, whereas non-glucogenic acetate and butyrate are sources for long-chain fatty acid synthesis. Higher NGR was related to a higher milk fat content (Abrahamse 2009). Too high NGR indicates a high loss of energy in the form of gases (Ørskov 1975). The type of saponin present in herb could be the reason for different response.

The supplementation of diet with saponin containing herbs at different levels, irrespective of type of herb and that of diet, H recovery, ratio of H consumed via methane to H via VFA increased (P<0.01) with increase in level of supplementation and these parameters were highest at 3% level of supplementation. The lowest value of NGGR, which indicates the best utilization of VFA, was achieved when diet was supplemented at 3% on DM basis, irrespective of type of herb used and the substrate used. Fermentation efficiency was also highest when diet was supplemented at 3% on DM basis.

Hydrogen consumption via methane or via VFA and fermentation efficiency was lowest in high concentrate diet. The VFA utilization index decreased with increase in level of concentrate in the diet (Table 7).

Herbs containing saponins were evaluated for their anti-methanogenic properties also and the data revealed that the methane production (as % NGP, ml/100 mg DM/DMD/OMD) was lowest (P<0.05) in diet supplemented with patha in comparison to diet supplemented with other HFAs (Table 8). The type of saponin present in herb could be the reason for different response. The presence of different substituents in the sapogenin such as hydroxyl, hydroxymethyl, carboxyl, and acyl groups, as well as differences in the composition, linkage and number of sugar chains accounts for significant structural variation and thus their bioactivity (Patra and Saxena 2009, Podolak et al. 2011). Glucogenic propionate contributes to energy deposition in body tissues, whereas non-glucogenic acetate and butyrate are sources for long-chain fatty acid synthesis. Higher NGR was related to a higher milk fat content (Abrahamse 2009). Too high NGR indicates a high loss of energy in the form of gases (Ørskov 1975). The type of saponin present in herb could be the reason for different response. The presence of different substituents in the sapogenin such as hydroxyl, hydroxymethyl, carboxyl, and acyl groups, as well as differences in the composition, linkage and number of sugar chains accounts for significant structural variation and thus their bioactivity (Patra and Saxena 2009, Podolak et al. 2011).
Development of Mitigation Strategies’ Emission under Different Feeding Systems and work under the project entitled providing the financial support to carry out the research

Agricultural Research (ICAR), New Delhi, India for diet, of supplementation; Irrespective of level of supplementation and type of herb. Yan et al. (2000) reported that methane suppressing effects of saponins in diet and methane emissions. Goel et al. (2008) also reported that methane suppressing effects of saponins from Sesbania sesban and fenugreek were profound in concentrate-based diets compared to roughage based diets.

It can be concluded that saponin containing herbal feed additives like kulthi and patha has great potential in mitigating enteric methane production when supplemented @ 2% on dry matter basis in high concentrate based diet (roughage to concentrate ratio of 65:35).

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**Table 7. Fermentation parameter, hydrogen balance of TMRs supplemented with saponin containing herbs, level of supplementation and type of diet**

| Parameter | Herbal feed additives | PSE Levels of HFA (%) | PSE Roughage to Concentrate ratio | PSE |
|-----------|-----------------------|-----------------------|---------------------------------|-----|
| Kulthi    | Patha                 | Aritha                | 0 1 2 3                         | 80:20 75:25 70:30 65:35 |
| HR (%)**  | 99.7a                 | 109.5b                | 114.7c                          | 0.18 0.20 0.20 0.20 0.20 0.20 |
| HC, vit   | 2.40a                 | 2.65c                 | 2.54b                           | 0.006 0.007 2.51b 2.69b 2.48b 2.45a 2.007 |
| CH₄/VFA** | 73.48a                | 73.70b                | 74.56c                          | 0.004 0.005 73.84a 73.88b 73.87b 74.06c 0.005 |
| FE (%)**  | 4.18c                 | 4.07b                 | 3.71a                           | 0.01 0.01 4.03c 3.99b 4.00a 3.92a 0.01 |

**Table 8. Methane production from TMRs supplemented with saponin containing herbs**

| Parameter | Herbal feed additives | PSE Levels of HFA (%) | PSE Roughage to Concentrate ratio | PSE |
|-----------|-----------------------|-----------------------|---------------------------------|-----|
| Kulthi    | Patha                 | Aritha                | 0 1 2 3                         | 80:20 75:25 70:30 65:35 |
| CH₄ (% of NGP) ** | 46.1b | 35.1a | 47.6b | 0.68 0.79 | 44.7b 40.9b 40.9b 45.2b 41.5a 47.4a 42.4a 40.4a 0.79 |
| CH₄ (ml/100 mg DMD)** | 8.82c | 6.84a | 7.46b | 0.06 0.08 | 8.00b 7.27a 7.29a 8.28b 7.48b 8.76c 7.61b 6.98a 0.08 |
| CH₄ (ml/100 mg OMD)** | 4.72c | 3.70a | 4.12b | 0.07 0.08 | 4.22a 3.98a 3.94a 4.58b 3.86a 4.73c 4.16b 3.98ab 0.08 |
| CH₄ (ml/100 mg DM)** | 4.80c | 3.89a | 4.12b | 0.04 0.04 | 4.29b 4.07b 4.11a 4.60c 3.95a 4.76c 4.35b 4.01a 0.04 |

Figures with different superscripts in row differ significantly (**P<0.01); 1Irrespective of level of supplementation and type of diet; 2Irrespective of type of herb and diet, of supplementation; 3Irrespective of level of supplementation and type of herb.
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