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In-sacco nutritional worth and *in-vitro* methane production potential of leguminous forages

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ABSTRACT

The present study was undertaken to assess the *in-vitro* methane production potential of multi-cut berseem, lucerne and shaftal at different cuts. The CP and EE content declined, while the cell wall constituents increased linearly with the increase in number of cuts. The *in-sacco* studies revealed that the degradation rate and effective degradability (ED) of DM was highest ($P<0.01$), while the rumen un-degradable fraction (UDF) and rumen fill values were the lowest in shaftal. The degradation rate and ED of DM declined, while the UDF and rumen fill values increased with the increase in number of cuts. The digestion kinetic parameter of CP revealed that the UDP as per cent of CP was higher in berseem followed by that in shaftal. The ED and UDP as per cent of CP declined linearly with the increase in number of cuts. The digestion kinetic parameter of NDF revealed that shaftal had the highest ED and lowest UDF, while reverse trend was observed in berseem. The rumen fill values also confirmed that shaftal had better palatability than that of lucerne and berseem. The ED declined, while the UDF and rumen fill values increased with the increase in number of cuts.

The *in vitro* gas production analysis revealed that irrespective of cuts, the tested leguminous forages produced almost same amount of gas even though the digestibility of NDF and OM of berseem was lower than that of lucerne and shaftal. The methane production at $t^{1/2}$ (expressed as ml/g digestible OM) was lowest in shaftal as compared to berseem and lucerne. The digestibility of NDF and OM was lowest in the fourth cut fodders. Accordingly, the methane production at t½ increased linearly with the number of cuts and was highest in the fourth cut fodders. Irrespective of cuts, total VFAs production varied from 6.6 mM/dl in shaftal to 7.95 mM/dl in lucerne. Lucerne produced highest amount of acetate, propionate and butyrate, followed by that produced by berseem. Irrespective of fodder species, the relative proportion of propionate declined with the number of cuts. It was concluded that amongst the tested multi-cut leguminous forages, the use of shaftal in the diet of ruminants may help in mitigating enteric methane production and that the enteric methane production increased with the number of cuts of the forage.

Key words: *In-sacco*, *In-vitro* gas production, Methane, Multi-cut leguminous fodders

The projections for the next 20 years for the agricultural greenhouse gas emissions revealed that enteric methane emissions are expected to increase by 30%, while from manure by 24% and that from rice fields by 22% (USEPA 2006). The enteric methane generated by ruminants is an important greenhouse gas (GHG) which contributes 4–9% to the total greenhouse effect (Patra 2012b). Its concentrations have more than doubled over the last 150 years. Enteric fermentation accounts for 89.2% of the total GHG emissions, followed by that generated from manure (9.49%, Patra 2012a). Methane remains in the atmosphere for only 10 years, but trap 21 times more heat than CO_2. Therefore, it is considered a highly potent GHG and requires immediate interventions to restrict its alarming emissions from livestock. The primary substrates for ruminal methanogenesis are H_2 and CO_2, produced during fermentation of dietary carbohydrates. Significant quantities of ruminal methane, particularly with high-protein diets, can also arise from microbial fermentation of amino acids (Mills *et al*. 2001). The amount of methane released depends on dietary factors like soluble sugars, dietary lipids, level of feeding, roughage to concentrate ratio, type of forage, stage of maturity of forage, rate of passage of digesta, efficiency of feed conversion, processing and supplementation (Benchaar *et al*. 2001, Bakshi and Wadhwa 2009), plant secondary metabolites (Bakshi and Wadhwa 2010, 2012), feed additives (Wadhwa and Bakshi 2009), phytogenics (Bakshi and Wadhwa 2011) and ambient temperature (McAllister *et al*.

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1996). Mitigating the enteric methane production in ruminants without altering animal production is desirable both as a strategy to reduce global greenhouse gases emission and as a means of improving feed conversion efficiency (Dong *et al*. 2010, Martin *et al*. 2010). Keeping in view the potential of quality and type of forages used in dairy ration in mitigating enteric methane production, the present study was conducted with the objective of evaluating methane production potential of multi-cut leguminous forages.

MATERIALS AND METHODS

The samples of multi cut (first to fourth cut) leguminous forages like berseem (*Trifolium alexandrinum*), shaftal (*Trifolium resupinatum*) and lucerne (*Medicago sativa*) were procured from Forage Section of Department of Plant Breeding and Genetics, PAU, Ludhiana.

In-sacco degradability: The *in-sacco* degradability and kinetic parameters were determined (Mehraz and Orskov 1977) by using the above mentioned rumen fistulated buffalo calves. Finely ground (1mm) sample (leguminous fodder, 3g) was placed in the nylon bag (8×17cm) stitched with monofilament polyester thread with pore size of 50±10μ. The bags were incubated in the rumen for 2, 6, 9, 12, 24, 36, 48, 60, 72, 84 and 90 h in triplicate. The bags were removed after the stipulated period, washed until rinsing water became colourless; bags were dried in a forced air oven at 60°C for 48 h. The 'zero' h samples were not incubated in the rumen but the bags containing samples as such were washed in the same manner as incubated bags. The disappearance of DM was assessed as the loss in weight of the bag content. The residue was analyzed for DM, CP and NDF content. The different physical constants characterizing extent and rate of ruminal degradation, i.e., 'a' rapidly soluble fraction, 'b' insoluble but potentially degradable fraction and 'c' degradation rate constant of 'b' were worked out. The effective degradability (ED) was calculated by using the equation of McDonald (1981). The rumen fill was calculated according to Van Eys (1982).

In-vitro *gas production studies:* The nutritional value of above multi-cut leguminous forages was evaluated by *in vitro* gas production technique (Menke *et al*. 1979, Menke and Steingass 1988). About 375 mg of the forage sample (on DM basis) was incubated at 39°C for 24h in triplicate in 100 ml calibrated glass syringes with buffered rumen fluid. Blank and sample of standard hay were run in triplicate with each set. Rumen contents were collected from three buffalo calves fitted with permanent rumen fistulae, which were maintained on 10 kg green fodder and 4 kg wheat straw supplemented with 2 kg concentrate mixture (wheat 25, mustard cake 10, de-oiled mustard cake 20, rice bran 15, de-oiled rice bran 15, wheat bran 12, mineral mixture 2, common salt 1 part each).

Gas production profile (2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 84 and 96h) was worked out by IVGP technique. The rate and extent of gas production were calculated by graph pad prism to work out $t^{1/2}$. Methane at $t^{1/2}$ was estimated by gas chromatograph equipped with stainless steel column packed with porapak-Q and flame ionization detector. Standard calibration gas 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, 100ml gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane. Digestible OM was determined at $t^{1/2}$ and accordingly methane production in ml/g digestible OM at $t^{1/2}$ was worked out.

Volatile fatty acids (VFAs) were also estimated by using GLC equipped with a glass column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 (Cottoyn and Boucque 1968). Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor, allowing it to stand for 2 h, followed by centrifugation at 4000 rpm for 7 min. Supernatant was used for estimation of VFA. Standard VFA mixture was prepared by mixing stock solutions (each of 25 mg/ml concentration) of standard acetic acid, propionic acid, butyric acid and distilled water in 1.68, 0.48, 0.24 and 7.24 ml respectively to obtain final concentration of 7.0, 1.62, 0.68 mM/100 ml, respectively. The mixture was stored in deep freezer until further use.

Chemical and statistical analysis: Samples were analysed for proximate (AOAC, 2000) and cell wall components (Robertson and Van Soest 1981). Data were analysed by 3×4 factorial design (Snedecor and Cochran 1994) by using SPSS (2007) Version 16 and the differences in means by using Tukey's b test. All possible combinations of leguminous fodders and number of cuts were tested and interactions were worked out.

RESULTS AND DISCUSSION

Chemical composition of multi-cut leguminous forages: The chemical composition of leguminous fodders revealed that the CP content decreased as the number of cuts increased (Table 1). Katiæ *et al*. (2008, 2009) recorded the highest CP content in the first and fourth cuts and the lowest in the cut during the driest and warmest part of the year. The higher CP content in berseem was due to its relatively active vegetative growth in the last cut compare to shaftal which almost disappeared from the production. The cell wall constituents increased with increase in number of cuts. The cell wall gets lignified with the increase in number of cuts as indicated by increase in ADL content of leguminous fodders. In case of berseem, the hemi-cellulose content decreased, while in lucerne and shaftal it increased with increase in number of cuts. In case of berseem and lucerne the ash content decreased with number of cuts resulting in increased availability of OM, while in case of shaftal reverse trend was observed.

Table 1. Chemical composition of leguminous fodders % DM basis

Parameter	Berseem				Lucerne				Shaftal			
	1cut	2 cut	3 cut	4 cut	1cut	2 cut	3 cut	4 cut	1cut	2 cut	3 cut	4 cut
Ash	13.0	11.2	11.6	10.4	14.4	12.7	11.0	9.8	10.9	10.6	11.6	11.9
OM	87.0	88.8	88.4	89.6	85.6	87.3	89.0	90.2	89.1	89.4	88.4	88.1
CP	21.4	20.9	20.8	19.8	22.4	21.5	21.3	20.6	20.2	19.8	19.4	18.2
EE	2.5	2.5	2.3	2.2	2.7	2.5	2.4	2.3	2.4	2.5	2.4	2.3
Cellulose	19.5	22.5	26.5	28.0	15.5	17.5	20.5	19.5	15.5	18.5	21.5	22.5
NDF	38.5	44.7	45.9	46.1	33.4	38.2	41.4	43.1	36.2	40.2	42.8	44.7
ADF	26.0	34.1	38.0	38.4	20.5	22.9	25.0	25.9	22.8	25.3	27.1	28.4
Hemicellulose	12.5	10.7	7.9	7.8	12.9	15.4	16.5	17.2	13.5	15.0	15.8	16.4
ADL	7.3	9.6	10.7	10.9	4.2	4.7	5.1	5.3	5.7	6.3	6.8	7.2

Digestion kinetic parameters of nutrients of leguminous fodders

Dry matter degradability: Amongst the tested multi-cut leguminous fodders irrespective of cuts, berseem and lucerne had higher ($P<0.01$) amount of rapidly soluble fraction (Table 2), while shaftal had higher ($P<0.01$) insoluble but potentially degradable fraction, which was degraded at a very high ($P<0.01$) rate. However, the rumen un-degradable fraction was higher ($P<0.01$) in berseem as compared to other multi-cut leguminous fodders. The effective degradability and apparent extent of digestion of lucerne fall in between berseem (lowest, $P<0.01$) and shaftal (highest $P<0.01$). The true degradability and potential extent of digestion of berseem and lucerne was comparable but lower ($P<0.01$) than that of shaftal. Shaftal proved to be of better quality as also indicated by lowest ($P<0.01$) rumen fill value, followed by that lucerne and berseem, predicting high voluntary DM intake by animals in case of shaftal.

The leguminous multi-cut fodders during the first and second cut had the highest ($P<0.01$) amount of insoluble but potentially degradable fraction as compared to that in third and fourth cut (Table 2). The rapidly soluble fraction was observed to be higher ($P<0.01$) in third cut of leguminous multi-cut fodders. The leguminous forages were degraded at the fastest rate ($P<0.01$) in the first cut as compared to other cuts and the degradation rate varied from 6.1 (third cut) to 9.6%/h (first cut). The rumen un-degradable fraction increased ($P<0.01$) linearly with advancement of cuts. The effective degradability of leguminous fodder was observed to be highest ($P<0.01$) during first cut and lowest ($P<0.01$) in fourth cut. The true degradability and potential extent of degradation of dry matter was higher ($P<0.01$) in first cut fodders and further cuts had no effects on these parameters. The apparent extent of digestion decreased ($P<0.01$) as the number of cuts advanced. The lowest ($P<0.01$) rumen fill value in first cut fodders predicted a higher voluntary DM intake as compared to other cuts.

Crude protein degradability: The leguminous fodders are very good sources of quality protein and constitute the major chunk of complete feed. The *in sacco* digestion kinetic parameter of CP revealed that irrespective of cuts, lucerne had the highest ($P<0.01$) amount of rapidly soluble fraction and lowest ($P<0.01$) amount of insoluble but potentially degradable fraction (Table 3). Shaftal showed the reverse

Table 2. Digestion kinetic parameters for DM degradability (%) of different leguminous fodders with different number of cuts

Paramater	Leguminous fodder (LF)			Number of cuts (NOC)				PSE	Effects		
	Berseem	Lucerne	Shaftal	first	second	third	fourth		LF	NOC	LF*NOC
'a'	42.00^{b}	42.76^{b}	27.72^{a}	35.05^{A}	36.79^{A}	43.99^{B}	27.72^{A}	0.80	**	**	**
'b'	45.09^{a}	46.90^{a}	67.83^{b}	58.43^{C}	55.95^{C}	46.79^{A}	67.83^{B}	0.82	**	**	**
'c',/h	0.058^{a}	0.059^{a}	0.103^{b}	0.096^{C}	0.071^{B}	0.061^{A}	0.066^{AB}	0.002	**	**	**
UDF	15.78^{c}	12.72^{b}	4.44^{a}	6.78^{A}	10.00^{B}	11.56^{C}	15.60^{D}	0.15	**	**	**
ED	72.74^{a}	75.32^{b}	82.32^{c}	81.57^{C}	77.04^{B}	76.74^{B}	71.81^{A}	0.10	**	**	**
TD	68.36^{a}	68.78^{a}	80.19^{b}	79.16^{B}	70.65^{A}	69.09^{A}	70.37^{A}	0.46	**	**	**
PED	66.37^{a}	67.08^{a}	78.21^{b}	77.21^{B}	68.91^{A}	67.87^{A}	68.63^{A}	0.44	**	**	**
AED	60.96^{a}	63.27^{b}	78.60^{c}	75.92^{D}	67.35^{C}	64.74^{B}	62.42^{A}	0.38	**	**	**
RF, kg	17.64^{c}	16.56^{b}	9.53^{a}	10.68^{A}	15.10^{B}	15.96^{C}	16.57^{C}	0.21	**	**	**

'a', Rapidly soluble fraction; 'b', insoluble but potentially degradable fraction; 'c', degradation rate; ED, effective degradability; TD, true degradability; PED, potential extent of digestions; AED, apparent extent of digestion; RF, rumen fill, Figures with different superscripts^{a,b,c} for different leguminous fodders; different superscripts^{A,B,C,D} for different number of cuts in a row differ significantly $^{**}P<0.01$.

Table 3. Digestion kinetic parameters for CP degradability (%) of different leguminous fodders with different number of cuts

Paramater	Leguminous fodder (LF)			Number of cuts (NOC)				PSE	Effects		
	Berseem	Lucerne	Shaftal	first	second	third	fourth		LF	NOC	LF*NOC
'a'	36.33^{b}	42.99^{c}	8.58^{a}	20.19^{A}	31.65^{B}	30.36^{B}	35.00^{C}	0.58	**	**	**
'b'	56.15^{b}	52.43^{a}	86.13^{c}	75.73^{C}	63.24^{B}	63.24^{B}	57.41^{A}	0.60	**	**	**
'c'/h	0.057^{a}	0.066^{b}	0.106^{c}	0.101 ^{C}	0.07 ^{B}	0.071^{A}	0.064 ^{A}	0.001	**	**	**
ED	75.07^{a}	80.85^{c}	78.21^{b}	80.98 ^{C}	77.27^{B}	77.08^{AB}	76.83^{A}	0.08	**	**	**
TD	68.31^{a}	71.94^{b}	80.66^{c}	79.57^{D}	71.36^{B}	77.33^{C}	70.29^{A}	0.23	**	**	**
PED	66.62^{a}	70.17^{b}	78.66^{c}	77.61 ^{D}	69.60^{B}	71.52^{A}	68.56^{A}	0.22	**	**	**
AED	64.71^{a}	70.42^{b}	78.36^{c}	78.29^{D}	69.36^{B}	70.34^{C}	66.66^{A}	0.22	**	**	**
UDP	5.14^{c}	4.10^{a}	4.21^{b}	4.04^{A}	4.71 ^{C}	4.70^{C}	4.47^{B}	0.02	**	**	**
UDP%CP	24.94^{c}	19.15^{a}	21.79^{b}	19.02^{A}	22.73^{B}	22.92^{BC}	23.17^{C}	0.08	**	**	**

'a'- Rapidly soluble fraction, 'b'- Insoluble but potentially degradable fraction, 'c'- Degradation rate, UDP- Rumen undegradable protein; ED- Effective degradability, TD- True degradability, PED- Potential extent of digestion, AED- Apparent extent of digestion, Figures with different superscripts^{a,b,c} for different leguminous fodders; different superscripts^{A,B,C,D} for different number of cuts in a row differ significantly **P<0.01.

trend (P<0.01) for these parameters. The rate of degradation of shaftal was much faster (P<0.01), while that of berseem was much lower (P<0.01). Lucerne had higher (P<0.01) effective degradability followed by that in shaftal. True digestibility, potential extent and apparent extent of digestion were highest (P<0.01) in shaftal followed by lucerne and lowest (P<0.01) in berseem. The rumen undegradable protein (UDP) and UDP as per cent of total protein was higher (P<0.01) in berseem followed by that in shaftal.

Irrespective of type of leguminous forage, the amount of rapidly soluble CP fraction of multi-cut leguminous fodders was observed to be lower (P<0.01) in first cut fodder and higher (P<0.01) in fourth cut fodders, but during second and third cut it remained more or less constant (Table 3). Whereas the insoluble but potentially degradable fraction was higher (P<0.01) during the first cut and lower (P<0.01) in the fourth cut, similar trend was observed in the rate of degradation of potentially degradable fraction of these fodders. The rumen undegradable fraction increased with the increase in number of cut, being low (P<0.01) in first cut fodders and high (P<0.01) in fourth cut fodders, however rumen undegradable protein was observed to be highest (P<0.01) in second and third cut and lowest in first cut fodders. But rumen undegradable protein as percent of total protein content of fodders was higher (P<0.01) during third and fourth cut as compared to other cuts. The effective degradability of CP of multi-cut leguminous fodders decreased (P<0.01) as the number of cuts increased. The other digestion kinetic parameter like true digestibility, potential extent and apparent extent of digestion were observed to be higher (P<0.01) in first cut fodders and lower in (P<0.01) in fourth cut fodders.

Neutral detergent fiber (NDF) degradability: The NDF degradability pattern of multi-cut leguminous forages, irrespective of cuts, revealed that shaftal had the lowest (P<0.01) amount of rapidly soluble fraction and the highest (P<0.01) amount of insoluble but potentially degradable fraction (Table 4), which was degraded at the fastest rate (9.2%/h). Shaftal had the lowest (P<0.01) rumen un-degradable fraction and berseem had the highest (P<0.01), while reverse trend was observed in case of ED. The apparent extent of digestion of NDF followed the similar trend. The TD and PED of shaftal were higher (P<0.01) than that of berseem and lucerne, which were comparable. The lowest (P<0.01) rumen fill value also confirmed that shaftal had better palatability than that of lucerne and berseem.

The digestion kinetic parameter of NDF, irrespective of leguminous forage species in the first cut had the lowest amount (P<0.01) of rapidly soluble fraction and highest (P<0.01) amount of insoluble but potentially degradable fraction (Table 4), which degraded at the fastest rate (7.8%/hour). The rumen un-degradable fraction was observed to be highest and effective degradability lowest (P<0.01) in fourth cut. The true degradability and potential extent of digestion was higher (P<0.01) in the first cut of leguminous fodders, the increase in number of cuts showed no significant difference in these parameters. The apparent extent of digestion however decreased (P<0.01) with the advancement in number of cuts thereby decreasing the palatability as indicated by rumen fill values, which increase in number of cuts.

In vitro *gas and methane production studies:* The *in vitro* gas production analysis revealed that irrespective of cuts, different multi-cut leguminous forages produced almost same amount of net gas even though the digestibility of NDF and OM of berseem was lower (P<0.01) than that of lucerne and shaftal (Table 5). Shaftal produced the lowest (P<0.01) methane followed by that produced by berseem and highest in lucerne. There is also evidence that using clovers and grasses with high WSC in animal diets can directly reduce methane emission (Lovett *et al*. 2004). Ramirez-Restropo and Barry (2005) suggested that feeding forage legumes like lucerne or red clover also tends to decrease CH_4 losses

Table 4. Digestion kinetic parameters for NDF degradability (%) of different leguminous fodders with different number of cuts

Paramater	Leguminous fodder (LF)			Number of cuts (NOC)				PSE	Effects		
	Berseem	Lucerne	Shaftal	first	second	third	fourth		LF	NOC	LF*NOC
'a'	13.75^{b}	23.17^{c}	0.18^{a}	-2.68^{A}	33.53^{C}	18.82^{B}	-0.19^{A}	0.69	**	**	**
'b'	67.61^{b}	61.88^{a}	88.85^{c}	88.53^{D}	57.60^{A}	68.07^{B}	76.92^{C}	0.72	**	**	**
'c'/h	0.050^{a}	0.055^{b}	0.92^{c}	0.078^{C}	0.062^{B}	0.058^{A}	0.066^{B}	0.001	**	**	**
UDF	23.83^{c}	18.62^{b}	10.96^{a}	15.03^{B}	12.64^{A}	17.05^{C}	26.51^{D}	0.24	**	**	**
ED	58.48^{a}	65.70^{b}	70.11^{c}	64.42^{B}	73.66^{D}	65.45^{C}	55.53^{A}	0.12	**	**	**
TD	65.9^{a}	66.90^{a}	78.39^{b}	75.48^{B}	68.88^{A}	67.99^{A}	69.26^{A}	0.38	**	**	**
PED	64.29^{a}	65.25^{a}	76.46^{b}	73.62^{B}	67.18^{A}	66.31^{A}	67.55^{A}	0.37	**	**	**
AED	55.04^{a}	58.35^{b}	71.58^{c}	66.44^{D}	64.37^{C}	60.58^{B}	53.24^{A}	0.27	**	**	**
RF, kg	20.88^{c}	18.99^{b}	12.27^{a}	14.64^{A}	16.68^{B}	18.22^{C}	19.98^{D}	0.20	**	**	**

Figures with different superscripts^{a,b,c} for different leguminous fodders; different superscripts^{A,B,C,D} for different number of cuts in a row differ significantly **P<0.01.

Table 5. *In vitro* nutrient digestibility, methane production and ME availability from different leguminous fodders with different number of cuts

Paramater	Leguminous fodder (LF)			Number of cuts (NOC)				PSE	Effects		
	Berseem	Lucerne	Shaftal	first	second	third	fourth		LF	NOC	LF*NOC
NGP, ml/g DM	155.11	160.90	161.10	152.47^{A}	162.09^{B}	157.41^{AB}	164.17^{B}	2.08	NS	*	**
t½, h	8.86^{a}	8.94^{a}	11.80^{b}	9.18^{A}	9.96^{B}	10.58^{C}	9.75^{B}	0.06	**	**	**
CH_4, at ½, ml/g % DOM	9.90^{b}	11.35^{c}	8.36^{a}	8.57^{A}	9.51^{B}	10.09^{C}	11.31^{D}	0.08	*	**	**
NDFD,%	35.35^{a}	48.37^{b}	43.01^{b}	41.73^{A}	40.08^{A}	49.76^{B}	37.40^{A}	1.81	**	**	**
TOMD,%	68.94^{a}	77.57^{b}	75.15^{b}	76.71^{C}	73.24^{B}	75.95^{BC}	69.66^{A}	0.74	**	**	**
NH_3-N, mg/dl	19.79^{b}	27.52^{c}	18.81^{a}	22.72^{B}	20.25^{A}	24.58^{C}	20.61^{A}	0.22	**	**	**
PF	1.89	1.78	2.17	1.76	1.80	1.99	2.24	0.11	NS	NS	NS
ME, MJ/kg DM	7.67^{a}	7.88^{b}	7.78^{ab}	7.68	7.89	7.73	7.82	0.05	*	NS	NS

NGP-Net gas production, PF- Partitioning factor, Figures with different superscripts^{a,b,c} for different leguminous fodders; different superscripts^{A,B,C,D} for different number of cuts in a row differ significantly **P<0.01;* P<0.05; NS- Non significant.

compared to grass. This reduction can be further enhanced by legumes that contain condensed tannins. The partitioning factor varied (P>0.05) from 1.78 (lucerne) to 2.17 (shaftal). The degradation of protein revealed that lucerne produced higher (P<0.01) amount of ammonia nitrogen followed by that produced by berseem. The ME availability was the highest from lucerne which was comparable with that of shaftal but higher (P<0.05) than that of berseem.

Irrespective of leguminous fodder species, the net gas produced in the first and third cut was lower (P<0.01) than that produced during other cuts (Table 5). The digestibility of NDF and OM was lowest (P<0.01) in the fourth cut fodders. Accordingly, the methane production at t½ (expressed as ml/g digestible OM) increased (P<0.01) linearly with the number of cuts and was highest in the fourth cut. The partitioning factor was observed to be higher (P>0.05) in the fourth cut fodders followed by that in third cut fodders. The release of ammonia was higher (P<0.01) during the third cut of these fodders.

Irrespective of number of cuts, the total VFA's production varied (P<0.01) from 6.6 mM/dl in shaftal to 7.95 mM/dl in lucerne (Table 6). Lucerne produced highest (P<0.01) amount of acetate, propionate and butyrate followed by that produced by berseem. The acetate to propionate ratio however was higher (P<0.01) in berseem, closely followed by lucerne and lowest in shaftal. The relative proportion of acetate was higher (P<0.05) in shaftal and lucerne than that in berseem, while significantly higher (P<0.01) proportion of propionate was produced by shaftal in comparison to other fodders. The highest (P<0.01) amount of iso-valerate was produced by shaftal followed by that produced by lucerne. Both berseem and lucerne produced higher (P<0.01) valerate than the shaftal.

Irrespective of leguminous fodder species, the total VFA's, acetate and propionate production were higher (P<0.01) in fourth cut than first cut (Table 6). Fodders in the second cut produced the lowest (P<0.01) amount of butyrate. The A:P ratio was lowest (P<0.01) in the first cut fodders and highest in the fourth cut fodders. According to Russel (1998) and Lila *et al.* (2005), methane production was highly correlated with A:P ratio in the rumen. The relative proportion of propionate was higher (P<0.01) during first and second cut,

Table 6. *In vitro* volatile fatty acid production (mM/dl) from different leguminous fodders with different number of cuts

Paramater	Leguminous fodder (LF)			Number of cuts (NOC)				PSE	Effects		
	Berseem	Lucerne	Shaftal	first	second	third	fourth		LF	NOC	LF*NOC
TVFA	7.61^{b}	7.95^{c}	6.61^{a}	7.48^{C}	6.54^{A}	7.11^{B}	8.44^{D}	0.03	**	**	**
Acetate (A)	5.49^{b}	5.69^{c}	4.74^{a}	5.35^{C}	4.71^{A}	5.08^{B}	6.08^{D}	0.02	**	**	**
Propionate (P)	1.57^{b}	1.64^{c}	1.40^{a}	1.58^{C}	1.36^{A}	1.48^{B}	1.73^{D}	0.01	**	**	**
Butyrate	0.53^{b}	0.59^{c}	0.44^{a}	0.52^{B}	0.46^{A}	0.52^{B}	0.60^{C}	0.003	**	**	**
A:P ratio	3.52^{b}	3.46^{b}	3.38^{a}	3.39^{A}	3.46^{AB}	3.45^{AB}	3.53^{B}	0.02	**	*	NS
Relative proportion (%)											
Acetate	72.30^{b}	71.56^{a}	71.64^{a}	71.60	72.13	71.53	72.08	0.19	*	NS	**
Propionate	20.54^{a}	20.69^{a}	21.22^{b}	21.13^{B}	20.93^{B}	20.77^{AB}	20.45^{A}	0.11	**	**	**
Butyrate	6.95^{b}	7.38^{c}	6.75^{a}	6.88^{A}	6.93^{A}	7.22^{B}	7.06^{AB}	0.05	**	**	**
Isovalerate	0.10^{a}	0.16^{b}	0.18^{c}	0.22^{D}	0.08^{A}	0.17^{C}	0.13^{B}	0.01	**	**	**
Valerate	0.27^{b}	0.26^{b}	0.02^{a}	0.22	0.19	0.21	0.12	0.04	**	NS	**

Figures with different superscripts^{a,b,c} for different leguminous fodders; different superscripts^{A,B,C,D} for different number of cuts in a row differ significantly **P<0.01;* P<0.05; NS, Nonsignificant.

while that of butyrate was higher (P<0.01) in third cut. The proportion of branched chain volatile fatty acid was lower than that of other VFA's but was higher (P<0.01) in first cut fodders followed by that in third cut fodders. Based on these results it was concluded that use of shaftal in the diet of ruminants may help in alleviating enteric methane production.

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