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Effect of synchronization of ruminal energy and nitrogen supply on *in vitro* microbial protein synthesis and rumen fermentation*

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ABSTRACT

Present study was conducted to evaluate the effect of synchronization of ruminal energy and nitrogen (N) supply on *in vitro* microbial protein synthesis (MPS) and rumen fermentation pattern. Commonly used tropical feeds and fodders (sorghum grain, maize grain, wheat bran, mustard cake, groundnut cake, maize fodder, oat fodder and wheat straw) were first evaluated *in sacco* for their organic matter and N degradation characteristics. The protein sources (mustard and groundnut-cake) had faster N degradation rate while energy source (sorghum grain) had faster whereas the other (maize grain) had slower rate of energy release. By varying the proportion of feed ingredients, 6 isonitrogenous and isocaloric diets were formulated and their synchrony index (SI) was calculated using *in sacco* degradability kinetic data. The diets were classified as high (HS), medium (MS) and low (LS) synchronous based on their SI with 2 diets in each category. These diets were evaluated for rumen fermentation parameters by *in vitro* gas production technique. The total gas production from HS and MS diets was significantly higher indicating higher fermentation while any specific trend in methane production was not observed. Degradability of HS and MS diets was significantly higher than that of LS diets. The NH_3–N content in HS and MS diets was significantly lower indicating higher uptake of N from these diets while TCA-ppt N and total N content was significantly higher from these diets in comparison with LS diets. The TVFA and propionate production was higher in HS and MS diets while acetate production was higher in LS diets. Acetate: propionate ratio was significantly lower in HS and LS diets. The HS and MS diets produced significantly higher amount of microbial N with greater efficiency of MPS. It was concluded that due to synchronization of energy and N supply, the *in vitro* rumen fermentation was improved with higher MPS and efficiency of N capture.

Key words: Energy, Microbial protein synthesis, Nitrogen, Rumen fermentation, Synchronization

Growth of ruminal microbes and microbial protein synthesis (MPS) in the rumen depends on the supply of dietary energy from the organic matter (OM) degradation and N from the degradation of proteins and NPN. Generally, rumen microbes are capable of capturing the majority of NH_3-N; however, under certain dietary conditions such as surplus of rumen degradable protein (RDP) and/ or lack of available energy, rate of NH_3-N release in the rumen exceeds the rate of its uptake by rumen microbes and this excess NH_3 is excreted in the form of urea (Dewhurst *et al.* 2000). This results not only in wastage of N, the costly item in animal rations but also poses threat to the environment. Dietary ingredients vary in rate and extent of OM and N degradation in the rumen. Even for same level of RDP and energy supply, the efficiency of microbial protein synthesis varies because of variation in rate of release of N and energy from the diet. Synchronizing the energy and N supply to rumen microbes by selecting the specific type and levels of ingredients that are having more or less similar degradation characteristics will help increase the MPS (Richardson *et al.* 2003), which may ultimately get translated into improved animal performance. The aim of the present study was to investigate the effect of synchronizing the ruminal availability of energy and N on rumen fermentation characteristics and MPS.

MATERIALS AND METHODS

Representative samples of mustard cake, groundnut cake, wheat bran and sorghum and maize grain were collected from Central Store of the Institute. Samples of oat and maize fodder and wheat straw were collected from Farm Section of the Institute. Proximate and fibre composition of feed ingredients (Table 1) was estimated as per AOAC (2005) and Van Soest *et al.* (1991).

In sacco *study:* Samples of all the ingredients used for *in sacco* study were dried and ground using 2 mm sieve.

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Table 1. Chemical composition of feed ingredients (%, DM basis)

Feed ingredient	OM	CP	EE	Ash	NDF	ADF	ADL
Mustard cake	93.82	38.73	7.42	6.18	21.27	18.42	5.92
Groundnut cake	95.72	40.19	7.53	4.28	19.45	13.81	4.09
Sorghum grain	97.78	13.49	3.80	2.22	12.07	3.67	1.03
Maize grain	98.06	11.15	3.26	1.94	10.18	3.54	0.54
Wheat bran	94.97	15.21	2.68	5.03	43.15	9.48	4.68
Oat fodder	86.03	15.34	1.85	13.97	58.87	30.52	2.36
Maize fodder	92.72	8.24	1.51	7.28	62.43	31.86	3.63
Wheat straw	91.71	3.61	1.08	8.29	77.78	49.77	9.25

Approximately 5 g sample was taken in the nylon bags (size 17 cm × 8 cm, pore size 40 mm) and incubated in the rumen of three crossbred rumen fistulated bulls maintained on basal diet containing concentrate mixture (maize grain 49, wheat bran 30, groundnut cake 18, mineral mixture 2 and common salt 1 part each), oat/maize fodder and wheat straw. Nylon bags were incubated for 2, 4, 6, 8, 12, 24, 36, 48 h and washed under running tap water till the affluent became clear. Fodder and straw samples were incubated for additional 3 intervals (60, 72 and 96 h). Nylon bags for 0 h were washed along with other bags without incubation. After washing, bags were hanged to drain the excess water and then dried at 60°C in a hot air oven till constant weight. The N and OM in residual material in nylon bags were estimated as per the AOAC (2005) to calculate *in sacco* disappearance of OM and N. Degradability coefficients of each feed ingredients (Table 2) were calculated by fitting the data obtained for N and OM disappearance to the first order exponential model (Ørskov and McDonald, 1979). The quantity of N and OM degraded each hour for each feed ingredient of successive hours was calculated. From the hourly quantity of N and OM degraded from each feed ingredients, diets were formulated having various synchrony index (SI) as described by Sinclair *et al.* (1993).

Table 2. *In sacco* OM and N degradation kinetics of feed ingredients

Feed ingredient	OM			N		
	a (%)	b (%)	c (h^{-1})	a (%)	b (%)	c (h^{-1})
Mustard cake	61.22^{a}	25.24^{f}	0.115^{b}	73.40^{a}	19.21^{e}	0.192^{a}
Groundnut cake	41.52^{b}	47.06^{de}	0.167^{a}	48.93^{b}	$49.71b^{c}$	0.171^{ab}
Sorghum grain	26.40^{e}	66.09^{b}	0.133^{b}	25.92^{c}	57.06^{a}	0.065^{c}
Maize grain	15.02^{f}	83.33^{a}	0.047^{cd}	29.18^{c}	62.57^{a}	0.061^{cd}
Wheat bran	36.44^{c}	41.68^{e}	0.137^{b}	44.07^{b}	48.52^{bcd}	0.151^{b}
Oat fodder	37.62^{c}	48.08^{d}	0.067^{c}	29.42^{c}	44.55^{cd}	0.036^{cd}
Maize fodder	30.58^{d}	48.07^{d}	0.035^{d}	12.10^{d}	55.66^{ab}	0.028^{d}
Wheat straw	8.77^{g}	54.70^{c}	0.035^{d}	00.21^{c}	41.68^{d}	0.024^{d}
SEM	0.95	1.37	6.19	1.24	1.73	8.34

Means with different superscripts in a column differ significantly ($P<0.01$). a, rapidly soluble fraction, b, potentially degradable fraction, c, rate of degradation of fraction b.

Total 6 diets having 15% crude protein (CP) and approximately 65% TDN (using book values) were categorized as high synchronous (HS), medium synchronous (MS) and low synchronous (LS) with 2 diets in each category. The diets with SI closer to 1 (above 0.8) were defined as HS, similarly, closer to 0.5 (below 0.6) as LS and in between as MS. The ingredient and chemical composition of these diets are presented in Table 3. These rations were evaluated by *in vitro* gas production technique (Menke *et al.* 1979, Menke and Steingass 1988) for rumen fermentation parameters and MPS.

In vitro *study:* Incubations were carried out in 100 ml calibrated glass syringes in which 200 mg DM (1 mm particle size) of each diet was incubated in triplicate with the buffered rumen fluid for 24 h in a water bath at 39±0.5° C (Makkar *et al.* 1995, Blümmel *et al.* 1997). Incubations were repeated twice and average values are reported (total 6 observations). Mulberry leaves (*Morus alba*) were used as internal standard. Rumen inoculum was obtained before morning feeding from the same animals used for *in sacco* study. The rumen fluid from 3 animals was pooled before use and proportion of medium mixture solution to rumen fluid was 2:1. Volume of buffered rumen fluid used per syringe was 30 ml.

In vitro *study: In vitro* rumen fermentation parameters of the diets varying in SI are presented in Table 4.

After 24 h incubation, gas production was estimated from the displacement of piston during incubation. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing no substrate, but only the inoculum) from total gas produced in the syringe containing substrate and inoculum. Methane production was estimated, using Nucon–5700 gas chromatograph equipped with flame ionization detector (FID) and stainless steel column packed with Porapak-Q. A 50:50 mixture of methane and carbon dioxide was used as a standard. True digestibility was estimated after 24 h incubation as per Goering and Van Soest (1970) with little modifications. The partitioning factor (PF) was calculated as the ratio of TDOM (mg) to gas volume (ml) produced from it during 24 h of incubation (Makkar 2004). Nitrogen fractions were estimated using KEL PLUS-N analyzer and ammonia evolved was titrated against standard acid (AOAC 2005). Values of all the N fractions were reported after correcting for blank values. Individual volatile fatty acids (VFAs) were estimated using Nucon-5700 gas chromatograph equipped with double FID and chromosorb glass column.Total VFA (TVFA) concentration was obtained by summing up concentrations of these 3 VFAs.Total purines content (as yeast RNA equivalent) in apparent undegraded residue and microbial pellet was analyzed using spectrophotometer method (Obispo and Dehority, 1999) modified by Makkar and Becker (1999). The total purines

(as RNA equivalent) were calculated by subtracting purine content of blank residue from that of 24 h apparent undegraded residue.

Statistical analysis: Statistical analysis of the data was carried out by one-way ANOVA assuming diets with varying SI as treatment using SAS software package.

RESULTS AND DISCUSSION

Chemical composition of feed ingredients: The CP content of feed ingredients ranged from 3.61 to 40.10% with minimum and maximum values in wheat straw and groundnut cake, respectively. The ether extract (EE) content of oilseed cakes was higher than other ingredients. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) ranged from 10.18 to 77.78 and 3.54 to 49.77%, respectively. Maize grain had lowest while wheat straw had highest NDF and ADF values among all the feed ingredients. Acid detergent lignin (ADL) content was highest in wheat straw. Chemical composition of the feed ingredients was similar with the reports of Ranjhan (1998) and Kiran and Krishnamoorthy (2007). The green fodders were harvested in early flowering stage and their fiber content was within normal range. Both the oilseed cakes were expeller products and their ether extracts content was in accordance with Ranjhan (1998).

In sacco *study:* There was a large variation in the *in sacco* OM and N degradation kinetics among the ingredients (Table 2). The rapidly soluble OM fraction 'a' ranged from 8.77 to 61.22%. Fodders had lower 'a' fraction than concentrate ingredients. Mustard cake had highest while wheat straw had lowest 'a' fraction. Potentially degradable OM fraction 'b' ranged from 25.24 to 83.33% with mustard cake and maize grain having lowest and highest values, respectively. Ingredients with higher 'a' fractions generally had lower 'b' fraction and vice-versa. The rate of degradation (c) of potentially degradable OM fraction ranged from 0.035 to 0.167/h. Wheat straw and maize fodder had lowest rate of degradation among all ingredients while maize grain had lowest rate among concentrate ingredients.

The rapidly soluble N fraction 'a' ranged from 0.021 to 73.40% with lowest and highest values for wheat straw and mustard cake, respectively. Potentially degradable N fraction 'b' ranged from 19.21 to 62.57% similar to the trend observed for OM. The rate of degradation (c) of potentially degradable N fraction (b) was highest in mustard cake and lowest in wheat straw. The rate and extent of OM and N degradation were more or less similar in fodders but varied significantly for concentrate ingredients. The rate of N degradation from both the protein supplements (mustard and groundnut cake) was better parallel correlated to OM degradation from sorghum gain than maize grain. Therefore, diets containing sorghum grain as major energy source had higher SI than that of maize grain.

In sacco OM and N degradation kinetic parameters of the feed ingredients were consistent with the various earlier reports (Sharma and Singh 1997, Pattanaik 1997, Chatterjee and Walli 2003, Fardin 2005 and Ayyappan 2008). The oilseed cakes had very high instantly soluble 'a' fraction despite same particle size of all the ingredients. The higher solubility may not always be correlated with high degradation rate, a major drawback in nylon bag technique, and often this fraction is overestimated (Cone *et al.* 2009). Grains obviously had higher potentially degradable OM fraction due to their higher starch and lower fiber content. Maize grain had very low rate of OM degradation, which may be attributed to the physico-chemical characteristics of its starch protein matrix (Herrera-Saldana *et al.* 1990). As N degradation rate of both the major N sources (mustard and groundnut cake) was similar, difference in OM degradation rate of both grains (major energy sources) was the basis to formulate the diets of varying SI.

Proportion of ingredients in diets and their chemical composition: Proportion of different ingredients in the diets and chemical composition of diets is presented in Table 3. The HS and MS diets had sorghum grain, a fast energy releasing source, whereas LS diets had maize grain, a slow energy releasing source. Diets within same group varied in green fodder i.e. either oat or maize fodder. All the diets had 6% molasses so as to correspond the practical diets fed to animals in the form of complete feed blocks. All the diets had almost similar OM and CP content. The CP content of the diets was adjusted around 15%. The NDF and ADF content of diets was approximately around 45 and 25%, respectively.

Table 3. Ingredient and chemical composition of diets with different synchrony index

	HS_1	HS_2	MS_1	MS_2	LS_1	LS_2
Synchrony index (SI)	0.86	0.82	0.77	0.76	0.56	0.55
Ingredient composition (parts, DM basis)						
Maize grain	-	-	-	-	20.0	20.0
Sorghum grain	16.0	16.0	20.0	20.0	-	-
Wheat bran	14.0	14.0	10.0	10.0	10	10
GNC	10.0	10.0	4.0	4.0	5.0	5.0
Mustard cake	7.0	7.0	13.0	13.0	12.0	12.0
Molasses	6.0	6.0	6.0	6.0	6.0	6.0
Oat fodder	30.0	-	30.0	-	30.0	-
Maize fodder	-	30.0	-	30.0	-	30.0
Wheat straw	15.0	15.0	15.0	15.0	15.0	15.0
Common salt	1.0	1.0	1.0	1.0	1.0	1.0
Min. mixture	1.0	1.0	1.0	1.0	1.0	1.0
Chemical composition (%, DM)						
OM	91.42	93.06	91.38	93.03	91.85	93.22
CP	15.33	15.17	15.45	15.18	15.53	15.29
EE	03.13	04.06	03.15	03.72	04.15	03.54
Ash	08.58	06.94	08.62	06.97	08.15	06.78
NDF	41.95	43.41	44.88	47.26	45.39	47.16
ADF	24.67	24.74	26.61	27.72	27.20	27.39
ADL	04.04	04.13	04.17	04.24	04.52	04.64

Table 4. *In vitro* rumen fermentation parameters of diets with different synchrony index

Parameter	High synchronous		Medium synchronous		Low synchronous		SEM
	HS_1	HS_2	MS_1	MS_2	LS_1	LS_2	
Total gas, ml/200 mg DM	39.99^{d}	41.49^{de}	41.83^{de}	42.33^{e}	37.16^{f}	37.49^{f}	0.50
Methane, ml/200 mg DM	12.97^{ab}	13.62^{b}	12.75^{a}	13.63^{b}	12.57^{a}	12.38^{a}	0.26
Methane, ml/g TDOM	84.99^{a}	88.58^{ab}	85.80^{ab}	90.89^{ab}	91.97^{b}	88.70^{ab}	1.98
TDMD,%	74.32^{a}	76.32^{a}	75.07^{a}	74.60^{a}	68.19^{b}	68.04^{b}	1.67
TOMD,%	76.29^{a}	76.87^{a}	74.56^{a}	75.04^{a}	68.33^{b}	69.80^{b}	1.51
PF	3.81	3.71	3.57	3.55	3.68	3.73	0.09
NH_3-N,mg/100 ml	3.24^{de}	2.96^{de}	3.89^{ef}	2.59^{d}	5.39^{g}	4.73^{fg}	0.25
TCA-ppt N, mg/100 ml	19.60^{d}	19.79^{d}	19.32^{d}	19.04^{d}	16.33^{e}	15.96^{e}	0.24
TN, mg/100 ml	24.64^{d}	24.36^{de}	24.55^{d}	23.99^{e}	21.93^{f}	21.65^{f}	0.13
TVFA, mM	65.68^{de}	66.56^{d}	64.64^{e}	64.46^{e}	59.24^{f}	59.09^{f}	0.35
Acetate,%	68.98^{a}	68.99^{a}	69.08^{a}	69.47^{ab}	70.37^{c}	70.25^{bc}	0.26
Propionate,%	23.07^{a}	23.10^{a}	23.04^{a}	22.80^{ab}	21.76^{bc}	22.00^{c}	0.28
Butyrate,%	7.95	7.91	7.88	7.73	7.88	7.75	0.12
A:P	2.99^{a}	2.99^{a}	2.99^{a}	3.05^{a}	3.24^{b}	3.19^{b}	0.05
MN, mg/200 mg DM	3.44^{d}	3.21^{de}	3.30^{de}	3.08^{e}	2.78^{f}	2.63^{f}	0.06
EMPS, g MN/kg DOM	22.58^{a}	20.90^{ab}	22.26^{ab}	20.51^{bc}	20.38^{bc}	18.81^{c}	0.59

Means with different superscripts in a row differ significantly (^{a,b,c} P<0.05; $^{d,e\ f}$ P<0.01). SEM, standard error of difference between means; TDMD, true DM degradability, TOMD, True OM degradability, PF, partitioning factor, TN, total N, TVFA, total volatile fatty acids, MN, microbial N, EMPS, efficiency of microbial protein synthesis.

Total gas and methane production: Total gas production (ml/200 mg DM) was 39.99 and 41.49 in HS diets, 41.83 and 42.33 in MS diets while 37.16 and 37.49 in LS diets. The total gas production from fermentation of the diets of any particular group was similar. The HS and MS diets had significantly higher (P<0.01) total gas production compared with LS diets. This indicated that HS and MS diets were fermented better than LS diets possibly due to more synchronous supply of energy and N from these diets. Total gas production was influenced by the SI of the diets, possibly due to differences in supply of energy and N to the rumen microbes during incubation. As far as energy source in the diets was concerned, maize grain in LS diets resulted in lower gas production, which is in agreement with the results of Ayyappan (2008) who also reported lower total gas production from the diets containing maize grain. These observations showed that the SI of the diet was directly proportional to the gas production. Similar observations were recorded by Blümmel *et al.* (2003) and Ayyappan (2008).

Methane production (ml/200 mg DM) was 12.97 and 13.62 in HS diets, 12.75 and 13.63 in MS diets while 12.57 and 12.38 in LS diets. The absolute amount of methane produced from HS_2 and MS_2 diets was higher (P<0.05) obviously due to their higher total gas volume because of the higher feed degradability. Methane production expressed in terms of OM degradability (ml/g truly degradable OM, TDOM) were 84.99 and 88.58 in HS diets, 85.80 and 90.89 in MS diets while 91.97 and 88.70 in LS diets. LS_1 diet had highest while HS_1 diet had lowest methane production in terms of OM degradability. Slightly higher methane production (ml) from HS and MS diets than from LS diets may be attributed to higher degradability of these diets as a result of efficient utilization of released energy and N from synchronous diets. These observations were consistent with those reported by Pattanaik (1997). The inclusion of maize grains in the diets did not show any effect on methane production. These results differ from those of Herrera-Saldana *et al.* (1990) who reported higher while Tiwari and Yadav (1994), Pattanaik (1997) and Ayyappan (2008) reported lower methane production from maize grain based diets. There might be more complex interactions among nutrients involved than simply the physico-chemical structure and solubility in production of methane from rumen fermentation and could be related to the basal diet of donor animals.

Substrate degradability: True DM degradability (TDMD) (%) was 74.32 and 76.32 in HS diets, 75.07 and 74.60 in MS diets while 68.19 and 68.04 in LS diets. LS diets had significantly lower (P<0.05) TDMD than HS and MS diets. True OM degradability (TOMD) (%) was 76.29 and 76.87 in HS diets, 74.56 and 75.04 in MS diets while 68.33 and 69.80 in LS diets being significantly lower (P<0.05) in LS diets. It is clear from these results that diets with higher SI had higher degradability than that with lower SI. Partitioning factor ranged from 3.55 to 3.81 and there was no significant difference in PF among the diets.

Diets with higher SI had higher degradability than that with lower SI, which is in agreement with the observations of Blümmel *et al.* (2003) and Ayyappan (2008). Higher DM and OM degradability in the diets with higher SI was possibly due to synchronous release of energy and N from the diets, which might have been utilized more efficiently by the rumen

microbes for maintaining the optimum fermentation. The PF is considered as an index of efficiency of microbial biomass production assuming that the carbon from truly degradable OM is quantitatively distributed between microbial biomass and the sum of fermentative gas and short chain fatty acids (SCFA) and gas production is stoichiometrically related to SCFA (Kiran and Krishnamoorthy 2007). The PF calculated by concomitant true DM degradability and gas volume measurements after 24 h incubation are used to assess the partitioning of nutrients between gases and microbial cells (Blümmel *et al*. 1997) and the high PF value (low gas production per unit of substrate degraded) is indicative of proportionally higher microbial yield. As the PF values in present study are similar for all the diets, it is obvious that the lower total gas production from LS diets was due to corresponding decrease in the OM degradability of these diets.

Nitrogen fractions: The NH_3-N content (mg/100 ml; blank corrected) was 3.24 and 2.96 in HS diets, 3.89 and 2.59 in MS diets while 5.39 and 4.73 in LS diets. HS and MS diets had significantly lower ($P<0.01$) NH_3-N content than LS diets. The TCA-ppt N content (mg/100 ml; blank corrected) was 19.60 and 19.79 in HS diets, 19.32 and 19.04 in MS diets while 16.33 and 15.96 in LS diets. HS and MS diets resulted in higher ($P<0.01$) TCA-ppt N than LS diets, possibly due to efficient microbial utilization of NH_3-N, as evident from lower NH_3-N concentration. Total N content (mg/100 ml) was 24.64 and 24.36 in HS diets, 24.55 and 23.99 in MS diets while 21.93 and 21.65 in LS diets. Total N followed the same trend as recorded for TCA-ppt N and values were higher for HS and MS diets as compared with LS diets.

Higher NH_3 -N levels in LS diets were similar to that reported by Blümmel *et al.* (2001) and Blümmel *et al.* (2003). Minimum NH_3-N level recommended for optimum microbial growth is more than 5 mg/dl incubation medium (Satter and Slyter, 1974) but McCarthy *et al.* (1989) reported that the lower NH_3-N concentration (4 mg/dl) in dairy cows, irrespective of dietary combination of corn, barley, soybean and fish meal, did not affect their production and concluded that this concentration of ammonia N in rumen was sufficient to maintain normal rumen fermentation. The NH_3-N concentration in the rumen at any particular time is a balance between 2 entry sources, i.e., degradability of feed nitrogen and nitrogen recycling and 3 outputs, incorporation of NH_3-N into rumen microbes, NH_3-N absorption and outflow through omaso-reticular orifice (Doreau and Ferlay 1995). The decrease in rumen NH_3-N concentration could be attributed to lower degradability of feed nitrogen, lower bacterial nitrogen recycling and higher incorporation of NH_3-N into microbial cells (Alexander *et al.* 2008). The lower NH_3-N values of HS and MS diets in the present study correspond to the higher microbial N values of these diets. This was also supported by the higher TCA-ppt N values in HS and MS diets.

Total N followed the same trend as TCA-ppt N and values were similar to those reported by Ayyappan (2008). These observations showed that the fraction of TCA-ppt N in total N was almost similar, irrespective of the SI of the diets, which may be due to the fact that feed N is also accounted for in the total N. Higher total N content in HS and MS diets than LS diets may be attributed to the increased microbial proliferation due to synchronous availability of energy and N, and it was also supported by the decreased NH_3-N concentration and increased OM degradability during their *in vitro* fermentation.

Volatile fatty acids: Total VFA (mM) concentration was 65.68 and 65.66 in HS diets, 64.64 and 64.46 in MS diets while 59.24 and 59.09 in LS diets. Statistical analysis of data indicated significantly higher ($P<0.01$) TVFA concentration in the higher SI diets. Molar proportion of acetate (% of TVFA) ranged from 68.98 to 70.37 among all the diets; that of propionate from 21.76 to 23.10% while of butyrate from 7.73 to 7.95%. The molar proportion of acetate and propionate (% of TVFA) in LS diets was significantly different ($P<0.05$) from that of HS diets where acetate was higher while propionate was lower in LS diets. Acetate to propionate ratio ranged from 2.99 to 3.24 in all the diets. Although the ratio varied in a narrow range, LS diets had significantly higher ($P<0.05$) ratio than HS and LS diets. The values of total and individual VFAs recorded in the present study were within normal range (Ayyappan 2008) though towards lower side. Higher TVFA production from HS and MS diets than from LS diets was possibly due to their higher OM degradability. Similar observations were recorded by Blümmel *et al.* (2001) from *in vitro* study in which they reported higher TVFA concentration from diet having higher SI.

The results of present study are in agreement with the reports of Herrera-Saldana *et al.* (1990) and Witt *et al.* (1999) but contrary to those of Ayyappan (2008) who reported higher proportion of acetate in synchronized rations. Faster and extensive starch degradation from sorghum grains in HS and MS diets might have lowered acetate and increased propionate production. Lower acetate to propionate ratio from diets having higher SI was reported by many workers (Herrera-Saldana *et al.* 1990, Witt *et al.* 1999, Rymer and Givens 2002, Kaswari *et al.* 2007) however, Blümmel *et al.* (2001) and Ayyappan (2008) reported higher acetate to propionate ratio from diets having higher SI.

Microbial protein synthesis: Microbial N (mg) was 3.44 and 3.21 in HS diets, 3.30 and 3.08 in MS diets while 2.78 and 2.63 in LS diets. LS diets produced significantly lower ($P<0.01$) microbial N than HS and MS diets. In all the 3 groups, the diet containing maize fodder produced less microbial N than that of oat fodder but the difference was not significant. Efficiency of MPS (EMPS; g MN/kg DOM) was 22.58 and 20.90 in HS diets, 22.26 and 20.51 in MS diets while 20.38 and 18.81 in LS diets. Again the values for

maize fodder containing diets were lower than that of oat fodder containing diets. The reason behind this was not clear but probably more energy from the fermentation of maize fodder containing diets was wasted in the form of gas despite having higher OM degradability.

The mean EMPS of HS group was about 2 and 11% higher than that of MS and LS group, respectively. Though CP and the NH_3-N content in the incubation medium was also optimum in all the diets, the MPS was higher on HS and MS diets than on LS diets. Variation among the diets having different SI may be attributed to the differences in the release of energy and N during their fermentation. Diets with higher SI produced lower NH_3-N concentration and higher microbial N in agreement with Blümmel *et al.* (2003). The NH_3-N was effectively utilized by microbes in these diets to produce more microbial N leading to less wastage of dietary N and energy during fermentation process. The EMPS from HS and MS diets was similar to those reported by Stern and Satter (1982) for commonly used diets but higher than those reported by Oldham (1984) and Kaswari *et al.* (2007). Higher EMPS from HS and MS diets may be attributed to the availability of the peptides to microbes (Joo *et al.* 2005). Similar to the observations of Witt *et al.* (1999), higher MPS was recorded in present study from fast energy releasing grain (sorghum grain) than from slow energy releasing grain (maize grain). It appeared from these observations that MPS depends upon the pattern of nutrient release from the ingredients and also the amount of digestible DM/OM. McCarthy *et al.* (1989) and Casper *et al.* (1999) have reported higher microbial purine concentration on wheat grain based diets as compared to maize grain based diets, a trend observed in the present study also.

From present study it can be concluded that due to the synchronous release of energy and N from diets with higher SI, N was utilized more efficiently for MPS. The increase in microbial proliferation on these diets resulted in higher feed degradability and VFA concentration with lowered NH_3-N content in rumen fluid in *in vitro* condition. So, synchronization of ruminal supply of energy and N from tropical feeds and fodders can serve the purpose of improving animal performance along with reduction in environmental pollution.

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