# Influence of Diet Induced Changes in Rumen Microbial Characteristics on Gas Production Kinetics of Straw Substrates *In vitro*

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**ABSTRACT :** The effect of diets varying in level and source of nitrogen (N) and fermentable organic matter on dynamic characteristics of microbial populations in rumen liquor and their impact on substrate fermentation *in vitro* was studied. The diets tested were straw alone, straw+concentrate mixture and straw+urea molasses mineral block (UMMB) lick. The same diets were taken as substrates and tested on each inoculum collected from the diets. Diet had no effect on the amino acid (AA) composition of either bacteria or protozoa. Differences among the diets in intake, source of N and OM affected bacterial and protozoal characteristics in the rumen. Upper asymptote of gas production (Y$\alpha$) had a higher correlation with bacterial pool size and production rate than with protozoal pool size and production rate. Among the parameters of the gas production model, Y$\alpha$ and lag time in total gas has showed significant ($p<0.01$) correlation with bacterial characteristics. Though the rate constant of gas production significantly differed ($p<0.01$) between diet and type of straw, it was least influenced by the microbial characteristics. The regression coefficient of diet and type of straw for Y$\alpha$ indicated that the effect of diet on Y$\alpha$ was threefold higher than that of the straw. As microbial characteristics showed higher correlation with Y$\alpha$, and diet had more influence on the microbial characteristics, gas production on a straw diet could be used effectively to understand the microbial characteristics. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 990-996)***

**Key Words** : Rumen Microbes, Gas Production, Tracer Techniques, Straw, UMMB

## INTRODUCTION

Although the gas production measurements *in-vitro* (GPIV) was originally proposed for energy determination in ruminant feedstuffs (Menke et al., 1979), this can have a wide ranging potential for application in areas such as rumen studies and ruminant feed evaluation (Getachew et al., 1998). Since the nutritional value of feed is influenced by the feed characteristics and their influence on rumen microbial characteristics, knowledge of both and their interaction can contribute to a better understanding of the nutritional or incriminate qualities of ruminant feedstuffs.

If gas production parameters *in vitro* that are attributable to feed characteristics can be differentiated from those attributable to rumen microbes, the GPIV can perhaps be used to assess the diet induced changes in rumen microbial characteristics. Such an approach could be useful specially to evaluate the effect of nutritional supplements like concentrate, green fodder or urea molasses mineral block (UMMB) commonly used with low quality roughages in developing countries (De and Singh, 2002; Srinivas et al., 2002). Understanding the dynamics of rumen microbes and digestion process on such low nitrogen roughages is very essential. Therefore, this study was designed to elaborate the relationship of diet induced changes in microbial characteristics to gas production kinetics *in vitro* with diets differing in quality and physicochemical characteristics.

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## MATERIALS AND METHODS

### Animals and diets

Twelve crossbred steers fitted with large diameter flexible rumen canula were divided into three groups of four each of comparable age and body weights (31±0.8 months, 248±16 kg) and diets differing in source and amount of nitrogen (N) were assigned at random. The three diets were wheat straw ($D_1$), wheat straw+concentrate mixture ($D_2$) and wheat straw+urea molasses mineral block (UMMB; $D_3$). The compositions of the diets estimated in quadruplicates are presented in Table 1. After an adaptation period of 15 days on the respective diets, the total ration was divided into 12 equal parts and fed at 2 hourly intervals starting from 9:00 h and water was given 6 times a day for 5 days to attain a steady state condition in rumen environment (Chaturvedi et al., 1973). The steers were housed in a concrete floored, well ventilated and protected cattle shed in a single row individually in stalls.

### Rumen microbial attribute

*Amino acid (AA) composition* : Isolation of bacteria and protozoa from rumen liquor: Rumen fluid was collected from each animal at 11:00, 11:30, 12:00, 12:30, 13:30, 14:30 and 16:30 h. The rumen liquor was strained through single layered muslin cloth and centrifuged at 500×g for 3

min to separate protozoa and feed particles. Supernatant and precipitate were taken in separate test tubes. The precipitate was washed twice with normal saline and centrifuged at 500×g for 2 min to remove residual feed particles from protozoa. The supernatant was centrifuged at 15,000×g for 15 min to separate bacteria. Bacterial pellet was washed twice with normal saline. Both protozoal and bacterial samples were processed immediately.

*Amino acid analysis* : A weighed quantity (100±7 mg) of bacteria and protozoal biomass isolated from the rumen liquor from each animal was subjected to amino acid hydrolysis with 5 ml of 6 N HCl at 110°C for 24 h in sealed test tubes flushed with nitrogen. The hydrolysate was centrifuged at 3,000×g for 15 min and filtered through 0.22 µM Millipore filter. The filtrate was diluted ten fold with 400 mM sodium borate buffer. Pre-column derivatization was done using ortho-phthalaldehyde (OPA). Twenty µl of the sample was mixed with 20 µl of OPA/2-mercaptoethanol mixture (50 mg of OPA crystals in 1 ml of absolute methanol, adding 40 µl 2-mercaptoethanol and diluting to a final volume of 10 ml with 0.2 M di-Sodium tetraborate) and incubated for one minute at 39°C for reaction to take place to cause fluorescence. Twenty µl of the mixture was injected into HPLC with automatic sample injector (Millipore, USA, Model 231). Mobile phase used was A = 0.05 M sodium acetate (pH 6.8): methanol: tetrahydrofuran (80:19:1) B = methanol: 0.05 M sodium acetate (80:20) at a flow rate of 1 ml $min^{-1}$. Isoindole derivatives of AA were estimated at 360 nm excitation and 455 nm of emission on reversephase column (4.6 mm ID× 15 cm, octadesyl group as stationary phase) using fluorescence detector. Standard of known concentration (50 nano moles/ml) of each AA was run simultaneously to estimate the concentration of the corresponding AA in the test samples (Roach and Gehrke, 1970).

*Production rate of bacteria and protozoa* : Bacterial production rate (BPR) and protozoal production rate (PPR) were determined using isotope dilution technique with $^{35}S$ (sodium sulfate) as bacterial marker (Singh et al., 1974) and $^{14}C$ (choline methyl chloride) as a protozoal marker (Leng, 1982).

i) Preparation of labeled bacteria and protozoa

• Bacteria : Bacterial suspension was prepared *in vitro* using 150 ml of freshly drawn strained rumen liquor (SRL) incubated with 500 µCi of $^{35}S$ sodium sulfate for 12 h at 39°C under $CO_2$. After 12 h of incubation, bacterial pellet was separated from feed particles and protozoa as explained in section Amino acid (AA) composition. The bacterial pellets thus obtained were washed three times with centrifuged rumen liquor to remove free radioactivity and suspended in 100 ml of freshly drawn, centrifuged rumen liquor.

• Protozoa : Protozoal suspension was prepared *in vitro* using 150 ml of freshly drawn SRL incubated with 120 µCi of $^{14}C$ choline methyl chloride for 12 h at 39°C under $CO_2$. Subsequently, rumen liquor was centrifuged at 500×g for 3 min. Supernatant was discarded and the protozoal sediment was washed thrice using fresh rumen liquor. Finally the washed protozoal sediment was suspended in 100 ml of freshly drawn, centrifuged rumen liquor.

ii) Dosing and sampling

Out of 100 ml of labeled suspension of bacteria and protozoa, 90 ml was infused into the rumen of each animal through the canula in a single dose. The remaining 10 ml of the suspension was kept separate for counting radioactivity in the infusate. The rumen contents were thoroughly mixed by hand covered with long sleeved rubber gloves.

• Bacteria sampling : Samples of rumen fluid were drawn on placing the probes at four different sites in rumen. Samples in triplicate were drawn in 125 ml capacity polypropylene narrow mouth screw capped bottle at 11:00, 11:30, 12:00, 12:30, 13:30, 14:30 and 16:30 h after infusion at 9:00 h and preserved in icebox until processed. Bacterial pellet was isolated as explained before (section Amino acid (AA) composition) and then washed with brij detergent solution followed by 10% TCA and subsequently with ethnol: ether at the ratio of 3:1. Pellets were dried in oven at 60°C for 24 h to a constant weight under atmospheric pressure. Dried pellets were dissolved in soulene followed by addition of 7 ml scintillation fluid. The radioactivity was measured using Tricarb PLD liquid scintillation analyzer (M/s Packard Instruments, USA). Exponential decline in specific radioactivity with time was calculated for determination of BPR.

• Protozoa sampling : PPR was studied after an interval of 120 days from the estimation of BPR to avoid the residual effect of $^{35}S$ sodium sulphate. Samples of rumen fluid was collected after 2 h of post infusion at 9:00 h and continued at 1 h interval upto 17:00 h. Samples were processed immediately within one hour of collection. About 2 ml of rumen fluid samples in triplicates were centrifuged at 500×g for 3 min and supernatant was discarded. The protozoal sediment was resuspended in 20% formal saline and centrifuged again. The centrifugation and suspension were repeated twice. The samples were pooled and an aliquot of 0.5 ml each was used for counting radioactivity and N estimation. 5 ml of scintillation fluid was added to one part of the aliquot and radioactivity was measured as described earlier in this section. N was estimated in another part of aliquot by micro-Kjeldahl's method.

**Gas production kinetics *in vitro***

The influence of diet induced changes in rumen microbial characteristics on gas production kinetics *in*-vitro was measured with straw substrates differing in physicochemical characteristics. Untreated and urea-

**Table 1.** Ingredient and chemical composition of diets (% on dry matter basis)

| Component | $Diet_1$ | $Diet_2$ | $Diet_3$ |
|---|---|---|---|
| Diet ingredients (%): | | | |
| Wheat straw* | 100 | 84.1 | 90.2 |
| Concentrate mixture** | - | 15.9 | - |
| Ummb lick*** | - | - | 8.4 |
| Chemical composition | | | |
| Organic matter | 87.9 | 88.9 | 87.0 |
| Nitrogen | 0.6 | 1.1 | 1.4 |
| Ether extract | 0.8 | 1.4 | 0.9 |
| Neutral detergent fibre | 82.2 | 75.6 | 75.1 |
| Acid detergent fibre | 54.7 | 49.6 | 49.7 |
| Total ash | 12.1 | 11.2 | 12.7 |

* Mineral mixture 2 and common salt 1% as supplement.

** Maize 35, GNcake 27, wheat Bran 35, mineral mixture 2, Salt 1 on as in basis.

*** Urea 15, moolasses 40, groundnut extractions 14, cottons seed cake 14, mineral mixture 8, common salt 3, sodium bentonite 3 and calcite powder 3).

ammoniated straw (Dias-Da-Silva and Sundstol, 1986) of wheat and paddy were used as the substrates. The substrate fermentation rate was measured over time using *in vitro* gas production (Krishnamoorthy et al., 1991). Rumen fluid drawn from each animal on different diets was used as inoculum and maintained under $CO_2$ at 39°C before use. About 500±34 mg of air dry samples were weighed into 250 ml capacity Borosil glass bottles and incubated with rumen inoculum according to Menke and Steingass (1988). Incubations were continued for 48 h with recording of gas production at 2 h intervals upto 12 h and subsequently at 4 h intervals using water manometer (McBee, 1953). The gas production values were fitted to the following model for rate determination with lag (Krishnamoorthy et al., 1991). Methane proportion in the rumen gases was estimated by gas liquid chromatography (M/s Nucon Instruments, India. Series 5500) fitted with thermal conductivity detector and narrow mouth stainless steel columns packed with chromosorb 101 to serve as the stationary phase. Simultaneously standard gas (carbondioxide 2.2, ethylene 2.0, propylene 1.2, methane 27.4, carbonmonoxide 7.6 and ethane 1.1 and nitrogen 7.4% in hydrogen) was also run for comparison.

$$Y = Y\infty[1\text{-}e^{-k(t-\lambda)}]$$

Where,

Y = Total gas production

$Y\infty$ = Upper asymptote for total gas production

k = Rate of gas production

t = Time

$\lambda$ = Lag for initiation of gas production

**Statistical analysis**

Difference among the diets with respect to AA composition of bacteria and protozoa, BPR and PPR were tested by using randomized complete block design with the model,

**Table 2.** Amino acid composition of rumen bacteria and protozoa (μmol/g) on different diets

| Component | Diet -1 | | Diet - 2 | | Diet - 3 | | $SE_m$ | |
|---|---|---|---|---|---|---|---|---|
| | Bacteria[a] | Protozoa[b] | Bacteria[a] | Protozoa[b] | Bacteria[a] | Protozoa[b] | Bacteria | Protozoa |
| Essential: | | | | | | | | |
| Arginine | 151(3.8) | 113(3.4) | 512 (11.6) | 115 (3.4) | 156 (3.9) | 114 (3.4) | 2.45 | 2.32 |
| Histidine | 61 (1.5) | 48 (1.4) | 83 (1.9) | 48 (1.4) | 58 (1.5) | 48 (1.4) | 3.38 | 6.99 |
| Isoleucine | 210 (5.3) | 196 (5.9) | 221 (5.0) | 193 (5.9) | 203 (5.1) | 194 (5.8) | 3.62 | 7.22 |
| Leucine | 308 (7.7) | 219 (6.6) | 332 (7.5) | 224 (6.6) | 304 (7.7) | 219 (6.5) | 3.35 | 7.68 |
| Lysine | 317 (8.0) | 254 (7.6) | 306 (6.9) | 262 (7.7) | 318 (8.0) | 256 (7.6) | 3.28 | 5.80 |
| Methionine | 68 (1.7) | 56 (1.7) | 72 (1.6) | 56 (1.7) | 71 (1.8) | 56 (1.7) | 2.83 | 2.56 |
| Phenylalanine | 131 (3.3) | 148 (4.5) | 134 (3.0) | 152 (4.5) | 122 (3.1) | 145 (4.3) | 3.39 | 5.12 |
| Threonine | 301 (7.6) | 262 (7.9) | 287 (6.5) | 258 (7.6) | 290 (7.3) | 263 (7.8) | 2.86 | 5.76 |
| Tyrosine | 120 (3.0) | 135 (4.1) | 118 (2.7) | 139 (4.1) | 114 (2.9) | 136 (4.1) | 4.30 | 3.16 |
| Valine | 207 (5.2) | 144 (4.3) | 210 (4.8) | 148 (4.4) | 207 (5.2) | 148 (4.4) | 3.93 | 3.60 |
| Total | 187.40 | 157.50 | 227.50 | 159.50 | 184.30 | 157.90 | 28.94 | 1.01 |
| Non-essential: | | | | | | | | |
| Alanine | 415 (10.4) | 303 (9.1) | 412 (9.3) | 318 (9.4) | 414 (10.5) | 309 (9.2) | 5.76 | 3.41 |
| Aspartic acid | 408 (10.2) | 386 (11.6) | 412 (9.3) | 392 (11.6) | 417 (10.5) | 388 (11.6) | 3.96 | 4.06 |
| Cystein | 33 (0.8) | 28 (0.8) | 34 (0.8) | 28 (0.8) | 36 (0.9) | 30 (0.9) | 2.69 | 2.53 |
| Glutamic acid | 488 (12.2) | 404 (12.2) | 496 (11.3) | 414 (12.2) | 496 (12.5) | 410 (12.2) | 4.31 | 3.56 |
| Glysine | 467 (11.7) | 326 (9.8) | 463 (10.5) | 334 (9.8) | 457 (11.5) | 328 (9.8) | 3.57 | 6.50 |
| Proline | 121 (3.0) | 118 (3.6) | 126 (2.9) | 124 (3.7) | 116 (2.9) | 120 (3.6) | 3.55 | 4.72 |
| Serine | 180 (4.5) | 186 (5.6) | 192 (4.4 ) | 188 (5.5) | 181 (4.6 ) | 190 (5.6) | 3.14 | 5.03 |
| Total | 301.71 | 250.14 | 305.00 | 256.86 | 302.43 | 253.57 | 2.39 | 1.46 |

Values in parenthesis indicate percentage of total amino acids.

Components bearing different superscripts differ significantly at 0.01 level of significance but for significance level for total essential amino acids between bacteria and protozoa was at 0.05 level only.

**Table 3.** Feed intake, digestibility and dynamic characteristics of bacterial and protozoa in rumen

| Component | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|
| Intake and digestibility | | | |
| Body weight (kg) | 223±22 | 234±22 | 4.51[b]±0.55 |
| Straw DM intake (kg/d) | 3.47[a]±0.32 | 4.66[b]±0.38 | 0.42[b]±0.10 |
| Supplement DM intake (kg/d) | - | 0.85[a]±0.07 | 5.00[c]±0.56 |
| Total DM intake (kg/d) | 3.47[a]±0.32 | 5.54[b]±0.47 | 4.35[c]±0.85 |
| Total OM intake (kg/d) | 3.06[a]±0.43 | 4.93[b]±0.72 | 70.00[c]±13.77 |
| Total nitrogen intake (g/d) | 20.85[a]±2.91 | 60.94[b]±8.88 | 49.70[b]±0.35 |
| DM digestibility (%) | 43.72[a]±1.09 | 49.52[b]±0.40 | 51.59[b]±0.25 |
| OM digestibility (%) | 46.0[a]±0.90 | 51.72[b]±0.52 | 2.24[c]±0.42 |
| Digestible OM intake (kg/d) | 1.41[a]±0.24 | 2.55[b]±0.41 | |
| Bacterial production rate | | | |
| Dose injected (μCi) | 6.68±0.44 | 6.30±0.46 | 6.73±0.46 |
| Specific radio activity at zero time** ($\times 10^{-4}$ μCi/mg bacterial cells) | 2.06[a]±0.25 | 0.69[b]±0.04 | 0.80[b]±0.04 |
| Rate constant of decline radio activity per min ($\times 10^{-4}$) | 16.74[a]±0.70 | 20.44[b]±0.67 | 20.59[b]±0.56 |
| Pool size (g) | 33.50[a]±3.50[a] | 91.56[b]±2.14 | 84.82[c]±2.09 |
| Production rate (g $h^{-1}$) | 3.34[a]±0.28 | 11.23[b]±0.43 | 10.46[c]±0.24 |
| Turnover time (h) | 10.01[a]±0.42[a] | 7.80[b]±0.21 | 8.12[c]±0.22 |
| Protozoa production rate | | | |
| Dose injected (μCi) | 0.99±0.03 | 1.13±0.05 | 1.13±0.09 |
| Specific radio activity at zero time (μCi/g N) | 0.88[a]±0.02 | 0.61[b]±0.06 | 0.85[b]±0.03 |
| Rate constant of decline radio activity per min ($\times 10^{-4}$) | 3.68[a]±0.53 | 11.35[b]±1.42 | 10.40[b]±0.56 |
| Pool size (g N) | 1.14[a]±0.03 | 1.89[b]±0.16 | 1.37[b]±0.16 |
| Production rate (g N $h^{-1}$) | 41.77[a]±6.01 | 209.27[b]±14.96 | 138.65[c]±13.13 |
| Turnover time (h) | 29.56[a]±5.49 | 9.17[b]±0.94 | 9.71[b]±0.54 |

$$X_{ij} = \mu+\gamma_i+e_{ij}$$

Where, $X_{ij}$ = observation variable corresponding to the observation coming from i th replication and j the main treatment, $\mu$ = mean of the variable of population of either bacteria or protozoa, $\gamma_i$ = random variable denotes effect of the i th level of variable and, $e_{ij}$ was error component.

The difference in AA composition of bacteria and protozoa were tested with Student *t*-test. Effect of incoculum, substrate and interaction between both the factors on gas composition and parameter estimates of the model were analysed using randomized complete block design for factor $\alpha$ (inoculum) at three levels with factor $\beta$ (substrate) a split plot on $\alpha$ with four levels using following model.

$$X_{ijk} = \mu+\gamma_i+\alpha_j+\beta_k+e_{ijk}$$

Where, $X_{ijk}$ = observation variable corresponding to the observation $X_{ijk}$ coming from the k th subplot treatment of the j main plot treatment in the i th replication, $\mu$ = general mean, $\gamma_i$, $\alpha_j$ and $\beta_k$ = the fixed effect of the i th replication, j th main treatment and k th subplot treatment respectively. $e_{ijk}$ is the error component that assumed to be independently and normally distributed with zero mean and constant variance. Correlation between pool size, production rate and turnover time of bacteria or protozoa and characteristics of the model were calculated along with significance test (Snedecor and Cochran, 1967; Das and Giri, 1991).

## RESULTS AND DISCUSSION

Ingredient and chemical composition of diets were presented in Table 1. Supplements in $D_2$ and $D_3$ were characteristically different in source of nitrogen in rumen. The significant difference between $D_2$ or $D_3$ with $D_1$ reflected the deficiencies in the straw when not supported with supplement. AA composition of rumen bacteria and protozoa isolates from 3 diets did not vary significantly (Table 2). The amino acid composition of bacteria and protozoa remained unaffected regardless of the source and level of N and OM intake suggesting that what was observed by others (Greife et al., 1985; Clark et al., 1992) hold good for straw and straw based diet as well. However, the microbial biomass synthesis efficiency in $D_2$ and $D_3$ diets were close to the lower range of values reported in the literature (Singh et al., 1974; ARC, 1980). The differences among diets in intake and source of N and OM affected bacterial and protozoal characteristics significantly ($p<0.01$) in the rumen (Table 3). Specific radioactivity at time zero was significantly ($p<0.01$) higher for $D_1$. Significant ($p<0.01$) increase in bacterial and protozoal pool size was noticed in $D_2$ and $D_3$. Bacterial and protozoal pool size in

**Table 4.** Parameter estimates from the model

| Source of rumen inoculum | Type of straw | Y | Yα | K ($\times 10^{-2}$) | Lag time λ (h) | $CO_2$:$CH_4$ |
|---|---|---|---|---|---|---|
| $D_1$ | WS | 58.0 | 76.25 | 3.30 | 4.4 | 54.6:45.4 |
| | PS | 62.1 | 93.00 | 2.58 | 5.2 | 55.6:44.4 |
| | $WS_U$ | 83.0 | 108.58 | 3.29 | 4.0 | 58.6:41.4 |
| | $PS_U$ | 85.6 | 105.27 | 3.73 | 3.0 | 58.9:41.1 |
| $D_2$ | WS | 83.6 | 128.50 | 2.22 | 1.2 | 58.1:41.9 |
| | PS | 89.0 | 126.44 | 2.59 | 1.0 | 59.1:40.9 |
| | $WS_U$ | 116.6 | 145.06 | 3.44 | 0.6 | 61.1:38.9 |
| | $PS_U$ | 122.8 | 151.93 | 3.35 | 0.8 | 62.2:37.2 |
| $D_3$ | WS | 87.8 | 120.47 | 2.94 | 3.6 | 58.8:41.2 |
| | PS | 86.2 | 116.77 | 2.91 | 1.9 | 58.9:41.1 |
| | $WS_U$ | 113.6 | 151.48 | 3.23 | 3.0 | 62.1:37.9 |
| | $PS_U$ | 122.9 | 158.52 | 3.19 | 1.2 | 62.5:37.5 |
| $SE_m$ | Straw | 0.70** | 1.12** | 0.03** | 0.06** | 0.27** |
| | Diet | 0.60** | 0.92** | 0.03** | 0.05** | 0.24** |
| | Interaction | 1.21** | 1.94** | 0.06** | 0.10** | 0.47 |
| LSD | Straw | 1.03 | 2.68 | 0.07 | 0.14 | 0.40 |
| | Diet | 1.18 | 2.32 | 0.07 | 0.12 | 0.46 |
| | Interaction | 2.05 | 4.65 | 0.14 | 0.24 | NS |

** $p<0.01$, * $p<0.05$, NS = Non significant.
WS = Wheat straw, PS = Paddy straw, $WS_U$ = Urea treated wheat straw, $PS_U$ = Urea treated paddy straw.

$D_2$ was 9 and 28% higher than in $D_3$, respectively. BPR (g $h^{-1}$) and PPR (mg N $h^{-1}$) were lower in $D_1$ than in either $D_2$ or $D_3$. BPR and PPR were increased by 68-71 and 70-80%, respectively in the two groups. Turnover time of bacteria was decreased ($p<0.01$) by about 2 h and that of protozoa by about 20 h when animals were fed supplements along with basal roughage.

The bacterial and protozoal production efficiency (g N/ kg DOMI) with $D_1$, $D_2$ and $D_3$ were 5.60, 0.71, 10.57, 1.97 and, 11.21, 1.49, respectively. The differences between $D_1$ and $D_2$ or $D_3$ in bacteria and protozoal N is explainable by the difference in intake of N and OM whereas the differences between $D_2$ and $D_3$ in protozoal N is perhaps due to the source of N and OM. $D_2$ supplied more RDN in the form of proteins and DOM in the form of starch. While AA and peptides are known to stimulate bacterial synthesis (Orskove and Miller, 1988; Broderick et al., 1991), starch is reported to increase protozoal population (Leng, 1982; Williams and Coleman, 1988). In this experiment, assuming rumen degradability of OM as 0.65 (ARC, 1980) and N content of the microbial biomass as 0.1 of the dry matter (Vansoest, 1994), microbial biomass yield with $D_1$, $D_2$ and $D_3$ are 97, 192.0 and 195.4 g per kg of rumen digested organic matter. Blummel et al. (1997) reported a microbial biomass yield of about 40% of the rumen digested organic matter. Although reasons for low efficiency can not be explained from this experiment, identification of factors to improve this efficiency with straw based diets can have useful application in practical feeding situations with cereal crop residues.

The gas production parameters (Y∞, k and λ) obtained from the fitted model are presented in Table 4. Straw, diet and their interaction had significant ($p<0.01$) effect on all parameters tested. This indicated that no difference in the AA composition of bacteria or protozoa could reduce the variation in the kinetic parameters but quantitative difference in their pools had significant impact. Influence of concentrate and UMMB licks supplementation in general on nutrient intake and rumen fermentation was significant and thoroughly investigated (Thu and Uden, 2001; Mirza et al., 2002; Hosamani et al., 2003). The Yα of both untreated and urea treated straw substrates on $D_1$ were 30% less compared to the other 2 diets. Carbon dioxide to methane also differed significantly ($p<0.01$) among straw substrates and diets but interaction effect was non-significant.

Among the gas production parameters Yα, lag time and proportion of methane in the total gas showed significant correlation with rumen microbial characteristics and k remained least influenced (Table 5). However, significant difference in k among the straw substrates indicated that it represents the physicochemical characteristics of the substrates as suggested by Vansoest (1994). Digestible OM intake was positively ($p<0.01$) related to bacterial and protozoal pool size and production rate. Yα had a higher correlation with bacterial pool size and production rate than with protozoal pool size and production rate. Although the bacterial pool size and production rate showed a high correlation with Yα, the increase in Yα in $D_2$ was only for untreated straw but not of treated straw when compared with $D_3$. The lag time had a higher negative correlation with bacterial pool size and a lower negative correlation with bacterial production rate, when compared to protozoal pool size and production rate respectively. While higher

**Table 5.** Correlation between microbial characteristics of the rumen inoculum and gas production kinetics *in vitro*

| Attribute | Digestible Om intake | Bacterial population | | | Protozoal population | | |
|---|---|---|---|---|---|---|---|
| | | Pool size | Production rate | Trunover time | Pool size | Production rate | Turnover time |
| Digestible OM intake | 1.00 | | | | | | |
| Bacterial pool size | 0.85** | 1.00 | | | | | |
| Production rate | 0.85** | 0.99** | 1.00 | | | | |
| Turnover time | -0.67** | -0.83** | -0.87** | 1.00 | | | |
| Protozoal pool size | 0.68** | 0.89** | 0.88** | -0.85** | 1.00 | | |
| Production rate | 0.76** | 0.89** | 0.88** | -0.84** | 1.00** | 1.00 | |
| Turnover time | -0.76** | 0.90** | -0.87** | 0.65** | -0.81** | -0.81** | 1.00 |
| Yα | 0.39** | 0.96** | 0.96** | -0.83 | 0.56 | 0.86** | -0.86** |
| K | -0.19 | -0.26 | -0.26 | 0.19** | -0.20** | -0.29 | 0.56 |
| Lag time | -0.66** | -0.79** | -0.61** | -0.03 | 0.24 | 0.22 | -0.30 |
| Methane (%) | -0.35* | -0.56** | -0.56** | 0.52** | -0.28* | -0.50** | 0.46 |

Significance of 'R' value; * $p<0.05$, ** $p<0.01$.

microbial biomass contributed to shorten lag time and increase c with treated straw. higher Yα was observed with $D_3$ instead of with $D_2$. This may be suggestive of the influence of microbial species distribution on fermentation of straw. Lag time in gas production also reported to be reduced ($p<0.05$) by application of surfactants on alfalfa. corn and orchard grass silages but not the cumulative gas production as observed in this study (Wang et al.. 2003). The soluble carbohydrates in UMMB may favour a different group of microbial species. which may adapt better to easily available soluble carbohydrate fragments in the treated straw (Russel. 1983; Hobson. 1988). Such advantage might be yielded higher cumulative gas production in $D_3$ than even $D_2$. A lower proportion of methane in total gas from incubation with treated straw can perhaps lend support to this explanation. The proportion of methane in the gas had a higher negative correlation with bacterial pool size than with protozoal pool size. An equation for Yα derived with diet ($X_1$) and straw ($X_2$) as independent variable was: $-25.06+1.00\ X_1+0.30\ X_2$, $R^2 = 0.55$. $R^{2'} = 0.52$, Multiple R = 0.74. F = 26.98. $F_{Prob} = 0.001$.

S.E. t and $t_{prob}$ for $X_1$ was 0.14. 7.06 and 0.001, respectively and for $X_2$ they were 0.15. 2.00 and 0.05, respectively. Correlation between Yα with straw type was 0.20 and much smaller than the value of diet that was 0.71. The quantity of microbial characteristics in the rumen contributes to reduction in lag time and increase digestion as revealed by Yα. About 71% of the variability associated with Yα could be explained by the diet signifying the influence of quantitative characteristics of rumen inoculum. Blummel et al. (1999) reported that while 68% of variability in straw intake could be explained by cumulative gas production. 11% of variability is explainable by efficiency of microbial biomass synthesis expressed in terms of partitioning factor. Therefore, the avenues to increase microbial biomass yield in rumen inoculum can have useful application in practical feeding systems. Since Yα reflects to a larger extent the quantitative rumen microbial characteristics. cumulative gas production measurements can be used as an indirect index of microbial biomass while assessing the effect of various supplements to straw diet.

## CONCLUSIONS

The effect of diet on the AA composition of rumen bacteria and protozoa population in rumen liquor appeared to be none. Quantitative aspects of bacterial and protozoal population. such as pool size. production rate and turnover time in rumen liquor responded positively to the supplements but variability between supplements was due to N source and digestible OM intake. As gauged by the exponential model. the greatest influence of dynamic characteristics of microbial population on substrate fermentation was on lag time and upper asymptote and methane percent in gas. Substrate fermentation in rumen was dependent more on quantitative characteristics of microbial population rather than on qualitative characteristics such as amino acid composition. Rate of gas production was less related to microbial characteristics but dependent on physico-chemical characteristics of substrate. Quantitative aspects of fermentation curve obtained by using gas production technique thus help in explaining some properties of dynamic characteristics of microbial population and feed attributes like physico-chemical nature of the fibre complex. As diet had a large influence on Yα and related to microbial characteristics. gas production studies may be extended to assess dynamic characteristics of microbial population on straw diets differing in supplements. Since microbial biomass determination *in vivo* is rather expensive. adoption of indirect techniques such as this can prove useful in preliminary comparison of the effect of various supplements on rumen microbial biomass.

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