Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 124–129

Effect of supplementing the *Dicanthium annulatum* hay diet with tree leaves on microbial activity in the rumen of goats

S. Singh[1], S.S. Kundu and L.K. Karnani

Plant Animal Relationship Division, Indian Grassland and Fodder Research Institute Jhnasi-284003 (UP), India

ABSTRACT

Four adult goats (mean body weight 29.0±2.65 kg) were fed *Dicanthium annulatum - Leucaena leucocephala* (DA-LL*)* and *Dicanthium annulatum - Hardwickia binat*a (DA-HB) in 50:50 ratio in switch-over experiment to assess the effect of tree leaves supplementation on rumen concentration of protozoa, their generic distribution, bacteria and fungi. Animals were maintained on each diet for more than 3 months and rumen liquor was collected at 0 and 4 h post feeding. Mean protozoa counts were 5.98 and 5.03×10^5/ml in goats supplemented with LL and HB, respectively. *Entodiniomopha* were the most dominant (88.0%) rumen protozoa on both diets. Total viable, amylolytic and cellulolytic bacteria population was higher ($P<0.05$) in rumen of goats fed DA-LL (23.74, 11.22 and 29.66) than DA-HB diet (10.52×10^8/ml, 6.31×10^8/ml and 11.02×10^7/ml), while the concentration of proteolytic bacteria was 8.64 and 6.23×10^7/ml in rumen of goats fed earlier and later diets, respectively. Mean sporangia counts were comparable in rumen liquor of goats fed both types of diets (0.31 vs 0.33×10^5/ml), while total fungi was higher ($P<0.05$) in goats fed DA-LL (9.62) than DA-HB (5.04×10^4/ml). On both diets bacteria population was higher at 0 h post feeding, while population of fungi was higher at 4 than 0 h of feeding. Results revealed that goats had higher activities of bacteria and fungi on LL supplemented diet.

KEY WORDS: tree leaves, goats, microbial activity, grass

INTRODUCTION

Browse species (tree and shrubs foliage) with grasses constitute a major component of pasture and grazing systems and provide a major part of cellulosic biomass to ruminants particularly to goats and sheep. Tree foliages usually rich

[1] Corresponding author: email: singh.sultan@rediffmail.com

in protein and minerals are used as dry season supplement to poor quality natural pasture, grazing land and /or poor quality crop residues (Devendra, 1981; Kibon and Ørskov, 1993). Presence of lignin and other anti-nutritional components mainly the tannins and phenolics hampers their use as animal feed. Tannins and phenolics had bacteriostatic and bactericidal effect on rumen microbes (Henis et al., 1964; Martin and Akin, 1988), fungi (Akin and Rigsby, 1987) and protozoa (Akin, 1982). Work with *Leucaena leucocephala* and *Hadwickia binata* tropical trees on rumen microbial population (bacteria, protozoa and fungi) seems to be obscure. The present work was carried out to assess relative effect of these tree leaves supplementation to *Dicanthium annulatum* grass hay on rumen microbial activity in goats.

MATERIAL AND METHODS

Animal feeding and rumen sampling

Four adult local male goats (mean body weight 29.0±2.65 kg) were maintained on *Dicanthium annulatum* (DA) - *Leucaena leucocephala* (LL) and *Dicanthium annulatum* (DA) - *Hardwickia binata* (HB) diets (50:50 ratios on % DM basis) for more than 3 months on each diet in switch-over experiment. Animals were offered half grass (conserved as hay) and tree leaves (collected from whole plant canopy and wilted for 24 h before feeding) together once at 9 a.m. and remaining half at 12.00 noon. The animals maintained hygienically had free access to clean drinking water. The chemical composition of grass (DA) and tree leaves (LL and HB) is presented in Table 1. After more than 1 month of feeding rumen liquor samples were collected at 1 month interval for 2 consecutive days from animals at 0 and 4 h post parandial to estimate protozoa counts, their generic distribution and fungal sporangia numbers. Total viable bacteria, amylolytic, cellulolytic and proteolytic bacteria, and total fungal population were ascertained too in fresh rumen liquor samples collected 1 month interval twice for individual animal on each diet.

Table. 1 Chemical composition of grass-tree leaves, % DM basis

Chemical constituents	*Dicanthium annulatum*	*Leucaena leucocephala*	*Hardwickia binata*
Crude protein	5.35	22.50	7.80
NDF	69.90	28.50	36.80
ADF	42.00	17.50	28.00
Cellulose	29.05	10.00	16.20
Hemicellulose	27.90	12.30	10.60
Lignin	6.50	7.20	9.75

Analysis

Grass and tree leaves samples were analysed for proximate and cell wall polysaccharide according to AOAC (1980) and Goering and Van Soest (1970). Method described by Moir (1951) was followed for counting the total protozoa in formaldehyde-conserved rumen liquor. For generic identification, morphological features of Kudo (1960), Hungate (1966) and Ogimoto and Imai (1981) were used. For culturing bacteria pre-reduced anaerobic dilution fluid was prepared as described by Bryant and Burkey (1953). For starch hydrolysing bacteria estimation 0.1 ml iodine-potassium iodide solution (1:2) in the tubes was added. For culturing cellulolytic bacteria Whatman No. 1 filter paper strip was used and media was prepared as described by Mann (1968). For enumerating proteolytic bacteria and anaerobic fungi composition of media described by Abou-Akkada and Blackburn (1963) and method of Theodorou and Trinci (1988), respectively, were followed. Most probable number of proteolytic bacteria was computed using McCrady table based on the positive tubes.

Data were analysed according to Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The results revealed that goats supplemented with LL tended to have higher numbers of protozoa (5.98) than those from HB supplementation (5.03×10^5/ml; Table 2). *Entodiniomorpha* were the most dominant (88%) protozoa in the

Table 2. Protozoa concentration in goat rumen fed grass-tree leaves diet

Protozoa number [1]	h	DA-LL	DA-HB
Total protozoa × 10^5/*ml*	0	6.21	4.50
	4	5.74	5.57
Mean		5.98	5.03
Entodiniomorpha	0	5.43	4.00
	4	5.09	4.97
Mean		5.26	4.48
Holotricha	0	0.78	0.45
	4	0.62	0.60
Mean		0.70	0.52

[1] each mean has 16 values

rumen liquor of goats on both diets. Mean counts of *Entodiniomorha* and *Holotricha* were 5.26 and 0.70 on DA-LL vs 4.48 and 0.52×10^5/ml on HB supplemented diet. Percent generic distribution of protozoa revealed that *Isotricha* and *Entodinium* were predominant species and constituted 50 and 80% of the *Holotricha* and

Entodiniomorpha population, respectively (Figure 1). *Blepharoprosthium* and *Ophryoscolex* occurrence was lowest in rumen liquor of goats both on LL (0.4 and 0.4%) and HB (0.5 and 1.1%) supplemented diets, respectively. Variability in protozoa counts in rumen of sheep supplemented with different multipurpose tree leaves has been reported by Odenyo et al. (1997). They further reported that *Entodiniomorpha* are most dominant (93.3%) on all the tree leaves-supplemented diets being in accordance with the present results where *Entodiniomorpha* constitute 88% of total protozoa numbers.

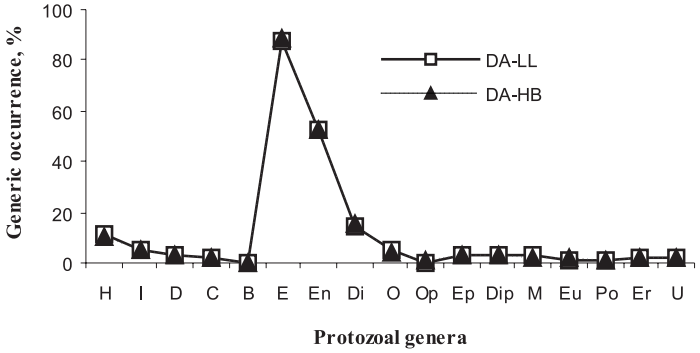

Figure 1. Generic distribution of protozoa in rumen of goats fed grass-tree leaves
H - *Holotricha*, I - *Isotricha*, D - *Dasytricha*, C - *Charon*, B - *Blepharoprosthium*, E - *Entodiniomorpha*, En - *Entodinium*, Di - *Diplodinium*, O - *Ostracodinium*, Op - *Ohryoscolex*, Ep - *Epidinium*, Dip - *Diploplastron*, M - *Metadinium*, Eu - *Eudiplodinium*, Po - *Polyplastron*, Er - *Eremoplastron*, U - unidentified

Mean total viable bacteria concentration was significantly ($P<0.05$) higher in rumen liquor of goats supplemented with LL (23.74) than with HB leaves (10.52×10^8/ml; Table 3). Similarly the mean activities of amylolytic, cellulolytic and proteolytic bacteria were significantly ($P<0.05$) higher in rumen of goats fed DA-LL (11.22, 29.66 and 8.64) than DA-HB based diet (6.31×10^8/ml, 11.02×10^7/ml and 6.23×10^7/ml, respectively). The higher bacterial activities in goats rumen on LL supplemented diet may be attributed to its higher CP content and degradability (Singh, 2004) resulting in optimum metabolites concentration for rumen environment conducive for multiplication and proliferation of bacteria.

Rumen liquor from goats fed diet supplemented with LL and HB had similar fungal sporangia counts (0.31 and 0.33 × 105/ml; Table 4). However, fungus activity (TFU) was significantly ($P<0.05$) higher in rumen liquor of goats fed LL (9.62) vs HB (5.04×104/ml) supplemented diet. The reason for higher fungal activity on LL supplemented diet is not known. However, higher mineral contents particularly of S in LL might be responsible for increased fungal activity in the rumen of goats.

Table 3. Bacterial activity in goat rumen fed grass-tree leaves diet

Bacteria population[1]	h	DA-LL	DA-HB
Total viable (× 10^8/ml)	0	26.03	11.39
	4	21.44	9.65
Mean		23.74[a]	10.52[b]
Amylolytic (× 10^8/ml)	0	11.27	6.22
	4	11.18	6.39
Mean		11.22[a]	6.31[b]
Cellulolytic (× 10^7/ml)	0	34.45	11.51
	4	24.87	10.53
Mean		29.66[a]	11.02[b]
Proteolytic (× 10^7/ml)	0	9.27	6.54
	4	8.01	5.92
Mean		8.64[a]	6.23[a]

[a,b] means within a row bearing different superscripts differ significantly at $P<0.05$
[1] each mean has 16 values

Table 4. Fungal sporangia counts and fungus activity in goat rumen fed grass-tree leaves diet

Fungus activity [1]	h	DA-LL	DA-HB
Sporangia count (× 10^5/ml)	0	0.31	0.29
	4	0.31	0.36
Mean		0.31	0.33
Fungi as TFU (× 10^4/ml)	0	8.39	3.90
	4	10.86	6.18
Mean		9.62	5.04

[1] each mean has 16 values

CONCLUSIONS

The results presented here indicate that activity of rumen microorganisms and particularly that of bacteria and fungi is higher in rumen of goats on *Leucaena lancocephala* supplemented diet and thus goats can be supplemented with up to 50% of total diet (dry matter basis) with *Leucaena lancocephala* for higher rumen microbial activity.

REFERENCES

AOAC, 1980. Association of Official Analytical Chemists, Official Methods of Analysis. 13th Edition. Washington, DC

Abou Akkada A.R., Blackbum T.H., 1963. Some observations on the nitrogen metabolism of rumen proteolytic bacteria. J. Gen. Microbiol. 31, 461-469

Akin D.E., 1982. Forage cell wall degradation and p-coumaric, ferulic and sinapic acids. Agron. J. 74, 424-428

Akin D.E., Rigsby L.L., 1987. Mixed fungal populations and lignocellulosic tissue degradation in the bovine rumen. Appl. Environ. Microbiol. 53, 1987-1995

Bryant M.P., Burkey L.A., 1953. Number of some predominant bacteria in the rumen of cows fed different rations. J. Dairy Sci. 36, 218-224

Devendra C., 1981. The utilization of forage from cassava pigeonpea, leucaena and groundnut by goats and sheep in Malaysia. In: Proceeding of the International Symposium on the Nutrition and System of Goat Feeding. ITOVIC-INRA. Tours (France) 1, 137-141

Goering H.K., Van Soest P.J., 1970. Forage Fiber Analysis. Agriculture Handbook No. 379. ARS-USDA. Washington, DC, pp. 20

Henis Y., Tagari H., Volcani R., 1964. Effect of water extract of carob pods tannic acid and their derivatives on the morphology and growth of microorganisms. Appl. Microbiol. 12, 204-211

Hungate R.E., 1966. The Rumen and its Microbes. Academic Press, London

Kibon A., Ørskov E.R., 1993. The use of degradation characteristics of browse plants to predict intake and digestibility by goats. Anim. Prod. 57, 247-251

Kudo R.R., 1960. Protozology. 4th Edition. Charles C. Thomas, Springfield, Il

Mann S.O., 1968. An improved method for determining cellulolytic activity in anaerobic bacteria. J. Appl. Bacteriol. 31, 241-244

Martin S.A., Akin D.E., 1988. Effect of phenolic monomers on the growth and β-glucosidase activity of Bacteriodes ruminicola and on carboxymethyl cellulase, β-glucosidae and xlanase activities of Bacteroides succinogenes. Appl. Environ. Microbiol. 54, 3019-3022

Moir R.J., 1951. The seasonal variation in the rumen microorganisms of grazing sheep. Aust. J. Agr. Res. 2, 322

Odenyo A.A., Osuji P.O., Karanfil O., 1997. Effect of multipurpose tree supplements on ruminal ciliate protozoa. Small Ruminant Res. 67, 169-180

Ogimoto K., Imai S., 1981. Atlas of Microbiology. Japan Scientific Societies Press, Tokyo

Singh S., 2004. Metabolic and rumen microbial activities in sheep and goats on grass-tree leaves/shrubs dietary regimens. Technical Bulletin, Indian Grassland and Fodder Research Institute, Jhansi (India)

Snedecor G.W., Cochran W.G., 1968. Statistical Methods. Oxford and IBH Publishing Company, Kolkatta (India)

Theodorou M.K., Trinci A.P.J., 1988. Procedure for the isolation and culture of anaerobic fungi. R.A. Leng, D.I. Demeyer (Editors). The Roles of Protozoa and Fungi. Penambul Books Armidale, NSW (Australia), pp. 145