RUMINAL FERMENTATION AND MICROBIAL NITROGEN SYNTHESIS IN BUFFALO FED FIBROUS FEEDS

Budi Santoso[1], Subur Priyono Sasmito Budhi[2],
Budi Prasetyo Widyobroto[2], Moehamad Soejono[2]

ABSTRACT

To investigate the ruminal fermentation status (N-NH_3, VFA, pH) and microbial nitrogen synthesis (allantoin and uric acid), three mature rumen cannulated buffaloes and six mature non rumen cannulated buffaloes were used respectively. All animals were fed peanut haulm (PH), king grass (KG) and corn stover (CS) *ad libitum* as sole feed. The data was analyzed using variance analysis and followed by Duncan's multiple range test. Ruminal fermentation data showed that ruminal N-NH_3 concentration during 24 h for PH was higher ($P<.01$) than that of CS and KG, of 18.89, 8.62, 5.18 mg/100 ml, respectively. Total VFA concentration during 24 h was not different among feed types, of 61.72, 55.44, 48.98 mmol/l for KG, CS and PH respectively. Ruminal pH was not influenced by feed types. Parameters collected on microbial nitrogen synthesis showed that allantoin and uric acid was not affected by feed types, on the other hand the total purine derivates of PH was higher ($P<.05$) than that of CS and KG, but there was not different between CS and KG. The results showed that purine derivates excretion has the tendency to be affected by DDMI or DOMR as showed by regression equation $Y=5.53 + 3.55X$ ($R^2=0.30$, $P<.05$) and $Y=6.13 + 5.51X$ ($R^2=0.31$, $P<.05$), respectively. The highest microbial nitrogen synthesis in the rumen was produced by PH followed by CS and KG.

Key words: Buffalo, Fermentation, Purine derivates, Microbial nitrogen, Fibrous feed

INTRODUCTION

Green feeds are the main feed for ruminant in the tropics, but green feeds in the tropics has low quality (Soetanto, 1984). On the other hand, the green feeds are very much fluctuated depend on the season, particularly in dry season, ruminant usually were fed with agricultural plant byproducts namely rice straws, corn stalks, cassava tops, sweet potato top, peanut vines and forage which usually called fibrous feeds.

In that case ruminant fed low quality feed, ruminal microbial protein is the main protein source for host animal, according to Merchen (1988) that one of protein entered through duodenum was originally from bacterial and protozoa protein, furthermore about 80 percent nitrogen amino acid from microbial were synthesized in the rumen could be absorbed by host animal (Ørskov, 1992).

The main product of structural carbohydrate fermented in the rumen are VFA, CH_4 and CO_2, meanwhile the protein will be hydrolyzed to ammonia as the end product (McDonald *et al.*, 1987). Furthermore the availability of energy as ATP, carbon chain and ammonia from those degradations will be used for microbial protein synthesis.

Several in vivo methods have been developed to estimate the microbial protein supply to the host animal such as a "protein free" purified diet, diamino pimelic acid (DAPA), amino acid profile in postruminal digesta, ^{35}S, ^{15}N and ^{32}P isotops. Those methods were not practicable in the field because there required rumen or duodenal cannulated animal (Ørskov, 1990).

[1] Faculty of Agriculture, Cenderawasih University, Indonesia
[2] Faculty of Animal Science, Gadjah Mada University, Indonesia

Now it has been developed the method to estimate the supply of the microbial protein by measuring urinary purine derivates (PD) excretion. According to Chen *et al* (1992), the absorption of microbial protein and nucleic acid are correlated, hence the amount of microbial protein absorbed can be estimated from urinary excretion of the purine derivates, on the otherhand Djouvinov and Todorov (1994) suggested that the purine derivates in the urine can be used for microbial protein estimation reaching the duodenum with relatively good accuracy.

The aim of this research was to determine the rumen fermentation status (N-NH_3, VFA, pH), purine derivates excretion and estimation of microbial synthesis in the rumen of buffalo fed fibrous feeds as sole feed.

MATERIALS AND METHODS

Animals and Feeding.

In this experiments were used nine female buffaloes, weighed 235,58 ± 21,98 kg randomly were divided into two groups. The first group consisted of three rumen fistulated buffaloes were used to determine rumen fermentation status (N-NH_3, VFA and pH) and the second group consisted of six non rumen fistulated buffaloes were used to determine purine derivates excretion, estimation of microbial nitrogen synthesis in the rumen.

The buffaloes were fed peanut haulm (PH), king grass (KG) and corn stove (CS) *ad libitum* as sole feed. The feeds were fed twice daily (08.00 and 16.00). The experiment was carried out in 3 period, and each period consisted of 2 weeks adaptation followed by 1 day collection for the measuring of rumen fermentation, while 3 weeks adaptation and 8 days collection for measuring purine derivates excretion and estimation of microbial nitrogen synthesis. Buffaloes were weighed at before and after collection pHase.

Sampling and analysis

For 1 day in each experimental period, 300 ml ruminal fluid was collected for each buffalo via the cannula at 0, 1, 2, 3, 4, 6, 8 h post feeding. The pH was then measured thereafter by pH meter WTW 320, and then strained through two layers of cheesecloth. Five ml of 20 % NaCl was added to 5 ml ruminal fluid for N-NH_3 analysis by method of Conway (1962) and 1 ml of $HgCl_2$-H_3PO_4 was added to 10 ml ruminal fluid for VFA analysis by gas chromatograpHy.

Urine collection was carried out using harnesses during 8 consecutive days. Urine was collected every 4 hours into 20 l of container in which 40 ml of 10 % H_2SO_4 was added. After that it was weighed and 2 % collected for samples. Samples were frozen and stored at –20 °C for uric acid analysis by Fujihara method (1987); allantoin analysis by Young and Conway method (1942) and gross energy by bomb calorimeter.

Feed, feed refusal and feces samples were taken 400 g, 10 % and 2 % respectively for DM, OM, CP analysis (AOAC, 1975); NDF, ADF and lignin (Van Soest and Goering, 1970); gross energy by bomb calorimeter.

Calculation and statistical analysis

In this experiment, the estimation of rumen microbial nitrogen synthesis used two methods, as follow:

1. The microbial purine absorbed (X, mmol/day) was calculated on the basis of purine derivates excretion (Y, mmol/day), (Chen *et al.*, 1992) as follows:

$$Y = 0.85\ X + 0.385\ W^{0.75}$$

where 0.85 is the recovery of absorbed purines as urinary purine derivates and 0.385 $W^{0.75}$ represents the endogenous contribution to purine excretion. The X values, calculated for each buffalo were converted to microbial nitrogen supply (EMNS) as follow:

$$EMNS\ (g/day) = (70\ X) / (0.83 \times 0.116 \times 1000)$$

where : 70 = N content of purines; 0.83 = digestibility of microbial protein and 0.116 = ratio of purine N to total microbial N.

The estimation of microbial synthesized in the rumen (EMNR) was expressed as grams of microbial N per kilogram of digestible OM apparently digested in the rumen (DOMR) by

assuming that 65 % of digestible OM intake (DOMI) was fermented.

2. Microbial N yield was calculated by dividing allantoin N excretion rate (coefficient b) from regression of allantoin excretion (Y, g N/day) and DDMI (X, kg/day) by 0.045 (Rys *et al.*, 1975 cited Liang *et al.*, 1994). Furthermore this value was converted to MJ^{-1} ME intake (Liang *et al.*, 1994).

Data for $N\text{-}NH_3$, VFA and pH were obtained using split block design (Little and Hills, 1979) where feed types were regarded as whole plot and time post feeding as subplot. Data for purine derivates excretion, EMNS and EMNR were obtained from complete block cross over design (Astuti, 1981). The relationship between purine derivates and DDMI or DOMI was analyzed by simple linear regression. All data were analyzed using the General Linear Models procedure of the SAS (SAS, 1982).

RESULTS AND DISCUSSION

Ruminal $N\text{-}NH_3$ kinetic in buffaloes fed fibrous feeds is presented in Table 1.

Based on Table 1, $N\text{-}NH_3$ concentration were affected ($P<.01$) or ($P<.05$) by feed types at every sampling time. Nitrogen ammonia concentration for pH was higher than that of CS and KG, because crude protein content and organic matter intake of pH was higher than that of KG and CS (13.87, 9.25, 8.61% for crude protein and 61.22, 49.52, 50.89 $g/BW^{0.75}$ for organic matter intake).

Highest ruminal $N\text{-}NH_3$ concentration reached at 0 h for pH, 1 h for CS and 2 h for KG respectively due to higher degradation rate of pH than CS and KG. Average ruminal $N\text{-}NH_3$ concentration during 24 h for all feed types were 18.89, 8.63, 5.18 mg/100 ml for pH, CS and KG, and still in the above minimal concentration requirement for microbial growth (2–5 mg/100 ml) (Slyter and Satter, 1974).

The effects of feed types on ruminal acetate proportion (% total) are shown in Table 2.

Table 2 showed that feed types were significant affect ($P<.05$) on acetate proportion at 2 h post feeding. Acetate proportion of PH was higher ($P<.05$) than that of KG and CS. This result was supported by NDF and ADF degradation (*in sacco*) during 2, 4 and 8 h were 19.70, 24.36, 35.52% vs. 6.80, 11.86, 19.86% vs. 9.04, 12,32, 19,03% for PH, KG and CS respectively (Budhi *et al.*, 1998). Carey *et al* (1993) suggested that acetate concentration in the rumen associated with digestible fiber. On the other hand, highest acetate

Table 1. Effect of feed types on ruminal $N\text{-}NH_3$ concentration (mmol/100 ml) at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	27.25[c]	6.11[d]	9.45[d]	1.099	••
1	27.11[c]	10.75[d]	12.43[d]	1.319	••
2	23.00[e]	13.17[f]	12.29[f]	1.622	•
3	22.86[e]	10.95[f]	9.57[f]	1.880	•
4	21.80[e]	8.20[f]	8.87[f]	2.396	•
6	20.60[e]	4.78[f]	10.01[f]	2.710	•
8	18.48[e]	3.56[f]	7.09[f]	2.577	•

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]• $P<.05$; •• $P<.01$
[c,d]Means in the same row with different superscripts differ significantly ($P<.01$)
[e,f]Means in the same row with different superscripts differ significantly ($P<.05$)

Table 2. Effect of feed types on ruminal acetate proportion (% total) at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	67.30	61.69	63.40	1.548	NS
1	65.60	61.46	62.22	1.672	NS
2	65.77[d]	59.23[e]	61.64[e]	0.928	*
3	66.11	60.43	60.00	1.766	NS
4	65.49	60.73	61.12	1.423	NS
6	64.74	60.07	59.95	2.298	NS
8	65.05	61.25	59.71	2.029	NS

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]* P<.05; NS = non significant
[d,e]Means in the same row with different superscripts differ significantly (P<.05)

concentration of pH was supported by cellulose content, as mentioned Dijkstra (1994) that fermentation of cellulose would produced high amount of acetate. Average acetate proportion during 24 h were 65.91, 61.25, 59.71% for pH, KG, CS, respectively. Ruminal propionate proportions in buffaloes fed fibrous feeds are presented in Table 3.

Table 3 showed that feed types were significant affect (P<.05) on propionate proportion only at 6 h post feeding. Propionate proportion of CS was higher (P<.05) than that of PH and KG, but between KG and PH were not different. This result was supported by NFE content of 47.93, 47.04, 44.01 % for CS, PH, KG respectively. McDonald (1987) reported that NFE fraction are sugar, fructans, starch, and organic acids where according to MurpHy (1984) cited Dijkstra (1988) said that fermentation of soluble carbohydrate in the rumen yielded high amount propionate. Average propionate proportions during 24 h were 23.47, 21.23, 19.88% for CS, KG, pH, respectively.

Ruminal butyrate proportions in buffaloes fed fibrous feeds are shown in Table 4.

Table 3. Effect of feed types on ruminal propionate proportion (% total) at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	19.01	21.04	21.86	0.828	NS
1	20.57	21.51	23.19	1.179	NS
2	20.72	22.00	23.19	1.248	NS
3	20.00	21.86	24.28	1.341	NS
4	20.48	21.51	23.76	0.994	NS
6	20.24[e]	21.55[de]	24.71[d]	0.871	*
8	20.46	21.55	24.57	1.002	NS

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]* P<.05; NS = non significant
[d,e]Means in the same row with different superscripts differ significantly (P<.05)

Table 4. Effect of feed types on ruminal butyrate proportion (% total) at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	8.22	12.27	9.74	1.090	NS
1	8.82	12.04	9.58	1.552	NS
2	8.51[e]	13.83[d]	10.17[e]	0.761	*
3	8.69	12.71	10.72	1.167	NS
4	9.02	12.76	10.12	1.116	NS
6	10.01	13.38	10.34	1.604	NS
8	9.72	12.20	10.72	1.343	NS

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]* P<.05; NS = non significant
[d,e]Means in the same row with different superscripts differ significantly (P< .05)

Table 4 showed that butyrate proportion were affected (P<.05) by feed types at 2 h post feeding. Butyrate proportion of KG was higher (P<.05) than that of CS and pH. This results was indicated figured by hemicellulose content were 32.64, 32.28, 21.08% for CS, KG, pH respectively. Finding of MurpHy (1984) cited Dijkstra (1994) showed that hemicellulose is one of carbohydrate substrate has high contributed to butyrate production.

Average butyrate proportions during 24 h were 12.90, 10.37, 9.21% for pH, KG and CS respectively.

Ruminal total VFA kinetic of buffaloes were fed fibrous feeds are presented in Table 5. Ruminal pH was not affected by feed types and time post feeding. Average ruminal pH during 24 h were 6.36, 6.29, 6.35 for PH, KG and CS respectively. Those results were still out of range of pH in which the growth of cellulolytic microor-ganism can be inhibited 6.2 ± 0.5 (Van Soest, 1994). Highest ruminal pH for PH because it has total VFA concentration was lower than that of KG and CS (Table 5).

Table 5. Effect of feed types on ruminal total VFA (mmol/l) at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	57.24[e]	45.55[e]	77.73[d]	4.755	*
1	57.21	61.34	75.80	6.593	NS
2	63.70	71.10	73.20	2.550	NS
3	62.99	70.26	74.58	7.663	NS
4	65.80	67.58	73.25	6.919	NS
6	53.17	57.50	68.59	9.492	NS
8	52.52	66.23	69.31	7.834	NS

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]* P<.01; NS = non significant
[d,e]Means in the same row with different superscripts differ significantly (P< .05)

Table 6. Effect of feed types on ruminal pH at various times post feeding

Time post feeding (h)	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
0	7.21	6.69	6.69	0.196	NS
1	7.12	6.64	6.61	0.150	NS
2	6.76	6.56	6.63	0.155	NS
3	6.67	6.62	6.63	0.178	NS
4	6.64	6.46	6.63	0.177	NS
6	6.47	6.44	6.58	0.188	NS
8	6.44	6.38	6.45	0.188	NS

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]NS = non significant

Purine derivates excretions in buffaloes fed fibrous feeds are shown in Table 7.

Table 7 showed that feed types was not different for N allantoin, allantoin and uric acid excretion but it was different (P<.05) for total purine derivate excretion. Total PD excretions of pH were higher (P<.05) than that of CS and KG, but between both treatments were not different. The allantoin contributions of the total purine were 78.11 %, 78.62 % and 78.03 % for KG, PH and CS respectively. These results were close those obtained by Liang *et al.* (1994): 75%. The rates of allantoin excretion in the present study were higher than the values have been reported by Varcoe (1975); Liang *et al.* (1994) were 0.37 and 0.59 g N day^{-1} respectively.The relationship between N allantoin excretion and digestible dry matter intake was Y = 0.16 + 0.18 X (R^2 = 0,28, P<.05). The uric acid contribution of the total purine between 21.39% and 21.92%. In this experiment, total PD excretion was tend to affected by DDMI and DOMR as seen in regression equation Y = 6,10 + 3,35X (R^2 = 0,30, P<.05) and Y= 6,60 + 5,24X (R^2 = 0,31, P<.05), respectively.

Estimate of microbial nitrogen supply and estimate of microbial nitrogen synthesized in the rumen in buffalo fed fibrous feeds are presented in Table 8.

In this experiment EMNS and EMNR values were negative. Table 8 showed that EMNS for pH was higher than that of KG and CS, meanwhile EMNR for pH was higher than CS and KG. Because the EMNS and EMNR have negative value, hence

Table 7. Effect of feed types on allantoin, uric acid and total purine derivates (PD) excretion

Variables	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
N allantoin (g/d)	0.81	0.59	0,62	0.061	NS
Allantoin (mmol/d)	14.49	10.59	11.02	1.102	NS
Uric acid (mmol/d)	3.94	3.01	3.16	0.428	NS
Total PD (mmol/d)	18.43[d]	13.51[e]	14.17[e]	1.167	*

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]* P<.05; NS = non significant
[d,e,f]Means in the same row with different superscripts differ significantly (P<.05)

Table 8. Effect of feed types on digestible dry matter intake (DDMI), digestibleorganic matter intake in the rumen (DOMR), estimate of microbial nitrogensupply (EMNS), estimate of microbial nitrogen synthesized in the rumen (EMNR)

Variables	Feed types[a]			SE[b]	Level of significance[c]
	PH	KG	CS		
DDMI (kg/d)	3.40[d]	2.20[f]	2.70[e]	0.102	**
DOMR (kg/d)	2.10[d]	1.44[f]	1.62[e]	0.193	**
EMNS (g/d)[g]	-7.99	-4.56	-8.45	-	.
EMNR (g/kg)[g]	-6.17	-2.18	-5.14	-	.

[a]PH = peanut haulm; KG = king grass; CS = corn stover.
[b]SE = standard error
[c]** P < .01
[d,e,f]Means in the same row with different superscripts differ significantly (P<.05)
[g]Calculated based on Chen *et al.* (1992)

researchers calculated the microbial N synthesis in the rumen using the relationship between DDMI and N allantoin excretion as mentioned by Liang *et al.*, 1994. The result showed that microbial N yield were 8.40, 6.20, 4.20 g/kg DDMI for pH, CS and KG respectively. The conversion of microbial N on ME intake were 0.49, 0.31, 0.27 g/MJ. Those results were lower than the value of reported Liang *et al.*, (1994) that 0.8 g/MJ ME intake.

CONCLUSION

The buffalo fed fibrous feeds had a good ruminal condition for microbial growth. Peanut haulm was the best fibrous feed for microbial N synthesis, hence it's EMNR was higher than that of corn stover and king grass.

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