Effects of Protein and Carbohydrate Supplementations on Fibre Digestion and Microbial Population of Sheep

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ABSTRACT : The effects of two types of protein, soybean meal (SBM) and fish meal (FM); and two types of energy supplements, corn flour (CF) and paper pulp (PP), on intake of guinea grass (*Panicum maximum*), fibre digestion and microbial activities in four Merino rams with an average weight of 54.4 ± 4.5 kg were studied. Each animal was fitted with a ruminal cannula and a duodenal cannula at the proximal position. The animals were fed twice daily with chopped guinea grass (5 cm) *ad libitum* and one of the four dietary supplements: 170 g FM+268 g PP; 170 g FM+268 g CF; 200 g SBM+ 200 g PP or 200 g SBM+200 g CF. All the supplements were mixed with 100 g molasses. *In sacco* and *in vivo* digestibilities, digesta flow rates, fermentation and microbial population were studied in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of dietary treatments.

The effects of energy or protein sources were not significant on grass intake of sheep. The potential degradabilities of NDF and ADF were not significantly affected by any of the supplements. However, the energy and protein sources had significant efects on disappearance rate of NDF and ADF. The disappearance rate of both NDF and ADF were significantly ($p < 0.05$) higher in animals fed PP when compared to animals fed CF. Animals fed FM also showed significantly ($p < 0.03$) higher disappearance rate of ADF than those fed SBM. Animals fed PP showed better digestion in the rumen and total tract. Total flow of NDF and ADF through the duodenum was not significantly affected by the various supplements.

The mean rumen pH values (5.8-6.1) were not significantly different among the four different diets. The concentration of rumen ammonia was significantly ($p < 0.0001$) higher in animals fed SBM (235-266.4 mg N/L) supplement than in animals fed FM (174.9-179.7 mg N/L), while total VFA concentration was not significantly affected by both energy and protein supplements. Mean values of total VFA ranged from 72.5-82.3 mM. Molar proportions of acetate, propionate and butyrate were typical of a roughage type fermentation. Molar proportion of acetate was significantly ($p < 0.0001$) higher in sheep fed PP when compared to sheep fed CF. Animals fed FM had higher total viable bacterial counts, while animals fed CF showed higher protozoal numbers. Proportions of cellulolytic bacteria were only slightly higher in animals fed SBM or PP.

(**Key Words:** Fibre Digestion, Energy and Protein Supplementations)

INTRODUCTION

The high fibre and low nitrogen (N) content of tropical grass are the main factors affecting digestibility and feed intake of animals. When the N content of feed is less than 1%, the animal's appetite will be depressed and voluntary intake reduced (Minson, 1990). Ruminants when fed with low-N forages or straw-based diets with continuous supplementation of urea show an increase in feed intake, digestibility and protein in the rumen (Preston and Leng, 1987). However, true protein supplementation was found to be more effective than non-protein supplementation. The rate of fibre digestion in animals supplemented with fish meal is higher than those supplemented with urea (McAllan and Smith, 1984). In energy-deficit diets, additional energy input is necessary to optimise rumen protein synthesis (Poppi and McLennan, 1995). Readily fermentable carbohydrate supplement also enhances cellulolytic activity in the

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rumen (William et al., 1984). However, high amount of soluble carbohydrate in diets may reduce both voluntary intakes of forages and fibre digestion (Khalili, 1993).

The objective of this study was to determine the effects of two types of protein (soybean meal and fish meal) and two types of energy (corn flour and paper pulp) supplements with varying degrees of degradability on fibre digestion and microbial activities.

MATERIALS AND METHODS

Animals

Four Merino rams with an average weight of 54.4 ± 4.5 kg were each fitted with a ruminal and duodenal cannula at the proximal position (4 cm posterior to the pylorous duodenum, Ivan and Johnston, 1981). The animals were dosed one month before surgery to kill gastrointestinal parasites and were used five weeks after surgery.

Experimental design and diets

The animals were fed twice daily with chopped (5 cm) guinea grass (*Panicum maximum*) *ad libitum* and four dietary supplements. The supplements were two types of protein (soybean meal and fish meal) and two types of energy (corn flour and paper pulp). The supplements offered per day per animal are presented in table 1.

Table 1. Supplements offered (g/d/animal)

Diet	Fish meal (FM)	Soybean meal (SBM)	Paper pulp (PP)	Corn flour (CF)	Molasses
1	170	0	268	0	100
2	170	0	0	268	100
3	0	200	200	0	100
4	0	200	0	200	100

Each supplement was formulated to contain the same amount of N and gross energy but with varying rates of rumen degradability. Corn flour (CF) was purchased from Glow San Sdn Bhd, Selangor, Malaysia. Paper pulp (PP) boards made from softwood (Scott Paper Sdn. Bhd.) were cut into small pieces (approximately 0.5 × 0.5 cm) by using an electric plane. Fish meal (FM) and soybean meal (SBM) were purchased from Mansura Trading, Selangor, Malaysia.

The experimental design was 4 × 4 Latin square with a 2 × 2 factorial arrangement of dietary treatments. The effects of protein and energy supplements on fibre digestion (*in sacco* and *in vivo*), digesta flow rates, fermentation pattern and microbial population were studied within four periods. Each period (31 days) consisted of 14 days for dietary adaptation and 17 days for experimentation. During the experimentation period, the animals were placed in individual crates fitted with containers for urine and faecal collections.

In sacco and *in vivo* digestibilities, fibre intake and digesta flow

In sacco degradability of guinea grass was determined by the nylon bag technique (Ørskov and McDonald, 1979). The nylon bags (9 cm × 12 cm, 44 μm mesh size) containing 5 g of fresh guinea grass, approximately 2.5 mm in lengths, were incubated in the rumen for 12, 24, 48, and 72 h. Percentage losses of ADF and NDF were plotted against incubation times, and by using the equation $p = a + b\ (1\text{-}e^{-ct})$ of Ørskov and McDonald (1979), the potential degradability (p) and the degradation rate (c) of NDF and ADF were estimated by the Gauss-Newton iterative procedure (nonlinear) in the SAS programme (1988).

The amounts of feed intake, and faeces and urine outputs were recorded twice daily for nine days for the *in vivo* digestibility study. Ten percent representatives of the supplements and fresh guinea grass offered and 15% representatives of guinea grass refusal and faecal outputs were collected and stored at −20℃ until used for analyses. There were no refusals for supplements. For analyses, the samples were dried at 60℃ for 72 h to determine their moisture content. They were then ground through a 2-mm screen to be used for neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses (Goering and Van Soest, 1970). The total tract digestibility, true and apparent digestibility of fibre in the rumen were calculated by using equations developed by Czerkawski (1986):

$$\text{Total tract fibre digestibility (\%)} = \frac{100 \times [\text{Intake fibre (g/d)} - \text{Faecal Fibre Output (g/d)}]}{\text{Intake fibre (g/d)}}$$

$$\text{Fibre digestion in the rumen} = \text{Intake fibre} - \text{Undigested fibre}$$

The digesta flow was estimated by the double marker technique of Faichney (1980). Chromium (Cr)-mordanted paper pulp (particulate marker) and cobalt (Co)-EDTA (liquid marker) were prepared according to the method described by Uden et al. (1980). Chromium-mordanted paper pulp (10 g/d/animal) was mixed with the supplement and fed to the animals for 11 days, while Co-EDTA (180 mg Co/d) was infused at 5 ml/h at the same time. Duodenal samples were collected during the last two days of markers administration. One hundred ml of duodenal digesta were collected from the cannula by letting the digesta to flow out (by gravity) through a plastic tube into a container at the following times of the day: 24:00, 06:00, 12:00, 18:00, and at 03:00, 09:00, 15:00 and 20:00 the next day. The samples were stored at −20℃ and later pooled. A total of 16 samples (4 animals, 4 periods) was obtained Each pooled sample was homogenized and then divided equally into two portions. One portion was used as a representative of pooled whole duodenal digesta phase, while the other portion was centrifuged at 1,000 g for 5 min, and the pellet obtained represented the pooled particulate duodenal digesta phase. Both phase samples were lyophilized and analysed for NDF, ADF (Goering and Van Soest, 1970), Cr and Co (Le Du and Penning, 1982). The computer programme developed by Nolan (pers. com.) based on the double marker method of Faichney (1980; 1993) was used for calculating the true fibre flow through the duodenum.

Fermentation pattern and bacterial population

Representative samples of rumen contents were obtained before feeding and at 3, 6 and 9 h after the onset of feeding. The samples were determined for pH, fixed with methyl green formalin-saline for protozoal counts (Ogimoto and Imai, 1981) and the rest acidifed with 24% metaphosphoric acid in 12 M sulphuric acid for ammonia and total volatile fatty acids (VFA) analyses (Abdullah, et al., 1995). Protozoal numbers were estimated by counting in the Neubauer Counting Chamber under the light microscope (Laborlux S, Leitzwetzlar, Germany).

For viable and cellulolytic bacterial counts, rumen contents were sampled once at 3 h after the morning feed. The anaerobic roll tube technique was used to determine total viable bacteria, while the most probable number (MPN) method was used to estimate total cellulolytic bacteria (Bryant and Burkey, 1953; Scott and Dehority, 1965). The percentage of cellulolytic bacteria was calculated by comparing the total cellulolytic bacterial population with the total viable bacterial population.

Statistical analyses

The means of each parameter measured in the degradability studies, fibre intake and flow and the microbial populations were analysed by analysis of variance (ANOVA) techniques according to the General Linear Model (GLM) procedures of the Statistical Analysis System Institute (SAS, 1988). Treatment means were compared by the least significant difference method.

The 2 × 2 factorial type of diets was used to examine diet effect, which was divided into the main effect of protein and energy sources and their interactions. According to the GLM procedures of SAS, the single degree of freedom orthogonal comparisons were 1) FM versus SBM, 2) PP versus CF and 3) the interaction of protein and energy sources. The interaction, when significant ($p < 0.05$) will be shown in the results.

Rumen fluid data (pH, ammonia-N and VFA) were analysed by split-plot analyses of variance (Snedecor and Cochran, 1967) using following the model:

$$Yijklm = \mu + Ai + Pj + Tk + eijk + Hi + (AH)il + (PH)jl + (TH)kl + eijklm$$

Where: μ is mean of A, P, T and H (animal, period, treatment and time effects, respectively); eijk is the main plot error; eijklm is the sub-plot error.

The main plot included dietary treatment period and animal effects: treatment × period × animal was used in the SAS (1988) model to calculate main-plot error, subplot error and was tested for time × treatment interaction. Time × treatment interaction when detected ($p < 0.05$) will be shown in the results.

RESULTS

Composition of feeds and diets

The chemical compositions of guinea grass, CF, PP, SBM and FM are presented in table 2. Guinea grass contained 1.7% N (10.4% CP). The energy supplements, CF and PP contained 85% starch and 92.9% NDF, respectively, but only trace amounts of N (< 0.1%). The protein supplements, SBM and FM contained 6.7 and 8.0 % N, soybean meal contained 35.6% NDF, respectively.

The amount of supplements (fresh weight) provided daily per animal is presented in table 3. The nitrogen contents (N) of the supplemental diets were quite similar. They were within the range of 12.7-13.1 g N/d. However, the starch content varied widely between the different diets, ranging from 0 in PP supplemented diet to 228.3 g in CF supplemented diet. Similarly, the NDF content also varied widely between the diets. It was high in PP (231.6-

Table 2. Chemical components of feeds (% based on DM basis)

Components	Guinea grass	Soybean meal	Fish meal	Paper pulp	Corn flour	Molasses
Dry matter[1]	91.9	95.2	93.2	92.8	88.9	63.1
Organic matter	93.4	93.3	63.3	99.7	99.9	91.6
Nitrogen	1.7	6.7	8.0	0.0	0.1	0.6
Neutral detergent fiber	73.6	33.6	0	92.9	0.3	0.9
Acid detergent fiber	42.2	11.3	0	83.7	0.1	0.1
Starch[2]	ND	ND	ND	ND	85.2	ND
Gross energy (joules/g)[3]	ND	18.0	14.8	15.0	14.4	8.5

ND : Not determined.
[1] : Based on DM at 60℃.
[2] : Starch was analysed by the method described by Southgate (1976).
[3] : Gross energy was determined by using the IKA-Bomb. Calorimeter System C 4000 Adiabatic (Germany).

Table 3. Amount of supplements (fresh weight) provided daily per animal

Component	Fish meal		Soybean meal	
	Paper pulp	Corn flour	Paper pulp	Corn flour
Amount of different constituents of supplements (composite) provided daily per animal (g)				
Dry matter	470.2	459.8	439.1	431.4
Organic matter	406.1	396.1	420.5	413.1
Nitrogen	13.0	12.7	13.1	12.8
NDF	231.6	1.3	236.9	65.1
ADF	208.2	0.3	176.9	21.8
Starch	0	228.3	0	170.4
Gross energy (joules/g)	15.7	15.7	16.9	17.0

236.9 g) diet but much lower in CF supplemented (1.3-65.1 g) diet. Dry matter of supplements was similar for all treatments (431.4-470.2 g), but OM of FM was slightly lower (396.1-406.1 g) than the OM of soybean meal (413.1-420.5 g).

In sacco and *in vivo* digestibilities and digesta flow

The results of the percentage losses and degradation parameters of ADF and NDF of guinea grass are presented in tables 4 and 5. At 12 h of incubation, percentage losses of ADF and NDF of guinea grass were significantly ($p < 0.008$) affected by energy sources. The percentage losses were highest in animals fed SBM+PP and lowest in animals fed SBM+CF. A significant ($p < 0.05$) interaction between protein and energy was observed at this incubation period. However, at 24, 48 and 72 h incubation, there were no significant differences in NDF and ADF percentage losses of guinea grass among the animals fed the different supplementary diets.

The potential degradability (p) of both NDF and ADF were not significantly different among sheep fed the various supplements (tables 4 and 5). However, the disappearance rate (c h^{-1}) of NDF and ADF was significantly ($p < 0.05$) affected by the energy sources. Animals supplemented with PP had higher rates than animals supplemented with CF. A significant ($p < 0.05$) interaction between protein and energy was observed for the rate of disappearance (c h^{-1}) for NDF and ADF (tables 4 and 5). The values were significantly ($p < 0.05$) higher in animals fed FM as the protein supplement (with both CF and PP) than those fed SBM with CF (tables 4 and 5).

Table 6 shows the intake, flow and digestion of NDF in sheep fed the various supplements. Total amount of NDF consumed by sheep tended ($p < 0.08$) to be affected by energy supplements as PP contained higher amount of fibre than CF. Sheep fed CF supplement had higher values of NDF flow through the duodenum, although the differences were not significant. However, digestion of NDF in the rumen and in the total tract were significantly

Table 4. Percentage disappearance and degradation parameters (p and c) of guinea grass NDF in sheep fed guinea grass as a basal diet with different protein and energy supplements

Items	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
Percentage disappearance at various incubation period (h)							
0	10.9	10.4	11.8	10.9	0.6	NS	NS
12	48.3^{b}*	48.0^{bc}	50.7^{a}	46.1^{c}	0.9	NS	0.008#
24	63.2	63.0	62.5	60.1	2.0	NS	NS
48	68.0	67.0	68.3	68.4	1.2	NS	NS
72	69.4	68.7	70.3	69.7	1.1	NS	NS
Degradation parameters							
p	69.5	68.6	69.6	69.4	1.5	NS	NS
c (h^{-1})	0.09^{a}*	0.1^{a}	0.1^{a}	0.08^{b}	0.004	NS	0.05#

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
p : Potential degradability.
c : Rate of disappearance.
* : Means in the same row with different superscripts are significantly different ($p < 0.05$).
[Means in the same row without superscripts are not significantly different ($p > 0.05$)].
: Interaction between protein and energy ($p < 0.05$).

Table 5. Percentage disappearance and degradation parameters (p and c) of guinea grass ADF in sheep fed guinea grass as a basal diet with different protein and energy supplements

Items	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
Percentage disappearance at various incubation period (h)							
0	7.0	6.6	6.3	6.6	0.4	NS	NS
12	44.9^{b}*	44.5^{bc}	47.4^{a}	42.5^{c}	0.9	NS	0.008#
24	55.0	54.9	54.2	51.2	2.5	NS	NS
48	60.7	59.4	61.0	61.1	1.5	NS	NS
72	62.8	61.9	63.8	63.1	1.4	NS	NS
Degradation parameters							
p	61.9	61.8	61.8	61.9	1.5	NS	NS
c (h^{-1})	0.1^{a}*	0.1^{a}	0.1^{a}	0.08^{b}	0.005	0.03	0.02#

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
p : Potential degradability.
c : Rate of disappearance.
* : Means in the same row with different superscripts are significantly different ($p < 0.05$).
[Means in the same row without superscripts are not significantly different ($p > 0.05$)].
: Interaction between protein and energy ($p < 0.05$).

($p < 0.04$, $p < 0.02$) higher in sheep fed PP supplement. Protein sources (SBM and FM) did not have a significant effect on the various paramenters measured.

The intake, flow and digestion of ADF in sheep fed the different supplementary diets are shown in table 7. Similar to NDF, PP supplementation also had a significant effect on ADF intake, the amount of ADF digested in the rumen and total tract digestibility. The other parameters measured were not significantly affected by the energy supplements. Protein supplements did not have any significant effect on any of the parameters measured. Although, the ADF flow through the duodenum was higher by about 12.5% in sheep fed FM than in those supplemented with SBM, the difference was not significant.

Table 6. Neutral detergent fibre (NDF) intake, digestion and flow of NDF from the rumen of sheep fed guinea grass as a basal diet with different protein and energy supplements

Parameters	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
NDF (supplement) (g/d)	231.6	1.3	236.9	65.1	–	–	–
NDF (guinea grass) (g/d)	437.9	496.1	461.5	464.9	110.8	NS	NS
Total NDF intake (g/d)	669.5	497.4	695.4	530.0	110.8	NS	0.08
Total NDF flow (g/d) at duodenum:	228.3	266.4	232.7	249.5	44.0	NS	NS
Faecal NDF out put (g/d)	218.3	241.5	216.9	230.6	35.5	NS	NS
NDF digested in the rumen (g/d)	441.1	247.6	462.8	278.3	102.7	NS	0.04
NDF digested in the rumen (%)	65.0	39.6	66.5	50.3	11.6	NS	0.05
Total tract NDF digestibility (%)	67.3^{a*}	47.6^{b}	69.1^{a}	55.4^{ab}	7.2	NS	0.02

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
* : Means in the same row with different superscripts are significantly different ($p < 0.05$).
[Means in the same row without superscripts are not significantly different ($p > 0.05$)].

Table 7. Acid detergent fibre (ADF) intake, digestion and flow of ADF from the rumen of sheep fed guinea grass as a basal diet with different protein and energy supplements

Parameters	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
ADF (supplement) (g/d)	208.2	0.3	176.9	21.8	–	–	–
ADF (guinea grass) (g/d)	243.0	282.3	260.4	271.6	66.6	NS	NS
Total ADF intake (g/d)	451.3^{a}	282.6^{b}	437.3^{ab}	293.4^{ab}	87.3	NS	0.02
Total ADF flow (g/d) at duodenum:	169.9	154.1	141.1	142.4	32.3	NS	NS
Faecal ADF out put (g/d)	146.9	158.5	143.9	152.3	24.6	NS	NS
ADF digested in the rumen (g/d)	281.4^{a*}	128.6^{b}	296.2^{a}	151.0^{ab}	62.3	NS	0.02
ADF digested in the rumen (%)	62.8^{ab}	34.0^{b}	67.7^{a}	51.0^{ab}	13.2	NS	0.08
Total tract ADF digestibility (%)	67.6^{a}	37.0^{b}	67.3^{a}	48.3^{ab}	8.6	NS	0.05

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
* : Means in the same row with different superscripts are significantly different ($p < 0.05$).
[Means in the same row without superscripts are not significantly different ($p > 0.05$)].

Fermentation pattern and microbial population

Rumen pH, ammonia and VFA concentrations and molar proportions were determined at various times before and after the onset of feeding. Rumen pHs were significantly affected ($p < 0.05$) by diets and times of sampling. The rumen pHs for all animals were low at 3 h after the onset of feeding (figure 1). When the values were compared for the effects of diets, the rumen pH for sheep supplemented with CF was significantly lower ($p < 0.05$) at 3 h after feeding and tended ($p < 0.08$) to be lower at 6 h after feeding (figure 1). However, when the means of rumen pH of the four dietary supplements were compared, no significant differences between the supplements were observed (table 8).

For all animals, the concentrations of ammonia-N reached a maximum at 3 h after feeding (figure 2). However, the ammonia-N concentrations were higher in animals fed SBM supplements at all sampling times. The mean values of ammonia-N concentrations were significantly ($p < 0.0001$) affected by protein sources (table 8). The concentrations of rumen ammonia-N were significantly higher in animals fed SBM supplement. On the other hand, energy sources did not have any significant effect on the mean ammonia-N concentration.

There were no significant interactions between diets and times of sampling in total VFA concentrations. Effects of diets on total VFA concentrations were also not significant. Total VFA ranged from 67-101 mM (figure 3). The lowest value (67 mM) was observed in animals fed FM+PP at 6 h after the onset of feeding. Molar proportions of acetate in sheep supplemented with PP

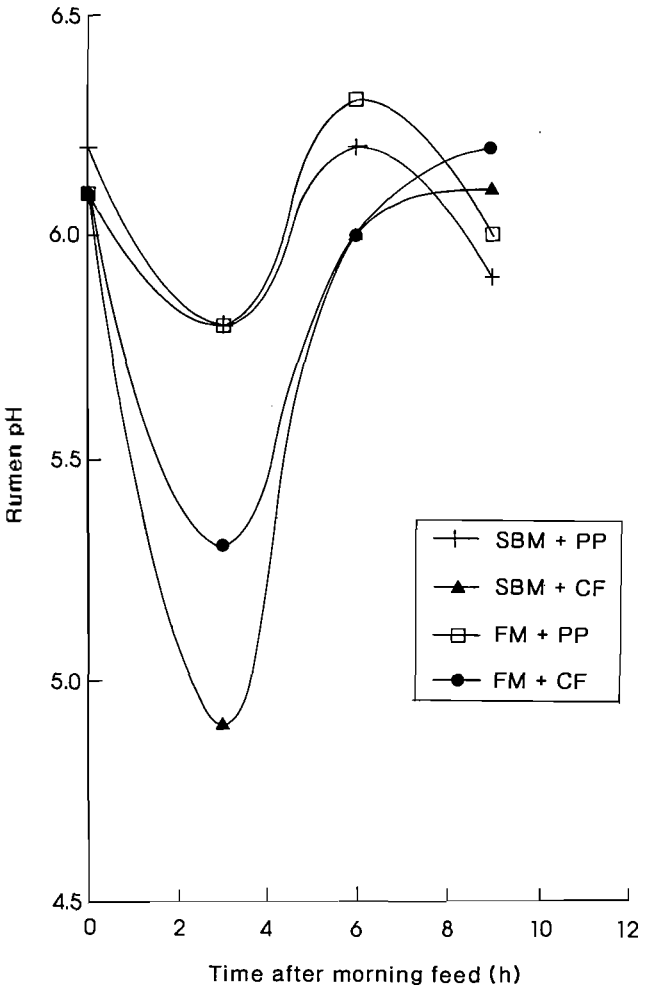

Figure 1. Rumen pH of sheep fed the four dietary treatments at various times after feeding.

Table 8. Means of rumen pH, ammonia-N (mg-N/L) and total VFA (mM) and molar proportions of VFA (%) in rumen fluid of sheep fed guinea grass as a basal diet with different protein and energy supplements

Rumen Parameters	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
pH	6.1	5.9	6.0	5.8	0.14	NS	NS
Ammonia-N (mg-N/L)	174.9^{b*}	179.7^{b}	266.4^{a}	235.3^{a}	17.2	0.0001	NS
Total VFA (mM)	72.5	72.8	81.7	82.3	7.4	NS	NS
Molar %:							
Acetate	74.8^{a}	69.8^{c}	72.8^{ab}	71.5^{bc}	1.1	NS	0.0001#
Propionate	17.5^{b}	19.1^{a}	19.0^{ab}	18.5^{ab}	0.8	NS	NS
Butyrate	7.4	9.0	7.6	7.9	0.9	NS	NS
Acetate : Propionate	4.4^{a}	3.7^{b}	3.9^{b}	3.9^{b}	0.19	NS	NS

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
* : Means in the same row with different superscripts are significantly different ($p < 0.05$). [Means in the same row without superscripts are not significantly different ($p > 0.05$)].
\# : Interaction between energy and protein sources ($p < 0.05$).

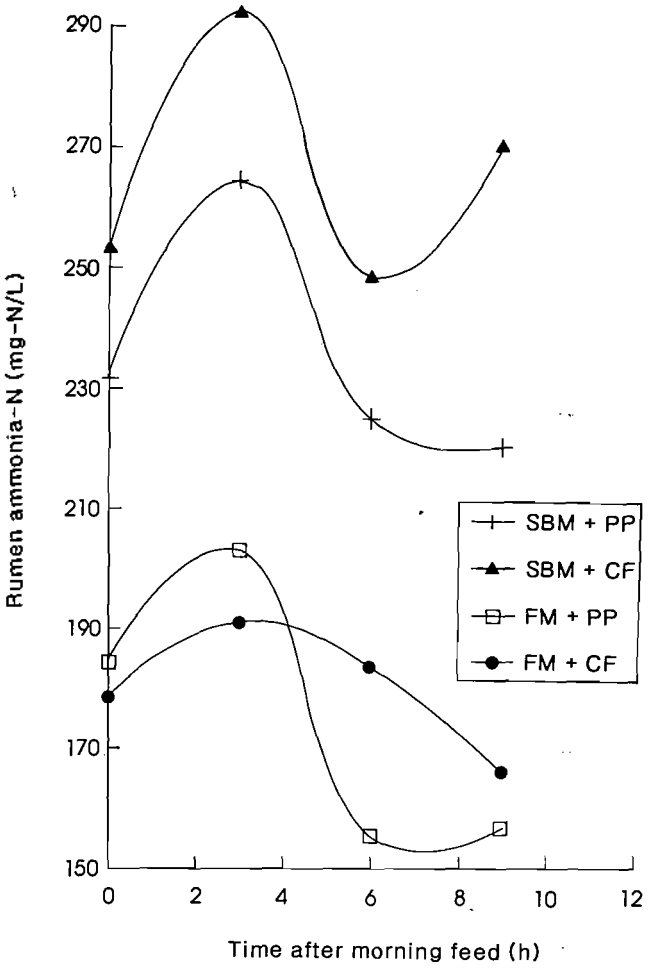

Figure 2. Rumen ammonia-N of sheep fed the four dietary treatments at various times after feeding.

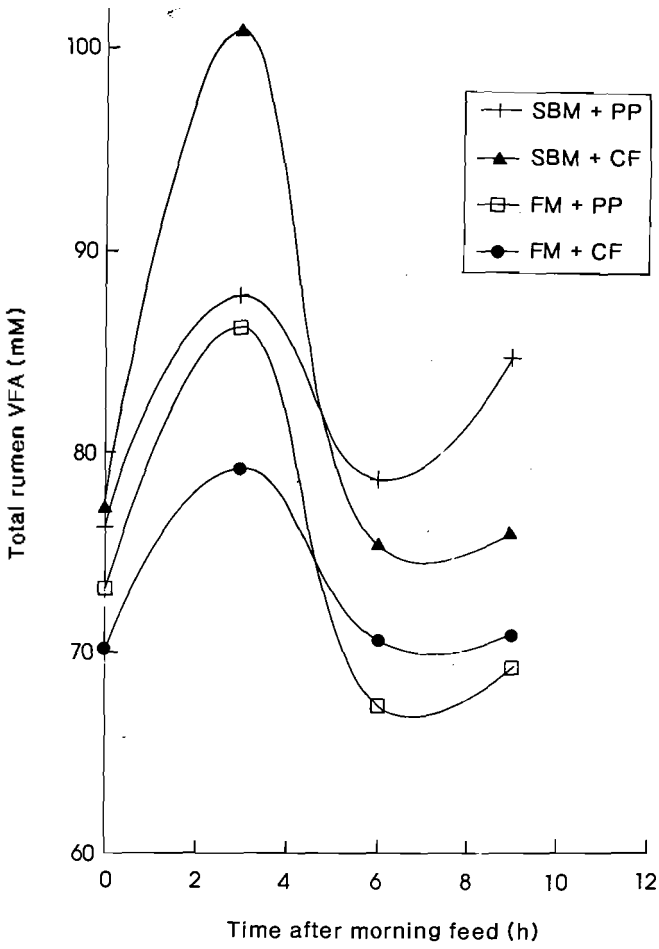

Figure 3. Total rumen VFA of sheep fed the four dietary treatments at various times after feeding.

were higher ($p < 0.0001$) than in those supplemented with CF (table 8). Propionate was lower in sheep supplemented with FM+PP when compared to sheep fed FM+CF, while butyrate proportions and acetate: propionate ratios were not significantly affected by both the energy and protein supplements.

Table 9 shows the protozoal counts in rumen fluid of sheep. Their numbers were significantly ($p < 0.05$) lower

Table 9. Protozoal counts ($\times 10^5$/ml) in rumen fluid of sheep fed guinea grass as a basal diet with different protein and energy supplements

Time after morning feed (h)	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
0	1.5	3.8	2.9	2.3	1.1	NS	NS
3	1.1	4.3	2.8	3.4	1.6	NS	NS
6	1.2^{b*}	3.2^{ab}	2.1^{ab}	3.4^{a}	0.9	NS	NS
9	0.9^{b}	3.1^{a}	3.4^{a}	3.7^{a}	1.1	NS	NS
Means	1.2^{b}	3.6^{a}	2.8^{a}	3.2^{a}	0.05	NS	0.001#

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
* : Means in the same row with different superscripts are significantly different ($p < 0.05$).
[Means in the same row without superscripts are not significantly different ($p > 0.05$)].
\# : Interaction between energy and protein sources ($p < 0.05$).

in sheep fed FM+PP supplements than in sheep fed other supplements. A significant interaction ($p < 0.05$) between protein and energy sources was observed. Animals fed CF had significantly ($p < 0.001$) higher protozoal counts than animals fed PP.

The total number of viable bacteria tended ($p < 0.07$) to be higher in sheep supplemented with FM, where their bacterial counts ranged from 27.2-30.2 $\times 10^9$/ml, when compared to that of sheep supplemented with SBM where counts ranged from 5.2-12.9 $\times 10^9$/ml of rumen fluid. Although the percentage of cellulolytic bacteria in sheep supplemented with PP or SBM was higher than those fed CF or FM, the difference was not significant. The proportion of cellulolytic bacteria ranged from 5.3 to 8.4% of the total viable bacteria (table 10).

Table 10. Total number of viable bacteria and percentage of cellulolytic bacteria in the rumen of sheep fed guinea grass as a basal diet with different protein and energy supplements

Parameters	Fish meal		Soybean meal		S.E.D	Probability for contrast between	
	Paper pulp	Corn flour	Paper pulp	Corn flour		Protein	Energy
Total viable bacteria ($\times 10^9$/ml)	30.2*	27.2	12.9	5.2	12.5	0.07	NS
Cellulolytic bacteria (%)	8.2	5.3	8.4	7.1	4.3	NS	NS

S.E.D. : Standard error differences.
NS : Not significantly different ($p > 0.05$).
* : Means in the same row without superscripts are not significantly different ($p > 0.05$).

DISCUSSION

The results of the present experiment showed that the intake of NDF and ADF of guinea grass by sheep did not differ significantly between the two energy supplements (CF and PP) or the two protein supplements (FM and SBM). Although the two energy supplements and the two protein supplements vary in rumen degradability, they had similar effect on the voluntary feed intake of sheep. Freeman et al. (1992) also observed that protein sources and levels of protein supplements had minimum effects on voluntary intakes in grazing cattle. Allden (1981) and Gaskins et al. (1990) indicated that protein supplementation probably had no effect on forage intake if crude protein content of forages was between 5-7%. Similarly, Hess et al. (1994) found that protein supplementation had no effect on the amount of forage consumed by grazing cows. Judkins et al. (1991) observed that forage intakes were unaffected by SBM and cracked corn supplementation in beef heifer fed low quality mixed grass hay. A number of workers (McCollum and Galyean, 1985; Minson 1990; Poppi and McLennan, 1995) have concluded that forage intakes in grazing animals may not be stimulated by protein supplementation when available N content in forage is above 1%.

In the nylon bag study, the percentage losses of NDF and ADF of guinea grass at various times of incubation (except at 12 h incubation) were similar among the four dietary treatments. This was probably due to the concentrate: forage ratios being similar among the treatments (44-47: 53-58). Mertens and Loften (1980) and Archimede et al. (1995) indicated that the extent of degradation was influenced by the ratio of concentrate to forage, whereby the extent of degradation changed concomitantly to the changes in the ratio of concentrate to forage. Generally, the addition of readily fermentable carbohydrates in excess decreases fibre digestion (Mertens and Loften, 1980; Hoover, 1986).

Although sheep fed SBM+CF showed lower percentage losses of NDF and ADF at 12 h of incubation, the effect was transient because at other incubation times, percentage losses of these parameters were not significantly affected by diets. On the other hand, Hussien et al. (1995) reported that a decrease in the in situ disappearance of DM, OM and fibre occurred when SBM was supplemented to steer fed ammoniated corn cobs+corn silage+oat hulls as basal diet.

The disappearance rate constants (c) of NDF and ADF were lowest in sheep fed SBM+CF. Similar results were reported by a number of workers (Veen, 1986; McCarthy et al., 1989; Hussein et al., 1995) who showed that the degradation rate of fibre decreased in animals supplemented with soluble protein (SBM) when compared to animals supplemented with slowly degradable protein like roasted SBM or FM. The results also indicated that

animals given PP supplementation had faster rates of degradation than those given CF. The effects of energy sources seemed to be similar to that of protein sources, where readily degradable feed (CF and SBM) reduced the rate of disappearance of fibre in the rumen.

Sheep supplemented with CF had significantly lower digestibilities of fibre (NDF and ADF) in the rumen and total tracts than sheep supplemented with PP. This result lends support to the other reports by Mertens and Loften (1980), Mould et al. (1983), England and Gill (1985) and Khalili (1993) who observed a reduction in fibre digestion when animals were supplemented with a readily fermentable carbohydrate.

It is possible that in sheep fed CF supplement, the slight increase in passage rate of NDF from the rumen resulted in a reduction of NDF digestion in the rumen. Similar observation was reported by Aitchison et al. (1985) in sheep fed hay and supplemented with maize starch. In these animals, passage rate of digesta and rumen pool size increased, but digestion in the rumen decreased.

There were no significant differences in the total fibre (NDF or ADF) digestions in sheep fed FM or SBM supplements. Dyness et al. (1994) and Kabre' et al. (1995) had also reported that the effects of FM supplement on apparent digestibility of fibre were small in sheep.

The rumen pH values before feeding were in the range of 5.8-6.2. However, 3 h after the onset of feeding, rumen pH of sheep declined as active fermentation of the newly ingested feed occurred. At this time the pH values ranged from 4.9-5.8. The greatest drop in pH occurred in animals supplemented with CF. Corn flour is a highly degradable source of carbohydrate and, as expected, would be rapidly fermented by the rumen microbes, resulting in a rapid decline in pH. However, this low pH was maintained for only a short while because three hours later the pH rose to 6.0. At 9 h after the onset of feeding, the pH increased to almost the same level as that at 0 h. Protein supplements did not seem to have any effect on rumen pHs. The rumen pHs were within the physiological range and a range of 5.8-6.0 may not have any effect on bacterial growth (Hoover, 1986).

Rumen fermentation pattern in all sheep fed the various diets was typical of a roughage fermentation, i.e., with high acetate and low propionate and butyrate proportions. However, molar proportion of acetate was highest in sheep supplemented with PP. A high acetate production in animals fed PP reflects a high content of fibre in their diets. Total rumen VFA concentrations tended to be high at 3 h after the onset of feeding as fermentation of newly ingested feed took place. Animals fed SBM supplement seemed to produce higher amounts of VFA compared to animals fed FM supplement. This is not surprising since SBM is a soluble protein source and would provide the rumen microbes with easily available amino acids and peptides. The microbes would thus be in a better growth condition to carry out more fermentation activity than those in animals fed FM which is a less degradable protein source (NRC, 1985).

The rumen ammonia concentrations in all animals were within the physiological ranges and should be adequate for microbial growth as the values were more than the optimum value required (100 mg-N/L) for microbial growth (Leng, 1990).

Rumen ammonia concentration was higher in sheep supplemented with SBM than in sheep supplemented with FM. This is propably due to SBM being more degradable than FM in the rumen.

The number of protozoa in sheep fed CF supplemented diets was higher than in those fed PP supplemented diets. This agrees with the finding of Chamberlain et al. (1985) and Jouany (1988) in which starch supplementation favoured the development of protozoa, in particular the entodiniomorphid species. Abe and Iriki (1978) also showed that protozoa were less in animals given cellulose diet, but more in animals fed starch, xylose and sucrose diets. Coleman (1960) reported that cellulose diets did not allow entodiniomorphid species to survive. Later, he (1986) showed that the growth of cellulolytic protozoa was greatly enhanced by starch and without starch in the ration, protozoal density was low and the rates of digestion were reduced. Protozoal growth is also influenced by the availability of proteins. Protozoa are able to utilise short chain peptides, dipeptides or tripeptides efficiently (Newbold et al., 1989). In the present study, protozoal numbers were higher in animals fed SBM+PP than in animals fed FM+PP. Soybean meal, being more soluble than FM, would probably provide a better niche for protozoal growth.

The number of viable bacteria was higher in sheep fed FM. This is expected as long chain peptides are more important in increasing the bacterial population than short chain peptides (Newbold et al., 1989). The non-celluloytic bacteria represent a major proportion of the viable bacterial population. Non-cellulolytic bacteria prefer peptides to amino acids, while cellulolytic bacteria prefer amino acids or ammonia for microbial protein synthesis (Chen et al., 1987). It has also been reported by Cruz Soto et al. (1994) that non-cellulolytic bacteria show faster growth when given peptides than when given amino acids. The higher proportion of cellulolytic bacteria in animals fed SBM is probably due to the availability of

amino acids from SBM. A higher proportion of cellulolytic bacteria was also observed in animals fed PP. It is possible that PP being non-soluble but highly degradable provides a better niche for cellulolytic bacteria. This small difference in cellulolytic bacterial population could be sufficient to cause a significant increase in rate and extent of fibre digestion in the rumen.

The present experiments indicated that intake of guinea grass by sheep did not differ when the animals were supplemented with different energy or protein sources. However, fibre digestion was decreased when the animal was supplemented with a more readily soluble carbohydrate (CF), but was enhanced when supplemented with a less readily soluble carbohydrate (PP). The rate of fibre disappearance was also enhanced in animals fed PP. Feeding twice daily may have resulted in the rumen pH being continuously low. Fermentation pattern was typical of a roughage diet, but acetate proportion was significantly ($p < 0.0001$) higher in animals supplemented with PP. Total VFA concentrations were similar for all sheep, but ammonia concentration was significantly ($p < 0.0001$) higher in animals fed SBM supplement. Fish meal supplement increased total bacterial numbers, while CF increased protozoal growth. Cellulolytic bacteria were slightly higher in sheep fed SBM or PP supplements.

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