

## Effects of Different Feeding Frequency of Faba Beans on Rumen Degradation Characteristics of Oaten Hay in the Rumen of Sheep

(Pengaruh Perbedaan Frekuensi Pemberian Bijian Faba terhadap Karakteristik Degradasi Hijauan Oat pada Rumen Domba)

Asmuddin Natsir\*

*Jurusan Nutrisi dan Makanan Ternak Fakultas Peternakan, Universitas Hasanuddin, Makassar, 90245*

**ABSTRAK :** Suatu penelitian dilaksanakan dengan tujuan untuk mengetahui pengaruh frekuensi pemberian yang berbeda dari bijian faba terhadap karakteristik degradasi hijauan oat pada rumen ternak domba. Empat ekor ternak domba jantan kastrasi yang telah dilengkapi dengan kanula pada rumen, dengan bobot badan rata-rata  $85 \pm 5,5$  kg, digunakan dalam percobaan ini. Kombinasi (85:15%) antara hijauan oat dan alfalfa digunakan sebagai ransum basal selama studi. Bijian faba digunakan sebagai pakan suplemen diberikan pada level 0,5% dari bobot hidup. Penelitian dilaksanakan berdasarkan pola rancangan bujur sangkar latin  $4 \times 4$ . Setiap periode berlangsung selama 20 hari. Pada masing-masing periode, setiap ternak mendapatkan salah satu dari 4 macam ransum perlakuan, yakni  $T_0$  = ransum basal tanpa suplemen,  $T_1$  =  $T_0$  + FB yang diberikan sekali dalam sehari pada pagi hari,  $T_2$  =  $T_0$  + FB yang pemberiannya dibagi dua dan diberikan pada pagi dan sore hari, dan  $T_3$  =  $T_0$  + FB yang pemberiannya dibagi atas 8 bagian yang sama dan diberikan setiap tiga jam. Hasil analisis statistik memperlihatkan bahwa suplementasi meningkatkan ( $P < 0,05$ ) kadar  $NH_3$  dan VFA rumen. Rataan kadar  $NH_3$  rumen untuk masing-masing perlakuan adalah 162,40; 264,45; 301,75; dan 293,00 mg/l, dan kadar VFA rumen rata-rata untuk setiap perlakuan adalah 65,81; 86,00; 77,63 dan 86,76 mmol/l. Akan tetapi, perlakuan tidak berpengaruh nyata ( $P > 0,05$ ) terhadap pH rumen, dengan rata-rata 6,36; 6,05; 6,15; dan 6,10 untuk masing-masing perlakuan  $T_0$ ,  $T_1$ ,  $T_2$ , dan  $T_3$ . Begitupula, pemberian suplemen bijian faba tidak berpengaruh ( $P > 0,05$ ) terhadap karakteristik degradasi hijauan oat dalam rumen ternak domba. Kesimpulan, suplemen bijian faba nyata memperbaiki pola fermentasi rumen ternak domba, dalam hal peningkatan kadar  $NH_3$  dan VFA rumen, tetapi pH rumen dan karakteristik degradasi rumen dari hijauan oat tidak dipengaruhi oleh pemberian suplemen bijian faba.

**Kata Kunci:** Feeding frequency, faba beans, rumen degradation characteristics, sheep

### Introduction

Among protein sources, the extent of degradation in the rumen varies greatly, ranging from less than 50% to as high as 100%. Faba beans (*Vicia faba*) are legume seeds, which are particularly high in crude protein concentrations (25-42%) (Cottle, 1991; Petterson and MacKintosh, 1994; Yu *et al.*, 2000), making them potentially useful protein supplements in ruminant diets. However, their rapid and extensive degradation (85-90%) (Yu *et al.*, 1999) by ruminal microbes can result in substantial and undesirable N loss from the rumen. If an imbalance between rate of feed protein breakdown and rate of microbial protein synthesis is unavoidable, utilisation as a protein supplement for ruminants is not efficient.

To overcome this problem, the legume seeds may be pretreated either chemically, such as by formaldehyde treatment (Virk *et al.*, 1994; Tewatia *et*

*al.*, 1995), or physically, such as by dry roasting (Yu *et al.*, 1999, 2000) before being offered to the animal. The main purpose of these treatments is to reduce the high degradation rate of protein and starch in the rumen and make them more available for post-ruminal digestion, conferring benefits for the host animal.

Another approach that is possible to apply is to alter the feeding frequency. Offering the faba beans in smaller portions more frequently than giving once per day can improve the time relationships for availability of nitrogen released from the faba beans and energy release from degradation of both readily fermentable carbohydrates from the supplements and fibre from the basal diet (roughages). This would in theory bring conditions closer to the optimum for microbial protein synthesis in the rumen (Owens *et al.*, 1984). In addition, frequent provision of faba beans can reduce the high amplitude of fluctuations of rumen conditions such as pH after feeding. Providing the animals with large amounts of readily fermentable carbohydrate in one rapidly ingested

\* Korespondensi penulis : email asmuddin@unhas.ac.id  
Telp. +62 411 587126, Hp. +6281543150878

meal produced dramatic changes in rumen conditions and the significant decrease in rumen pH markedly affected fibre degradation.

The objective of this study was to test the hypothesis that offering faba beans as a supplement more than once a day to sheep receiving a combination of oaten chaff and lucerne chaff as a basal diet will improve rumen conditions and in turn will increase the degradation rate of roughage (oaten chaff).

## Research Methods

### Animal and Feeding

Four mature merino sheep (all wethers) with existing rumen cannula (internal diameter 40 mm) were used for this study. The average body weight,  $BW \pm SD$ , was  $85 \pm 5.5$  kg. The sheep were penned and fed individually in the Animal House, School of Agriculture and Food system, Institute of Land and Food Resources, the University of Melbourne, Parkville, Victoria, throughout the study. The animals were cared for according to the guidelines on animal care established as standard operating procedure by NH&MRC/CSIRO.

A combination (85%:15%) of oaten chaff and lucerne chaff was given to the animal as the basal diet throughout the study. This diet was calculated to contain 12 g N/kg DM and was designed to simulate feed under grazing conditions during the summer-autumn period. FB were used as a supplement at the rate of approximately 0.5% of live body weight. The basal diet was given once per day at 09:00 in the

morning (20% in excess of the previous day's intake) while FB were delivered according to the treatment arrangement with roughage and supplements fed in separate feeders. Feed refusals were collected at 09:00 daily, weighed and samples bulked for subsequent analysis. No supplementary vitamins and minerals were given and animals had a free access to water. The dietary ingredients used in this experiment were purchased from a commercial company (Essendon Produce, Essendon, Victoria). The composition of dietary ingredients is shown in Table 1.

### Experimental Design

The experiment was conducted according to latin square design (4 x 4, Steel and Torrie, 1981) consisting of four treatments and four periods. In every period, each animal received one of four treatments:  $T_0$  = basal diet without supplement,  $T_1$  =  $T_0$  + FB given once per day,  $T_2$  =  $T_0$  + FB which was divided into two equal portions, and  $T_3$  =  $T_0$  + FB which was divided into 8 equal portions. The animal in  $T_0$  received only the basal diet. For animal in  $T_1$ , 450 g (air dry basis) FB was given in the morning (09:00) at the same time as the basal diet was offered. For  $T_2$ , 225 g FB was given at 09:00 and the other 225 g at 15:00. For the animal receiving  $T_3$ , an equal amount of 58.2 g of FB was delivered to the animal every 3 h starting at 09:00 using an automatic feeder. Each experimental period lasted 20 days, in which the last three days of each period were allocated for *in sacco* experiment and for taking rumen fluid sampling.

Table 1. Chemical composition of the experimental diets

Measurement	Oaten chaff	Lucerne chaff	Faba beans
DM (g/kg)	871.2	875.9	891.1
Composition (g/kg DM)			
Ash	68.8	103.3	28.8
OM	931.2	896.7	971.2
CP	49.5	201.9	265.1
NDF	714.8	419.2	260.6
ADF	413.7	332.3	146.6
*ME (MJ/kg DM)	6.8	8.5	12.8

\*Values from AFRC (1993).

### **In Sacco Experiment**

Rumen degradation characteristics of oaten chaff in the rumen of sheep were determined using the *in sacco* method described by Ørskov *et al.* (1980). Bags were made from a nitrogen-free, woven polyester cloth, have heat-sealed edges, pore size 35-65 µm with outer dimensions of 5 x 12 cm (Ankom technologies, USA). Prior to incubation, the nylon bag samples were prepared by coarsely milling it through a hammer mill to pass a 3-mm screen. The weight of the clean, dry, and labelled nylon bag was recorded before transferring approximately 2.5 g of air-dry sample into each bag. A duplicate bag for each treatment was incubated in the rumen of each animal.

A slight modification was introduced in conducting incubation in the rumen to obviate some difficulties faced when removing the bags from the rumen, especially those removed after incubation for the relatively short periods (6 and 12 h). PVC plastic tubes (approx. 35 cm long), instead of nylon cord, were used to prevent tangling during the incubation of the bags in the rumen. A total of 5 PVC tubes for each animal, with 2 bags attached to each tube, were simultaneously incubated in the rumen of each sheep just before the morning feeding. Bags held by individual tubes were then removed from the rumen at 6, 12, 24, 48, and 72 h after commencement of incubation. Immediately after withdrawal, the removed bags containing the residues were rinsed under cold running tap water to remove excess ruminal contents and microorganisms on the surface of the bags and then kept in the freezer (-20°C) for later analysis.

After completing the incubation program, all collected bags were washed in washing machine without spinning for about 15 minutes and then dried at 55°C for 48 h to determine dry weight of the residue in each bag (AOAC, 1990). To determine the zero time losses, duplicate bags containing the same samples were soaked in warm tap water (39°C) for 1 h followed by washing and drying as before.

### **Rumen Fluid Sampling**

Rumen conditions of the sheep were monitored by taking rumen fluid samples from each animal after completing the *in sacco* experiment. A plastic tube with an attached syringe covered by nylon cloth was inserted through the cannula into the mid ventral region of the rumen. Rumen fluid samples were withdrawn at 0h (before morning feeding) and at 3, 6, 9, 12 h and 24h for three consecutive days. The

fluid was withdrawn using a 20 ml disposable syringe. On each sampling time, the first 10 ml of the fluid was discarded. Approximately 40 ml of the rumen fluid was collected from each sheep, 20 ml of this fluid was dispensed into a tube and immediately tested for pH (measured within 1 minute) using a portable pH meter (HI 8424, Hanna Instruments Srl, Italy), and the rest for rumen fluid samples taken at 0 and 6 h was acidified with 5-6 drops of H<sub>2</sub>SO<sub>4</sub> concentrate before freezing it for later analysis for rumen NH<sub>3</sub> and VFA.

### **Laboratory Analysis**

Prior to chemical analysis all samples (diets and *in sacco* samples) were ground to pass a 1-mm screen. Sample DM content was determined by drying at 100°C in the oven for 24 h. The percentage of ash was determined by combustion of samples for 6 h at 550°C. Organic matter (OM) was calculated as 100-%ash (DM basis). Total N content was determined by the Kjeldahl procedure (AOAC, 1990) and percentage of crude protein (CP) was calculated as total N\*6.25. Fibre composition (NDF and ADF) was analysed according to the procedure of Goering and Van Soest (1970).

Total N content of feeds & rumen fluid samples were determined by the Kjeldahl procedure (AOAC, 1990) with automatic titration (Radiometer, Copenhagen, Denmark) while rumen VFA concentration was analysed by a gas chromatograph (GC; Hewlett-Packard, Model 5890, Series II).

### **Calculation of Rumen Degradation Characteristics**

Analysis of ruminal degradation pattern for DM was performed by fitting the degradation values to the equations of Ørskov and McDonald (1979):

$$P = a + b(1 - e^{-ct}), \text{ where}$$

$p$  = rumen degradation at time  $t$ ,

$a$  = intercept, which is highly correlated with the water soluble fraction (WSF),

$b$  = the portion of feed DM (other than WSF) which is degraded in time  $t$ ,

$c$  = the degradation rate of the insoluble ( $b$ ) fraction (%/h),

$t$  = incubation time,

In addition,  $a + b$  (asymptote) shows the value of rumen potential degradability.

The calculation of the rumen degradation characteristics was performed using the program NEWAY EXCELL (Chen, 1997).

## Statistical Analysis

All data were subjected to analysis of variance for a Latin square design (4 x 4) using the General Linear Model (GLM) procedure of MINITAB for Windows rel.13.1 (Minitab Inc., 2000). The difference among the treatment means was determined by Tukey test (Steel and Torrie, 1981).

## Results and Discussion

### Fermentation Conditions and Degradation Characteristics

Rumen fermentation pattern was monitored by measuring rumen pH, rumen NH<sub>3</sub> and rumen VFA. The average values for those parameters are presented in Table 2. Analysis of variances indicated that rumen pH was similar ( $P>0.05$ ) among the treatments, averaging 6.16 across treatments. However, rumen NH<sub>3</sub> and rumen VFA were affected ( $P<0.05$ ) by supplementation. The average rumen NH<sub>3</sub> was 286.4 mg/l for supplemented group compared to 162.4 mg/l for control group. Similarly, rumen VFA for supplemented group was 27% higher than the control group (83.46 vs 65.81 mmol/l).

In general, the characteristics of rumen degradation parameters of oaten chaff (Table 3) were not affected ( $P>0.05$ ) by the treatments. The average

values for the water soluble fraction (*a*), the portion of insoluble fraction (*b*), degradation rate of insoluble fraction (*c*), and potential degradability (*a* + *b*) across treatment was 29.57%, 39.81%, 0.0596%/h, and 69.38%, respectively.

It is well known that the maximum rate of ruminal degradation of forage dry matter and particularly fibrous constituents can be achieved when the rumen conditions are not a limiting factor for rumen microbial activities. Rumen pH, rumen NH<sub>3</sub> and rumen VFA are indicators commonly used to monitor rumen fermentation pattern. In this particular experiment, the average rumen pH for control group was 6.36 compared to 6.10 for the supplemented group. This value was much less than the range of pH 6.5 - 7.0 reported for optimum fibre digestion *in vitro* (Stewart, 1977; Hiltner and Dehority, 1983; Russell and Wilson, 1996) and *in vivo* (Mould and Ørskov, 1983). However, the average rumen pH in this experiment was relatively similar to that recommended by Dixon and Stocdale (1999) for optimum fibre digestion of pH 6.20. Moreover, the average rumen pH was slightly higher than rumen pH of 6.0, which is regarded as "fibrolytic threshold" (Mould and Ørskov, 1983).

Table 2. Rumen fermentation pattern of sheep according to the treatments

Measurement	Treatments				Means
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Rumen pH	6.36	6.05	6.15	6.10	6.16
Rumen NH <sub>3</sub> (mg/l)	162.40 <sup>a</sup>	264.45 <sup>b</sup>	301.75 <sup>b</sup>	293.00 <sup>b</sup>	255.40
Rumen VFA (mmol/l)	65.81 <sup>a</sup>	86.00 <sup>b</sup>	77.63 <sup>b</sup>	86.76 <sup>b</sup>	79.05

<sup>a,b</sup> Means sharing similar superscript at the same row were different ( $P < 0.05$ )

Table 3. Rumen degradation characteristics of DM of oaten chaff according to the treatment

Degradation parameters	Treatments				s.e.m*	Diff. (P<)
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		
<i>a</i>	0.3224	0.2592	0.2869	0.3143	0.024	0.329
<i>b</i>	0.3732	0.4283	0.4010	0.3899	0.024	0.488
<i>a</i> + <i>b</i>	0.6956	0.6876	0.6879	0.7042	0.006	0.253
<i>c</i>	0.0655	0.0553	0.0581	0.0595	0.004	0.483

\*) s.e.m = standard error of means

The importance of rumen pH for rumen metabolism is difficult to assess, and depends on the relative importance of minimum pH, mean pH or the length of time that pH remains below a critical value. Certainly, cellulolysis is known to be sensitive to pH; a value of 6.0 is frequently suggested as the minimum below which cellulolysis may be inhibited (Mould and Ørskov, 1983) and most culturable cellulolytic rumen microbes will cease activity below a pH of 5.5 (Van Es, 1983). However, in some instance, NDF in fresh pasture is highly digestible (Van Vuuren *et al.*, 1992) and *in vitro* studies that have incubated highly digestible pasture indicate that these pastures may have a cellulolytic threshold lower than 6 (De Veth and Klover, 1999).

The purpose of offering faba beans in smaller portions more frequently than once per day is intended to improve the time relationships for availability of nitrogen released from the faba beans and energy release from degradation of both readily fermentable carbohydrates from the supplements and fibre from the basal diet (roughages). Frequent provision of faba beans, rather than providing it at once, can reduce the high amplitude of fluctuations of rumen conditions such as pH after feeding. The influence of large amounts of readily fermentable carbohydrate given to the animal in one rapidly ingested meal produced dramatic changes in rumen conditions and the significant decrease in rumen pH due to accumulation of VFA (Mould *et al.*, 1983; Chamberlain and Wilkinson, 1996; Beauchemin, 2000; Natsir *et al.* 2002; Natsir, 2004, 2005). In this experiment, that purpose was quiet achievable. Rumen pH of sheep receiving faba beans in smaller portion more than once per day experienced shorter period where rumen pH dropped below the threshold point of 6.0.

Similar rumen pH across treatments is might be one reason why the rumen degradation characteristics for oaten chaff among treatments are not different, even though other rumen fermentation parameters, i.e.  $\text{NH}_3$  and rumen VFA are higher for animals receiving supplements. Improved levels of rumen  $\text{NH}_3$  and rumen VFA concentration on the animals receiving faba beans supplement are not expressed further in terms of improved rumen degradation characteristics, i.e. rumen potential degradability and degradation rate of oaten chaff. One of possible reason is that concentration of rumen  $\text{NH}_3$  for control group is already sufficient to support optimum growth for rumen microorganisms. Based on the published work, all diets used in this experiment,

including the control one, would have provided sufficient rumen  $\text{NH}_3\text{-N}$  to support good levels of microbial activity since the average rumen  $\text{NH}_3\text{-N}$  concentration exceeded the threshold point of 50-80 mg/l indicated as a sufficiency level (Satter and Slyter, 1974), and still in agreement with another study reported that the minimum rumen  $\text{NH}_3\text{-N}$  required for optimising rumen microbial fermentation was in the range of 100 to 200 mg N/l (Perdok *et al.*, 1988). Use of oaten chaff mixed with a small amount of lucerne chaff (15% of the total roughage) thus seems to be sufficient to create a sound rumen environment in terms of availability of rumen  $\text{NH}_3\text{-N}$  to support microbial synthesis.

## Conclusion

Even though provision of faba bans supplements on the rate of 0,5% of body weight of sheep could improve rumen conditions of sheep in terms of higher rumen  $\text{NH}_3$  and rumen VFA, but it did not give any benefits in improving rumen degradation characteristics of oaten chaff, suggesting that combination of oaten chaff (low quality roughage) with small portion of lucerne chaff (medium quality roughage) was sufficient in creating a sound rumen conditions for rumen microorganisms.

## Acknowledgments

The author is grateful for the financial support from the government of Australia through the Australian Development Scholarship during his postgraduate study at the University of Melbourne, Melbourne, Australia. Warm appreciations are also extended to Mr. Andre Thalen, *Animal House Manager* and Ms. Fahi Nowniaz, *Animal Production Laboratory Manager*, Institute of Land and Food Resources, Melbourne University, for their invaluable technical assistances during the study.

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