Rumen Microbial Protein Supply as Estimated from Purine Derivative Excretion on Sheep Receiving Faba Beans (*Vicia faba*) as Supplement Delivered at Different Feeding Frequencies

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ABSTRAK

NATSIR, A. 2008. Suplai protein mikroba rumen yang diestimasi berdasarkan ekskresi turunan purin pada domba yang diberi bijian faba (*Vicia faba*) sebagai suplemen dengan frekuensi pemberian berbeda. *JITV* 13(2): 103-108.

Tingkat degradasi protein yang cepat dari bijian faba (*Vicia faba*) oleh mikroba rumen dapat menyebabkan kehilangan nitrogen yang berlebihan dari rumen. Penelitian ini bertujuan untuk menguji hipotesis bahwa pemberian suplemen bijian faba lebih dari satu kali sehari pada ternak domba yang mengkonsumsi kombinasi hijauan oat dan hijauan alfalfa sebagai ransum basal, akan meningkatkan suplai protein mikroba ke usus halus. Penelitian dilaksanakan berdasarkan rancangan bujur sangkar latin 4 x 4 menggunakan empat ekor ternak domba dewasa. Perlakuan adalah: T0 = ransum basal *ad libitum* tanpa suplemen, T1 = T0 + bijian faba (FB) yang diberikan sekali dalam sehari, T2 = T0 + FB yang diberikan dua kali dalam sehari, T3 = T0 + FB ditawarkan 8 kali sehari. Ransum basal diberikan pada jam 09.00, sementara FB diberikan dengan level 0,5% dari bobot badan berdasarkan protokol perlakuan. Ekskresi turunan purin (PD) dalam urine digunakan sebagai indikator untuk mengestimasi suplai protein mikroba. Hasil penelitian memperlihatkan bahwa walaupun secara statistik, perlakuan tidak berpengaruh nyata (P > 0,05) terhadap ekskresi PD dalam urine, PD yang diserap, estimasi suplai mikroba N, serta efisiensi sintesis protein mikroba rumen, suplementasi meningkatkan estimasi suplai protein mikroba hingga 92% dibanding dengan kontrol. Akan tetapi, untuk kelompok ternak yang diberi suplemen, frekuensi pemberian suplemen yang berbeda tidak berpengaruh terhadap estimasi tersebut.

Kata Kunci: Turuna Purin, Sintesis Protein Mikroba, Ternak Domba

ABSTRACT

NATSIR, A. 2008. Rumen microbial protein supply as estimated from purine derivative excretion on sheep receiving faba beans (*vicia faba*) as supplement delivered at different feeding frequencies. *JITV* 13(2): 103-108.

Rapid and extensive degradation of faba beans (*Vicia faba*) by ruminal microbes can result in substantial and undesirable N loss from the rumen. The purpose 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 increase microbial protein supply to the intestines. The experiment was conducted in a Latin square design (4 x 4) using four mature merino sheep. The treatments were: T0 = basal diet *ad libitum* + nil supplements, T1 = T0 + faba beans (FB) fed once daily, T2 = T0 + FB fed twice daily, T3 = T0 + FB fed 8 times daily. The basal diet was given once per day at 09:00 in the morning while FB were given at the rate of approximately 0.5% of live body weight and delivered according to the treatment protocol. Urinary excretion of purine derivative (PD) was used to estimate microbial protein supply. The results indicated that even though treatment statistically had no effects on total urine output, PD excretion in the urine, PD absorbed, estimated microbial N supply, and the efficiency of rumen microbial protein synthesis, provision of supplement to sheep numerically improved microbial N supply by 92% compared to that of control group. However, there were no differences within the supplemented group. Therefore, it is concluded that feeding faba beans more than once a day was unnecessary.

Key Words: Purine Derivatives, Microbial Protein Synthesis, Sheep

INTRODUCTION

The rate of rumen degradation of protein from dietary feed ingredients is one of the important factors influencing the supply of nitrogen for the synthesis of microbial protein. The rate of degradation determined the availability of NH_3, amino acids, peptides and branched chained fatty acids that influenced microbial growth rate in the rumen (DEWHURST *et al*., 2000)

Faba beans (*Vicia faba*) are legume seeds, which are particularly high in crude protein (25-42%) (COTTLE, 1991), making them a potentially useful protein supplement for ruminants. 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.

Therefore, legume seeds may be pre-treated either chemically, such as by formaldehyde treatment (TEWATIA *et al*., 1995), or physically, such as by dry roasting (YU *et al*., 2000) before being fed to the animal. The main purpose of these treatments is to reduce the degradation rate of protein and starch in the rumen and to make them more available for post-ruminal digestion, conferring benefits for the host animal.

Other approach, which is possible to apply in some management circumstances, is to alter the feeding frequency. Offering the faba beans in smaller portions more frequently than 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; HOOVER and STOKES, 1991).

In addition, frequent provision of faba beans can reduce the high amplitude of fluctuations of rumen conditions such as pH after feeding (NATSIR, 2004). 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 (BEAUCHEMIN, 2000; NATSIR *et al.*, 2001; NATSIR, 2005) with one consequence is a decrease in digestibility of fibre fraction of diet (MOULD *et al*., 1983).

The objective of this study was to test the hypothesis that offering whole 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 increase the microbial protein supply for the sheep.

MATERIALS AND METHODS

Animal and feeding

Four mature merino sheep with an average body weight of 85 ± 5.5 kg used in this study, penned and fed individually. 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. Faba beans (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 was delivered according to the treatment arrangement with roughage and supplements fed in separate feeders. No supplementary vitamins and minerals were given, and the animals had a free access to water.

Experimental design

The experiment was conducted according to a Latin square design 4 x 4 (STEEL and TORRIE, 1981) consisting of four treatments and four periods. In each period, each animal received one of four treatments: T0 = basal diet *ad libitum* + nil supplement, T1 = T0 + faba beans (FB) fed once daily, T2 = T0 + FB fed twice daily, T3 = T0 + FB fed 8 times daily. Each period was lasted for 21 days.

The animal in T0 received only the basal diet. For T1, 450 g FB (air dry basis) was given in the morning (09:00) at the same time as the basal diet was offered. For T2, 225 g FB was given at 09:00 and the other 225 g at 15:00. For the animal receiving T3, an equal amount of 58.2 g of FB was delivered to the animal every 3 hours starting at 09:00 using an automatic feeder.

Sample collection

During the sampling period the (last five days of each period), the quantity of a mixture of oaten hay, lucerne chaff and supplements offered and refused (if any) were recorded every morning and a sub-sample was bulked for analysis.

The daily faecal collection was put in plastic bags and weighed. The bags, with faeces, were labelled and stored at 5^{o}C. At the end of each collection faeces collected daily from each sheep over the 5-day period were bulked on an individual animal basis, mixed thoroughly and a sub-sample oven dried (100^{0}C) to determine dry matter of the faeces. Another sub sample (10%) was taken and kept frozen for subsequent laboratory analysis.

Total urine output was collected and removed each day in the morning just before feeding. Immediately after collection, urine volume was measured and 1% aliquot of undiluted urine was taken, after thorough mixing, for total N analysis. The remaining urine was then diluted 15 times with fresh tap water, and concentrated H_2SO_4 was then added and stirred, to bring the pH below 3. A 1% aliquot of the diluted urine was sub-sampled for each animal and kept in the freezer for purine derivatives analysis.

Chemical analysis

The feed samples (oaten chaff, lucerne chaff, and faba beans) and faeces were analyzed for dry matter (DM), ash, total N, neutral detergent fibre (NDF), and acid detergent fibre (ADF). All samples were ground to pass 1-mm screen prior to analysis. Urine samples were analyzed for total N content and purine derivative (PD).

DM content was determined by drying at 100^{o}C in the oven for 24 h (Qualtex, Universal series 2000, Australia). The percentage of ash was determined by igniting the samples for 6 h at 550^{o}C. Organic matter (OM) was calculated as 100 %ash (DM). Total N content of feeds, rumen fluid, and urine samples were determined by the Kjeldahl procedure (AOAC, 1990) with automatic titration (Radiometer, Copenhagen, Denmark). Fibre composition (ADF and NDF) was analysed according to the procedure of GOERING and VAN SOEST (1970). Chemical composition of experimental diets is presented in Table 1.

The urinary concentration of creatinine and PD (allantoin, uric acid, hypoxanthine, and xanthine) was measured on each urine sample collected daily for 3 days. Allantoin was determined using high-performance liquid chromatography (HPLC) with pre-column derivatization as described by CHEN *et al.* (1993). Uric acid, xanthine and hypoxanthine were determined with a RA/XT Autoanalyzer (Technicon Ltd., Swords Co., Dublin, Ireland) using commercial kits according to the procedure of CHEN and GOMES (1992). The principle of the method is that the xanthine oxidase converts xanthine and hypoxanthine into uric acid and the xanthine and hypoxanthine are measured as uric acid. Therefore, the uric acid data represents the sum of uric acid, xanthine and hypoxanthine, while total PD is the sum of allantoin, uric acid, xanthine and hypoxanthine.

Calculation and statistical analysis

The amount of microbial PD absorbed (X, mmol per day) corresponding to the PD excreted (Y, mmol per day) was calculated from the relationship derived by CHEN and GOMES (1992) as follows:

$$Y = 0.84X + (0.150W^{0.75}e^{-0.25X})$$

The supply of microbial N (MN) in grams per day based on total PD was estimated as follows:

$$MN_{pd} = \frac{70\ X}{0{,}116 \times 0{,}83 \times 1000} = 0{,}72X$$

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

In general, supplementation did not affect (P>0.05) total urine output, as well as allantoin, uric acid, and total PD concentration excreted in the urine, averaging: 840.2 g/day, 8.20 mmol/day, 1.89 mmol/day, and 10.08 mmol/day, respectively (Table 2). The estimated PD absorbed was similar (P>0.05) across treatments, averaging 11.40 mmol per day. Supply of microbial N, based on the PD absorbed, was also similar (P>0.05) for each treatment, averaging: 8.28 g N or 51.76 g CP/day. In addition, there was no significant differences observed for the estimated efficiency of rumen microbial synthesis based either on rumen

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 (l993)

Table 2. Urinary excretion of purine derivatives and microbial N supply to sheep fed oaten: lucerne chaff with or without faba beans supplementation

Measurement	Treatment				Overall mean	s.e.m*	Diff (P<)
	T0	T1	T2	T3			
Urinary products							
Urinary output (g/day)	868.30	702.80	852.30	937.20	840.20	116.90	0.60
Allantoin (mmol/day)	4.64	7.34	8.71	12.08	8.20	1.67	0.09
Uric acid (mmol/day)[a]	1.49	1.90	1.88	2.29	1.89	0.36	0.52
Total PD (mmol/day) [b]	6.13	9.24	10.60	14.38	10.08	2.02	0.13
Estimated supply of microbial N							
PD (mmol/day)	5.66	10.61	12.31	16.98	11.40	2.66	0.11
N (g/day)[c]	4.11	7.72	8.95	12.35	8.28	1.93	0.11
Estimated efficiency of rumen microbial N synthesis							
Microbial N g/kg DOMR[d]	8.77	14.88	15.42	20.3	14.84	3.58	0.26
Microbial N g/g N intake	0.29	0.29	0.32	0.42	0.33	0.08	0.63

*Standard error means
[a] Sum of uric acid, xanthine and hypoxanthine
[b] Sum of allantoin, uric acid, xanthine and hypoxanthine excretion
[c]Microbial N (g/day) = 0.727 x (where x = total absorption of purine, mmol per day)
[d]DOMR: 0.65 DOMI (ARC, 1984)

fermented digestible organic matter (DOMR) or on the nitrogen intake (NI). The range for the former was between 8.77 and 20.30 g N/kg DOMR with a mean of 14.84 g of N/kg DOMR, and for the latter ranging from 0.29 to 0.42 g N/g NI, averaging 0.33 g N/g NI.

The concentration of purine in ruminant feed is generally low and most is subject to extensive degradation in the rumen due to microbial fermentation. Therefore, the nucleic acids leaving the rumen are mainly of microbial origin. Absorbed nucleic acid purines are degraded and excreted in the urine as their derivatives, allantoin, uric acid, hypoxanthine and xanthine. The urinary excretion of the purine derivatives is directly related to the microbial purine absorption. With a knowledge of the purine-N: total N ratio in microbial biomass, microbial N absorption can be estimated from the amount of purine absorbed which is estimated from the urinary purine excretion (CHEN and GOMES, 1992; DIPU *et al*. 2006; FUJIHARA *et al.*, 2007).

There are many factors involved in determining the efficiency of microbial protein synthesis in the rumen. Even though the source of dietary protein markedly influences the total amount of microbial protein entering the small intestine, the effects on the efficiency of microbial protein synthesis appear to be limited (THEURER, 1979). Some of the major factors influencing microbial growth efficiency include the availability of nitrogen precursors (SATTER and ROFFLER, 1975), the nature of the dietary carbohydrate (HAGEMEISTER *et al*., 1981) and the availability of other essential elements such as sulphur (ARC, 1984). Thus microbial protein synthesis in the rumen has been shown to respond to provision of specific nutrients such as sulphur, branched chain fatty acids, and trace nutrient elements (HESPELL and BRYANT, 1979). However, under most dietary conditions, the nutrient supply to the microbes is considered largely in terms of ruminal availability of nitrogen and of carbohydrate that can be fermented in the rumen to provide both carbon skeletons and energy in the form of ATP for microbial protein synthesis (HOOVER and STOKES, 1991).

Statistically, no differences were observed in purine derivatives excreted in the urine, the estimated supply of microbial N (g/day) or the estimated efficiency of rumen microbial N synthesis. The failure to detect any effects of treatments was probably related to high variability between animals and/or between periods as indicated by the high standard error of means (s.e.m). However, the estimated efficiency of microbial protein synthesis (g MN/kg DOMR) was numerically higher in the supplemented groups compared to the control group, regardless of the time of provision of faba beans (Table 2). The average efficiency for animals receiving

supplements was 16.90 g MN/kg DOMR, ranging from 14.90 to 20.30 g MN/kg DOMR. These results lie within the range of 14-49 suggested by ARC (1984). The value for sheep on the control diet was 8.8 g N/kg DOMR, thus being below the value proposed by ARC. From published data, there was a wide range of values for efficiency of microbial protein synthesis. The yield and efficiency of synthesis of microbial protein has been recorded as high as 30-45 g MN/kg DOMR when high quality grass was grazed (BEEVER *et al*., 1986; CARRUTHERS *et al*., 1997; ELIZALDE, 1998). Much lower efficiencies (<20) have been noted with lower-quality autumn grass (DOVE and MILNE, 1994; CARRUTHERS *et al*., 1997). In fact, the efficiency of reported microbial growth was quite variable, being in the range of 20 – 50 g of MN/kg DOMR (CHEN and GOMEZ, 1992; BOONEK, 2002; NATSIR, 2007).

These results demonstrate, across all diets, a significant relationship between provision of rumen degradable true protein to sheep and the microbial N supply to animals as estimated by purine derivative excretion in urine. *In vitro* studies indicated that peptides and amino acids were known to stimulate growth of rumen bacteria in pure cultures (CRUZ SOTO *et al.*, 1994). However, under *in vivo* conditions the effects of manipulation can be masked by other factors, especially those relating to interactions between more than 200 species of rumen microbes (DEWHURST *et al*., 2000).

CONCLUSION

Even though faba beans supplements statistically failed to produce significant impact on the efficiency of microbial protein synthesis, it numerically increased the estimate of microbial N flow to duodenum by 92% compared to that of the control group. However for supplemented group, there was no significant benefit of altering frequency of faba beans provision, as the estimated N supply to animal were similar when supplements provided to animals either once, twice, or eight times a day.

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