QUANTIFICATION OF THE EFFICIENCY OF RUMEN MICROBIAL PROTEIN SYNTHESIS IN STEERS FED GREEN TROPICAL GRASS

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ABSTRACT

The rate of rumen microbial crude protein (MCP) supply to the intestines is a crucial element in the current rumen models to predict respond of ruminants to a certain diet. Data from tropical pastures always below predicted results from the existing rumen models. Thus, quantification of the rumen MCP supply from tropical grass will improve predictive rate under tropical feeding conditions. Four Brahman crossbred steers (457±20.1 kg) were used in a metabolism study. Pangola grass (*Digitaria erianthe* cv. Steudal) was harvested every morning and fed to the animals soon after. Parameters measured were E_{MPS}, intake, fractional passage rates, and rumen ammonia concentration. The E_{MPS} was estimated using purine derivative excretion in urine. Crude protein and water soluble carbohydrates content were 6.3 and 7.4% of dry matter (DM) respectively. DM intake was 1.6% live weight. Average rumen ammonia concentration was 69 mg/L whilst rumen passage rates were 7.84 and 6.92 %/h for fluid and solids respectively. E_{MPS} was only 72 g MCP/kg digestible organic matter. It might be concluded that E_{MPS} in steers consuming green pangola grass was below the minimum level for forage diets adopted in the current feeding standards.

Keywords: microbial protein, efficiency, tropical grass, cattle.

ABSTRAK

KUALIFIKASI EFISIENSI SINTESIS MIKROBA RUMEN PADA SAPI JANTAN MUDA YANG DIBERI RUMPUT TROPIS SEGAR

Tingkat pasokan protein mikroba rumen (MCP) ke usus halus merupakan salah satu unsur kunci dalam meramal respon pertumbuhan ruminan terhadap ransum tertentu. Data MCP hijauan tropis selalu berada di bawah nilai prediksi model rumen yang dipakai saat ini. Dengan demikian, kuantifikasi pasokan MCP rumput tropis diharapkan menjadi masukan untuk meningkatkan kemampuan prediksi model rumen untuk pakan daerah tropis. Empat sapi jantan muda Brahman persilangan (457±20,1 kg) digunakan dalam sebuah penelitian metabolisme. Rumput pangola (*Digitaria erianthe* cv. Steudal) dipanen setiap pagi dan langsung diberikan kepada ternak dalam kandang metabolis. Parameter yang diukur adalah produksi MCP dan efisiensi sintesis MCP (E_{mps}), konsumsi, laju alir digesta, dan konsentrasi amonia rumen. Nilai E_{MPS} diestimasi menggunakan turunan purin dalam urin. Kandungan protein kasar dan karbohidrat mudah larut adalah 6.3 % and 7.4%. Rata-rata konsumsi BK adalah 1.6% berat badan. Konsentrasi amonia rumen 69 mg/L, sedangkan laju alir digesta cair sebesar 7.84 %/jam and padat sebesar 6.92 %/jam. Rata-rata E_{MPS} hanya 72 g MCP/kg bahan organik tercerna. Disimpulkan bahwa nilai E_{MPS} untuk rumput tropis segar yang dikonsumsi oleh sapi jantan berada di bawah nilai standar hijauan yang dipedomani dewasa ini.

Kata kunci: protein mikroba, efisiensi sintesis, rumput tropis, sapi.

INTRODUCTION

One vital factor in the current rumen models to predict respond of ruminants to a certain feeding regime is microbial crude protein (MCP) supply. The amount of MCP available for ruminants is dictated by the efficiency of its synthesis (E_{MPS}) in the rumen. The E_{MPS} is affected by many factors, such as diet quality, level of intake and rumen dilution rate, and varies considerably across diets. The E_{MPS} values for tropical grass hay reported in literatures ranged from 33 to 117 g MCP/kg DOM (McMeniman *et al.*, 1986; Poppi *et al.*, 1997; Prior *et al.*, 1998; Bowen, 2003; Mullik, 2006) which is lower than the values adopted in the current feeding standards

(SCA, 2007; AFRC, 1992; NRC, 2000). Although a higher efficiency value from high quality pangola grass (176 g MCP/kg DOM) was reported by Mullik (1999) but it may have not been accurate since pasture intake and digestibility were indirectly measured. There are also methodological problems in measuring urinary purine output through spot sample technique as used by Mullik (1999) and assuming that creatinine:purine derivative ratio is constant across diets and animals is still debatable. The present experiment was designed to measure the E_{MPS} of tropical grass (pangola) during the wet season and managed to provide high amounts of green leaf hence nutrient content.

MATERIALS AND METHODS

Experimental animals

Four Brahman crossbred steers (457±20.1 kg) were used in this study. The steers were vaccinated and drenched against internal and external parasites prior to the commencement of the study. They were held in feedlot pens and housed in metabolism crates on site for the duration of the study.

Experimental Design, Diet, and Treatment

There was only 1 treatment with 4 replicates (steers) to estimate the parameter, efficiency of MCP production, and compare it to the feeding standards. The steers were randomly allocated into metabolic crates. There was a two week preliminary and one week data collection period. The feed was freshly cut pangola grass. The grass was harvested daily and fed at 10% above voluntary intake determined in the last week of the adaptation period, and offered in 3 periods daily. Drinking water and mineral block were freely available at all times. Approximately 0.5 ha permanent pangola grass pasture was used to provide feed for the steers. The paddock was slashed, approximately 8 cm above ground, and fertilized with 320 kg diammonium phosphate/ha (18% N and 20% Phosphorus) and 130 kg urea/ha 6 weeks before the study commenced. Approximately 17 mm irrigation was applied after slashing and fertilizing. There was no more irrigation because of an adequate rainfall throughout the study (88.3 mm).

The steers were held in individual concrete pens (feedlot pens) during the first 11 d of the preliminary period and were moved onto metabolic crates on day 12. The steers were given 3 days to adapt to the metabolic crates before data collection began.

Experimental Procedures

Feed Intake

The freshly cut pangola grass was offered at 10% above voluntary intake, based on the intake during the last week in the preliminary period, three times daily at 08.00, 13.00 and 19.00 h. The morning portion was given soon after cutting and two other portions were spread on a large plastic sheet in a cool room at 4°C and fed at 14.00 and 19.00 h. Two samples were taken at morning feeding. One sample was weighed into a plastic bag, sealed and frozen. Another sample was dried in the oven at 55°C for dry matter (DM) and bulked at the end of the collection period. The same procedures applied for the refusal but daily refusals were taken and processed separately between animals. One sample of forage was also taken at each time of feeding (afternoon and evening), weighed and frozen. At the end of the collection period, frozen samples of feed offered at each time and refused were bulked within the sample times (without thawing), mixed and one sub-sample was taken, weighed, freeze dried, ground through 1 mm screen and stored for analysis of organic matter (OM), crude protein (CP), water soluble carbohydrates (WSC), and neutral detergent fibre (NDF).

Digestibility

Digestibility of DM, OM, CP, and NDF was calculated from intake and faecal data. Daily faecal output was measured by total collection into individual buckets placed under metabolism crates. The collection was done for 7 days in each treatment period. A 24 h faecal collection was homogenised, and approximately 5% of faeces produced by each animal was taken and bulked individually in plastic containers in a freezer. At the end of the collection period, the bulked samples were thawed at room temperature and 2 sub-samples were taken from each animal. One sub-sample was dried in an oven at 60^{o}C until constant weight (5 days) to obtain DM content, and discarded. Another sub-sample was frozen followed by freeze drying, and grinding prior to Nitrogen, OM and NDF analysis.

Passage Rates

Passage rates were estimated during the period in which digestibilities were measured. Fluid and particulate passage rate from the rumen were estimated using chromium-ethylenediamine tetraacetic acid (Cr-EDTA; 2 g Cr/animal) and Ytterbium trichloride hexahydrate ($YbCl_3.6H_2O$; 1 g Yb/animal) as external markers. A single dose of markers were done at Day 1 of the collection period. Dosing was done a few minutes prior to morning feeding. A faecal sample from each animal was taken before dosing to serve as a blank or base line in marker analysis and calculation. Subsequent faecal sampling (freshly voided faeces) was taken approximately at the following times : 12, 24, 32, 48, 56, 72, 80,

96, 104, 120, 132, and 144 h post dosing. The samples were oven-dried at 65° C, ground through 1 mm screen, and stored at room temperature prior to processing for maker analysis. The fractional and fluid passage rates were calculated from the slope of natural log of marker concentration against time. Only samples taken from 12 h to 84 h were used in the regression as they did not deviate from linearity determined by visual observation.

Rumen Ammonia-nitrogen Concentration

Two rumen fluid samples, collected on different occasions, were taken from each animal on the last day of the collection period. The first collection was done 3 to 4 h after morning feeding and the second sample was collected before morning feeding the next day (24 h after feeding).

Urine Sampling for Predicting Microbial Protein Synthesis

The MCP production was estimated by reference to PD (allantoin, uric acid, xanthine, and hypoxanthine) excretion in total urine and creatinine (Ct) excretion was also measured. Daily urine output of individual animals was measured by total collection into trays covered with a cloth filter to stop faecal contamination. pH of the urine was kept below 3 by adding approximately 200 mL 10% H_2SO_4 into individual trays prior to collection. Urine collected over 24 h was mixed and 5% was taken, bulked into a plastic container in a refrigerator over the collection period. Immediately at the end of each treatment period, 5 mL of the acidified sample was measured into a red cap plastic tube where 1 mL allopurinol (internal standard) had been added. The solution was made up to 50 mL using 0.1M $NH_4H_2PO_4$ buffer. This solution was then transferred into a clean labelled plastic container and frozen prior to analysis for Ct and PD.

Analytical Procedures

Analytical procedures for DM, OM, CP, NDF, using the method of Van Soest. Ammonia concentration determined by distillation technique. Purine derivatives and Creatinine were analysed using High pressure Liquid chromatography based on method proposed by Ballcell *et al.,* (1991). Concentration of WSC was determined by cold water extraction method (Thomas, 1977).

Concentrations of Cr and Yb in faecal samples were determined using the digestion method. Approximately 0.3 to 0.4 g dried ground sample was measured into 50 mL individual erlenmeyer flasks. A 15 mL solution of 5:1 nitric:perchloric acid was added and left to stand for 24 h. After standing, the flasks were placed on a preheated frypan (150^{o}C) and were allowed to digest at this temperature until all brown smoke was dissipated. The temperature was then increased to 300^{o}C and the samples were digested at this temperature for 1 h and followed by digestion at 400^{o}C for about 20 min. The flasks were removed and cooled. The residues were then transferred into 25 mL volumetric flasks and diluted to the mark using distilled water and marker concentration was determined using an ICP (Inductively Coupling Plasma Emission Spectrometer, M+P, Spectro Analytical).

Calculations

Microbial protein production was estimated from the excretion of PD in the urine based on formula of Chen and Gomez (1995) as described in Mullik (2006). Fractional passage rate was calculated by regressing the natural log of marker concentration in faecal samples against time and determining the slope which is the fractional passage rate (Grovum and Williams, 1973).

Statistical Methods

There was no statistical analysis as there were no treatments to compare. Rather standard deviation from the mean was calculated and results were compared to the literature. In particular the efficiency of MCP synthesis was compared to that adopted in the SCA (2007).

RESULTS AND DISCUSSION

Herbage Composition

Chemical composition is listed in Table 1. It appears that CP and WSC content of forage used in this experiment was quite low (only 63 and 74 g/kg DM for CP and WSC respectively). It was noticed that soil contamination in the forage occurred during harvesting.

The low CP content of freshly harvested pangola grass observed here (6.3%) is markedly lower than the values (in a range of 8.1% to 15.8%) reported by Mullik (1999) for the same grass and location. This is surprising because the pasture was fertilized with DAP and urea after slashing. The grass was harvested only once a day at 0745 h, and the morning portion was fed to the animals within 15 min after harvesting whereas the afternoon portions were stored in a cool room at a temperature of 4 °C and fed at 1400 and 1900 h. This feeding method seems to have had no effect on chemical analysis as

Table1. Chemical composition per kilogram dry matter (DM) of freshly harvested pangola grass fed to steers in metabolism crates over 7 day

Nutrients	
Dry matter (g/kg feed)	247
Organic matter (g/kg DM)	922
Crude protein (g/kg DM)	63
Water soluble carbohydrates (g/kg DM)	74
Neutral detergent fibre (g/kg DM)	680

there was only a small difference in CP content between morning and afternoon feeding.

The WSC content of the grass used here was also low (74 g WSC/kg DM). The low WSC observed here is consistent with values for pangola grass and 2 other tropical grasses (setaria and buffel grass) cut during summer reported by Hunter *et al.* (1970). Among the samples analysed by these authors only GS of setaria grass contained 95 g WSC/kg DM, which is above the minimum value (90 g WSC/kg DM) suggested to affect net energy value of forage (Corbett *et al.,* 1966). The WSC content of temperate grasses is usually much higher (Davies *et al.,* 1991; Fulkerson and Trevaskis, 1997).

The WSC content is influenced by solar radiation and balance of photosynthesis and respiration processes within plant, so its level fluctuates markedly within a day with the lowest concentration observed in the early morning due to the respiration process during the night (Humpreys, 1991; Fulkerson *et al.*, 1994). This is probably one of the factors contributing to the low WSC observed here because the grass was harvested early in the morning (0745 h). Fulkerson and Trevaski (1997) showed that the highest WSC content was around 1800h in the afternoon.

The objective in harvesting fresh pangola grass and feeding in pens was to obtain pasture of high quality which would provide data comparable to that from grazing animals. On the basis of chemical composition this was not successful yet the results are very interesting in that they confirm that very low values of E_{MPS} occur in tropical pastures.

Intake and Digestibility

Data of intake and digestibility is shown in Table 2. Dry matter intake was only 1.5% of the body weight (W). The intake of CP was only 469 g/d equal to 71 g CP/kg OM. Digestibility of DM (60%) and OM (69%) was quite high for this grass.

The extent of voluntary feed intake is determined by interplay between plant properties, activity of rumen microbes, and passage of particles from the rumen. This interrelationship suggests that using simple general relationships between intake and measures of feed chemical composition, feed digestibility, or feed physical properties are most likely to be less than satisfactory (Wilson and Kennedy, 1996). However, it has been well established that there is a close relationship between intake and chemical and physical characteristics of the forage (Milford and Minson, 1966; Hodgson, 1982; 1984). The mean DM intake of steers in the present study was only 1.6% W, a value similar to that recorded previously with forage of this quality (Minson, 1982; 1990).

Table 2. Intake and digestibility of nutrients by steers given freshly harvested pangola grass in metabolism crates over 7 d. The values are the mean of 4 animals. Standard deviation (SD) from the mean is shown

Nutrients	Mean	SD
Intake :		
Dry matter (kg/d)	7.05	1.070
Dry matter (% W)	1.57	0.218
Organic matter (kg/d)	6.56	0.992
Digestible organic matter (kg/d)	4.49	0.579
Crude protein (g/d)	469	71.2
Water soluble carbohydrates (g/d)	522	86.8
Neutral detergent fibre (kg/d)	4.79	0.152
Digestibility :		
Dry matter (%)	59.7	1.71
Organic matter (%)	68.6	2.06
Crude protein (%)	52.3	0.92
Neutral detergent fibre (%)	69.9	1.21

Rumen Fermentation

Mean concentration of NH_3-N in the rumen fluid of steers in this experiment measured 3 and 24 h after morning feeding were 58,2 and 60,7 mg NH_3-N/L respectively. These values were above the minimum level (50 mg NH_3-N/L) for effective rumen microbial activity as suggested by Satter and Slyter (1974). Since CP content of the grass was quite low, there might be a significant contribution of recycled urea into the rumen. Evidence suggests that for ruminants consuming low quality forages (<6% CP/kg DM) urea recycling plays an important part in meeting requirement of N in the rumen (Norton, 1982; 1984). A stable rumen NH_3-N concentration as found here might be explained by the fact that the steers were fed 3 times a day and the feed refusals were usually greater than 2 kg/d so there appeared to be no times that food was not present.

Fractional Passage Rate

Mean passage rates of fluid and particulate markers (Cr and Yb respectively) from the rumen estimated from their concentration in faecal samples is illustrated Table 3. Estimated fluid passage rate from the rumen was 10.0% /h which was higher than that of particulate passage rates (6.7% /h).

Table 3. The slope, intercept and coefficient determination of regression lines of fluid and particulate passage rate in the rumen of steers given freshly harvested pangola grass in metabolism crates over 7 d. Standard deviation (SD) from the mean is shown

Parameter	Mean	SD
Fluid passage rate:		
Intercept (a)	7.84	0.382
Slope (b)	0.100	0.022
Coefficient determination (r^2)	99.1	0.559
Particulate passage rate:		
Intercept (a)	6.92	0.336
Slope (b)	0.067	0.018
Coefficient determination (r^2)	98.6	1.249

Table 4. Creatinine and purine derivative(PD) excretion, and estimated microbial crude protein (MCP) synthesis in steers given freshly harvested pangola grass in metabolism crates over 7 d. Only uric acid and allantoin were used in the total PD since concentration of xanthine and hypoxanthine in urine samples was very small. The values are the mean of 4 animals. Standard deviation (SD) from the mean is shown

Parameter	Mean	SD
Excreted :		
Creatinine (mmol/d)	115	6.4
Creatinine (mmol/kg metabolic weight)	1.17	0.060
Total purine derivatives (mmol/d)	101	3.9
Allantoin (mmol/d)	93	3.4
Uric acid (mmol/d)	7	0.4
Molar ratio of PD/Creatinine	0.88	0.102
Molar ratio of allantoin/creatinine	0.80	0.104
PD Absorbed (mmol/d)	69.6	24.90.
Estimated MCP production:		
g MCP/ d	316	113.5
g MCP/kg metabolic weight	3.20	1.118
g MCP/kg digestible organic matter	71.8	15.44

The rate of particulate (6.7 %/h) and fluid (10.0 %/h) dilution observed here was reasonably high and this is usually associated with a high E_{MPS} (AFRC, 1992) but this did not occur here. Fractional flow rates observed in this study were similar to fast fractional outflow rates observed by De Vega and Poppi (1997; 6.7 and 10.1 %/h for particulate and fluid respectively) in sheep fed pangola hay and administered with labelled undigested pangola particles and Cr-EDTA. It appears that the predominant limiting factor for this experiment was RDP adequacy (see below).

Rumen Microbial Crude Protein

Excretion of Ct and PD, and estimated MCP synthesis are listed in Table 4. Daily Ct excretion was 115.26 mmol/d or 1.17 mmol/kg $W^{0.75}$. Allantoin was the predominant compound (91%) in the total PD excreted. The remaining (9%) was uric acid. The molar ratio of PD/Ct was 0.88. The mean value of E_{MPS} was only 71.8 g MCP/kg DOM.

Quantitative data on MCP supply, as affected by E_{MPS}, is crucial in predicting the growth response of cattle more accurately under different feeding strategies. However, the complexity and high cost of the methods employed for quantification of MCP in the past resulted in a very limited available database particularly for tropical forages. With the development of a new method using excretion of PD in the urine to estimate MCP supply, quantifying MCP synthesis over a wide variety of feeds can now be done easily and cheaply (Chen and Gomes, 1995).

The main objective of this experiment was to quantify the E_{MPS} of pangola grass fed in a fresh state in a cut and carry system in an attempt to simulate conditions of wet season growth rather than the low quality hay. Pangola grass is one of the tropical grasses used extensively in tropical areas. It should be stressed that mechanically harvesting the grass and feeding to the animals in pens, as in this experiment might not represent the real situation for grazing animals which can select high amounts of green leaf of higher nutritive value than in the cut and carry system (Minson, 1981). However, an attempt was made to provide the highest quality material by harvesting regrowth grass at 5 to 6 weeks after slashing and fertilizing where the proportion of GL was high (51 % of DM) and total green material (GL and GS) was 90% of the available forage so selectivity would be minimum. The main reason underlying the decision to use a pen feeding system in this study was to accurately predict MCP synthesis by collecting all urine as the spot sampling methodology had major limitations.

The E_{MPS} observed here was only 71.8 g MCP/kg DOM which was only 55% of the minimum value (130 g MCP/kg DOM) suggested for forage based diets (SCA, 2007). The E_{MPS} reported here was similar to those of tropical hays (Prior *et al.,* 1998; Bolam *et al.,* 1998; Bowen, 2003; Mullik, 2006; Marsetyo, 2007). This is surprising because green forages are expected to have a much better E_{MPS} than dried ones. The E_{MPS} value under this experimental condition even lower than the value (90 g/kg DOM) reported by Marsetyo (2007) for the same breed of cattle given green panic hay (5.7%).

The probable argument for this low E_{MPS} is inadequacy of RDP and energy particularly WSC. The CP content of the grass used here was only 6.3% (Table 1). It is clear from intake data (Table 2) that CP intake was only 71 g CP/kg OM or 104 g MCP/kg DOM. Assuming that degradability of CP in the rumen is 75% (McLennan *et al.,* 1997) then the RDP availability would be only 53 g RDP/kg OM or 78 g RDP/kg DOM. This calculation clearly shows that RDP supply was far below the recommended level (130 to 170 g RDP/kg DOM) by the current feeding systems (SCA, 2007; NRC, 1996). So, any feeding strategies to provide extra RDP is likely to be effective in improving E_{MPS} under this feeding condition. Predicted E_{MPS} in the current study, according to the above feeding standards, is around 78 g MCP/kg DOM which is close to the actual value (72 g MCP/kg DOM) observed here.

The importance of WSC in determining microbial growth has been proposed (Corbett *et al.*, 1966; Beever *et al.*, 1978; Dove and Milne, 1994). Whilst quantitative aspects of WSC have not been established, particularly the ratio of WSC and RDP, earlier experiments (e.g. Corbett *et al.*, 1966) indicated that diets containing WSC lower than 90 g/kg DM had a lower net energy value. A recent study (Dove and Milne, 1994) observed a two

fold increased in E_{MPS} in sheep grazing spring/summer pasture above those grazing autumn pasture. These authors related this improvement to the WSC of pasture though WSC was not directly measured. The WSC content of the grass used in the present experiment was only 74 g WSC/kg DM. This value agreed with values for fresh pangola grass reported by Hunter *et al*. (1970). These researchers showed total sugars in the stem fraction of pangola grass was 70 g/kg DM whereas green leaf contained only 25 g/kg DM.

The E_{MPS} in steers given freshly harvested pangola grass used in this study was only 71.8 g MCP/kg DOM which is much lower than the values set for forage diets in the current feeding standards. This low E_{MPS} most probably stems from deficiency of RDP and WSC in this diet.

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