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## **Efficiency of Microbial Protein Synthesis in Steers Fed Freshly Harvested Tropical Grass**

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### **INTRODUCTION**

Rumen microbial crude protein (MCP) supply is a vital element in the rumen models to predict response of ruminants to a certain feeding regime. The MCP production is dictated by the efficiency of MCP synthesis ( $E_{MPS}$ ) calculated as g MCP/kg digestible organic matter (DOM). Data from tropical pastures ranged from 33 to 117 g MCP/kg DOM (Poppi *et al.*, 1997; Prior *et al.*, 1998; Mullik, 1998; Mullik, 2007) which is lower than the values adopted in the current feeding standards (SCA, 1990; AFRC, 1992; NRC, 2000). This is due to the fact that database used in the models are derived mainly from temperate pastures. Although a higher efficiency value from high quality tropical 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  $E_{MPS}$  for fertilized tropical grass (pangola) during the wet (growing) season and managed to provide high amounts of green leaf.

### **MATERIALS AND METHODS**

#### **Experimental animals**

Four Brahman crossbred steers ( $457 \pm 20.1$  kg) were used in this study. The steers were vaccinated and drenched against internal and external parasites prior to the commencement of the study.

#### **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. Diet 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. 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.

## **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), NSC, neutral detergent fibre (NDF), and acid detergent fibre (ADF). Another sub-sample of feed offered and refused was also taken, freeze-dried and sorted to green leaf (GL), green stem (GS), and dead matter (D).

### ***Digestibility***

Digestibility of DM, OM, crude protein (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. 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°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 N, 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<sub>3</sub>.6H<sub>2</sub>O; 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 marker 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 pH and 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***

Microbial protein 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<sub>2</sub>SO<sub>4</sub> 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<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 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). The principles for ADF analysis were similar to NDF but acid detergent reagent was used instead of neutral detergent reagent. In addition, sodium nitrate was not used in ADF analysis. Concentration of WSC (total sucrose) was measured 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 frying pan (150°C) and were allowed to digest at this temperature until all brown smoke was dissipated. The temperature was then increased to 300°C and the samples were digested at this temperature for 1 h and followed by digestion at 400°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 as described by Chen and Gomez (1995). 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 (Grovmum 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 (1990).

## **RESULTS**

### ***Herbage composition***

Herbage fraction composition in the harvested grass offered was 50.8% green leaf (GL), 39.1% green stem (GS) and 10.1% dead material (D) on a DM basis. 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). Mean CP content in individual herbage fractions (GL, GS and D) was similar to the value obtained from the whole plant subsample (63.9 vs 63.4 g CP/kg DM for the former and the latter respectively) so only the latter is presented. It was noticed that soil contamination in the forage occurred during harvesting.

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

<b>Nutrients</b>	<b>Content</b>
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

### **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.

**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
<b>Intake :</b>		
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
<b>Digestibility :</b>		
Dry matter (%)	59.7	1.71
Organic matter (%)	68.6	2.06
Neutral detergent fibre (%)	69.9	1.21

***Rumen pH and ammonia concentration***

The pH was a slightly higher in the rumen liquor taken at 3 (7.14) than 24 h (7.43) after the morning feeding. The mean concentration of the rumen NH<sub>3</sub>-N (Table 3) indicated that the values were above the minimum level (50 mg NH<sub>3</sub>-N/L) for effective rumen microbial activity.

***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**

<b>Parameter</b>	<b>Mean</b>	<b>SD</b>
Rumen NH <sub>3</sub> -N (mg NH <sub>3</sub> -N/L)	59.4	17.05
Fluid passage rate (%/h)	10.0	0.22
Particulate passage rate (%/h)	7.7	0.18
Production of Microbial Crude Protein (g/ d)	316	113.5
Efficiency of rumen microbial Protein synthesis (g/kg DOM)	71.8	15.44

### ***Microbial protein synthesis***

Excretion of Ct and PD, and estimated MCP synthesis are listed in Table 3. Daily Ct excretion was 115.26 mmol/d or 1.17 mmol/kg W<sup>0.75</sup>. 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<sub>MPS</sub> was only 71.8 g MCP/kg DOM.

## **DISCUSSION**

### ***Efficiency of microbial protein synthesis***

Quantitative data on MCP supply, as affected by E<sub>MPS</sub>, 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<sub>MPS</sub> 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, 1990). The  $E_{MPS}$  reported here was similar to those of tropical hays (Prior *et al.*, 1998; Bolam *et al.*, 1998). This is unexpected since green forages are expected to have a much better  $E_{MPS}$  than dried ones.

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, 1990; 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 increase 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 GL contained only 25 g/kg DM.

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 as discussed earlier.

#### ***Nutrient composition, intake and digestibility***

The low CP content of freshly harvested pangola grass observed here (9.0, 3.6, and 4.1% for GL, GS and D respectively). 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 1300 and 1900 h. This feeding method seems to have had no effect on chemical analysis as there was only a small difference in CP content between morning and afternoon feeding

(9.9 vs 8.1%, 4.2 vs 3.0%, and 4.5 vs 3.7% for GL, GS, and D of morning vs afternoon samples respectively).

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 (Humphreys, 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.

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; 1990a).

### ***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 (58 and 61 mg  $NH_3-N/L$  respectively) was slightly above the minimum value suggested (Satter and Slyter, 1974) to be the baseline for optimum rumen function. As 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.

## CONCLUSION

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.

## REFERENCES

- AFRC. 1992. Nutritive requirements of ruminants: protein nutrition. *Abstracts and Reviews*, 62:787-835.
- Balcells, J., Guada, J.A., Castrillo, C., and Gasa, J. 1991. Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum. *J. Agric. Sci.*, 116:309-317.
- Beever, D.E., Terry, R.A., Cammell, S.B., and Wallace, A.S. 1978. The digestion of spring and autumn harvested perennial ryegrass by sheep. *J. Agric. Sci.*, 90:463-470. Cambridge.
- Bolam, M.J., Connors, M.T., McLennan, S.R., Poppi, D.P. 1998. Variability in microbial protein supply under different supplementation strategies. *Anim. Prod. Aust.*, 22:398.
- Chen, X.B., and M.J. Gomes. 1995. *Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of the Technical Details*. Occasional Publication, Rowett Research Institute, UK.
- Corbett, J.L., Langlands, J.P., McDonald, I., and Pullar, J.D. 1966. Comparison by direct energy calorimetry of net energy values of an early and late season growth of herbage. *Anim. Prod.*, 8:13-27.
- Daveis, D.A., Forthergill, M., and Jones, D. 1991. Assessment of contrasting perennial ryegrass, with and without white clover, under continuous sheep stocking in the uplands. 3. Herbage production, quality and intake. *Grass and Forage Sci.*, 46:39-49.
- De Vega, A., and Poppi, D.P. 1997. Extent of digestion and rumen condition as factor affection passage of liquid and digesta particles in sheep. *J. Agri. Sci.*, 128:207-215. Cambridge.
- Dove, H., and Milne, J.A. 1994. Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. *Aust. J. Agric. Res.*, 45:1229-1245.
- Fulkerson, W.J., and Trevaskis, L. 1997. Limitations to milk production from tropical pasture. *Recent Advances in Animal Nutrition in Australia*, pp 159-165. Dept. Anim. Sci., UNE, Armidale. Australia.
- Fulkerson, W.J., Slack, K., and Lowe, K.J. 1994. Variations in the response of lolium genotypes to defoliation. *Aust. J. Agric. Res.*, 45:1309-1317.
- Grovum, W.L., and Williams, V.J. 1973. Rate of passage of digesta in sheep: IV passage of marker through elementary tract and the biological relevance of rate constant derived from the change in concentration of marker in the faeces. *Br.J. Nutr.*, 30:313-329.
- Hodgson, J. 1982. Influenced of sward characteristics on diet selection and herbage intake by grazing animals. In *Nutritional Limits to Animals Production From Pastures*, pp. 153-166. Editor: J.B. Hacker. CAB. Franham Royal, U.K.
- Humphries, L. R. 1991. *Tropical Pasture Utilization*. Cambridge University Press. Cambridge, U.K.
- Hunter, R.A., McIntyre, B.L., and McIlroy, R.J. 1970. Water-soluble carbohydrates of tropical pasture grasses and legumes. *J. Sci. Food Agric*, 21:400-405.
- McLennan, S.R., Kidd, J.F., Hendricksen, R.E., Jeffery, M., and Poppi, D.P. 1997. Seasonal changes in the degradability of protein in a tropical grass pasture. In *Recent Advances in Animal*

- Nutrition in Australia*, p. 252. Editors: J.J. Corbett, J.V. Nolan and J.B. Rowe. Dept. Anim. Sci., The University of New England, Armidale, Australia.
- McMeniman, N.P., Beale, I.F., and Murphy, G.M. 1986. Nutritional evaluation of south east Queensland pastures. II. The intake and digestion of organic matter and nitrogen by sheep grazing on mitchell grass and mulga grassland association. *Aust. J. Agric. Res.*, 37:303-314.
- Milford, R. and Minson, D.J. 1966. Intake of tropical pasture species. *Proc. 9<sup>th</sup> Int. Grassland Cong.*, pp.814-822. Sao Paolo.
- Minson, D.J. 1981. The measurement of digestibility and voluntary intake of forage with confined animals. In *Forage Evaluation : Concepts and Techniques*, pp.159-174. Editors: J.L. Wheeler and R.D. Mochrie. Melbourne.
- Minson, D.J. 1982. Effects of chemical and physical composition of forage eaten upon intake. In *Nutritional Limits to Animals Production From Pastures*, pp. 167-182. Editor: J.B. Hacker. CAB. Franham Royal, U.K.
- Minson, D.J. 1990a. *Forage in ruminant nutrition*. Academic Press.
- Mullik, M.L. 1998. Efficiency of Rumen Microbial Protein Synthesis in Steers Fed a Tropical Grass Hay and Supplemented With Whole Cottonseed. *Bul.Nutr.* 1(3):1-6
- Mullik, M.L. 1999. Strategies to Increase Efficiency of Rumen Microbial Protein Synthesis and Productivity of Cattle on A Tropical Grass. Ph.D Thesis. School of Land and Food. The University of Queensland.
- Mullik, M.L. 2007. Strategic Supplementation to Increase Efficiency of Rumen Microbial Protein Synthesis in Steer Fed Tropical Grass Hay. *Journal Ilmu Ternak dan Veterinar*, 11(1):15-23.
- National Research Council (NRC). 2000. *Nutrient Requirement of Beef Cattle*. 8<sup>th</sup> Edition. National Academy Press
- Norton, B.W. 1982. Differences between species in forage quality. In *Nutritional Limits to Animals Production From Pastures*, pp. 89-110. Editor: J.B. Hacker. CAB. Franham Royal, U.K.
- Norton, B.W. 1984. The practical significance of nitrogen recycling in ruminants. *Proc. Soc. Anim. Prod. Aust.*, 15:108-111.
- Poppi, D.P., McLennan, S.R., Badiye, S., de Vega, A., and Zoririlla-Rios, J. 1997. Forage quality and utilization, *Proc. Int. Grassland Cong.* Canada. In Press.
- Prior, S.J., Poppi, D.P., and McLennan, S.R. 1998. Microbial protein production in cattle fed ryegrass, buffel grass, and spear grass. *Anim. Prod. Aust.*, 22:315.
- Satter, L.D., and Slyter, L.L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *B. J. Nutr.*, 32:199-208.
- SCA. 1990. *Feeding Standards For Australian Livestock*. Standing Committee on Agriculture, CSIRO, publications. Melbourne, Australia.
- Thomas, A. 1977. An automated procedure for the determination of soluble carbohydrates in herbage. *J. Sci., Food Agric.*, 28:639-642.
- Wilson, J.R., and Kennedy, P.M. 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. *Aust. J. Agric. Res.*, 47:199-225.