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Digestion of forages in the rumen is increased by the amount but not the type of protein supplement

T. Panjaitan[A,E], *S. P. Quigley*[B], *S. R. McLennan*[C], *A. J. Swain*[D] *and D. P. Poppi*[B]

[A]Balai Pengkajian Teknologi Pertanian Nusa Tenggara Barat, Jl. Raya Peninjauan Narmada, Nusa Tenggara Barat 83371, Indonesia.
[B]School of Agriculture and Food Sciences, The University of Queensland, Gatton, Qld 4343, Australia.
[C]The University of Queensland, Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, Ecosciences Precinct, GPO Box 267, Brisbane, Qld 4001, Australia.
[D]Formerly Queensland Department of Agriculture, Fisheries and Forestry, Dutton Park, Qld 4102, Australia.
[E]Corresponding author. Email: tanda_panjaitan@yahoo.com

Abstract. Three polyester bag experiments were conducted with fistulated *Bos indicus* steers to determine the effect of the amount and type of nitrogen (N) supplement on the digestion rate of forages different in quality. In Experiment 1, test substrates were incubated in polyester bags in the rumen of steers fed ryegrass, pangola grass, speargrass and Mitchell grass hays in a 4 by 4 Latin-square design. In Experiment 2, test substrates were incubated in polyester bags in the rumen of steers fed speargrass hay supplemented with urea and ammonium sulfate (US), branched-chain amino acids with US (USAA), casein, cottonseed meal, yeast and Chlorella algae in a 7 by 3 incomplete Latin-square design. In Experiment 3, test substrates were incubated in polyester bags in the rumen of steers fed Mitchell grass hay supplemented with increasing amounts of US or Spirulina algae (*Spirulina platensis*). The test substrates used in all experiments were speargrass, Mitchell grass, pangola grass or ryegrass hays. Digestion rate of the ryegrass substrate was higher than that of the speargrass substrate ($P < 0.05$) in Experiment 1. Supplementation with various N sources increased the degradation rate and effective degradability of all incubated substrates above that apparent in Control steers ($P < 0.05$; Experiment 2). Supplementation of US and Spirulina increased degradation rate and effective degradability of ryegrass, pangola grass and Mitchell grass substrates above that apparent in Control steers ($P < 0.05$; Experiment 3). However, there was no further response on digestion rate of the substrates in increasing supplementation levels either for US or Spirulina. In conclusion, rate of digestion was affected by forage physical and anatomical properties. Supplementation with various N sources increased rate of digestion when the Control forage ration was very low in N but once a minimum level of N supplementation was reached, irrespective of form of N or other potential growth factors, there was no further increase in rate of digestion.

Additional keywords: feed quality, polyester bag, rumen function, tropical forages.

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Introduction

Beef cattle production from tropical pasture is subject to extreme climatic conditions, which impact on the quantity and quality of forage supply. The pastures grow rapidly to maturity following the commencement of seasonal rainfall resulting in a decline in crude protein (CP) content and dry matter (DM) digestibility as the dry season approaches. Tropical grasses are characterised by a distinct anatomical structure and architecture to allow rapid growth. This rapid growth is associated with rapid stem development and a composition high in cell wall content of both leaf and stem and low in nitrogen (N) content as part of the anatomical structure associated with the C_4 photosynthetic pathway. It is also high in lignified cell wall content and high in N bound to lignin thus high in insoluble N (Humphreys 1980; Wilson 1991; Van Soest 1994; Coleman *et al.* 2004). In addition, as tropical grasses mature the cell wall proportion increases while N, phosphorus and sulfur content declines (Minson 1990). Low N or low rumen-degradable protein (RDP) content of the diets result in low rumen ammonia concentration. Low N with subsequent low rumen ammonia-N are known to affect digestion rate (Ortiz-Rubio *et al*. 2007).

Ammonia is the main source of N utilised by rumen microbes. Satter and Slyter (1974) reported that the minimum rumen ammonia-N concentration for optimal rumen function is 50 mg NH_3-N/L. Boniface *et al*. (1986) reported that in cattle consuming low-quality speargrass hay infused with urea the maximum DM disappearance of speargrass hay from polyester bags occurred at a rumen ammonia concentration of ~45 mg NH_3-N/L. True protein supplements can stimulate growth of rumen bacteria and enhance fibre digestion more than simple

inorganic N (Cruz Soto *et al*. 1994; Van Soest 1994). In addition to protein, other organic molecules such as vitamins, minerals and other growth factors may enhance fibre digestion. Algae have many favourable nutrients such as protein, nucleic acids, fatty acids, fermentable carbohydrates, vitamins, minerals and possible growth factors that have the potential to improve digestion rate. Thus digestion rate of high and low digestibility forage types may respond differently to the source of N used as a supplement. This study examined different N sources for their effects on digestion rate of substrates, which varied from low to high digestibility characteristics.

Materials and methods

These experiments were conducted at The University of Queensland, Mt Cotton Research Farm, Queensland, Australia. All procedures used in these experiments were conducted in accordance with the guidelines of the Australia Code of Practice for the Care and Use of Animals for Scientific Purpose and were reviewed and approved by The University of Queensland Animal Ethics Committee.

Animals and experimental design

Fistulated *Bos indicus* steers were used in each experiment and were maintained in individual pens with feed intake, digestibility, microbial protein production and rumen ammonia-N concentration measured before the commencement of each polyester bag study. A full description of the animals, feeding procedures and results pertaining to intake, digestibility, microbial protein production and rumen NH_3-N concentration has been published elsewhere for Experiments 1 (Panjaitan *et al*. 2010) and 3 (Panjaitan *et al*. 2014). The description of the materials and methods presented here refers specifically to the polyester bag studies conducted within each of the three larger experiments.

In each of the three experiments, polyester bags (24 cm × 10 cm, with a pore size of 45 μm, Allied Filter Fabrics; Sydney, NSW, Australia) were incubated in the rumen of steers for between 0 and 144 h and were withdrawn from the rumen at designated times. The bags were incubated in duplicate at each time, except for 0 h, which was in quadruplicate. At the end of each incubation period, polyester bags were removed from the rumen, washed, oven-dried at 65°C and weighed to determine DM disappearance. The test substrates that were weighed into the polyester bags and dried at 65°C before incubation were speargrass [923 g organic matter (OM), 24 g CP, 746 g neutral detergent fibre (NDF)/kg DM], Mitchell grass (898 g OM, 47 g CP, 603 g NDF/kg DM), pangola grass (926 g OM, 158 g CP, 784 g NDF/kg DM) and ryegrass (891 g OM, 311 g CP, 599 g NDF/kg DM) hays. Not all test substrates were evaluated in all three experiments, and the specific details are provided below. The test substrates were from the same source and batch of hay as the treatment diets used in some of the experiments and were therefore the same quality as the basal diet offered.

In Experiment 1, four steers were fed speargrass, Mitchell grass, pangola grass and ryegrass hays *ad libitum* in a 4 by 4 Latin-square design. The steers were offered their daily feed allowance at 0800 hours each day. The test substrates used in this experiment were speargrass and ryegrass hays both of which were incubated in polyester bags in the rumen of each steer for 0, 4, 8, 14, 24, 48, 72 and 96 h within the 4 by 4 Latin-square design for animal allocation to diet.

In Experiment 2, seven steers were fed speargrass hay (Control) or speargrass hay supplemented with non-protein N, (urea) and ammonium sulfate were mixed at 4 : 1 (w/w) (US), USAA (Urea and ammonium sulfate) plus a mixture of amino acids including branched-chain amino acids (BCAA; leucine, isoleucine, valine and phenylalanine) and the BCAA were mixed in the same proportion as present in casein, casein (sodium caseinate), cottonseed meal (CSM; 926 g OM, 478 g CP, 283 g NDF/kg DM), yeast (931 g OM, 516 g CP, 7 g NDF/kg DM) and Chlorella algae (943 g OM, 649 g CP, 35 g NDF/kg DM) in a 7 by 3 incomplete Latin-square design. The basal diet was offered at 0.5 kg digestible organic matter (DOM)/100 kg liveweight (W).day and supplements were administered through the cannula in amounts so as to supply 170 g RDP/kg DOM. Daily feed and supplement allowances were divided in two and were offered at 0830 hours and 1500 hours, with hay offered immediately after supplement administration at both times. The test substrates used in this experiment were speargrass, Mitchell grass and ryegrass hays, which were incubated in polyester bags in the rumen of each steer for 0, 3, 7, 10, 14, 24, 48, 72 and 96 h.

In Experiment 3, seven steers were fed Mitchell grass hay supplemented with increasing amounts of non-protein N, in the form of US (4 : 1) or Spirulina algae (S) in an incomplete Latin-square design. Rate of digestion was measured in steers (n =7/experimental period) offered Mitchell grass hay only (Control), and in steers that received the US supplement at estimated 90, 170 and 210 g RDP/kg DOM (US90, US170, US210) and in steers that received the S supplement at estimated 90, 170 and 290 g RDP/kg DOM (S90, S170, S290) in a 7 by 3 incomplete Latin-square design. The US was not supplied at the comparable highest amount relative to S due to potential risks of urea toxicity. The US supplement was diluted in water and sprayed evenly throughout the daily hay allowance. The S supplement was administered directly through the cannula before feeding each day. Hay was offered at 90% of *ad libitum* intake, determined during a 2-week preliminary period during each experimental run, once daily at 0800 hours. The test substrates were Mitchell grass, pangola grass and ryegrass. Mitchell grass and pangola grass were incubated in polyester bags in the rumen of steers for 0, 3, 6, 9, 12, 24, 48, 96, 120 and 144 h while ryegrass was incubated in the rumen of steers for 0, 3, 6, 9, 12, 24, 48 and 96 h.

Calculations

Dry matter disappearance of incubated substrate in the rumen was determined by measuring the residual substrate of the sample. Dry matter disappearance at each time was plotted against time and the disappearance data was fitted to the equation

$$Y = a + b[1 - \exp(-ct)],$$

where a = immediately soluble fraction, b = slowly fermentable fraction, $a + b$ = potential degradable fraction, c = degradation rate per h, and t = incubation time (h).

The effective degradability (ED) in the rumen is the function of the size of pools a and b, rate of degradation (c) of pool b and

the fractional rate of passage of pool *b* from the rumen and assumed to be either 0.02 or 0.05 (Ørskov and McDonald 1979).

Statistical analyses

Each variable derived from the fitted curve was then analysed with a mixed model using restricted maximum likelihood. The fixed effects were hay (Experiment 1) or type and amount of supplement (Experiments 2 and 3), incubation substrate and their interaction. Approximate *F*-tests were used to assess the significance of the fixed effects. If the interaction was not significant ($P > 0.05$) it was removed and the model refitted. Approximate pair-wise comparisons were made between means for significant fixed effects. The 5% level was used to assess statistical significance in all cases. All analyses were done using the statistical package Genstat 2007 (Genstat for Windows, 10th edition, VSN International, Hemel Hempstead, UK).

Results

Experiment 1

The potential degradability ($a + b$) of the ryegrass substrate was higher than that of speargrass substrate ($P < 0.05$) in all treatment diets. There were no differences in the slowly fermentable fraction (b) and the potential degradability of each of the substrates when incubated in the four forage diets. In general, *a*, *b*, *c* and ED of DM of the ryegrass substrate were higher in steers fed better quality forages ($P < 0.05$; Table 1); these parameters did not vary for the speargrass substrate with the exception of ED, which was higher when the steers were consuming pangola and ryegrass hays. Steers fed ryegrass hay had a higher rumen NH_3-N concentration (191.0 mg/L) than the steers fed pangola grass (57.1 mg/L), speargrass (48.5 mg/L) and Mitchell grass (31.3 mg/L). The rumen NH_3-N concentration of steers fed speargrass and Mitchell grass hays were both below the minimum concentration suggested for microbial function (50 mg/L).

Experiment 2

The slowly fermentable fraction (*b*), potential degradable fraction ($a + b$), degradation rate and ED were all greatest in ryegrass, followed by Mitchell grass and speargrass substrates for all treatments ($P < 0.05$; Table 2). All N supplements increased the degradation rate and ED of all incubated substrates above that apparent in Control steers ($P < 0.05$), with the exception of the US-supplemented steers where the degradation rate was not different to the Control ($P > 0.05$). There was no difference in ED of substrates in the rumen of steers administered the USAA, casein, algae, yeast or CSM supplements ($P > 0.05$) except that the yeast supplement significantly increased ED over USAA. The Control steers had significantly lower rumen NH_3-N concentration (30.6 mg/L) than all supplementation treatments, which ranged between 93.7 mg/L and 270.2 mg/L.

Experiment 3

The potential degradable fraction ($a + b$) and ED of DM were greatest in ryegrass, followed by pangola grass and Mitchell grass substrates (Table 3). Both US and S supplementation increased degradation rate and ED of ryegrass, pangola grass and Mitchell grass substrates above that apparent in the unsupplemented steers ($P < 0.05$). The response in ED was greater in the rumen of steers offered the S290 than the other S supplement levels and the US supplement ($P < 0.05$). An interaction between substrates and supplements was observed for degradation rate. However, there was no difference in degradation rate between supplements supplied at the same level of RDP within each substrate. The mean degradation rate (*c* value, per h) for ryegrass, pangola grass and Mitchell grass in

Table 1. The immediately soluble fraction (*a*), slowly fermentable fraction (*b*), fractional degradation rate of *b* (*c*) and effective degradability (ED) of the dry matter (DM) of speargrass and ryegrass hay substrates incubated in the rumen of steers fed speargrass, Mitchell grass, pangola grass and ryegrass hay *ad libitum*

ED, effective degradability at rumen outflow rate 0.05/h. Means within a column followed by the same letter are not significantly different (at $P = 0.05$)

Incubated substrate	Diet	*a* (%)	*b* (%)	*c* (per h)	ED (% DM)
Speargrass	Speargrass	7.6b	55.5c	0.021d	23.0e
Speargrass	Mitchell grass	8.5b	67.8bc	0.014d	22.2e
Speargrass	Pangola grass	7.7b	62.8c	0.021d	26.5d
Speargrass	Ryegrass	7.5b	58.4c	0.026d	27.7d
Ryegrass	Speargrass	11.9ab	81.2b	0.078c	62.8c
Ryegrass	Mitchell grass	15.9a	77.8b	0.075c	63.8c
Ryegrass	Pangola grass	15.0a	79.9b	0.101b	69.3b
Ryegrass	Ryegrass	14.3ab	79.6b	0.174a	74.7b
s.e.m.	–	2.13	4.51	0.0068	1.02

Table 2. The effect of urea-ammonium sulfate (US), US plus amino acids (USAA), casein, cottonseed meal (CSM), yeast and algae supplementation on the immediately soluble fraction (*a*), slowly fermentable fraction (*b*), fractional degradation rate of *b* (*c*) and effective degradability (ED) of the dry matter (DM) of speargrass, Mitchell grass and ryegrass hay substrates incubated in the rumen of steers fed a speargrass hay basal diet

AA, mixture of leucine (33.6%), isoleucine (22.1%), valine (26.2%) and phenylalanine (18.0%). ED, effective degradability at rumen outflow rate of 0.02/h or 0.05/h. US, mixture of urea (80%) and ammonium sulfate (20%). Value of each treatment and each parameter is mean of the three substrates. Means within a column followed by the same letter are not significantly different, among substrates or supplements (at $P = 0.05$)

	a (%)	*b* (%)	*c* (per h)	ED (% DM)	
				0.02	0.05
Substrate					
Speargrass	6.3c	49.6c	0.027b	34.9c	24.0c
Mitchell grass	16.0a	54.1b	0.030b	48.1b	36.1b
Ryegrass	9.5b	76.4a	0.092a	72.6a	60.1a
s.e.m.	1.79	0.82	0.003	1.08	1.14
Supplement treatment					
Control	12.2	60.2	0.036b	49.3d	36.9d
Casein	11.4	60.0	0.051a	52.7ab	41.1ab
Algae	9.1	62.0	0.057a	53.3a	41.5ab
Yeast	11.9	59.0	0.053a	53.6a	41.7a
CSM	11.0	59.9	0.051a	52.7a	40.9abc
USAA	9.8	59.2	0.052a	51.1bc	39.5bc
US	8.8	59.8	0.050ab	50.4cd	38.8cd
s.e.m.	1.99	1.25	0.004	1.15	1.26

Table 3. The effect of urea-ammonium sulfate (US) or Spirulina(S) supplementation on the immediately soluble fraction (*a*), slowly fermentable fraction (*b*) and effective degradability (ED) of the dry matter (DM) of Mitchell grass, pangola grass and ryegrass substrates incubated in the rumen of steers fed Mitchell grass hay at 90% *ad libitum* intake (see text for description of amounts of supplement offered)

US, mixture of urea (80%) and ammonium sulfate (20%). ED, effective degradability at rumen outflow rate of 0.02/h or 0.05/h. Value of each treatment and each parameter is mean of the three substrates. Means in the same column followed by the same letter are not significantly different from each other, among substrates or supplements (at $P = 0.05$)

	a (%)	*b* (%)	*c* (per h)	ED (% DM)	
				0.02	0.05
Substrate					
Mitchell grass	8.6	42.7c	0.032a	36.5c	26.5c
Pangola grass	7.3	56.0a	0.041b	50.7b	37.0b
Ryegrass	6.9	54.3b	0.076c	62.4a	49.9a
s.e.m.	0.43	1.00	0.004	0.53	0.60
Supplement treatment (*rumen-degradable protein g/kg digestible organic matter*)					
Control	9.7a	52.4a	0.035a	46.2c	33.6d
US90	8.6ab	52.2a	0.042b	49.7b	37.1c
US170	6.3c	50.0b	0.054c	49.9b	38.2bc
US210	7.3bc	50.0b	0.053c	49.8b	38.2bc
S90	6.6c	51.1ab	0.052c	50.9ab	38.7b
S170	8.3ab	50.2b	0.053c	50.5ab	38.7b
S290	6.2c	51.0ab	0.060c	52.0a	40.3a
s.e.m.	0.53	1.07	0.004	0.67	0.75

steers not receiving an N supplement (Control steers) was 0.052, 0.029 and 0.023 respectively while the mean for all levels and form of N supplement (US or algae) was 0.080, 0.043 and 0.033 for the same substrates, respectively. The highest degradation rate for the ryegrass substrate was recorded in S at the 290 g RDP/kg DOM treatment but did not differ to US at the 210 g RDP/kg DOM treatment. Similar trends occurred with the pangola and Mitchell grass substrates where the highest rate of digestion was for the S290 g RDP/kg DOM treatment but did not differ to US at RDP 170 g RDP/kg DOM. However, overall no differences in degradation rate occurred at the same RDP for both supplements. The Control steers had very low rumen NH_3-N concentration (5 mg/L). Both US and S supplementation increased rumen NH_3-N concentration above the minimal level suggested for microbial function (50 mg/L) with the highest rumen NH_3-N concentration reaching 132.5 mg/L from US at the 210 g RDP/kg DOM treatment and 246.5 mg/L from S at the 210 g RDP/kg DOM treatment.

Discussion

The ability of ruminants to consume forage is the most important factor controlling animal production. Intake, to some extent, is affected by rate of digestion of material in the rumen. In the first experiment, the rate of digestion of a high-quality ryegrass substrate was above that of a low CP/high NDF speargrass substrate irrespective of the treatment diets consumed by the steer. The differences in digestion rates were presumably related to physical and anatomical properties of the forage and were expected (Wilson 1994). However, rumen conditions, as set by the forage type being consumed by the steers, markedly affected digestion rate of the high CP/low NDF ryegrass substrate but not the low CP/high NDF speargrass substrate, except for the ED (Table 1). Low digestion rate of the low CP/high NDF speargrass substrate incubated in the rumen of steers that received speargrass and Mitchell grass diets may be associated with low rumen NH_3-N concentration from both diets, which is below the minimal concentration suggested for microbial function (50 mg/L) (Satter and Slyter 1974) as well as the inherent low digestion rate of these C_4 tropical grasses (Wilson 1994). The results of Experiment 2 confirmed that various N supplements offered to cattle fed a low CP basal diet of speargrass hay improved the rate of digestion of either the highly degradable forage substrate (ryegrass) or the lowly degradable forage substrates (speargrass and Mitchell grass) above that of the same substrates incubated in the rumen of unsupplemented steers (Table 2). The NH_3-N concentration in the rumen of Control steers were well below the minimal concentration suggested for microbial function while in the rumen of supplemented steers they were at least twice the minimal concentration. This supports the well accepted principle of NH_3-N availability being important for fibre digestion in the rumen. However, there was no difference in degradation rate of each of the three substrates between the different sources of N suggesting that the form of N was not important especially the forms which might have provided other growth factors such as nucleic acids, minerals and vitamin (algae and yeast). A greater ED occurred with algae and yeast supplementation than that of inorganic N (US) or USAA (BCAA). Algae is a single cell protein source that contains other organic molecules such as vitamins, minerals and other growth factors in addition to N. Experiment 3 validated the finding that N supplementation improved the rate of digestion of a highly degradable forage substrate (ryegrass), a medium degradable forage substrate (pangola grass) and a low degradable forage substrate (Mitchell grass) in the rumen of supplemented steers above that in the rumen of unsupplemented steers (Table 3). However, there was no further improvement on digestion rate of the substrates when incubated in the rumen of steers receiving either US or S in an increasing amount from the lowest amount (90 g RDP/kg DOMI) up to the highest amount (210 or 290 g RDP/kg DOMI for US and S supplements, respectively). The NH_3-N concentration in the rumen of Control steers were extremely low (5 mg/L), which is well below the minimal concentration for optimal rumen function (50 mg/L). Increasing amount of both US and S supplement increased rumen NH_3-N concentration over and above the minimal concentration required for optimal rumen function and resulted in an increase of the digestion rate of all test substrates irrespective of their quality. However, there was no further improvement on digestion rate of the substrates when incubated in the rumen of steers receiving either US or S in an increasing amount from the lowest amount (90 g RDP/kg DOMI) up to the highest amount (210 or 290 g RDP/kg DOMI for US and S supplements, respectively). This further supports the principle that the form of N was not important.

The overall result of the current series of experiments indicates that apart from physical and anatomical properties

of the forage, rate of digestion is associated with the N content of the forage which influences the rumen conditions, particularly the ammonia-N concentration of the rumen. When the ammonia-N concentration was low, digestion rate was low, and increasing the ammonia-N concentration in the rumen up to a critical concentration increased the rate of DM digestion. However, further increases in supplementary N above the amount required to reach the critical concentration, irrespective of the source of N, had no effect on digestion rate. In this respect, the form of N and other potential growth factors (nucleic acids, minerals, vitamins) in the supplement do not appear to be important in affecting digestion rate and the amount of N in the diet, which results in a rumen ammonia-N concentration above a minimal critical level as the most important factor. Novel protein supplements such as algae with a range of nutrients required by bacteria in addition to simply N do not increase the rate of digestion over and above the N supplied by the supplement. This supports the accepted principle of a minimal rumen ammonia concentration which must be achieved, although the determination of that concentration was not tested here. This study examined a wide range of substrates and supplement types, some of which have not previously been studied for their effects on rate of digestion.

Conclusions

Digestion rate of forages is related to their inherent physical and anatomical properties as well as the total N content. Nitrogen supplementation, irrespective of N source, increased the rate of DM digestion when ammonia-N was inadequate but supplementation at higher amounts had no effect on the rate of digestion in the rumen. Algae are a novel protein supplement that were comparable to traditionally used protein supplements (CSM) in their effect on rate of digestion, and as such may be of value as a supplement for beef cattle where production is often constrained by low intakes associated with low rates of digestion.

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