

Faecal near infrared reflectance spectroscopy estimates of diet quality and responses to nitrogen supplements by cattle grazing *Bothriochloa pertusa* pastures

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Abstract. A grazing experiment in the seasonally dry tropics of north Queensland examined the diet selected and the growth responses of *Bos indicus* steers to urea supplement over two dry seasons and one wet season, from August 2001 to January 2003. There were two groups of 10 steers (control and urea-supplemented) and each group comprised two age cohorts, A and B, of five steers each with an age difference of ~1 year. In June 2002, cohort A steers were replaced with steers 2 years younger (cohort C). The steers grazed *Bothriochloa pertusa* pastures on a low fertility Red Chromosol soil. The groups were switched between two adjoining paddocks at fortnightly intervals when they were weighed and faecal samples were collected for faecal near infrared reflectance spectroscopy (F.NIRS) estimates of diet quality and growth rate. Rainfall and diet quality followed the expected seasonal pattern, but the 2001–02 wet season was very short (November–January) with only 65% of the long-term average rainfall. There was no rain during the 2001 dry season (DS-1) from August to mid November, and no effective rain (17 mm) between February and December 2002. Non-grass (i.e. herbaceous dicot plants) made only a small contribution to the diet, averaging 13%. In DS-1 and in the dry season of 2002 (DS-2) diet crude protein (CP) averaged 2.5% and 2.9%, and DMD/CP (ratio of dry matter digestibility to CP) averaged 18.6 and 17.1, respectively. Liveweight (LW) loss in control steers during DS-1 averaged 32 kg. Urea supplement reduced LW loss by 18 kg ($P < 0.001$), but most of the benefit was lost during the following growing season. During the extended dry period in 2002, average LW losses of control steers were 85 and 47 kg in the older and younger cohorts, respectively, and supplementation with urea reduced weight losses by 53 and 31 kg, respectively ($P < 0.001$). F.NIRS predicted the cumulative LW of the unsupplemented steers in cohorts A and B with acceptable accuracy, the difference between the observed and predicted final LW being 6 kg for cohort A after 10 months, 12 kg for cohort B after 17 months, and 27 kg for cohort C after 7 months. The results demonstrated that F.NIRS can be effectively used to monitor dietary CP and DMD levels in grazing cattle, to help develop an understanding of cattle responses to urea supplement relative to the quality of the basal forage diet, and to provide useful decision support information for the nutritional management of grazing cattle.

Introduction

The first limiting nutrient in the diet of grazing cattle during the dry season in the seasonally dry tropics is usually nitrogen (N) (Winks 1984; Minson 1990). Numerous studies have reported that the provision of N supplements, including non-protein N supplements such as urea, increases forage intake and liveweight (LW) by pen fed cattle consuming low quality forages (Hennessy and Williamson 1990; Dixon and Doyle 1996). Experiments reporting the responses of grazing cattle to N supplements in northern Australia are limited to few sites (Winks *et al.* 1972, 1979; McLennan *et al.* 1981; Graham *et al.* 1983; Foster and Blight 1984) and did not include concurrent diet quality measurements, particularly protein and digestibility levels. Faecal near infrared reflectance spectroscopy (F.NIRS), which relates diet and animal attributes to measurements of the NIR spectra of the faeces, can provide reliable estimates of dietary parameters (e.g. protein, digestibility, grass to non-grass proportions) in free-grazing cattle (Lyons and Stuth 1992; Stuth *et al.* 1999; Coates 2004; Dixon and Coates 2005). This

technology, therefore, provides an opportunity to relate growth of cattle to the diet selected, thus enhancing our understanding of the performance of grazing cattle relative to constantly changing nutrient intake. Similarly, the technology has obvious potential as a decision support tool in the nutritional management of grazing cattle, especially in relation to the feeding of supplements with regard to timing, nutrients provided, and target intakes. This experiment aimed to use F.NIRS to profile dietary parameters through the annual cycle in cattle grazing semi-cleared savannah near Charters Towers in north Queensland, and to examine the LW responses of cattle fed urea based supplement during the dry season in relation to the quality of the forage ingested.

Materials and methods

The experimental site was on Forest Home Station (19°56'S, 146°31'E) located east of Charters Towers and consisted of semi-cleared woodland of narrow leaf ironbark and bloodwood growing on Red Chromosols derived from granodiorite. The

area has been classified in the black speargrass pasture region (Weston 1988). The pasture was Indian couch grass (*Bothriochloa pertusa*) that had completely replaced the native grass species. A native legume, *Indigofera colutea*, was a minor component of the pasture and there were isolated plants of currant bush (*Carissa lanceolata*).

The trial site comprised two adjoining 40-ha paddocks, each grazed by 10 *Bos indicus* steers over a period of 1.5 years, August 2001–January 2003. Steers consisted initially of two age cohorts each of 10 steers (five steers per paddock): 2.5-year-old steers (cohort A) and 1.5-year-old steers (cohort B). In June 2002, the older cohort (A) was replaced by a new cohort (C) of 1.5-year-old steers. At entry to the experiment, steers weighed (mean \pm s.d.) 351 ± 13 kg, 275 ± 34 kg, and 249 ± 34 kg for cohorts A, B, and C, respectively. Steers in each cohort were allocated at random to an unsupplemented treatment (control group) and a supplemented treatment (urea group) where N and sulfur (S) were provided by mixing urea and ammonium sulfate (N : S ratio of 10 : 1) in the drinking water at the rate of 1 g urea/L for the first fortnight, thereafter at 1.5 g/L. The urea supplement was provided from 30 August 2001 to 17 January 2002, and then from 28 March 2002 through to the end of the experiment in January 2003. Because intake of supplement was often lower than the target rate of 40 g urea/day, a loose mineral mix of salt (25 kg), urea (1.4 kg) and calcium phosphate (Kynophos, 1.4 kg) was offered each fortnight from 19 July 2002 to 3 January 2003 to provide an additional 10 g urea/steer.day. The control group was fed salt and Kynophos during the same interval because faecal phosphorus (P) concentrations suggested the possibility of a marginal P deficiency.

To eliminate possible paddock differences the herds were switched between the paddocks fortnightly when they were also weighed (unfasted) and faecal sampled per rectum. Faeces were bulked within treatment and age group. The water troughs, which provided the only water supply, were arranged so that the treatments were maintained in the designated herds when the steers were switched between paddocks. Samples of Indian couch, plucked to simulate material selected by the grazing cattle, were collected fortnightly from both paddocks from August 2001 until October 2002, and then less regularly from only one paddock until January 2003. The experiment terminated in January 2003 due to low pasture availability in the absence of rain, exacerbated by damage from army worms.

Laboratory, NIRS, and statistical analysis

Faecal samples were oven-dried (65°C) and then ground (1 mm-screen, Model 1093 Cyclotec mill, Foss Tecator AB, Hoganas, Sweden). Prior to analysis, samples were redried (65°C) and scanned (400–2500 nm range) using a monochromator fitted with a spinning cup module (Foss 6500, NIRSystems, Inc., Silver Spring, MD, USA). Chemometric analysis used ISI software (Infrasoft International, Port Matilda, PA, USA). The Coates (2004) F.NIRS calibration equations, or updated versions where relevant, were used to estimate the non-grass proportion [$n=1736$, standard error of cross validation (SECV)=6%, $R^2=0.94$]; crude protein (CP) ($n=1202$, SECV=1.1%, $R^2=0.95$); and dry matter digestibility (DMD) ($n=637$, SECV=1.7%, $R^2=0.93$) of the diet selected; faecal N

concentrations ($n=987$, SECV=0.08%, $R^2=0.96$), and also daily weight gain (DWG) ($n=808$, SECV=133 g/day, $R^2=0.92$) of unsupplemented steers. Current F.NIRS calibration equations for predicting DWG cannot be usefully applied to supplemented cattle. To make F.NIRS predictions of DWG independent of the dataset used to develop the calibration, all samples from the present experiment were deleted from the Coates (2004) ADGSGBA calibration dataset, and a modified calibration equation (ADG-mod) was computed. The same spectral transformations and model structure were used for the ADGSGBA and the ADG-mod calibration models. Calibration statistics of the modified calibration were $R^2=0.89$, SECV=141 g/day.

CP and estimated *in vivo* DMD of plucked Indian couch samples were measured using in-house NIRS forage calibration equations. Some samples (23 and 65% for CP and DMD, respectively) were also analysed by conventional laboratory techniques to validate the forage NIRS predictions for the present experiment. CP analyses were out-sourced while DMD analyses were conducted on-site using the pepsin-cellulase *in vitro* digestibility technique of McLeod and Minson (1978). *In vitro* DM disappearance was adjusted to estimated *in vivo* DMD using Indian couch standards of known *in vivo* DMD. Where laboratory analyses were available these determinations were used; otherwise the NIRS measurements, corrected for bias where appropriate, were used.

The experiment was divided into four seasonal intervals as follows: (i) 2001 dry season from 13 August to 22 November 2001 (DS-1); (ii) wet season from 22 November 2001 to 18 April 2002 (WS); (iii) wet–dry transition season from 18 April to 5 July 2002 (TS); and (iv) a second dry season from 5 July 2002 to 17 January 2003 (DS-2). The cumulative LW of unsupplemented steers was calculated in fortnightly steps using F.NIRS predictions of DWG. The effects of supplement and steer age, and the interaction, on diet attributes and cumulative LW were computed for the four seasons by ANOVA. Because the two groups were switched between paddocks each fortnight it was assumed that paddock effects would not have influenced the treatment means, and that samples obtained at intervals from a specific group were independent. For LW change, individual animals were the experimental unit; for diet attributes the sequential measurements within a season were considered as independent observations.

Results

Rainfall (see Fig. 2 for monthly totals) from 1 July 2001 to 30 June 2002 was 432 mm or only 65% of the long-term mean (660 mm) and, apart from 7 mm in May 2002, this fell from mid November 2001 to the end of January 2002. The 2002 dry season extended through to January 2003 when the trial was terminated, the only rain being 10 mm in August and 26 mm on the 3 January. Visual assessment of standing forage suggested that DM on offer was not limiting until shortly before the experiment was terminated.

The average urea intake of supplemented steers was 29 g/day during DS-1. In 2002, urea intakes averaged 23 g/day during TS and 46 g/day during DS-2.

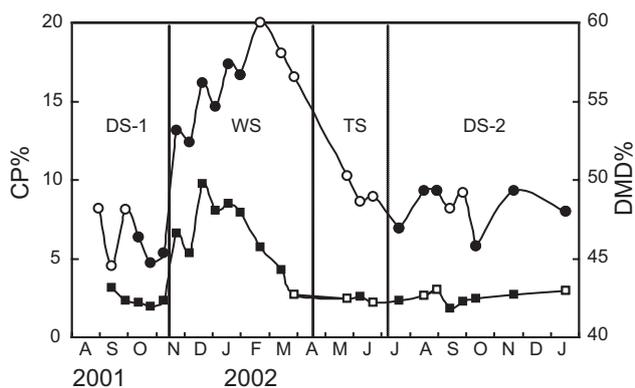


Fig. 1. Crude protein concentration (CP, □, ■) and dry matter digestibility (DMD, ○, ●) of plucked samples of Indian couch grass for the period August 2001 to January 2003. Open symbols represent samples analysed by conventional laboratory procedures; closed symbols represent samples analysed by near infrared reflectance spectroscopy. Seasonal boundaries are delineated by vertical lines.

Quality of plucked grass

The quality of the plucked grass samples (Fig. 1) reflected the below average rainfall and short duration of the 2001–02 WS. Mean CP was only 2.4% during DS-1. It increased to a peak of 9.7% in December during the WS, and then declined rapidly to average 2.5% through TS and DS-2. Digestibility measurements followed a similar pattern with values <50% during DS-1, TS, and DS-2, and a maximum value of 60% in February 2002. DMD/CP ratios averaged 19.7, 20.5, and 19.6 during DS-1, TS, and DS-2, respectively, with a minimum value of 5.8 recorded in December 2001.

Diet quality

F.NIRS estimates of diet CP and DMD (Fig. 2) were generally not influenced by steer age, by supplement, or by the interaction between these factors. Therefore, most of the results presented are the means over age groups and treatments. The seasonal patterns and concentrations of diet CP and DMD were similar to those observed for plucked grass. Diet CP averaged (±s.d.) 2.5% (±0.41) during DS-1, 2.9% (±0.36) during DS-2, and 6.5% (±2.2) during WS, with a maximum value of 11% in January 2002. F.NIRS estimated DMD tended to be slightly lower than for the plucked grass and averaged 46% during DS-1, peaked during WS at 59% in January 2002, and averaged 48% during DS-2. CP and DMD of the diet were correlated with CP and DMD of plucked grass, and regression equations for the relationships were as follows:

$$\text{Diet CP\%} = 0.735(\text{forage CP\%}) + 1.11$$

$$(n = 25, R^2 = 0.70) \tag{1}$$

$$\text{Diet DMD\%} = 0.730(\text{forage DMD\%}) + 13.0$$

$$(n = 26, R^2 = 0.75) \tag{2}$$

The regression relationship between CP of the diet and plucked grass indicated that F.NIRS diet estimates were

slightly higher than those of plucked grass when protein in the grass was very low during the dry seasons. Conversely, diet CP estimates were lower than CP of plucked grass when forage protein levels were moderate to good during the growing season. Nevertheless, the average difference between diet CP estimates and CP of plucked grass (n=25) was only 0.85% (range of 0.05–3.9%) and differences for 21 of the 25 paired estimates were <0.9% CP. The larger differences occurred during the WS. Similarly, the regression relationship between DMD of the diet and plucked grass indicated F.NIRS estimates of diet DMD were slightly higher than DMD of plucked grass when that of plucked grass was below 48% while the former was increasingly less than the latter as DMD of plucked grass rose above 48%. Despite the differences indicated by the regression equation, the means of 16 dry season measurements for DMD were almost identical at 47.5 and 47.6% for diet and plucked grass respectively, while the maximum and mean differences for dry season samples were 2.4 and 1.6%, respectively. For all seasons combined, the differences ranged between 0.5 and 4.4% with a mean difference of 2.0%.

DMD/CP ratios ranged between 5.3 and 21.0 during the experiment (Fig. 2). Values below 10 were recorded only during the relatively short interval mid December to mid March. Average values during DS-1, TS and DS-2 were 18.6, 15.5 and 17.1, respectively.

Dietary non-grass predictions remained low throughout the experiment, the means being between 12 and 13% during the four seasons. There was no discernable seasonal pattern in diet non-grass proportions.

Faecal N averaged 0.99, 1.39, 1.04 and 0.97% during DS-1, WS, TS and DS-2, respectively. In DS-2 there was a significant treatment × steer age interaction (P < 0.05) such that faecal N of the unsupplemented younger (cohort C) steers (0.89%N) was lower than that of the unsupplemented older steers (cohort B) or supplemented steers of both ages (0.99–1.01%N). The seasonal pattern of faecal N was similar to that for diet CP. Also, diet CP and faecal N were closely correlated (P < 0.001, n = 35, R² = 0.80).

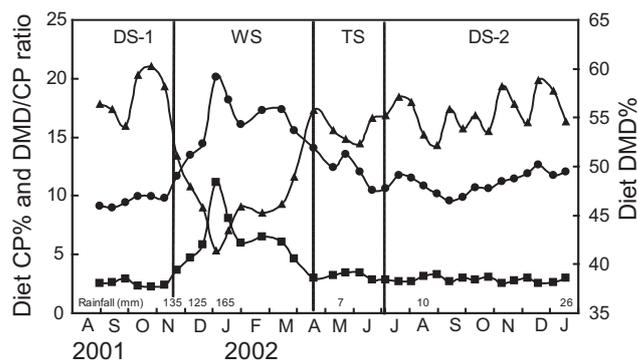


Fig. 2. Faecal near infrared reflectance spectroscopy predictions of diet crude protein (CP) (■) and dry matter digestibility (DMD) (●), and the ratio of DMD/CP (▲) for the period August 2001 to January 2003. Monthly rainfall totals are shown below the plots. Seasonal boundaries are delineated by vertical lines.

Animal LW change

Measured LW change (Fig. 3) reflected the low rainfall and poor diet quality during most of the experiment. Provision of urea supplement had a significant ($P < 0.001$) effect on reducing LW loss during both DS-1 and DS-2, and also on LW gain during WS, but not during TS. During DS-1 the urea supplement reduced LW loss by 18 kg for both age groups. However, DS-1 losses were significantly higher ($P < 0.001$) for the older steers (38 kg) compared with younger steers (28 kg) ($P < 0.05$). During WS, steers which were not supplemented during DS-1 gained at 0.53 kg/day compared with 0.43 kg/day for steers that had been supplemented during DS-1 demonstrating greater compensatory gain in the control steers ($P < 0.001$). Compensatory gain removed any benefit from the supplement by the time cohort A steers were removed from the experiment in June while the supplemented cohort B steers retained an advantage of 11 kg. There was a trend ($P = 0.06$) for older steers to gain more rapidly than younger steers during the WS (0.58 and 0.49 kg/day). During the TS and DS-2 the unsupplemented B cohort lost 85 kg over 9 months while the

supplemented B cohort lost only 32 kg, representing a 53-kg response to urea. The unsupplemented younger steers (cohort C) lost 47 kg over 7 months while their supplemented counterparts lost only 16 kg, representing a 31-kg response. In DS-2, the main effect of age on weight change was not significant ($P > 0.05$) but there was a significant supplement \times age interaction where the older steers lost more weight than younger steers in the control treatment but not in the supplemented treatment.

Prediction of steer LW using F.NIRS

Overall, there was good agreement between predicted cumulative LW (calculated from F.NIRS predictions of DWG) and actual LW for each age group of unsupplemented steers (Fig. 4). Predicted LW based on ADGSGBA was generally higher than that based on ADG-mod though there was negligible difference during the dry season and early WS of 2001–02. It was notable that the maximum deviations of predicted LW from actual LW over the 17-month trial period for cohort B were only 21 and 20 kg for the ADGSGBA and ADG-mod calibration equations, respectively.

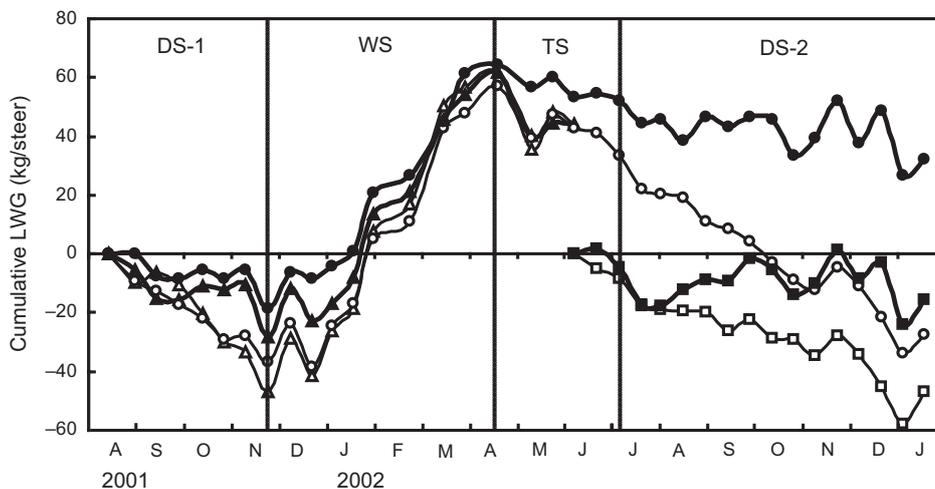


Fig. 3. Effect of nitrogen + sulfur supplement on the cumulative liveweight gain (LWG) or loss of three steer cohorts (■, △ cohort A steers; ●, ○ cohort B steers; ■, □ cohort C steers) for the period August 2001 to January 2003. Closed symbols represent control steers and open symbols represent supplemented steers. Seasonal boundaries are delineated by vertical lines.

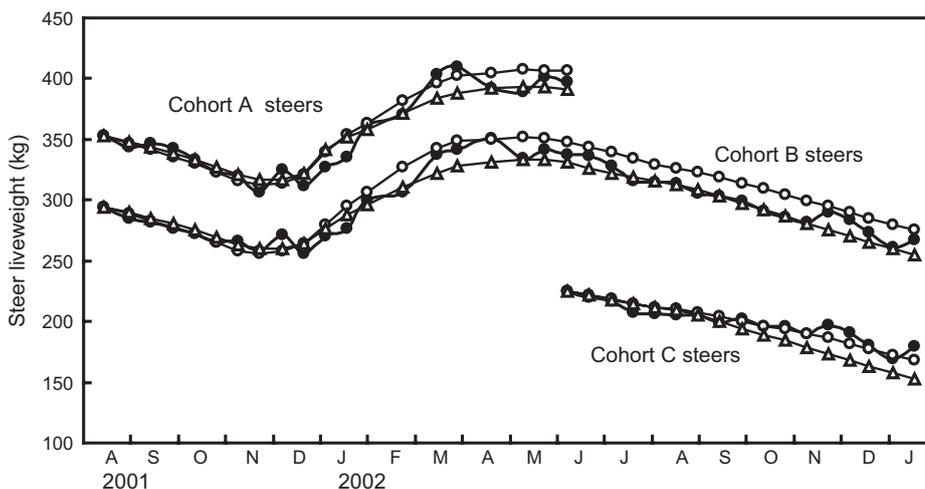


Fig. 4. Actual and predicted cumulative liveweight (LW) of three steer cohorts (● actual LW; ○ predicted LW using equation ADGSGBA; △ predicted LW using equation ADG-mod).

Discussion

Nutritional conditions during most of the experiment were very poor due to the very short WS, below average rainfall, and loss of water due to runoff in high intensity storms. The experiment had to be prematurely terminated because of drought and army worm infestation – both of which severely hindered the recovery of pasture in 2003. Nevertheless, the results were informative and demonstrated the capacity of F.NIRS as a research and management tool.

F.NIRS for monitoring diet quality

F.NIRS predictions indicated that diet quality was very low throughout most of the experiment. In the absence of diet : faecal sample pairs obtained during the experiment it was not possible to directly validate the predictions of diet CP and DMD. However, the calibration sets used to develop the Coates (2004) calibration equations contained a substantial number of native grass and Indian couch diets from the same region in north Queensland. Moreover, the relatively good agreement between F.NIRS predictions of diet CP and DMD with those measured in plucked grass, indicated that the F.NIRS predictions were probably reliable. Although in most grazing situations the analysis of plucked pasture provides an unreliable estimate of the diet selected, in this experiment the reliability of plucked grass as an indicator of diet quality was enhanced due to: (i) the dominance of Indian couch over the whole site; and (ii) the sward characteristics of this stoloniferous species, viz. a uniform, short mat of grass (under 10 cm high for most of the experiment) as well as the absence of coarse stems of high tensile resistance. Although there were differences between estimates of CP in the diet and in plucked grass, the higher diet CP estimates in the dry seasons (e.g. 2.9% compared with 2.5% in DS-2) were consistent with the presence of some non-grass in the diet (average of 13%) during the same intervals. When feed is mature and/or dry, non-grass is usually higher in CP than grass and, therefore, elevates diet CP (Coates and Dixon 2007). The lower estimates of diet CP compared with CP of plucked grass when the latter was moderate to high may have been due to prediction errors in the diet CP estimates or to differences between the quality of plucked grass and the diet selected by the steers, or a combination of the two. Nevertheless, on most sampling occasions there was good agreement between CP estimates in the diet and of plucked grass.

Differences between estimates of DMD in the diet and plucked grass followed the same directional trend as diet CP and were more pronounced at the higher digestibility levels. Notwithstanding the existence of some error in F.NIRS predictions, these differences may also have been the result of differences in leaf/stem ratio between plucked grass (less stem) and the actual diet selected (more stem). Similar to the results for CP, the agreement between DMD estimates for diet and plucked grass was most satisfactory for the large majority of samples. We conclude that F.NIRS provided reliable and useful estimates of both dietary protein and digestibility levels and that the technology offers a simple, practical and cost effective means of monitoring diet quality in free-grazing cattle at regular intervals over any desired period. Reliable

profiling of diet quality in grazing cattle was impractical, often impossible, before the development of robust F.NIRS calibration equations.

Liveweight responses to urea supplement

There was a positive LW response to feeding urea supplement during both dry seasons. Dixon and Coates (2005) suggested that when DMD/CP ratios are above 10, a response to urea supplement can be expected in *B. indicus* cattle grazing tropical pastures. In this experiment, DMD/CP ratios reached very high levels, averaging 18.6 and 17.1 through DS-1 and DS-2. The response to feeding urea was 0.18 kg/day in both A and B cohorts over 101 days in DS-1, while in 2002, the responses averaged over TS and DS-2 were 0.19 kg/day over 275 days for the B cohort, and 0.14 kg/day over 224 days for the C cohort. The response by cohort B was clearly evident from mid April 2002 while a definite response by cohort C occurred only from early August even though DMD/CP ratios were over 15 from the time they entered the experiment in June. This delayed response resulted in a lesser overall response by cohort C steers compared with cohort B. The change in diet quality during the late WS and TS was abrupt and atypical; there was a rapid decline in diet CP concentrations from above maintenance levels in early March to severe submaintenance levels by mid April and little change thereafter. The decline in DMD occurred more gradually and as a consequence the increase in the DMD/CP ratio was more rapid than the decline in diet CP. Moreover, despite large fluctuations in DMD/CP through DS-2, the overall trend was for little further increase in DMD/CP after mid April. Importantly, because of the very rapid increase in the DMD/CP ratio, it was not possible to determine precisely at what DMD/CP ratio a response to urea supplement commenced. Faecal N concentration has also been used as an indicator to when a response to urea can be expected. For northern speargrass pastures, Winks (1984) recommended a threshold value of 1.3% faecal N below which cattle would respond to urea supplementation. As with the DMD/CP ratio, it was not possible to determine a precise threshold value appropriate for Indian couch pasture because of the rapid change in faecal N from 1.43% in mid March to 1.16% by mid April.

The responses to urea in the current experiment were comparable with published responses in grazing cattle where the supplement was provided as molasses-urea mixtures in roller drums. Winks *et al.* (1972) reported that urea supplements reduced LW loss in eight drafts of cattle by an average 0.15 kg/day, and up to 0.27 kg/day, in cattle grazing speargrass pastures in the same region as the present experiment, and Foster and Blight (1984) reported similar responses on speargrass pastures in southern Queensland. McLennan *et al.* (1981) reported an average response over 4 years of only 0.05 kg/day when the urea was provided in loose mineral mixes for speargrass pastures, but the lesser response was likely associated with benign dry seasons. In these earlier experiments, however, there were no concurrent diet quality estimates to which responses could be related.

The benefit from feeding urea supplement in DS-1 was either completely (cohort A) or partly (cohort B) eroded by compensatory gain in the control steers during the following

short WS. This was consistent with reports by Winks *et al.* (1972, 1979), whereas in other situations little compensatory growth was observed in the postsupplementation WS (Foster and Blight 1984). In the seasonally dry tropics, compensatory growth effects have been quite variable, but the reasons are not well understood (Winks 1984). We suggest that a high proportion of the benefit from feeding supplement during DS-2 would have been retained once conditions improved due to the magnitude of the response and the long duration of the dry season.

The agreement between either ADGSGBA or ADG-mod predicted cumulative LW and measured LW of unsupplemented steers was most satisfactory, especially for cohorts A and B, and despite the unusual seasonal conditions where weight losses occurred for more than 70% of the experiment. Inspection of the LW curves (Fig. 4) showed obvious differences between actual and predicted LW change with respect to short-term changes; those in predicted LW were gradual, those in measured LW were often abrupt. It seems likely that the short-term fluctuations in measured LW were often due to changes in gut fill and/or body water while the more uniform, progressive changes in F.NIRS predicted LW probably provided a better indication of tissue accumulation or loss. Reference values for 'growth rate' determined by weighing cattle and used in developing calibration sets for predicting DWG, will frequently contain substantial errors due to changes in gut fill between successive weighings. This in turn will lead to calibration statistics that appear poorer than would otherwise be the case. The satisfactory agreement between measured and F.NIRS predicted LW in this experiment was consistent with similar results reported for the same region by Dixon *et al.* (2007).

This experiment is one of just a few trials (Dixon 2008) where responses to supplementation by grazing cattle have been measured in conjunction with continuous and reliable monitoring of diet quality made possible through the development and application of F.NIRS. We suggest that many more such experiments are needed to build up datasets of quantitative responses to various supplements in relation to diet quality and for a range of pasture types. Such information used in conjunction with F.NIRS would allow producers to make better informed, economically-based decisions on supplementation options. In this respect, F.NIRS is valuable as a decision support tool in the nutritional management of grazing cattle.

In conclusion, the present study demonstrated the usefulness of F.NIRS for monitoring diet quality in grazing cattle, and for providing information to understand animal responses to supplementation. Furthermore, for this experiment, F.NIRS was able to estimate animal LW change in unsupplemented cattle through the annual cycle with a degree of accuracy acceptable for many purposes.

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