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# Diet quality estimated with faecal near infrared reflectance spectroscopy and responses to N supplementation by cattle grazing buffel grass pastures

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#### ABSTRACT

In the seasonally dry tropics the productivity of grazing cattle is often constrained by nutrition, particularly during the dry season. An experiment during 2 annual cycles estimated the diet selected by grazing Bos indicus heifers, and their growth and growth responses to a non-protein N supplement. Two drafts (groups) of 2 herds (10 heifers per herd) grazed a pasture consisting of buffel grass (Cenchrus ciliaris) with some legume and edible browse, and herds were offered loose mineral mix (LMM) supplements either without (control) or with urea. Near infrared reflectance spectroscopy measurement of faeces (F.NIRS) was used to estimate each 2 weeks the non-grass and crude protein (CP) concentrations, and DM digestibility (DMD) of the diet selected. Rainfall and diet quality generally followed the seasonal pattern expected for the region and for the seasonally dry tropics. Non-grass (i.e. dicotyledonous species) content averaged 0.13 (S.D. 0.053) of the diet and did not appear to be related to season, but during the first dry season and wet season the urea supplemented heifers selected diets lower (P<0.01 and P<0.05, respectively) in non-grass than the control heifers. Diet quality was generally low during the dry seasons, increased sharply after the seasonal break, and later declined gradually through the wet seasons and wet-dry transition seasons. During the dry seasons average diet CP ranged from 33 to 49 g/kg and diet DMD from 0.480 to 0.507. During the 2 wet seasons CP averaged 94 and 100 g/kg, and the DMD 0.591 and 0.601. The control treatment heifers lost 30 and 32 kg LW during the late dry seasons, and gained 143 and 136 kg during the wet, wet-dry transition and early dry seasons of Drafts 1 and 2, respectively. F.NIRS predicted the daily weight gain (DWG) of the heifers with accuracy useful for management purposes except during the early wet season of Draft 2 when intermittent rains increased diet quality but were insufficient for substantial pasture growth. The urea supplement reduced (P<0.01) heifer LW loss during the dry seasons, but much of this benefit subsequently disappeared due to compensatory growth; net benefit of the urea supplement over the annual cycle was 24 and 14 kg in the 2 drafts. This study demonstrated the capacity of F.NIRS to estimate the diet selected, liveweight change and liveweight responses to a N supplement in grazing cattle.

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Abbreviations: CP, crude protein; DMD, dry matter digestibility; DS, dry season; DWG<sub>Actual</sub>, actual daily weight change; DWG<sub>Direct</sub>, daily weight change predicted directly by F.NIRS; DWG<sub>DMD</sub>, daily weight change calculated from diet DMD; F.NIRS, faecal near infrared reflectance spectroscopy; LW, liveweight; LMM, loose mineral mix; ME, metabolizable energy; N, nitrogen; rsd, residual standard deviation; TS, transition season; WS, wet season;  $\delta^{13}$ , C ratio of  $^{13}$ C to  $^{12}$ C.

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## 1. Introduction

In the seasonally dry tropical rangelands such as occur in Australia, Africa and South America, undernutrition is a major cause of low growth and productivity of grazing cattle. Tropical pasture species are usually more fibrous, contain lower concentrations of nitrogen (N) and metabolizable energy (ME), and mature more rapidly than temperate pasture species. A summer dominant rainfall distribution typically leads to an annual cycle of adequate to good nutrition during a short rainy season changing progressively to maintenance or sub-maintenance nutrition from mature pastures during a prolonged dry season (Minson, 1990). N is usually the first limiting nutrient in the diet selected by cattle grazing such pastures during the dry season. Although pastures often contain low concentrations of minerals such as phosphorus and sulphur, these are usually first limiting to animal growth only during the wet season (McDowell et al., 1984; McDowell, 1996). Numerous studies have reported intake and growth responses of pen-fed cattle consuming low quality tropical forages to N supplements, including to N provided as urea non-protein N (Hennessy and Williamson, 1990; Minson, 1990; Dixon and Doyle, 1996). However, there is limited published information on the responses of grazing cattle to non-protein N supplements. For example, in the rangelands of northern Australia studies are limited to a few sites (Winks et al., 1972, 1979; McLennan et al., 1981; Graham et al., 1983; Foster and Blight, 1984; Winter, 1987) and did not include concurrent measurements of pasture quality or the diet selected to relate the animal response to nutrient supply.

The responses of grazing cattle to supplements clearly depend on the quality of the forage selected as well as the quantity of pasture available. Information on the nutrient content of diets selected by grazing cattle in the seasonally dry tropics has depended on anecdotes, observations, measurements of the utilization of pasture components, faecal histology and the use of oesophageal fistulated cattle. These approaches all require major resources and may involve large errors (Holechek et al., 1982; Coates et al., 1987; Gordon, 1995). Furthermore such studies with cattle in the seasonally dry tropics are limited to only a few measurements at a few sites. The development of faecal near infrared reflectance spectroscopy (F.NIRS), which relates dietary attributes to measurements of the near infrared spectra of the faeces which can be collected in the field, allows estimation of the diet selected by grazing cattle with little or no disturbance (Lyons and Stuth, 1992; Coates, 2004; Dixon and Coates, 2005, 2009; Coates and Dixon, 2007; Decruyenaere et al., 2009), and thereby provides a procedure to relate growth and other responses of cattle to the diet ingested and to manipulations such as supplementation. F.NIRS calibration equations have been developed to estimate the proportion of dicotyledonous non-grass, crude protein (CP) concentration and DM digestibility (DMD) of the diet selected, and animal liveweight (LW) change, of cattle grazing tropical pastures in northern Australia (Coates, 2004). By providing rapid, economical and objective evaluation of nutrient intake by grazing cattle, F.NIRS provides a practical technology to understand nutrient intake, responses of cattle to supplements and productivity of cattle grazing under various circumstances.

The present experiment was undertaken to utilize F.NIRS to examine the diet ingested through the annual cycle by cattle grazing a buffel grass (*Cenchrus ciliaris*) based pasture in the seasonally dry tropics of northern Australia and to improve knowledge of the diet selected by cattle grazing such pastures. Also we wished to examine the capacity of F.NIRS to directly or indirectly predict LW change of the cattle. A further objective was to examine the LW change, and LW responses to a non-protein N supplement fed through the dry season, in the heifers in relation to diet quality. Such non-protein N supplements constitute one of the few nutritional manipulations which are possible in extensive rangeland systems. However, since such supplements involve substantial cost input, it is important to understand the pasture conditions and the diet selected by grazing cattle when animal responses are likely to be economically viable.

#### 2. Materials and methods

#### 2.1. Experimental site, cattle and supplementation treatments

An experiment was conducted on Fletcherview Research Station, James Cook University, situated about 20 km north of Charters Towers, North Queensland, Australia (lat. 19° 53′ S, long. 146° 11′ E). The site consisted of an area of black basalt soil in two similar and adjacent 50 ha paddocks. The pasture was predominantly buffel grass (*C. ciliaris*) with some stylo (*Stylosanthes scabra* cv *seca*), native herbaceous dicotyledonous species, and edible browse of whitewood (*Atalaya hemiglauca*) and Bauhinia (*Lysiphyllum caronii*). Average long-term annual rainfall is 651 mm, 0.69 of which occurs during the summer from December to March. There were 2 drafts (groups) of cattle, Draft 1 from the 1 August 2001 until the 19 September 2002, and Draft 2 from the 11 October 2002 until the 20 October 2003. Each draft comprised 20 *Bos indicus* spayed heifers (initially 1.5 years old; LW mean 279 (S.D. 27) kg for Draft 1 and LW mean 307 (S.D. 23) kg for Draft 2). The heifers were allocated at random to 2 herds which comprised 2 supplement treatment groups. To eliminate confounding due to any differences between paddocks, the 2 herds were switched between the 2 paddocks every 4 weeks, with the supplement treatments being imposed on the designated herd irrespective of paddock.

The two treatments consisted of loose mineral mix (LMM) supplements offered *ad libitum* through the dry season until the seasonal break. The control supplement contained P, S and Na (g/kg: 472 salt, 472 calcium phosphate and 57 elemental sulphur), while the urea supplement contained N, P, S and Na (g/kg: 275 salt, 275 urea, 175 limestone, 125 cottonseed meal, 125 calcium phosphate and 25 elemental sulphur). Draft 1 heifers were given the urea supplements from 2 August 2001 through to 15 November 2001, and from the 16 April 2002 until the 19 September 2002. Draft 2 heifers were given urea supplements from the 11 October 2002 until 26 January 2003, and from 17 March 2003 until 20 October 2003. Heifers were

weighed without fasting every 4–5 weeks. Faecal samples were obtained per rectum when the heifers were weighed, or were collected from fresh dung pats in the paddock midway between weighings, and were bulked within treatment groups.

# 2.2. F.NIRS measurements, calculations 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). Samples were redried (65 °C) prior to analysis and then 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 F.NIRS calibration equations developed by Coates (2004) were used to estimate the crude protein (CP) concentration and DM digestibility (DMD) of the diet, the ratio of <sup>13</sup>C to <sup>12</sup>C in faeces (faecal  $\delta^{13}$ C) and faecal N concentration. These calibrations were developed for tropical forages in northern Australia. The DMD/CP ratio of the diet was calculated from the respective estimates of these attributes. Diet non-grass was calculated from the  $\delta^{13}$ C of faeces (Coates and Dixon, 2008a). In order to make F.NIRS predictions of daily weight gain (DWG) independent of the data set used by Coates (2004) to develop the calibration equation, a new DWG calibration equation was developed. Data from the present experiment were deleted from the Coates (2004) data set, and a revised calibration was calculated using the same spectral transformations and model structure. Calibration statistics for this revised calibration (*R*<sup>2</sup> = 0.86, standard error of cross validation = 191 g/day, *n* = 1138) were similar to those reported by Coates (2004).

The experiment was divided into 8 seasonal intervals as follows:

- (i) the 2001 dry season from 1 August 2001 to the seasonal break on the 11 November 2001 (DS-1),
- (ii) the 2001/2002 wet season from the seasonal break until the 31 March 2002 (WS-1),
- (iii) the 2002 wet-dry transition season from 1 April 2002 to 30 June 2002 (TS-1),
- (iv) the 2002 dry season from 1 July 2002 to the termination of Draft 1 on the 19 September 2002 (DS-2A),
- (v) the 2002 dry season from the commencement of Draft 2 on the 11 October 2002 until the seasonal break on the 21 December 2002 (DS-2B),
- (vi) the 2002/2003 wet season from the seasonal break until the 31 March 2003 (WS-2),
- (vii) the 2003 wet-dry transition season from 1 April 2003 until the 30 June 2003 (TS-2), and
- (viii) the 2003 dry season from 1 July 2003 until the termination of Draft 2 on the 20 October 2003 (DS-3).

However, because the F.NIRS estimates of diet quality from the samples obtained on the 15 November 2001 (*i.e.* 4 days after the 40 mm rain on the 11 November 2001 considered as the seasonal break) were similar to those during the dry season preceding the rain, the predictions of diet quality on this date were considered as DS-1 rather than WS-1. This was considered appropriate since at the seasonal break there are time lags of several days between rainfall and new pasture growth, and between ingestion of new growth pasture and appearance of the undigested plant residues in faeces.

The measured daily weight gain (DWG<sub>Actual</sub>) of the heifers at any specific time was calculated as the tangent at that time to the fitted polynomial regressions of measured LW with time. In addition DWG was predicted by F.NIRS following 2 procedures. First, DWG of control treatment heifers for each faecal sampling date was predicted from the revised F.NIRS calibration equation described above (DWG<sub>Direct</sub>). Since the Coates (2004) F.NIRS calibrations for predicting DWG cannot be applied to cattle where N supplements are fed to correct a deficiency, this prediction was not applied to the urea treatment heifers. Second, DWG was predicted from the diet DMD and CP estimated by F.NIRS and using procedures described by Dixon and Coates (2008) and based on CSIRO (2007) (DWG<sub>DMD</sub>). For this calculation voluntary intake of forage DM by the control treatment heifers was calculated from the F.NIRS predicted DMD with adjustment when necessary for N deficiency using the estimated CP content of the diet, and by assuming rumen degradability of the forage protein was 0.8 (Bowen et al., 2008) and that 7 g rumen degradable protein were required per MJ metabolizable energy (ME) ingested. ME intake was then calculated assuming that digestible DM contained 15.5 MJ ME/kg, and finally the DWG<sub>DMD</sub> was calculated from the ME intake. The cumulative predicted LW of the control treatment heifers in each draft was calculated in 2-weekly steps from the LW at the commencement of each draft with both the DWG<sub>Direct</sub> and the DWG<sub>DMD</sub> predictions.

Effects of urea treatment on measurements of diet attributes and on the cumulative change in measured heifer LW within each of the 8 seasonal intervals were analysed by analysis of variance. Measurement dates were assumed to be independent within a season and were treated as a blocking factor in an analysis of variance. Residual plots were used to assess the normality of the data and indicated that no transformations were required. For LW each heifer was considered the observational unit, and the changes in heifer LW at each monthly measurement from the commencement of the draft were analysed by analysis of variance in which each measurement day was considered as a block.

# 3. Results

### 3.1. Rainfall and supplement intake

Rainfall was negligible during DS-1 from the 1 August 2001 until the effective seasonal break on the 11 November 2001 (Table 1). Rainfall totalled 511 mm during WS-1 from 11 November 2001 through to the 31 March 2002, and this was comparable to the long-term average wet season rainfall for the site of 450 mm. There was negligible rain through the

#### Table 1

Rainfall (mm) preceding and during the experiment at the site, and the long-term average at Charters Towers 20 km from the site.

Month	2001/2002	2002/2003	2003	123-year mean
July	6	0	0	16
August	0	22	0	13
September	0	0	0	14
October	11	0	14	22
November	73	2	0	45
December	77	24	61	87
January	137	18		138
February	215	284		127
March	9	21		98
April	0	1		42
May	10	23		24
June	8	17		25
Total	546	412		651
Break	11 Nov 01 <sup>a</sup>	21 Dec 02 <sup>b</sup>		27 Dec <sup>c</sup>

<sup>a</sup> The seasonal break occurred with 40 mm of rain on the 11 November 2001; this was followed by 151 mm in scattered falls (each  $\leq$  28 mm) between the 12 November and the 11 January 2002, and then 83 mm on the 13 January 2002.

<sup>b</sup> The seasonal break occurred with 24 mm on the 21 December 2002. This was followed by intermittent rain (each  $\leq$  12 mm, total 52 mm) between the 2 January and the 18 February, and by 84 mm of rain on the 23 February 2003.

<sup>c</sup> Median date of the seasonal break defined as at least 50 mm in 3 days.

TS-1, DS-2A and DS-2B seasons. The seasonal break for WS-2 occurred with 24 mm on the 21 December 2002, followed by a total of 52 mm in intermittent falls in January and early February, and 84 mm on the 23 February 2003. The rain from the 21 December through to mid February was sufficient to initiate new grass growth and to provide a 'green pick', but there was only a small amount of this new growth of grass. Substantial growth of pasture did not occur until early March 2003, and thus the 2002/03 pasture growing season was shorter than in the previous year or as generally observed for the region. Total rainfall during WS-2 (347 mm) was 0.77 of the long-term average. Intake of the urea treatment LMM was about 140 g/heifer day during DS-1, DS-2A and DS-2B, and about 120 g/heifer day during DS-3. Thus the urea treatment heifers ingested 16–19 g supplementary N/day, 0.94 of which was urea N.

#### 3.2. F.NIRS predictions of diet quality

During the entire experiment the non-grass content of the diet selected by the 2 herds of heifers ranged from 0.00 to 0.36, averaged 0.13 (S.D. 0.053) of the diet, and appeared not to be related to the season (Fig. 1). Diet non-grass was lower in the urea treatment heifers than in the control treatment heifers during DS-1 (0.11 and 0.20, respectively, *P*<0.01) and during WS-1 (0.09 and 0.15, respectively, *P*<0.05), but not during the remainder of the experiment. Thus the urea supplementation during DS-1 reduced the selection of non-grass during the first 7 months of the experiment. During DS-1 diet DMD tended (*P*<0.10) to be lower in urea treatment than in the control treatment heifers (0.474 and 0.485, respectively). Also during DS-1 the predictions for diet CP, which estimated the CP of the forage component of the diet, were 29 and 37 g/kg in the urea and control treatment heifers, respectively, but these means were not different (*P*>0.10). The lower non-grass content selected by the urea treatment heifers during DS-1 and WS-1 did not have any discernable effect on other attributes of the diet estimated with F.NIRS.

Since F.NIRS estimates of diet CP, DMD and faecal N are not influenced (*P*>0.05) by ingestion of LMM supplements *per se* (Dixon and Coates, 2005, 2009), the mean values for the 2 supplement treatments are presented. Diet CP of Draft 1 heifers averaged 33 g/kg during DS-1, increased to average 94 g/kg during WS-1, and declined through the TS-1 to







**Fig. 2.** The crude protein content (CP) ( $\triangle$ ) and DM digestibility ( $\blacksquare$ ) of the diet (Fig. 1A), the ratio DMD/CP ( $\blacktriangle$ ) in the diet, and the concentration of N in faces ( $\Diamond$ ) (Fig. 1B), of heifers grazing a buffel grass pasture. The average values for 2 herds offered LMM supplement containing, or not containing, urea are shown. For heifers fed the urea supplement the F.NIRS predictions of diet CP represented the forage component of the diet.



**Fig. 3.** The liveweights of 2 drafts of heifers grazing a buffel grass pasture and offered the control treatment ( $\bigcirc$ ) or the urea treatment ( $\triangle$ ) LMM supplement, and the LW response due to the urea supplement (**X**) as the cumulative difference in LW in 2 drafts of grazing heifers.

average 41 g/kg during DS-2A (Fig. 2). In Draft 2 heifers the diet CP averaged 35 g/kg in DS-2B, increased on average to 100 g/kg during WS-2, and then declined through TS-2 to average 49 g/kg in DS-3. Average DMD was high during WS-1 (0.591) and WS-2 (0.601), and declined progressively through each of the transition seasons (TS-1 0.521, TS-2 0.557 g/kg) into the dry seasons (0.477–0.506). The average DMD/CP ratio during the dry seasons ranged from 11 to 15, but was 6 to 7 during the wet seasons (Fig. 2). Average faecal N concentration during the dry seasons ranged from 10 to 11 g/kg (Fig. 2).

In control treatment heifers both diet DMD and faecal N concentration were correlated with the diet CP as follows:

Diet DMD = 0.00154(Diet CPg/kg) +  $0.442(n = 43, R^2 = 0.83, P < 0.001, rsd = 0.0191)$ 

Faecal N(g/kg) = 0.0867(Diet CPg/kg) + 6.7(n = 43,  $R^2 = 0.78$ , P < 0.001, rsd = 1.26)

In addition, the diet DMD/CP ratio (*Y*) was related curvilinearly to the faecal N concentration (*X*) as follows:

 $Y = 3.85 + 54.2 \times 0.827^{X} (n = 43; R^{2} = 0.56; P < 0.01).$ 

However, diet CP was a better predictor of the DMD/CP ( $Y = 4.68 + 28.4 \times 0.728^{X}$ , n = 43,  $R^{2} = 0.99$ , P < 0.01).

#### 3.3. Heifer LW and LW response to supplement

In general through the 2 years of the experiment the heifers maintained LW during the early and mid dry seasons, lost LW during the late dry season, and gained LW during the wet and transition seasons (Fig. 3). The Draft 1 control treatment heifers lost 30 kg during DS-1 and until shortly after the seasonal break (1 August to 15 November 2001), gained 143 kg (0.94 kg/day)

during WS-1 and early TS-1 (15 November 2001 to 16 April 2002), and then maintained LW through the remainder of TS-1 and during DS-2A. The Draft 2 control treatment heifers lost 32 kg during DS-2B and until shortly after the seasonal break (11 October to 1 January 2003), gained 136 kg (0.64 kg/day) from the seasonal break through to the mid dry season (1 January to 5 August 2003), and then lost 9 kg through to 20 October 2003. Overall the Draft 1 control supplement heifers gained 107 kg during the 12 months from 1 August 2001, while the Draft 2 heifers gained 96 kg during the 12 months from 11 October 2002.

The urea supplement reduced heifer LW loss during the dry seasons, and also during TS-1 (Fig. 3), but in both drafts much of the LW benefit of the urea supplement was lost due to subsequent compensatory growth. Briefly, the benefit of the urea supplement on LW change was 15 kg during DS-1 (P<0.05), 26 kg during TS-1 (P<0.10) and DS-2A (P<0.01), and 13 kg during DS-3 (P<0.01). However, the LW benefits accrued during DS-1 and DS-2B were largely lost during the subsequent growing seasons. Over the entire annual cycle, the urea supplement resulted in a LW benefit in the Draft 1 heifers of 24 kg (P<0.01) in September 2002, and in Draft 2 heifers of 14 kg (P<0.05) in October 2003 at the end of the draft.

### 3.4. F.NIRS prediction of changes in heifer LW

The relationship between the DWG calculated from the monthly LW of the heifers ( $DWG_{Actual}$ ) and that predicted from the revised F.NIRS calibration ( $DWG_{Direct}$ ) for the control treatment heifers is shown in Fig. 4A. Two observations in Draft 2, one 13 days after the seasonal break on the 21 December 2002 and one 9 days after the substantial rains on the 23 February 2003, appeared to be outliers from the general relationship. These observations were during the interval after the



**Fig. 4.** (A) The relationship between the actual daily weight gain (DWG<sub>Actual</sub>) calculated from the monthly measurements of liveweight and the DWG predicted by F.NIRS (DWG<sub>Direct</sub>) in heifers given the control supplementation treatment. The values shown ( $\triangle$ ) were derived from faecal samples obtained within 4 weeks after the seasonal break on the 11 November 2001 in Draft 1, or from the seasonal break on the 21 December 2002 and until 4 weeks after the substantial rain of the 23 February 2003 during the 2002/03 wet season for Draft 2. The values measured during the remainder of the annual cycle are shown as ( $\bigcirc$ ). Values represent faecal samples obtained on each occasion the heifers were weighed. The regression relationship for all values was: Y = 0.52X + 215 (*n* = 24, *R*<sup>2</sup> = 0.43, *P*<0.001, rsd = 332, ---). The regression relationship for the ( $\bigcirc$ ) values and excluding the ( $\triangle$ ) values was: Y = 0.74X + 66 (*n* = 20, *R*<sup>2</sup> = 0.78, *P*<0.001, rsd 197, –).

In (B) the relationships between DWG<sub>Actual</sub> and the DWG (DWG<sub>DMD</sub>) calculated from the diet DM digestibility estimated with F.NIRS and following CSIRO (2007) in heifers given the control supplementation treatment. The regression relationship for all values was: Y = 0.29X + 177 (n = 24,  $R^2 = 0.25$ , P < 0.01, rsd 279, ---). The regression relationship for the ( $\bigcirc$ ) values and excluding the ( $\triangle$ ) values was: Y = 0.45X + 58 (n = 20,  $R^2 = 0.63$ , P < 0.001, rsd 173, –).



**Fig. 5.** Measured LW ( $\bigcirc$ ) and the cumulative predicted LW ( $\triangle$ ) calculated from the initial LW of each draft and the F.NIRS estimates each 2 weeks of daily weight gain in the heifers given the control supplementation treatment.

seasonal break when intermittent rains led to 'green pick' but little pasture growth. To identify such apparent outlier values in Fig. 4A all of the observations obtained within 4 weeks after the seasonal break on the 11 November 2001, or from the 21 December 2002 through to 4 weeks after the substantial rain of the 23 February 2003, are shown separately. The DWG<sub>Actual</sub> and the DWG<sub>Direct</sub> were related ( $R^2 = 0.43$ ; P<0.001) when all observations were included, but were more closely related ( $R^2 = 0.78$ ; P<0.001) if the early wet season observations were excluded (Fig. 4A). In the relationship between the DWG<sub>Actual</sub> and DWG<sub>DMD</sub> (Fig. 4B) the observations obtained on the same dates during the early wet season also appeared to be outliers. Again the DWG<sub>Actual</sub> and DWG<sub>DMD</sub> were related ( $R^2 = 0.25$ ; P<0.01) when all observations were included, but were more closely related ( $R^2 = 0.63$ ; P<0.001) if the early wet season observations were excluded. Thus DWG<sub>Direct</sub> was more closely related to DWG<sub>Actual</sub> than was DWG<sub>DMD</sub>, although the two F.NIRS predictions of DWG (g/day units) were closely related as follows:

DWG<sub>DMD</sub> = 0.70DWG<sub>Direct</sub> + 6(n = 24,  $R^2 = 0.90$ , P < 0.001, rsd = 101).

It appeared that neither estimate of DWG derived from F.NIRS could provide useful predictions of the actual DWG during the early wet season and until there was substantial availability of new pasture growth. However both such estimates of DWG provided useful predictions of the actual DWG during the remainder of the annual cycle.

The cumulative predicted LW of the heifers, calculated from the LW at the commencement of each draft and the summative effects of DWG<sub>Direct</sub> measured in fortnightly increments, are shown in Fig. 5. In Draft 1 agreement between the measured and predicted cumulative LW of the control treatment heifers was generally good, although predicted LW overestimated actual LW by, on average, 12 kg during WS-1 and 17 kg during DS-2A. In Draft 2 heifer LW was predicted satisfactorily during DS-2B, but was substantially overestimated, by on average 42 kg, from the seasonal break on the 21 December 2002 until the end of the draft; this difference was principally due to the overestimation of DWG by F.NIRS during the early wet season as discussed above. Because DWG<sub>Direct</sub> predictions through the remainder of Draft 2 provided reasonable estimates of DWG<sub>Actual</sub>, the overestimation of LW continued through the TS-2 and DS-3 seasonal intervals. If the calculation of predicted LW had been reset with the actual heifer LW at the end of the WS-2 seasonal interval, the heifer LW would have been predicted more satisfactorily through TS-2 and DS-3.

### 4. Discussion

# 4.1. The reliability of the F.NIRS measurements of diet quality and LW change

Numerous studies have demonstrated that in ruminants F.NIRS can reliably estimate dietary attributes of forage diets (Lyons and Stuth, 1992; Coates, 2004; Landau et al., 2006; Dixon and Coates, 2009; Decruyenaere et al., 2009). In addition several considerations enhance confidence in the reliability of the F.NIRS predictions of diet quality for the cattle and pastures in the present study. First, the Coates (2004) F.NIRS calibrations used to predict diet CP concentration and DMD were likely particularly appropriate since the majority of the data set (n = 360 diets) used to develop these calibrations had been derived from comparable forages and cattle in the same region of NE Australia, and included many diets (n = 131) consisting predominantly or entirely of buffel grass. The bias for prediction of diet CP (observed – predicted) of these latter buffel grass diets within the Coates (2004) calibration was, on average, -3.5 (S.D. = 6.7) g/kg units for those with diet CP  $\leq 50$  g/kg (n = 54), and -4.5 (S.D. = 9.3) g/kg units for all of the diets; thus the prediction errors were small and the predictions tended to overestimate the actual CP concentration. Second, the Mahalanobis distance values (expressed as global H in the ISI software) during prediction of faecal samples were (mean  $\pm$  S.D.)  $1.4 \pm 0.8$  for diet CP concentration,  $1.8 \pm 0.9$  for DMD,  $0.8 \pm 0.3$  for  $\delta^{13}$ C, and  $0.6 \pm 0.3$  for faecal N concentration, with 6%, 11%, 0% and 0% of samples with GH values > 3.0, respectively. Thus the desirable maximum global H value of 3.0 for predictive reliability (Shenk and Westerhaus, 1991; Landau et al., 2005) was exceeded for only a small proportion of samples. Third, faecal samples (n = 17) selected at random from the present

study were analysed for total N and  $\delta^{13}$ C contents by conventional laboratory procedures in order to directly examine the errors in these predictions. The bias in prediction (mean ± S.D.) was 0.3 ± 0.6 g/kg units for faecal N, and 0.007 ± 0.043 for non-grass. Thus there is considerable evidence that the F.NIRS predictions of diet attributes were reliable, and in particular diet CP concentrations were more likely to have been slightly overestimated rather than underestimated.

Several studies have indicated that, provided diet selection remains unchanged, ingestion of LMM supplements containing urea and minerals does not have any discernable effect on F.NIRS predictions of diet attributes from current calibration equations (Dixon and Coates, 2005, 2009). Thus, in the present study, the predicted diet CP and DMD of both the control and the urea supplemented treatment heifers would have represented the forage component of the diet. Calculations from the amount of urea ingested and estimated DM intake indicated that the CP concentration of the entire diet would have been increased by 12–19 g CP/kg by the urea supplement when it was fed during the transition and dry seasons.

Because the heifers were weighed regularly in the present study it was possible to compare F.NIRS predictions of DWG with the measured DWG (Fig. 4), and the cumulative predicted LW with the actual LW through each annual cycle (Fig. 5), and thus directly examine the errors associated with these F.NIRS predictions. The prediction of DWG directly from an appropriate F.NIRS calibration (DWG<sub>Direct</sub>) was more satisfactory than the indirect prediction from diet DMD estimated by F.NIRS and then calculation of DWG from estimated ME intake (DWG<sub>DMD</sub>). The latter approach has the advantage that the F.NIRS prediction of DMD is likely to be more reliable across a variety of pasture situations than prediction of DWG<sub>Direct</sub>, but it has the disadvantage that many assumptions are required in the calculation. In the present study this included the assumption that voluntary DM intake was decreased during the late dry season by, on average, 38% due to rumen degradable protein deficiency. The Coates (2004) calibration data set for DWG<sub>Direct</sub> was particularly appropriate to the present study since comparable pastures and cattle in the same region were well represented in the data set. Furthermore this DWG calibration has performed well in other studies in the speargrass regions of NE Australia (Dixon, 2008; Dixon et al., 2007; Coates and Dixon, 2008b). It should, however, be pointed out that this calibration did not perform satisfactorily in cattle grazing some other pasture systems such as Astrebla spp. pastures (Dixon, 2008), or Leucaena-grass pasture until the calibration was modified with local data (Dixon and Coates, 2008). It seems likely that in pasture systems not well represented in the DWG calibration data set, but where nevertheless diet DMD is likely to be predicted robustly, the DWG<sub>DMD</sub> is likely to provide more reliable predictions than DWG<sub>Direct</sub>.

During the majority of the annual seasonal cycle the errors in prediction of DWG<sub>Direct</sub> would be acceptable for many purposes such as monitoring growth. However for 3 months after the seasonal break in the 2002/2003 wet season these predictions often differed greatly from the actual DWG. The DWG<sub>DMD</sub> predictions were also not useful during this interval. The lack of agreement between the observed and predicted values during this interval was likely associated with difficulties in the interpretation of measured DWG, as well as errors in the F.NIRS predictions. First, because there are often large decreases in digesta load and body water of grazing cattle at the onset of the wet season in seasonally dry tropical environments (Payne, 1965; Norman, 1967; Walker, 1969; McLean et al., 1983), the animal body tissues and body energy reserves usually decrease to a much lesser extent than indicated by the loss in animal LW. Second, in the present study there was increased error associated with calculating the measured DWG soon after the seasonal break. Third, DWG<sub>Direct</sub> calibrations are known to overestimate DWG where voluntary intake is constrained by availability of pasture *i.e.* when voluntary intake is constrained by the quantity rather than the quality of pasture available (Dixon and Coates, 2009). As discussed above, one factor likely contributing to the large difference between actual LW and predicted cumulative LW in the 2002/2003 wet season was that the small intermittent rains from the 21 December 2002 until 23 February 2003 initiated new pasture growth and increased diet quality, but were insufficient for substantial pasture growth. Therefore, despite diet quality being high, the amount of new growth pasture available was low and voluntary intake of such new season pasture was likely constrained by the quantity available. Thus the heifers only maintained LW rather than gaining LW rapidly. This phenomenon is often observed in the seasonally dry tropics when there is intermittent but not substantial rain during the early wet season (Norman, 1967; McLean et al., 1983). Thus several factors may explain the results observed in the present study. It is also of interest that in some other studies (Dixon et al., 2007; Dixon, 2008) F.NIRS predictions of cattle DWG have deviated markedly from the actual DWG in the early wet season.

F.NIRS estimates of the cumulative predicted LW over time appeared to be better than the individual predictions of DWG. As for any repeated sampling, the error of F.NIRS predictions from a sequence of faecal samples will be expected to decrease in proportion to the number of samples, by  $1/\sqrt{n}$  if the samples are truly independent. Thus, in the absence of bias, the error of the cumulative predicted LW derived from a sequence of faecal samples should be lower than that of DWG from a single faecal sample since under- and overestimation errors from individual samples will often cancel. Furthermore estimates of actual animal LW derived from weighing the animals on specific days will also be associated with error such as from deviations in digesta load, and repeated weighing of animals will reduce error from this source.

#### 4.2. Diet selection, diet quality, heifer LW and the response to the N supplement

The F.NIRS estimates of diet CP content and DMD were generally consistent with the distribution and amounts of rainfall, current knowledge of the diet selected by grazing cattle, and observed LW cycles of the cattle in comparable environments. The average 0.13 diet non-grass was comparable with, or not much greater than, the amounts reported previously for cattle grazing tropical native pastures without introduced legume augmentation in the same region (Ash et al., 1995; Coates and

Dixon, 2007, 2008b; Dixon, 2008). Thus the diet selected consisted primarily of buffel grass. In DS-1 the higher proportion of non-grass in the diet selected by the control treatment than the urea treatment heifers indicated that selection of one or more of the browse, legume and forb components of the pasture, which would have been higher in N concentration than the buffel grass (Coates and Dixon, 2007), was increased during N deficiency. Ruminants can often modify grazing behaviour to select plant species containing higher concentrations of a limiting nutrient (Provenza, 1996; Kyriazakis et al., 1999). For example, supplements which increase the availability of N from high-tannin browses can increase the selection of browse by grazing goats (Titus et al., 2001; Landau et al., 2002; Decandia et al., 2008). Similarly, in cattle grazing phosphorus-deficient pastures, phosphorus supplements have increased the proportion of stylo legume selected likely because high calcium concentrations in the stylo exacerbated the phosphorus deficiency in cattle not fed phosphorus supplement (Coates and Le Feuvre, 1998). The observation in the present study that urea supplemented heifers continued to select less non-grass during the 2001/2002 wet season (WS-1) when no supplementary urea was provided and when the diet was no longer N deficient was unexpected, and must have been a carry-over effect from provision of the urea supplement during the previous dry season. This is in accord with previous observations that experience with LMM supplements containing urea can influence voluntary intake of similar LMM supplements during subsequent seasons (Dixon et al., 2001, 2003). Urea supplementation did not affect the proportion of non-grass selected by the Draft 2 heifers, but there did not appear to be any obvious reason for this difference between the drafts of heifers.

The observed patterns and rates of LW gain and loss of the control treatment heifers and annual LW gains for the 2 drafts of heifers (Fig. 3), were within expectations for the soil, region, pasture, and the poorer than average seasonal conditions. The LW losses during the late dry season of the control treatment heifers (30 kg in Draft 1, and 9 kg in Drafts 2, respectively) were moderate and substantially lower than are often observed in the seasonally dry tropics (Foster and Blight, 1984; Dixon, 1998; Coates and Dixon, 2008). As discussed above the poor LW performance of the heifers during the early and mid 2002/2003 wet season, despite high dietary CP and DMD during this interval, was presumably because ME intake was constrained by the amount of high quality pasture available.

The effect of urea supplementation during the dry season on LW change of cattle in comparable seasonally dry environments has been reported to vary widely, from negligible or small responses in some years with benign dry season conditions (McLennan et al., 1981; Dixon and Doyle, 1996) to large effects (*e.g.* 0.2 kg/day) with cattle grazing native pastures in the speargrass regions of northern Australia (Winks et al., 1972, 1979; Foster and Blight, 1984; Dixon, 1998; Coates and Dixon, 2008b). The reduction of heifer LW loss by 0.11–0.15 kg/day during the dry season in both drafts of the present study was thus comparable with the middle of this range. However because each draft in the present study commenced in the mid dry season and compensatory growth occurred during the wet and transition seasons, the overall effect on animal LW during each draft was appreciably lower than that often observed during the entire dry season. We are aware of only one previous study examining urea supplementation of cattle grazing buffel grass pasture (Graham et al., 1983); this was in a region with fertile clay soils and greater winter rainfall, and there was only a minor response.

Compensatory growth effects in the present study resulted in the benefits of the urea supplement on LW being largely lost during the subsequent wet and transition seasons. Similar compensatory growth has often been observed in previous urea supplementation experiments in comparable environments (Winks et al., 1979; Foster and Blight, 1984; Coates and Dixon, 2008b). In the present study, with a benefit in heifer LW at the end of an annual cycle of only about 24 and 14 kg in Drafts 1 and 2, respectively, the efficacy of urea supplementation was marginal. However, comparisons between studies need to consider that in the present study the drafts of heifers were measured from the mid dry season to the next mid dry season, whereas previous experiments have usually reported changes in animal LW through a dry season although subsequent compensatory growth often reduced the annual LW benefit of the urea supplement.

It has been suggested that in cattle ingesting low quality forages responses to supplementary CP can be expected when the forage CP is less than 60 g/kg (Minson, 1990), or when the DMD/CP is greater than 8–10 (Dixon and Coates, 2005), or when faeces contain less than 13 g N/kg (Winks et al., 1979). In the present study F.NIRS indicated that the diet averaged 33–35 g CP/kg in the late dry season and 41–49 g CP/kg in the early to mid dry season, that during the same intervals the DMD/CP was usually greater than 11, and that faecal N concentration was less than 11 g N/kg. Therefore according to these criteria the responses by the heifers to the urea supplement were expected. Several factors may have contributed to the generally smaller change in animal LW when urea supplements were fed in the present study than sometimes observed previously. First in the present study the intervals during which diet CP concentration was low, and DMD/CP was high, were briefer than observed in a number of the other experiments in the same region with cattle grazing senesced native pastures during the dry season (Dixon et al., 2007; Coates and Dixon, 2008b; Dixon, 2008). Second, *Bos indicus* cattle have a greater ability to conserve N and a lower response to supplementary N than *Bos taurus* cattle (Winks et al., 1972; Hunter and Siebert, 1987; Hennessy et al., 2000); thus the heifers used in the present study may have had a lower potential to respond to supplementary urea than the *Bos indicus* × *Bos taurus* crossbreds used in most of the previous studies. Third, the voluntary intake of the urea supplement was lower than expected, and the intake of 16–19 g supplementary N/day may have been less than that required for the maximum animal response (Winks et al., 1979; Dixon and Doyle, 1996).

In conclusion, the F.NIRS estimates of diet quality as crude protein content, digestibility and non-grass proportion were generally consistent with previous studies and knowledge of the diet selected by cattle in such environments following various rainfall events, and the observed performance of the cattle. The study demonstrated that F.NIRS can be easily used to provide detailed nutritional information on the seasonal changes in the diet selected by grazing cattle in the seasonally

dry tropics, and to estimate cattle growth. This is clearly valuable information to evaluate animal productivity and the likely economic responses to nutritional and management interventions in grazing cattle.

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