

**ILC2018 Keynote paper\***

## **Evaluating crude protein concentration of leucaena forage and the dietary legume content selected by cattle grazing leucaena and C4 grasses in northern Australia**

*Evaluando la concentración de proteína cruda de forraje de leucaena y la proporción de la leguminosa en la dieta seleccionada por ganado pastoreando mezclas de leucaena con gramíneas C4 en el norte de Australia*

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### **Abstract**

In Australia's central and southern Queensland regions, *Leucaena leucocephala*-grass pastures produce substantially more beef and higher profits than grass-only pastures and annual forage crops. Near infrared reflectance spectroscopy (NIRS) provides a rapid and cost-effective approach to assessing quality of available forage as well as the quality of the diet selected by cattle, but existing calibrations have not been comprehensively validated for leucaena-grass pastures. This study examined the reliability of existing northern Australian calibrations for NIRS to predict the crude protein (CP) concentration of the edible fraction of the leucaena plant, and the proportion of leucaena in the diet of grazing cattle. Samples of edible leucaena and cattle feces were analyzed by NIRS and the predictions plotted in a linear regression and fitted to a 1:1 line with Dumas analysis of CP for leucaena forage, and mass spectrometry of  $\delta^{13}\text{C}$  for cattle feces. Results demonstrated that prediction of the CP concentration of leucaena forage and the proportion of leucaena in the diet of grazing cattle using current broad northern Australian NIRS forage calibrations were associated with substantial error. However, it is likely that these errors can be reduced with the inclusion in the calibration data set of more samples representing leucaena forage and feces of cattle grazing leucaena from varying locations, seasonal conditions and management strategies.

**Keywords:** Diet quality, forage quality, near infrared reflectance spectroscopy, tree legumes, tropical pastures.

### **Resumen**

En las regiones central y sur de Queensland, Australia, pasturas con *Leucaena leucocephala* y gramíneas producen sustancialmente más carne y mayores ingresos en comparación con pasturas de solo gramíneas o con cultivos forrajeros anuales. La espectroscopía de reflectancia en el infrarrojo cercano (NIRS) es un método rápido y económico para evaluar la calidad de forraje disponible, así como la calidad de la dieta seleccionada por el ganado. Sin embargo, las calibraciones disponibles no se han validado de manera exhaustiva para las pasturas de asociaciones de leucaena con gramíneas. En este estudio se examinó la confiabilidad de las calibraciones actualmente existentes en el norte de Australia para NIRS, para predecir la concentración de proteína cruda (PC) en la fracción comestible de plantas de leucaena y la proporción de leucaena en la dieta seleccionada por ganado pastando mezclas con gramíneas. Se analizaron por NIRS muestras de

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leucaena y de heces de ganado y se proyectaron las predicciones en una regresión lineal ajustadas a una línea 1:1 usando el método de análisis de Dumas para CP en el forraje de leucaena y la espectrometría de masas de  $\delta^{13}\text{C}$  para las heces de ganado. Los resultados demostraron que usando las calibraciones de NIRS que actualmente existen para forraje en el norte de Australia, las predicciones de la concentración de PC en el forraje de leucaena y de la proporción de leucaena en la dieta del ganado en pastoreo, estaban asociadas con errores sustanciales. Sin embargo, es probable que estos errores se puedan reducir si en el conjunto de datos de calibración se incluyen muestras adicionales representativas de forraje de leucaena y de heces de animales pastoreando leucaena provenientes de diferentes lugares, condiciones estacionales y estrategias de manejo.

**Palabras clave:** Calidad de dieta, leguminosas arbóreas, NIRS, pastos tropicales, valor nutritivo.

## Introduction

The ~123,500 ha of established *Leucaena leucocephala*-grass pastures is important to the beef industry in central and southern Queensland (Beutel et al. 2018), as it provides opportunity to substantially increase beef production and profitability compared with perennial grass pastures and other sown forages (Bowen et al. 2018). However their optimal management requires knowledge of available quantity and quality of both the leucaena and grass pasture components, especially crude protein (CP) concentration, dry matter digestibility (DMD) and the proportion of leucaena in the diet selected by grazing cattle (Bowen et al. 2015).

Near infrared reflectance spectroscopy (NIRS) provides a rapid and cost-effective approach to not only assess the quality of the forage (plant) material presented to cattle, but also the quality of the diet selected by grazing cattle by testing their feces. NIRS predictions depend on the availability of reliable and robust calibration equations appropriate to the forages and grazing systems of interest. Broad NIRS calibrations have been developed for most common pastures in northern Australia (Coates 2004; Dixon and Coates 2009), but have not been comprehensively validated for leucaena-grass pasture systems. This study examined the reliability of these northern Australian NIRS calibrations to predict the CP concentration of the edible fraction of leucaena forage and the proportion of leucaena in the diets of grazing cattle.

## Materials and Methods

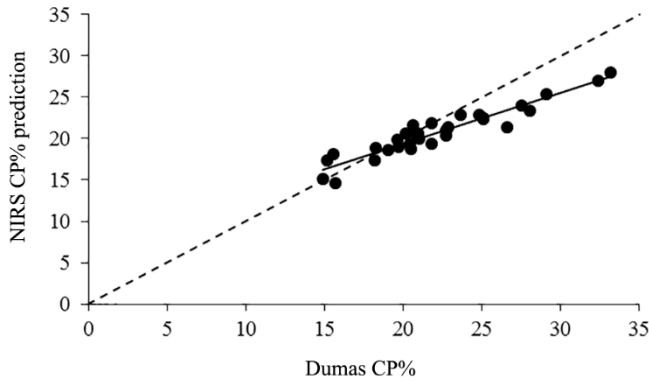
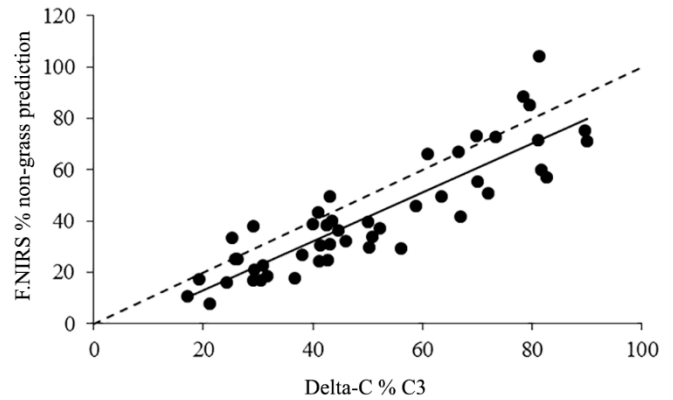
Samples of the leucaena forage selected by grazing cattle (leaf and stem <5 mm in diameter, considered the 'edible' fraction of leucaena forage), and feces of cattle grazing leucaena-grass pastures were collected as described by

Bowen et al. (2015) from 4 commercial producer sites in the Fitzroy River Basin. These samples represented a range of environments, seasonal conditions and management strategies.

Edible leucaena forage samples (n = 31) were analyzed for CP by both wet chemistry (Dumas) and by NIRS (Dixon and Coates 2009), with CP predicted from established 'in-house' calibrations suitable for northern Australian forages (Coates and Dixon unpublished data). Fecal samples (n = 48) from cattle grazing these leucaena-grass pastures were analyzed for  $\delta^{13}\text{C}$  by mass spectrometry and the proportions of C3 species in the diets calculated, with corrections for diet-tissue discrimination and differences in digestibility and  $\delta^{13}\text{C}$  values between the C3 and C4 species (Bowen et al. 2018). NIRS of feces (F.NIRS) was used to predict the non-grass proportion of the diet using calibrations for northern Australian tropical pastures (Dixon and Coates 2008). Linear regressions between NIRS predictions of CP in forage and that measured by Dumas, and F.NIRS predictions of non-grass in the diet and that measured by mass spectrometry, were fitted and compared with the 1:1 line.

## Results

There was a strong linear relationship between the NIRS-predicted CP concentrations of edible leucaena forage and those measured by wet chemistry ( $R^2 = 0.90$ ), but the regression differed ( $P < 0.05$ ) from the 1:1 relationship (Figure 1a); samples containing >ca. 22% CP were under-predicted. The relationship between the proportion of leucaena in the diet, predicted by F.NIRS as % non-grass, and that calculated from the  $\delta^{13}\text{C}$  measured by mass spectrometry, did not differ from a 1:1 line (Figure 1b), but there was considerable variation about the regression line ( $R^2 = 0.78$ ).

**a) Forage NIRS****(b) Fecal NIRS**

**Figure 1.** **a)** The relationship between edible leucaena CP% (Y) predicted by NIRS and that measured by Dumas (X);  $Y = 0.61X + 7.2$  ( $n = 31$ ;  $R^2 = 0.90$ ); intercept  $>0$  ( $P < 0.05$ ) and slope  $<1$  ( $P < 0.05$ ). **b)** The relationship between proportion of leucaena in the diet (Y) predicted by F.NIRS and that calculated from  $\delta^{13}\text{C}$  in feces (X):  $Y = 0.95X - 6.1$  ( $n = 48$ ;  $R^2 = 0.78$ ); the relationship did not differ ( $P > 0.05$ ) from the 1:1 line. The 1:1 relationships are indicated by a dashed line (---).

**Discussion**

The broad NIRS calibration equation for forage samples used to predict the CP concentration of the ‘edible’ fraction of leucaena forage was developed from a large calibration data set dominated by tropical grasses and containing only a few samples of leucaena forage. Thus the observed deviation of CP% of leucaena forage as predicted by NIRS from the 1:1 relationship (Figure 1a) was not unexpected. While this error was minor for the range ca. 17–22% CP, the equation substantially underestimated the CP concentration in samples above this range. For an NIRS calibration to be reliable, it must include samples applicable to the forage type, location and season of those being analyzed. The existing calibration for northern Australian tropical pasture systems proved unsatisfactory in predicting the CP% of leucaena forage. However, inclusion of additional samples of leucaena forage into the calibration sample set, particularly those with CP outside of the range ca. 17–22%, is likely to reduce the errors associated with predicting CP% in leucaena forages containing low or high concentrations of CP. This is supported by the study of Wheeler et al. (1996) which showed that satisfactory calibrations with a validation  $R^2 = 0.89$  can be developed for prediction of the CP concentration of leucaena forage.

The F.NIRS calibration equation used to predict the proportion of leucaena in the diet of grazing animals was based on a large sample set of feces from cattle grazing northern Australian pasture systems which included few samples ( $n = 9$ ) from leucaena-grass pastures. Within that calibration set there was a close relationship between the

reference and predicted values [ $R^2 = 0.90$ , standard error of cross-validation (SECV = 6.6% units)] and this calibration satisfactorily predicted the leucaena % in the diet in a previous study ( $R^2 = 0.92$ ;  $n = 15$ ; relative standard deviation = 8.1 % units; Dixon and Coates 2008). However in the present study, the relationship between the measured  $\delta^{13}\text{C}$  reference values and those predicted by F.NIRS using the above mentioned calibration were poor with  $R^2 = 0.78$  (Figure 1b). As discussed above for NIRS predictions of CP% in forage, it is likely that the errors in prediction of non-grass (% C3 or leucaena) content of diets of cattle grazing such pastures can be reduced by including in the calibration data set more samples representing these diets from varying locations, seasonal conditions and management strategies. It must also be noted that F.NIRS calibration sets do not currently account for the difference in digestibility between C3 and C4 forage species; it is possible that the errors in prediction may be further reduced by accounting for this factor.

Improvement of F.NIRS calibrations to predict the diet of cattle grazing leucaena-grass pastures can be expected in the future. However, until such improvements can be made to the NIRS predictions of dietary non-grass,  $\delta^{13}\text{C}$  should be used for scientific experiments.

In conclusion, measurement of the CP concentration of leucaena forage using current broad northern Australian NIRS forage calibrations was associated with substantial error, when CP concentrations were above ca. 22%. In addition, measurement of the leucaena content of the diet of cattle grazing leucaena-grass pastures using F.NIRS and the current broad northern Australian F.NIRS calibration equations was associated with substantially larger errors

than those for most grass and grass-stylo pastures. Given the economic importance of leucaena-grass pastures in northern Australia and the advantages of the NIRS technology for measurement of forage and diet attributes in grazing cattle, it is important that the northern Australian NIRS calibrations are refined to more accurately and reliably measure the quality of forages and that of diets selected by cattle in leucaena-grass pasture systems.

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