Determining the quality of diets of grazing animals

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Abstract: *Predicting growth rates or determining the needs for supplementary feeding of grazing animals requires knowledge of the nutritive value of the diet the animals are consuming. Faecal analyses are non-invasive and effective methods for compiling information about the diets of animals. In this study the usefulness of faecal chemistry and near infrared spectroscopy (fNIRS), either used individually or in combination to predict the quality of mixed diets fed to sheep, was investigated. Faecal nitrogen, ash, neutral detergent fibre and lignin contents can be successfully used to predict the metabolisable energy content and the organic matter digestibility of the diet as well as the type of rumen fermentation (in terms of short chain fatty acids) whilst fNIRS calibration equations can be successfully used to predict the crude protein, total phenolic and total tannins contents of mixed diets consumed by sheep.*

Introduction

Ultimately the nutritive value of the diet of grazing animals depends on what they eat, and determination of the nutritive value of the diet of grazing ruminants is still one of the major challenges of grazing scientists and managers, especially when the grazing environment is highly diverse, as is the case for the Australian grasslands and rangelands. In these environments the pastures are heterogeneous and, frequently, the plant assortment is highly complex.

Faecal analyses are effective methods for compiling information about the diets of animals. Sample collections are easy and fast to carry out and unlimited in the number of samples collected, which is ideal for grassland and rangeland environments. Faecal fractions such as faecal fibre, lignin and nitrogen (N) or faecal near infrared spectroscopy (*f*NIRS) have been used to predict the quality of the diet of grazing animals. Vera (1973) and Hodgman *et al.* (1996) showed the potential of faecal fibre fractions to predict digestibility and energy contents of ruminant diets. Faecal N has been found to be closely associated with dietary N (Holecheck *et al.* 1982; Mubanga *et al.* 1985), organic matter digestibility (OMD, Boval *et al.* 2003) and metabolisable energy (ME) content (Kamler & Homolka, 2005) of typical (grass/legume) ruminant diets. However, faecal N is less useful for predicting the quality of diets containing forages high in soluble phenolics and tannins (as is typical of many plants found in rangeland grazing environments). Condensed tannins (CT) bind to fibre and protein in the ruminant digestive tract (Degan *et al.* 1995; Makkar *et al.* 1995), increasing the excretion of faecal N and fibre (Ben Salem *et al.* 2005; Kaitho *et al.* 1998; Krebs *et al.* 2007). The increased faecal N values may misleadingly suggest diets are high in protein.

Faecal NIRS equations have been used to predict various nutritional parameters of mixed diets fed to ruminants (Boval *et al.* 2004; Landau *et al.* 2004; Landau *et al.* 2008; Li *et al.* 2007). Each livestock species is unique in its digestive physiology (Huston *et al.* 1986) and, therefore, *f*NIRS equations derived for one livestock species may not be applicable for another, essentially due to spectral differences. Generally, *f*NIRS requires calibration equations to be developed for each of the diet types under consideration. However, Decruyenaere *et al.* (2009) derived a *f*NIRS calibration to predict *in vivo* organic matter digestibility (OMD) of sheep diets containing a wide range of temperate forages.

In this study the usefulness of faecal chemistry and *f*NIRS, either used individually or in combination to predict the quality of mixed diets fed to sheep was investigated.

Method

Data and faecal samples collected from five sheep digestibility trials using mixed (browse and grass) diets conducted during the period 2006–2008 were used for the study. In total, 40 experimental diets consisting of varying levels of (fresh) browses (*Acacia saligna* [saligna], *Chamaecytisus palmensis* [tagagaste], *Atriplex amnicola* [river saltbush], *Atriplex nummularia* [Oldman saltbush] and *Rhagodia eremaea*) and oaten (*Avena sativa*) chaff were fed to individually penned sheep at a daily forage DM intake equivalent to 2% of the body weight of the sheep.

Feed and faecal samples collected from each sheep were analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, ash and crude protein (CP) contents. Total phenolics (TP) and tannin (TT) contents and *in vitro* gas production of the feed samples were also measured, according to the procedures described by Makkar (2003). Organic matter digestibility (OMD), short chain fatty acid production (SCFA) and metabolisable energy (ME) content of the diets were determined using the net 24 h gas volume (GV), CP and ash contents (Getachew *et al.* 2002; Menke & Steingass 1988).

Feed and faecal data were pooled. Pearson correlation coefficients (r) among feed and faecal variables were estimated. The predictive regression models of dietary CP, TP, TT, OMD, SCFA, and ME were developed from faecal indices by a stepwise regression procedure. Faecal N, ash, lignin/NDF and lignin/ADF were specified independent variables. The stepwise regression procedure adds independent variables one by one to the model and proceeds towards increasing of the precision. The model was specified to retain those coefficient of independent variables significant at $P<0.05$ level. The significant ($P<0.05$) predictive model with the highest R^2 and lowest residual standard deviation (RSD) was selected (i.e. best-fit predictive model). This model was then used to predict nutritional characteristics of diets included in a validation trial from respective faecal indices.

Ground faecal samples were scanned over the range 1100–2498 nm in 2 nm increments using a Foss 6500 NIR system. Results of dietary chemical analysis (CP, TP, TT), digestibility (DM digestibility, OMD, CP digestibility) and ME were used as reference values. Calibration equations were developed using version 1.04a of WinISI (II) software. The calibration relies on modified partial least squares (MPLS) procedure (Martens & Naes 1987). First order derivatives were used in the calibrations, with scatter correction. A global H (GH) factor of 3 was applied to eliminate outliers. GH, the standardised Mahalanobis distance, ascertains the degree of difference of the result in the data set – those with a GH of 3 or greater are eliminated, to retain the rigor and accuracy of the calibration data set. The precision of calibrations was evaluated by the coefficient of determination (R^2_c) and standard error of calibration (SEC). The predictive ability of calibrations was internally evaluated by standard error of cross-validation (SECV) and standard error of prediction (SEP) (Landau *et al.* 2006; Stuth *et al.* 2003). The slope of the validation regression (Landau *et al.* 2006) and the ratio of the standard deviation of the original data to the SECV (RPD, Williams & Sobering 1993) were used to evaluate accuracy of calibrations. The *f*NIRS equations with R^2_c and RPD greater than 0.80 and 3, respectively were considered as acceptable predictive equations (Williams 2004).

Results

None of the investigated faecal chemical properties had strong (significant) correlation with dietary CP content. The best-fit regression models predicting dietary TT, TP and ME contents, OMD and SCFA from faecal(*f*) N, *f*ash and *f*lignin/*f*NDF had high R^2 and low RSD (Table 1). However, the regressions between measured and predicted TP and TT contents

Table 1. Best-fit regression models

Diet characteristic	Predictive regression model (P<0.05)	Adjusted R2	RSD
Crude protein	CP= 58.82729 + 107.83681(*f*Lignin/*f*NDF)	0.21	2.15
Total phenolics	TP = 2.2036 + 1.55408(*f*N) – 0.14331 (*f*Ash) + 32.22348 (*f*Lignin/*f*NDF)	0.89	0.45
Total phenolics	TT = –17.43746 + 1.36504 (*f*N) + 50.37654 (*f*Lignin/*f*NDF) – 0.04475 (*f*Ash)	0.87	0.44
Organic matter digestibility	OMD = 814.55657 – 514.4869(*f*Lignin/fcNDF) – 0.93196(*f*Ash) – 5.4971(*f*N)	0.78	3.45
Short chain fatty acids	SCFA = 1.64152 – 0.00256(*f*Ash) – 1.55683(*f*Lignin/*f*NDF) – 0.01352 (*f*N)	0.78	0.01
Metabolisable energy	ME = 12.32323 – 8.25181(*f*Lignin/*f*NDF) – 0.01522(*f*Ash) – 0.07933(*f*N)	0.78	0.05

f: faecal

had positive intercepts (13.90 and 11.62, respectively; P<0.05) and low R^2 (0.62 and 0.65, respectively). In addition, the slopes of these regressions were much lower than 1.0 (0.50 for TP and 0.61 for TT). Regressions between measured and predicted OMD, SCFA and ME contents did not have significant intercepts and both the slope and R^2 of these regressions were close to 1.

Calibration and validation of the *f*NIRS prediction equations for chemical and functional nutritive attributes of the mixed (browse and grass) sheep diets are presented in Table 2. The R^2_c was greater than 0.80 for all *f*NIRS calibrations. The SEC was close to the respective SECV. The slope of the validation regressions (i.e. predicted values against respective reference data) of chemical attributes did not deviate from 1 while that of functional attributes (except SCFA) deviated from 1. The RPD of DMD and OMD was less than 3 but was greater than 3 for other (i.e. CP, TP, TT, PPC, P, CPD, IVOMD, ME, SCFA) calibrations.

Discussion

If a predictive regression model is perfect, the validation regression will have an insignificant intercept, a slope of 1 and a R^2 of 1. The positive (P<0.05) intercept recorded for validation regression for TP and TT indicated an over estimation of the variables by the predictive models. On the other hand, the considerably low slope (compared to a perfect prediction) indicated underestimation of the predicted value.

Table 2. fNIRS calibration performance of chemical and functional nutritive attributes of mixed (browse and grass) sheep diets

Attribute	Calibration statistics			Validation statistics				RPD
	R^2_C	R^2_C	R^2_{CV}	SECV	SEP	Slope	Bias	
CP†	0.96	3.97	0.94	4.91	6.69	0.95	0.87	4.19
TP†	0.96	3.09	0.92	4.34	9.13	0.85	1.28	3.45
TT†	0.93	2.89	0.90	3.44	5.83	1.04	0.44	3.13
DMD†	0.83	21.6	0.79	24.3	40.7	0.74	3.23	2.18
CPD†	0.95	38.5	0.94	42.7	175.9	0.51	17.3	3.95
OMD†	0.85	21.6	0.80	25.0	46.6	0.65	5.11	2.21
ME††	0.95	0.24	0.93	0.30	0.98	0.62	0.09	3.80
SCFA†††	0.94	0.05	0.92	0.06	0.12	0.80	–0.02	3.49

R^2_c, R^2 of calibration; SEC, Stranded error of calibration; R^2_{cv}: R^2 of cross validation; SECV: Stranded error of cross validation; SEP: Stranded error of prediction; RPD: SD/SECV; †: g/kg DM; ††: MJ/kg DM; †††: mL/0.2 g DM

Such association is undesirable in a predictive equation (Draper & Smith 1981). Therefore, the validity of the predictive regression models of TP and TT is less. Insignificant intercept and slope close to 100% with very high R^2 of validation regressions for OMD, SCFA and ME imply that the predictive models approached a highly accurate prediction. Therefore, the best-fit predictive regression models for OMD, SCFA production and ME derived from faecal chemical components will be useful in the field where sheep are consuming mixed diets.

Faecal NIRS calibrations developed to estimate dietary chemical attributes (CP, TP and TT) had excellent performance. The R^2_c (0.93–0.98) and RPD (3.10–5.9) were well above the minimum acceptable levels for NIR calibrations (Stuth *et al.* 2003; Williams 2004). The slope of validation regressions being close to 1 (0.85–1.07) confirmed that the calibrations derived for dietary chemical attributes do not under- or over-estimate the true values. In addition, the estimates of SEC, SECV and SEP were small, and the difference between SEC and SECV was marginal.

The low RPD (<3) of DMD and OMD calibrations indicated low accuracy due to greater variability in predicted data compared to reference data (Williams 2004). Low slopes (0.51–0.65) of OMD and ME calibrations would seriously underestimate the respective predictions.

In conclusion, whilst neither method (faecal chemical composition or *f*NIRS) could be used exclusively to predict the nutritive value of the diet, combining both methodologies would be useful for application in the field. Faecal chemistry is appropriate for predicting the OMD and ME content whilst *f*NIRS can be used to predict the CP, TP and TT contents of mixed diets consumed by sheep.

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