Predicting N-alkane Concentration in Pastures and Deer Faeces for Dietary Composition and Digestibility Measurement Using Near Infrared Spectroscopy

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ABSTRACT : The alkanes in plant materials can potentially be used as markers to estimate composition and digestibility of diet of deer for the development of feeding strategies, but the analysis of alkanes in plant materials and deer faeces is time-consuming and expensive. In this study, 242 faecal samples and 119 pasture samples were scanned using near infrared spectroscopy (NIR) and the concentrations of alkanes in these samples were analysed to develop calibrations for predicting alkane concentrations in pastures and deer faeces. The R^2 values for NIR calibrations were <0.6 for C_{24}, C_{26}, C_{32} and C_{36}, but were >0.8 for other alkanes for faecal samples. The R^2 values were >0.87 for alkanes with chains from C_{27} to C_{35} for pasture samples. However, NIR was unable to predict concentrations of alkanes with chains of C_{24}, C_{26}, C_{32} and C_{36} in faecal samples and C_{24}, C_{25}, C_{26} and C_{36} in pasture samples. While the use of these NIR calibrations will accelerate the estimation of diet digestibility, dietary components and botanical composition of pastures, the influence of the type of pasture on NIR calibration will require further examination. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1611-1616)***

Key Words : NIR Calibration, Grazing, Alkane Capsule, Fallow Deer

INTRODUCTION

To develop a cost-effective supplementation strategy for grazing deer under a Mediterranean environment, it is essential to know the seasonal feed intake. While there are a number of methods used for estimating feed intake of grazing ruminants (Dove and Mayes, 1996), the most advanced technique is the alkane method. The principle of this technique is that n-alkanes, long-chain (C_{25-35}) hydrocarbons, predominantly with odd-numbered carbon chains, occur in the cuticular wax of most plant materials and are substantially indigestible. These alkanes can be used, in combination with orally dosed even-chain alkanes to estimate intake. Dove and Moore (1995) also showed that diet composition of individual animals could be accurately estimated from the pattern of alkanes in each component of the diet and the faeces. With the wide adoption of this method, a commercial alkane capsule has been developed for estimating pasture intake in sheep and cattle. These capsules were tested for estimating feed intake of deer indoors (Ru et al., 2002). The results clearly indicated that intake of individual deer could be accurately predicted although the recovery of alkanes in deer faeces was incomplete, and the amount of dosed alkanes as commercial capsules excreted from faeces was lower than recommended for sheep. Through this study, it was found that the high cost of alkane capsules and alkane analysis is a key factor limiting the wide adoption of this technique in deer research.

Near infrared spectroscopy (NIR) has been used successfully for rapid estimation of chemical composition and nutritive value of feed and food (Ru and Glatz, 2000). The NIR analysis is simple, non-destructive and accurate. This is particularly valuable for faecal samples of deer, which are difficult to collect under grazing conditions. The objective of this study was to develop and subsequently validate NIR calibrations for the prediction of alkane contents in deer faecal samples and in various pasture species grazed by deer.

MATERIALS AND METHODS

Samples

Samples of faeces and pastures were obtained from two experiments conducted at SARDI-Livestock System Alliance at Roseworthy, South Australia. Briefly, in Exp. 1, 6 fallow deer, 8 months-of-age, were housed in individual stalls with dimensions of 1,200 mm long×1,950 mm high× 900 mm wide. Holes with a diameter of 100 mm were cut in the stalls to allow deer to view each other in the next stall and reduce fractious behaviour. The feeder was fixed on the door with the water bucket next to the feeder. Deer were fed a diet comprising straw and lucerne chaff. Once the deer were housed in the individual stalls, artificial slow-release alkane capsules were dosed via the oesophagus. The deer were fed *ad libitum* and water was available at all times. All faeces were collected daily (9:00 am) for 24 days from day one after dosing. Hair was removed manually from the faeces and 10% of the faeces were subsampled and freeze-dried. All samples were milled through a 1 mm screen for alkane analysis and NIR scanning. Details of the

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experimental procedure are described by Ru et al. (2002).

In Exp. 2, 36 fallow deer were grazed on medic and ryegrass-based pastures in three groups on the Roseworthy Deer Farm from May to October. Three groups were supplemented with a concentrate based on barley and lupin (*Lupinus angustifolius*) from May to August 2000. Deer were dosed with artificial slow-release alkane capsules in May, June, July, September and October. From day 10 after dosing, faeces were collected every second day over 6 days. During each faecal collection, pasture samples were taken by cutting 3 cm above ground level and separated into legume and grass. Samples of faeces, mixed pastures, legume and grass were freeze-dried and milled through a 1 mm screen for alkane analysis and NIR scanning.

After scanning the faecal samples from the two experiments, it was found that all samples fell into the same population, thus faecal samples from both experiments were pooled for the development of NIR calibration. Total number of faecal and pasture samples were 242 and 119, respectively.

Alkane analysis

Alkane concentrations in faeces and pastures were analysed at University of New England in Armidale, New South Wales. The analytical method used to determine alkane contents in the samples was a modified method of Dove (1992). In brief, to a dry sample of 100-500 mg, an appropriate amount (50-200 mg) of internal standard ($C_{34}H_{70}$ in dodecane) was added. The samples were then subjected to 1.5M ethanolic KOH in a heating-block at 90°C for 1 h with stirring. After cooling, the hydrocarbons were extracted in n-hexane several times, filtered, purified and quantified by gas chromatography. The alkanes were analysed including the following: C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{35} and C_{36}.

A relatively small percentage of analyses were performed in duplicate. These duplicate reference data were used to determine the standard error of the reference method which was used for comparison to primarily assess the validity of the calibration. Based on the available laboratory data, the calculation of the error was only possible for faecal samples. The formula used was for Standard Error of Laboratory (SEL) which is a standard error of variance between replicates analysed by the reference method and is defined as :

$$SEL = \sqrt{\frac{\sum_{i=1}^{N} (Y_{i1} - Y_{i2})^2}{N}}$$

The calculated SEL values for all measured constituents ranged from 0.30 to 13.56 ppm.

NIR scanning

NIR reflectance spectra of all available samples were recorded using a Foss NIRSystem Model 6500 Spectrophotometer (FossNIRSystem Inc., Silver Spring, MD, USA) and Intrasoft International (ISI) WINISI software (FossNIRSystem Inc., Silver Spring, MD, USA). Scanning was performed via a transport module in reflectance mode over the wavelength range 400-2,500 nm at 2 nm intervals using a small ring cup. Examination of final spectra was conducted in second derivative using SNV and Detrend scatter correction. An identical scanning procedure was applied to faecal and pasture samples.

Population structuring

Both sample sets were examined using the population structuring software in order to identify spectral outliers. To identify patterns in the group of spectra that contribute the most to the variation among the spectra, Principal Component Analysis (PCA) was used. An average Mahalanobis distance (Global H) was calculated and H values for individual samples were standardised by dividing by the average H value. Any sample with a spectrum more than 3.0 standardised units above the mean of the sample set was regarded as a spectral outlier. An identical population structuring procedure was applied to both types of samples.

For development of the calibration, both sample sets were randomly divided into calibration and validation groups. As a result, 212 faecal samples were used for the calibration, with the remaining 30 randomly selected faecal samples used in the validation. Similarly, the number of pasture samples for the calibration modelling was 99 with 20 remaining samples used for the validation.

Calibration development

The applied calibration technique involved SNV and Detrend scatter correction method and modified partial least squares (MPLS) regression of derivatised spectra. The superlative math treatment was 2,5,5,1. The same calibration procedure was used for both calibration sample sets. The calibration equations were produced for the 1,100 -2,500 nm segment of wavelengths.

The Standard Error of Cross Validation (SECV) was used as a measure of accuracy of calibrations in each case. Final equations were chosen according to a combination of the lowest SECV and the highest 1-VR value (coefficient of determination for cross validation). Calibration equations were developed for each of the 12 analysed constituents for both faecal and pasture samples.

Validation

The validation sample sets were used to test the performance of the calibration equations. The monitoring program within ISI software was used to test the calibration equations against independent sample sets. The NIR predicted constituent content was compared with laboratory measured values using a t-test.

The Standard Error of Prediction (SEP) was used as a calibration performance indicator. In addition, a ratio of SEP to Standard Deviation (SEP/SD) was used in the test. For superior calibrations this ratio should ideally be less than 0.3, although calibrations with the value below 0.6 are still regarded auspicious.

RESULTS

No spectral outliers were found for faecal and pasture samples, so all samples were included in the calibration and validation sample sets. There were differences in the second derivatives in the spectra between faecal and pasture samples over the wavelength of 1.977-2.498 nm (Figure 1 and 2).

The 1-VR and R^2 values for NIR calibrations were <0.6 for alkanes C_{24}, C_{26}, C_{32} and C_{36} in faecal samples, but were >0.8 for other alkanes examined. The SECV value was relatively high for alkane C_{31} (Table 1). The NIR validation also showed lower R^2 values for alkanes C_{24}, C_{26}, C_{32} and C_{36} with rather high SEP/SD ratios, although statistically there was no difference between NIR predicted and laboratory measured values for all alkanes (Table 2). The correlation results tended to be higher for long chain alkanes except for C_{32} and C_{36}, which were dosed with artificial capsules. Alkane C_{25} had a reasonable R^2 value for calibration, but a relatively poor one for validation.

Both 1-VR and R^2 results of the calibrations developed on pasture samples were favourable for alkanes with chains from C_{27} to C_{35}. Alkanes C_{24}, C_{25}, C_{26} and C_{36} had lower coefficients of determination for cross validation (1-VR, Table 3). The validation results also showed these alkanes had lower R^2 values and higher SEP/SD ratios. However, the alkane concentrations between the NIR predicted and laboratory measured values did not differ significantly (p-value, Table 4).

DISCUSSION

The outcomes of this study indicate that NIR calibrations can be used to estimate the concentrations of some alkanes in faecal and pasture samples. These calibrations will facilitate the measurement of dietary composition and digestibility of grazing fallow deer, which consequently can be used for the development of feeding strategies.

The successful prediction of alkanes using NIR calibrations developed in this study will stimulate the application of this technology in deer research. Compared with traditional laboratory alkane determination, a NIR-based assay offers lower cost of analysis and is less time consuming. More importantly, NIR assay will not destroy samples, especially faeces, which are extremely difficult to collect from grazing deer due to the difficulty in handling deer. After NIR scanning, the material can be used for other chemical analyses. However, the application of these calibrations to other types of pastures requires further examination. The calibrations for the faecal samples developed in this study may only apply to deer, and their relevance to sheep is unknown.

In the current study, NIR was unable to predict the

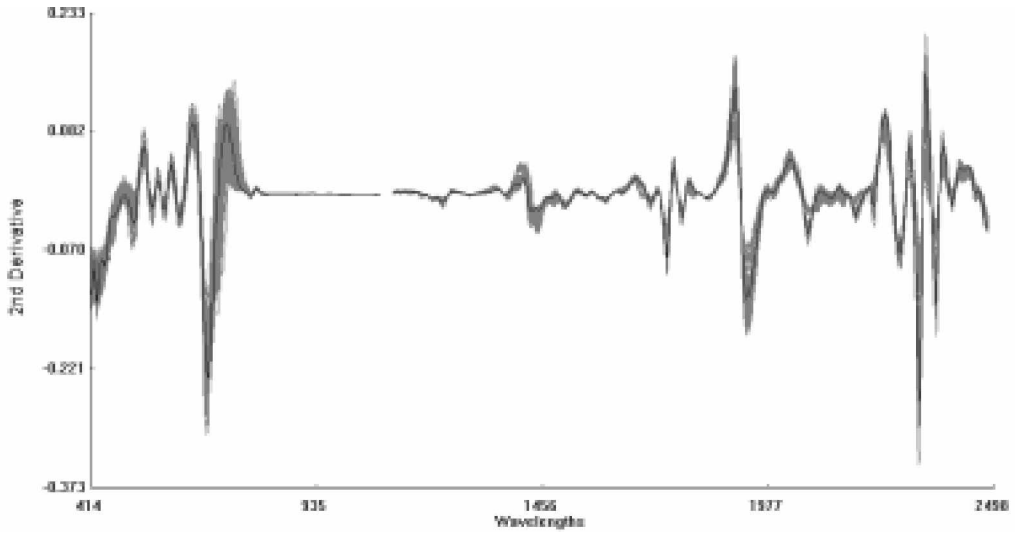

Figure 1. NIR spectra of deer faecal samples (calibration set).

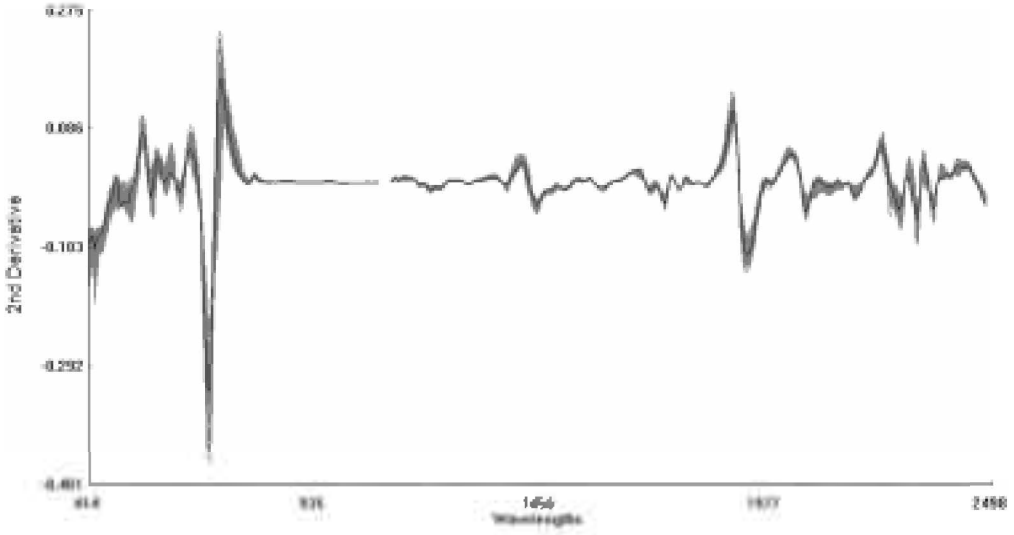

Figure 2. NIR spectra of pasture samples (calibration set).

Table 1. NIR calibration equations statistics developed for determination of alkane content in deer faeces (N=212)

Constituent	Mean (ppm)	Range (ppm)	SD	R^2	SECV	1-VR
C_{24}	4.21	1.44-28.14	2.62	0.01	2.67	-0.04
C_{25}	15.67	2.96-48.20	9.36	0.82	4.21	0.80
C_{26}	4.74	1.86-32.11	1.69	0.44	1.51	0.21
C_{27}	55.55	10.57-144.67	33.27	0.96	8.17	0.94
C_{28}	17.45	1.31-36.26	9.46	0.94	2.73	0.92
C_{29}	397.82	53.49-948.44	248.28	0.97	48.96	0.96
C_{30}	20.77	3.52-69.93	19.57	0.98	3.86	0.96
C_{31}	524.15	1.10-3,752.88	414.80	0.89	183.56	0.80
C_{32}	175.39	11.55*-320.75	44.79	0.46	33.72	0.43
C_{33}	83.34	20.29-235.48	45.90	0.97	9.99	0.95
C_{35}	6.83	2.72-17.51	2.78	0.93	0.92	0.89
C_{36}	161.30	3.59*-326.49	42.59	0.56	29.24	0.53

SD, standard deviation; SECV, standard error of cross validation; 1-VR, coefficient of determination for cross validation.
* Faecal samples without dosing slow-release alkane capsules.

concentrations of alkanes C_{24} and C_{26} in both faecal and pasture samples. It appears that the ability of NIR to predict the concentration of a particular natural alkane in a sample was related to the level of that alkane. Thus when the concentration of the alkane was high, its predictability was also high. The concentrations of C_{24} and C_{26} in grass and faecal samples were less than 5 mg/kg. Determination of such trace levels of alkanes using the current chemical method was probably not appropriate since the error level associated with the chemical determination was extremely high. The R^2 values of the calibrations and validations tended to be higher for longer chain alkanes. However, the C_{32} and C_{36} alkanes (dosed alkanes) in faecal samples cannot be estimated using NIR, presumably due to the lack of structural interactions between artificially dosed alkanes and other chemical components in the samples or other properties of the samples used in these calibrations.

The poor prediction of C_{32} and C_{36} in faecal samples may limit the application of these calibrations in the forage

Table 2. NIR validation statistics for determination of alkane content in deer faeces performed on 30 randomly selected samples

Constituent	Range (ppm)	Mean value (ppm)		SEP	R^2	SD	SEP/SD ratio	P-value
		Laboratory	NIR					
C_{24}	3.59-4.58	5.67	4.22	5.60	0.05	0.31	18.06	0.24
C_{25}	5.45-21.07	16.24	14.97	6.27	0.60	8.38	0.75	0.28
C_{26}	1.29-6.93	6.92	4.52	7.06	0.01	1.20	5.88	0.11
C_{27}	12.88-117.13	54.17	53.31	9.36	0.93	35.03	0.27	0.62
C_{28}	1.92-31.03	15.63	15.98	4.35	0.86	10.34	0.42	0.71
C_{29}	54.78-827.71	395.47	389.70	49.85	0.96	253.16	0.20	0.54
C_{30}	3.11-68.66	22.28	21.81	3.10	0.98	20.10	0.15	0.49
C_{31}	139.76-1796.98	716.58	589.35	540.89	0.80	465.31	1.16	0.21
C_{32}	117.16-228.72	161.81	171.20	46.67	0.22	29.74	1.57	0.28
C_{33}	32.07-193.98	83.85	83.63	10.02	0.96	46.63	0.21	0.91
C_{35}	2.95-13.28	6.46	6.36	0.87	0.92	2.60	0.33	0.62
C_{36}	111.88-222.76	145.87	157.09	46.75	0.18	31.67	1.48	0.19

SEP, standard error of prediction; SD, standard deviation.

Table 3. NIR calibration equations statistics developed for determination of alkane content in medic and ryegrass based pastures (N=99)

Constituent	Mean (ppm)	Range (ppm)	SD	R^2	SECV	1-VR
C_{24}	4.61	1.20-22.76	2.51	0.80	1.69	0.55
C_{25}	17.37	0.62-67.01	8.95	0.79	5.82	0.58
C_{26}	3.67	0.80-31.39	1.81	0.09	1.78	0.04
C_{27}	43.96	7.18-119.65	23.43	0.89	10.68	0.79
C_{28}	10.96	1.05-24.98	5.69	0.87	2.30	0.84
C_{29}	239.78	1.00-494.49	141.11	0.97	31.79	0.95
C_{30}	9.36	0.67-19.73	5.37	0.94	1.81	0.89
C_{31}	226.14	0.80-530.52	130.21	0.97	30.98	0.94
C_{32}	4.42	0.52-16.99	2.71	0.93	1.02	0.86
C_{33}	47.01	8.94-114.98	28.13	0.98	6.16	0.95
C_{35}	4.30	1.09-9.10	2.10	0.95	0.70	0.89
C_{36}	1.83	1.03-5.14	0.38	0.22	0.36	0.17

SD, standard deviation; SECV, standard error of cross validation; 1-VR, coefficient of determination for cross validation.

Table 4. NIR validation statistics for determination of alkane content in medic and ryegrass based pastures performed on 20 randomly selected samples

Constituent	Range (ppm)	Mean value (ppm)		SEP	R^2	SD	SEP/SD ratio	P-value
		Laboratory	NIR					
C_{24}	1.26-15.84	5.09	4.33	3.47	0.25	2.21	1.57	0.35
C_{25}	2.96-55.59	19.15	16.83	7.55	0.71	9.45	0.80	0.17
C_{26}	1.99-23.94	4.93	3.72	5.18	0.00	0.60	8.63	0.34
C_{27}	8.44-118.16	48.63	50.85	15.27	0.71	26.50	0.58	0.53
C_{28}	2.47-22.15	11.14	11.47	2.73	0.73	4.87	0.56	0.60
C_{29}	43.83-464.43	253.40	257.58	28.23	0.95	124.23	0.23	0.52
C_{30}	2.56-19.73	11.74	11.42	2.27	0.84	5.46	0.42	0.54
C_{31}	70.90-512.76	294.67	294.35	20.59	0.98	135.21	0.15	0.95
C_{32}	2.07-13.08	6.85	6.24	1.51	0.92	2.64	0.57	0.07
C_{33}	11.29-114.98	46.02	45.26	4.61	0.97	27.15	0.17	0.48
C_{35}	1.38-9.10	3.94	3.84	0.40	0.98	2.35	0.17	0.43
C_{36}	1.60-3.81	2.07	1.84	0.62	0.04	0.18	3.44	0.10

SEP, standard error of prediction; SD, standard deviation.

intake measurement because C_{32} and C_{36} alkanes dosed to animals are essential for intake estimate. However, these calibrations are useful when natural alkanes in plant materials are used to determine the botanical composition of available or consumed herbage by grazing animals. Under field conditions, the understanding of pasture species selected by grazing animals and their utilisation is of great importance for the development of pasture management strategies. Research reviewed by Dove and Mayes (1991) suggests that cuticular wax alkanes can be used to estimate the botanical composition of mixed herbage or of the whole diet using simultaneous equations, assuming that the faecal recovery of an individual alkane is constant across a range of herbage species.

The application of NIR calibrations will accelerate the estimation of diet digestibility of grazing deer. Dove and Moore (1995) developed an Eatwhat ® software to estimate the proportion of pasture species consumed by grazing animals to produce 1 kg dry faeces. Based on this software, dry matter digestibility of the diet can be estimated without using the dosed artificial alkanes. It is expected that these predictions can be performed rapidly and cheaply for deer if these NIR calibrations are used for the measurement of alkanes in pasture and deer faeces.

In conclusion, NIR can be used to predict alkanes in pasture and deer faeces with chain lengths of C_{27}-C_{35}, except for C_{32} in faecal samples. The application of these calibrations will assist the rapid measurement of diet composition and digestibility of grazing deer for the development of feeding strategies. However, the influence of type of pastures on the accuracy of these calibrations requires further examination.

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