Near infrared spectroscopy applied to faeces to predict botanical composition of sheep intake

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Introduction

Diet selection is a key process affecting both animal productions and pasture structure. This is particularly relevant when pasture is composed of several plant communities. By preferring some plant in a rangeland or in a common pasture, ruminants have a great impact on the ecosystem and the development of some plant population.[1,2]

Actually adequate evaluation procedure for determining diet selection on a large number of individuals and on grazing situation is not available. Study of animal behaviour with conventional methods as direct observations of the animals, utilization techniques, automatic recording of animal movements, oesophageal fistula techniques, microhistological analysis of faeces or digestive tract content is labour intensive and time consuming.[3] Recent developments with indigestible plant cuticular wax components, especially n-alkanes method, have opened new techniques to estimate herbage intake and botanical composition of ingested diet of free grazing animals.[4–6] Nevertheless this methodology is limited by the number of items researched.

Near infrared (NIR) reflectance spectroscopy may become an interesting tool to asses diet preferences at pasture. These last years, NIR has been used successfully to determine species composition of mixed forage samples[7–10] and oesophageal fistula samples.[11] NIR has been used also to assess the plant composition in term of stems and leaves.[12,13] Finally NIR analysis of faeces have been developed to estimate both nutritive value of ingested grass and dietary nutrient content of free ranging ruminants.[14,15]

Indeed faeces are composite materials that contain undigested residues and in this way, they can provide NIR spectral information highly correlated with the nature of diet.

Recently, Walker[16,17] have demonstrated that NIR applied to faeces can predict the proportion of respectively leafy spurge (*Euphorbia esula* L.) and mountain big sagebrush (*Artemisia tridentate* Nutt. Ssp. *vaseyana* (Rydb) Beetle) in sheep diet on range land situation.

The objective of this study is to evaluate the potential of NIR applied to faeces to predict botanical composition of diet ingested by sheep grazing temperate pasture.

Materials and methods

This research was conducted in summer 2001 (August and September) at the Agricultural research centre of Gembloux, Farming Systems section located in Libramont (Ardenne, Belgium)

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(altitude 480 m; raining 1550 mm for the year 2001). Faecal materials and diet samples were obtained from indoor and outdoor feeding trials.

Experiment 1: development of faecal NIR data base

This experiment was conducted on nine castrated Texel × Bleu du Maine sheep (82.2 ± 8.1 kg of live weight). During the experiment sheep were housed in individual box with continuously available water. Three forage plant species (*Lolium perenne* (LP), *Trifolium repen*s (TR) and *Holcus lanatus* (HL)) have been offered in separate feeding trough. These plant species, which could be selected freely, were given at *ad libitum* level intake but in different proportions (Table 1).

At the beginning of the experimental period, each sheep was weighed and during the trial, each forage species was cut daily, stocked at 4°C and offered to sheep the next day. During the experimental period (seven days of diet adaptation and seven days of data collection), sheep were fed twice daily and forage species were individually weighed and subsampled for moisture determination (oven dried at 60°C during 48 hours) and for purity of sward. In each plant specie sward, weeds proportions were determined every day by hand separation. So that botanical composition of sheep intake was obtained in term of legume, grass and weeds (proportion in weight on a dry matter basis).

Individual forage residues were collected daily, weighted and samples were oven dried (60°C during 48 hours) for moisture determination. Dried forage and residues samples were finally grounded in an hammer mill and in a cyclotec mill (1 mm) screen.

Faeces were collected individually three times a day on the pen floor. Faecal samples were oven dried (60°C during 48 hours) and grounded in a hammer mill (1 mm screen). Each samples of grounded forage, residues and faeces were stored in plastic bags for future analysis.

Table 1. Theoretical plant composition (% of DM) of diet tested.

Diet	Number of sheep	LP	HL	TR
Diet 1	2	50	25	25
Diet 2	2	25	50	25
Diet 3	2	30	30	40
Diet 4	3	33.3	33.3	33.3

Experiment 2 : prediction of diet composition at pasture

The second experiment was conducted on six castrated Texel × Bleu du Maine sheep (83.2 ± 6.1 kg live weight) grazed a pasture divided in three paddocks (6 × 45 m). The first one was a LP sward, the second a TR sward and the third a HL sward. Sheep were conducted in a one day grazing system, each day a new part of the three paddocks was placed at sheep's disposal.

During the experimental period of eight days, faeces were sampled individually at morning and afternoon. Faecal samples were oven dried (60°C during 48 hours), grounded (hammer mill 1 mm screen) and stored into plastic bags for future analyses. Forage species were individually sampled for moisture determination (oven dried at 60°C during 48 hours) and for purity of sward. In each plant specie sward, weeds proportions were determined every day by hand separation

NIR data acquisition

Offered forages, forages residues and faeces were submitted to NIR analysis (NIR system monochromator 5000). In experiment 1, all individual spectral data (three spectra by day by sheep) in the range of 1100–2500 nm by 2 nm steps were recorded as log $1/R$ and correlated with the proportion of grasses, legume and weeds ingested by sheep one day before or two days before. Calibrations were developed according to the MPLS procedure with cross validation of ISI software. Before calibrations, each faecal spectrum was transformed with a (2,5,5) derivative and scatter correction was done with the standard normal variance and detrend procedure. Evaluation of calibrations was done with the coefficient of determination (R^2), standard error of calibration (*SEC*), standard error of cross-validation (*SECV*) and standard error of validation (*SEP*). In Experiment 2, individual selected diets were predicted using faecal calibration developed in Experiment 1.

Results and discussion

Level intake and diet composition at feeding trough (experiment 1)

Over the experimental period, voluntary intake measured was 57.4 g/kg metabolic weight (MW) with a maximum of 73.5 g kg^{-1} MW and a minimum of 41.3 g kg^{-1} MW. Concerning diet composition, values varied respectively between 30.7% and 65.2% for grasses (LP + HL) and between 12.8% and 34.6% for legume (TR).

Voluntary intake decreased when the proportion of HL increased in offered diet (Table 2). The same results have been observed by Morton.[18] HL was a softly hairy grass less palatable than LP or TR[19] and Penning[20] showed that Clover and Ryegrass are preferred by sheep, when they can choose.

Table 2. Level (g kg^{-1} MW) and composition (% DM) of intake.

Diet	LP	HL	TR	weeds	Voluntary intake
Diet 1	37.0	14.3	16.0	32.8	58.4
Diet 2	20.3	35.9	17.8	26.0	55.7
Diet 3	22.5	18.7	26.6	32.8	56.7
Diet 4	21.6	14.0	27.1	37.3	57.7

Faecal NIR calibrations

Prior to develop final NIR calibrations, data from diet 1, 2 and 3 were analysed to determine which lag time (24 hours or 48 hours) between diet consumption and faecal spectra provided the best calibrations (Table 3).

Table 3. Lag time (24–48 hours) between NIR spectra and reference values.

Variable	24 hours					48 hours				
(% DM)	*n*	mean	*SEC*	R^2	*SECV*	*n*	mean	*SEC*	R^2	*SECV*
HL	97	22.8	4.9	0.74	6.7	73	23.7	4.2	0.84	5.6
LP	99	27.4	3.9	0.80	5.5	74	28.1	3.7	0.81	5.9
TR	95	19.1	1.7	0.87	2.5	75	18.9	2.1	0.83	3.1
Weeds	100	30.1	2.2	0.76	3.3	75	29.1	1.6	0.88	2.5

n = number of observations with outliers excluded (Mahalonobis distance >3)

According to the results described in Table 3, it was not possible to distinguish the two graminaceae. Indeed, statistics of calibration for HL and LP were poor with high *SECV* and low coefficients of determination. TR could be predicted with a relatively good accuracy. It appeared that statistics of TR calibrations were better for the 24 hours lag time. Walker[16] reported that for both sheep and goats, the best calibration results were found between NIR analysis of faeces and percent of leafy spurge in diets consumed 48 hours early. Lyons[21] found the same delay. In these studies, species proposed to animals were fibrous and less digestible than temperate grass. In our

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experiment, temperate forage proposed to sheep stayed probably less long in the digestible tract and a lag time of 24 hours should seem more appropriate.

On the total database, faecal NIR analysis can predict species composition of diet with success (table 4). Coefficients of determination were good with $R^2 = 0.87$ for TR and $R^2 = 0.88$ for grass (LP+HL). Calibration was less accurate to predict the part of others plants in diets. When a set of independent samples ($n = 20$) was predicted, relations between predicted values and measured values were sufficient excepted for weeds ($R^2 = 0.52$). For the other parameters tested, *SEP* were close to *SEC* with a good slope (Table 4).

Walker[16] could predict leafy spurge in diet of sheep and goat with a comparable accuracy ($R^2 = 0.85$ and *SEC* = 6.4%). On forage samples, Pitman[9] and Mika[10] developed NIR models to predict percent of grasses, legumes and herbs from analysis of forage samples. Statistics of their calibrations were close to those obtained from faecal samples and percentages of grasses, legumes and herbs were predicted with *SEP* lower than 6%. Faecal NIR could predict species composition of ingested forage with the same accuracy. Moreover, in grazing situation, necessity to have a representative sample of ingested diet remains a real problem. Consequently working on faecal samples to predict composition of diet ingested could better reflect the consumed diet.

Table 4. Statistics of faecal NIR calibrations.

Variable	Calibration					Validation				
(% DM)	*n*	mean	*SEC*	R^2	*SECV*	*N*	*SEP*	R^2	slope	bias
TR	122	22.0	2.0	0.87	2.9	20	2.7	0.80	0.92	-0.05
LP+HL	127	45.0	3.3	0.88	4.4	20	3.6	0.90	1.09	1.93
Weeds	122	33.1	2.3	0.74	2.6	20	4.3	0.52	1.09	-2.21

n = number of observations with outliers excluded (Mahalonobis distance >3)

Estimation of diet composition at pasture

According to the experimental procedure (availability of grasses = 66.6%, availability of legume = 33.3%) and to the purity of sward determined by hand separation (LP = 72%; HL = 69.7% and TR = 85.2%), theoretical intake of sheep should be 28% of legume, 47% of grasses and 24% of herbs. Over an eight day period, NIR predicted grass mixtures ingested by sheep contained 60.5 ± 14.1% of grasses; 14.0 ± 8.4% of legume and 28.8 ± 7.8% of weeds and if predicted percent of grasses, legumes and weeds were toted up, total percent was 103.4 ± 5.2% which was close to 100%. TR proportion predicted by faecal NIR should be lower but grasses and weeds predicted values should be close to theoretical values. To test the robustness of NIR models, predicted values of morning and afternoon sampled faeces were correlated. There was a very good relation between morning and afternoon predicted values as illustrated at Figure 1. Therefore, those predicted values could be considered as a good estimation of diet truly consumed.

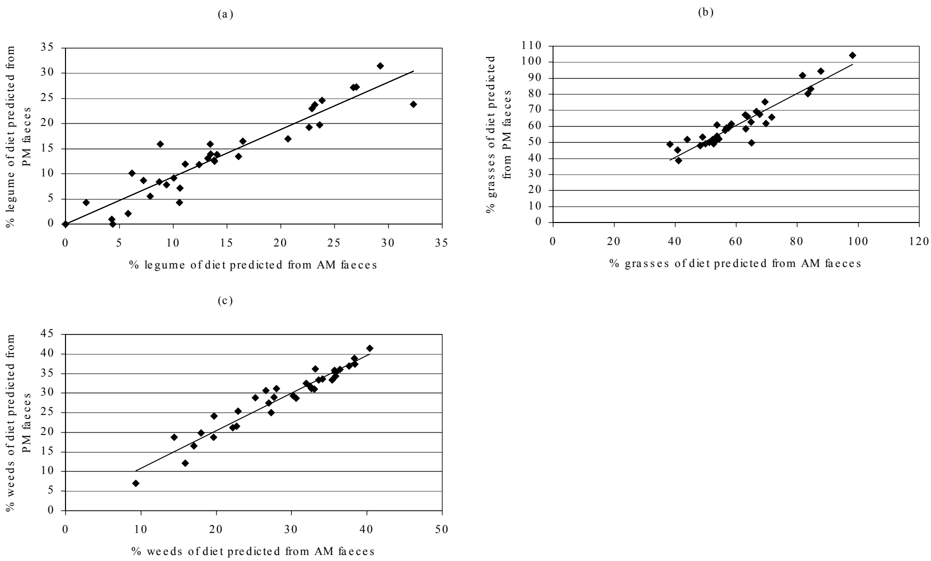

Figure 1. Relation between morning and afternoon predictions for each species of ingested diet (a) legume; (b) grasses; (c) weeds.

Conclusions

NIR applied to faeces gives good results in the prediction of animal choose at pasture. A major drawback of this method is the development of robust calibrations. Indeed, the value of a NIR calibration is directly linked to the accuracy of the reference method and actually, the problem is mainly the acquisition of reference values because accurate procedures for determining diet selection individually were not available. To be robust, such calibrations need the integration of reference data as diverse as possible to be applied in conditions as diverse as possible.

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