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Use of near infrared reflectance spectroscopy (NIRS) for the prediction of the chemical composition and nutritional attributes of green crop cereals

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Abstract

The NIR spectra of 135 samples of the following green crop oats (*Avena sativa*), barley (*Hordeum vulgare*), triticale (*x Triticosecale*), wheat (*Triticum durum*), ryegrass (*Lolium multiflorum*) and sorghum (*x Sorghum sudanense*) grown in Portugal, were obtained by reflectance from oven-dried, milled whole plant material. Samples were cut at various stages of development (viz. stem elongation, boot, early heading, milk and dough stage) and were analysed by standard laboratory methods. The nutritive value was evaluated in vivo using rams in metabolic cages. The green crops showed values of ASH varying from 5.5 to 14.9%, crude protein (CP) from 5.0 to 22.3%, crude fibre (CF) from 25.3 to 40.4%, neutral detergent fibre (NDF) from 45.9 to 77.2%, acid detergent fibre (ADF) from 23.6 to 49.9%, acid detergent lignin (ADL) from 1.7 to 8.0%, dry matter digestibility (DMD) from 53.2 to 82.9%, organic matter digestibility (OMD) from 56.2 to 85.0% and dry matter intake (IVDMI) from 35.8 to 88.6 g DM kg^{-1} $W^{0.75}$. Spectra were used to develop NIR calibration models for ASH (SEC= 0.72; r^2=0.90), CP (SEC=0.63; r^2=0.98), CF (SEC=1.02; r^2=0.94), NDF (SEC=1.41; r^2=0.97), ADF (SEC=1.43; r^2=0.96), ADL (SEC=0.49; r^2=0.87) and in vivo attributes DMD (SEC=2.61; r^2=0.86), OMD (SEC=2.36; r^2=0.88) and IVDMI (SEC=6.05; r^2=0.41). Calibration models were successful for all parameters except for IVDMI. However, animal attributes were less successfully predicted than chemical composition. Calibration models were generated omitting one of the six plant species to assess their ability to accommodate unknown green crop species not represented in the calibration model. In general, NIRS was shown to provide acceptable prediction of composition across different species of green crop cereals whether or not these were included in the calibration process. © 1998 Elsevier Science B.V.

Keywords: NIRS; Green crop cereals; Chemical composition; Digestibility; Ruminants

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1. Introduction

Conventional analytical methods and animal feeding trials have been used to evaluate feed and forage materials. However, the cost and labour requirements are excessive resulting in difficulties in performing cost analysis for research or advisory purposes.

Near Infrared Reflectance Spectroscopy (NIRS) is a physical technique with the potential of allowing rapid and accurate determination of the chemical composition and nutritive value of different feeds.

NIRS is well established in the analysis of forages (Dardenne et al., 1991; Waters and Givens, 1992; Shenk and Westerbaus, 1994) cereals and feedstuffs (Murray and Garrido, 1990; Givens et al., 1991; Dardenne et al., 1993) particularly cereal grains (Krishnan et al., 1994), forages grasses (Paul and Mika, 1989; Herrero et al., 1996) and silage (Biston et al., 1989; Murray et al., 1990; De Boever et al., 1996). However, less information exists about the application of NIRS on green crop cereals cut at various stages of maturity.

Green crop cereals may be grown alone or in mixtures with more than one species of plants. In the Mediterranean region such green crops may be cut and conserved as hay or silage.

A systematic study was carried out to characterise legume forages at various stages of development (Abreu et al., 1982; CIHEAM, 1990) with respect to their chemical composition and nutritive value. Less work has been done with reference to green crop cereals. Knowledge of such attributes at various stages of maturity is necessary in order to establish the relationship among stage of maturity, composition and nutritive value.

The aim of the work presented was: (i) to develop NIR calibration models for chemical composition parameters and in vivo parameters of selected green crop cereals; (ii) to evaluate if there were patterns of change in spectra corresponding to changes in the maturity of the crops; (iii) to assess if NIRS could discriminate between crop species and, (iv) to ascertain to what extent universal calibration could accommodate all plant species at various stages of maturity, even if some species were not represented in the calibration set.

2. Materials and methods

2.1. Green crop cereals and laboratory procedures

The green crop cereals used consisted of *Avena sativa* (cv. Avon and Boa Fé), *Hordeum vulgare* (cv. Ancora and Caravela), *Triticale* (cv. Arabiand and Borba), *Triticum durum* (cv. Tejo and Almocreve), *Lolium multiflorum* (cv. Elsa) and *Sorghum*, which were grown in central and southern Portugal.

Green crops grown in replicated experimental plots were cut on several cutting dates (7–10) to show the changes in composition with increasing plant maturity along the growing season. Cutting was conducted during the following months and included the phenological stage of development: February (ryegrass) – stem elongation and tillering stage; March, April and May (oats, barley, triticale, wheat and ryegrass) – boot, early heading, full heading and pre-milk stage; June and July (oats, barley and sorghum) – stem elongation, boot stage, milk and dough stage. The population studied included 135 samples: 32 (oats), 31 (barley), 20 (triticale) and 20 (wheat), 9 (ryegrass) and 23 (sorghum). The freshly cut samples were dried in a forced air oven for 24 h at 65°C and ground to pass a 1 mm screen prior to analysis. Ash was determined by combustion at 550°C overnight (18 h), crude protein (CP) using Kjeldahl method ($N\times 6.25$); crude fibre (CF) was analysed with a Fibertec apparatus (Tecator, Sweden). Cell walls were analysed with a Tecator apparatus by sequentially adding neutral detergent (for NDF), acid detergent (for ADF) and 72% H_2SO_4 (for ADL) according to Robertson and Van Soest (1981).

2.2. Animal experimentation and statistical analysis

In vivo parameters were evaluated for all crops except for triticale and wheat. Dry matter digestibility (DMD), organic matter digestibility (OMD) and dry matter intake (IVDMI) were obtained using rams ($n=6$) confined to metabolic cages, fed ad libitum with the green crop cereals as sole feedstuff. After an adaptation period of 2 weeks, faeces and refusals were collected for 8 days. Complete details of the animal experiments used to measure DMD, OMD and IVDMI have been given by Abreu et al. (1982).

One way ANOVA was used to test differences among chemical composition, in vivo parameters of the species and phenological stages of development, using the Duncan (1955) test for comparison of means.

The NIR reflectance spectrum of each dried ground sample was obtained using 50 mm quartz cuvettes in an 'NIRSystems' 6500 scanning VISIBLE-NIR Monochromator (NIRSystems, Silver Spring MD) scanning the region 400–2500 nm at 2 nm intervals to give 1200 data points for each sample spectrum.

ISI NIRS2 software version 3.0 was used (Infrasoft International, Port Matilda, PA) to manipulate and transform spectra prior to calibration modelling using either discrete wavelengths (stepwise multiple linear regression, SMLR) or whole wavelength regions using Partial Least Squares (PLS).

Sample spectra were examined both in large sets and in sub-groups as show in Table 1.

Spectra of samples having different cutting dates were also examined to look for patterns of change in spectra corresponding to changes in the maturity of the crop in large sets and in sub-groups ($n=80$, included barley, oats, triticale and wheat).

To seek spectral differences between species, spectra of samples of different crop species were also examined in large sets and in sub-groups (included barley $n=20$, oats $n=20$, triticale $n=20$ and wheat $n=20$).

3. Results

The range, mean and standard deviation values for ASH, CP, CF, NDF, ADF, ADL, DMD, OMD and IVDMI in the samples used in the calibration set, are shown in Table 2.

A wide variation in studied components studied was expected because samples included a large degree of phenological variability. In fact, the green crop cereals

Table 1
Feeds and number data used in the large sets and in sub-groups

	Chemical data	Biological data	
Green crop	No.	DMD and OMD	IVDMI
Barley	31	11	11
Oats	32	12	12
Ryegrass	9	6	—
Sorghum	23	23	23
Triticale	20	—	—
Wheat	20	—	—
Total	135	52	46

Table 2
Mean, range and standard deviation (SD) of analytical values for calibration sets of green crop cereals

Parameters	No samples	Mean	Range	SD
ASH (%)	131	9.3	5.0–14.3	2.30
CP	127	10.6	5.0–22.3	4.25
CF	54	33.0	25.3–40.4	4.01
NDF	128	60.6	46.9–77.2	7.62
ADF	130	34.3	23.6–49.9	7.46
ADL	123	3.9	1.8–8.0	1.35
DMD	50	64.6	52.4–82.9	7.04
OMD	50	67.7	56.2–85.0	6.79
IVDMI (g DM kg^{-1} $W^{0.75}$)	44	49.7	35.6–70.7	7.87

$W^{0.75}$= metabolic weight.

included samples taken at development stages as different as stem elongation, full heading stage, milk and dough stage.

The relative content of ADL varied the most, followed by CP and ash. The ADL content was low (mean=3.9%) and typical for grasses (Van Soest, 1982). The wide range observed in ASH levels may be explained by soil contamination of the samples cut during winter (Abreu and Bruno-Soares, 1996).

The decrease concentration of CP and increase concentration of fibre fractions, equivalent to the physiological variation in green crop cereals, is corroborated by the work of Demarquilly and Weiss (1970) and Abreu et al. (1982).

In fact, the results clearly show the relationship between these parameters and the stage of maturity of the green crops: significant correlation was observed between the ASH ($r=-0.77$, $p\leq0.05$), CP ($r=-0.76$, $p\leq0.01$), ADL ($r=0.60$, $p\leq0.01$) and the stage of maturity of the green crops.

The absence of correlation between the phenological stage of the forage and the NDF fraction may be explained by the percentage of gramineous grain in the final phase of the development cycle, higher or at least equal to other parts of the plant (stems and leaves). Values of 8.6, 15.6, 22.9, 33.4 and 47.4% were observed as percentages of the reproductive organs in barley, respectively, for the early heading stage, full heading stage, pre-milk stage, milk stage and dough stage by, Abreu et al. (1982).

The large variability of in vivo parameters reflects the differences among growth stage of green crop cereals and between taxonomic groups.

Included in this work were values of OMD and IVDMI of 60.2% and 44 g DM kg^{-1} $W^{0.75}$ for barley in dough stage, 80.5% and 70 g DM kg^{-1} $W^{0.75}$ for ryegrass in tillering stage and 70.5% and 64 g DM kg^{-1} $W^{0.75}$ for oat in the early heading stage, respectively.

Similar values to the mean of OMD and DMI (OMD=67.7%; IVDMI=50 g DM kg^{-1} $W^{0.75}$) were mentioned by Demarquilly and Jarrige (1973) for grasses.

Concerning the variation to the studied species, significant differences ($p \leq 0.05$) among the chemical compositions were found with exception to the CP ($p > 0.05$). Oats showed a significant ($p \leq 0.001$) lower cell wall content (NDF, ADF and ADL) in relation to others species.

Regarding in vivo parameters (DMD, OMD and IVDMI)) significant differences ($p \leq 0.001$) were also observed among species. Ryegrass showed significantly higher values in relation to the other green crop cereals.

Statistics for calibration and cross-validation (Table 3), include standard error of calibration (SEC) and the coefficient of determination (r^2). Cross-validation performance is expressed as coefficient of determination (1-VR) and standard error of cross validation (SECV).

The log 1/R and second derivated mathematical treatment were used to analyse the spectra of green crop samples. Unimportant pattern changes in spectra of samples of different cutting data (Fig. 1) and green crop species (Fig. 2) were observed.

Six calibration models were established for the population of green crops in which just one species was omitted from the model. The calibration model thus obtained was then used to predict chemical parameters and animal attributes of the omitted sample species to determine to what extent the NIRS model can accommodate unknown species not represented in the calibration model. r^2 values for calibrations with one omitted species are shown in Table 4.

Table 3
Statistics for calibration (SEC, r^2) and cross-validation (SECV, 1-VR)

Parameters	SEC	r^2	SECV	1-VR
ASH (%)	0.72	0.90	0.80	0.88
CP	0.63	0.98	0.76	0.97
CF	1.02	0.94	1.23	0.90
NDF	1.41	0.97	1.77	0.95
ADF	1.43	0.96	1.68	0.95
ADL	0.49	0.87	0.56	0.83
DMD	2.61	0.86	3.74	0.72
OMD	2.36	0.88	3.49	0.73
VI (g DM kg^{-1} $W^{0.75}$)	6.05	0.41	7.05	0.20

$W^{0.75}$= metabolic weight.

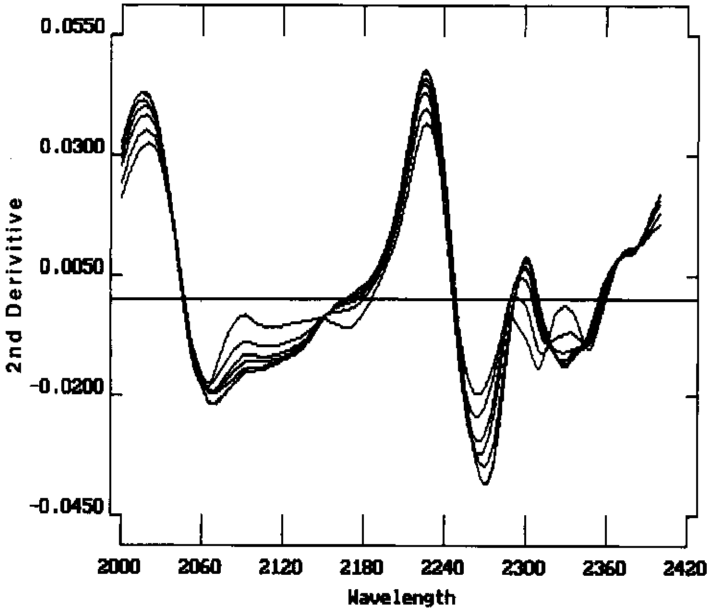

Fig. 1. NIRS absorption bands in different cuttings of green crop cereals.

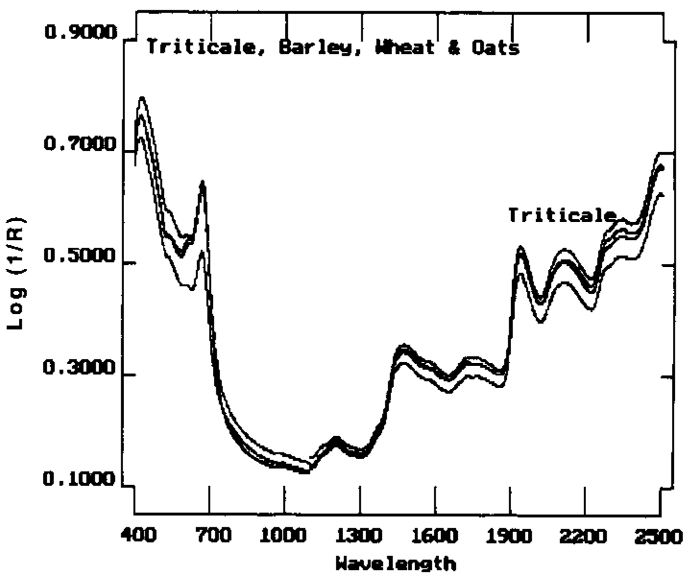

Fig. 2. NIRS absorption spectra of different green crop species.

Table 4
Coefficient of determination of calibration (r^2) for the green crops with one species omitted

Species omitted	Oat		Barley		Triticale		Wheat		Ryegrass		Sorghum		None omitted	
	r^2	n	r^2	n	r^2	n	r^2	n	r^2	n	r^2	n	r^2	n
Attribute														
ASH	0.94	103	0.91	104	0.92	115	0.94	115	0.93	126	0.94	112	0.90	135
CP	0.98	103	0.98	104	0.98	115	0.97	115	0.98	126	0.98	112	0.98	135
CF	0.94	103	0.93	104	0.94	115	0.94	115	0.91	126	0.92	112	0.94	135
NDF	0.97	104	0.96	104	0.96	113	0.95	113	0.95	124	0.93	110	0.97	133
ADF	0.97	103	0.98	103	0.97	112	0.98	112	0.98	123	0.95	109	0.96	132
ADL	0.86	103	0.87	103	0.87	112	0.86	112	0.89	123	0.73	109	0.87	132
DMD	0.82	40	0.91	41	0.86	52	0.86	52	0.84	43	0.86	29	0.86	52
OMD	0.84	40	0.93	41	0.87	52	0.87	52	0.86	43	0.87	29	0.88	52
IVDMI	0.51	34	0.46	35	0.40	46	0.44	46	0.44	37	0.22	23	0.41	46

n= Number of observations.

4. Discussion

Results of NIR calibration indicated good correlations (Fig. 3) with all chemical components showing r^2 values higher than 0.94, except for ADL (r^2=0.87). The nitrogen content of forage is one of the most successful measurements performed by NIR, because it varies considerably and the Kjeldalh nitrogen determination is quite precise. The measurement of detergent fibre fractions, namely, NDF and ADF of plant material, are also successful NIR calibrations demonstrated with SEC values of 20 g kg^{-1}. Several authors (Shenk et al., 1979; Abreu et al., 1991; Smith and Flinn, 1991; Herrero et al., 1996) reported accurate predictions of both constituents by NIRS, which confirm the findings of the present study.

DMD and OMD results also indicated acceptable correlations, (r^2=0.86 and r^2=0.88, respectively), showing a rather high standard error of calibration (2.61 and 2.36, respectively), which is typical of such biological measures (Norris et al., 1976 and Shenk et al., 1977). The different feeding levels used to measure digestibility parameters (where OMD and DMD were measured ad libitum), may contribute to the inaccuracy of the NIRS (Clark, 1989); therefore, the intake level, especially for a roughage, may significantly affect its digestibility (Raymond et al., 1959). A large number of reports has been published citing successful NIR prediction of digestibility such as Abreu et al. (1991), Berardo (1991), and Dardenne et al. (1991) who reported standard errors of DMD of 2.0% with r^2 values close to 0.90.

The IVDMI calibration (r^2=0.49) was inadequate for prediction purposes. Norris et al. (1976) were the first to show the extension of NIR spectroscopy to provide estimates of voluntary intake. They used a group of 87 forages and obtained r^2 values close to 0.60. The small number (n=46) of samples used and the dependence of this parameter on animal factors, which are external to the chemical information (Windham and Coleman, 1989) may confound the obtained results, since these animal factors may not be represented in the NIRS spectra. According to Givens et al. (1991) there is now evidence that the measurement of rumen degradability characteristics as digestion rate and

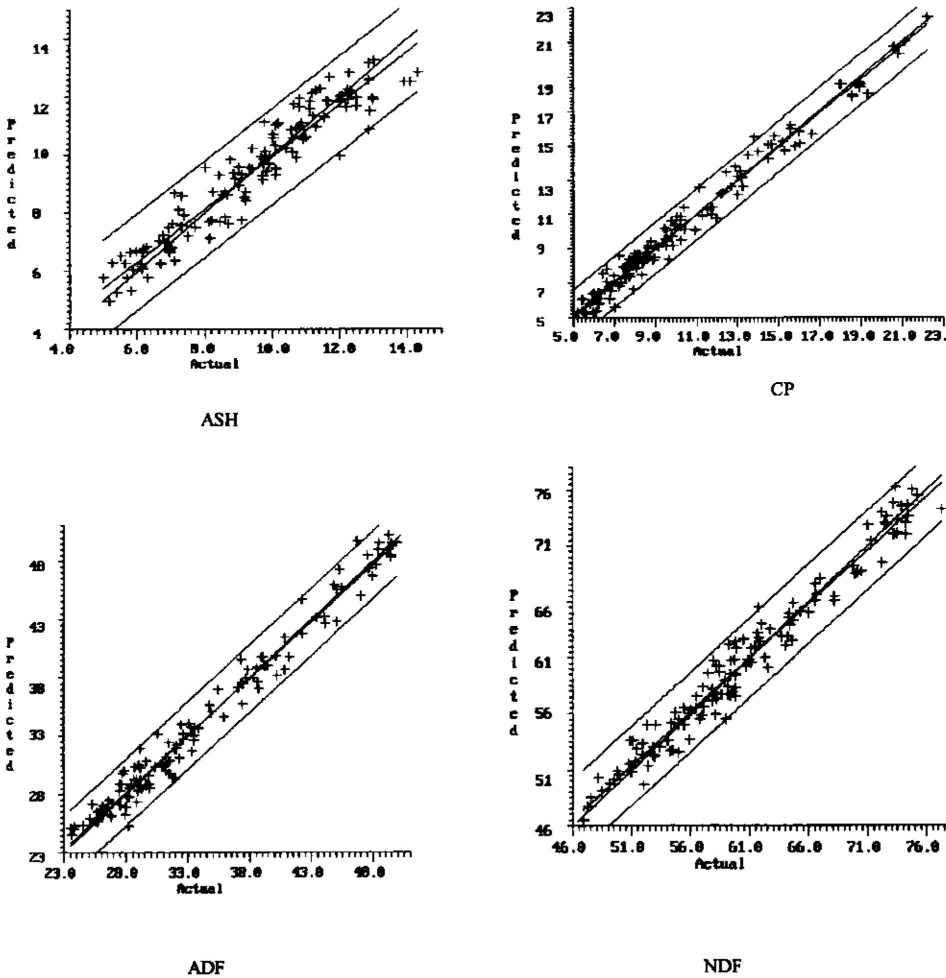

Fig. 3. Scatter diagrams of chemical vs. NIR predicted composition of green crop cereals.

potential degradability can be helpful as an indicator of the roughages IVDMI (Orskov and Reid, 1989). Therefore, it would seem logical to also use further NIRS calibration for other variables (e.g. degradation rate, potential degradability), correlated with voluntary intake, to improve NIRS.

Murray et al. (1990) reported that plant maturity shows very significant seasonal changes in NIR spectra which can be used to measure plant age as well as composition (Blakeney and Batten, 1991). Moreover, Paul (1989) observed small changes in pattern spectra when increased concentration of lignin equivalent to physiological variation in forage grasses. Meuret et al. (1993) obtained poor quality results in the calibration age of the current year's leaves and stems of DMD mediterranean shrubs, referring to insufficient number of data as the probable cause.

This work did not present important patterns of changes in spectra of samples having different cutting dates, although significant correlations were observed between some chemical attributes (ASH, CP and ADL) and the stage of maturity of the green crops. The contribution for this fact may be found in the weak variation of the chemical parameter values of the green crop cereals under study.

Spectra of samples of different green crop species show unimportant pattern changes. Paul (1989), considering numerous species of gramineae, leguminisae, brassicaceae, and compositae, established discriminant analytical models for separating samples according to their botanical identity on the basis of their NIR spectra. For CF and CP, the coefficient of determination and error of prediction between predicted NIRS and laboratory values were 0.91 and 1.86% and 0.95 and 1.17%, respectively. The patterns changes observed by this author are subjacent to the known differences between botanical groups, namely, in what concerns the fibre fraction composition of the gramineae and legumes (Aman, 1993).

As a first approach to develop a universal green crop calibration suitable to be valid across all species of green crops, six calibration models are proposed in which just one species is omitted from the model. The calibration models thus obtained are used to predict the omitted species. Therefore, the chemical attributes were generally unaffected by the omission of one species; occasionally, a slight benefit was caused by omission.

Animal attributes were generally unaffected, but DMD and OMD benefitted 5% when barley was omitted.

IVDMI was benefitted by omission of oats, but sorghum omission seriously spoiled the prediction. Therefore, the developed calibration models work well on the omitted species for chemical attributes and, in a lesser extension, for animal attributes (DMD and OMD).

5. Conclusions

Although working with insufficient data of green crop cereals, mainly in animal parameters, some conclusions can be drawn. Calibration models were successful for most of the chemical and animal attributes listed except IVDMI. The chemical attributes were more successfully predicted than animal attributes. NIRS calibration is appropriate and successful for the quality determination of green crop cereals. In order to evaluate patterns of change in spectra for discrimination between green crop species as well as changes in maturity of green crop cereals, further work is desirable over a more diversified plant population, as a contribution to improve the acceptance of NIRS methods in forage quality.

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