Prediction of the chemical composition and nutritive value of lucerne (*Medicago sativa L.*) by Near Infrared Spectroscopy

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RIASSUNTO – Previsione della composizione chimica e del valore nutritivo dell'erba medica con la spettroscopia nel vicino infrarosso (NIRS). *I foraggi di leguminose rappresentano un'importante risorsa alimentare per i bovini da latte allevati in Italia. Tra le leguminose l'erba medica, caratterizzata da ottime caratteristiche nutrizionali e buone rese agronomiche, è quella maggiormente coltivata nelle aziende agro-zootecniche padane, per l'impiego come fieno o insilato. L'obiettivo del lavoro è stato di predisporre equazioni di calibrazione NIR capaci di stimare accuratamente in erba medica il tenore in proteina grezza, ceneri, NDF, digeribilità della sostanza organica, energia grezza, carboidrati solubili in acqua e potere tampone. I risultati analitici ottenuti con 302 campioni di erba medica raccolti a diversi stadi, hanno consentito di predisporre buone calibrazioni, confermate da coefficienti di determinazione in validazione (r^2) superiori a 0,90, per la stima del tenore in ceneri, proteine grezze, NDF, digeribilità della sostanza organica ed energia grezza, mentre per carboidrati solubili e potere tampone si sono ottenuti valori di R^2 di 0,81 e di 0,78. Si può quindi confermare la validità del metodo NIRS per una stima precisa dei principali parametri qualitativi del foraggio di erba medica, ottenendo inoltre stime delle caratteristiche di insilabilità.*

Key words: lucerne, NIRS, nutritive value.

INTRODUCTION – Lucerne is a low input energy efficient crop that improves soil fertility, and its importance is rising with the increase of public interest in sustainable agriculture. Furthermore, it occupies a significant economic position in the animal feed market (i.e. hay, dehydrated forage, pellets and silage products) and deserves a particular interest in the Parmigiano-Reggiano and Grana Padano cheese production areas of Northern Italy (Torricelli *et al.*, 2000). Particularly for these productions the feeding strategy based on lucerne forage needs a rapid assessment of its nutritive value. Near-infrared reflectance spectroscopy –NIRS- methods are now widely used in animal science to predict the chemical composition of forages and other foods, the digestibility and other nutritional characteristics (Coates 2000; Stuth and Tolleson, 2000). For determining adaptation and quality traits of the new lucerne cultivars to the Italian environmental conditions, NIR spectroscopy was already proposed and used (Odoardi *et al.*, 2001). The aim of the present work was: i) to confirm the ability of NIR spectroscopy for a rapid estimation of quality parameters of lucerne samples with a wide range of maturity and growth stages; ii) to assess the ability of NIRS to predict water soluble carbohydrates (WSC) content and buffering capacity (BC) as important ensilability characteristics for lucerne.

MATERIAL AND METHODS – At the Research Centre of the University of Turin two cultivars of lucerne were sown on September 1995 and September 1996. The herbage was harvested following a 5 cuts per year schedule from April to November. To provide large variability of quality parameters, 4 to 5 cuts were performed at progressive morphological stages from early vegetative to late flowering. For each sampling date, one sample was dried in a forced-draft oven at 65°C, and a second fresh sample stored at -18°C. Dried samples were milled and chemically analysed to determine: crude protein (CP), from total N by combustion, NDF (Robertson and Van Soest, 1981), gross energy (GE) with an adiabatic calorimeter bomb and organic matter digestibility (OMD) according to the two-stage rumen fluid technique. OMD values were expressed *in vivo* using the regression equation of Goldman *et al.* (1987). WSC (Deriaz, 1961) and BC (Playne and McDonald, 1966) were analysed on the water extract from frozen herbage samples.
NIRS analyses of the 302 lucerne samples were carried out with a FossNIRSystem 5000 monochromator, in the spectral range 1098-2500 nm, using the spinning ring cup cell. All spectra and reference data were recorded and managed with the software WinISI II (Intrasoft International). The 302 samples were divided into two independent sets. To determine similar or redundant samples in the full set, the Select option was used (cut-off standardised H distance of 0.6) to select 200 samples for calibration; the remaining 102 samples were used for validation. The Step-up, stepwise and Modified Partial Least squares (MPLS) regression techniques were used to develop the NIRS calibrations. The statistics used for equation development and evaluation were: standard error of calibration (SEC), standard error of cross validation (SECV), coefficient of determination in calibration (R^2) and in cross validation (r^2), according to Shenk and Westerhaus (1996).

RESULTS AND CONCLUSIONS – The calibration set of samples (table 1) covers a great variability as demonstrated by the broad ranges observed in the reference data for ash, CP, NDF, OMD and GE. In the same table the calibration and cross-validation statistics for the best calibration equations obtained are reported.

Table 1. Mean, range of reference values and NIRS calibration statistics for ash, CP, NDF, OMD and GE.

	n.	Mean	Range	SEC	R^2	SECV
Ash (% DM)	185	10.8	6.9-30.3	0.40	0.98	0.50
CP (% DM)	190	20.9	15.3-30.5	0.55	0.96	0.61
NDF (% DM)	184	42.5	21.1-55.9	1.43	0.95	1.55
OMD (%)	141	68	55-86	2	0.89	2
GE (MJ kg^{-1} DM)	193	18.3	13.8-20.7	0.12	0.94	0.18

Table 2 summarises for the validation set of samples the mean value, range and validation statistics of the best calibration equation models. The high R^2 and r^2 values obtained confirm NIRS as an excellent predictor of these parameters (Shenk and Westerhaus, 1996). The R^2 values obtained for WSC (0.81) and BC (0.78) indicate a good prediction capacity also for these parameters. The equation for the prediction of OMD explains 92% of the variability existing in digestibility, and the standard error of the estimate (SECV = 2%) is lower than that (3.15%) reported by Andueza *et al.* (2001) for the prediction of IVDMD in lucerne. These good results can be explained by the chemical characteristics of the samples analysed: the wide range of variation in chemical composition seems adequate to cover the whole variation found in this species. The accuracy of the NIRS equations obtained depended on the adherence to critical imperatives outlined by Shenk and Westerhaus (1996), one of the critical factors being that the calibration samples adequately represent all the variation sources (e.g. cultivars, year of cultivation, harvest time, etc.) associated to a given forage. Even with the high variability observed, NIR spectroscopy is able to predict lucerne feeding value and provide relevant information for its ensilability.

Table 2. Mean, range of reference values and NIRS validation statistics for ash, CP, NDF, OMD and GE with best calibration equations.

	n.	Mean	Range	r^2	SECV
Ash (% DM)	97	10.7	7.2-32.3	0.99	0.50
CP (% DM)	97	20.2	15.2-31.2	0.95	0.65
NDF (% DM)	97	42.1	22.5-56.5	0.93	2.07
OMD (%)	72	69	54-84	0.92	2
GE (MJ kg^{-1} DM)	96	18.3	13.6-19.2	0.94	0.19

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