Prediction of purine nitrogen and total nitrogen content of duodenal digesta of sheep by near infrared spectroscopy

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Introduction

The quantity of microbial and undegraded feed protein reaching the small intestine is essential for the new protein evaluation systems for ruminants. Most often, the microbial protein is distinguished from the feed protein by microbial markers such as nucleic acids, some amino acids as diaminopimelic acid,[1] or by ^{32}P, ^{35}S and ^{15}N labelled feeds.[2] The analytical procedures are labour and time-consuming.

The measurements of forage nitrogen content are the most successful ones made by near infrared (NIR) spectroscopy. At present, there are few reports on the use of NIR spectroscopy to measure protein quality or protein degradation kinetics. Abrams *et al.*[3] showed promising calibrations for total, soluble and insoluble nitrogen in silages. Waters and Givens[4] and Todorov *et al.*[5] tested NIR spectroscopy for prediction of nitrogen degradation characteristics. It could be suggested that it was possible to distinguish microbial and forage protein by NIR spectroscopy because there are differences between the chemical composition of feeds and bacteria and protozoa—differences between feed protein and microbial protein, different level of lipids, different carbohydrate fraction etc.

The aim of this study was to examine the possibility for prediction of total nitrogen (N) and purine nitrogen content as a marker of microbial protein in duodenal digesta samples of sheep.

Materials and methods

Duodenal digesta samples were obtained by using three mature wethers, fitted with rumen cannula and duodenal re-entrant cannulas. The diet of the animals consisted of alfalfa hay or maize silage and concentrate mixture in a 50 : 50 ratio (on dry matter basis) at maintenance level of energy intake and nitrogen intake. Following an initial 14-day diet adjustment period samples of rumen liquor were removed via the rumen cannula and rumen bacteria and protozoa were isolated by differential centrifugation as described by Kurilov.[6] The duodenal digesta was collected during a 72 h period, measured and 3% samples were taken. The samples of rumen microbes and duodenal digesta were oven dried at 65°C for at least 72 h. The total nitrogen of digesta was determined by Kjeldahl following AOAC[7] procedures and the purine N (RNA equivalent) content—by the method of Zinn and Owens.[8]

NIR spectra of 34 duodenal digesta samples, 10 samples of ruminal bacteria and 8 protozoa samples were obtained on dried samples using a NIRSystems 4250 spectrophotometer (NIRSystems Inc., Silver Spring, MD) and InfraAlyzer 450 (Bran+Luebbe, Nordestedt, Germany). For

Table 1. Mean, range and standard deviation of tested parameters in the air dry samples, and obtained calibration and cross-validation statistics.

					NIRSystems 4250				IA-450[a]	
Parameter	Range	Mean	*SD*	Math. method	R^2	*SEC*	*SECV*	CVR^2	R^2	*SEC*
Purine N	5.55–17.50	12.89	2.78	MLR	0.96	0.59	0.63	0.95	0.93	0.80
mg g^{-1}				PLS	0.94	0.69	0.71	0.94		
Total N	12.0–47.0	35.10	7.90	MLR	0.93	2.2	2.3	0.92	0.92	2.3
g kg^{-1}				PLS	0.91	2.4	2.9	0.87		

[a]InfraAlyzer 450.

each sample, two cups were filled up and measured and spectral data were recorded as log (1/*R*). Calibration equations for NIRSystems 4250 spectrophotometer were obtained by ISI Software (Infrasoft International, Port Matilda, PA) using multiple linear regression (MLR) and modified partial least square (PLS) as the regression method. The calibration equations were tested in cross-validation, using four cross-validation groups. The calibration equations for InfraAlyzer 450 were done by MLR using log (1/*R*) data.

Results and discussion

The range of purine N and total N content of the tested samples and the statistical results from calibration procedure and the cross-validation–calibration coefficient of determination R^2, standard error of calibration *(SEC)*, standard error of cross-validation *(SECV)* and cross-validation coefficient of determination CVR^2 are presented in Table 1. The best calibration equations for NIRSystems 4250 were obtained when the spectral data were converted into first derivative—1.5.5.1. mathematical treatment. The results showed that the prediction of purine N by the NIRSystems 4250 was successful—the obtained *SEC* and *SECV* were from 0.59 to 0.71 mg g^{-1}.

The NIR spectral data explained a great part of the variability in the purine N content—the coefficients of determination were higher than 0.94. Figure 1 also graphically illustrates the relationships between laboratory determined and NIR spectroscopy predicted values of purine N content. There was a strong positive correlation between purine N content and spectral data at 1630–1643, 1709–1716, 1961, 2040–2060, 2117, 2167–2197, 2262 and 2280–2307 nm—for each individual wavelength the correlation coefficient was higher than 0.5. Some of these wavelengths are very close to a characteristic point in the spectra of pure ruminal bacteria and protozoa (Figure 2). These wavelengths might be associated with absorption features of pure proteins at 2040–2060, 2167, 2197 and 2300 nm, as well as with oil bands at 1709–1716, 2117 and 2307 nm, and starch bands at 1961 and 2280 nm.[9] Spectral data at 1629, 1643, 1798, 2060 2115, 2167 and 2270 nm were included in the calibration equation for predicting purine N by MLR. It might be concluded that the estimation of purine N by NIR spectroscopy was based not only on microbial protein, but also on other microbial components such as lipids, carbohydrates etc.

The calibration equation for prediction of purine N by the InfraAlyzer 450 has almost the same accuracy as these for the NIRSystems 4250—*SEC* = 0.80 mg g^{-1} and includes spectral data at 1445, 1734, 1778, 1940, 2190, 2208 and 2270 nm.

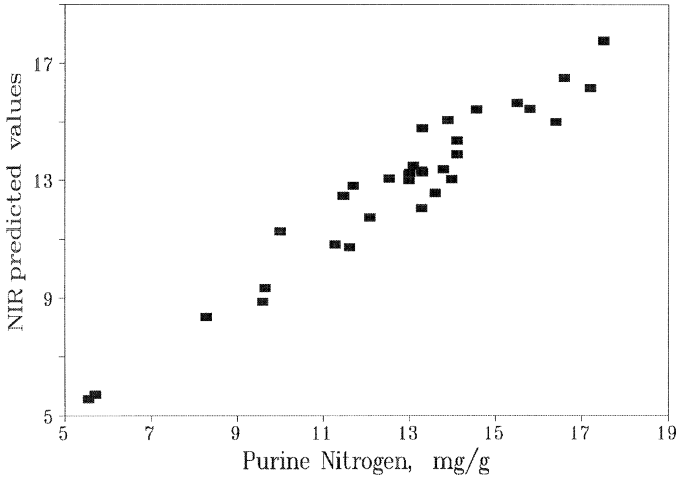

Figure 1. Relationship between NIR predicted and laboratory determined values of purine nitrogen content by the NIRSystems 4250.

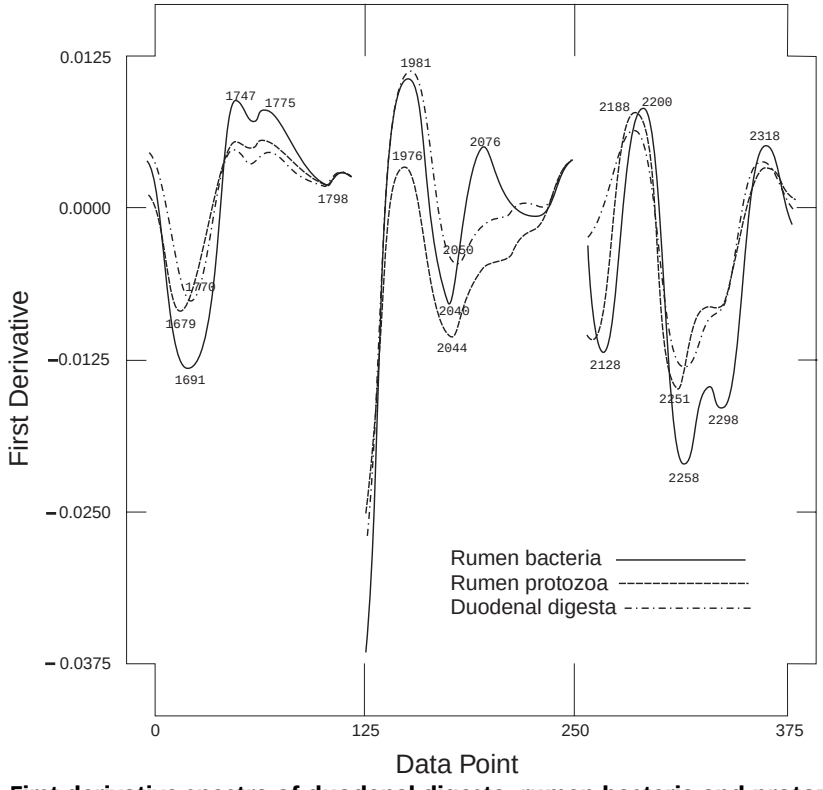

Figure 2. First derivative spectra of duodenal digesta, rumen bacteria and protozoa.

The prediction of the total N content of the samples was successful and the estimation accuracy was similar for both instruments used.

These initial results indicates that NIR spectroscopy has the potential for rapid and simultaneous prediction of purine N and total N duodenal digesta concentration in sheep and for differentiation of microbial N from total N content, which is needed for protein evaluation of feeds according to the new systems.

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