Near infrared spectroscopy for measuring purine derivatives in urine and estimation of microbial nitrogen synthesis in the rumen for sheep

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Introduction

The efficiency of the ruminal fermentation process influences overall efficiency of ruminal production, animal health and reproduction. Ruminants' production systems have a significant impact on the global environment, as well. Animal waste contributes to pollution of the environment as ammonia volatilised to the air and nitrate leached to ground water.

Microbial protein synthesis in the rumen satisfies a large proportion of the protein requirements of animals. Quantifying the microbial synthesis is possible by using markers for rumen bacteria and protozoa such as nucleic acids, purine bases, some specific amino acids, or by isotopic ^{15}N-, ^{32}P- and ^{35}S-labelled feeds.[1–4] All those methods require cannulated animals, they are time-consuming and some methods are very expensive as well. Many attempts have been made to find an alternative method for indirect measurement of microbial synthesis in intact animals. One of them is based on measurement of purine derivatives (allantoin and uric acid in ruminants) excreted in urine and milk. Several authors demonstrated a direct relationship between urinary purine metabolite excretion and microbial protein production in the rumen.[5–7]

Near infrared (NIR) spectroscopy has been demonstrated as a successful toll for compositional analysis of feedstuff and other agricultural products.[8,9] At present there are a number of studies on the use of NIR spectroscopy in animal physiology, for example, brain research, Hb research, bacterial nitrogen in duodenal content[10,11] and in feed residue after ruminal incubation in nylon bags.[12,13]

The present investigation aimed to assess possibilities of usingNIR spectroscopy for the prediction of purine nitrogen excretion and ruminal microbial nitrogen synthesis by NIR spectra of urine.

Materials and methods

Eighty urine samples were collected from 12 growing sheep, six male and six female. The sheep were included in a feeding experiment which consisted of sorghum silage and protein supplements—70 : 30 on a dry matter (DM) basis. The protein supplements were chosen to differ in protein degradability: urea, soybean meal and soybean meal/fish meal (40 : 60 DM basis). The urine samples were collected daily in a vessel containing 60 mL10% sulphuric acid to reduce pH below 3 and diluted with tap water to 4 L. The samples were stored in plastic bottles and frozen at –20°C until chemical and NIR analyses. The samples were analysed for allantoin, uric acid, xantine and hypoxantine content. All methods of analyis were described in detail by Chen and Gomes.[14] Microbial nitrogen synthesis in

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the rumen was calculated based on the urinary excretion of purine derivatives, according to Chen and Gomes.[14]

NIR transmittance (*T*) spectra of urine samples were obtained by an NIRSystem 6500 spectrophotometer (Foss NIRSystems, Silver Spring, MD, USA). A cell 1 mm thick was used for the NIR measurements. The spectra were taken in the wavelength range from 1100 to 2500 nm at 2 nm intervals and were recorded in the linked computer as absorbance, i.e. log(1/*T*). Prior to spectral analysis each sample was warmed up to 40°C in a water bath.

A commercial software package, ISI NIRS 3 (Infrasoft International, Port Matilda, PA, USA), was used to process the data and to develop models for determination of allantoin, total purine derivatives and purine derivatives nitrogen content in urine and microbial nitrogen synthesis in the rumen.

The calibration was performed using partial least square (PLS) regression. In the development of all calibration models, ten PLS factors were set up as a maximum. The optimum number of PLS factors used in the models was determined by a cross-validation method. In cross-validation, five samples were temporarily removed from the calibration set to be used for validation. With the rest of the samples a PLS model was developed and applied to predict the content of tested parameters of the group of five samples. The results were compared to the respective reference values. This procedure was repeated several times until a prediction for all samples was obtained. Performance statistics were accumulated for each group of removed samples. The validation errors were combined into a standard error of cross-validation (*SECV*). The optimum number of PLS factors in each model was defined to be the one that corresponded to the lowest *SECV*.

Selection of the best calibration equations was made on the basis of the lowest standard error of calibration (*SEC*) and standard error of cross-validation (*SECV*), and the highest calibration coefficient of determination (R^2) and cross-variation coefficient of determination CVr^2, respectively.

Results and discussion

The ranges, mean values and standard deviation of allantoin, total purine derivatives and total purine nitrogen content of urine samples and calculated microbial nitrogen synthesis in the rumen are presented in Table 1. The statistical parameters of calibration and cross-validation procedures for purine derivatives content and microbial protein synthesis determination are shown in Table 2. Figures 1 and 2 graphically illustrate the relationships between determined and NIR predicted values of allantoin content and microbial nitrogen synthesis, respectively.

The results of estimating the purine derivatives, excretion and microbial protein synthesis of urine using NIR spectra showed promising accuracy. The ratio of standard deviation of population and standard error of calibration was 2.96 for allantoin content, 2.65 for total purine derivatives, 2.53 for total purine nitrogen and 2.87 for microbial nitrogen, respectively. These values were considered adequate for fast and noninvasive evaluation of microbial nitrogen synthesis in the rumen.

Table 1. Mean, range and standard deviation (SD) of tested parameters in urine samples.

Parameter	Average	Min	Max	SD
Allantoin, mg L^{-1}	33	849.5	403.68	125.20
Total purine derivatives, mmol d^{-1}	2.36	26.67	13.33	3.75
Purine derivatives nitrogen, mg d^{-1}	165	1867	935.80	259.14
Microbial nitrogen, g d^{-1}	0.77	23.08	11.40	3.36

Table 2. The statistical results of NIR calibration for prediction of purine derivatives and microbial protein synthesis.

Parameter	Math. Transf.	PLS factors	*SEC*	R^2	*SECV*	CVR^2
Allantoin, mg L^{-1}	log(1/*T*)	9	42.25	0.848	52.13	0.770
Total purine derivatives, mmol d^{-1}	first derivative	7	1.42	0.843	1.68	0.776
Purine derivatives nitrogen, mg d^{-1}	log(1/*T*)	8	102.20	0.821	118.63	0.760
Microbial nitrogen, g d^{-1}	first derivative	8	1.17	0.861	1.37	0.810

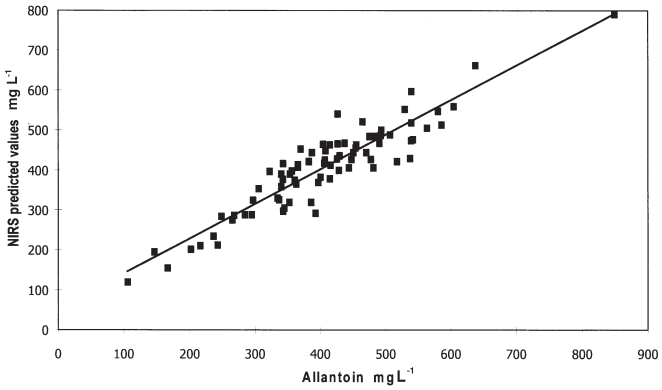

Figure 1. Relationship between actual and NIR predicted values of allantoin content in urine.

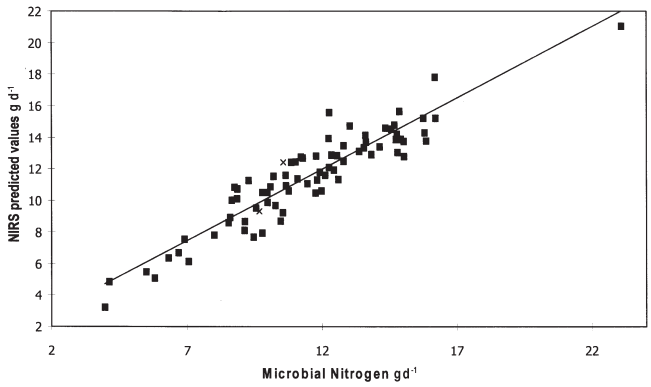

Figure 2. Relationship between actual and NIR predicted values of microbial nitrogen synthesis in the rumen.

The PLS modelling not only aided the development of quantitative models but also was used as a tool for discerning the location of spectral information related to purine derivatives. The regression vector of the calibration equation for allantoin content, based on log(1/*T*) spectral data, was studied. High positive coefficients in the regression vector were found at 1368, 1484, 1838, 1956, 2024, 2190,

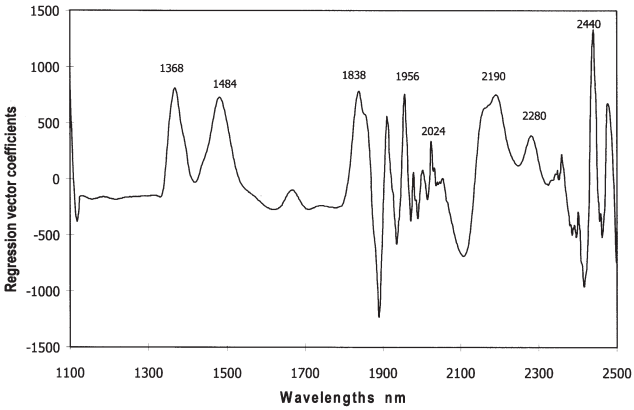

Figure 3. Plot of regression vector coefficients of equation for determination of allantoin content.

2280 and 2440 nm (Figure 3). The NIR absorption around 1484 and 1956 nm may be due to the first overtone in the N–H stretching mode and combination of N–H stretching and amide II vibration, respectively.[8,15] In the regions around 2190, 2280 and 2440 nm dominated absorption of C–H bands and absorption at 2024 nm was connected with C=O absorption.[8,15] It might be concluded that the determination of purine derivatives by NIR spectroscopy was connected with their absorption in the NIR region.

Conclusion

The results indicate that the advantages of the NIR technology can be extended into animal physiological studies. The fast and low cost NIR analyses could be used with no significant loss of accuracy when microbial protein synthesis in the rumen are to be assessed by NIR spectroscopy.

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