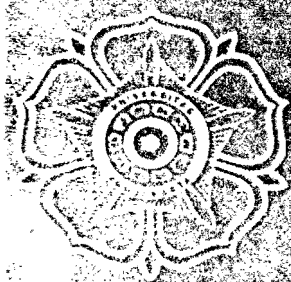


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COMPARISON OF SHORT AND LONG WAVELENGTH OF NEAR INFRARED SPECTROSCOPY FOR PREDICTING NITROGEN AND ALLANTOIN IN URINE

Agung Purnomoadi, I. Nonaka, K. Higuchi, K. Ueda, O. Enishi, T. Nishida, M. Kurihara, F. Terada and A. Abe¹

ABSTRACT

Total 130 urine samples collected from dairy cattle were studied for knowing the possibility and accuracy of the use of long and short wavelength of near infrared spectroscopy (NIRS) for nitrogen and allantoin content in urine. The samples were randomly separated for the calibration (n=94) and the validation samples (n=36). Short and long wavelength of NIRS was selected in the range of 700-1350 and 1350-2500 nm, respectively. Four wavelengths selected from short wavelength were found mainly related to the N-H, C-H or O-H structure in both for nitrogen at 1225, 970, 1060 and 806 nm and for allantoin at 990, 1030, 1170 and 815 nm, respectively. Meanwhile, the selection in long wavelength was found mainly related to the N-H bound or protein for each component at 1430, 1530, 2242 and 1960 nm for nitrogen and 2180, 2242, 1978 and 1530 nm for allantoin, respectively. Coefficient correlation (and standard error) of prediction with calibration equations using short wavelength were 0.85 (0.19), and 0.85 (47.7) for nitrogen and allantoin, respectively. Those values obtained from long wavelength were found to be 0.91 (0.13) and 0.92 (42.3), respectively. Judgement of validation with RPD values (the ratio of standard deviation of reference data in prediction sample to the standard error of prediction by NIR) for nitrogen and allantoin predicted by short wavelength were found 2.06 and 2.42, respectively. These values were lower than 2.5 the limit value recommended available for application. However, the use of long wavelength of NIRS showed an possibility to predict nitrogen and allantoin in urine as shown by high RPD values to be 2.94 and 2.70, respectively.

Key words: Near infrared, Allantoin, Urine.

INTRODUCTION

The rapid analytical method using near infrared (NIR) spectroscopy have been widely used for feeds analysis in farm service since this method successfully studied in forages (Norris *et al.*, 1976). This method shows many advantages for the future analytical tools due to the rapid, accuracy and no reagents so that no pollutant. This method is based on the information included in the NIR spectra of the samples that expressed the content of organic substances in the sample. This fact leads the studies in many field such as on milk quality and detection of udder health (Tsenkova *et al.*, 1994), cerebral

oxygen consumption and energy expenditure (Takahashi and Eda, 1998). Now, the big research project for developing the continuous measurement system on the living system in animals using NIR has been done in Japan. A part of this project was aimed at providing the monitoring system for nutritional status in animal. Since allantoin, the purine derivatives can be used as an estimator of rumen microbial protein supplied to the host animal (Verbic *et al.*, 1990), and showing an animal response to protein supply (Chen *et al.*, 1992) and has a relationship with dry matter intake (Gonda and Lindberg, 1994), it may be worth to study the possibility of the use of NIR spectroscopy for determining allantoin and

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also nitrogen content in urine.

MATERIALS AND METHODS

Total 130 urine samples collected from lactating cows and one month post partum dairy cows were used in this study. Samples were spot collected after morning milking at 10:00 and filtered to separate solid contaminant. Reference data of nitrogen content and allantoin were determined using Kjeldahl methods and the procedure described by Young and Conway (1942) as modified by Matsumoto *et al.* (1995), respectively. Ninety-four samples were randomly chosen for developing calibration equation, named calibration samples. The remaining thirty-six samples were used for validating that equation named validation samples.

Near infrared spectra of the urine were collected with a Pacific Scientific model 6500 instrument (Perstorp Analytical, Silver Spring, MD) using a transmittance cell sample (thickness : 1 mm). Spectra were read over a range of wavelengths between 400 and 2500 nm at 2 nm intervals. The spectra were then calculated on second derivative of $\log I/T$, where T is transmittance. The data from the points of wavelength recorded were used to develop calibration equation using multiple step-wise linear regression with ISI (InfraSoft International, Port Matilda, PA) to obtain the highest correlation regression and lowest standard error. The final form of calibration equation will be :

$$Y = a + b(X_1) + c(X_2) + d(X_3) + e(X_4),$$

where ; the Y is variable to be predicted which commonly obtained by reference method; the a, b, c, d and e are the coefficients; and $X_{1,2,3,4}$ are the absorbance at selected wavelength.

Two methods based on the range of wavelength used for developing calibration equations were compared. First method was the use of short wavelength of NIR in range from 700 to 1350 nm, while the second was the use of long wavelength of NIR in range from 1350 to 2500 nm. Shorter wavelengths

contain a higher level of the overtones of same chemical structure with that appeared in longer wavelength. The peaks in shorter wavelength are more clearly separated but the signals are weaker than that in longer wavelength. This comparison is important to find the most available wavelength for determination.

Judgement of availability was done based on the coefficient correlation, standard error and the value of RPD (the ratio of standard deviation of reference data in validation sample to the standard error of prediction by NIR) as introduced by Williams (1996) obtained from validation samples.

RESULT AND DISCUSSION

The range, mean and standard deviation of urine samples used for the calibration and for validation are presented in Table 1. The mean value of nitrogen in calibration samples was slightly higher than that of the validation, but standard deviation of these samples were similar. For allantoin, the mean and standard deviation in calibration samples were similar with the validation. Only a few samples of the validation were lying on the below of minimum value of the calibration, but since the range of the calibration was wider than of the validation, this separation is available to support the reliability of calibration equation to determine the validation sample.

Calibration equation for nitrogen and allantoin were developed using combination of four wavelengths in respective wavelength range as presented in Table 2. In the use of short wavelength, four wavelengths at 1225, 970, 1060 and 806 nm, and 990, 1030, 1170 and 815 nm were obtained as the most available for calibration equation of urinary nitrogen and allantoin, respectively. Those wavelengths are mainly correlated with the RNHR' structure (815 nm), RNH₂ structure (806, 1030 and 1060 nm), C-H bound (1170 and 1225 nm) and O-H bound (970 and 990 nm). In the use of long wavelengths, the calibration equation for both nitrogen and

Table 1. Separation of urine samples for the calibration and for validation of nitrogen and allantoin contents.

	Calibration (n=94)			Validation (n=36)		
	Range	Mean	SD	Range	Mean	SD
Nitrogen, %	0.27 - 1.80	1.03	0.36	0.22 - 1.60	0.93	0.39
Allantoin, mg/dl	73.4 - 557.2	274.2	109.0	49.6 - 439.6	275.1	114.1

n : number of observation; SD : standard deviation

allantoin were correlated with N-H bound.

The wavelength at 1430 and 1960 nm are absorbance of CONH₂ structure, 1530 nm is absorbance of RNH₂ structure and 2242 nm is related to amino acid absorbance. The second and the fourth wavelengths used for calibration equation of allantoin were same with the third and second wavelengths used for nitrogen calibration. The first and third were absorbance of amide (Osborne and Fearn, 1986). Those selected wavelengths were in agreement with the own chemical structure. These calibration equations developed with two ranges of wavelengths were then used to determine the nitrogen and allantoin in the validation samples. The results are presented in Table 2 and illustrated in Figure 1.

Calibration equation for nitrogen and allantoin in urine developed using long wavelength showed better accuracy than that

of short wavelength as shown by high correlation coefficient and low standard error. The use of long wavelength also shows stability of prediction compared to the short wavelength. Moreover, the judgement by using RPD value showed that for nitrogen and allantoin, the long wavelength were higher than that of the short wavelength. The RPD values obtained in the short wavelength were lower than 2.5 the limit value for availability of measurement. Meanwhile, the RPD values obtained in the long wavelength were higher than that the limit value.

From this result, the conclusion can be made on the possibility and reliability of the use long wavelength from 1350 to 2500 nm of NIR spectroscopy method for urinary nitrogen and allantoin measurement. In connection with the previous study on urine for monitoring the urea in milk (Purnomoadi *et al.*, 1998), this study shows the potential to

Table 2. Wavelength used for developing calibration equations and statistical summary in calibration and validation.

	Wavelength (nm)				Calibration				Validation
	1st	2nd	3rd	4th	R	SEC	r	SEP	RPD
	700 - 1350 nm								
Nitrogen	1225	970	1060	806	0.90	0.14	0.85	0.19	2.06
Allantoin	990	1030	1170	815	0.82	50.6	0.85	47.7	2.42
	1350 - 2500 nm								
Nitrogen	1430	1530	2242	1960	0.93	0.12	0.91	0.13	2.94
Allantoin	2180	2242	1978	1530	0.90	42.8	0.92	42.3	2.70

R : correlation coefficient of multiple regression, r : correlation coefficient of simple regression ; SEC : standard error of calibration, SEP : standard error of prediction ; RPD : ratio of standard deviation of reference data in validation samples to the SEP.

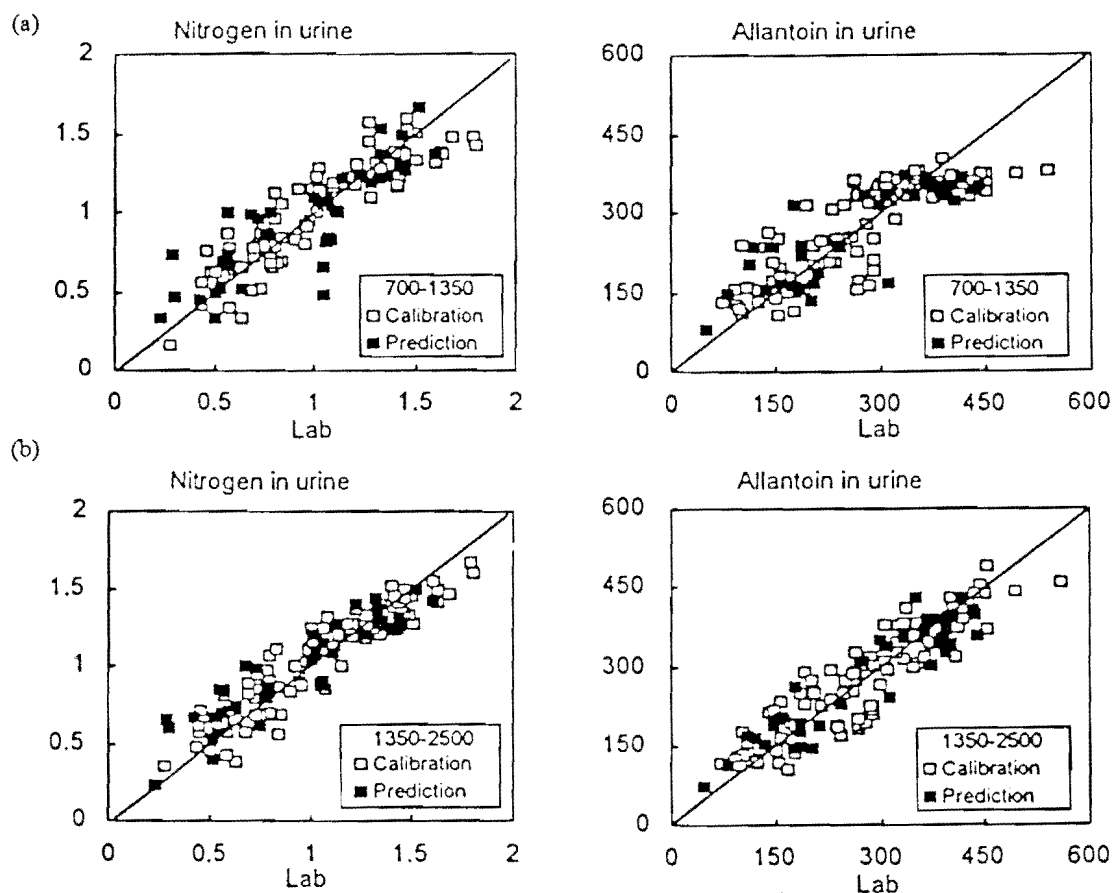


FIGURE 1. Regression between the reference value (Lab) and NIR predicted value of nitrogen and allantoin in urine using wavelengths selected in (a) 700-1350 nm and (b) 1350-2500 nm.

make a monitoring system of animal based on NIR spectra of urine.

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