Preliminary study on the use of near infrared spectroscopy for determination of plasma deuterium oxide in dairy cows

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Introduction

Information on body composition (fat and protein) in live animals is important in the determination of the nutrient requirement of the animal. The change of body composition due to mobilisation and reserves of body tissues in dairy cows occurs during the whole lactation period.[1] Significant changes in body composition especially occurs during early lactation because of the insufficiency of nutrient intake to meet the requirement for lactation, and it is reported[1–4] that empty body fat was reduced by 42.4 kg for early lactation cows compared with that of prepartum cows.

The conventional method for measuring body composition is direct slaughter, a method that is expensive, laborious and unrepeatable. Non-destructive methods such as various body water dilution procedures, have been proposed to estimate body composition and one of them is the deuterium oxide (D_2O) dilution technique.[5–7] The D_2O dilution technique has also been validated as a direct method, and shown its ability to detect changes in body fat and protein across physiological stages in dairy cows[6] and in fat-tailed Barbary ewes.[8] The usefulness of this technique, however, is limited by the time consuming use of special equipment for liophilisation to extract water from plasma prior to the D_2O concentration measurement. This study was carried out to determine if the rapid analytical method of near infrared spectroscopy had the potential to solve this problem.

Materials and methods

Four dairy cows (Cows #474, 478, 550, 942; mean body weight 575 kg) in early lactation were used. They were fed total mixed rations consisting of corn silage, timothy hay and concentrates to make 17.0% CP and 14.0 MJDE kg DM^{-1}. At weeks 1, 3 and 5 after parturition, D_2O with 0.9% NaCl was infused into the jugular vein at a dosage rate of 250 mg kg BW^{-1}. Blood samples were collected at 0, 5, 10, 15, 20, 25, 30, 40, 50, 65, 80, 100, 120, 150 min, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h after infusion. The samples were then centrifuged at 3,000 rpm for 10 minutes to obtain plasma that was then stored in a freezer at –35°C for chemical and spectral analysis.

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D_2O analysis, chemically

Plasma samples were thawed at room temperature (23°C) prior to the determination of D_2O. The D_2O concentration was analysed by gas chromatography (deuterium oxide analysis system, HK102, Shokotsusyou) after extraction from plasma by liophilisation.

D_2O analysis with near infrared spectroscopy

The rest of the blood plasma sample remaining after chemical analysis was used for near infrared analysis. The NIR spectra of plasma samples were recorded by a Pacific Scientific (Neotec) model 6500 (Perstorp Analytical, Silver Spring, MD, USA) using transmittance cell samples (1 mm thickness). Spectra were read at 2 nm interval over the wavelength range from 1100 to 2500 nm, which were then converted to second derivative of log A^{-1}; where A is the absorbance, using ISI software (InfraSoft international, Port Matilda, PA, USA). A calibration equation was developed by multiple linear regression using a combination of four wavelengths.

The calibration equation and wavelength selections were developed using samples from one animal (calibration set; cow #550; *n*: 74), while the rest of the samples from three animals (cows #474, $n = 44$; #478, $n = 62$; #942, $n = 68$) were used for validating the developed calibration equation (validation set). This methology was used to make it possible to judge whether the calibration equation (including the selected wavelengths) was valid for D_2O determination while allowing for variation between individual animals. Judgement of accuracy was done on averaged values from three weeks collection for each cow for replication of NIR measurements, ignoring the effect of the sample collection period.

Results and discussion

The range and average of D_2O concentration in blood samples collected in this study is presented in Table 1. The lowest values in each animal is considered as the base level of D_2O in blood at zero time collection, while the highest concentration of D_2O in blood occurred in five or ten minutes after infusion. Development of the calibration equation using four wavelengths in the 1100–2500 nm range wavelengths from the spectra of blood samples from cow #550 showed a high correlation ($R = 0.93$) with a standard error 48.1 ppm. The four wavelengths used were 2128, 1636, 1190 and 2210 nm. These wavelengths were correlated with bonds of N–H (amide) and C–H.[9] This calibration result is presented in Figure 1.

Table 1. The deuterium oxide (D_2O) concentration in blood samples from four cows used in this study and the statistical summary of calibration and validation set on average value of NIR predicted value from three weeks.

Cow no	*n*	D_2O (ppm)	Average (ppm)	*R*	*SEP*	RPD
Calibration set sample s						
#550	74	135 – 925	535	0.93	48.1	—
Validation set samples						
#474	44	303 – 840	530	0.94	53.1	2.34
#478	62	303 – 984	544	0.98	23.5	6.64
#942	68	283 – 919	525	0.95	37.2	3.36

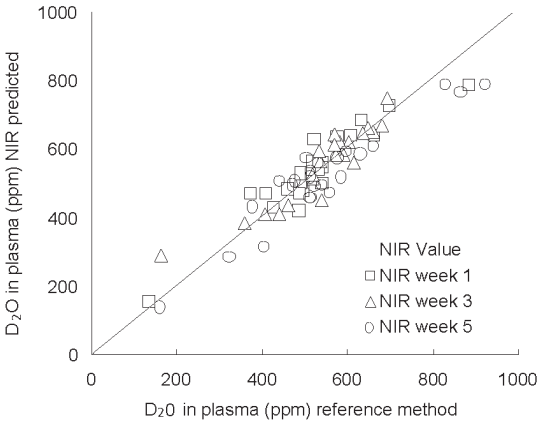

Figure 1. Correlation between deuterium oxide (D_2O) in blood plasma determined by conventional method and the NIR predicted in the calibration set samples using cow #550 at three weeks collections; NIR predicted at week 1, 3 and 5.

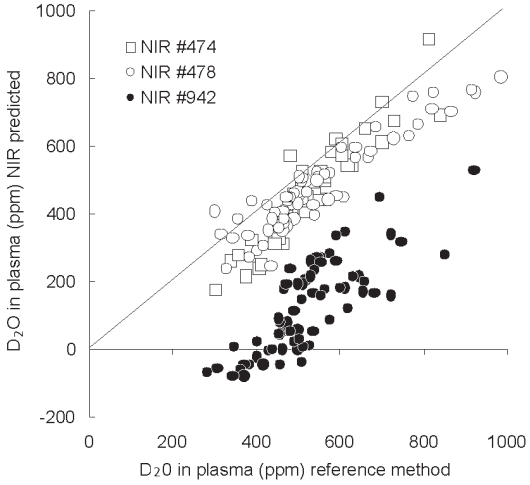

Figure 2. Correlation of NIR predicted value and Lab value of three cows used for validating the calibration equation.

Validation of the calibration equation for three individual cows was done on the average of NIR predicted value of D_2O at each collection time from the three weeks injection. The results showed a high correlation as presented in Table 1 and Figure 2. The *r* and *SEP* for plasma from cows in #474 were 0.94 and 51.8 ppm; cow #478 were 0.98 and 23.5 ppm; cow #942 were 0.95 and 37.2 ppm, respectively. Judgement of the accuracy based on the ratio of standard deviation and standard error in validation set samples (RPD) for cows #474, #478 and #942 were 2.3, 6.6 and 3.4, respectively. Based on RPD values, the results were higher than 2.5, the limit value available for screening[10] while cow # 474 was just below the limit. From this figure, the most interesting is cow #942. The predicted value

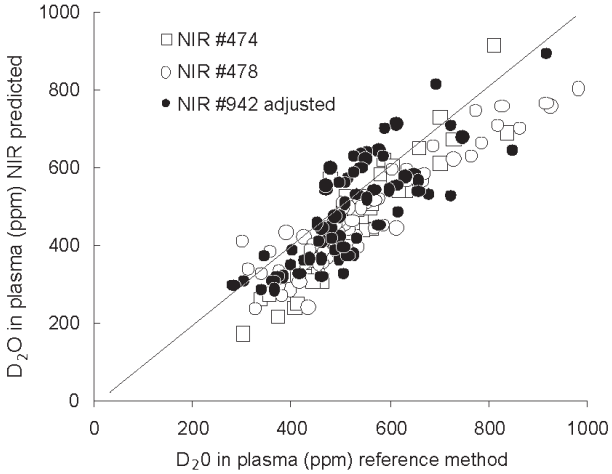

Figure 3. Correlation of NIR predicted value and Lab value of three cows used for validating the calibration equation. The value from cow #942 was adjusted using the difference value on deuterium oxide (D_2O) concentration at the 72 h collection period.

was much lower that the Lab values that resulted in a bias in prediction because the *r* and *SEP* showed high accuracy. This was considered as an effect of the lower level in NIR absorbance compared to those of the other three cows. By converting the lowest value (concentration at 72h) of cow #942 to the point of Lab determined value, the predicted values were lying in the area of NIR predicted values, as shown in Figure 3. These facts showed that the existence of variation between animals in their blood composition possibly biases the prediction values. However, this problem can be overcome by providing standard samples at known concentrations to allow calculation of a correction factor.

Conclusion

The high accuracy in prediction found in this preliminary study on the use of NIR spectroscopy for determining deuterium in plasma suggests that this is a very promising application. The bias measurement may come from the variation between individuals, but this can be overcome with provision of a correction. Further studies on various physiological stages of animals should be done to measure any other factors that biases the measurement.

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