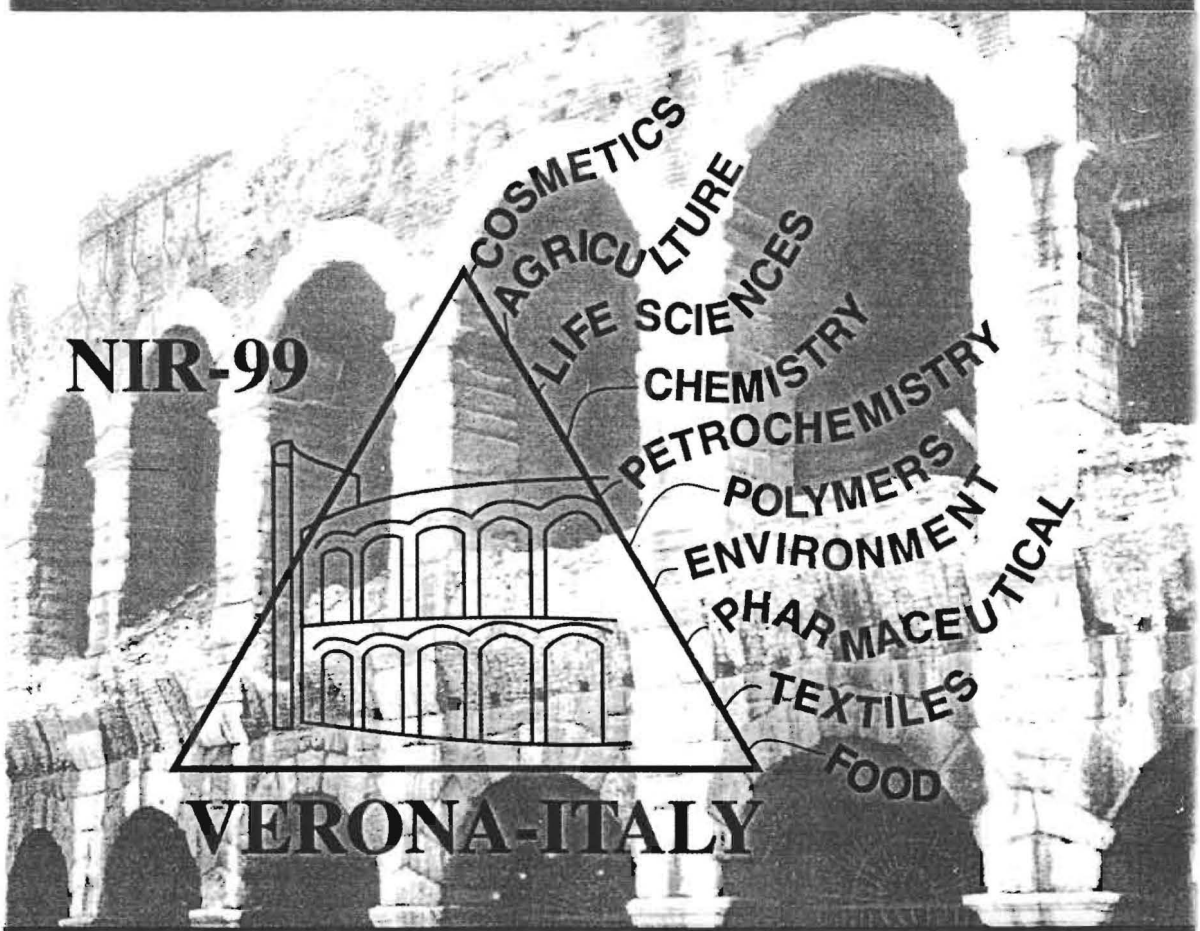
Near Infrared Spectroscopy:

Proceedings of the 9th International Conference



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Animals and animal food

Monitoring blood urea nitrogen through near infrared spectra of urine in dairy cows

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Introduction

Studies on urea N-content in body fluids of cows have been given increased attention in the dairy industry and in research.[1] This interest has followed the increased achievements in improving efficiency of N-utilisation and to reduce losses of N to the environment from dairy farms.

It has been reported that the high urea N in body fluids in dairy cows was associated with impaired fertility,[2–4] reduced energy availability,[5] low protein utilisation[1,6] and increased environmental pollution of N.[7] The level of urea N in body fluids is mainly affected by dietary factors, such as percentage of dietary protein,[8] the ratio of dietary protein to ruminally fermentable organic matter,[9] post-ruminal protein metabolism[10] and balance of protein to energy.[8,11] In other words, urea N concentration in body fluids can be used as a useful tool for monitoring the nutritional status in animals.[12]

Primarily, the best information about urea status in animals is provided from blood, but blood collection requires invasive techniques and is difficult for daily analysis. There is a high correlation between urea N in milk (MUN) and urea in blood (BUN).[11]Thus, urea N in milk is generally used for estimating body urea levels due to ease of milk collection. Since controlling the balance of protein to energy is important in many herds, the problem from the use of milk for urea N measurement will arise for cattle in the dry and prepartum periods because they do not give the milk. So, there is a need to find an alternative medium for urea status measurement. Urine may become an ideal medium for assessing the nutritional status because of its easy collection methods and the amount of urea excreted in urine is directly proportional to the concentration of urea in blood[13] and there is a significant relationship between milk urea and urinary nitrogen.[14]

Despite the analysis method for urea N becoming simpler due to commercial kits, this method does not seem suitable for daily measurement because of the need for sample preparation prior to analysis. Near infrared (NIR) spectroscopy, a rapid and accurate method, may become an alternative tool to reduce the analytical time. This present study attempted to use NIR spectra of urine for monitoring the BUN, because blood gives the best information for urea N levels in the body. This work is a part of a research project for developing a continuous measurement system for a live animal using NIR techniques.

Materials and methods

Animal experiment

Four multiparous Holstein cows in mid-lactation (average body weight 576 kg) were used. The mean milk yield of these cows before the experiment was 37.4 kg d^{-1}. The cows were fed Timothy grass hay, beetpulp and commercial concentrate containing 13.4% CP and 11.6 MJME kg^{-1} DM, according to the requirements of maintenance and milk production.[15] The feed was given four times a day at 08.00, 10.00, 16.00 and 18.30 h. Daily intake of dry matter (DMI) was measured by collecting the orts and drying them in an airdraft oven at 105°C overnight.

The cows were housed in a climatically-controlled room over four weeks with an adjustment period of a week prior to the collection period. The room was exposed to a temperature of 18°C for the first two weeks and then to 28°C for the following two weeks. The temperature elevation was performed to obtain the significant trend of BUN as the increased temperature might cause a decrease in DMI resulting in underfeeding of the animals. Blood and spot urine samples were collected daily from week 2 to week 4.

The milk was collected daily at 08.30 and 18.00 h and the production was recorded. Blood and urine samples were collected at 08.00 h. Blood samples were taken using a catheter inserted into the jugular vein. The blood samples were centrifuged at 3000 rpm for ten minutes to obtain the plasma and then the plasma was stored in – 35°C until chemically analysed. Spot urine samples were collected by vulval stimulation and then filtered to separate the solid contaminants and stored at – 35°C until chemically analysed. Urea N concentration in blood was analysed with the Urease Indophenol method using a commercial kit (Wako pure chemical Ltd).

NIR measurements

Urine samples were thawed at room temperature (around 23°C) prior to the spectra collection. NIR spectra of urine samples were measured with a Pacific Scientific (Neotec) model 6500 instrument (Perstorp Analytical, MD, USA) using a transmittance cell sample (thickness: 1 mm). Spectra were read at 2 nm intervals over the range of wavelength between 1100 and 2500 nm. The spectra were then calculated into a second derivative of $\log A^{-1}$; where A is the absorbance, using ISI software (InfraSoft International, Port Matilda, PA, USA). The spectra obtained from daily morning urine were analysed to find the wavelength that highly correlated with BUN.

Correlation between BUN and the absorbance of urine was analysed using linear regression and the Spearman correlation test.[16] Linear regression was carried out for determining the correlation between the absorbance of urine in near infrared spectra and BUN, while the Spearman test was carried out for examining the synchronise in the daily change of urine absorbance and the BUN.

Results

Animal performance

The DMI, milk yield and BUN concentrations are presented in Figure 1. DMI started to decrease at d2 of temperature elevation and become relatively steady at around d6. The trend of milk yield was quite similar with DMI and the correlation coefficients between DMI and milk yield was found to be 0.98. After the temperature was

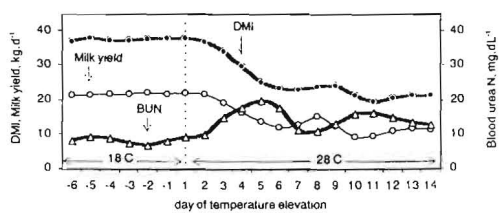

Figure 1. Daily change of DMI kg d^{-1}, milk yield kg d^{-1} and BUN mg dL^{-1} during heat exposure.

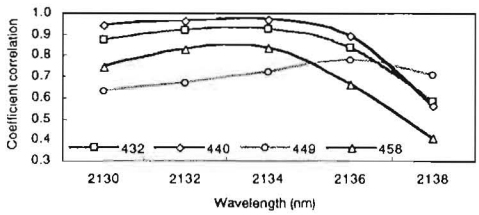

Figure 2. Coefficient correlation between the BUN concentratioon and urine specra at each wavelength in the range 2130–2138 nm of individual cows. The four cows were nos, 432, 440, 449 and 458.

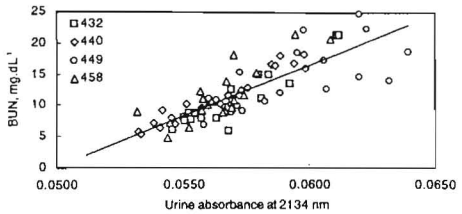

Figure 3. Correlation between BUN mg dL^{-1} and urine absorbance at 2134 nm from four dairy cows. Linear regression equations was found to be BUN mg dL^{-1} = 1631.3 (U-2134)—81.3; r^2 = 0.69, Residual standard deviation = 2.62.

increased to 28°C, the concentrations of BUN increased significantly at d2, d3, d4 and d5, before returning to the original level (the level of BUN before temperature was increased) at d7 and increased again at d9 and d10, which may be due to the fitting of the head cage for balance trials. Figure 1 shows that the daily change of BUN concentration was in contrast with that of DMI with the correlation coefficients being – 0.74.

Correlation of NIR spectra of urine with BUN

Observation of wavelengths on individual dairy cattle

Spectra of urine collected from four dairy cattle during temperature elevation were individually calculated. Highly correlated wavelengths were found in the region of 2130–2136 nm. Coefficient of correlation was calculated using linear regression analysis for four cattle and the patterns of correlation between the wavelengths and BUN varied, as presented in Figure 2. The highest correlation between BUN concentration and urine spectra appeared at 2134 nm (U-2134) for three cows (Cow nos 432, 440 and 458), while the spectra from the cow no. 449 was found at 2136 nm (U-2136). The level of highest correlation also varied and ranged between 0.78 and 0.97. Almost all coefficient correlation value of cows started to drop sharply at 2138 nm. The linear regression of U-2134 and BUN from four cows are presented in Figure 3.

Monitoring in dairy cattle herds

For the purpose of monitoring, the trend of daily urine absorbance and daily BUN must be analysed for synchronism. For that, the daily NIR absorbance of urine and BUN concentration of four animals were averaged. Linear regression analysis showed the highest coefficient of correlation at U-2134 nm with the coefficient correlation being 0.96. The linear regression equation was found to be, BUN (mg dl^{-1}) = 2163 (U-2134) – 111.5 ; (r^2 = 0.91; RSD = 1.16). Evaluation of synchronism using Spearman rank correlation test[16] showed that the urine absorbance at 2134 nm was highly synchronised with BUN and the value was 0.90. The trends of daily BUN and U-2134 during the increase in temperature from 18°C to 28°C is shown in Figure 4.

Discussion

Many works have been attempted to estimate the urea level in blood by employing the correlation between BUN and MUN or urinary nitrogen.[13,14,17] The present study showed that the spectra of urine at the wavelength region of 2130–2136 nm was highly correlated with BUN. Based on the reference of

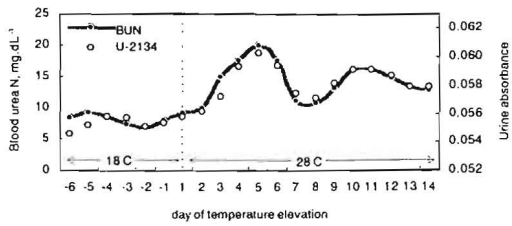

Figure 4. Daily change of BUN concentration mg dL^{-1} in comparison with daily change of urine absorbance at 2134 nm (U-2134).

assignments of bands in NIR regions, that region represents N–H bond component.[18] In this study, the chemical composition of urine was not determined. Therefore, it cannot be confirmed that the substance appeared in that wavelength. However, since the urea in blood excretes through milk and urine, and they are closely correlated, it was considered that U-2134 represents the urea concentration in urine.

In the present study, the coefficient of determination (r^2) of BUN, using U-2134 calculated on individual animals ($r^2 = 0.69$), was lower than if the group average was calculated ($r^2 = 0.91$). These results were similar to other findings[6,13,19] that body urea levels (BUN or MUN) from group averages of animal were more reliable than from individual cows. However, the r^2 values of BUN and U-2134 obtained in this study are comparable to the findings reported by Ciszuk and Gebregziabher[13] and Gonda and Linberg[14] (0. 67 and 0. 78, respectively), on their studies on BUN and urinary urea. Those values were also comparable with other studies on estimation of BUN from MUN which reported the values of 0.82,[9] 0.77[6] and 0.93.[20]

The most important finding in this study was the accuracy of urine spectra as a medium for monitoring BUN. Daily change of BUN was highly synchronised with the daily change of urine absorbance [U-2134 mn (Figure 4)]. Our observations on individual animals also showed the high synchronism between the daily BUN and U-2134, although there are variations between animals in their level of urine absorbance. The availability of urine spectra for monitoring BUN, as shown in this study, is very helpful in dairy farm management, since the animal performance in BUN level is affected by physiological status, feedstuff replacement and change of environment in the farm. However, because of the individual variation on the NIR absorbance and its response to BUN (Figure 2), a further study should be carried out on developing suitable methods for BUN prediction.

In this study, urine showed its capacity as a suitable medium for monitoring body urea levels in dairy cows, either in individual or in herd levels. Combined with NIR technology, urine is much more superior than blood or milk due to ease of collection (non-invasive), no sample preparation (prior to analysis), anytime collectable (compared with milk which depends on milking time). Moreover, with this method we could maintain the nutritional balance of animals of any herds and any stage of production.

Conclusion

Urine absorbance in the region of 2130–2136 nm showed an availability for monitoring urea nitrogen levels in blood. Observation from four cows indicated that there were individual variations between the animals in their urine spectra to pronounce BUN. However, in comparison with other similar studies, this method showed the possibilities either for individual or groups measurement.

Acknowledgments

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