Non-destructive determination of bovine milk progesterone concentration during milking using near-infrared spectroscopy

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Abstract： In the current dairy industry, an intensive demand for estrus detection and early diagnosis of pregnancy has been increasing. Progesterone concentration in bovine milk is used as an important indicator of estrus detection and early pregnancy diagnosis. Current method for milk progesterone determination is destructive in nature. In contrast, near-infrared spectroscopy (NIRS) is a non-destructive analytical method that can be used for milk quality determination but there has been limited study on using NIRS for milk progesterone concentration during milking. Thus, the objective of this study was to develop an online real-time NIR spectroscopic sensing system for milk progesterone determination during milking by using a specific enzyme immunosorbent assay as a reference (chemical) method. Milk spectra with a wavelength range of 700 to 1050 nm and milk samples were collected every 20 s during milking from four lactating Holstein cows for 28 days using the NIR spectroscopic sensing system. Calibration models were developed using partial least squares analytical method and the precision and accuracy of the models was validated. Milk progesterone concentration for each milking was calculated by taking the progesterone concentration of the milk predicted values and milk yield obtained every 20 s, and was compared with the milk progesterone concentration chemical analysis value for one milking (bucket milk). The results obtained show that one milking time measurement accuracy of progesterone concentration was reasonably good. By installing the NIR spectroscopic sensing system developed in this study into a milking robot, it could predict milk progesterone concentration for one milking with almost the same accuracy as chemical analysis. Thus, recording this predicted value every milking and monitoring the continuous transition of the milk progesterone concentrations, it becomes possible to use it for the detection of estrus status and for the diagnosis of pregnancy of each cow.

Keywords: bovine milk, progesterone, estrus status, pregnancy diagnosis, trend monitoring, near-infrared spectroscopy, milking robot

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1 Introduction

Progesterone is a steroid hormone that is secreted in milk by the female mammals. This kind of hormone is produced in the corpus luteum and placenta and its plays an important physiological role in the luteal phase of the menstrual cycle and in the maintenance of pregnancy. Its concentration in milk has a characteristic variation along the estrus cycle and so progesterone is accepted as an ideal

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indicator to control cow reproductive status, to detect the animal heat and for the diagnosis of cows' pregnancy (Käppel et al., 2007; Posthuma-Trumpie et al., 2009). Usually, the onset of the heat is indicated by a rapid fall in the concentration of progesterone in milk to below 2-5 ng mL^{-1}, hence, once the cow is pregnant the progesterone concentration remains high and constant (Posthuma-Trumpie et al., 2009). Accurate estrus detection is crucial for timed and successful artificial insemination and early detection of the pregnancy. An early detection of a failed insemination is critical for maximizing reproductive efficiency, as it could allow a meaningful elapsed time delay before a new repeated insemination.

Furthermore, the poor ability to detect female animals in heat results in longer calving intervals and lowers milk production (Posthuma-Trumpie et al., 2009). Due to those reasons, reliable analytical methods to detect timely and accurately the occurrence of estrus cycle and other reproductive states are needed (Friggens and Chagunda, 2005). Several methods, including measurement of milk temperature and radio-telemetric measurement of vaginal temperature have been used for estrus prediction. However, the most effective and reliable method for the purpose is the direct determination of the level of progesterone in plasma or bovine milk (Simersky et al., 2007; Tolleson et al., 2003). Different methods have been developed for accurate determination of progesterone in milk, including strategies based on thin layer, gas or liquid chromatography coupled to mass spectrometry detection (Díaz-Cruz et al., 2003) but such techniques are limited by several drawbacks including substantial equipment costs and/or extensive and time-consuming sample pre-treatments, rendering progesterone routine determination an expensive analysis. Alternatively, immunochemical assays are the most popular approach nowadays for the determination of progesterone (Gillis et al., 2006).

Similarly, near-infrared spectroscopy (NIRS) methods have been historically very successful at evaluating the quality of agricultural commodities, especially food such as rice, wheat and milk (Sato et al., 1987; Tsenkova et al., 2001; Natsuga et al., 2006; Kawamura et al., 2007; Kawasaki et al., 2008 and Iweka et al., 2016). These NIRS approaches are most preferable for analysis of food components because they are rapid, non-destructive, usually require little or no sample preparation, pretreatment free, have the potential to run multiple tests on a single sample and good for on-line analysis (Nawrocka and Lamorsk, 2013). Garnsworthy and Mann (2001) have reported the novel approach to online milk progesterone level using NIRS. NIRS has also been used for early pregnancy detection and gender of hair sheep in the tropics using near-infrared (NIR) reflectance spectroscopy in feces (Andueza et al., 2014). However, there has been limited study on using NIRS for online real-time determination of progesterone concentration in bovine milk during milking.

For this reason, the objective of this study was to develop an online real-time NIR spectroscopic sensing system for bovine milk progesterone determination during milking. The success of using NIR spectroscopic sensing system for bovine milk progesterone determination could guarantee non-destructive, accurate estrus detection, timely and successful artificial insemination, and early pregnancy detection.

2 Materials and methods

2.1 NIR Spectroscopic sensing system

An empirical online NIR spectroscopic sensing system was designed for analyzing milk progesterone concentration of each cow during milking. The system consisted of an NIR spectrum sensor, NIR spectrometer, milk flow meter, milk sampler and a laptop computer (Figures 1 and 2). The system was fixed between a teatcup cluster and a milk bucket of the milking system. Non-homogenized milk from the teatcup cluster flowed continuously across a bypass into the milk chamber of the NIR spectrum sensor. Excess raw milk flowed past the milk flow meter and was then released through a line tube into the milk bucket (Figure 2). The NIR spectrum sensor consisted of three Halogen lamps namely; halogen lamps A, B and C. The optical axes of halogen lamps A and B and the optical fiber were set at the same level, but the optical axis for halogen lamp C was set at 5 mm higher than the optical fiber (Figure 3). The volume of milk sample in the milk chamber was approximately 30 mL. The spectrum sensor acquired absorbance spectra through the milk. Spectra were obtained in the wavelength range of 700 nm to 1050 nm at 1 nm intervals every 20 s during milking (Table 1). The milk flow rate was simultaneously recorded.

2.2 Cow and milk samples

In this study, milk samples were obtained from four Holstein cows at the Experimental Farm of Field Science of Northern Biosphere, Hokkaido University, Japan. The cows (cow number 1221, 1239, 1250 and 1263) were used in the experiment during different lactation periods (Table 2). Cow number 1263 was treated by prostaglandin to promote estrus artificially. Measurements were performed in two consecutive milkings, milking in the evening and milking the following morning from July to August 2016. This experiment was carried out for 28 days because it covers the beginning of one period of estrus (period of sexual receptivity and fertility) to the beginning of the next period of estrus, which is known as 21 days of estrus cycle. The ovulation date was determined using rectal palpation method by a veterinarian. A pipeline milking system was used for milking the cows. Two cows were milked at the same milking time and each cow was examined for about 23 milking times. Milk spectra data were recorded and then milk samples were collected from the milk sampler every 20 s during milking. The milk samples obtained were then stored in a freezer at a temperature of -80oC and were later thawed and used for progesterone chemical determination. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times and lactation stage.

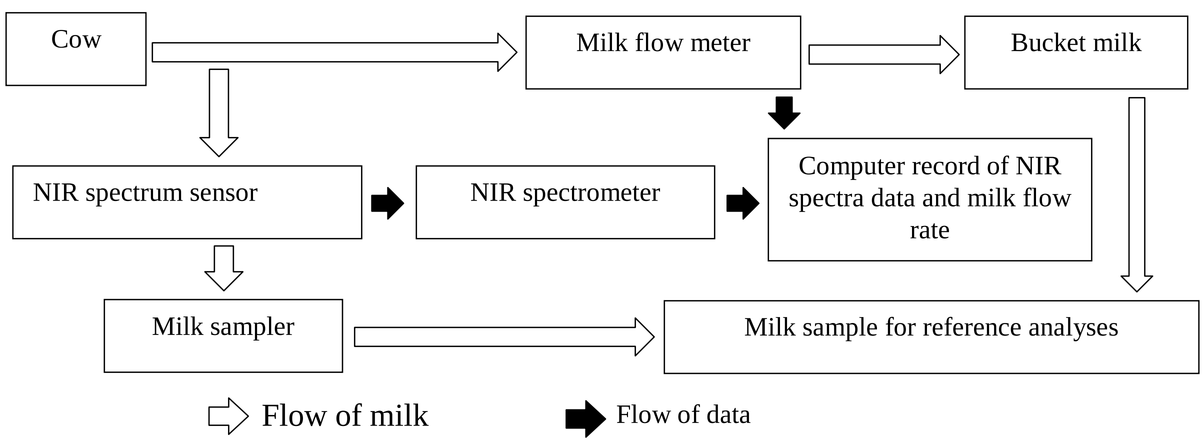

Figure 1 Flow chart of an on-line near-infrared spectroscopic sensing system for determining milk progesterone concentration during milking

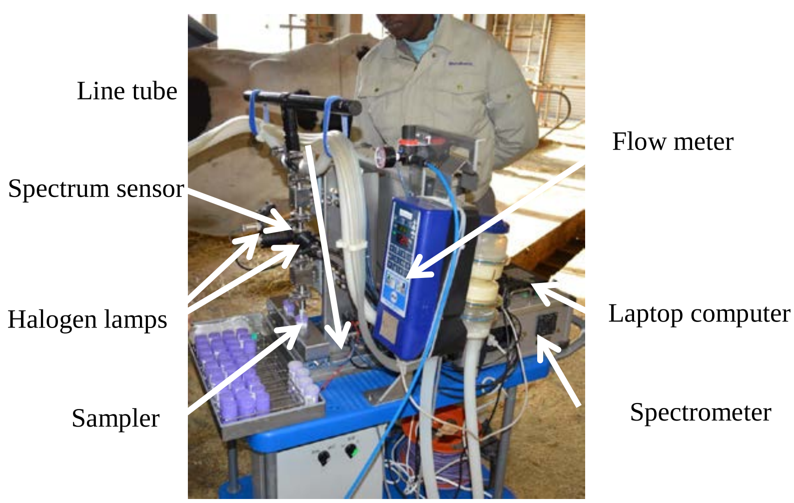

Figure 2 Near-infrared spectroscopic sensing system

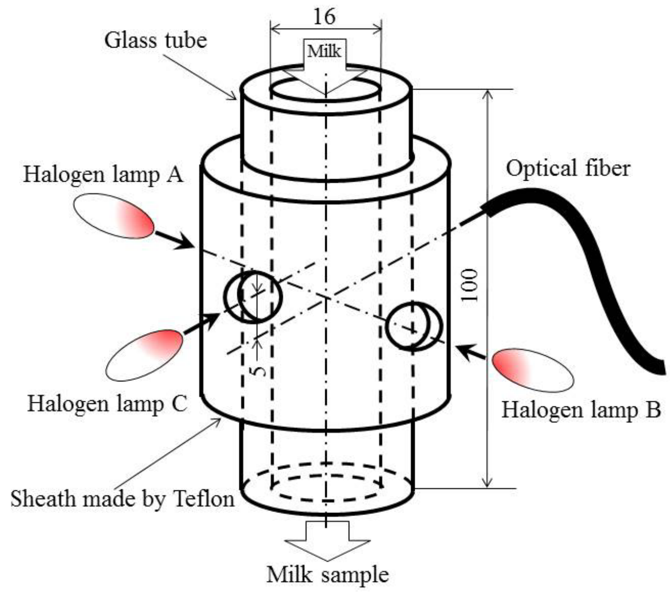

Figure 3 Schematic of the optical system of milk chamber of the near-infrared spectrum sensor

Table 1 Specifications of the near-infrared spectroscopic instrument

Devices	Specifications
NIR spectrum sensor	Absorbance spectrum sensor
Light source	Three halogen lamps
Optical fiber	Quartz Fiber
Milk chamber surface	Glass
Volume of milk sample	Approx. 30 mL
Distance between optical axis and milk level	55 mm
NIR spectrometer	Diffraction grating spectrometer
Optical density	Absorbance
Wavelength range	700–1050 nm, 1 nm internal
Wavelength resolution	Approx. 6.4 nm
Photocell	CMOS linear array, 512 pixels
Thermal controller	Heater and cooling fan
Data processing computer	Windows 7
A/D converter	16 bit
Spectrum data acquisition	Every 20 s

Table 2 Information on cows used in the experiment

Cow number	Date of birth	Date of latest calving	Calving times	Ovulation date during experiment
1221	Oct. 22, 2006	Mar. 31, 2016	6	Jul. 24, 2016 and Aug. 15, 2016
1239	Nov. 25, 2008	Nov. 22, 2015	5	Aug. 12-13, 2016
1250	Jul. 05, 2012	May 15, 2016	2	Aug. 4, 2016
1263	Mar. 02, 2014	May 08, 2016	1	Aug. 1, 2016

2.3 Hormone assay for reference (chemical) analyses

Progesterone concentration in bovine milk was evaluated using competitive double-antibody enzyme immunoassays (EIA) according to the method described by Yanagawa et al. (2015) modified to bovine milk. The primary antisera used assay were rabbit anti-progesterone-3-CMO-BSA serum (KZ-HS-P13, Cosmo Bio, Tokyo, Japan). Goat anti-rabbit IgG antiserum (111-005-003, Jackson ImmunoResearch, PA, USA) was used as the secondary antiserum. All samples were assayed in duplicates. The intra- and inter-assay coefficients of variation were 2.2% and 3.3%, respectively. The concentration of progesterone under 0.1 ng mL^{-1} was regarded as 0.1 ng mL^{-1} in this assay. The results obtained were used as reference (chemical) data for chemometric analyses.

2.4 Chemometric analyses

Chemometric analyses were carried out to develop calibration models for progesterone concentration of each cow and to validate the precision and accuracy of the models. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. One data set was obtained for each cow from the experiment conducted using four cows. Full cross validation method was used to validate the calibration models. The data set for each cow was used to develop calibration models and the same data set was used for validation of the calibration models. The best model was obtained when we used the original milk spectra data. Thus, pretreatment techniques such as multiplicative scatter correction, 2nd derivative and smoothing were not used.

2.5 Progesterone concentration at one milking time

Bucket milk progesterone concentration at one milking time was calculated by taking NIR-predicted progesterone value and milk flow rate obtained every 20 s and then compared it with the reference chemical values of bulk milk progesterone per each milking time.

3 Results and discussion

3.1 Near-infrared spectra

An example of original NIR spectra of raw milk at one milking time is shown in Figure 4. The NIR spectra showed two bands peaks at around 740 nm and 840 nm indicating the overtone absorptions by C-H strings and C-C strings that are related to the distinctive absorption bands of milk constituents such as fat, protein, lactose and progesterone. There was a strong absorption peak of O-H functional group in water such that band around 960 nm were prominent spectra.

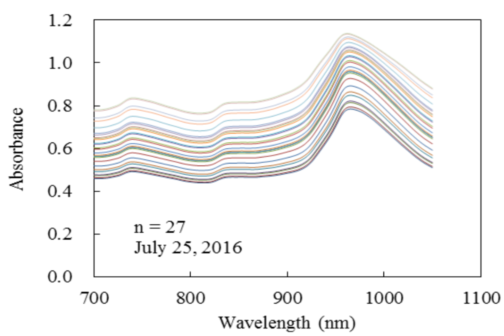

Figure 4 Typical spectra of non-homogenized milk from cow number 1221 during milking

3.2 Precision and accuracy of calibration models

The validation statistics of the NIR sensing system for the determination of progesterone concentration at every 20 s during milking for each of four cows are shown in Table 3.

Progesterone is a steroid hormone that is produced in bovine milk. It plays vital role in cow’s reproductive cycle such that it prepares the uterus for pregnancy and so progesterone has been globally accepted as the ideal cow reproductive status indicator. The coefficient of determination (r^2), standard error of prediction (SEP) and bias of the validation set for progesterone for cow number 1221, 1239, 1250 and 1263 were 0.73, 1.12 ng mL^{-1} and 0.05 ng mL; 0.62, 1.78 ng mL^{-1} and 0.02 ng mL^{-1}; 0.64, 1.64 ng mL^{-1} and -0.04 ng mL^{-1}; and 0.60, 3.05 ng mL^{-1} and -0.02 ng mL^{-1} respectively. The r^2 values obtained for each cow showed a good fit with the points lying close to the straight line and the SEP values were considered to be sufficiently low. These validation statistics indicated that this calibration models were much more accurate compared to that reported by Garnsworthy and Mann (2001). These results obtained indicate that there were sufficient levels of precision and accuracy for predicting the progesterone concentration of each cow. Thus, these values obtained by NIR sensing system for progesterone concentration indicate the ability of the calibration models to predict cow ovulation (Table 3).

3.3 Progesterone concentration at one milking time

The validation statistics of the NIR sensing system for determination of progesterone concentration at one milking time of each cow are summarized in Table 4. The r^2, SEP and bias of the validation set for progesterone for cow number 1221, 1239, 1250 and 1263 were 0.70, 1.35 ng mL^{-1} and -0.30 ng mL^{-1}; 0.69, 1.85 ng mL^{-1} and -0.13 ng mL^{-1}; 0.58, 1.94 ng mL^{-1} and -0.39 ng mL^{-1} and 0.70, 2.76 ng mL^{-1} and -0.38 ng mL^{-1} respectively. This validation statistic showed that these calibration models were sufficiently accurate for predicting milk progesterone concentration at one milking time.

The precision and accuracy of progesterone concentration at every 20 s during milking and at one milking time were almost the same. In other words, we were able to determine progesterone concentration at each milking time with almost the same accuracy as the predicted progesterone concentration in real-time milking. This means, by taking records of this predicted progesterone value at every milking time and monitoring the continuous changes in progesterone concentration, it is possible to predict each cow ovulation status and diagnose the early pregnancy of each cow.

Table 3 Validation statistics of near-infrared sensing system for the determination of progesterone concentration at every 20 s during milking

Cow number	n	Range (ng mL^{-1})	r²	SEP (ng mL^{-1})	Bias (ng mL^{-1})	Regression line
1221	194	0.10 - 9.07	0.73	1.12	0.05	y = 0.99 x - 0.03
1239	172	0.10 - 13.42	0.62	1.78	0.02	y = 0.97 x + 0.08
1250	212	0.10 - 11.33	0.64	1.64	-0.04	y = 0.99 x + 0.07
1263	178	0.10 - 17.74	0.60	3.05	-0.02	y = 0.96 x + 0.21

Note: n: number of validation samples. r^2: coefficient of determination.

SEP: standard error of prediction.

Regression line: Regression line from predicted value (x) to reference value (y).

Table 4 Validation statistics of near-infrared sensing system for the determination of progesterone concentration at one milking time

Cow number	n	Range (ng mL^{-1})	r²	SEP (ng mL^{-1})	Bias (ng mL^{-1})	Regression line
1221	22	0.10 - 8.67	0.70	1.35	-0.30	y = 1.01 x + 0.28
1239	22	0.10 - 12.00	0.69	1.85	-0.13	y = 1.14 x - 0.38
1250	18	0.10 - 10.56	0.58	1.94	-0.39	y = 1.09 x + 0.13
1263	23	0.10 - 16.28	0.70	2.76	-0.38	y = 1.09x - 0.07

Note: n: number of validation samples. r^2: coefficient of determination.

SEP: standard error of prediction.

Regression line: Regression line from predicted value (x) to reference value (y).

3.4 Comparison of chemical and NIR-predicted progesterone concentration trend

The calculated results by the calibration models having negative NIR-predicted values were changed to zero since progesterone values are always positive values. Figures 5 and 6 show chemical and NIR-predicted milk progesterone concentration trend for cow 1221. Figures 7 and 8 show the same for cow 1239.

The changes of (trend in) the chemical and NIR-predicted progesterone were almost the same for each cow. There were similar chemical and NIR-predicted progesterone concentration reductions on the day of ovulation (Jul. 24 and Aug. 15, 2016; Aug. 12-13, 2016) for cow number 1221 and 1239 respectively. The similarity of chemical and NIR-predicted progesterone concentration trend was good for each cow. The trend monitoring of progesterone concentration of each cow was much better than that previously reported by Garnsworthy and Mann (2001). These results suggested that NIR spectroscopic sensing system developed in this study could be used for online real-time monitoring of milk progesterone concentration of each cow during milking.

The results in this study are important because for the first time, an online real-time NIR spectroscopic sensing system could predict progesterone concentration (cow ovulation) of each cow during milking. Thus, by installing the NIR spectroscopic sensing system developed in this study into a milking robot, it could monitor milk progesterone concentration every day.

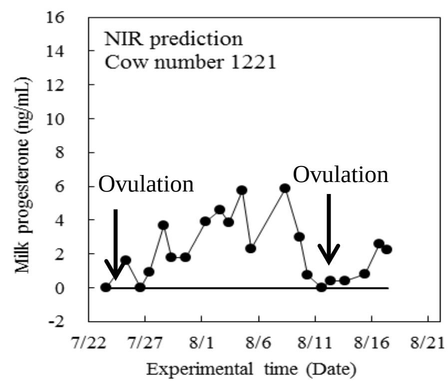

Figure 5 Milk progesterone trend for cow number 1221 by chemical analysis Figure

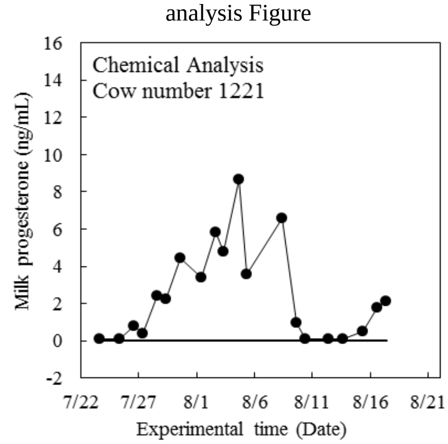

Figure 6 Milk progesterone trend for cow number 1221 by NIR sensing system

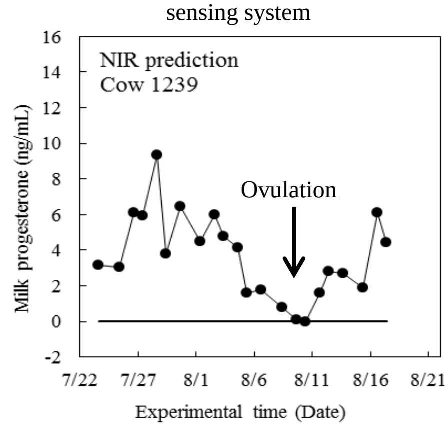

Figure 7 Milk progesterone trend for cow number 1239 by chemical analysis

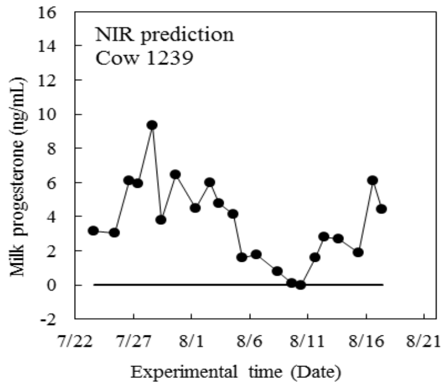

Figure 8 Milk progesterone trend for cow number 1239 by NIR sensing system

4 Conclusions

This study suggested that the NIRS sensing system developed has the potentials to predict milk progesterone concentration of each cow during milking. The results obtained in this study could provide screening capability for progesterone determination in bovine milk. Thus, the results obtained show the possibility of using NIRS methodology for the determination of milk progesterone concentration during milking.

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