Detection of Estrus in Dairy Cows by Means of Near Infrared Spectroscopy and Aquaphotomics

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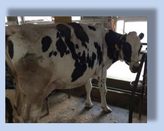
Introduction

Â Background

Reproduction performance of cows has declined worldwide
⇒ Economical loss to dairy farming

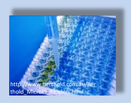
Accurate detection of estrus is a basic requirement for an effective management program




Â Conventional estrus detection methods

V **Visual observation** [1]
Non-effective, time & labor-consuming, requiring good knowledge

V **Sex hormones measurement** [2]
(e.g. RIA or EIA)
Accurate, time & labor-consuming, producing chemical waste



Â To develop a new method

V **NIRS**
Rapid, easy, chemical free

V **Aquaphotomics** [3]
Extract biosystem information from water spectral dynamics
⇒Detection of estrus in Giant panda [4,5]

É Objective of this study

To develop a method to detect estrus of dairy cows by means of NIRS and Aquaphotomics

Materials & Methods

Â Cows

Number of cow : 7 cows (Cow A - F & G (control))
Number of lactation : cow A, G (3), D, E, F (2), B, C (1)
V Estrus was induced by hormone treatment in Cow A - F
V Cow G got no hormone treatment and was not in estrus

Â Spectra Measurement

Spectrometer : XDS (FOSS)
Mode : transmittance
Temperature : serum 30 ℃, milk & foremilk 40 ℃
Technical Path length : 1 mm
Wavelength range : 400 - 2499.5 nm (0.5 nm step)
Consecutive scans : serum 6, milk & foremilk 3 / sample

Â Samples

Sample types : Serum, foremilk (am / pm), and raw milk (am / pm)
V Estrus and ovulation was synchronized at the middle of the sampling period

Â Hormone Measurement ; Enzyme Immunoassay (EIA)

Micro plate reader : iMark (Bio Rad)
Sample : serum
Hormone : **progesterone (P_4) (ng/mL)**
V Cow shows estrus ca. 72 hours after sharp decrease of P_4 concentration from high level to low one

Â Aquagram

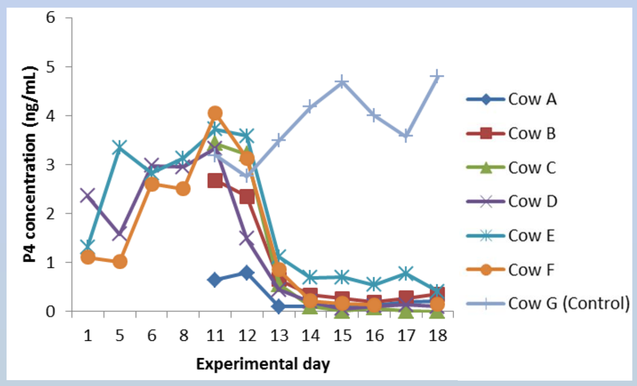
V Star chart to visualize the relationship among the groups of spectra of samples with high P_4 and low P_4 at characteristic wavelengths
V Formula to calculate absorbance at specific wavelengths in the aquagram

$$\text{öή''}\Omega_{ف} \text{ سي } \frac{\omega_{ف} \text{ طي } \overline{\omega_{ف}}}{\text{„}_{ف}}$$

öή''Ω$_{ف}$: Aquagram value at λ nm
ω$_{ف}$: Absorbance of a sample at λ nm
$\overline{\omega_{ف}}$: Absorbance average of a group at λ nm
„$_{ف}$: Absorbance standard deviation of a group at λ nm
(Transform : MSC (1300-1600 nm))

Results & Discussion

Â P_4 movements



V Serum samples were obtained on day 1, 5, 6, 8 and 11 - 18
V P_4 changed according to hormone treatment

Â Important bands of hormone investigation

V Analysis were done in 1300 – 1600 nm spectral range (water 1st overtone range)
V Cows and sample kinds (serum, foremilk am / pm, milk am / pm) were applied separately

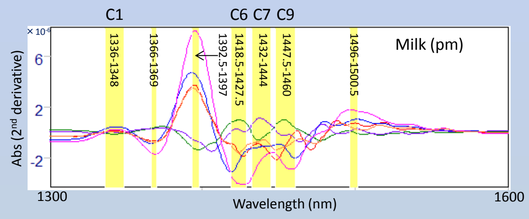
ü Basic bands

Bands proposed by R. Tsenkova [3,4,5]

C1 : 1336-1348 nm
C2 : 1360-1366
C3 : 1370-1376
C4 : 1380-1388
C5 : 1398-1418
C6 : 1421-1430
C7 : 1432-1444
C8 : 1448-1454
C9 : 1458-1468
C10 : 1472-1482
C11 : 1482-1495
C12 : 1506-1516

ü 2nd derivative Subtracted spectra

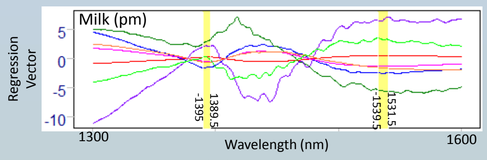
(High P_4 period averaged spectrum) – (Low P_4 period averaged spectrum)
High P_4 period : day 9 - 12
Low P_4 day 13 - 16
Transform :
Before subtraction : SNV, Smooth (25 points)
After subtraction : 2nd derivative (45 points)



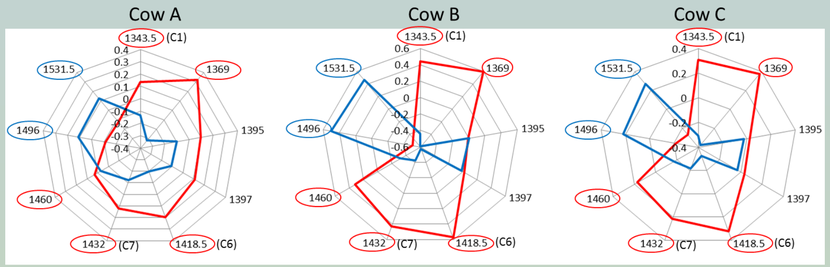
ü Regression Vector

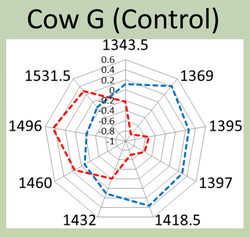
Regression vector of PLS regression (objective variable : P_4 concentration (ng/ml))

Transform : orthogonal signal correction
Smooth (25 points)
SNV (milk, foremilk)
2nd derivative (serum (cow A 25 points, other cows 55 points))



Milk (pm) Specific pattern





High P_4 period
Control
Low P_4 period
Control
Specific wavelength of high P_4 period
Specific wavelength of low P_4 period

V In case of milk (pm), all treated cows showed similar specific spectral pattern and the control didn't show the same pattern

V In all types of samples, more than half the numbers of the cows showed similar specific patterns

WAMACS [4]	Wavelength (nm)	High P_4 period	Low P_4 period	Sample type
C1	1342 – 1344	+		F (am / pm), M (am / pm)
	1348	+		S
	1366 – 1369	+		M (am / pm)
	1378 – 1380	+		S
C4	1388		+	F (am)
	1395.5		+	F (pm)
C5	1411.5	+		S
	1418.5 – 1421	+		F (pm), M (am / pm), S
C7	1432	+		All
C8	1454	+		F (am / pm), M (am), S
C9	1460 – 1465.5	+		F (am / pm), M (am / pm)
C10	1472	+		F (am / pm)
C11	1495 – 1497		+	F (pm), M (pm), S
C12	1506 – 1510.5		+	F (am / pm), M (am), S
	1515 – 1516		+	M (am), S
	1531.5		+	M (pm)
	1538		+	F (pm)

F : Foremilk, M : milk, S : Serum

1342-1472 nm (except for 1388, 1395.5 nm) : $S_0 \sim S_3$ [4]
1388, 1395.5 nm : v_1, v_1 and v_2
After 1495 nm : S_4 and v_1, v_2 [4]

If the two periods are compared, then in low P_4 period (estrus) the bands of strongly H-bonded water structures are dominant and signals of weakly H-bonded structures decrease.

Conclusion

This work showed that
- dairy cow's progesterone level can be monitored by milk NIR spectra based on Aquaphotomics.
- evaluating the changes of water molecular structures when progesterone concentration changes is an effective tool for estrus detection.

References[1] Dochi, O., Kabeya, S., Koyama, H. (2010), *Journal of Reproduction and Development* **56**: 561-565 [2] Saint-Dizier, M., Chastant-Maillard, S. (2012), *Reproduction in Domestic Animals* **47**: 1056-1061 [3] Tsenkova, R. (2009), *Journal of Near Infrared Spectroscopy* **17**: 303-314. [4] Kinoshita, K., Miyazaki, M., Morita, H., Vassileva, M., Tang, C., Li, D., Ishikawa, O., Kusunoki, H., Tsenkova, R. (2012) , *Scientific Reports* **2**: 856; doi:10.1038/srep00856 [5] Kinoshita, K., Morita, H., Miyazaki, M., Hama, N., Kanemitsu, H., Kawakami, H., Wang, P., Ishikawa, O., Kusunoki, H.,Tsenkova, R. (2010): *Analytical Methods*, **2**: 1671-1675.
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