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An attempt to detect oestrus from changes in Fourier transform infrared spectra of milk from dairy heifers

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Abstract

This study was carried out to investigate if there were systematic changes in milk Fourier transform infrared (FT-IR) spectra relative to stage of the oestrous cycle in cattle. Oestrous cycles of 22 lactating heifers were synchronized with two injections of prostaglandin F2α (PGF) administered 11 days apart. The heifers were milked twice daily, and milk samples were collected from each heifer at each milking for a period of 70 days, starting on the day of the second PGF injection. Oestrus was diagnosed by visual detection in conjunction with monitoring rectal temperature. Milk samples were analyzed by FT-IR spectroscopy and the spectra data were analyzed using partial least squares (PLS) methods in relation to time of observed oestrus in heifers. In this investigation, it was not possible to identify reliable changes in milk FT-IR spectra in relation to oestrus on a single heifer basis, though there was a weak correlation between FT-IR spectra and expected time of oestrus when the analysis was carried out across all the heifers.

Keywords: Cattle oestrus; Oestrus detection; Fourier transform infared spectra

1. Introduction

Detection of oestrus can be a problem in cows. It is compounded by the fact that the duration of oestrus may be as short as 6 h, and that successful insemination is partly dependent on timing relative to oestrus (Nebel et al., 2000). Progesterone levels are very low (<1 ng/ml) immediately around oestrus relative to levels during the rest of the oestrous cycle. Its measurement in milk provides an indicator of the peri-oestrus period, and can assist the timing of artificial insemination (Abeyawardene et al., 1984). Whilst milk is conveniently sampled, progesterone requires specialized assay procedures. Recent advances in the technology of infrared spectrophotometers routinely used to measure milk composition, now allow the full spectrum to be measured and analyzed. Using

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Fourier transform infrared (FT-IR) analysis, it has been possible to develop calibrations for components such as acetone which are present in milk in concentrations of approximately 1 mM (Hansen, 1999). The use of multivariate methods for data analyses has facilitated the extraction of minor features from the spectra, so that correlations between the milk components can be derived. These methods include principal component analyses (PCA) and partial least squares (PLS) regression (Martens and Næs, 1992). If a further dimension is introduced into the FT-IR data (e.g. by collecting spectra as a function of time), higher order methods, such as parallel factor (PARAFAC) analysis (Bro, 1997), are useful, since they can be used to deduct common time profiles in the analyzed data.

The detection limits of FT-IR are not likely to allow direct measurement of progesterone (milk progesterone normal range: 0–100 nM), but do present the possibility of detecting systematic changes in the micro-composition of milk correlated with oestrus. As there is little information on whether such measurable changes occur, the objective of this investigation was to examine whether oestrus caused detectable and reliable changes in milk FT-IR spectra, and if oestrus could be detected from milk samples using FT-IR spectroscopy.

2. Materials and methods

2.1. Animals and management

The 22 Holstein heifers used in this study had been calved 88 days (S.D. = 22.3) when enrolled. They had each been observed in oestrus at least once, but had not been inseminated. They were stalled in tie-stalls, and had free and continuous access to a total mixed ration based on grass silage and containing 35% concentrate in dry matter. They were milked twice daily at 5.00 h and 17.00 h throughout the investigation. Oestrous cycles were synchronized using two injections of prostaglandin (Estrumat; Coopers Pitman-Moore, Holger Danskes Vej 91, 2000 Frederiksberg, Denmark; PGF) administered 11 days apart. The experiment started on the day of the second PGF injection and continued for 70 days. This period included the first post-treatment oestrus and at least two spontaneous periods of likely oestrus.

2.2. Milk samples

A milk sample was collected at each milking from every animal using a Trutest proportional sampler, which sampled 2.2% of each animal's total yield. It was preserved with Broad Spectrum Microtabs (containing Bronopol; 2-bromo-2-nitro-1,3-propanediol; from D&F Control Systems Inc., CA, US), and stored at 4°C until analysis which were carried out within 4 days of sampling. Each sample was analyzed on a MilkoScan FT 120 (Foss Electric A/S, Slangerupgade 69, 3400 Hillerød, Denmark) which was slope and intercept adjusted against the reference methods for milk fat, protein, and lactose at the start of the experiment. This adjustment was used throughout the experiment and

the stability of the instrument was controlled by the use of pilot samples (IDF Standard 141B, 1996). The MilkoScan FT 120 was standardized once weekly during the study period.

2.3. Other measures

Milk yield was recorded at each milking. Heifers were observed for signs of oestrus three times daily in the period from 3 days before an expected oestrus up to and including the day after oestrus was detected. Oestrus was observed by increased stringy mucus secretion from vagina together with observation of hyperemia of the vulva (Peters and Ball, 1987), increased restlessness (observation of increased positional changes and stepping), and lowing (Peters and Ball, 1987) as well as reduced milk yield. Rectal temperature was measured twice daily at milking from 3 days before an expected oestrus and until 3 days after observed oestrus. Environmental temperature (T_e) was recorded continuously for the whole period. Any disease incidence or veterinary treatment carried out during the investigation was registered. Body weight was measured at the start and at the end of the experiment (509 ± 39 and 542 ± 37 kg, respectively).

2.4. Statistical analyses

A number of statistical methods were used to determine relationships between FT-IR spectra data and observed or expected oestrus. PCA and PLS regression were carried out to reveal any correlation between observed time of oestrus and the spectral data. PARAFAC analysis were used to detect any systematic changes in total milk composition which occurred at repeated time intervals. PARAFAC analyses create factors which are orthogonal statistical constructs that account for successive portions of the time-related variation. The analyses were performed in three stages. First, the spectra arising from the milk samples were analyzed independently of the non-milk oestrus detection measures. The across cow, combined PARAFAC profile was obtained from 44 individual profiles across days (22 from morning and 22 from afternoon milkings). Secondly, the across cow analysis was repeated including the day of oestrus. The day of oestrus was defined on the basis of visual detection in combination with changes in the adjusted rectal temperature. Days when oestrus was clearly shown by changes in vaginal mucus and at least one other indicator were designated potential oestrus days. If there was only one potential oestrus day within the time window of expected oestrus, then that day was designated as the day of oestrus. This occurred in 52% of the cases. When there were two consecutive potential oestrus days (33% of the cases), the day associated with an elevation in rectal temperature was designated the day of oestrus. In 15% of the cases, there were two consecutive potential oestrus days, but no marked elevation in temperature. In those cases, the first day was designated the day of oestrus. There was only one case where no visual signs of oestrus were observed, in this case there was a well-defined increase in rectal temperature. Because rectal temperature was found to be influenced by environmental temperature (T_e), adjusted rectal temperature was derived as the residuals from regression of rectal temperature on T_e and $(T_e)^2$. Thirdly, the PARAFAC factors were used on the individual milk profiles of each heifer, and the resulting cycles compared with the observed oestrus.

3. Results and discussion

Of 3080 milk samples, 11 were missing and 6 excluded from the FT-IR spectra data set as extreme outliers on the basis of a PCA of the data.

Changes in the main components of milk (fat, protein, and lactose) relative to days from the first spontaneous oestrus are shown in Fig. 1. Over the whole experiment, there were no significant relations to time of expected oestrus, although there tended ($P < 0.1$) to be a small depression in milk lactose and milk yield just before expected oestrus.

To account for possible differences in time of visual and spectral signs of oestrus, changes in spectra were looked for at the day of observed oestrus and three days before and after oestrus. PCA was carried out on spectral data for all heifers together or for the individual heifers, and no changes in spectral data were revealed neither on the day of observed oestrus nor on the days around oestrus. A PLS regression was carried out with a variable indicating oestrus; 1 when heifers were in oestrus and 0 when not in oestrus. The PLS regression was carried out for single heifers or for all heifers together. Like the PCA, no correlation was seen using this analysis neither at the day of oestrus nor when "oestrus" was set 1, 2, or 3 days before or after observed oestrus. Furthermore, the PLS regression was used on heifers to try to determine pregnancy, but still no correlations were detected.

A PARAFAC analysis using the time-dependent spectral data from all 22 heifers, but not including any other measures, was carried out. The PARAFAC model described 99.87% of the variations in the data on the basis of two factors. These factors are orthogonal (statistical) constructs to describe the time series. This revealed a factor with a weak systematic variation with minima at day 1, 21–23, 39–41, and 61–62, and with maxima at day 8, 33, and 51 (Fig. 2). Observed oestrus was, on average, on days 3, 24, and 44 (S.D. 0.7, 1.2, and 1.7 days, respectively). The minima and maxima had a tendency to become weaker as time progressed. This would be expected if the minima and maxima were related to oestrus, as heifers were less synchronized with time. When rectal temperatures (Fig. 3) and observed day of oestrus were included in the PARAFAC analysis, no improvement in the fit was seen. Whether the systematic variation is truly caused by oestrus is not clear. However, when the PARAFAC factors calculated from the combined data were applied to the milk data from the individual heifers, the predicted minimum values were variable among heifers. For seven heifers, no minima could be identified. There was no significant relationship between observed oestrus and individually-derived minima in the PARAFAC analysis (Fig. 4). The difference between the individual minima and the combined minima in the PARAFAC analysis clearly suggests that the cyclicity associated with oestrus is of low amplitude relative to the interferences from other random effectors. As such, it is only when data are combined across animals that a stable pattern emerges. In this study, the individually-derived minima had little predictive value for determining day of oestrus. Any effect of oestrus on FT-IR spectra was small relative to within cow, day-to-day variation in these spectra. This variation was not reduced by accounting for day-to-day variation in milk yield and milk composition.

An alternative explanation for not finding a significant relationship between observed oestrus and the individual minima of the PARAFAC analysis is that detection of oestrus was not sufficiently precise. In the absence of progesterone analyses, this explanation cannot be discounted. However, we do not consider precision of oestrus detection to have been a problem in this study. Changes in milk composition (Fig. 1) were similar to those reported

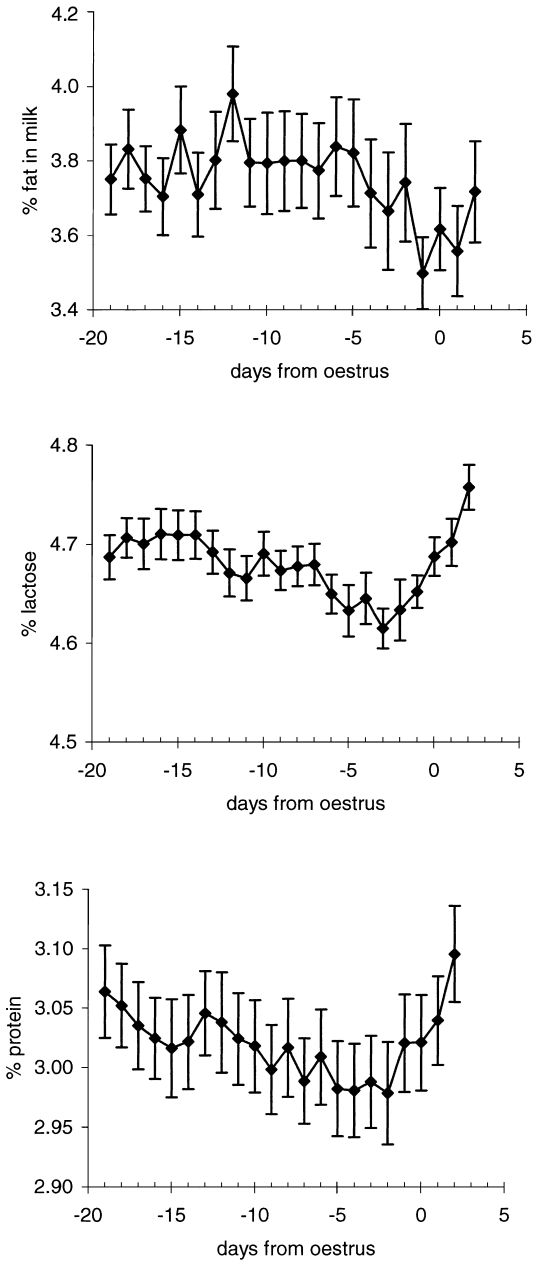

Fig. 1. The changes in milk composition (average ± S.E.) relative to days around oestrus.

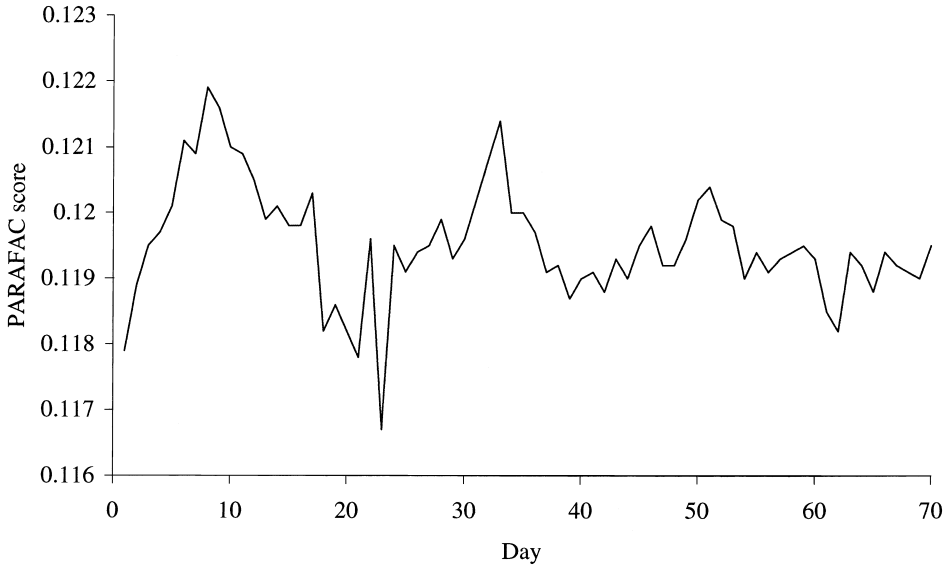

Fig. 2. A PARAFAC profile factor showing the daily variation in milk spectral data with minima at day 1, 21–23, 39–41, and 61–62, and with maxima at day 8, 33, and 51.

by Peters and Ball (1987). Changes in rectal temperature (Fig. 3) were comparable to the changes in vaginal temperature reported by Gil et al. (1997). Although, it is of greater practical convenience, there was more variation from observation-to-observation in rectal temperature than there would appear to be for vaginal temperature (Gil et al., 1997). The success rate for visual detection alone is substantially improved when cows are housed in tie-stalls where close examination of the vaginal opening is possible. In the present study,

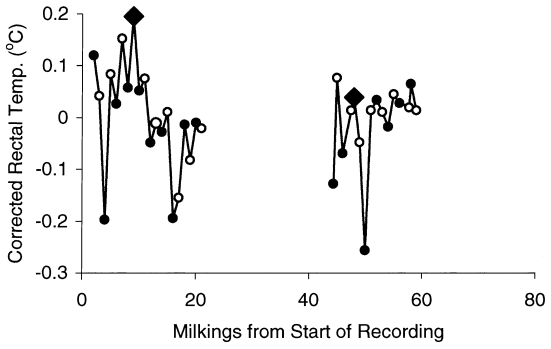

Fig. 3. Two examples of rectal temperature changes relative to observed oestrus (◆): a.m. measurements (○); p.m. measurements (●). Rectal temperatures are given as within cow residuals from the equation describing systematic changes in rectal temperature (RT) as a function of environmental temperature (T_e): $RT = 2.15 - 0.0784T_e + 0.000415(T_e)^2$.

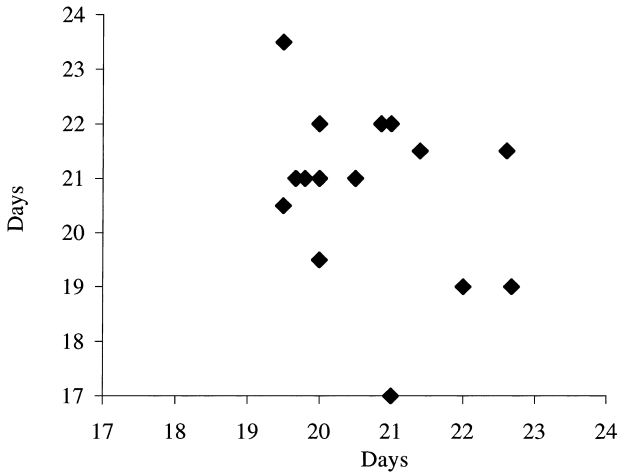

Fig. 4. Relationship between oestrous cycle length determined from milk spectra (*y*-axis) and observed oestrous cycle length (*x*-axis). The correlation coefficient was −0.336 ($P = 0.282$). It was not possible to identify cycle length from milk spectra for seven of the heifers; these are not shown.

using tie-stalls, visual signs of oestrus were seen in 65 out of 66 cases. Further, day of oestrus was designated on the basis of combining visual signs, changes in milk, and changes in rectal temperature. It has been shown that combining such indicators can give oestrus detection percentages (relative to progesterone) of 93% (Rossing and Spahr, 1992). Thus, we have no reason to believe that the precision of oestrus detection had any significant bearing on the relationship between milk FT-IR spectra and oestrus found in this study.

4. Conclusion

Whilst there were systematic changes in milk FT-IR spectra, which coincided with changes in the oestrus cycle of a group of oestrus synchronized heifers, it would appear that such changes in milk FT-IR spectra are not strongly enough related to changes in the oestrous cycle to allow individual detection of oestrus at the present time.

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