VOLUME 11 NUMBER 1–2 2001

ISSN 0958-6946

INTERNATIONAL DAIRY JOURNAL

Incorporating

NETHERLANDS MILK AND DAIRY JOURNAL

ELSEVIER

00400-00024645-6

Number 7

Number 8

ELSEVIER

International Dairy Journal 9 (1999) 1

INTERNATIONAL DAIRY JOURNAL

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Editorial

This special issue of the International Dairy Journal contains the invited papers of two sessions from the Ninth European Congress on Biotechnology (ECB9) held in Brussels (Belgium) on 11–15 July 1999. The first eight lectures address scientific, technological, and regulatory problems related to the use of microorganisms, in particular lactic acid bacteria, either as starters for the manufacture of dairy products (first session), or as probiotics (second session). The last paper from the second session extends the reflection on probiotic strains by presenting recent progresses in the field of prebiotic dietary adjuncts. The common link between these various topics is that in all cases these strains or substances will come in close contact with the gastro-intestinal mucosal surface and encounter the local microflora. Therefore, their biosafety ought to be constantly kept in mind, in particular when recombinant DNA technology is being used for the construction of strains with improved technological, organoleptic or health-promoting properties. Another reason which justifies cover-to-cover publication of 'starters' and 'probiotics' papers in a journal dedicated to dairy science, is that most probiotic strains are grown in milk and marketed as 'health' or 'bio' dairy products, and therefore offer interesting perspectives to the dairy industry at large.

The session on recombinant lactic acid bacteria as dairy starters was organized as a special ECB9 event with the support of the European Commission, which also partly covered the costs of publication of this special issue. These actions are part of the communication tasks from the EU BIOTECH Demonstration Project 'Demonstration of improved dairy products through the application of starter strains of lactic acid bacteria with engineered fermentation pathways', in short GEMOLAB (grant BIO4-98-0118). GEMOLAB aims at demonstrating that present-day metabolic engineering technology can be implemented using food-grade tools and methods for the development of novel industrial dairy starters producing less acid, more flavour (diacetyl), and a natural sweet taste (alanine). It also aims at demonstrating that the engineered strains allow a better control of the process of emmental cheese production and reduce the ammonium concentration of whey. Any interested reader may contact the coordinator (J. Delcour: Tel.: +32-10-473484; fax: +32-10-473109; e-mail: delcour@gene.ucl.ac.be).

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0958-6946/99/$ - see front matter
PII: S0958-6946(99)00037-0

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International Dairy Journal 9 (1999) 447-452

INTERNATIONAL DAIRY JOURNAL

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Influence of feed source on determination of fat and protein in milk by near-infrared spectroscopy

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Received 9 September 1998; accepted 10 April 1999

Abstract

Milk samples from two experiments (n = 506 in total) were analyzed by measurement of near-infrared (NIR) spectra to investigate the effect of feed source on milk fat and protein content. Milk samples from the first experiment (n = 300) were used for development of the calibration equation and validation of the equation. The calibration equations thus developed in the first experiment were then used in the second experiment to examine the applications of NIR measurement. All feeding trials used the same basal rations, which consisted of corn silage, Italian ryegrass, Alfalfa haycube, corn flake and commercial concentrate. The first experiment was comprised of three rations: (1) basal ration, (2) basal ration with soybean meal (48% of total crude protein (CP)) and (3) basal ration with soybean meal (19% of total CP) and fish meal (25% of total CP). The second experiment was comprised of five supplement alternatives: (1) no supplement (NS), (2) corn gluten meal (CGM, 26% of total CP), (3) fish meal (FM, 26% of total CP), (4) defatted soybean meal (SBM, 28% of total CP) and (5) roasted soybean meal (RSBM, 26% of total CP). Feeding regimes in both experiments were adjusted to fulfill the maintenance and production requirements. The results showed that NIR prediction of milk fat content was not influenced by the feed of animals, while the accuracy of protein prediction was significantly affected by the kind of feedstuff used in the ration. Thus, a wide range of milk samples from cows on clearly defined feeding regimes is necessary for developing a satisfactory calibration for NIR systems.

Keywords: NIR analysis; Milk fat; Milk protein; Feed supplementation

1. Introduction

Daily monitoring of fat and protein content in milk is important in the dairy industry because the value of raw milk depends largely on these two components. Rapid instruments based on middle infrared (mid-IR) spectroscopy have been developed and are routinely and widely used to determine the composition of milk from individual cows. IR spectroscopy technology has been used also for feed composition analysis (Norris, Barnes, Moore & Shenk, 1976), using the near-infrared (NIR) region. The usefulness of NIR technology in feed quality determination has been confirmed many times (Smith & Flinn, 1991; Tremblay, Broderick & Aorams, 1996; De Boever, Cottyn, DeBrabander, Vanacker & Boucque, 1996); this technology is now employed to determine feed composition in many experimental stations in Japan. A research project on the use of NIR for monitoring feed utilization in animals from the change of rumen juice, blood composition, milk, urine and fecal composition, has been progressing in Japan. The first step was the use of NIR measurement for analysis of liquid samples, since mid-IR absorption spectroscopy is not suitable for non-destructive analysis of solid bulk materials (Frank & Birth, 1982). Sato et al. (1987) showed the suitability of NIR spectroscopy for prediction of milk composition; while Tsenkova, Yordanov, Itoh, Shinde and Nishibu (1994) reported the successful measurement of milk quality and monitoring of udder health by detection of pattern of milk spectra. These studies involved milk samples collected from the same farm feeding management. However, feeding management and feed composition may vary on different farms. Because milk composition is influenced by feed composition (DePeters & Cant, 1992; Palmquist, Beaulieu & Barbano, 1993), NIR studies should account for variability of feed composition in rations used on different farms.

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0958-6946/99/$ - see front matter
PII: S0958-6946(99)00059-3

The objective of the present study was to investigate whether different feed sources affect the accuracy of NIR spectroscopy using the multiple linear regression (MLR) method for the prediction of milk fat and protein contents.

2. Materials and methods

2.1. Milk samples

A total of 506 milk samples collected from two feeding experiments using 66 multiparous Holstein cows raised in experimental barn environment were used in this study. Animals were fed a basal ration containing corn silage, Italian ryegrass, Alfalfa haycube, corn flake and commercial concentrate twice daily. Different feeding regimes were varied by using four proportions and types of crude protein (CP) sources in the ration. Rations in both experiments were adjusted to fulfill the maintenance and production requirements level (AFFRCS, 1994). Milk samples were collected from the evening and morning milkings, and analyzed by Milkoscan (134 A/B series equipment, Foss Electric, Denmark) for determination of milk fat and protein content. The experiments were conducted as explained below.

Experiment 1. Three hundred milk samples were collected from 31 cows (average body weight: 576 kg) raised under three feeding management regimes. The rations were (1) basal ration only, (2) basal ration supplemented with soybean meal which provided 48% of total CP, and (3) basal ration supplemented with mixture of soybean (20% of total CP) and fish meal (25% of total CP). Rations 1, 2 and 3 were fed to 17, 7 and 7 cows, respectively. Milk samples from cows given ration 1 were collected in two days per week, while milk from rations 2 and 3 were collected for three days a week. Collection was carried out over a period of two weeks.

Experiment 2. This experiment was carried out using 35 cows (average body weight: 585 kg) fed five kinds of rations. Each subgroup of seven cows received supplemented ration as follows: (1) no supplementation (NS, basal ration), (2) corn gluten meal (CGM, provided 26% of total CP), (3) fish meal (FM, 26% of total CP), (4) roasted soybean meal (RSBM, 26% of total CP), and (5) defatted soybean meal (SBM, 28% of total CP). Milk samples were collected consecutively in three days.

The composition of rations and the dry matter intake for both experiments are presented in Table 1. Metabolizable energy value was calculated using the value in Japanese Feeding Standard for dairy cattle (AFFRCS, 1994).

2.2. Measurements of NIR spectra and data analysis

NIR spectra of the milk samples were measured with a Pacific Scientific (Neotec) model 6500 instrument (Perstorp Analytical, Silver Spring, MD), within, at most, 3 h from collection. Milk samples were agitated at 2000 rpm for 10 s (IKA Labortechnik Staufen, Janke & Kunkel GmbH & Co., KG, Germany) and incubated in 40°C waterbath prior to the NIR measurements. A transmittance cell (thickness: 1 mm) was used. The spectral data were analyzed by ISI (InfraSoft International, Port Matilda, PA) software. Measurements were made using the second derivative of $\log(1/A)$, where A is absorbance. The spectra were recorded for the range between 1100 and 2500 nm and read at 2 nm intervals. The calibration equation was developed by stepwise multiple linear regression (MLR) using four wavelength combination only. The form of calibration equation used was:

$$Y = a + b(X_1) + c(X_2) + d(X_3) + e(X_4),$$

where Y is a variable to be predicted (milk protein or fat); for developing the calibration equations, the reference

Table 1
Average of dry matter intake and composition of the rations used in two feeding experiments[a]

Composition	DM intake kg d^{-1}	CP	NDF	EE	ME MJ kg^{-1} DM
		% DM			
Exp. 1. Basal ration	20.1	14.3	32.3	3.0	10.2
+ Soybean	19.7	16.4	34.8	4.4	11.9
+ Soybean + Fish meal	21.6	16.4	34.0	4.4	11.8
Exp 2. Supplemented ration					
+ No supplement	20.1	14.3	32.3	3.0	10.2
+ Corn gluten meal	20.6	15.2	31.6	2.9	10.1
+ Fish meal	21.0	15.2	28.6	3.3	10.9
+ Roasted soybean	20.4	14.6	30.9	4.9	10.2
+ Soybean meal	20.8	14.9	29.8	2.9	10.1

[a]DM: dry matter, CP: crude protein, NDF neutral detergent fiber, EE: ether extracts, ME: metabolisable energy calculated based on Japanese Feeding Standard (AFFRCS, 1994).

Table 2
Range, mean and standard deviation of fat and protein content of milk used in the calibration, validation and the validation samples as analyzed by Milkoscan 134 A/B

	n	Fat			Protein		
		Range (%)	Mean	SD	Range (%)	Mean	SD
Exp. 1. Calibration	170	0.85–7.40	3.47	1.23	2.65–3.97	3.20	0.26
Validation	130	0.92–6.46	3.21	1.08	2.77–3.71	3.17	0.21
Exp. 2. Validation							
+ No supplement	42	1.43–5.11	3.40	0.88	2.79–3.76	3.14	0.22
+ Corn gluten meal	42	1.62–6.80	3.82	1.06	3.06–3.91	3.46	0.21
+ Fish meal	40	1.21–5.18	3.14	1.02	2.98–3.91	3.32	0.26
+ Roasted soybean	42	1.38–5.29	3.42	1.08	2.81–3.40	3.14	0.16
+ Soybean meal	40	1.29–6.15	3.63	1.15	2.86–3.73	3.28	0.22

n: number of samples; SD: standard deviation.

data for 'Y' were obtained by Milkoscan; a, b, c, d, and e, are coefficients; and X_n is a level of absorbance at wavelength 'n'. The wavelengths were selected to give a suitable prediction of 'Y'.

2.3. Developing and validation of NIR calibration equations

Milk samples from Experiment 1 were randomly separated into two groups for NIR spectroscopy analysis. The first group was the set of calibration samples (n = 170), which was used for developing the calibration equation. The second group was the validation set (n = 130), which was used for validating the calibration equation developed from the first set of samples. The predicted values by NIR were then compared with the reference method (Milkoscan) for examining the accuracy.

The reliability of calibration equations prior to further application was established based on the values of correlation coefficients (r), standard error of prediction (SEP) and RPD (the ratio of standard deviation of reference data in validation set samples to SEP). Williams (1996) introduced a limit value of RPD adequate for screening of 2.5 or higher, with higher value indicating higher accuracy. The validated calibration equations were then applied to measure the milk samples from Experiment 2. To obtain a successful prediction, the value of milk components to be predicted (validation and prediction set) should be in the range of values of calibration set samples (Shenk, Westerhaus & Hoover, 1979); thus, some samples from Experiment 2 had to be eliminated from prediction set samples.

3. Results and discussion

Compositional data for milk samples collected in both experiments are presented in Table 2. The ranges of components were wide due to the wide range of lactation periods of the cattle in the barn. Milk samples from Experiment 1 were separated into two groups, calibration and validation.

3.1. Development of calibration equations

From the calibration samples, the wavelengths used for developing the calibration equations for milk fat and protein contents are presented in Table 3. For milk fat, the first and the second selected wavelengths are located around the 1720 nm and in the 2300–2350 nm region, respectively. These regions are where the signals of C–H bands of carbonyl compounds are expected to appear (Murray & Williams, 1990). The fundamental absorbance of 1720 nm region can be calculated to appear at about 3.5 μm of the IR region, which is the wavelength used to determine milk fat in measurement by Milkoscan (Barbano & Clark, 1989; Foss Electric, 1993).

The calibration equation for milk protein was developed using four specified wavelengths; 1518, 2172, 2226 and 1748 nm. The first, second and third

Table 3
Wavelengths used for developing the calibration equations of fat and protein content of milk and statistical results for the validation[a]

	Wavelength (nm)				Calibration			Validation	
					R	SEC	r	SEP	RPD
	1st	2nd	3rd	4th	n = 170			n = 130	
Fat	1718	2310	—	—	0.971	0.29	0.970	0.25	4.32
Protein	1518	2172	2226	1748	0.936	0.09	0.914	0.08	2.59

[a] R, r: correlation coefficients; SEC, SEP: standard error of calibration and prediction, respectively; RPD: ratio of standard deviation of reference data in validation sample set to SEP.

Table 4
Accuracy of prediction of fat and protein content for milk samples from cattle fed the supplemented ration used in experiment 2[a]

	r	SEP	RPD
+ No supplement (NS, n = 42)	0.964	0.22	4.03
Fat	0.904	0.07	3.02
Protein			
+ Corn gluten meal (CGM, n = 42)			
Fat	0.966	0.28	3.82
Protein	0.901	0.09	2.34
+ Fish meal (FM, n = 40)			
Fat	0.980	0.19	5.43
Protein	0.902	0.09	2.73
+ Roasted Soybean meal (RSBM, n = 42)			
Fat	0.971	0.24	4.43
Protein	0.834	0.09	1.76
+ Soybean meal (SBM, n = 40)			
Fat	0.970	0.29	3.91
Protein	0.902	0.08	2.70

[a] n: number of samples; r: correlation coefficients; SEP: standard error of prediction; RPD: ratio of standard deviation of reference data in validation sample set to SEP.

wavelengths are considered to relate to the absorbance of protein at ~1520 nm (Osborne & Fearn, 1986), the absorbance of the N–H peptide, and the absorbance of amino acids (Murray & Williams, 1990), respectively. The fourth wavelength of 1748 nm may correspond to the signal of fat absorbance which occurs near 3.5 μm of IR region. A study of the wavelength effects reported that 1720–1760 nm was the region of CH_2 absorbance (Wang et al., 1998). This fourth wavelength may be used to strengthen the calibration equation for milk protein, revealing the relationship between protein and fat concentration in milk in performance of milk spectra (Wang et al., 1998).

Correlation coefficients of multiple regressions (R) of fat and protein in milk for the calibration samples were found to be 0.971 and 0.936, respectively. For the validation group, those values were calculated to be 0.970 and 0.914, respectively. The calibration equation for milk fat was suitable for prediction as shown by the same level of correlation coefficients obtained in the calibration and in the validation. For milk protein, the value of correlation coefficient observed was lower than that observed for the calibration set. However, the standard error of predictions (SEP) for both milk fat (0.25%) and for milk protein (0.08%) were at the level similar to that obtained in the calibration (SEC).

The estimations of reliability of calibration equations were based on the RPD values for milk fat and protein content; these were 4.32 and 2.59, respectively, and indicated that both equations were adequate for practical measurement (Williams, 1996).

3.2. Determination of fat and protein in milk from cattle offered supplemented rations

Predictions of fat and protein content in milk from the cattle fed five kinds of supplementation in Experiment 2, using the calibration equations developed in Experiment 1, are presented in Table 4. The results of determinations of milk fat and milk protein in NS ration showed the repeatability of the calibration equation for the measurement. The values of the correlation coefficients (r) were lower especially for milk protein, but the SEP and RPD were similar compared to those found in Experiment 1.

On determination of milk fat and protein content in milk obtained from the five supplemented rations, the results showed that the prediction for milk fat was highly accurate, with the r values ranging between 0.96 and 0.98, but the r values for milk protein were lower and varied, with the range being between 0.83 and 0.90. The SEP for milk fat and milk protein were in the range 0.19–0.29 and 0.07–0.09, respectively. These errors are very small and similar to those obtained for the calibration data.

3.3. Effect of feed supplementation on the NIR-MLR prediction of milk composition

3.3.1. Milk fat

The fat contents in milk from the cattle fed the five supplemented rations were predicted accurately. The supplementation protein source did not influence the accuracy of milk fat prediction, as shown by the r, SEP and RPD values for each kind and proportion of supplement. Hence, NIR-MLR prediction for milk fat was not influenced by feed source of CP supplementation. This may be because fat intakes in the rations used in this study were similar, except for the roasted soybean meal (RSBM) ration. This result was in agreement with the finding of Palmquist et al. (1993), who reported that milk fat content is changed more by the amount and composition of rationary fat than other dietary components. In the present study, RSBM provided more fat than other feeding regimes, however, the increased fat content was not sufficiently high (only 0.5% DM) to differentiate the fatty acid composition in milk fat significantly from that of the feeding regimes used in the calibration set samples. As shown by Grummer (1991), the proportion of fatty acid synthesis changed as supplemental dietary fat changed from 1 to 5% DM. With regard to feed protein, Wu and Palmquist (1991) reported that protein intake may have indirect effects on milk fatty acids by providing precursors for synthesis of various branched-chain fatty acids through ruminal degradation of dietary protein.

3.3.2. Milk protein

Prediction results for milk protein are also presented in Table 4. The r values of prediction for milk protein were lower than those of prediction for milk fat and

ranged from 0.83 to 0.90. The r value for RSBM was the lowest, but the SEP was similar to those for FM and CGM. If the judgement were to be based on the RPD value, good predictions were obtained only for NS, FM and SBM rations. Predictions for milk protein for RSBM and CGM groups were lower than 2.5, the limit value of RPD. The possible reason for the low RPD or the low accuracy of prediction of RSBM and CGM could be that neither ration was included in developing the calibration equations.

The variation of NIR measurement of protein in milk from supplemented rations may be due to the different proportions of protein fractions. Several studies have shown that dietary protein intake influences milk protein, and that different proteins sources in diet may cause different proportions of nitrogen in milk (Wohlt, Chmiel, Zajac, Backer, Blethen & Evans, 1991; DePeters & Cant, 1992). Yousef, Huber and Emery (1970) reported that the increase in protein content on the high concentrate rations increases total casein (as α- and β-casein), but decreases γ-casein and non-protein nitrogen contents. Supplementation given in the form of CGM, FM, SBM and RSBM was characterized by varying level of degradability and N utilization in rumen, and associated with milk urea nitrogen (MUN) and milk non-protein nitrogen (NPN) (Roseler, Ferguson, Sniffen & Herrema, 1993; Wohlt et al., 1991). SBM is a protein source degraded rapidly in rumen, while CGM is degraded more slowly (Robinson, McQueen & Burgess, 1991). Similarly, FM is also degraded slowly and may contain mainly protein which is passing directly through to the intestine (Broderick, 1992). However, while FM and CGM contain similar levels of protein of similar degradability, they have a different effect on percentage protein in milk (Blauwiekel, Hoover, Slider & Miller, 1990). SBM tends to produce a higher plasma urea N than CGM (Robinson et al., 1991) which highly correlates with MUN (DePeters & Ferguson, 1992). In comparison with FM, SBM contains lower protein passing directly through to the intestine than FM (Broderick, 1992) and results in a significantly lower milk protein but the contents of milk urea are similar. RSBM results in lower ruminal ammonia concentrations (Annexstad, Stern, Otterby, Linn & Hansen, 1987; Tice, Eastridge & Firkins, 1991) than soybean. The ruminal ammonia has a strong correlation with BUN and MUN (Oltner & Wiktorsson, 1983).

In this study, different supplementation affected the proportion of N fractions in total milk protein. Data reported by Batajoo et al. (1998) are showed that the analyses of true protein (12% TCA), casein and MUN contents related to the feeding regimes were significantly different ($P < 0.05$). The ratios of true protein and casein to total milk protein and MUN content varied, from high to low, the order of effect being SBM, FM, RSBM and CGM for true protein and casein, but FM, RSBM, SBM and CGM for MUN contents. Because statistical calculations for developing calibration equations by MLR are based on absorbance over all samples that are most highly correlated with the laboratory reference value, these different concentrations of nitrogen fractions in milk protein may be the reason for the lower accuracy of prediction for RSBM and CGM, as these two were not represented in the calibration set samples. The main factor for poorly fitting calibration equations points to the constants for the absorbance at selected wavelengths which are representing the level of milk protein fractions rather than the selected wavelengths. Thus, application of the same milk protein calibration equation for milk containing various level of N compounds was not appropriate.

4. Conclusion

Determination of milk fat and protein by NIR-multiple linear regression in milk from feeding regimes not represented in the calibration set samples was suitable for milk fat, but not for milk protein. Therefore, because of the influence of diet on composition of milk protein, a wide range of milk from various rations is needed for developing an applicable calibration.

Acknowledgements

The authors are grateful to Dr. Y. Ozaki, professor of Kwansei Gakkuin University for reading the manuscript and valuable comments relating to the NIR. Also, the authors are indebted to I. Nonaka for providing a part of milk samples, to Y. Someya for the milk analysis by Milkoscan and to Dr. Chindo Hicks for comments in writing. This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

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