Breed effects for lactoferrin concentration determined by Fourier transform infrared spectroscopy

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ABSTRACT

A calibration equation based on partial least squares using Fourier transform infrared spectroscopy (FTIR) data was derived to predict concentration of lactoferrin (Lf) in bovine milk. The concordance correlation coefficient of the calibration equation was estimated at 0.81 using 246 milk samples collected in the 2003-04 season from second-parity crossbred cows during late lactation. The calibration equation was then used to predict the concentration of Lf (pLf) in 9,742 milk samples from 814 Holstein-Friesian (HF), 955 Jersey (J) and 842 crossbred HF x J cows sampled on average 3.7 times during the 2007-08 season. A repeatability animal model was used to obtain breed effects and estimates of variance components for pLf. Concentrations of pLF (mg/L milk) were significantly ($P < 0.001$) different between breed groups (HF = 163 ± 3.8, HF x J = 175 ± 5.0 and J = 193 ± 6.3). Heritability and repeatability of pLf concentration were 0.16 ± 0.036 and 0.35 ± 0.012, respectively. These results confirm previous studies showing that Lf concentration in milk can be predicted using FTIR spectroscopy and that there are significant breed differences and animal variation that can be exploited in a breeding program.

Keywords: lactoferrin; infrared spectroscopy; crossbreeding.

INTRODUCTION

Lactoferrin (Lf) is a glycoprotein that is present in milk (Brooker, 1978) and other body fluids including tears and blood (Farnaud & Evans, 2003). Reportedly, it has growth-promoting actions in dairy calves (Joslin *et al.*, 2002), and bacteriostatic (Bushe & Oliver, 1987) and iron-transporting (Maneva *et al.*, 1983) properties. The potential benefits of Lf on human and animal health have created a market for Lf enriched products, including nutritional supplements, sports drinks and animal and infant formulas (Turner *et al.*, 2003).

The concentration of Lf in colostrum immediately after calving is about 2,400 mg/L (Nonnecke & Smith, 1984), this level decreases rapidly with concentrations levels in normal milk ranging from 45 to 245 mg/L (Kawai *et al.*, 1999; Back & Thomson, 2005; Soyeurt *et al.*, 2007). Concentrations of Lf in milk can reach as high as 2,300 mg/L during clinical mastitis (Kawai *et al.*, 1999). Lactoferrin serves an important function in the mammary gland as a part of the defence mechanism against bacteria invading the intramammary ducts and is released in higher concentrations by the epithelial cells in response to inflammatory stimuli (Kawai *et al.*, 1999).

There are few data available with regards to the effect of breed on Lf concentration in milk and these are contradictory. Tsuji *et al.* (1990) reported that colostrum from Jersey (J) cows had higher concentration of Lf than colostrum from Holstein cows. Farr *et al.* (2002) found that Lf concentrations in milk collected at the evening (pm) milking from J and Holstein-Friesian (HF) cows during mid-lactation were not different. However, HF cows produced milk with higher Lf concentrations and Lf yields than J cows at the morning (am) milking. Back and Thomson (2005) reported no significant differences in Lf concentration throughout lactation between HF, J and crossbred HF x J cows. In the study of Soyeurt *et al.* (2007) concentration of Lf in J milk was significantly greater than in Holstein milk. However, the heritability of Lf concentration has been reported to be moderate (0.20; Soyeurt *et al.*, 2007) or relatively high (0.43; Gaunt *et al.* 1980) suggesting that selection for an increased Lf concentration in milk is possible.

Infrared spectroscopy provides rapid, cost-effective determinations of concentrations of fat, protein, lactose and somatic cell count in milk. This technique has been found to be effective for the determination of concentration of other specific milk components including α_s-casein, β-casein and κ-casein (Diaz-Carrillo *et al.* 1993), fatty acids (Soyeurt *et al.*, 2006), lactoferrin (Soyeurt *et al.*, 2006), urea (Hansen, 1998) and the quantification of acetone for

the detection of subclinical ketosis (Heuer *et al.*, 2001). The objectives of this study were, to derivate a calibration equation based on partial least squares using Fourier transform infrared spectroscopy (FTIR) data, and to use the calibration equation to predict Lf concentration in a representative sample of milk from HF, J and HF x J crossbred cows.

MATERIALS AND METHODS

Calibration equation

Milk samples were collected in 2004 from 246 second-parity crossbred HF x J cows in late lactation. These cows were part of a crossbreeding experiment designed for the identification of quantitative trait loci determining traits of economic importance in New Zealand dairy cattle (Spelman *et al.*, 2001). The herd was managed as a conventional spring calving herd grazing on rye grass/white clover pastures and milked twice a day in a rotary milk harvesting system.

Concentrations of Lf (mg/L milk) in these 246 milk samples were determined by high performance liquid chromatography (HPLC) as described by Palmano & Elgar (2002). The same milk samples were analysed on a Foss MilkoScan FT6000 (Foss, Hillerød, Denmark) to provide the FTIR spectrum. The calibration equation for Lf was determined using partial least squares (PLS) (Haaland & Thomas, 1988) using SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA). The optimum number of PLS factors was determined by cross-validation from a maximum of 15 PLS factors allowed in the model. Wavelengths related to water absorbance were removed from the spectra as these bands were found to have high noise levels. Absorbance values at each wave length were standardised with mean 0 and standard deviation 1. Measures of fitness to select the best calibration equation were the following:

Mean square prediction error (MSPE)

$$= \frac{1}{n}\sum_{i=1}^{n}(A_i - P_i)^2$$

Mean prediction error (MPE) = $\sqrt{MSPE}$

Relative prediction error (RPE) = MPE/ $\overline{A}$

Ratio performance deviation (RPD) = S_A/MPE

Concordance correlation coefficient (CCC)

(Lin, 1989) = $$\frac{2S_{AP}}{S_A^2 + S_P^2 + (\overline{A} - \overline{P})^2}$$

where A_i is the i^{th} concentration of Lf in milk determined by the HPLC procedure, Pi is the ith concentration of Lf in milk predicted by the calibration equation. Means, variances, standard deviations and covariances of A_i and P_i are calculated in the usual way:

$$\overline{A} = \frac{1}{n}\sum_{i=1}^{n}A_i \text{ , } \overline{P} = \frac{1}{n}\sum_{i=1}^{n}P_i \text{ , } S_A^2 = \frac{1}{n}\sum_{i=1}^{n}(A_i - \overline{A})^2 \text{ ,}$$

$$S_P^2 = \frac{1}{n}\sum_{i=1}^{n}(P_i - \overline{P})^2 \text{ ,}$$

$$S_{AP} = \frac{1}{n}\sum_{i=1}^{n}(A_i - \overline{A})(P_i - \overline{P}) \text{ and } S_A = \sqrt{S_A^2}$$

Fuentes-Pila *et al.* (1995) suggested that a RPE value lower than 10% is an indication of satisfactory prediction, whereas a RPE between 10% and 20 % indicates a relatively acceptable prediction, and a REP greater than 20% indicates poor prediction. Following Sinnaeve *et al.* (1994), a RPD value greater than 2 indicates that the calibration equation has good prediction and a RPD value indicates that predicted values are of poor quality and the equation cannot be used in practice. Values corresponding to the CCC and their significance are as follow: from 0.21 to 0.40, fair prediction; from 0.41 to 0.60, moderate prediction; from 0.61 to 0.80, substantial prediction; and from 0.81 to 1.00, almost perfect prediction.

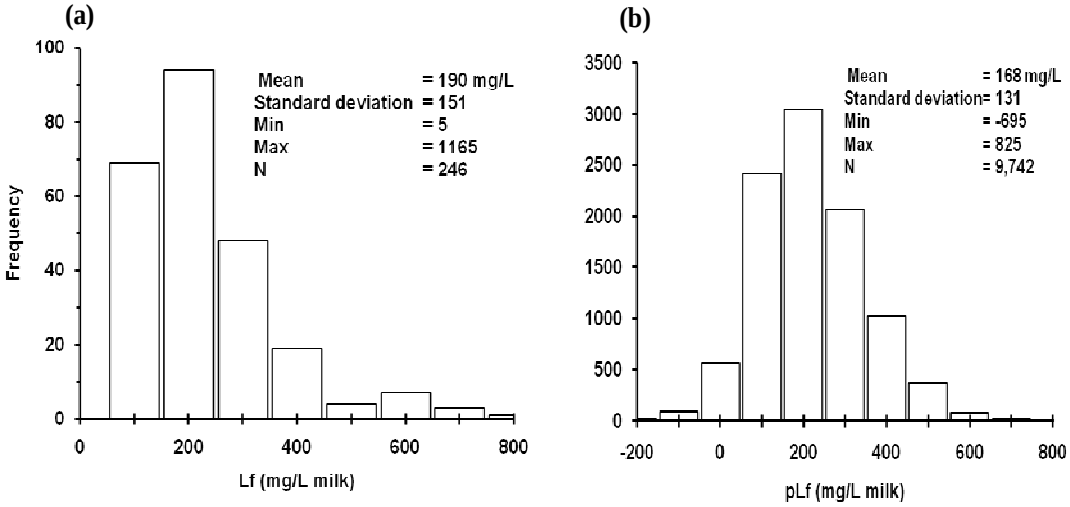
Breed effects and genetic parameters

The calibration equation was used to predict the Lf concentration in milk (pLf) (mg/L milk) in 9,742 milk samples from 2,611 cows from three breed groups; HF, JE and crossbred HF x J. These milk samples were part of the herd-testing program used for determination of concentrations of fat, protein, lactose and somatic cell counts in a Foss MilkoScan FT6000 instrument (Foss, Hillerød, Denmark). The FTIR spectrum for each milk sample was provided by Livestock Improvement Corporation. There were 814 HF, 955 J and 842 crossbred HF x J cows and each cow was sampled on average 3.7 times during the 2007-08 season. The cows were of different parities and distributed in 251 herds used for progeny testing of young bulls.

The pLf values were analysed with a linear mixed model using the statistical package SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA). The model included the fixed effect of herd-year-test-day, parity, month of lactation, breed group, the interaction between month of lactation and breed, and the random effect of cow to account for repeated samples in the same cow. Least squares means for breed groups and combinations of month of lactation and breed groups were obtained and used for multiple mean comparisons.

Variance components to estimate heritability and repeatability for pLf were obtained using the statistical package ASREML (Gilmour *et al.*, 2002) with the same mixed linear model plus the random additive genetic animal effect. The pedigree file included only sire and dam of the cow. Heritability was calculated as $[\sigma^2_a/(\sigma^2_a + \sigma^2_c + \sigma^2_e)]$ and repeatability was calculated as $[(\sigma^2_a + \sigma^2_c)/(\sigma^2_a + \sigma^2_c + \sigma^2_e)]$ where σ^2_a, σ^2_c, and σ^2_e are the additive genetic, cow and residual variances, respectively.

FIGURE 1: Distributions and descriptive statistics of lactoferrin (Lf) concentration in milk, with (a) Lf concentration determined by high performance liquid chromatography in the reference cow population, and (b) Lf concentration predicted by the calibration equation (pLf) in the cows for the progeny testing of young bulls.



RESULTS AND DISCUSSION

Descriptive statistics and distribution of Lf concentrations in milk samples used for the calibration equation and pLf concentrations in milk samples from the sire proving scheme cows are shown in Figure 1.The mean and standard deviation of Lf concentration in milk in the reference population were 190 and 151 mg/L with a range from 5 to 1165 mg/L (Figure 1a). The best calibration equation comprised = 45.3%, RPD = 1.8 and CCC = 0.81 (Figure 2). The RPE value is greater than 20% indicating 5 PLS factors explaining 96.9% of the total variation. Measures of fitness were RPE that the calibration equation had a poor predictive value (Fuentes-Pila *et al.*, 1995), however the value of RPD is close to 2, suggesting that the calibration equation can be considered to be of practical use for ranking purposes (Sinnaeve *et al.*, 1994). The estimated value of CCC re-affirmed the RPD values suggesting that the calibration equation can be considered to have substantial predictive ability. For the purposes of ranking animals based on pLf concentration the calibration equation can be considered to have practical utility.

FIGURE 2: Linear regression of predicted concentration in milk of lactoferrin (pLf) using Fourier transform infrared spectra on actual concentration in milk lactoferrin (Lf) determined by high performance liquid chromatography. Dashed line = Line of perfect prediction, N = Number of milk samples, RPE = Relative prediction error, RPD = Ratio performance deviation, CCC = Concordance correlation coefficient.

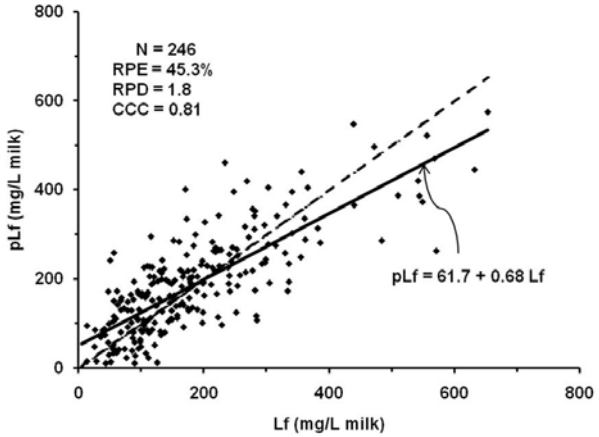


Figure 1b shows the distribution of the pLf values in the cows for the progeny testing of young bulls. The mean and standard deviation were 168 and 131 mg/L milk, respectively. These values were lower than the reference values but this was influenced by the range which was from -695.4 to 825.3 mg/L. The total frequency of pLf values that were less than zero was 7.0%. The regression equation of pLf on Lf is shown in Figure 2 with an intercept = 61. ± 7.3 and slope = 0.68 ± 0.3; these estimates are significantly ($P < 0.001$) different from zero witht the slope was also significantly different from 1. Figure 2 shows that the calibration equation tended to under predict Lf concentration for actual Lf concentration above 200 mg/L and over predict Lf concentration for actual Lf concentration below 200 mg/L of milk.

Concentration of pLF was significantly ($P < 0.05$) different between the three breed groups (HF = 163 ± 3.8, HF x J = 175 ± 5.0 and J = 193 ± 6.3). In this study the J cows had higher pLf concentration as found by Soyeurt *et al.* (2007), however in the other two studies with New Zealand dairy cattle (Farr *et al.* 2002; Back & Thomson, 2005) HF cows tended to have higher Lf concentration compared to J cows in mid and late

lactation. Crossbred HF x J cows were intermediate between the purebred HF and J cows.

Parity number had a significant effect on pLf concentration in milk ($P < 0.001$) but there was not a defined pattern. Least squares means and their standard errors were: first-parity, 191 ± 5.4; second-parity, 176 ± 4.8; third-parity, 184 ± 5.0; fourth-parity, 174 ± 5.0; fifth-parity, 171 ± 5.4; sixth-parity, 174 ± 5.7; and seventh-parity, 168 ± 5.0. This is consistent with the studies of Soyeurt *et al.* (2007) and Back and Thomson (2005) who reported a positive impact of parity number on the production of Lf by dairy cows but disagrees with results of the study of Hagiwara *et al.* (2003) who observed a negative effect of parity number on the Lf concentration in milk.

Month of lactation also had a significant effect on pLf ($P < 0.001$) following the same trend as found in other studies (Kawai *et al.*, 1999; Back & Thomson, 2005; Soyeurt *et al.*, 2007). Least squares means and their standard errors for breed groups in each month of lactation are shown in Figure 3. The concentration of pLf during the first month of lactation was 67 ± 8.6 mg/L milk, declining to 31 ± 7.2 in the second month of lactation. Starting from the fourth month of lactation, the pLf concentration in milk from J cows was significantly ($P < 0.001$) higher than from HF cows (164 ± 6.5 vs 209 ± 7.8 mg/L), these differences remained until the ninth moth of lactation. Concentration of pLf in crossbred cows was intermediate between HF and J cows.

Estimates of heritability and repeatability for pLF concentration during lactation were 0.16 ± 0.036 and 0.35 ± 0.012, respectively. A repeatability of 0.35 found in this study suggests that cows with high Lf concentration can be found within the population by screening after the fourth month of lactation. The estimated value of heritability in this study is similar to the value estimated by Soyeurt *et al.* (2007) of 0.20 but lower than 0.43, estimated by Gaunt *et al.* (1980). The heritability value of 0.16 for pLF concentration in cow's milk indicated that selection for an increased concentration of Lf in bovine milk is possible. Further studies are required to estimate the genetic correlation between Lf and somatic cell score and clinical mastitis to devise selection strategies using levels of lactoferrin as an indicator of resistance to intramammary infections.

CONCLUSIONS

Results from this study confirm those from previous studies from other countries showing that Lf concentration in milk can be predicted using FTIR spectroscopy. The results also highlight that there are significant breed differences and animal variation that could be exploited in a breeding program to provide Lf enriched milk for specialty products.

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