

Mid-Infrared Predictions of Lactoferrin Content in Bovine Milk

Potential Indicator of Mastitis

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- **Lactoferrin:**
 - Iron-binding glycoprotein naturally present in milk
 - Secreted mainly by the mammary cells:
 - Lower content at the early lactation stage
 - Various effects on the immune system
- **Interests:**
 - Potential indicator of mastitis
 - Human health:
 - Lactoferrin extraction from milk
 - Improvement of the nutritional quality of milk



- **Quantification:**
 - Enzyme-Linked ImmunoSorbent Assay (**ELISA**)
 - Immunodiffusion method
- **Inconvenient:**
 - Time consuming
 - Skilled staff
 - Not easy to implement in milk labs



- (1) Rapid quantification of lactoferrin by mid-infrared spectrometry (MIR)
 - Already used to measure the contents of fat, protein, lactose, urea, and fatty acids in milk
 - Implemented all around the world
 - Non destructive, non-polluting, and fast method
 - Previous study in 2007 from 69 samples
- (2) Test the interest to detect the presence of mastitis

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- Lactoferrin content was quantified by ELISA in at least duplicates
 - Only ELISA data with a repeatability $\leq 5\%$ were kept
- Samples were analyzed by MIR using 2 MilkoScan FT6000 spectrometers
- Milk samples collected between April 2005 until now in different countries from several dairy breeds:
 - 110 samples came from the Walloon Region of Belgium
 - 1,658 Irish samples
 - 731 Scottish samples



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2,499 samples : 163.00 ± 103.40 mg/L of milk
min. = 4.56 mg/L of milk
max. = 813.91 mg/L of milk

- Internal validation by **cross-validation**
 - 50 groups
- External validation from **Walloon milk samples**:
 - Samples composed of 50% of morning and 50% of evening milk
 - MIR analysis using a MilkoScan FT6000
 - Lactoferrin content measured by ELISA
 - Repeatability estimated from at least 2 measurements
 - Samples with repeatability $\leq 5\%$ were deleted

274 samples : 108.02 ± 88.33 mg/L of milk
min. = 7.69 mg/L of milk
max. = 597.73 g/L of milk

- 6 methods were tested:
 - PLS and **no pre-treatment** on the spectral data
 - PLS + the use of a **repeatability file**
 - PLS + the use of a **first derivative** pre-treatment on the spectral data
 - PLS + the use of a **first derivative** pre-treatment + **repeatability file**
 - PLS + the use of a **second derivative** pre-treatment
 - PLS + the use of a **second derivative** pre-treatment + **repeatability file**
- Interests:
 - Use of **derivatives** permits to correct the **baseline drift**
 - The **repeatability file** contained spectra from the same samples analysis on different spectrometers in order to improve the **reproducibility** of the MIR prediction

- Calibration and validation results for the six methods used

PLS + ...	N	R ² _c	R ² _{cv}	RPD	R ² _v
No pre-treatment	2,445	0.71	0.70	1.83	0.29
First derivative	2,463	0.74	0.73	1.91	0.43
First derivative + repeatability file	2,442	0.72	0.71	1.86	0.60
Second derivative	2,459	0.73	0.72	1.90	0.53
Second derivative + repeatability file	2,438	0.70	0.69	1.81	0.51
Repeatability file	2,445	0.69	0.69	1.79	0.27

R²_c = calibration coefficient of determination; R²_{cv} = cross-validation coefficient of determination; RPD = the ratio of standard deviation of reference values to the standard error of cross-validation; R²_v = validation coefficient of determination estimated from 274 samples;

A t-outlier test was used to correct the potential outliers from ELISA data → the maximum of deleted samples was 61 (2,499-2,438 = 61)

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Standard error of calibration (**SEC**) = **49.90** mg/L of milk
 Standard error of cross-validation (**SECV**) = **50.55** mg/L of milk
 Standard error of prediction (**SEP**) = **58.98** mg/L of milk

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- **5,886 milk samples** were collected:
 - from the Research herd of the University of Wisconsin - Madison
 - between January 2009 and February 2011
 - from 800 Holstein cows
 - **93 mastitis events** were recorded and related to spectral data (date of treatment \pm 7 days)
- All samples were analyzed by MIR using MilkoScan FT6000 spectrometer
 - All spectral data were recorded
- Lactoferrin content was measured by applying the developed equation on the recorded spectral data



	Mean	SD	Skewness	Kurtosis
SCC (*1000)	211.71	560.30	7.63	77.37
SCS	2.59	1.84	0.67	0.33
Lactoferrin (mg/L milk)	185.07	105.04	1.31	4.39

As expected, SCC was not normally distributed

Correlation	Lactoferrin
SCS	0.54 <i>P-value < 0.0001</i>



Dataset: 93 mastitis events and 230 no-mastitis (mastitis treatment date \pm 90 days; n1/n = 40%)

	Mean	SD	Skewness	Kurtosis
SCS	3.80	2.30	0.17	-0.57
Lactoferrin (mg/L milk)	182.50	96.48	0.61	2.03

Detection of mastitis by using logistic regression



	1 st dataset (93/230)	
	SCS	SCS + lactof.
Deviance	P = 0.05	P=0.06
Pearson	P = 0.73	P = 0.39
AIC	366.46	365.72
R ²	0.11	0.12
Wald	P < 0.01	P < 0.01
SCS	P < 0.01	P < 0.01
lactoferrin		P = 0.10
ROC area	67.3%	67.8%
% Concordant	67.1%	67.6%
% Disconcordant	32.5 %	32.0%
% Tied	0.4%	0.4%



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The inclusion of predicted lactoferrin content in the predictive model slightly improved the model.

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Based on a limitation of P-value of 0.20, the lactoferrin effect is relevant.



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The validation of the specificity of the model (the ability to predict the absence of mastitis) was evaluated based on **samples independent** of the calibration set (=the remaining samples of the initial dataset).

	1 st dataset (93/230)	
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Hosmer and Lemeshow (*)	P = 0.48	P = 0.72
Validation of Specificity (5,643 samples)		
Equations from 1 st dataset	98.67%	98.60%

(*) the P-value must be close to 1

Results provided by the Hosmer and Lemeshow test should be considered with caution because the dataset had less than 400 records.

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The best results for the Hosmer and Lemeshow test were obtained from the model including the lactoferrin content. This suggests that the introduction of **lactoferrin effect** could **improve the sensibility** of the model.

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The **equations** had a **high specificity**. However the sensibility of the model (the ability to predict the presence of mastitis) could be low !!

→ Need a validation dataset with mastitis information

- The MIR spectrum of milk is an useful indicator of milk lactoferrin content
 - RPD was close to 2
- Moderate correlation with SCS
 - The introduction of lactoferrin content in breeding animal selection in combination with SCS could be interesting to improve the mastitis resistance
- The inclusion of lactoferrin in the predictive model slightly improved the prediction of mastitis
 - Need to have a validation dataset containing the mastitis information

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