Mid-Infrared Predictions of Lactoferrin Content in Bovine Milk

Potential Indicator of Mastitis

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Introduction

- 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

Introduction

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

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Objectives

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- (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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Calibration Dataset

- Lactoferrin content was quantified by ELISA in at least duplicates
  - Only ELISA data with a repeatability ≤ 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

Validation Dataset

- 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 ≤ 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

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Methods

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- 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 Equations

- Calibration and validation results for the six methods used

PLS + …	N	R^2c	R^2cv	RPD	R^2v
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

R^2c = calibration coefficient of determination; R^2cv = cross-validation coefficient of determination;
RPD= the ratio of standard deviation of reference values to the standard error of cross-validation;
R^2v = 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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Dataset

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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 ± 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

Somatic cells - Lactoferrin

	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 *P-value < 0.0001*

Datasets « Mastitis »

Dataset: 93 mastitis events and 230 no-mastitis (mastitis treatment date ± 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

Detection of Mastitis

	1st 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 SCS lactoferrin	P < 0.01 P < 0.01	P < 0.01 P < 0.01 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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Detection of Mastitis

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).

	1st dataset (93/230)	
	SCS	SCS + lactof.
Hosmer and Lemeshow (*)	P = 0.48	**P = 0.72**
Validation of Specificity (5,643 samples)		
Equations from 1st 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**

Conclusions

- 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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