Mid-infrared predictions of milk titratable acidity and its genetic variability in first-parity cows

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Context

- Titratable acidity (TA)
  - Fresh milk
    - ✓Some components: carbon dioxide, citrates, casein, albumin/globulin and phosphates
    - ✓Buffer action
  - Developed acidity results from bacterial activity
    - ✓Lactic acid
    - ✓Collection, transportation, and transformation of milk
  - Influence on rennet-coagulation properties

(Formaggioni et al., 2001; Summer et al., 2002)

Context

- Titratable acidity (TA)
  - Measured TA
    - ✓ Some studies
    - ✓ Moderate heritability
    - ✓ Genetically correlated to coagulation properties

(Cassandro et al., 2008; Cecchinato et al., 2012; Penasa et al., 2010)

  - Prediction by mid-infrared (MIR) spectrometry
    - ✓ Few studies showed its feasibility.

(De Marchi et al., 2009; Colinet et al., 2010)

Context

- Titratable acidity (TA)
  - Expressed in Dornic Degree (D°)
  - Classification of milk according to D°
    - ✓ < 15 Mastitis milk or late lactation milk
    - ✓16-18 Normal fresh milk
    - ✓19-20 Early lactation milk or colostrum
    - ✓20-22 Heat-coagulation during sterilization (115°C)
    - ✓> 22 Heat-coagulation during pasteurization (72°C)

Objectives

- To determine TA of fresh milk at large scale
  - Fast method using small quantity of milk
  - Adapted to Walloon dairy cattle (multi-breed)
  - MIR spectrometry already implemented in milk labs

➔ MIR chemometric method for TA prediction

- To study the genetic variability of predicted TA
  - First-parity Holstein cows in Wallonia (Belgium)

MIR chemometric method

- Sampling
  - Wallonia (Belgium)
  - Variability of spectra: several criteria
    - ✓ Milk sampling: individual or bulk milk
    - ✓ Breed: Dual-Purpose Belgian Blue, Holstein, Red-Holstein, Montbeliarde and Jersey
    - ✓ Time of sampling: morning milking, evening milking or mix of 50 % morning & 50 % evening milk samples

➔ 507 fresh samples collected (October 2009 – June 2010)

MIR chemometric method

- Analysis
  - Milk Lab (Comité du Lait, Battice, Belgium)
    - ✓ FT-MIR
  - Titratable acidity
    - ✓ Recorded as Dornic degree (D°)
    - ✓ N/9 NaOH solution
    - ✓ Indicator: Phenolphthalein

MIR chemometric method

- Methods
  - Modified Partial Least Square regressions *(Shenk & Westerhaus, 1991)*
  - Use of a first derivative pretreatment
    - ✓ To correct the baseline drift
  - Detection of spectral outliers
    - ✓ Based on Mahalanobis distance → 7 samples discarded
  - Use of a repeatability file
    - ✓ Spectra from the same samples analysis on different spectrometers

MIR chemometric method

- Methods
  - Internal cross-validation (100 groups)
    - ✓ To determine the number of factors
    - ✓ To assess the robustness of equation
  - T-outlier test
    - ✓ Compare observed and predicted values
    - ✓ Samples with T-outlier value > 2.5 were discarded
    - ✓ Maximum 5 tests performed
    - → 41 additional samples discarded

MIR chemometric method

- Calibration equation
  - Statistical parameters of final dataset
    - ✓ Mean = 16.63 D°
    - ✓ Standard deviation (SD) = 1.80 D°
    - ✓ Range = 12 D° (from 10.50 to 22.50 D°)
  - Calibration
    - ✓ Standard error of calibration = 0.77 D°
    - ✓ Calibration coefficient of determination (R^2_C) = 0.82

MIR chemometric method

- Calibration equation
  - Statistical parameters to assess the accuracy
    - ✓ Standard error of cross-validation (SE_{CV}) = 0.80 D°
    - ✓ Cross-validation coefficient of determination (R^2_{CV}) = 0.80
    - ✓ RPD (= SD / SE_{CV}) = 2.25 > 2
    - ✓ RER (= Range / SE_{CV}) = 14.99 > 10

→ Calibration equation: good practical utility

Genetic variability study

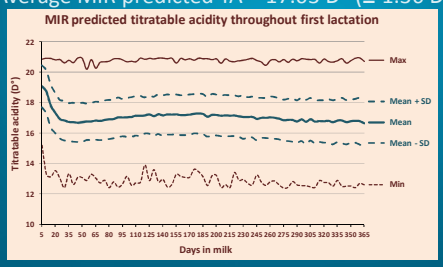
- Data editing
  - Walloon MIR spectral database
    - ✓ > 2 000 000 spectra
    - ✓ Routinely collected since 2007 by milk recording
  - Outliers discarding
    - ✓ Based on Mahalanobis distance computing using 451 MIR spectra of the final calibration dataset as reference
    - ✓ Below 0.5 percentile and above 99.5 percentile

Genetic variability study

- Data editing
  - After edits:
    - ✓ 10 457 first-parity Holstein cows from 153 herds
      cows with ≥ 3 TA predictions and known parents
      > 65 000 animals in pedigree file
    - ✓ > 93 000 records for milk, fat, and protein traits
    - ✓ > 92 000 records for somatic cell score (SCS)
    - ✓ > 64 000 records for MIR predicted TA
    - ✓ > 64 000 records for lactose content
    - ✓ > 46 000 records for pH

Genetic variability study

- Data
  - Average MIR predicted TA = 17.03 D° (± 1.36 D°)



Genetic variability study

- Correlations among observations at the same day

Correlations between predicted TA and:	
Milk yield	**0.08**
Fat content	**0.16**
Protein content	**0.35**
Lactose content	0.08
SCS	**- 0.08**
pH	- 0.33

Similar to Cassandro *et al.*, 2008

Genetic variability study

- Correlations among observations at the same day

Correlations between predicted TA and:	
Milk yield	0.08
Fat content	0.16
Protein content	0.35
Lactose content	0.08
SCS	- 0.08
pH	**- 0.33**

Lower than expected

Ongoing researches on genetic correlations

Genetic variability study

- Single-trait random regression animal test-day model

$$y = X\beta + Q(Zp + Za) + e$$

- β = fixed effects
  - ✓ herd x test day
  - ✓ lactation stage (classes of 5 days)
  - ✓ gestation stage
  - ✓ age at calving x season of calving x lactation stage

Genetic variability study

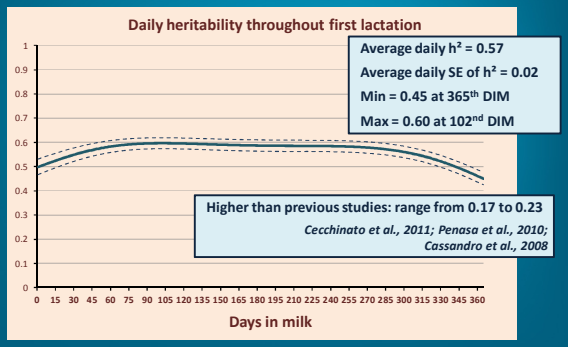
- Single-trait random regression animal test-day model

$$y = X\beta + Q(Zp + Za) + e$$

- p = permanent environment random effect
  a = additive genetic random effect
  - ✓ regression curves modelled with 2nd order Legendre polynomial

- Variances components estimated by AIREMLF90 *(Misztal, 2012)*

TA heritability



Conclusions

- MIR chemometric methods
  - Developed equation
    - ✓ R^2_{CV} = 0.80
    - ✓ RPD > 2 and RER > 10

➔ Good practical utility
➔ Results are promising for the prediction of titratable acidity from MIR spectrum

Conclusions

- Genetic variability study
  - Moderate daily heritability

➔ Potential of selection for TA

Next steps

- Improvement with new samples
- Study of genetic correlations of TA with
  - milk production traits
  - other milk components
  - milk properties
- Optimum for TA in milk ?

Thank you for your attention

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