



## 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
- (Formaggiani et al., 2001; Summer et al., 2002)*

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## 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)*

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## 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)

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## 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)

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## 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)

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## MIR chemometric method

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

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## 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

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## 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**

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## MIR chemometric method

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

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## MIR chemometric method

- Calibration equation
  - Statistical parameters to assess the accuracy
    - ✓ Standard error of cross-validation ( $SE_{cv}$ ) =  $0.80 D^\circ$
    - ✓ 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**

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## 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

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## Genetic variability study

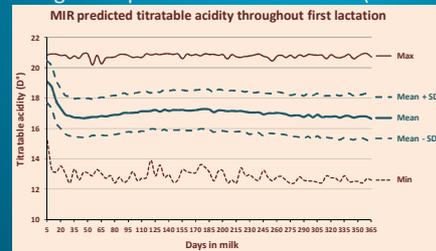
- Data editing
  - After edits:
    - ✓ 10 457 first-parity Holstein cows from 153 herds  
cows with  $\geq 3$  TA predictions and known parents  
> 65 000 animals in pedigree file
    - ✓ > 93 000 records for milk, fat, and protein traits
    - ✓ > 92 000 records for somatic cell score (SCS)
    - ✓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

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## Genetic variability study

- Data
  - Average MIR predicted TA = 17.03 D° ( $\pm 1.36$  D°)



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## 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

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## 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

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## Genetic variability study

- Single-trait random regression animal test-day model

$$y = X\beta + Q(Z_p + Z_a) + e$$

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

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## Genetic variability study

- Single-trait random regression animal test-day model

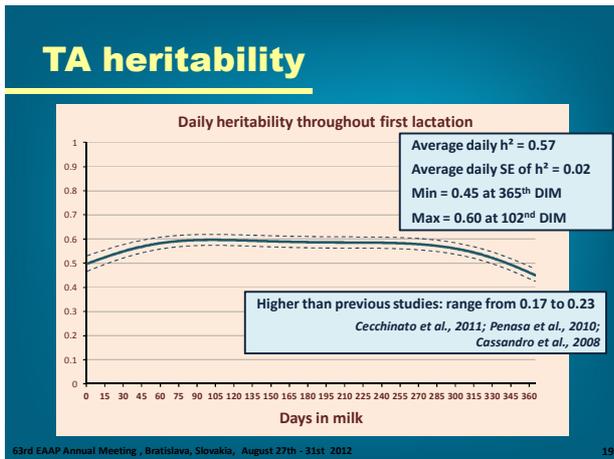
$$y = X\beta + Q(Z_p + Z_a) + e$$

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

- Variances components estimated by AIREMLF90 (Misztal, 2012)

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### 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

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### Conclusions

- Genetic variability study
  - Moderate daily heritability

→ Potential of selection for TA

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### 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 ?

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### Thank you for your attention

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  - Walloon dairy breeders

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