Prediction of blood B-hydroxybutyrate content in early-lactation **New Zealand dairy cows** using milk infrared spectra

V. Bonfatti¹, S.-A. Turner², B. Kuhn-Sherlock², C. Phyn², J. Pryce^{3,4}

🖂 valentina.bonfatti@unipd.it

Dept. Comparative Biomedicine & Food Science, University of Padova, Italy
DairyNZ Ltd., New Zealand
Agriculture Victoria, AgriBio, Australia
La Trobe University, Australia

Hyperketonemia

Blood β-hydroxybutyrate (BHB) concentration ≥ 1.2 mmol/L

- Increased occurrence of clinical ketosis, other health disorders, reduced fertility (Compton et al., 2015)
- Herd-level incidence ~68% in the first 5 wk of lactation (Compton et al., 2015)



- National infrared predictions of milk BHB not yet available in NZ
- An alternative is to predict blood BHB concentration

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Objective

To evaluate the ability of milk IR spectra to predict

the concentration of BHB in blood

the occurrence of hyperketonemia

in pasture-grazed, early-lactation New Zealand dairy cows

For large-scale phenotyping for selective breeding

► for on-farm management purposes



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Dataset

- ▶ 553 cows (HO and HOxJE)
- > 2 farms (seasonal-calving, pasture-based dairy system)
- Milk infrared spectra collected once a week → Milko-Scan FT1 (Foss Electric A/S, Hillerød, Denmark)
- Blood "prick" sample taken 3 times/wk (1-5 wk of lactation)
- Sampling at 7 am, before fresh allocation of pasture and supplementary feed
- ► BHB in blood measured using FreeStyle Optimum[™] Blood Glucose Monitoring System (Abbott Diabetes Care Ltd., UK)
- June October 2016



Data analysis

- Average of the 2 measures of blood BHB closest to spectra acquisition
- The regions of the spectra between 1,628 and 1,658 cm⁻¹, 3,105 cm⁻¹ and 3,444 cm⁻¹, and 2,966 to 5,010 cm⁻¹ were removed
- After outlier elimination
 - 1,910 spectra + BHB
 - from 542 cows

Spectra transformed using EMSC + 1st derivative

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Quantitative prediction models

- PLS regression with a 10-fold cross-validation (R package PLS, Mevik & Wehrens, 2007)...
- ► 2/3 of the cows in calibration and 1/3 in validation, randomly selected → 10 replicates
- All the records from a cow were in either the calibration or the validation subset

	N Cows N spectra	
Calibration	360	1,267
Validation	182	643

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Fitting of quantitative models

Average fitting statistics (SD) obtained across 10 calibration-validation partitions

	Ν	#terms	RMSEP	R ²
Calibration	1,267 (11)	24 (4.1)	0.28 (0.01)	0.56 (0.03)
Validation	643 (11)		0.32 (0.03)	0.50 (0.05)

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- N: number of records in the datasets
- #terms: number of optimal partial least square components
- RMSEP: root mean squared error of prediction

Discriminant models

- Partial least squares discriminant analysis (PLS-DA; Lê Cao et al., 2011), using the R package mixOmics (Rohart et al., 2017)
- 1.2 or 1.4 mmol/L used as a diagnostic reference
- Models developed and tested on the same calibration-validation sets created for testing the quantitative prediction models

Statistics:

- Global accuracy
- Sensitivity
- Specificity

- Area under the curve (AUC)
- Positive predicted value
- Negative predicted value

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Thresholds BHB > 1.2 mmol/L $BHB \ge 1.4 \text{ mmol/L}$ Calibration Validation Calibration Validation Prevalence, % 10.1 10.8 6.3 7.1 Global accuracy, % 84.9 84.0 81.8 87.4 Sensitivity, % 81.9 76.2 85.2 76.3 Specificity, % 84.3 82.5 87.5 85.4 Positive predicted value, % 34.6 31.5 28.2 37.1 Negative predicted value, % 97.6 98.9 98.0 98.0 AUC, % 94.8 90.9 91.6 88.0

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	Thresholds				
	BHB \geq 1.2 mmol/L		BHB \geq 1.4 mmol/L		
	Calibration	Validation	Calibration	Validation	
Prevalence, %	10.1	10.8	6.3	7.1	
Global accuracy, %	84.0	81.8	87.4	84.9	
Sensitivity, %	81.9	76.2	85.2	76.3	
Specificity, %	84.3	82.5	87.5	85.4	
Positive predicted value, %	37.1	34.6	31.5	28.2	
Negative predicted value, %	97.6	98.9	98.0	98.0	
AUC, %	91.6	88.0	94.8	90.9	

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Limitations

- Blood metabolites vary considerably in time (reliability of the reference measures)
- Possible time lag between the release of metabolites in blood and modification of milk composition
- Reference values produced by the ketone meter are one-digit values (discrete vs continuous variation)
- The dataset included samples from 2 farms and 1 season only (pasture quality and quantity impact on cow performance)



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Conclusions

The developed prediction models might be used to provide breeding organizations with indicator traits for ketosis

Potential use as a management tool in New Zealand

Infrared spectroscopy will not provide accurate measurements at an individual level, but it can provide information at herd level Dairy

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What's next

- More samples and farms!
- Measures of other blood metabolites
- Estimates of genetic parameters of the predicted blood BHB and its relationship with production and reproduction
- Genomic predictions for BHB concentration
- Application of other existing calibration equations for milk or blood ketone bodies to New Zealand milk samples ?
- Join reference data from different countries to create more robust equations ?

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