Genotype plus Environment
Integration for a more sustainable dairy production system

A first approach to predict nitrogen efficiency of dairy cows through milk FT-MIR spectra

C. Grelet[1], E. Froidmont[1], M. Hostens[2], A. Vanlierde[1], L. Foldager[3], M. Salavati[4], K.L. Ingvartsen[3], M.T. Sorensen[3], M. Crowe[5], C.P. Ferris[6], C. Marchitelli[7], F. Becker[8], GplusE consortium[9] and F. Dehareng[1]

[1] CRA-W, Gembloux, Belgium
[2] Ghent University, Merelbeke, Belgium
[3] Aarhus University, Tjele, Denmark
[4] RVC, London, United Kingdom
[5] UCD, Dublin, Ireland
[6] AFBI, Belfast, Northern Ireland
[7] CREA, Roma, Italy
[8] FBN, Dummerstorf, Germany
[9] http://www.gpluse.eu/index.php/project/partners/

c.grelet@cra.wallonie.be

Global objective of GplusE project

Optimise efficiency, fertility, health and environmental footprint of dairy cows:

First step:

Develop models predicting **phenotypes of interest** based on easily-measured and large scale **biomarkers in milk**:

→ **Milk MIR spectra**

→ **...**

Final steps:

Genomic study : to relate genotype to phenotypes of interest
~15 000 cows

→ **Breeding**

Management study: new management strategies at herd and cow level to improve phenotypes of interest
~600 cows

→ **Advisory tools**

Phenotype of interest : N efficiency

- High cost of protein
  → Poor N efficiency is affecting profitability (Powell et al., 2010)

- Environmental impact : ammonia and oxides → GHG (Muck and Steenhuis, 1992)
  leaching in water resources → Eutrophication (Ledgard et al., 1998)

- Potential negative impact on reproductive performances (Butler, 2000)

- Potential negative impact on milk processing quality (Hermansen et al., 1999)

Biomarker : MIR

- Used in routine to predict composition for milk recording and milk payment
- Fast
- Cheap

Milk recording

MIR analysis

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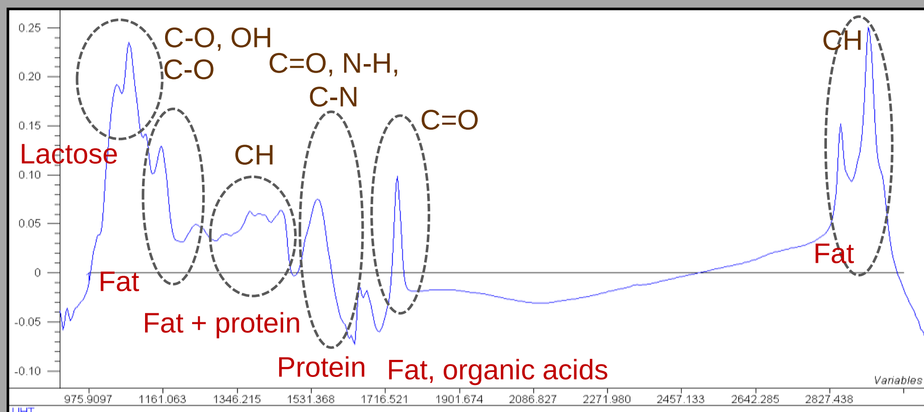
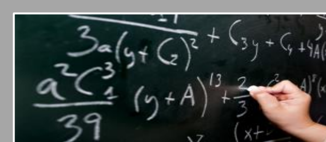


+ Models



Milk composition

- **-Fat**
- **-Proteins**
- **-Urea**
- **-Lactose**

...

Predict N efficiency by milk MIR?

$$\text{N Efficiency} = \frac{N\ Out\ Milk}{N\ In\ Feed}$$

✓ MIR

✓ MIR

Materials & Methods

Experiments

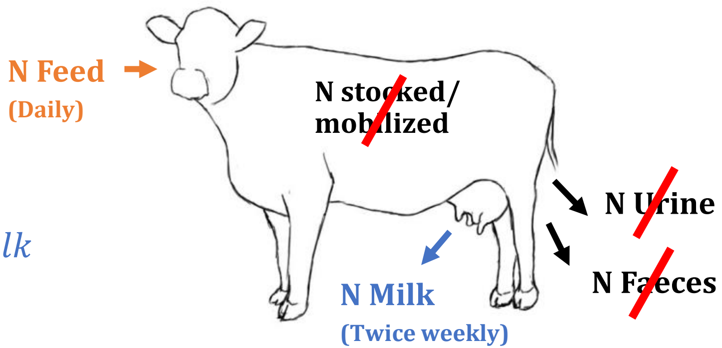
- 3 experimental farms
- Common sampling protocol
- 136 cows
- Holsteins
- **Early lactation:** Calving to DIM 50

	# Cows	PP	MP	MY	Diet 1	Diet 2	Diet 3
AFBI (UK)	62	18	44	31.6	Standard (50% Cc)	High Cc (70% Cc)	Low Cc (30% Cc)
AU (Denmark)	35	11	24	35.5	Standard	Ketosis (High sugar)	Acidosis (High starch)
UCD (Ireland)	39	3	36	30.5	Standard		
TOTAL	**136**	**32**	**104**	**32.3**			

N Data

- Nitrogen in feed = crude protein content/6.25 (kg/day)
- Nitrogen in milk = total nitrogen/6.38 (kg/day)

$$\text{N Efficiency (\%)} = \frac{\textit{N Out Milk}}{\textit{N In Feed}} * 100$$



$$\text{N losses (kg/day)} = \textit{N In Feed} - \textit{N Out Milk}$$

N mobilized unknown : efficiency potentially overestimated (and losses underestimated*) for cows mobilizing body proteins in early lactation

*(But ***N Feed*** – ***N Milk*** correlated at r=0.98 with real ***N Urine + N Faeces*** in Olmos-Colmeneros et al., 2006)

MIR data

- Milk samples analyzed locally or at CRA-W (Belgium)
- Twice per week
- AM & PM (weighted average)

- Foss and Delta (standardized*: Grelet et al.,2015*)

- Merging of spectral data with N data of the same day

MIR calibrations

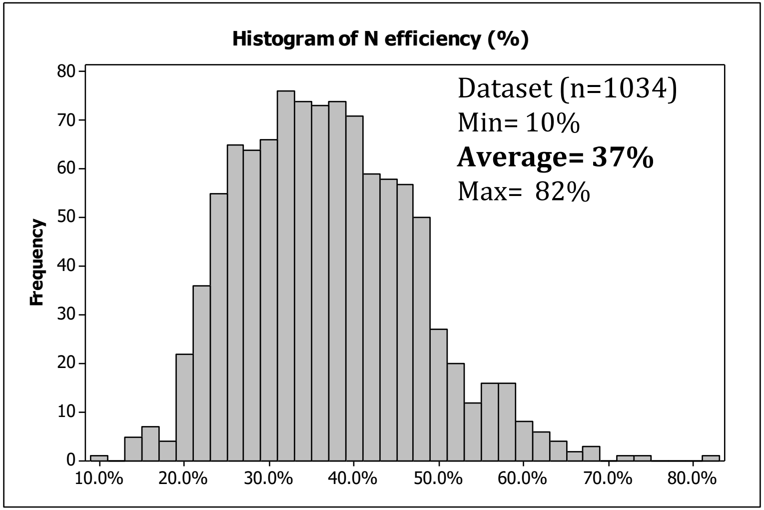
- Dataset : 1034 data from 131 cows (≈ 8 samplings from DIM0-DIM50)
- No removing of outliers
- Predictors
  - MIR spectra
  - Parity
  - MY
- Method
  - PLS: Partial Least Square - Linear method
  - SVM: Support vector machine - Nonlinear Method
- Evaluation of the model
  - Cross-validation: 10% of data removed randomly *(A cow can be in calibraton and in validation dataset)*
  - External-cow-validation: 25% of the cows randomly removed *(still similar diets in calibraton and in validation dataset)*
  - External-diet-validation: diets removed one by one.

Results

Descriptive statistics

Descriptive statistics

- **N efficiency distribution (individual data)**



Literature averages:
Castillo et al., 2000: **8 - 42%**
Olmos Colmenero et al., 2006: **25 - 37%**
Nadeau et al., 2007: **18 - 40%**

Early lactation:
Cowan et al., 1981 (DIM 1 – 112): **35%**
Law et al., 2009 (DIM 1 – 151): **39%**

Extreme data→ Individual data vs means at herd/period levels?

High efficiency → **Negative N balance in early lactation?** (Cowan et al., 1981; Komaragiri & Erdman., 1997; Sutter & Beever., 2000;)

Descriptive statistics

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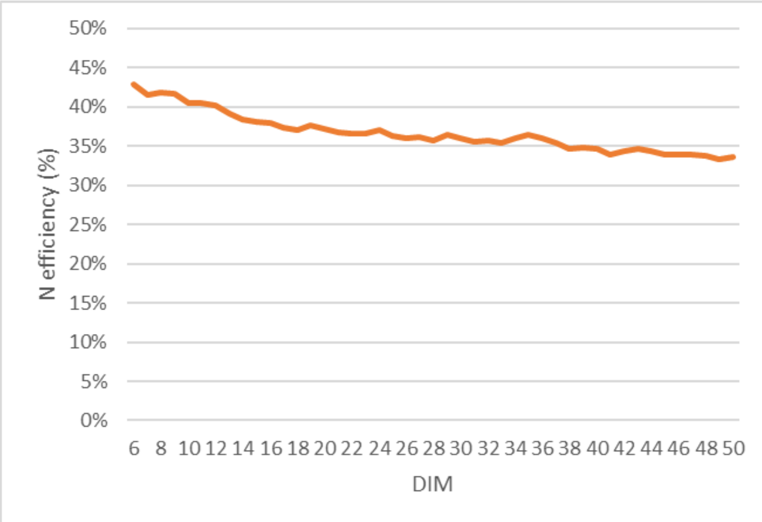

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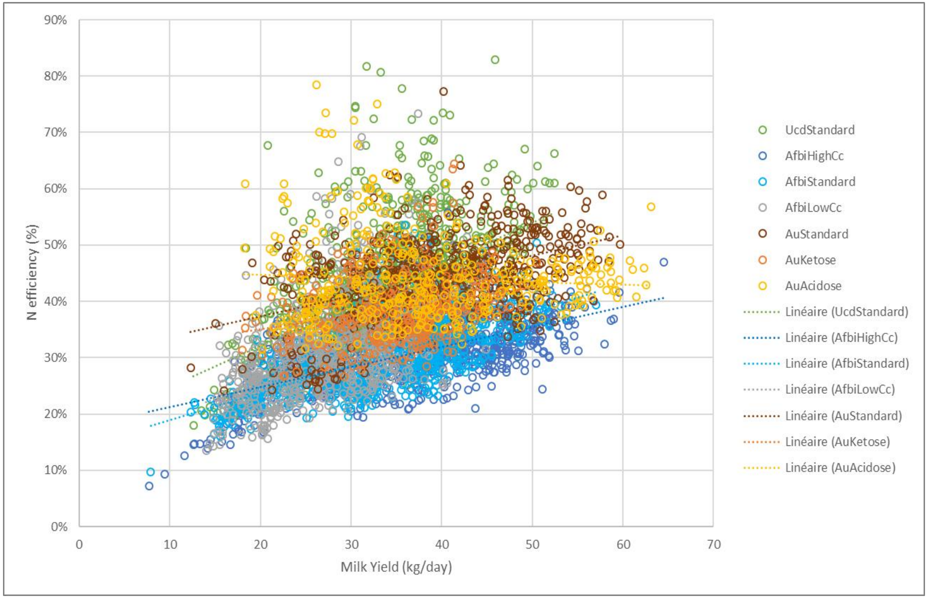
Descriptive statistics – main correlations

r	N efficiency %
Parity	0.23
DIM	-0.24
Weight (kg)	0.27
Milk Yield (kg)	0.44
Fat milk (%)	-0.04
Protein milk (%)	0.19
N Milk (kg/day)	0.49
DMI (kg/day)	-0.19
Energy Intake (Mcal/day)	-0.15
N Intake (kg/day)	-0.41
Crude Protein in Feed (%)	-0.60
Energy Balance (Mcal/day)	-0.65



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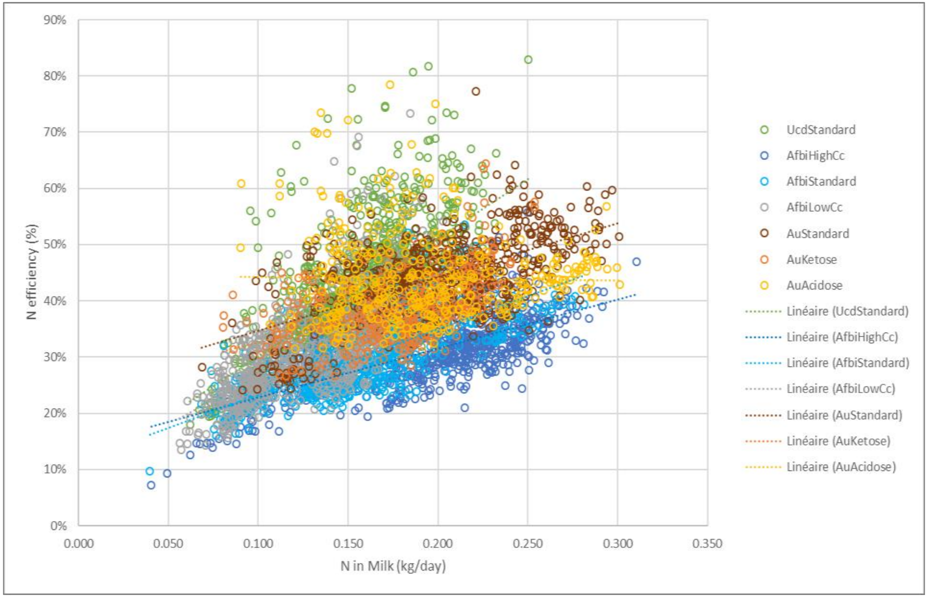

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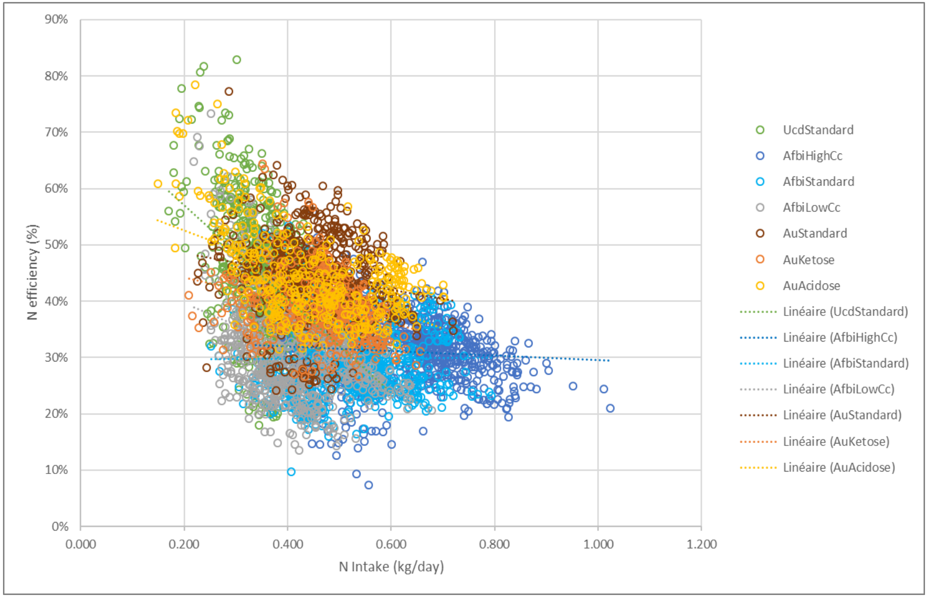

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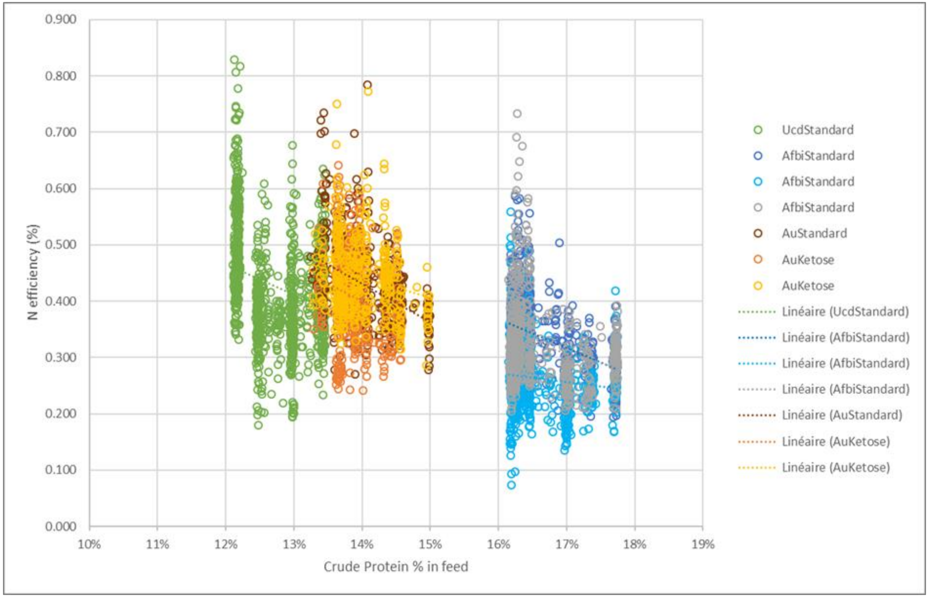

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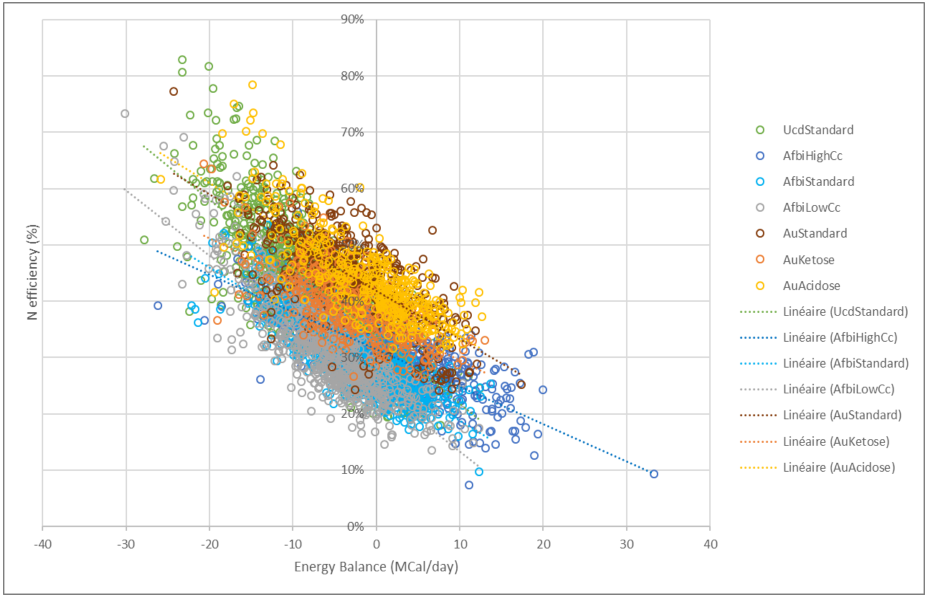
Low DMI + High MY → NEB

→ Real N Efficiency or Negative N balance ?

Need for N balance data (urine & faeces)



Results

MIR calibrations

MIR calibrations – N efficiency (%)

- **Cross-validation** (removing randomly 10% of the data)

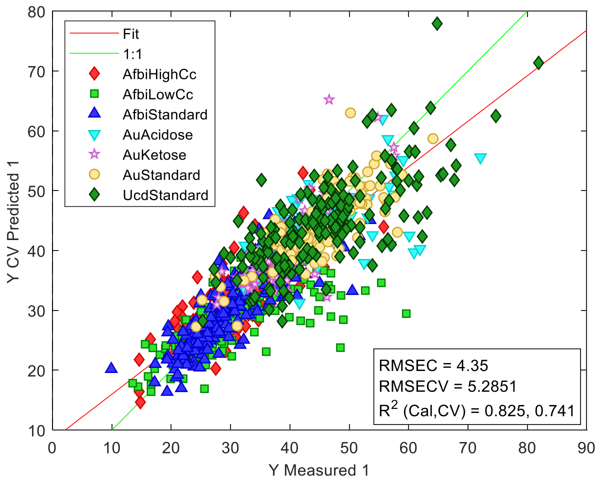
  → *A cow can be in calibration and in validation dataset*

- 1034 data

Method	X predictors	R^2cv	Error (RMSEcv)	Relative error (RMSEcv/mean)
PLS	MIR	0.59	6.6	**18%**
PLS	MIR+Parity	0.62	6.4	**17%**
PLS	MIR+Parity+MY	0.72	5.5	**15%**
SVM	MIR+Parity+MY	0.74	5.3	**14%**

→ Possibility to estimate N efficiency with fair accuracy

SVM model



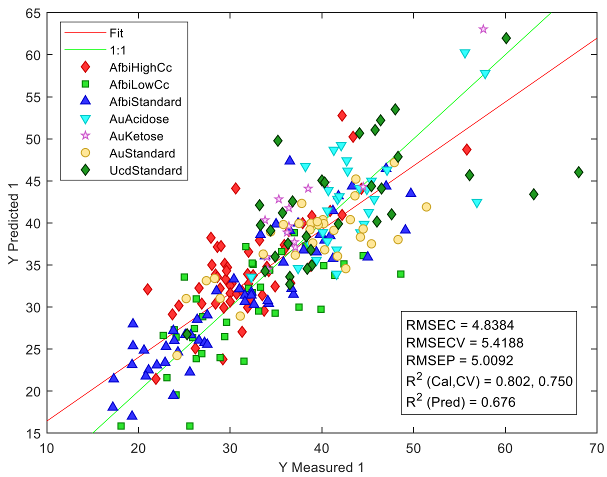
MIR calibrations – N efficiency (%)

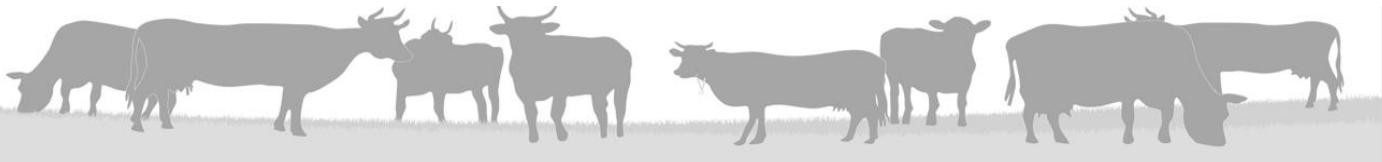
- **External-cow-validation** (removing randomly 25% of the cows)
- Calibration dataset : 779 data
- External dataset : 255 data

Method	X predictors	R^2cv	Error (RMSEcv)	Relative error (RMSEcv/mean)
SVM	MIR+Parity+MY	0.68	5.0	**14%**

→ Performances still good for other cows.
It confirms the potential of the method

SVM model





MIR calibrations – N efficiency (%)

- **External-diet-validation** (removing each diet when performing the model)
- Calibration dataset : 6 diets
- External dataset : the removed diet

	Error (RMSEcv)	Relative error (RMSEcv/mean)
Afbi HighCc	6.67	18%
Afbi LowCc	8.13	22%
Afbi Standard	4.38	12%
Au Acidose	6.95	19%
Au Ketose	7.51	20%
Au Standard	5.96	16%
Ucd Standard	12.58	34%
	7.45	20%

→ Models fairly succeed to predict AFBI and AU Standard diets

→ Increased errors for special diets and for UCD herd. Robustness to be increased

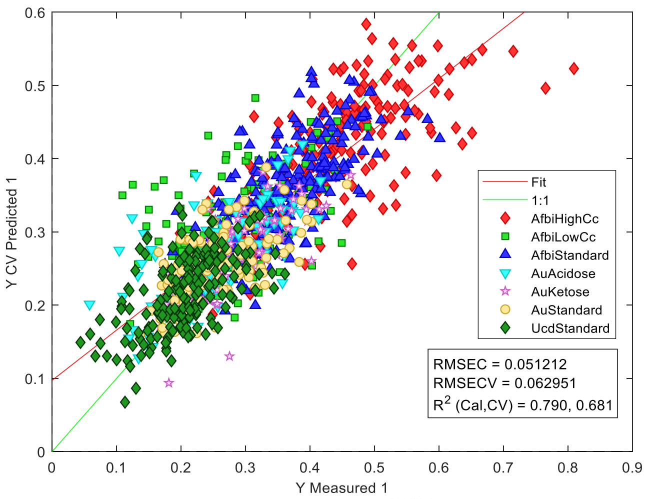
MIR calibrations – N losses (kg/day)

- **Cross-validation** (removing randomly 10% of the data)
- → A cow can be in calibration and in validation dataset
- No removing of outlier
- 1034 data

Method	X predictors	R^2cv	Error (RMSEcv)	Relative error (RMSEcv/mean)
PLS	MIR	0.60	0.071	23%
PLS	MIR+Parity	0.62	0.069	22%
PLS	MIR+Parity+MY	0.62	0.069	22%
SVM	MIR+Parity+MY	0.68	0.063	20%

→ Possibility to estimate N losses with fair error

SVM model



Conclusions

- Preliminary study: results to be validated
- Very early lactation: N balance to validate with urine and faeces data

- Seems possible to predict fairly N efficiency & N losses ($R^2cv \approx 0.74$ & 0.68) from FT-MIR spectra of milk

  BUT:

  - Robustness to be increased with other herds → collaboration?

- Test in genomic studies
- Test for management strategies

  BUT:

  - Need to take N (and energy) balance into account

Thank you for your attention!

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Descriptive statistics

- **Characteristics of herds and diets (means)**

	FPCM (kg/day)	Protein milk %	DMI (kg/day)	Weight (kg)	EnergyBalance (Mcal/day)	N Intake (kg/day)	N Feed %	N efficiency %	N Losses (kg/day)
Afbi									
HighCc	38.1	3.33	23.5	633	0.73	0.629	2.7%	31%	0.434
LowCc	27.1	2.94	15.4	596	-5.72	0.408	2.6%	32%	0.282
Standard	32.8	3.09	19.8	604	-2.22	0.523	2.6%	30%	0.367
Au									
Acidose	35.7	3.29	20.3	600	-1.21	0.448	2.2%	44%	0.256
Ketose	35.9	3.21	20.2	607	-3.00	0.462	2.3%	39%	0.284
Standard	37.8	3.27	20.6	594	-2.94	0.456	2.2%	44%	0.255
Ucd									
Standard	34.5	2.99	18.5	655	-6.87	0.380	2.0%	42%	0.223

Descriptive statistics

- **Really efficient cows of negative N balance?**

N Efficiency group	DIM	FPCM (kg/day)	N Milk (kg/day)	DMI (kg/day)	Weight loss w2-w6 (kg)	Energy Balance (Mcal/day)	Protein Feed %	N Intake (kg/day)
- 30%	30	27.31	0.129	19.54	0.83	1.99	16%	0.513
30 -40%	27	35.33	0.173	20.66	1.63	-2.74	15%	0.498
40 -50%	24	37.61	0.189	19.24	-24.96	-6.31	14%	0.427
+50%	21	39.42	0.197	16.48	-18.18	-12.44	13%	0.354

→Need for N balance data (urine and faeces)