Natural ^{15}N abundances in plasma and urea-N concentration in milk as biomarkers of urinary N excretion in dairy cows: a meta-analysis

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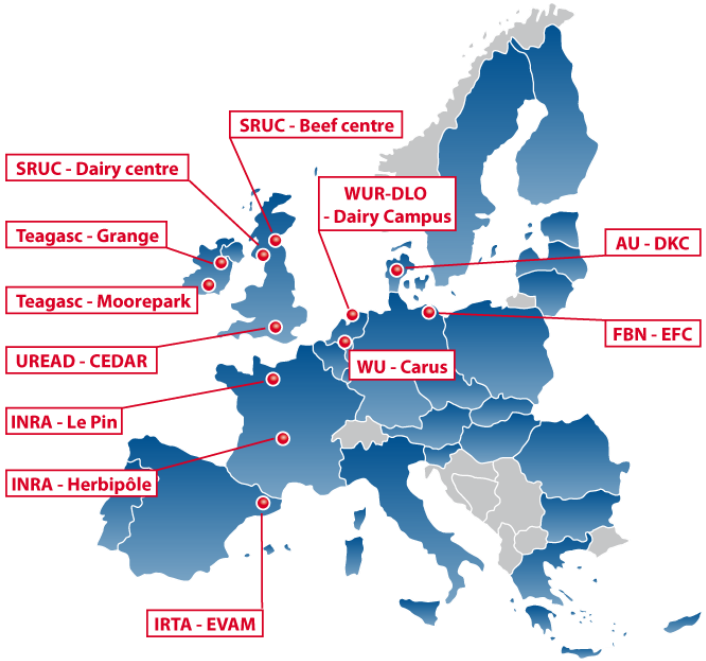
an integrated infrastructure for increased research capability and innovation in the European cattle sector



Domain:

7 countries; 11 research infrastructures; 3500 cattle

Objective:

Integration of research infrastructure for the EU cattle sector

Work package 6:

Developing and evaluating promising biomarkers to predict gold standard methods

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Introduction

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Nitrogen utilization in ruminants

- **Ruminants play a critical role in converting low-quality nitrogen (N) sources into high quality protein**
- **The efficiency of conversion depends on the balance between nutrient supply and animal requirements**
- **Low efficiency results in high N excretion having environmental and economical consequences**

How N excretion can be measured?

Gold standard method (GSM): Total collection of separated urine and feces

But with several constraints:

- **Costly and labor intensive**
- **Error of measurements**
- **Not be able to conduct on large numbers of animals (genetic objective) and for long time**
- **Animal welfare issues**

Prediction by promising biomarkers as alternative for GSM?

Potential biomarkers for urinary N excretion

BIOMARKERS	Mechanisms	Advantages	Drawbacks
MUN: milk Urea-N **BUN: blood Urea-N** ***(Hof et al., 1997)*** ***(Huhtanen et al., 2015)***	**N from rumen or body catabolism converted to urea** **Urea is soluble in blood, milk and urine**	**Easy to measure** **Evaluation of N excess in the diet**	**Diurnal variations** **Not detecting individual variability**
$\Delta^{15}N_{animal-diet}$: **$^{15}N$ enrichment of animal proteins over the diet** **(N isotopic discrimination)** *New/Promising*	**Common pathways:** **Hepatic transaminase** ***(Cantalapiedra-Hijar et al., 2015)*** **External transport of NH3 by rumen bacteria** ***(Wattiaux and Reeds, 1997)***	**No diurnal variations (stable)** **Detection of Individual variability**	**Time consuming analysis** **Diet dependent responses?** **(i. e. High rumen NH3)**

Potential of combination?

Objective

Evaluation of $\Delta^{15}N_{animal\text{-}diet}$ alone or in combination with urea in milk or blood as a biomarker of urinary N excretion in dairy cows

Materials and Methods

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Preliminary dataset

Criteria

1. **Dairy cows**
2. **Measuring urinary N excretion (UNE) : Total collection for urine for ≥3 d**
3. **Measuring either $\Delta^{15}N$ or both $\Delta^{15}N$ and MUN (BUN)**

Value	Experiment	diet	observation
n	9	34	177

Cows and diets description

Item	Mean	SD	Min	Max
Cow performances				
DIM, d	103	55.1	61	221
MY, kg/d	26.6	7.0	13	33
BW, kg	583.6	182.5	200	754
Diet characteristics, % of DM				
Forage	71.1	18.3	56	100
CP	16.3	3.3	11.0	24.5
NDF	41.8	7.5	25.5	58.9

Gold standard measurement and biomarkers description

Item	Mean	SD	Min	Max
Urinary N, g/g N intake	**0.33**	0.12	0.13	0.86
$\Delta^{15}N$, ‰	**3.27**	1.06	1.65	6.15
MUN, g/L	**0.12**	0.06	0.006	0.27

- **To increase comparability among experiments, values of UNE were adjusted for the amount of N intake**
- **Values with >3 SD difference from mean were remove if biological reason justified their elimination**

Statistical analysis: Mixed-model regression

$$\text{Urinary } N_{ij} = (\beta 0 + b0_i) + (\beta 1 + b1_i)\,\Delta^{15}N_{ij} + (\beta 2 + b2_i)\,MUN_{ij} + \varepsilon_{ij}$$

- $\beta 0$ and $\beta 1$, $\beta 2$ are the fixed effects for the intercept and slopes, respectively.
- The b_i are the random effects of grouping factors (experiment/period/diet).
- ε_{ijk} is errors of the model.

- Random effects were tested on intercept, slope or both and models compared based on AIC/BIC and log likelihood ratio criteria.
- Collinearity between both biomarkers was checked using variance inflation factor (VIF)

Results

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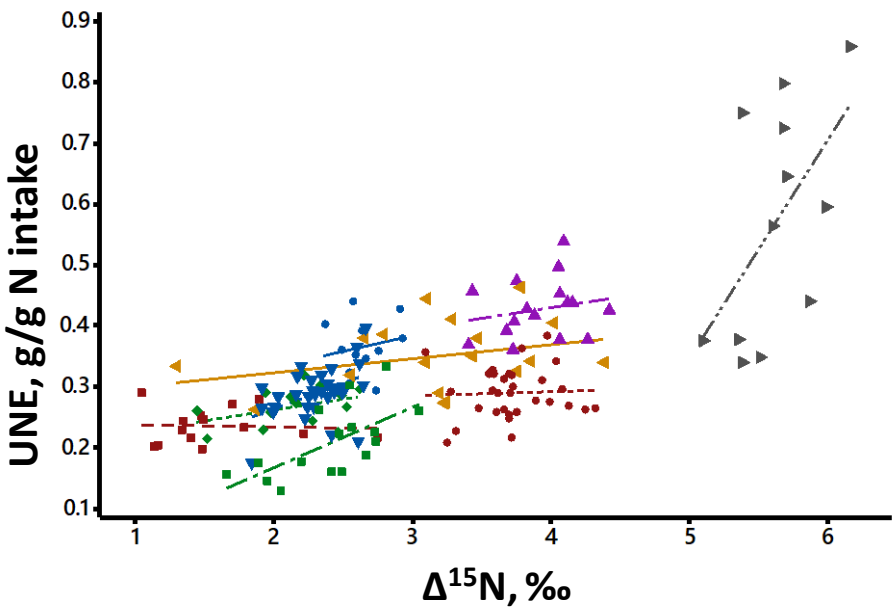
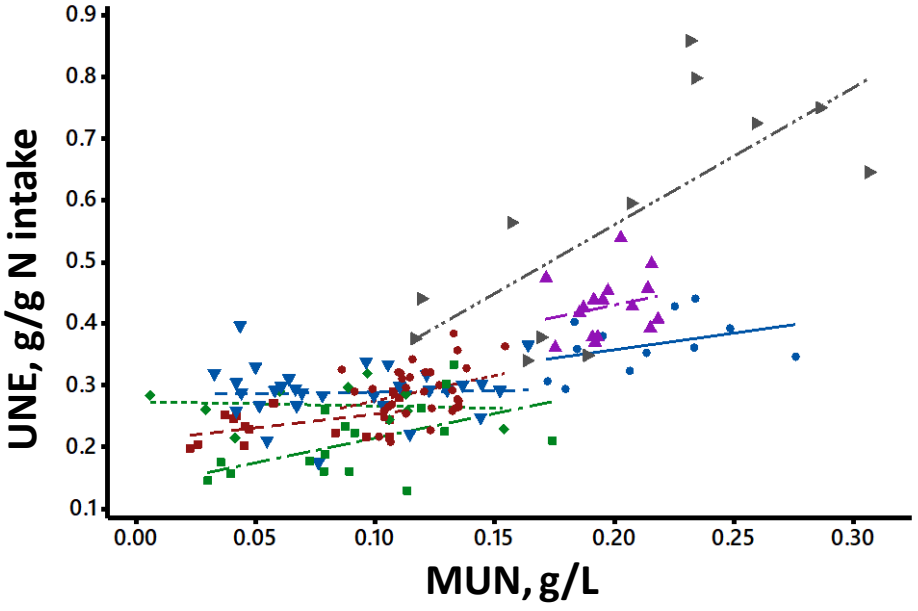
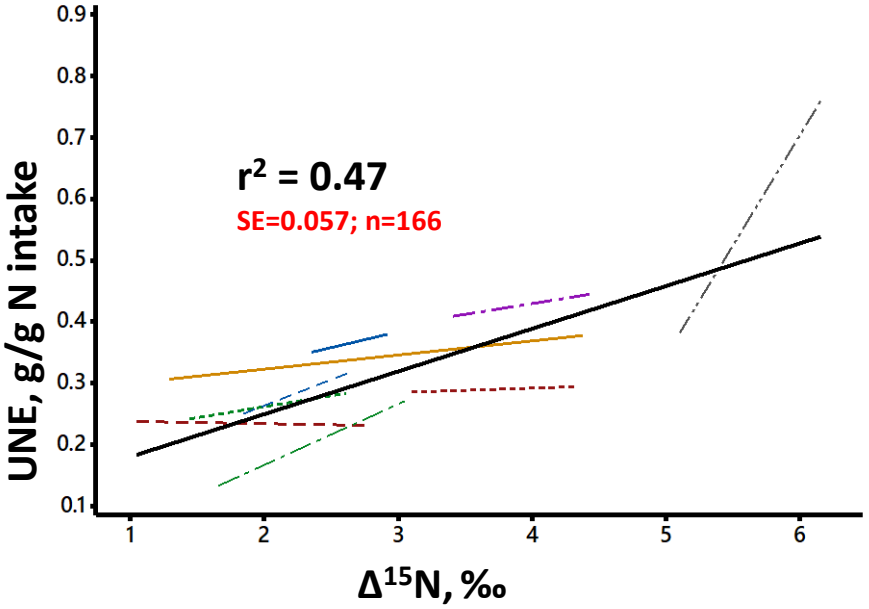
Relationship between urinary N excretion (UNE) and $\Delta^{15}N$ or MUN

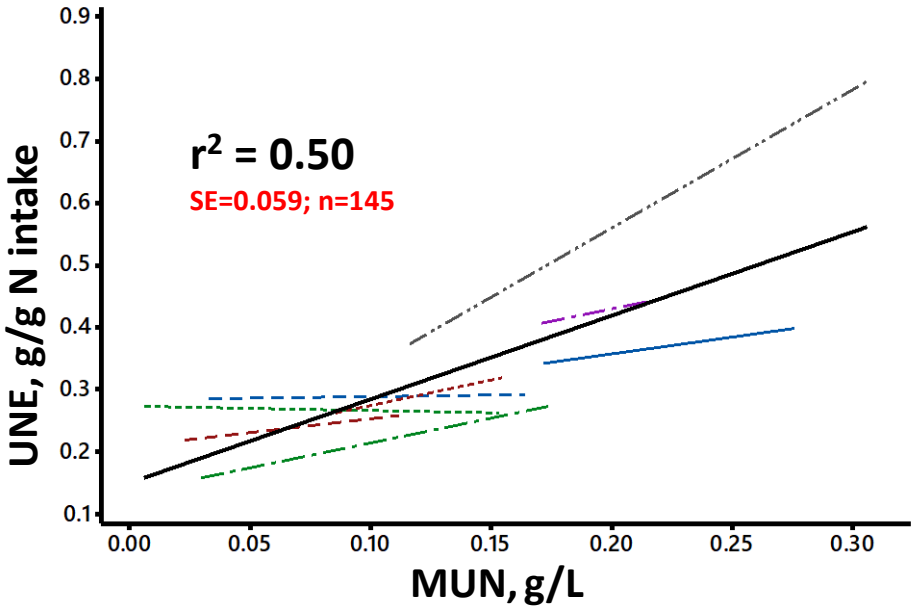




Colored lines are individual experiments; colored symbols are individual observations

Relationship between urinary N excretion (UNE) and $\Delta^{15}N$ or MUN





Colored lines are individual experiments; The solid line is the overall relationship

Fitted equations for urinary N excretion (UNE): the output of mixed model analysis

1) Urinary N $_{(g/g\ of\ N\ intake)}$ **= 0.183 + 0.045** $\Delta^{15}N$ $_{(‰)}$

residual SD%= 13.9; P<0.01

2) Urinary N $_{(g/g\ of\ N\ intake)}$ **= 0.288 + 0.313 MUN** $_{(g/L)}$

residual SD%= 14.6; P<0.01

3) Urinary N $_{(g/g\ of\ N\ intake)}$ **= 0.146 + 0.043** $\Delta^{15}N$ $_{(‰)}$ **+ 0.339 MUN** $_{(g/L)}$

residual SD% = 13.6; P<0.01

Conclusions

- Both $\Delta^{15}N$ and MUN had a potential to predict urinary N
- Combination of $\Delta^{15}N$ and MUN may strengthen the prediction capacity of the model

Perspectives

- Increasing of the dataset size for 3-4 times with new experiments
- Analyze interfering factors
- Model validation for its use in field conditions: guidelines

Thank you for your attention

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement n°730924.