# PREDICTING GLUTAMATE CONCENTRATION IN MILK USING MID-INFRARED SPECTROMETRY FOR ROUTINE DETECTION OF ENERGY-DEFICIENT COWS


***M. El Jabri, A. Leduc, M. Boutinaud, F. Le Provost, A. Fischer, C. Leroux, J. Pires and M. Gelé***

***Marine.gele@idele.fr***



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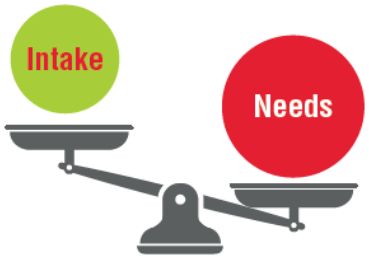
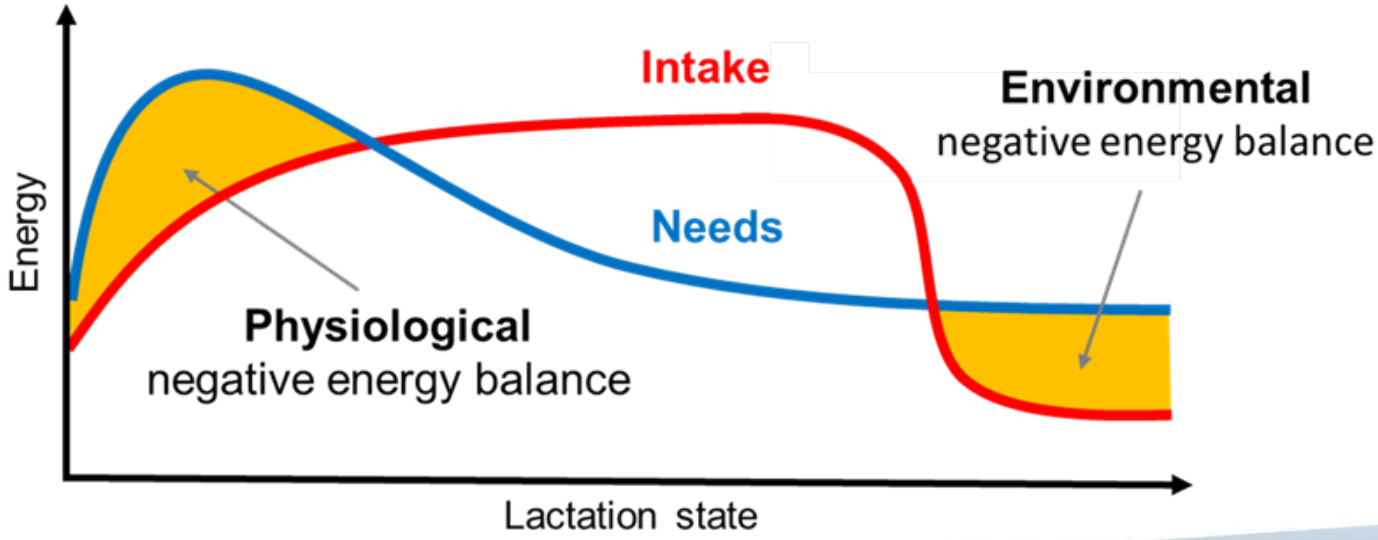
# ENERGY DEFICIT IN DAIRY COWS

**Negative energy balance**

**= Energy deficit**




State reached by the cow when the **energy provided by its feed** does not cover **its energy requirements**.



# ENERGY DEFICIT IN DAIRY COWS

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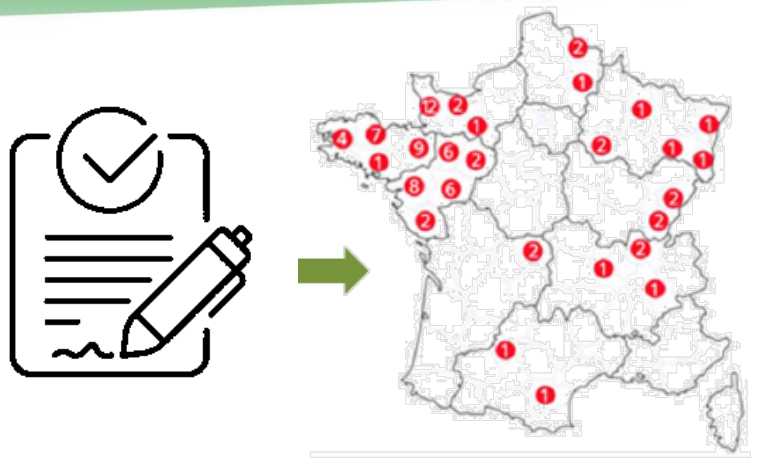
**Negative impact** on dairy farm's profitability when the deficit is **severe** or **long-lasting**

- Reduced milk production
- Impaired health
- Reproductive problems

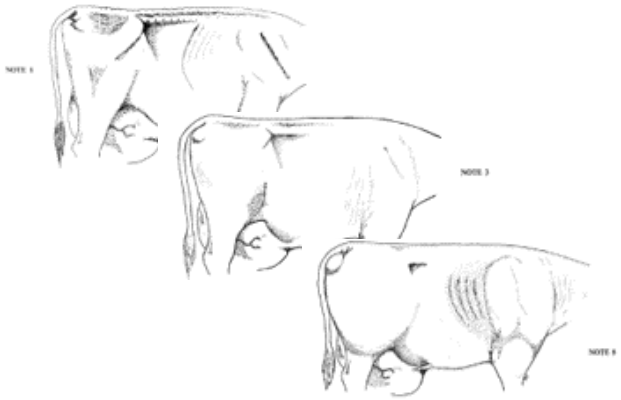


**Survey of 67 dairy farmers**

- Half of the farmers have observed cows in energy deficit
- Prevention and technical support help avoid severe deficits

*Pénasse et al., 2019*

# DETECTION OF ENERGY-DEFICIENT COWS



**Observation of body condition score:** used by farmers but too late

**Blood tests (non esterified fatty acids, β-hydroxybutyrate…):** accurate but invasive

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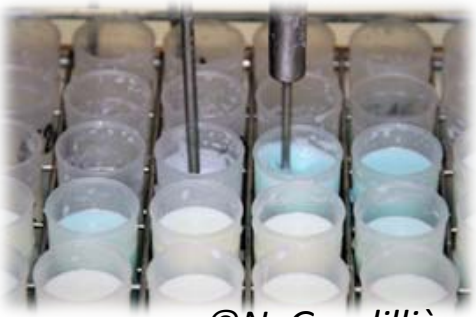

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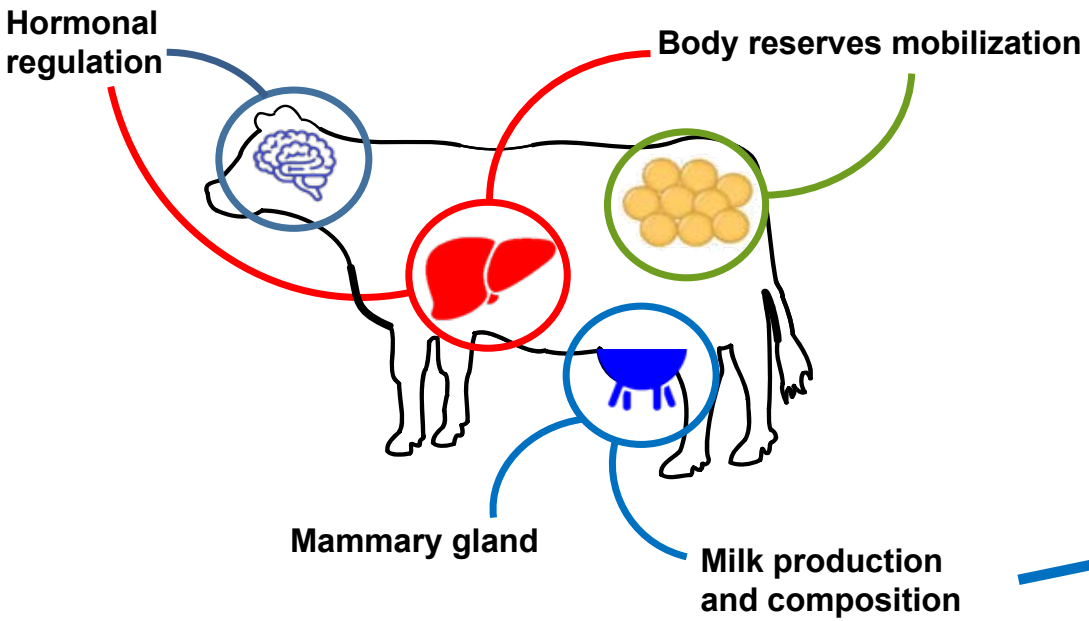
**Milk analysis:** easy-to-access but no specific indicator of energy deficit

→ **No individual and effective detection of energy-deficient cows**

## Negative energy balance affects metabolism and health

*Leduc et al., 2021*

*Journal of Animal Science*, 2021, Vol. 99, No. 7, 1–12




**Which milk components are affected by an energy deficit and could be interesting biomarkers?**

**Can we use these biomarkers to detect energy-deficient cows in routine?**

**Milk appears to be a source of potential energy-deficiency biomarkers**

# METHODOLOGICAL APPROACH

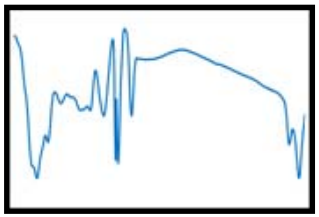
## 1. Identification of molecules affected by feed restriction in milk

✓Trials on INRAE experimental farms

✓Exploratory research on numerous milk components (milk metabolites, fatty acids, proteins and microRNAs…)

## 2. Selection of biomarkers of energy deficit

## 3. Prediction of biomarkers using mid-infrared spectrometry



✓Based on data collected during trials

✓Exploration of potential applications

## 3 feed restriction trials to identify milk components potentially biomarkers of energy deficit

### Short & Intense (SI)


Day : -3 ……13

n = 8
~165 days in milk

6-day restriction
-64 % DMI

INRAE UMR H
UE Herbipôle of Marcenat
*Billa et al., 2020*

### Long & Moderate (LM)

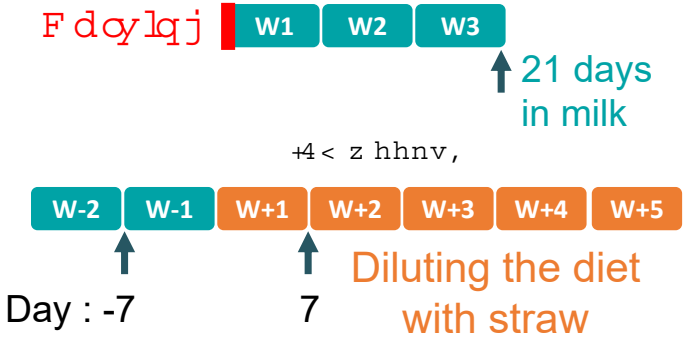

Day : -7 5 9 27 37

n = 10 restricted + 9 control
~77 days in milk

29-day restriction
-20 % DMI

INRAE UMR PEGASE
IEPL of Méjusseaume
*Hervé et al., 2019*

### Deffilait




n = 30

INRAE UMR PEGASE
IEPL of Méjusseaume
*Leduc et al., 2022*

Standard feeding  Restricted feeding  Milk and blood sampling  W: week

# IDENTIFICATION OF BIOMARKERS OF INTEREST

1. Trial-by-trial and component-by-component descriptive analysis
2. Integrative kinetic and multi-omics analysis

***Full results to be published*** *in a scientific article currently being written (Leduc et al.)*

**Identification of a panel of biomarkers including:**

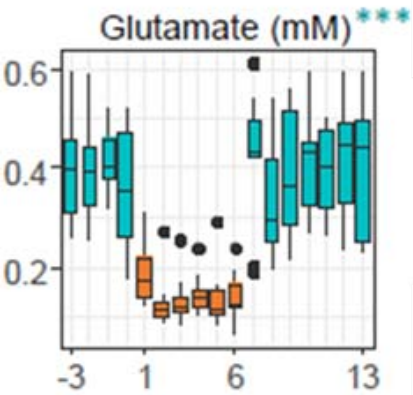
- milk macro-components,
- Proteins
- microRNAs
- metabolites, **including milk glutamate concentration**

## A potential biomarker of energy deficit

- ✓ Rapid **decrease during feed restriction**, then return to basal levels on ad libitum refeeding
- ✓ Positive **correlation with energy balance** (0.59)
- ✓ Effect of intensity of restriction on adaptive response
- ✓ No variation during an energy deficit at the **start of lactation**

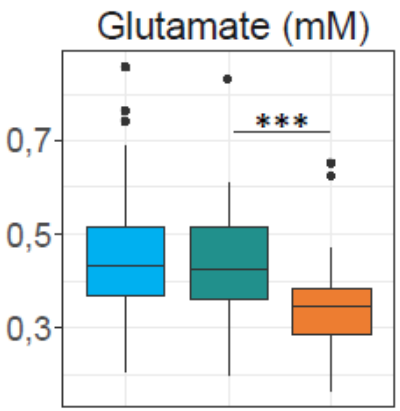
*Leduc et al., 2022*

**Short & Intense Trial**




Days from start of restriction

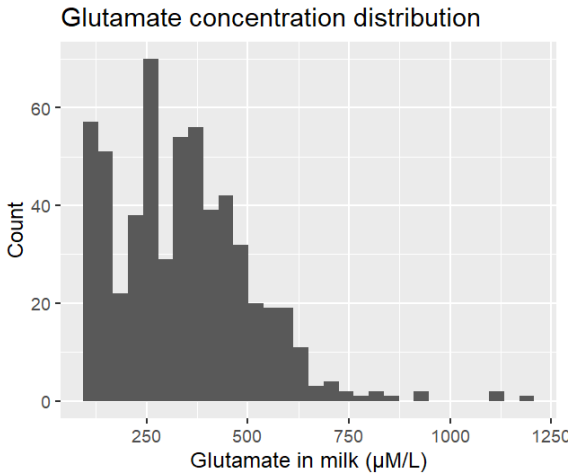
**Deffilait Trial**



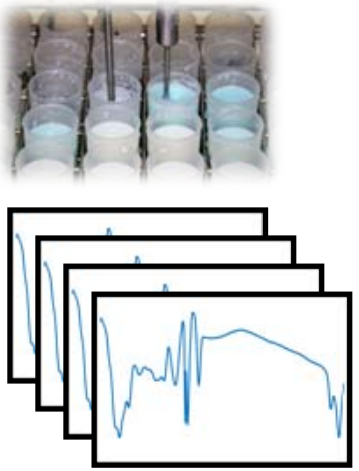

from start of restriction

## 577 MILK SAMPLES

| | n data | n cows | lactation number | days in milk |
|---|---|---|---|---|
| **Short & Intense trial** | 514 | 10 Montbeliarde + 8 Holstein cows | 2 to 7 | 114 to 215 days |
| **DEFFILAIT trial** | 63 | 26 Holstein cows | 1 to 6 | 22 to 205 days |



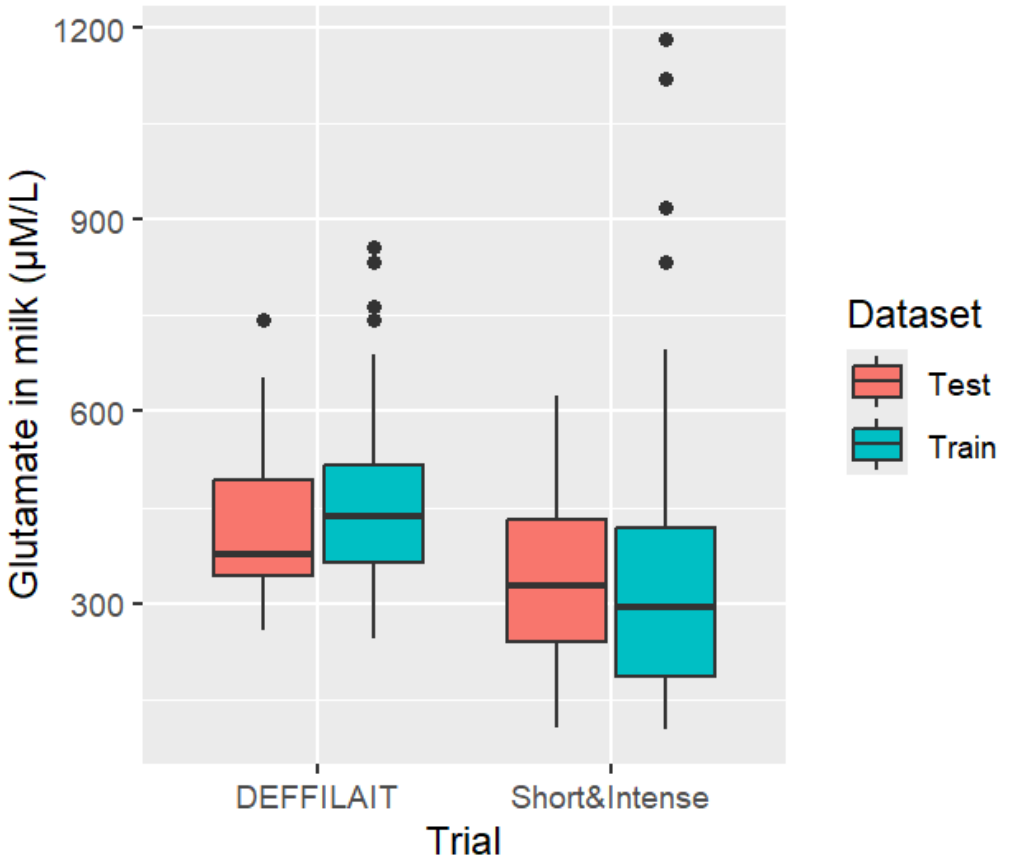

Enzymatic-fluorometric analysis of glutamate *(Larsen and Fernández, 2017)*



Extraction & standardisation of MIR spectra *(Grelet et al., 2015)*

## Random constitution of 2 datasets

- **Training set: 70 % of the data**
  - N = 406
  - Used to calibrate the PLS regression equation
- **Testing set: 30 % of the data**
  - N = 171
  - Used to validate the performances of equation

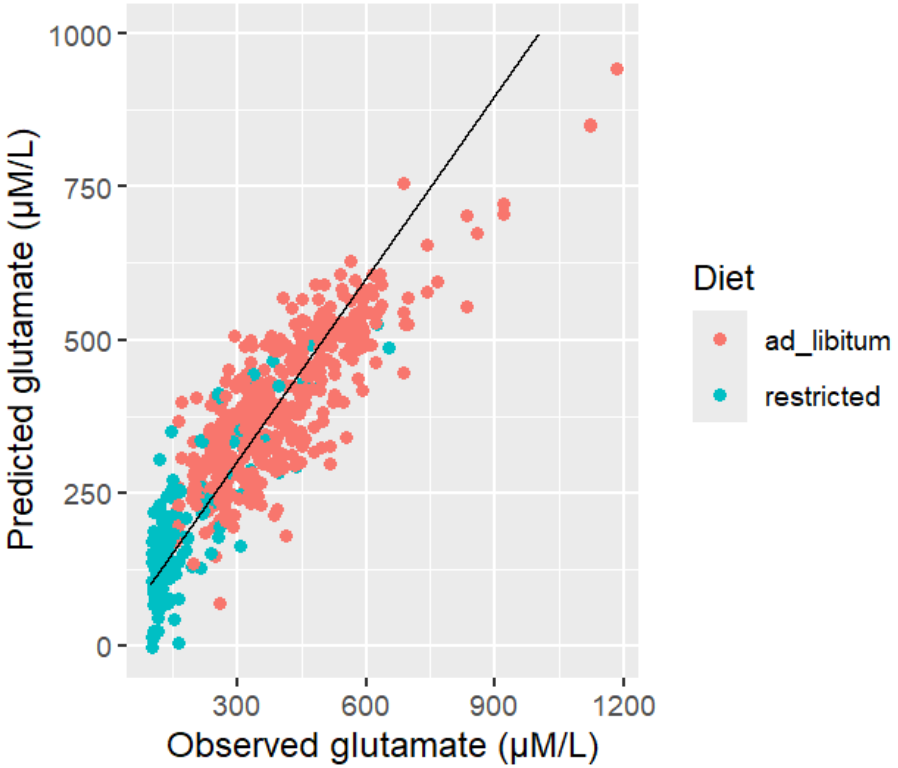


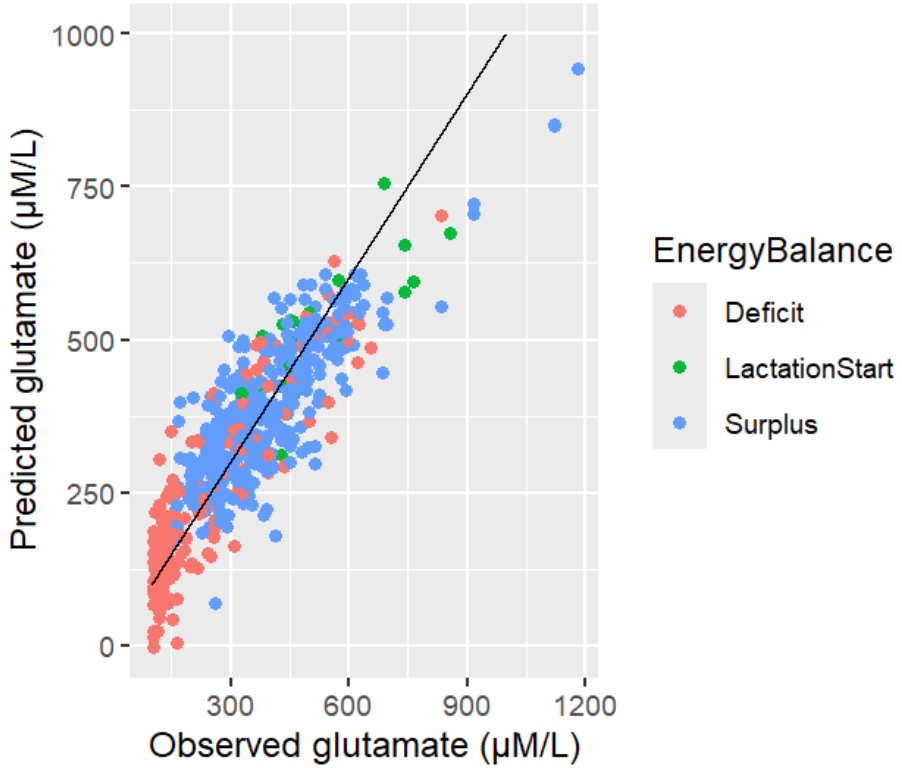
## Equation performances

| Method | Dataset | N | MEAN | SD | MEAN PRED | SD PRED | SD RESID | R2 | RPD |
|---|---|---|---|---|---|---|---|---|---|
| **SPLS** | **TRAIN** | 406 | 338.7 | 177.6 | 340.6 | 152.2 | 82.7 | **0.78** | **2.15** |
| **SPLS** | **TEST** | 171 | 346.0 | 138.9 | 348.2 | 123.4 | 82.5 | **0.65** | **1.68** |

- No similar equation in the literature

## Interesting prospects for detecting cows with an energy deficit due to feed restriction



## Predicting glutamate concentration in milk, a promising way of detecting energy-deficient cows

- ✓Milk glutamate appears to be an interesting biomarker of energy deficit in dairy cows that is caused by dietary restriction
- ✓It is possible to predict glutamate in milk using MIR spectrometry
- ✓This indicator could be used as a panel with other MIR-based indicators (BHB, acetone, C18:1*c9*, citrate, lactose, etc.) to provide more accurate information.

# Thank you for your attention

## Any questions?