Complementarity of NMR and MS methods in metabolome analysis of dairy cows' urine

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INTRODUCTION

In ruminant production systems, it is important to optimise the incorporation of dietary nitrogen (N) in milk and meat while minimising excretion losses contributing to water and air pollution. The excess or the deficiency in dietary N may affect animal's metabolism and, in this context, a metabolomic approach may be a strategy for discovering markers of N use efficiency.

Metabolites produced by organisms have diverse chemical structures and properties. Due to this complexity, efforts to survey the entire metabolome rely on the implementation of multiplatform approaches. In our study, urinary fingerprint was measured simultaneously with mass spectrometry (MS) and ^{1}H NMR for finding new biomarkers for N utilization.

EXPERIMENTAL

1. Design: Four Holstein cows were used in a 4 x 4 Latin Square. Animals received different rations in terms of nitrogen level: Low (LN) *vs* high (HN) and Energy supply: Starch (S) *vs* Fibre (F). Urine was collected over 5-day periods during the last week of treatment.

2. Fingerprint analysis: Urine samples were analysed simultaneously by LC-MS and NMR

- *HILIC-MS analysis*: Separation was performed on a HILIC-Kinetex column (Phenomenex, 150*2.1, 2.6 µm) (**A:** 5 mM Ammonium formate, pH 6-7; **B:** ACN + 5 mM Ammonium formate; flow rate**:** 0.3 mL/min): The MS-System (MicrOTOF, Bruker, Germany) with an Electrospray source ion operated in positive mode.
- *1H-NMR analysis*: All spectra were recorded at 300 K on a Bruker (AVANCE III Ultrashield Plus) spectrometer operating at 500 MHz.

3. Data processing: NMR spectra were manually corrected and buckets intensities were normalized to the total intensity in each spectrum. MS data were extracted and converted in NetCDF formats. The resulting NMR and MS data were exported and analysed by chemometric tools (SIMCA-P software).

RESULTS

- Multivariate data analysis showed that metabolic profiles of MS (Fig. 1a) and NMR (Fig. 1b) discriminate the N diets.

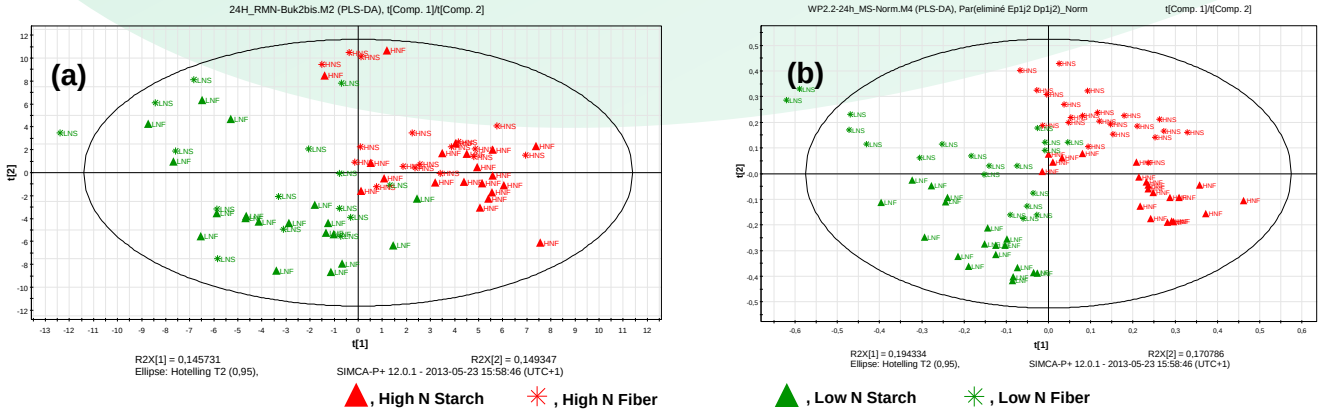

Fig. 1: PLS-DA plot of MS (a) and NMR (b) data

- For each technique, more than 50 markers candidates with a variable of importance plot VIP (variable important plot) higher than 1 could be ascribed to diet discrimination. Nine urinary differential metabolites were identified and confirmed by 2D NMR. For LC-MS, several discriminant ions were observed and are currently analysed for identification. However, only 3 metabolites, i.e. hippuric acid, phenylacetylglycine and allatoin are common markers to both techniques.
- In conclusion, better coverage of the metabolome is achieved by using both MS and NMR platforms.