



Barcelona, February 5th, 2016

DairyCare COST Action
Scientific Report for Short-term Scientific Mission (STSM)

COST STSM Reference Number: COST_STSM_FA1308-30900

Dates: 11.01.2016 up to 05.02.2016

COST Action: FA1308

STSM type: Regular (from Italy to Spain)

STSM applicant: Dr Carlotta Giromini, University of Milan, Italy,
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STSM Topic: A metabolomic investigation of choline supplementation using ¹H NMR spectroscopy of bovine blood plasma

Host institution: Dr. Ahmed Salama, Universitat Autònoma de Barcelona, Spain

Purpose of the STSM:

The aim of my STSM at the Universitat Autònoma de Barcelona was to acquire new knowledge on the use of nuclear magnetic resonance as a tool to detect biological markers for the response of animals to different treatments and physiological conditions. To achieve this purpose, blood plasma samples from dairy cows supplemented with rumen-protected choline (RPC) were used.

Description of the work carried out during the STSM

Preparation and packing samples in NMR tubes:

Blood plasma samples (200 µL) were mixed with 400 µL of cold saline solution (1% D₂O) in eppendorf tube and centrifuged at 12000 x g for 5 min at 4^oC. After centrifugation, 550µL of each sample were transferred to the ice-cold NMR tube and were analyzed with ¹H NMR spectroscopy operating at a frequency of 600 MHz.

Data analysis and markers detection:

More than 32,000 of frequencies were obtained in each sample spectra. The package “ChemoSpec” in the R software (version 3.2.3) was used for the inspection of all spectra. Then, unwanted frequency regions were excluded and data binning was carried out. The data were then subjected to multivariate statistical analysis using the R program and MetaboAnalyst 3.0. Initially, principal component analyses (PCA) were performed in order to get a general overview of the data and search for outliers. Visualization of the data was accomplished by inspection of the PCA score plot. Partial Least Squares-Discriminant Analysis (PLS-DA) was also performed to identify possible metabolite markers in blood plasma.

Description of the main results obtained:

The main aim of the STSM was fully achieved, which is learning new technique and its application for markers detection.

The NMR experiment carried out during my stay in Barcelona revealed that RPC supplementation resulted in greater plasma concentration of choline, indicating that choline in the rumen-protected form was absorbed in dairy cows. On the other hand, both PCA and PLS-DA revealed distinct metabolomics profile according to the stage of lactation (**Figure 1**). Lipids (mainly LDL/VLDL) were greater at week 12 compared to week 4 of lactation; whereas proline and tyrosine were significantly reduced over the weeks of lactation.

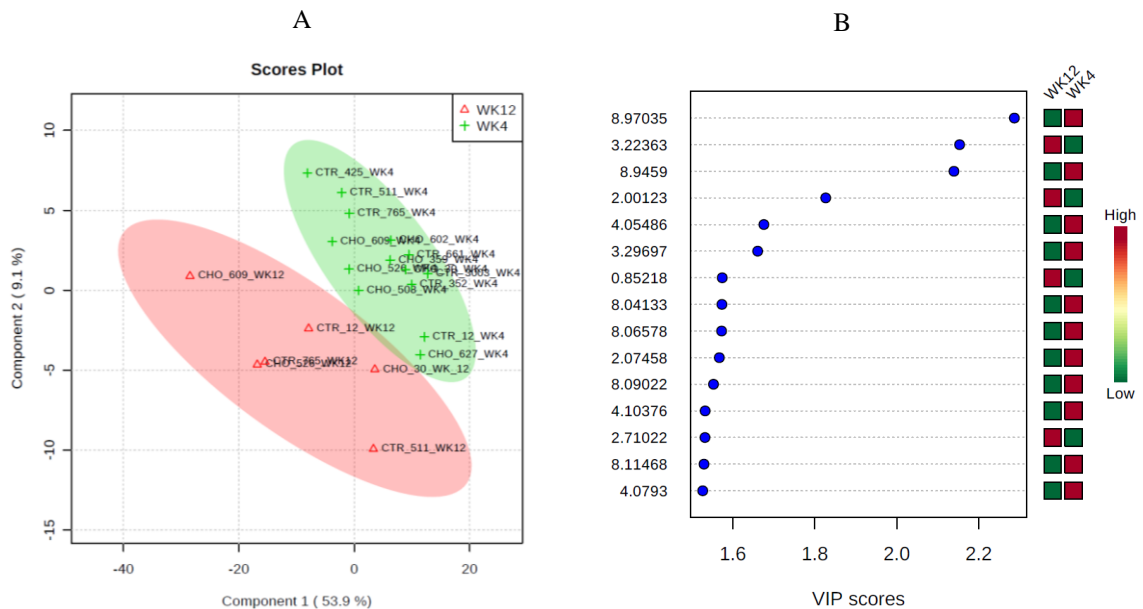


Figure 1: (A) PLS-discriminant analysis of cows at different week of lactation (week 4 vs week 12) showing 2 clusters for 2 groups and (B) metabolites ranked by variable important in projection (VIP).

Future collaboration with the host institution (if applicable):

The STSM represented an essential way to create a new collaboration with the Ruminant Research Group of Universitat Autònoma de Barcelona. The collaboration will continue by the way of further NMR data analysis, paper preparation and with further activities. The STSM in Barcelona has been a valuable experience to acquire experience with metabolomic techniques i.e NMR, but also to improve my background in the field of animal nutrition.

Foreseen publications/articles resulting from the STSM (if applicable):

The obtained results will be presented in the next EAAP Meeting, Belfast (UK). We will also consider the opportunity of presenting the data in the next Joint Annual Meeting (ADSA-ASAS), Salt Lake City, Utah (USA). Furthermore, a manuscript will be prepared and sent for publication in an ISI journal.

Confirmation by the host institution of the successful execution of the STSM:

Confirmation letter from the host institution is attached to this report.

Carlotta Giromini