SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: FA1308 – DairyCare
STSM title: Proteomic analysis of blood plasma samples in dairy cows predisposed for greater body condition versus normal condition cows

STSM start and end date: 04/03/2018 to 17/03/2018
(STSM was postponed from the original dates: 11/02 – 24/02/2018)
Grantee name: Katharina Schuh

PURPOSE OF THE STSM

(max.500 words)

This STSM is built on a completed animal trial aimed to establish a model of high versus normal mobilization of body reserves in dairy cows around calving (n = 19/group). Based on data from classical variables already assessed in blood samples to characterize metabolic status, and also on zoo-technical records, the success of our experimental approach, i.e., preselecting the cows according to their history of body condition well before dry off (15 weeks before anticipated calving) and pushing the difference by diets differing in energy content until dry-off, was already confirmed. In addition, we have already assessed several hormones and inflammatory markers in blood, and also have metabolome data from a targeted analysis as well as mRNA abundance of several genes of interest (mainly related to steroid biosynthesis and metabolism, protein and amino acid metabolism and data from biopsies (liver, subcutaneous adipose tissue and skeletal muscle).

We are now aiming to extend the analytical spectrum towards proteomics for a holistic characterization of metabolic health as affected by the extent of fat mobilization after calving. Protein expression levels are not strictly determined by cell gene regulation system but are also influenced by environmental stimuli (e.g. nutrition) and cells physiological or pathological state (e.g. disease).

I am very much interested to gain and insight and experience into the methodological aspects of proteomics and to search for biomarker candidates for metabolic health. In addition, the development of the calves from the dams in the two different groups is an ongoing and future research interest.

The work plan was to subject plasma samples obtained parallel at the times of tissue biopsies (liver, fat and muscle), i.e. on days -49, +3, and +21 relative to calving to proteomic analysis to be able to relate the serum proteome with tissue data. Plasma samples of 5 cows per group were selected for proteomic analysis according to different blood metabolites obtained within the project before (i.e., ß-hydroxybutyrate, non-esterified fatty acids) and also stratified for equal milk yield. Hence the chosen samples were shipped on dry ice to the VetMedZg project, in the Faculty of Veterinary Medicine in Zagreb, University of Zagreb prior to the STMS.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

Preparation of the samples started right at the beginning of STSM at the Laboratory of Proteomics in the Faculty of Veterinary Medicine in Zagreb. At first, we conducted a bis-cinchinonic acid **(BCA) assay for a total protein quantification** in the bovine serum samples. This protein quantification ensures that the amount of protein to be separated is appropriate for the further analysis and allows comparison among similar samples. The BCA assay is a spectrophotometric assay based on the alkaline reduction of the cupric ion to the cuprous ion by the protein, followed by chelation and colour development by the BCA reagent using the BCA Protein assay kit (Thermo Scientific). The final protein concentration in bovine serum is plotted against bovine serum albumin (BSA). Serum albumin accounts for about 50% of the total protein concentration. A set of standards were prepared with the final BSA concentrations: 0, 25, 125, 250, 500, 750, 1000, 1500, and 2000 µg/mL. In order to meet the right range of protein concentration of the BSA standard curve, all samples were diluted (1:50 and 1:100). A working reagent (WR) with BCA was prepared according to the protocol. All diluted samples (in duplicate) were mixed with the WR on a microplate well and incubated for 30 minutes at room temperature. The absorbance (colour) was plotted against BSA by spectrophotometric measurement at 630 nm [GEN5, Version 1.05]. Finally, all individual standards and samples were subtracted with the blank standard at 630 nm. Results were calculated with standard curve plotting while the final concentrations were given in µg/mL.

Afterwards, samples were prepared for peptide labelling using TMT Mass Tagging Kits and Reagents (TMT10plex™ Label Reagent Set, Thermo Scientific) according to the manufacturer's instructions. Briefly, based on the known protein concentrations, all samples were diluted 10 times and adjusted to exactly 35 µg protein per sample in a new tube to **prepare whole protein extracts**. In addition, the same volume (to get 35 µg protein) of each sample was mixed together to get an internal standard (IS). Samples were reduced (20 mM DTT) for 1 h at 55°C and alkylated (30 mM IAA) for 30 min at room temperature in the dark. Then, app. 300 µL of pre-chilled (-20°C) acetone was added and samples were allowed to precipitate to obtain a pellet of pure protein. Hence, a trypsin solution (1:30, w/w) was added to **digest the proteins** into the single peptides at 37°C overnight on a shaker. In the last preparation step, the single peptides were labeled using the TMT10plex™ Label Reagent Set with 10 different mass tags, and samples were randomized into 4 templates with one (homogenized) IS in each template. All samples of each template were combined in a new collection tube and stored at -80°C until analysis.

Protein identification (resolving of peptides) should have been conducted using a **nano-flow liquid chromatography system, coupled to a tandem mass spectrometer (nLC-MS/MS)**. Unfortunately, there was a problem with the nLC system so we could not finalize the analysis during my STMS stay.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max. 500 words)

The quantity of total protein in the bovine serum samples obtained from the BCA assay were calculated conferring to BSA standard curve (Figure 1) and results are shown in Figure 2.

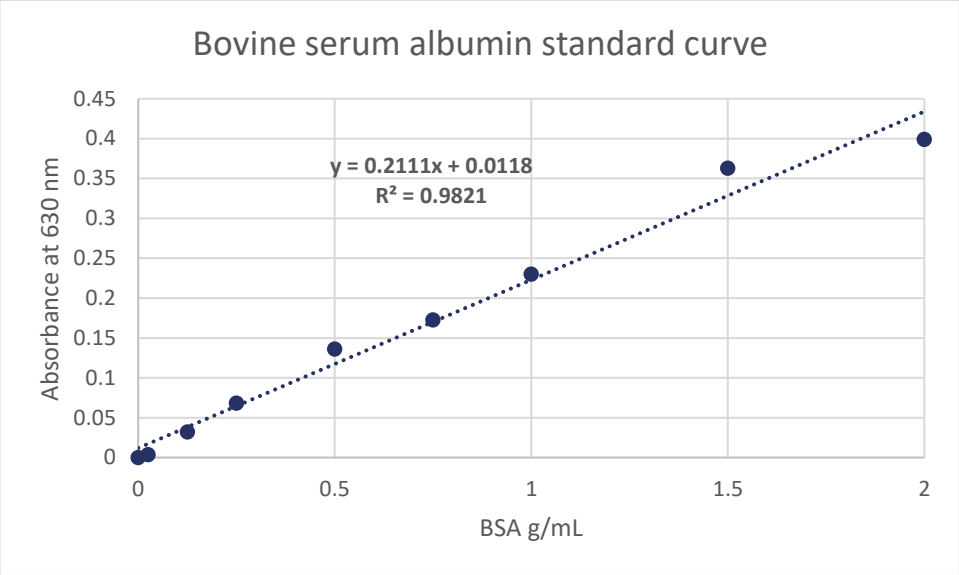

Figure 1: Bovine serum albumin standard curve. Each sample was measured for absorbance at 630 nm against a blank.

Due to more accuracy in the results, the absorbance in those samples diluted 50 times was used. In general, the total protein concentration in bovine serum samples from our study declined after calving, and NBCS cows showed numerically increased total protein concentration compared to HBCS cows. However, these are just preliminary results and have to be validated by the measurement via nLC-MS/MS. Since we could not use the nLC-MS/MS due to a technical error in my STMS stay, these results are still pending. Nevertheless, analysis will be done by the scientific partners at the host institution as soon as the system is fixed again and the results will be delivered.

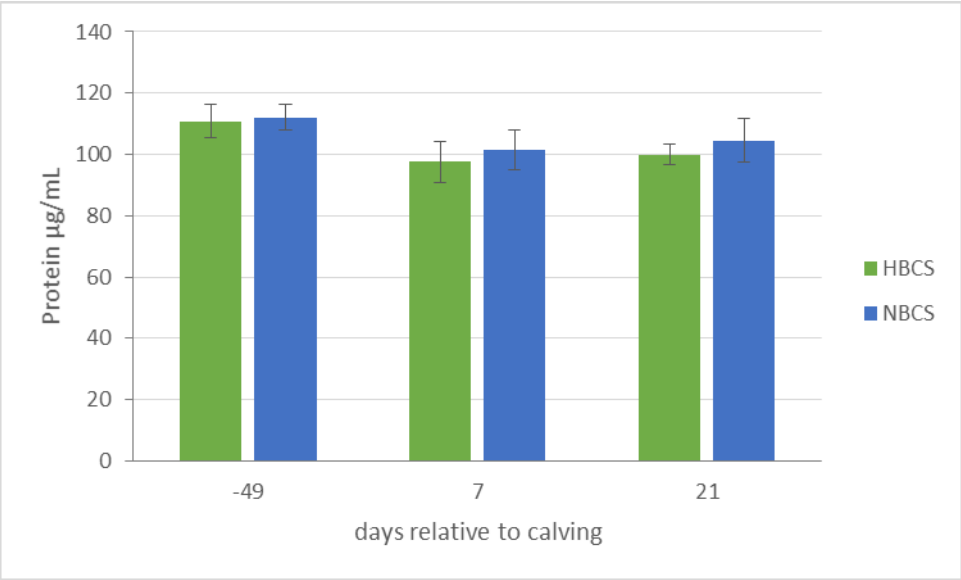

Figure 2: Total protein concentration in bovine serum samples from cows with either a high (HBCS) or normal body condition score (NBCS) before calving, according to BSA assay (results are shown as mean ± SEM)

FUTURE COLLABORATIONS (if applicable)

(max.500 words)

Evaluation of the data obtained will be transferred to the University of Bonn. Further data processing like statistical analysis, testing for relationships with other data available from the project and discussion of the results will be continued at the home institution after the STMS in collaboration with the host institution.

The final results for proteomic analysis might also be presented in a collaboration manuscript to be submitted in an international scientific manuscript or on international scientific conferences.