

## A Brief Work Plan of

### “Metabolomic Study of Amino Acids Supplementation in Dairy Calves”

#### 1. Background

The required levels of amino acids in the nutrition of weaning calves for optimal growth are seldom studied especially with the use of metabolomics. The metabolome, the complete set of small-molecules found within a biological sample, is directly related to gene expression, protein activity and/or the metabolic activity (1-3), and is highly complementary to other Omics-based studies namely Proteomics and Transcriptomics. NMR-based metabolomic studies have many advantages since this technique is quantitative, qualitative, non-destructive, non-equilibrium disturbing, highly reproducible and it has the ability to detect compounds within a wide range of physicochemical properties in the micromolar range (3, 4).

The project presented here is complementary to a wider project related to animal production, biochemical analysis and skeletal muscle proteomics (AGL2015-68463-C2-2-P from the Spanish Government). Altogether, the STSM will contribute to a more comprehensive overview of the animal physiology and production potential (5) of dairy calves. The identification of the metabolomic profiles of weaning calves will lead to a better understanding of biochemical pathway regulation during growth, and help to improve dairy farm productivity and auto-monitoring.

#### 2. Experimental Design and Working Hypothesis

Skeletal muscle samples are being collected through biopsy in the context of the project mentioned above. In this study, 32 dairy breed calves (7 days old) are included in the experiment. They are divided into 4 treatment groups (n=8) with different compositions of diet: **a) control diet:** 15% whey protein concentrate (WPC) with 35% crude protein (CP), 39% skim milk protein (SMP), 39% fatted whey (with 50% fat), 4% WPC 60 (whey protein with 60% protein) and 3% Premix (vitamins and minerals mixture); **b) supplementation with proline and glycine:** 15% WPC, 35% CP, 40% SMP, 39% fatted whey that contains 50% fat, 2.6% of WPC 60, 0.3% proline, 0.1% glycine and 3% Premix; **c) supplementation with phenylalanine and tyrosine:** 15.7% WPC (with 35%CP), 40% SMP, 38.85% fatted whey (with 50% fat), 2.05% WPC 60, 0.2% phenylalanine, 0.2% tyrosine and 3% Premix; **d) supplementation with lysine and methionine:** 15.6% WPC (with 35%CP), 40% SMP, 39% fatted whey (with 50% fat), 0.95% WPC 60, 0.62% lysine, 0.22% methionine and 3% Premix. After 8 weeks of treatment, muscle biopsies are obtained. All animal procedures have been approved by Institutional Animal Care and Use Committee.

There are three hypotheses in this project: **1)** the calf milk replacer with amino acids can improve the nitrogen effectiveness and thus will contribute to a key effect to the first 8 weeks growth of calves; **2)** using a metabolomic tool can lead to a better understanding of the biochemical pathways regulation on growth and the identification of adequate biomarkers; **3)** the metabolomic study can help to the design of an automated feeding behaviour monitoring system.

### 3. Nuclear Magnetic Resonance (NMR) techniques in Metabolomics Study

The host research teams led by Dr Almeida and Dr. Matzapetakis have successfully used NMR spectrometry to study the ovine muscle metabolome (6). The techniques for sample preparation and for the characterization of the NMR-metabolome in skeletal muscle of ruminants are currently being carried out by this research team (7).

### 4. NMR Data acquisition and statistical analysis

The host laboratory has the equipment and the expertise necessary to carry out this project in a 2-month stage. Briefly, the NMR-spectrometry protocol will be:  $^1\text{H}$  1D-NOESY (Nuclear Overhauser Effect Spectroscopy), J-Resolved and 2D 1H-1H COSY (Correlation Spectroscopy) spectra will be acquired on an 800MHz Bruker Avancell<sup>+</sup> (Ettlingen, Germany) spectrometer. Metabolite profiling will be performed using CHenomx NMR Suite 8.0 software and other public databases. The statistical analysis will be performed using SIMCA-P 13.0.3.0 software (Umetrics AB, Umeå, Sweden) for Univariate and Multivariate analysis. Multivariate tests will be used to evaluate any clustering behaviour of the samples' groups, as recently published (6).

### 5. From laboratory to deliverable: metabolome as a reliable technique for biomarker identification to be used in the automated monitoring systems in dairy industry

As previously described, one of the ultimate aims of this study is to establish an automated farm monitoring system using metabolites as biomarkers. We will select the potential metabolomic "candidates" according to Dr. Almeida and Dr. Matzapetakis's guidance as well as other publications, study their profiles in different treatments and compare their general metabolites concentration using multivariate analysis.

The data obtained in the metabolomic study will be correlated to measures of growth, fat and muscle content, plasma biochemical parameters and skeletal muscle histological characteristics. The goal is to find a set of metabolites which could be markers for growth in dairy calves that will be used for meat production. Such qualified "candidates" can be selected as a biomarkers or tools in automated monitoring systems of applied use to the dairy industry.

### References:

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