Welcome to the Farm Animal Proteomics Session
What is COST action FA1002 Farm Animal Proteomics?

• A network of Scientists working in the field of Farm Animal Proteomics (FAP)
• Involves 29 countries in Europe, Israel, Argentina, Australia and N. Zealand
• Running from 2010-2014
• Establish the European Research Area as the global leader in Farm Animal Proteomics with a coordinated network of expertise
Main activities

• Conferences on Farm Animal Proteomics
• Publishing scientific literature on FAP - Dissemination
• Organization of Training Schools
• Short Term Scientific Missions
• Website
COST-FAProteomics.org

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Working Group 1
Proteomics and Animal Health - The focus of Working Group 1 will be on research relevant to animal health covering biomarkers of infectious, parasitic and metabolic diseases, and genetic phenotype detection for breeding toward production goals as well as for resistance to disease. It will be expected to interact with and be fully integrated with investigations into other aspects of farm animal science such as reproduction, metabolic, microbiology, parasitology, virology, immunology and nutrition. Learn More
4th Conference on Farm Animal Proteomics

• From the 17-18th November

• In Milan, Italy

• Abstract deadline: 8th September 2014

• www.cost-faproteomics.org
And Today…

• Discovery Session on FAP

• Examples of the use of Proteomics in Animal Production:
  – Seasonal weight loss physiology (A Almeida)
  – Poultry production (D Eckersall)
  – Stress / Welfare livestock (A Bassols)
  – Quality in aquaculture (P Rodriguez)
  – Beef tenderness (E Veiseth-Kent)
  – Meat authenticity (M Sentandreu)
  – Dairy production (P Roncada)
  – Wool Production (J Plowman/ A Almeida)
Seasonal weight loss tolerance in Farm Animals: a proteomics and systems biology approach

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(August, 2014)
Introduction: What is Proteomics and what is Systems Biology?
Introduction to Proteomics

Proteome may be defined as the proteins present in a given cell, fluid, tissue, organ, organism or population

In order to understand how Biological Systems function, it is of the utmost importance to know how the proteome changes as a consequence of a stimulus

PROTEOMICS:

Study of the proteome;
Description and explanation of quantitative and qualitative changes in the proteome as a consequence of a certain stimulus
Introduction to Proteomics

Proteomics and other OMICs

Systems Biology

Genomics
- Functional Genomics
- Transcriptomics

Proteomics
- Phosphoproteomics; Peptidomics, Glycomics

Metabolomics

Adapted from Lovric (2011)
Proteomics workflow in Animal/Veterinary Sciences

Disease / Physiological condition

Proteomics Approach

Proteomics Study

2D Electrophoresis and Gel analysis

EXTENDED USE IN ANIMAL AND VETERINARY SCIENCES

Wide range of Applications:
- Biomarkers of disease / parasitism
- Biomarkers of Physiological condition
- Biomarkers of production traits
- Biomarkers of Meat quality

PUTATIVE BIOMARKER ESTABLISHED

Protein ID using Mass Spectrometry
During the last six years we have been working essentially in the application of Proteomics and Mass Spectrometry to **Animal** and also **Plant Sciences**, particularly to SWL adaptation.
Introduction

• Seasonal weight loss is a serious limitation to animal production in Tropical and Mediterranean areas.

• Due to poor quality of pastures in the dry season, animals may lose up to 30% of their initial body weight – constraint to ruminant production sectors with special relevance to ruminant extensive production.

• In contrast, rainy season pastures are usually considered of adequate quality and availability.

• To control Seasonal Weight Loss, supplementation is often implemented during dry season – Unavailable in undeveloped countries and Expensive in developed countries.
Animal species and breeds show different levels of adaptation to harsh environments as a consequence of the selection process and adaptation: diseases, parasites, pasture and water availability, etc.

Trypanotolerant dwarf cattle and goats of West Africa

*Bos indicus* – tropical climate conditions
Rabbit Proteomics Experiments:

Mapping the proteome of the Gastrocnemius muscle in the rabbit;

Comparison of fed and underfed rabbit gastrocnemius muscle profile
Experiment with rabbits – Basic Design

Normal Nutritional Level

Expression differences

MS Protein Characterization

Low Nutritional Level
Structural Proteins

Animal Genetics
Almeida et al., 2010

Metabolism
proteins
Interesting results on both structural and glycolytic enzymes.

↓

Conduct a proteomics study on the effects of weight loss in a production animal (sheep)
Protein expression in muscle tissue of three domestic sheep breeds influenced by weight loss: a study using 2DE and MALDI-TOF/TOF – Work in progress
• Objective: protein expression study in the muscle of three sheep breeds with different levels of adaptation to seasonal weight loss:

• Damara: Semi-Desert **fat tail** sheep - tolerant;
• Aus. Merino: European origin breed, Susceptible;
• Dorper – Intermediate type selected for high muscular development
Live weight parameters and feed intake in Dorper, Damara and Australian Merino lambs exposed to restricted feeding

T.T. Scanlon\textsuperscript{a,1}, A.M. Almeida\textsuperscript{b,1}, A. van Burgel\textsuperscript{a}, T. Kilminster\textsuperscript{a}, J. Milton\textsuperscript{c}, J.C. Greeff\textsuperscript{a}, C. Oldham\textsuperscript{a}

Assessing carcass and meat characteristics of Damara, Dorper and Australian Merino lambs under restricted feeding

André M. Almeida, Tanya Kilminster, Tim Scanlon, Susana S. Araújo, John Milton, Chris Oldham, Johan C. Greeff

Gene expression of regulatory enzymes involved in the intermediate metabolism of sheep subjected to feed restriction

S. van Harten\textsuperscript{1,2}, R. Brito\textsuperscript{1,2}, A. M. Almeida\textsuperscript{1,2}, T. Scanlon\textsuperscript{3}, T. Kilminster\textsuperscript{3}, J. Milton\textsuperscript{4}, J. Greeff\textsuperscript{3}, C. Oldham\textsuperscript{3} and L. A. Cardoso\textsuperscript{1,2}
Basic Trial Overview

Normal Nutritional Level

UP Regulated / Down Regulated proteins

Protein Identification

Low Nutritional Level
Preliminary results: muscle tissue – 2D gel
Preliminary Results regarding muscle tissue – protein expressions – examples…

Actin

Histidine triad nucleotide-binding protein 1

Troponin
In Summary:

• Weight loss significantly affects production parameters and biochemical/physiological profiles in domestic animals;

• The study on how does SWL affects Nitrogen metabolism plays a key role in farm animal selection, particularly concerning adaptation to SWL;

• Our research has aimed to establish biomarkers of tolerance/adaptation to weight loss that in conjunction with genomics and transcriptomics, may be of interest as selection tools;

• Possible next steps: Interpretation of the sheep experiment results and possible validation
Dairy goats Proteomics studies

Seasonal Weigh Loss Physiology studies: focusing on the mammary gland in dairy goats from the Canary Islands through the use of Omics (Proteomics, Transcriptomics and Metabolomics)

**Project Objective:** Establish Molecular Markers of Tolerance to SWL in dairy goats of use in selection programs
Proteomics – whole mammary gland and mitochondrial proteomes

Metabolomics – NMR

Lipidomics: Fatty Acid Profiling

Transcriptomics - NGS
(See Poster on RNA extraction)

Metabolomics – NMR
(See Poster)
General Overview & Objectives

2 groups subjected to weight loss -15-20% decrease

2 control groups

42 day trial

OBJECTIVES

Proteome Characterization

Diff. Proteome Study

Lérias et al. (2013). Trop Anim Health Prod. 2013 Nov;45(8):1731-6
Results: Optimization of the Blue Native Protocol and Proteome Characterization

Similar band Profile – Eight Putative Mitochondrial Protein Complexes?

Apparent better extraction yield at 1:5 Protein / Detergent ratio

Eight bands were selected for protein ID using LC-MS/MS
Results: Optimization of the Blue Native Protocol and Proteome Characterization

We detected several subunits of the main mitochondrial membrane protein complexes: respiratory complexes I, II, III, IV and V, as well as glutamate dehydrogenase complex and NAD(P) transhydrogenase complexes.
Results: Blue Native Page vs. 2DE & Proteome Characterization

88 different spots

Proteome characterization
(66% ID success rate)

188 different spots
Results: Proteome Characterization

- cellular component organization or biogenesis (GO:0071840)
- cellular process (GO:0009987)
- localization (GO:0051179)
- apoptotic process (GO:0006915)
- biological regulation (GO:0065007)
- response to stimulus (GO:0050896)
- developmental process (GO:0032502)
- multicellular organismal process (GO:0032501)
- biological adhesion (GO:0022610)
- metabolic process (GO:0008152)
- immune system process (GO:0002376)
Results: Proteome Characterization

Molecular function

- Translation regulator activity (GO:0045182)
- Binding (GO:0005488)
- Receptor activity (GO:0004872)
- Enzyme regulator activity (GO:0030234)
- Structural molecule activity (GO:0005198)
- Catalytic activity (GO:0003824)
- Antioxidant activity (GO:0016209)
- Transporter activity (GO:0005215)
Results: Comparative study

Comparative proteomics analysis enabled the identification of Succinyl-CoA synthetase, Guanine nucleotide-binding protein, NADH-ubiquinone oxidoreductase, in majorera, and ACTA2 protein in Palmera, as being over-expressed as a consequence of SWL.
Future Prospects:

1. New approach on the 2DE analysis
2. Repeat ID for differentially expressed spots
3. Interpret results of the differentially expressed spots

Physiological Interpretation

Integration with other Omics

Establishment of Biomarkers relating SWL and milk production at the level of the mammary gland
Major advantages of Proteomics in Animal Science:

• It is a high-throughput technology;

• Provides key information on product characteristics and changes – ex: protein profiles of meat and processed products;

• Allows the comprehension of essential and determinant events underlying specific metabolic pathways as affected by specific factors;

• Broad use in all areas of animal science
Major Drawbacks of Proteomics in Animal Science:

• It is a very expensive technology – powerful deterrent;

• Animal Science researchers have little knowledge on Proteomics achievements and principles;

• Animal Science researchers have little access to proteomics and Mass Spec equipment;

• Strong technical limitations: Protein separation and fractionation (low abundance, low molecular weight, several contaminants)

• Dependence on Mass Spec: – Double Edge Sword
• Dependence on Mass Spec: – Double Edge Sword
• Even the best Mass Spectrometer in the world is of limited use when proteins are not present in the database
Solving poor representation in databases…

- Homology identifications
- Increase the number of entries in public databases
- Construct particular species database
- Have access to private databases by international consortia
- De novo sequencing?
Take home message:

Animal Production and food of animal origin are directly linked to proteins;
• Proteomics is therefore a worthy approach to the study of all areas in animal and food science;
• It is therefore vital to increase the use of proteomics-based studies on all aspects of animal science, while integrating it with other large scale disciplines (genomics, transcriptomics, metabolomics, lipidomics, etc)

International Collaboration and Networking in application to funds and research activities
Acknowledgements

ITQB/UNL & IBET:
José Ricardo
Mariana Carvalho
Rui Palhinhias
Joana Lérias
Lorenzo Hernandez (ULPGC)
Kamila Koci
Renata Soares
Gonçalo Costa (now FCUL)
Alexandre Campos (now CIIMAR)
Ana Varela Coelho
Rita Francisco
Jorge Paiva
Carlota Vaz Patto
Pedro Fevereiro

IICT:
Sofia van Harten
Susana Araújo
Luís Alfaro Cardoso

DAFWA (Australia):
Johan Greeff
Tanya Kilminster
Tim Scanlon
Chris Oldham

ULPGC & ICIA (Canary Is., Spain)
Noemi Castro
Anastasio Arguello
Juan capote

(Fundação para a Ciência e a Tecnologia)
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INSSUNHO SUPERIOR

(PTDC/CVT/116499/2010)