Welcome to the Farm Animal Proteomics Session

cost

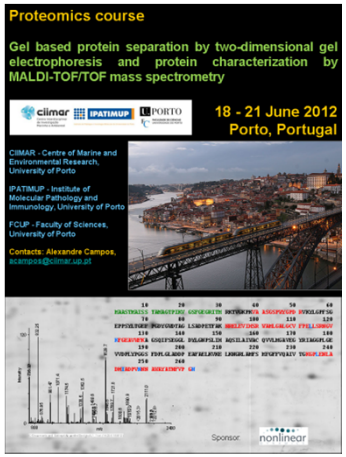
EAX · EVT · FEZ · EAAP · EUROPA

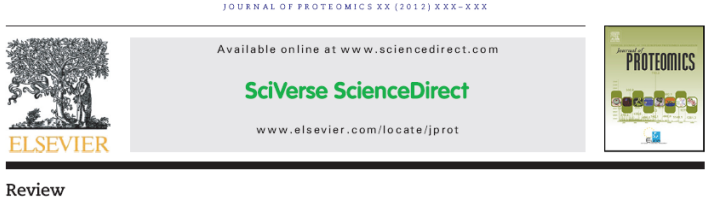
What is COST action FA1002 Farm Animal Proteomics?

- 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 - cost-faproteomics - Windows Internet Explorer

PT Português (Portugal)

http://174.122.126.220/~proteo/joom/

Google

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COST-FAProteomics.org

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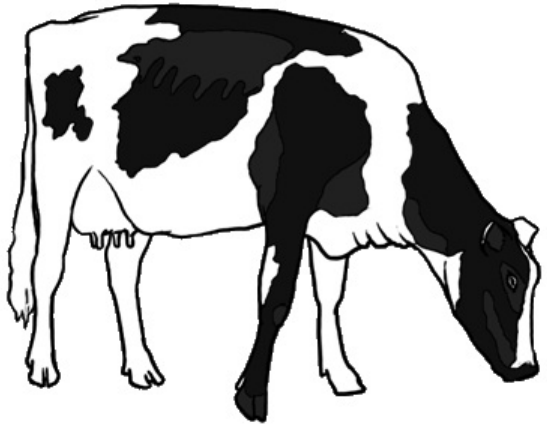
» Home » About » Management » Working Groups » Meetings » STSMs » Training Schools » Documents » Publications » Opportunities



Main Menu

- Home
- About
- Management

Working Group 1 | Working Group 2 | Working Group 3

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

Concluído

Internet | Modo Protegido: Activado

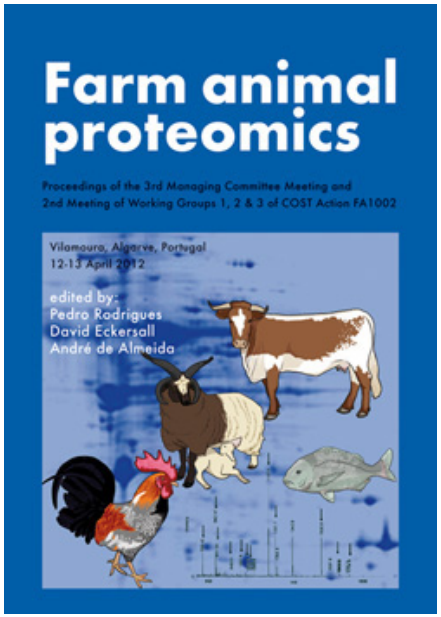
100%

cost-faproteomics -... | BCN 2013 - Almeida... | Main papers Andre

18:14

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

André Martinho de Almeida

aalmeida@fmv.utl.pt

Copenhagen, Denmark

(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

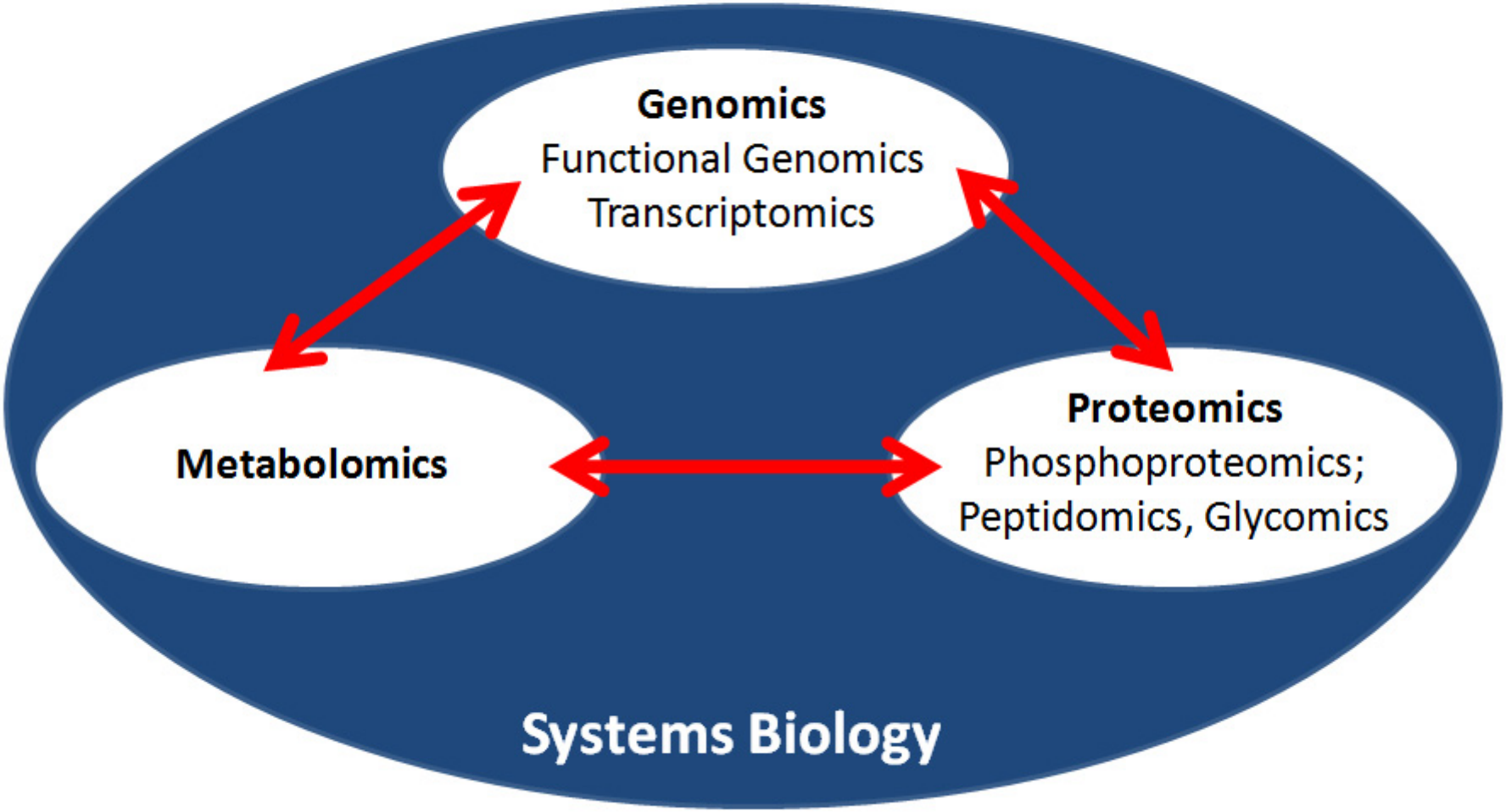
PROTEOMICS:

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



Adapted from Lovric (2011

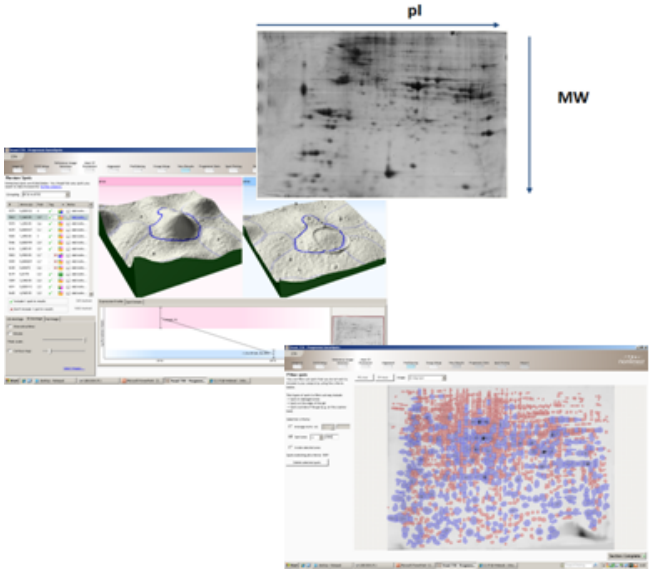
Proteomics workflow in Animal/Veterinary Sciences

Disease / Physiological condition

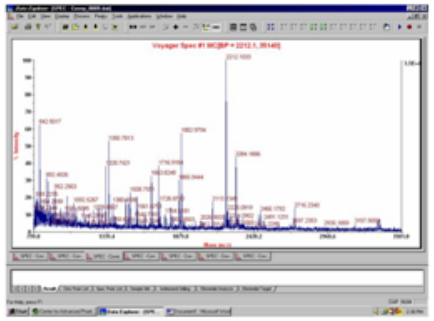
Proteomics Approach

Proteomics Study

2D Electrophoresis and Gel analysis



PUTATIVE BIOMARKER ESTABLISHED



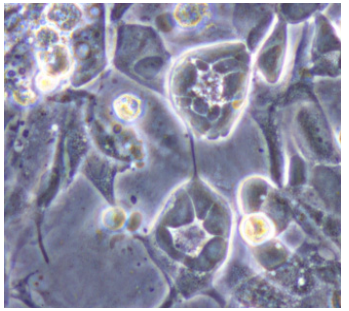
Protein ID using Mass Spectrometry

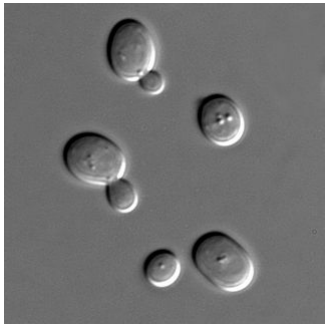
EXTENDED USE IN ANIMAL AND VETERINARY SCIENCES

Wide range of Applications:

- Biomarkers of disease /parasitism
- Biomarkers of Physiological condition
- Biomarkers of production traits
- Biomarkers of Meat quality

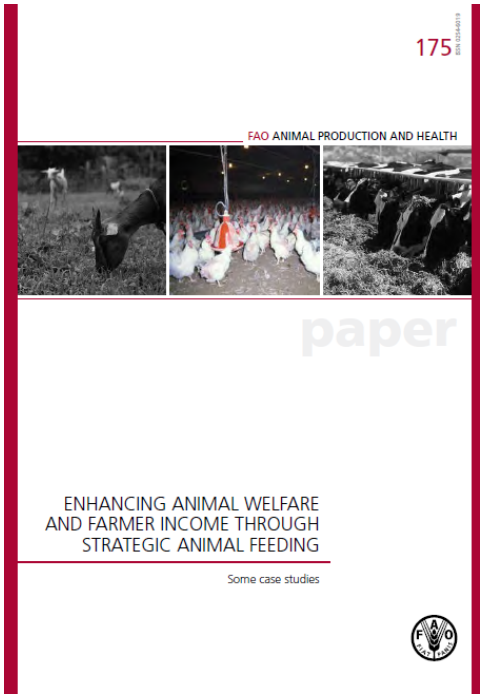
During the last six years we have been working essentially in the application of Proteomics and Mass Spectrometry to **Animal** and also **Plant Sciences, particularly to SWL adaptation**





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 loose 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



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Seasonal weight loss – an assessment of losses and implications for animal welfare and production in the tropics: Southern Africa and Western Australia as case studies

Luis Alfaro Cardoso and André M. de Almeida
Instituto de Investigação Científica Tropical, Centro de Veterinária e Zootecnia, Faculdade de Medicina Veterinária, Av. Univ. Técnica, 1300-477 Lisboa, Portugal & AIA - Associação Tropical Agrária (NGO), Lisboa, Portugal.
E-mail: aalmeida@fmv.utl.pt

MAIN MESSAGES

- Weight loss has a strong impact on animal productivity compromising the animal welfare and income of farmers worldwide.
- Susceptibility to weight loss may be decreased if indigenous stock, adapted to local conditions, are used and bred towards the increase of their productive traits.
- The molecular study and definition of the molecular mechanisms behind adaptive traits should be the object of research programmes.

ACRONYMS

SWL	seasonal weight loss
WH group	veld hay group
WH+S group	veld hay supplemented group
3MH	trimethylhistidine

INTRODUCTION

Animal production in the tropics is severely limited by several constraints that reduce output and consequently affect productivity and farmers' livelihoods. We have recently reviewed this subject (Lamy *et al.*, 2012) and identified three major categories of production constraints: diseases, parasites and nutritional factors.

Nutritional factors and in particular seasonal weight loss (SWL) are the most relevant of such conditions. Tropical and subtropical climates, including those in the Mediterranean basin, are characterized by the existence of a dry season, during which pasture quality and

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

Research in Veterinary Science 87 (2009) 196–199

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Establishment of a proteomic reference map for the *gastrocnemius* muscle in the rabbit (*Oryctolagus cuniculus*)

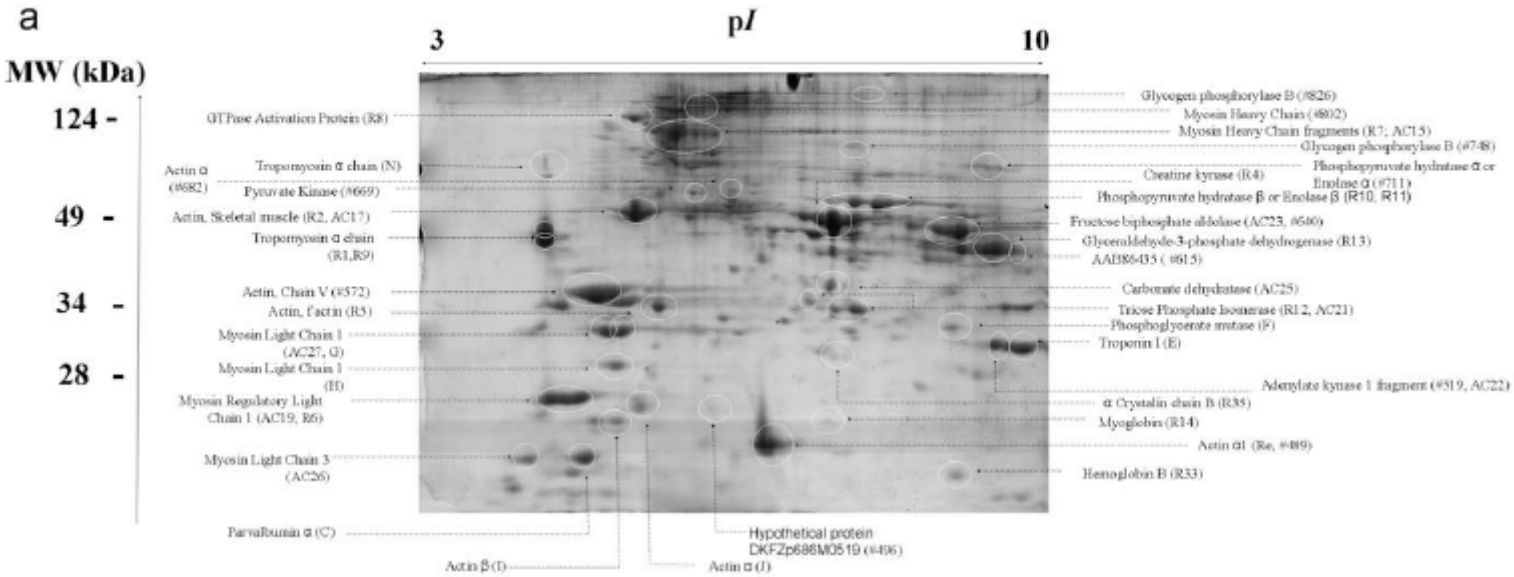
André M. Almeida [a,b,*], Alexandre Campos [b], Sofia van Harten [a], Luís Alfaro Cardoso [a], Ana Varela Coelho [b,c]

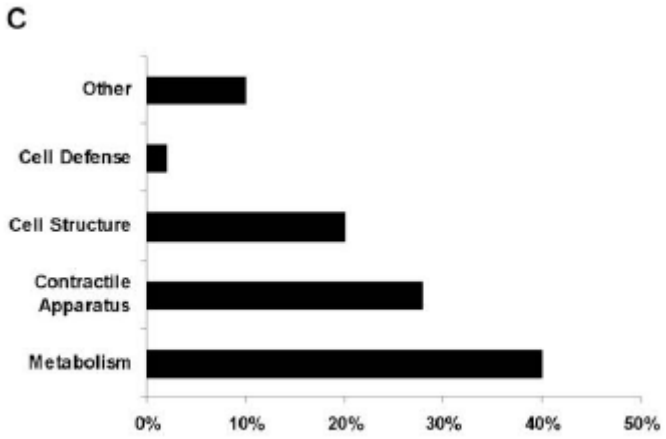
ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi:10.1111/j.1365-2052.2009.01994.x

Proteomic investigation of the effects of weight loss in the gastrocnemius muscle of wild and NZW rabbits via 2D-electrophoresis and MALDI-TOF MS

A. M. Almeida*, A. Campos†, R. Francisco†, S. van Harten*, L. A. Cardoso* and A. V. Coelho†,‡



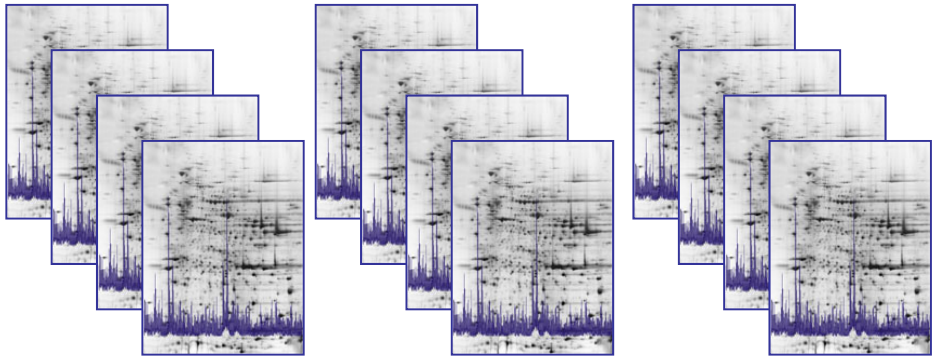


Almeida et al., 2009

Experiment with rabbits – Basic Design

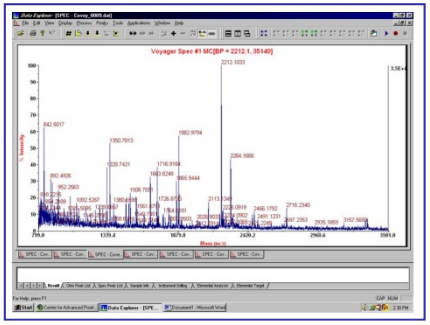
Normal Nutritional Level

Low Nutritional Level

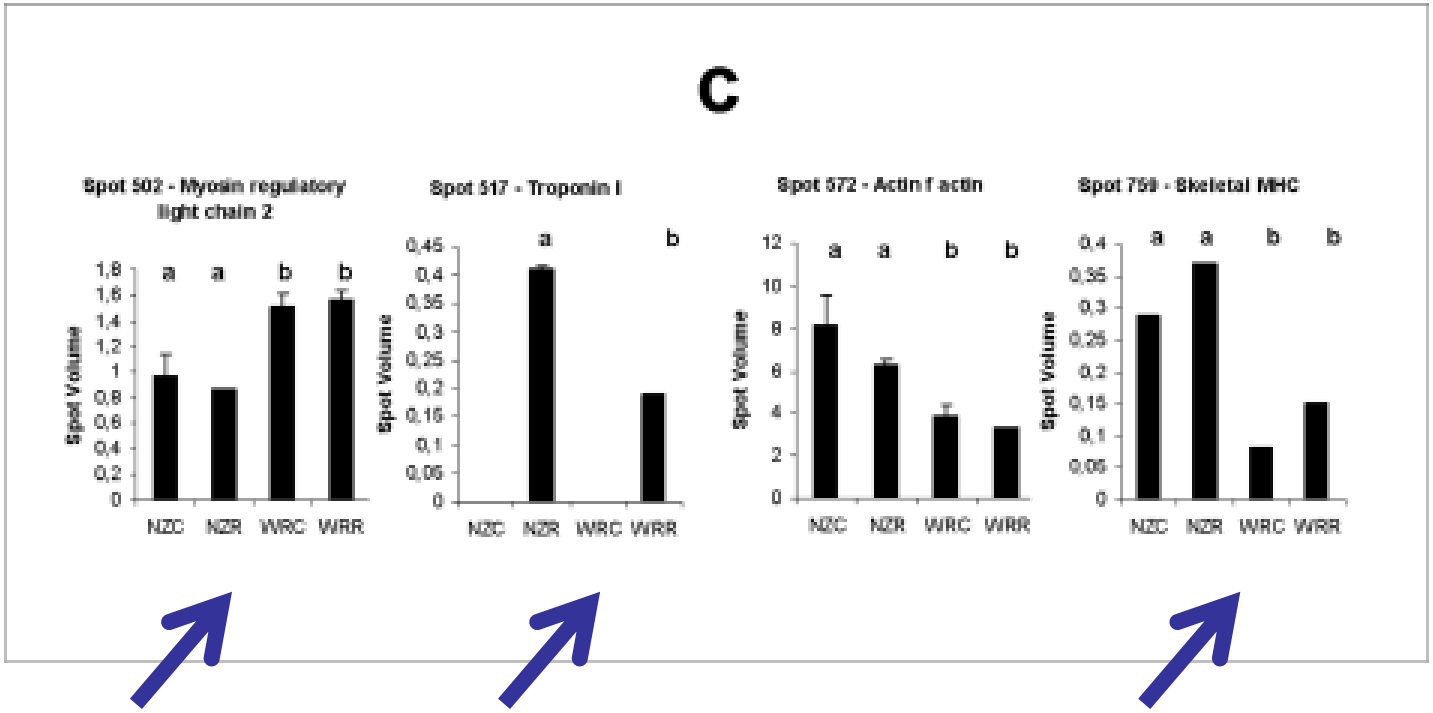


Expression differences

MS Protein Characterization

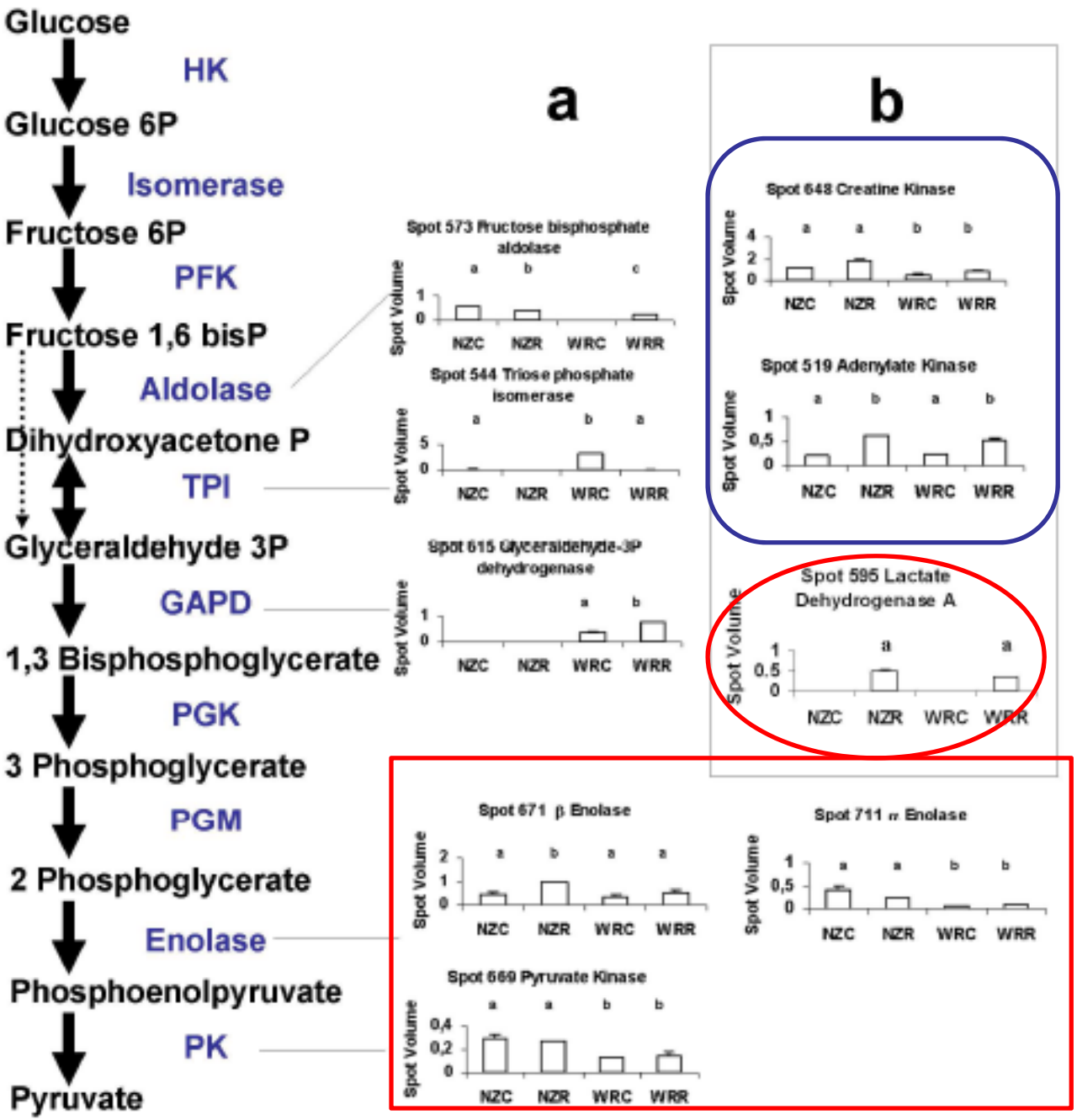


Structural Proteins



Almeida et al., 2010

Metabolism proteins



Almeida et al., 2010

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

Small Ruminant Research 109 (2013) 101–106

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Live weight parameters and feed intake in Dorper, Damara and Australian Merino lambs exposed to restricted feeding

T.T. Scanlon[a,1], A.M. Almeida[b,*,1], A. van Burgel[a], T. Kilminster[a], J. Milton[c], J.C. Greeff[a], C. Oldham[a]

361-z

REGULAR ARTICLES

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

Animal, page 1 of 7 © The Animal Consortium 2012
doi:10.1017/S1751731112001589

animal

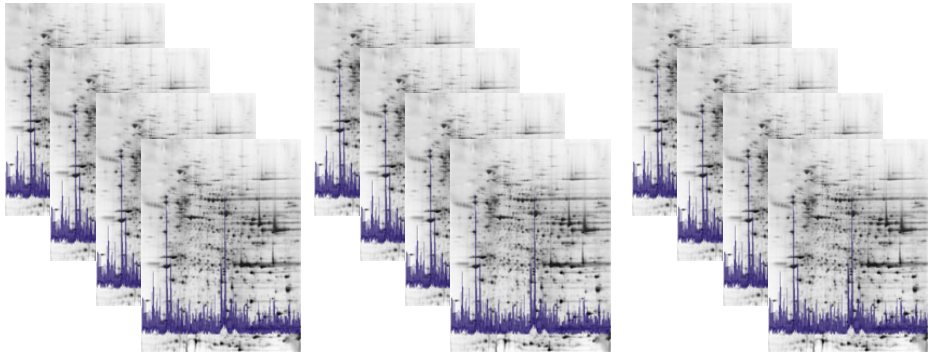
Gene expression of regulatory enzymes involved in the intermediate metabolism of sheep subjected to feed restriction

S. van Harten[1,2†], R. Brito[1,2], A. M. Almeida[1,2], T. Scanlon[3], T. Kilminster[3], J. Milton[4], J. Greeff[3], C. Oldham[3] and L. A. Cardoso[1,2]

Basic Trial Overview

Normal Nutritional Level

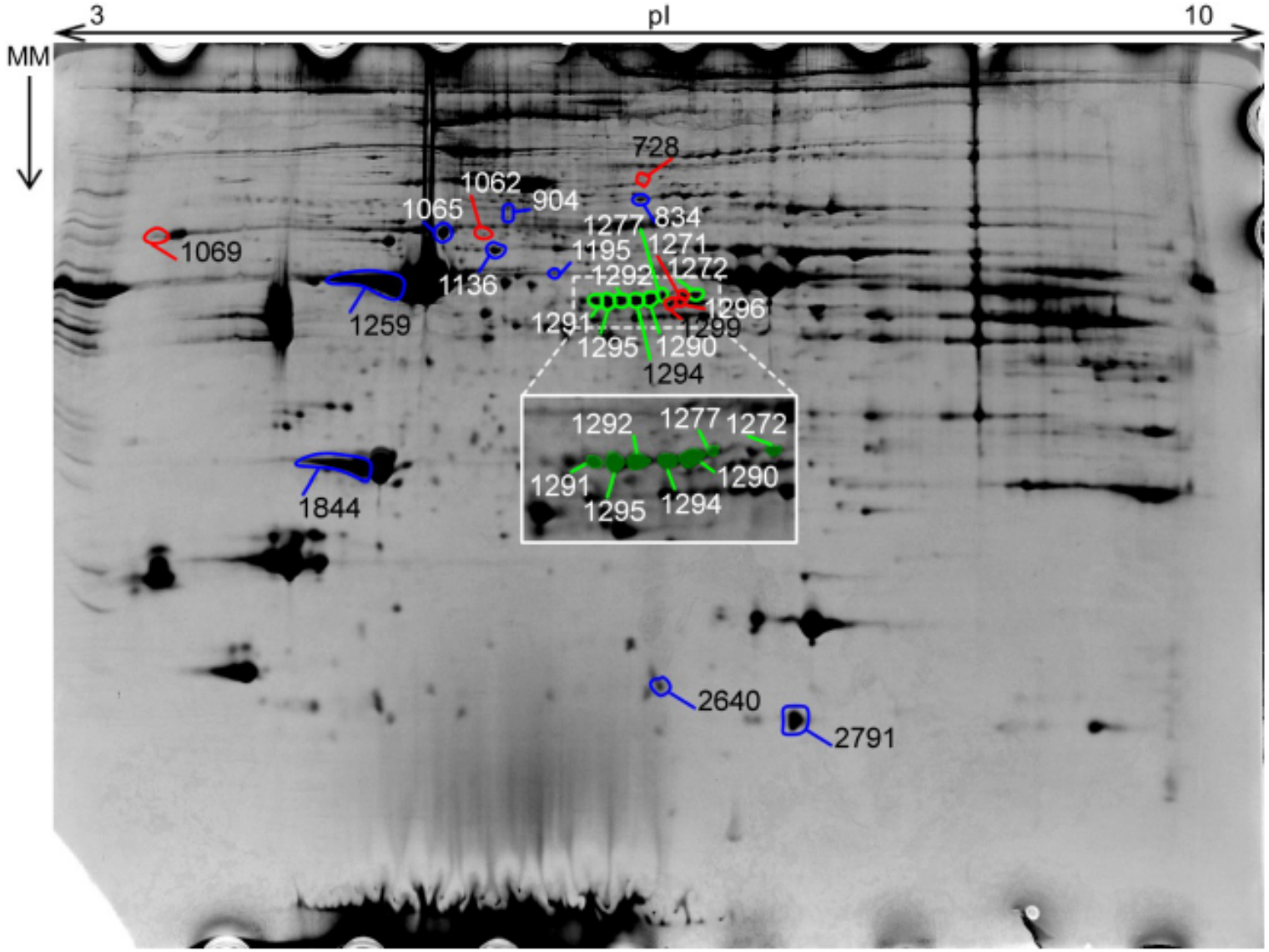
Low Nutritional Level



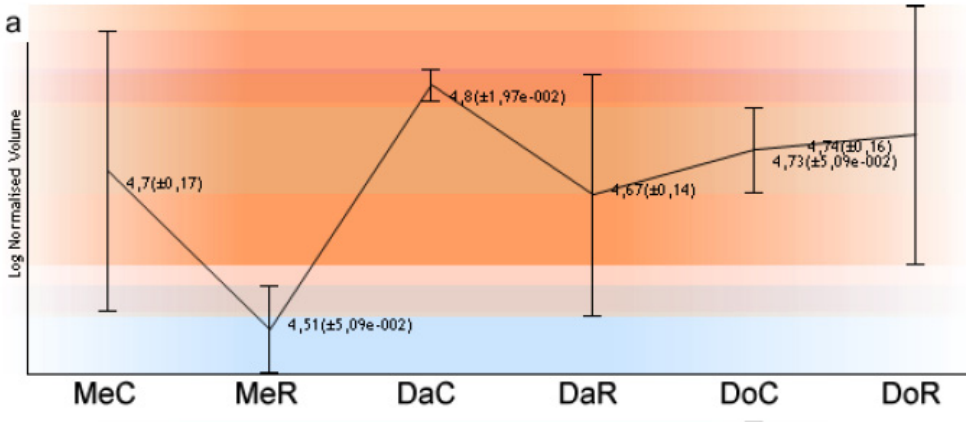
UP Regulated / Down Regulated proteins

Protein Identification

Preliminary results: muscle tissue – 2D gel

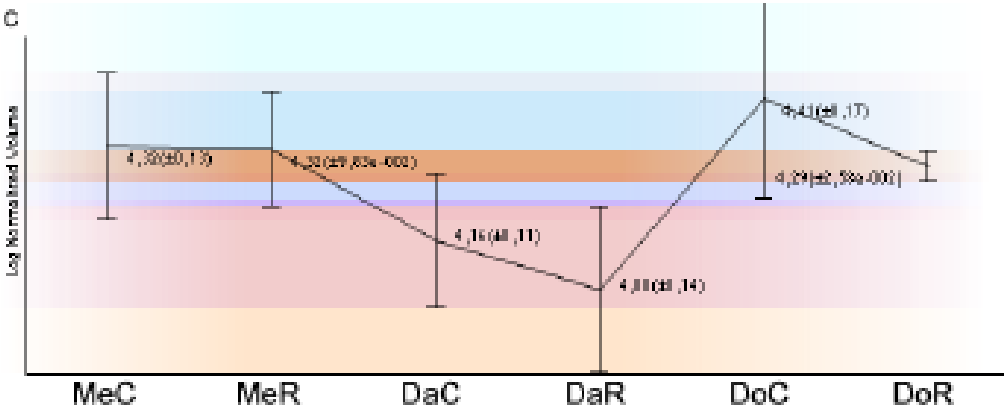


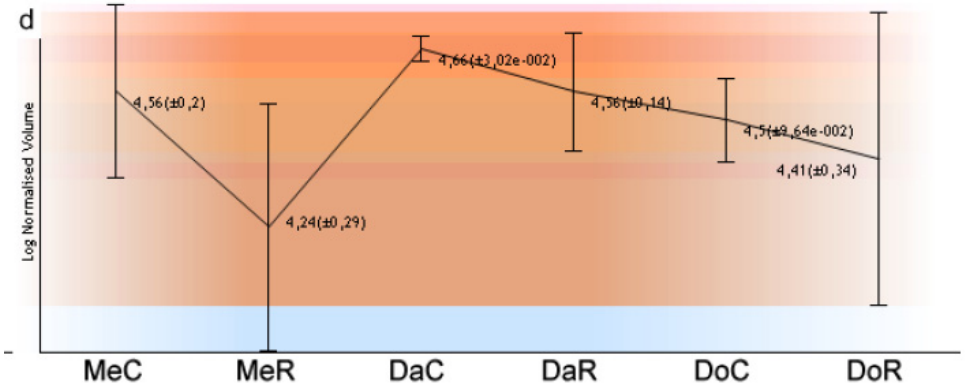
Preliminary Results regarding muscle tissue – protein expressions – examples…



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;

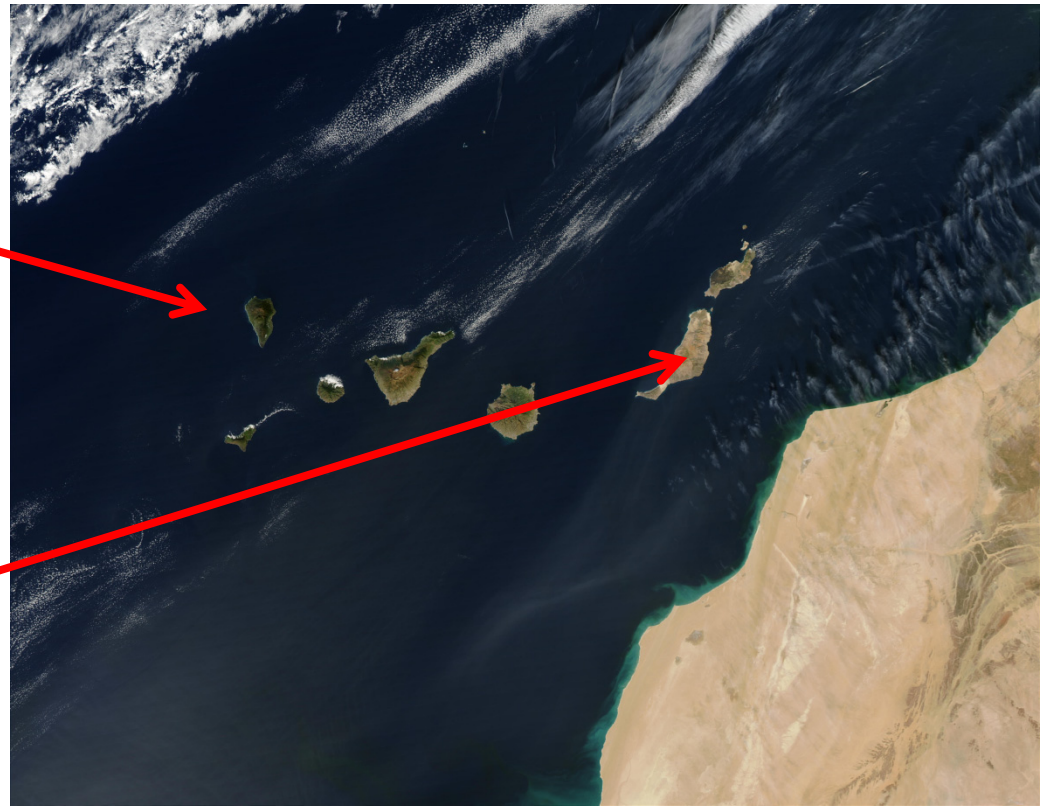
• Our research has aimed to establish biomarkers of tolerance/adaptation to weight loss that in conjunction with genomics and transcriptomics, may be of interest as selection tools;

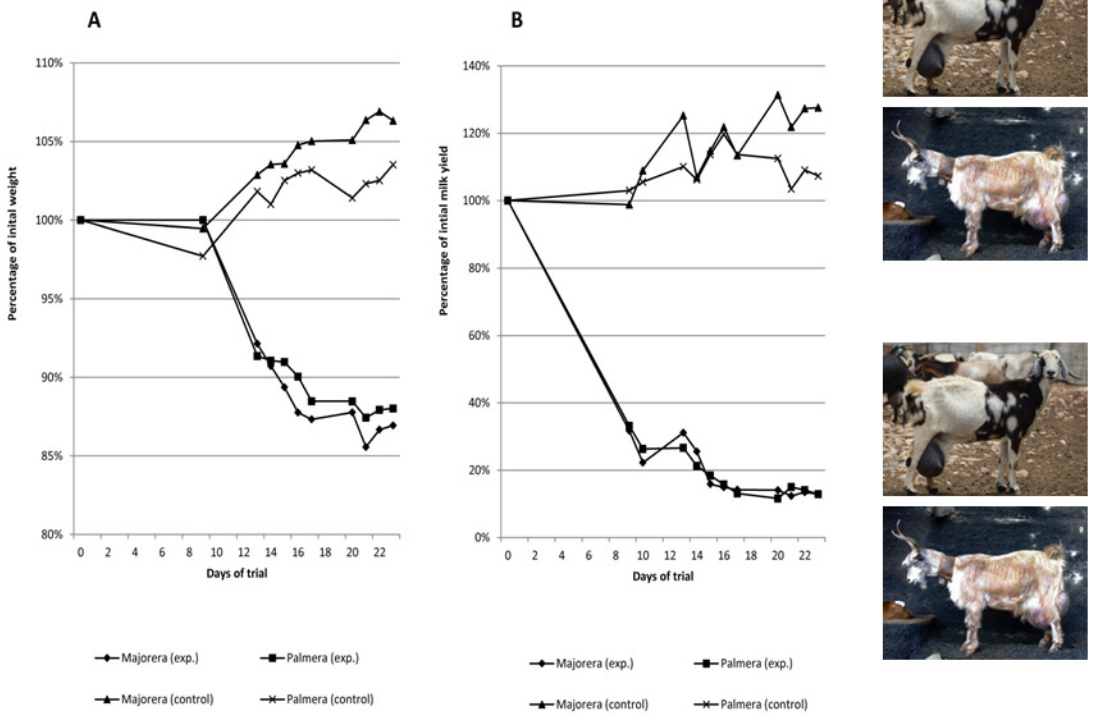
• Possible next steps: Interpretation of the sheep experiment results and possible validation

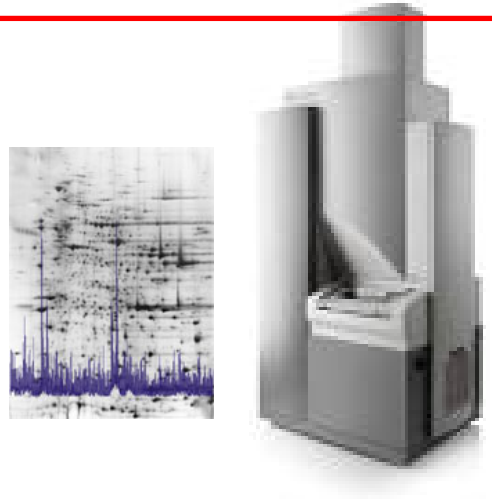
Dairy goats Proteomics studies

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)

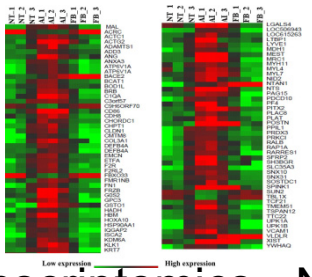
Project Objective: Establish Molecular Markers of Tolerance to SWL in dairy goats of use in selection programs







Proteomics – whole mammary gland and mitochodrial proteomes



Transcriptomics - NGS (See Poster on RNA extraction)

Lipidomics: Fatty Acid Profiling

Metabolomics – NMR (See Poster)

General Overview & Objectives

Majorera

Palmera

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

Majorera

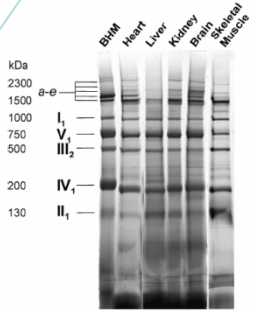
Palmera

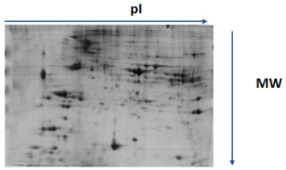
2 control groups

Mammary gland Biopsies (Secretory Tissue)

Mitochondria isolation

BN & 2DE





Protein ID using Mass Spectrometry

42 day trial

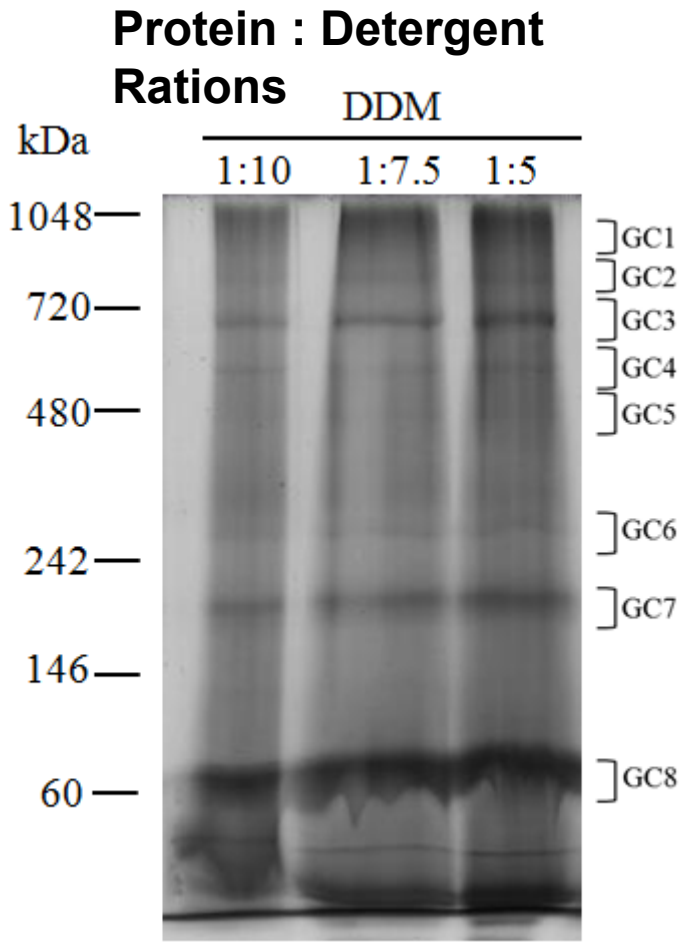
OBJECTIVES

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

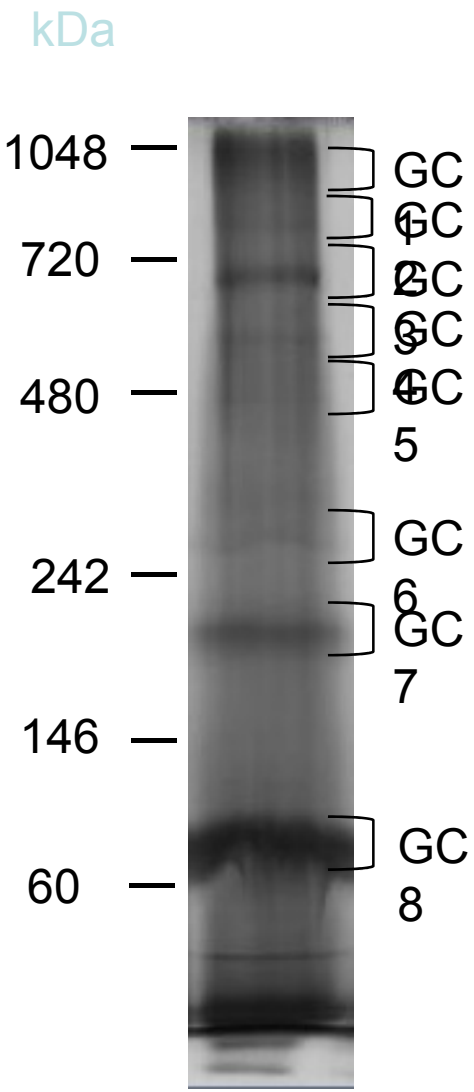


Similar band Profile – Eight Putative Mitochondrial Protein Complexes?

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

Eight bands were selected for protein ID using LC-MS/MS

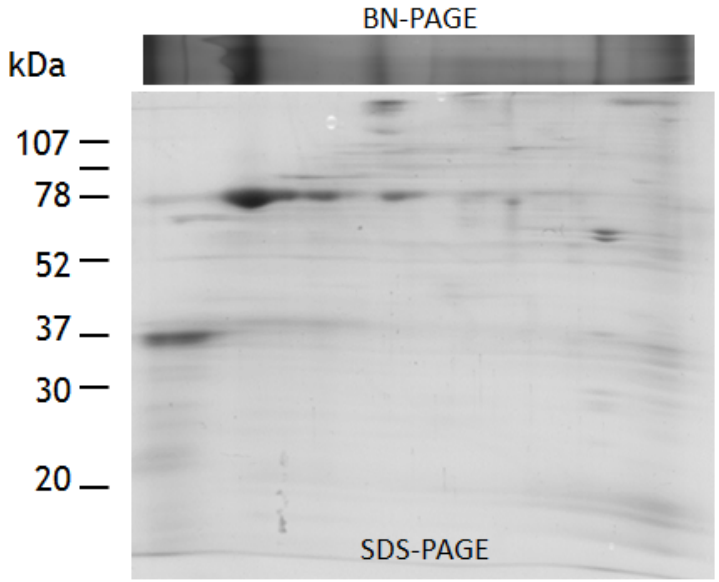
Results: Optimization of the Blue Native Protocol and Proteome Characterization



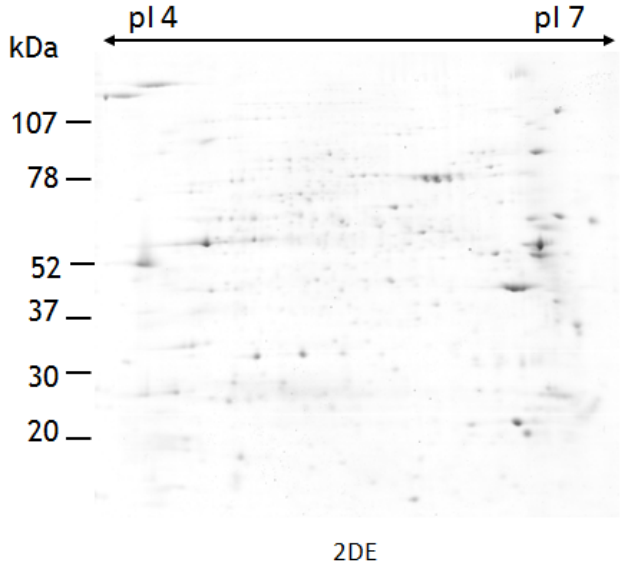
Protein band	membrane	type of complex	subunits
G8	y	complex V	2
	y	Voltage-dependent anion-selective channel	3
GC7	y	complex V	2
GC6	y	cation transport ATPase	1
	y	complex IV	2
GC5	n	glutamate dehydrogenase	3
	y	complex V	3
	y	complex II	2
	y	NAD(P) transhydrogenase	1
GC4	y	ATPase, complex V	2
	n	glutamate dehydrogenase	2
GC3	y	complex V	5
	y	complex III	2
	y	NAD(P) transhydrogenase	1
	y	complex IV	1
	y	Creatine kinase U-type	1
GC2	y	complex I	8
	y	complex V	3
	y	complex II	2
GC1	y	complex V	3
	y	complex I	2
		Complex II	1

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

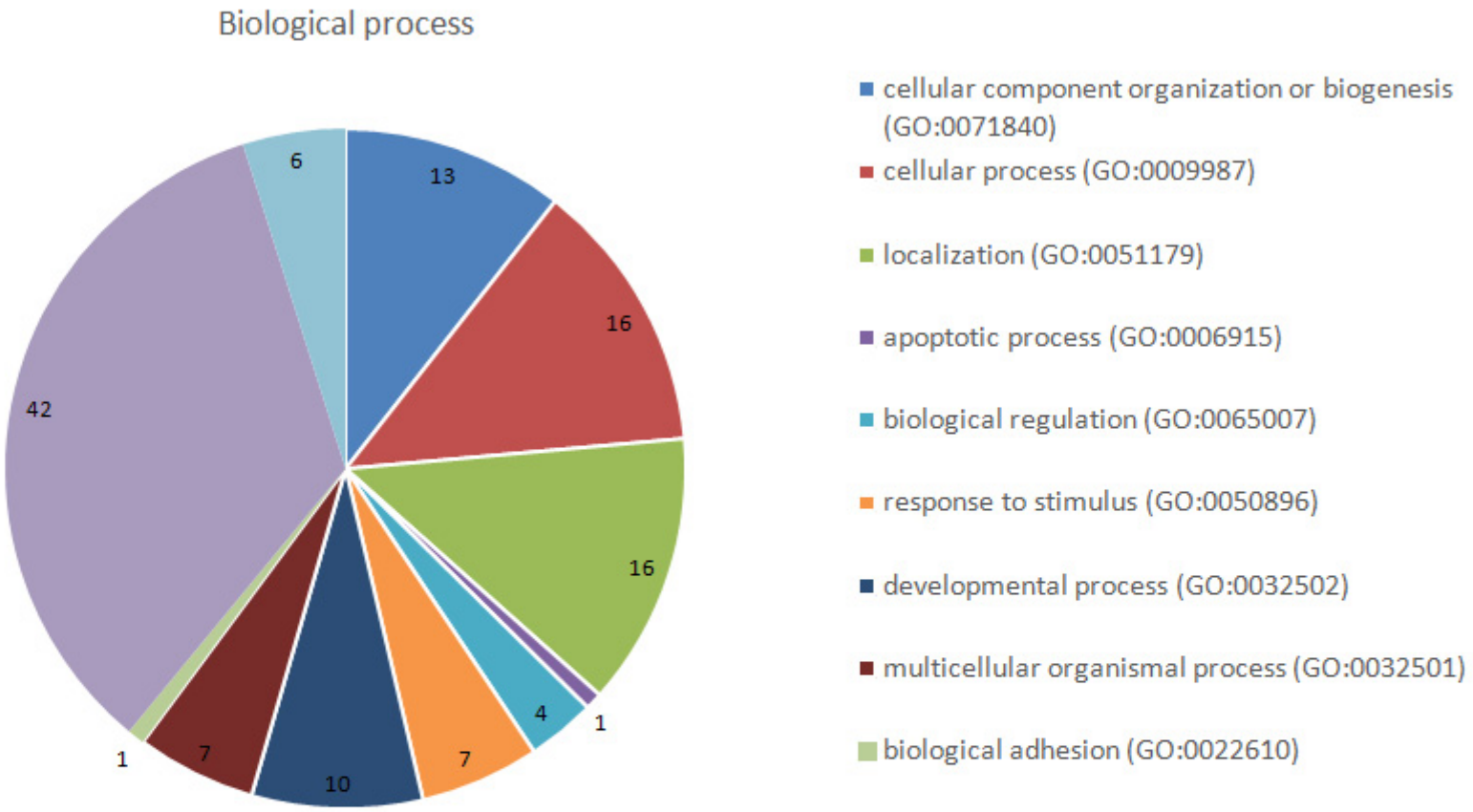


188 different spots

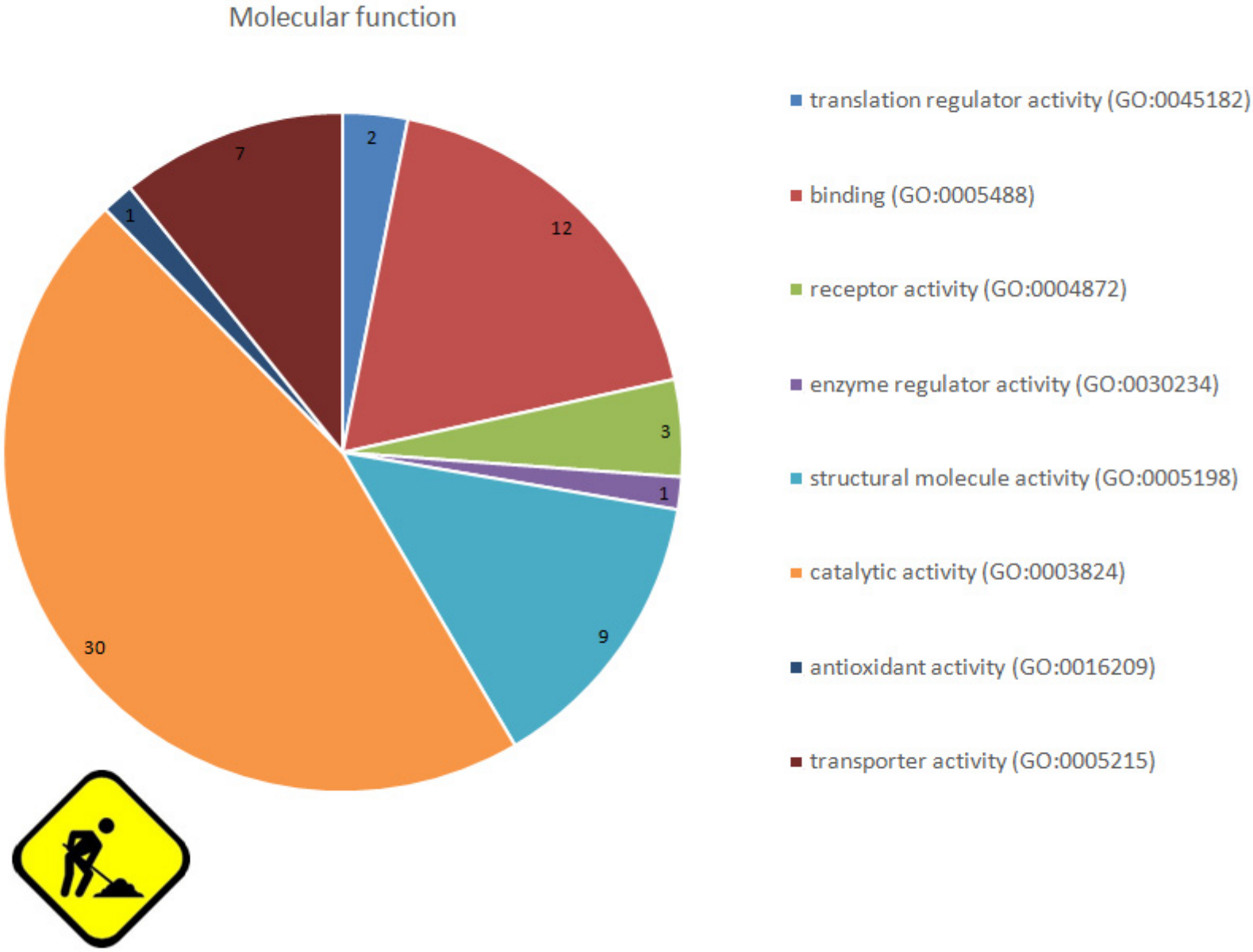
Proteome characterization

(66% ID success rate)

Results: Proteome Characterization



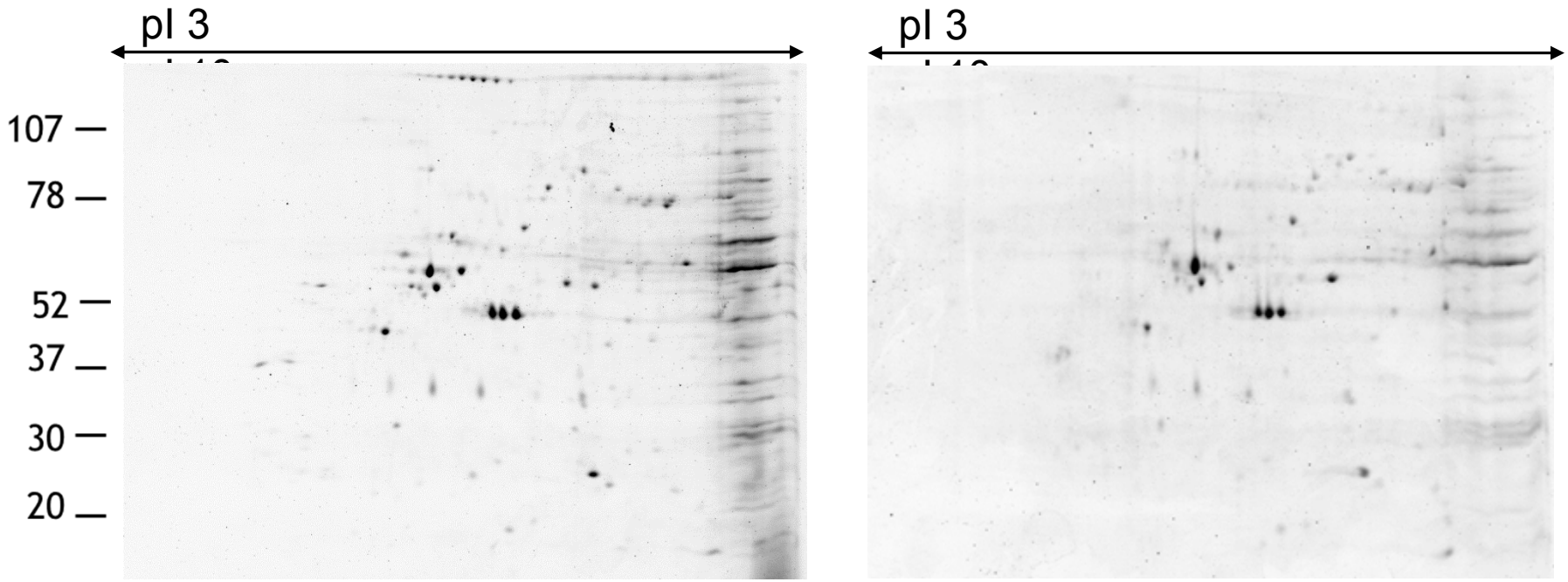
Results: Proteome Characterization



Results: Comparative study

majorera

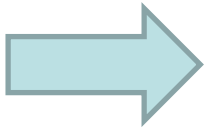
palmera



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;

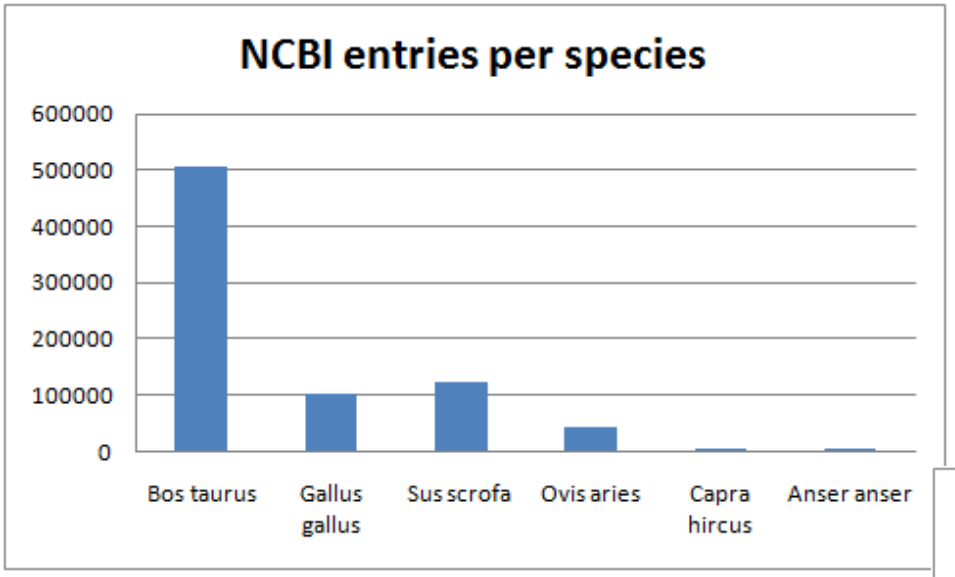
- Animal Science researchers have little knowledge on Proteomics achievements and principles;

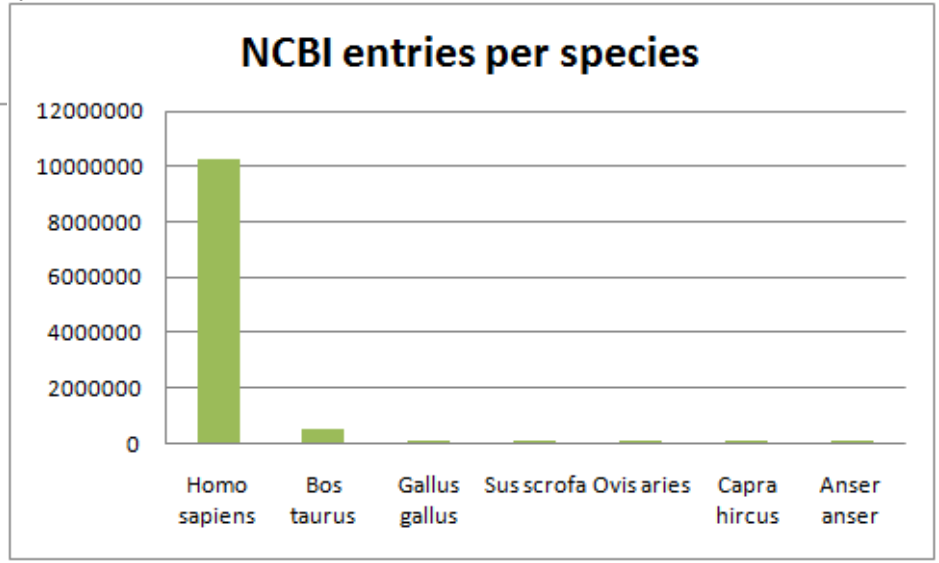
- Animal Science researchers have little access to proteomics and Mass Spec equipment;

- 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?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Review

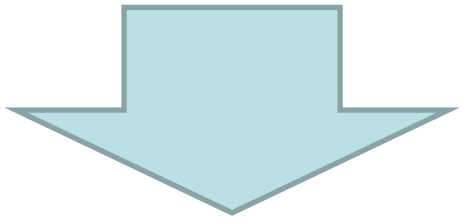
Mass spectrometry and animal science: Protein identification strategies and particularities of farm animal species☆

Renata Soares[a], *Catarina Franco*[a], *Elisabete Pires*[a], *Miguel Ventosa*[a,b], *Rui Palhinhas*[a], *Kamila Koci*[a], *André Martinho de Almeida*[a,c,d], *Ana Varela Coelho*[a,*]

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, metabolomice, lipidomics, etc)



International Collaboration and Networking in application to funds and research activities

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