First characterization of the goat mammary gland mitochondrial proteome: A gel based approach on the study of tolerance to weight loss in two breeds from the Canary Islands

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Introduction

- Seasonal weight loss poses 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
- To control Seasonal Weight Loss, supplementation is often implemented– Unavailable in undeveloped countries and Expensive in developed countries

Introduction

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.

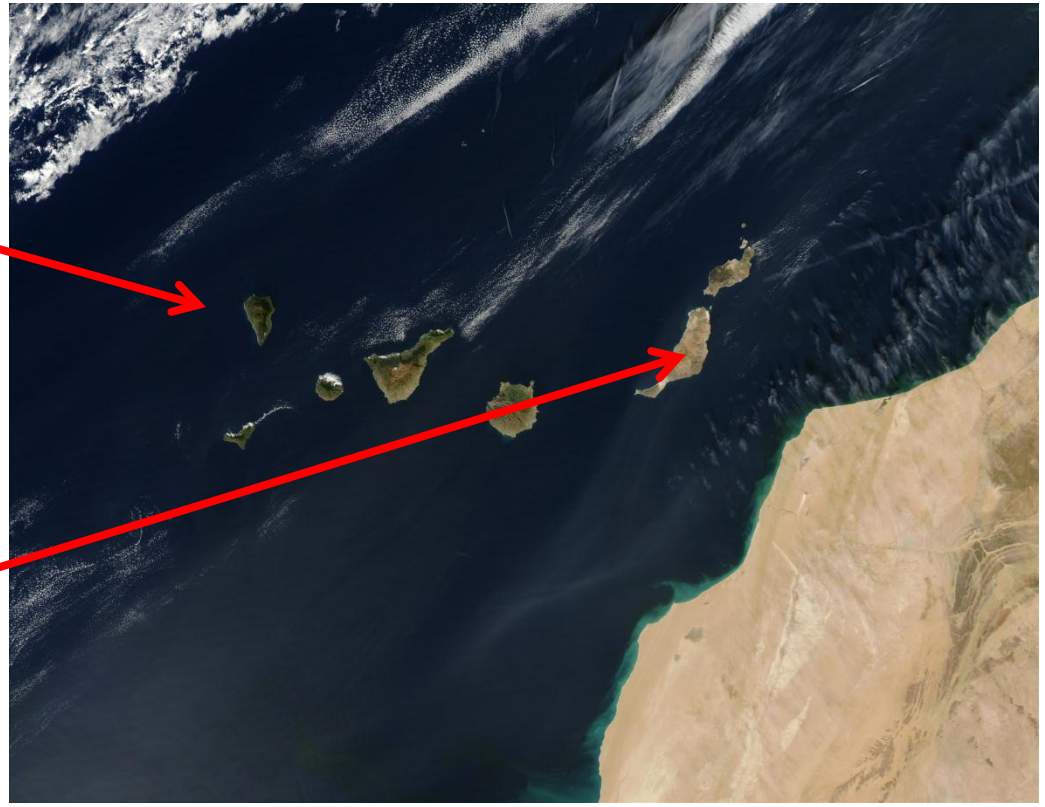
Trypanotolerant dwarf cattle and goats of West Africa

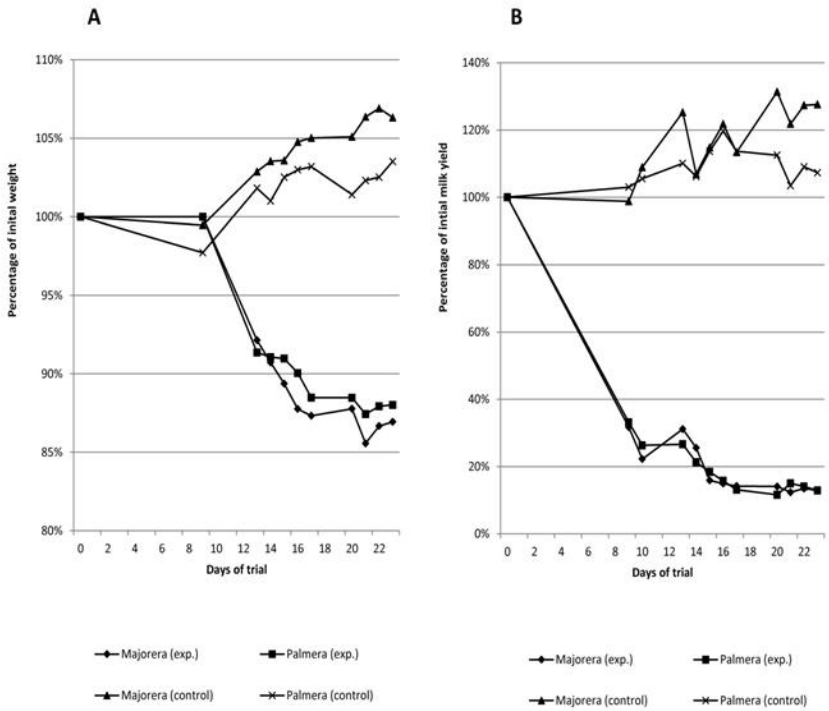
Bos indicus – tropical climate conditions

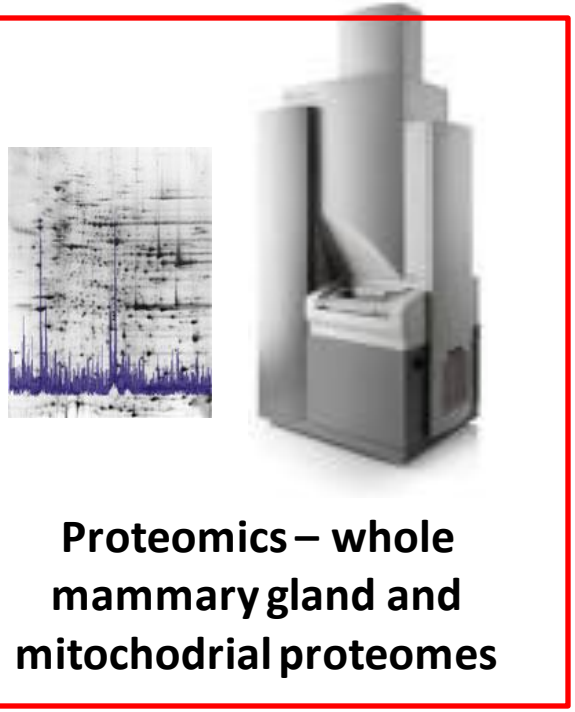
Introduction

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)

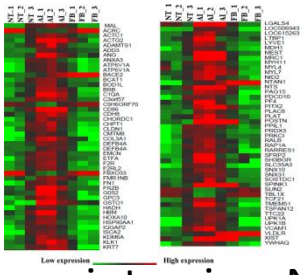
Project Objective: Establish Molecular Markers of Tolerance to SWL in dairy goats of use in selection programs







Proteomics – whole mammary gland and mitochodrial proteomes



Transcriptomics - NGS (See Poster on RNA extraction)

Lipidomics: Fatty Acid Profiling

Metabolomics – NMR (See Poster)

Introduction to Proteomics

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

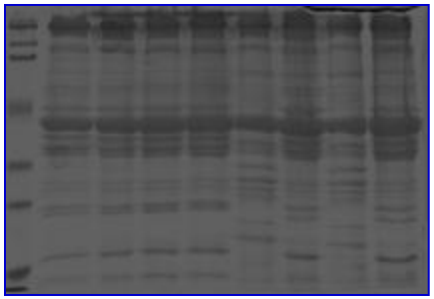
In order to understand how Biological Systems function, it is of the utmost importance to know how the proteome changes as a consequence of a stimulus

PROTEOMICS:

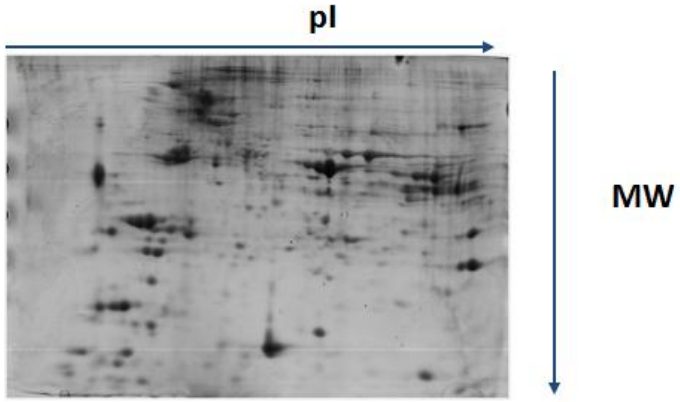
Study of the proteome;

Description and explanation of quantitative and qualitative changes in the proteome as a consequence of a certain stimulus

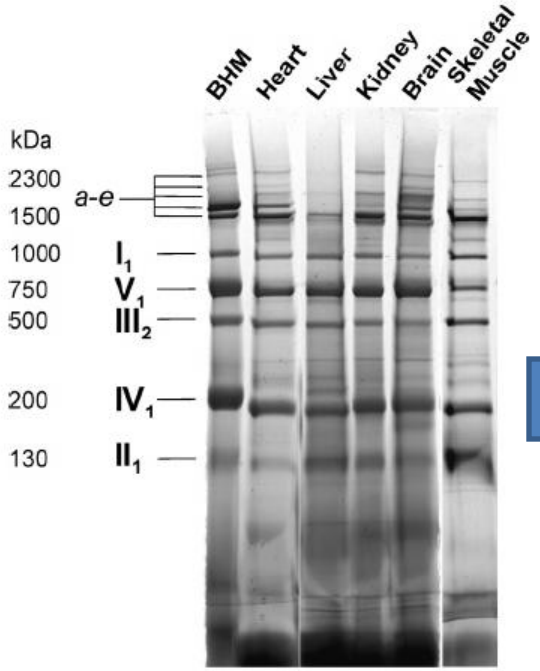
Introduction



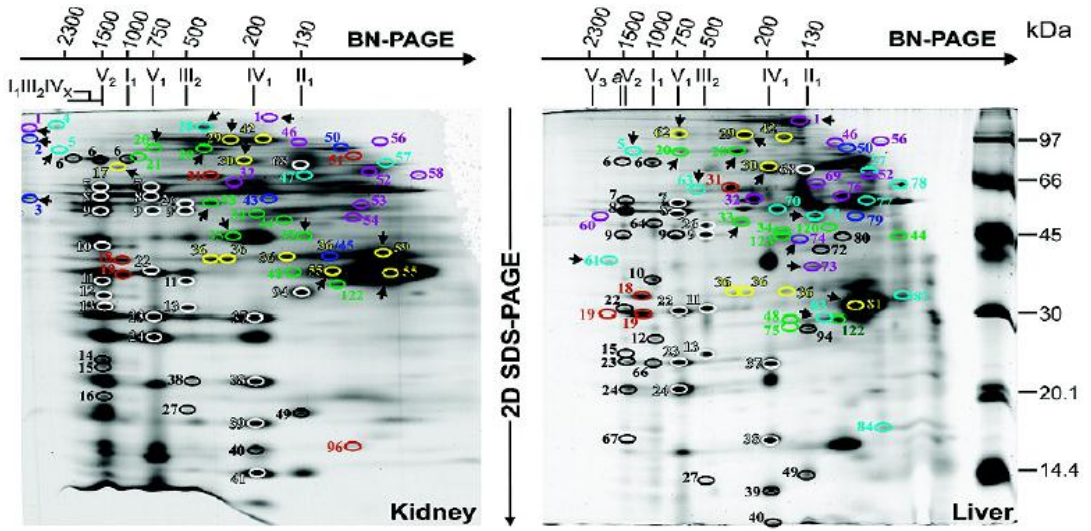
One Dimensional gel



Two Dimensional gel



Blue Native Gel – complex separation



Blue Native Gel – SDS separation

General Overview & Objectives

Majorera

Palmera

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

Majorera

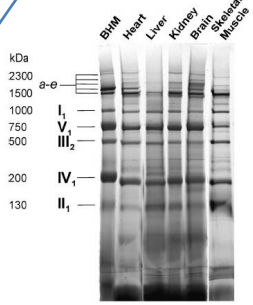
Palmera

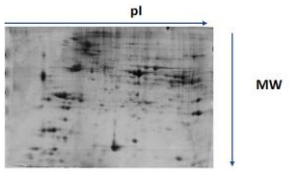
2 control groups

Mammary gland Biopsies (Secretory Tissue)

Mitochondria isolation

BN & 2DE





Protein ID using Mass Spectrometry

42 day trial

OBJECTIVES

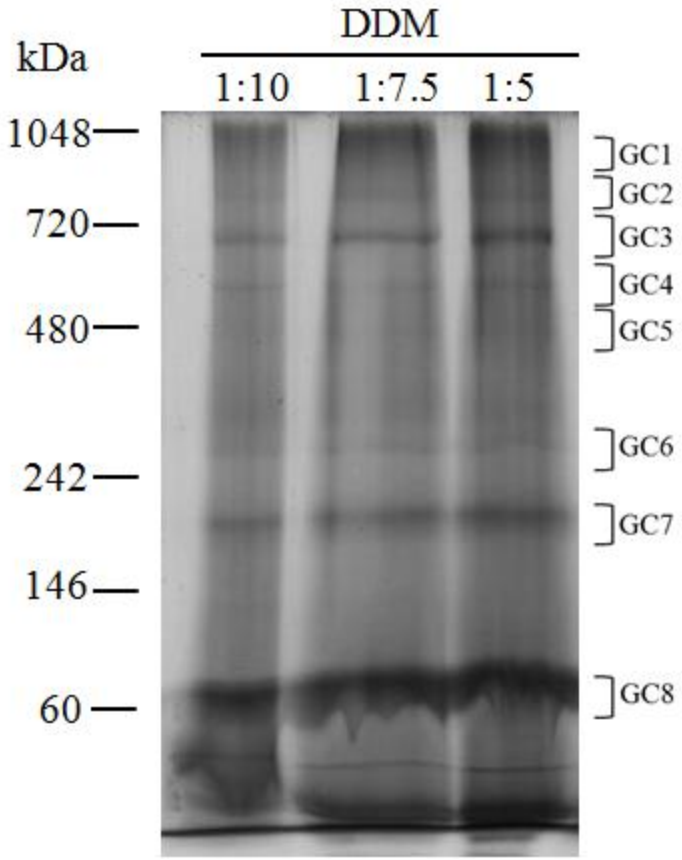
Proteome Characterization

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

Protein : Detergent Rations

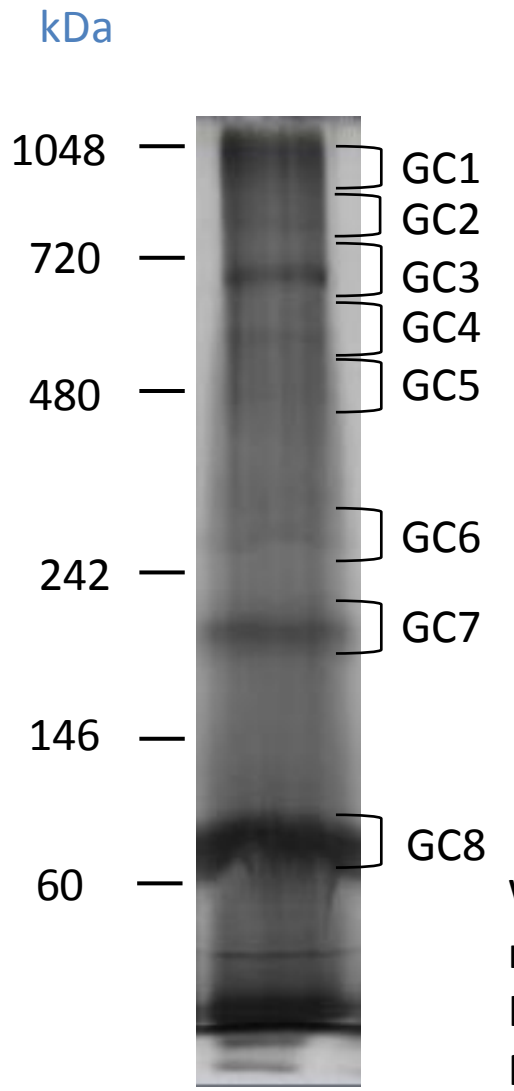


Similar band Profile – Eight Putative Mitochondrial Protein Complexes?

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

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

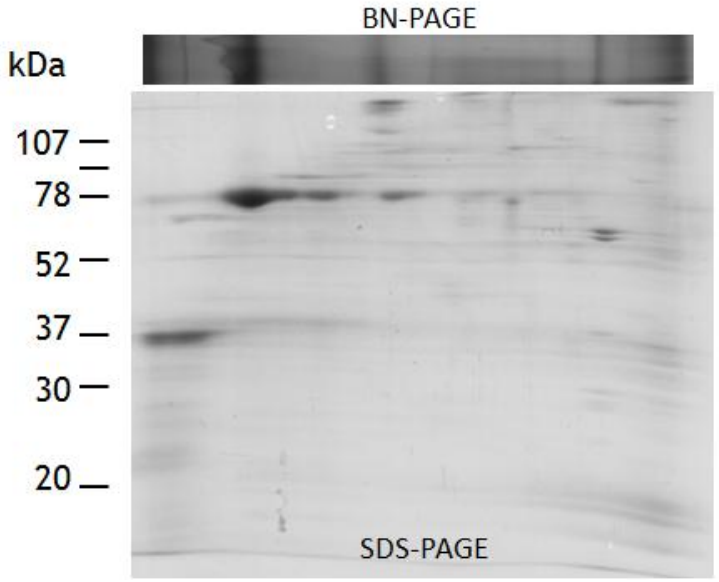
Results: Optimization of the Blue Native Protocol and Proteome Characterization



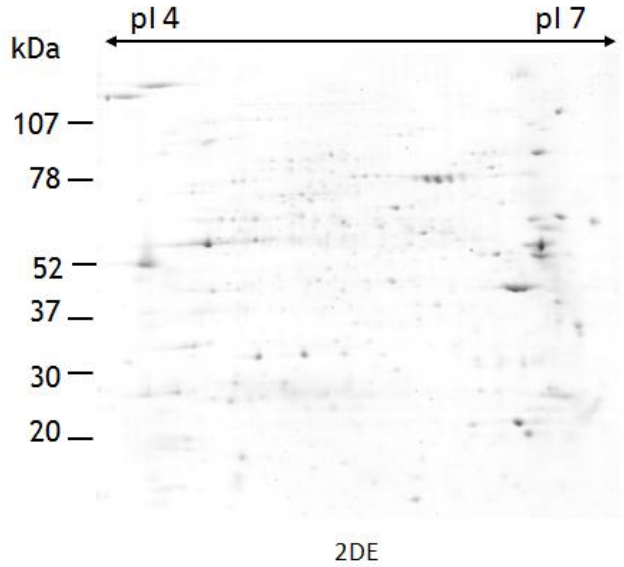
Protein band	membrane	type of complex	subunits
G8	y	complex V	2
	y	Voltage-dependent anion-selective channel	3
GC7	y	complex V	2
GC6	y	cation transport ATPase	1
	y	complex IV	2
GC5	n	glutamate dehydrogenase	3
	y	complex V	3
	y	complex II	2
	y	NAD(P) transhydrogenase	1
GC4	y	ATPase, complex V	2
	n	glutamate 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

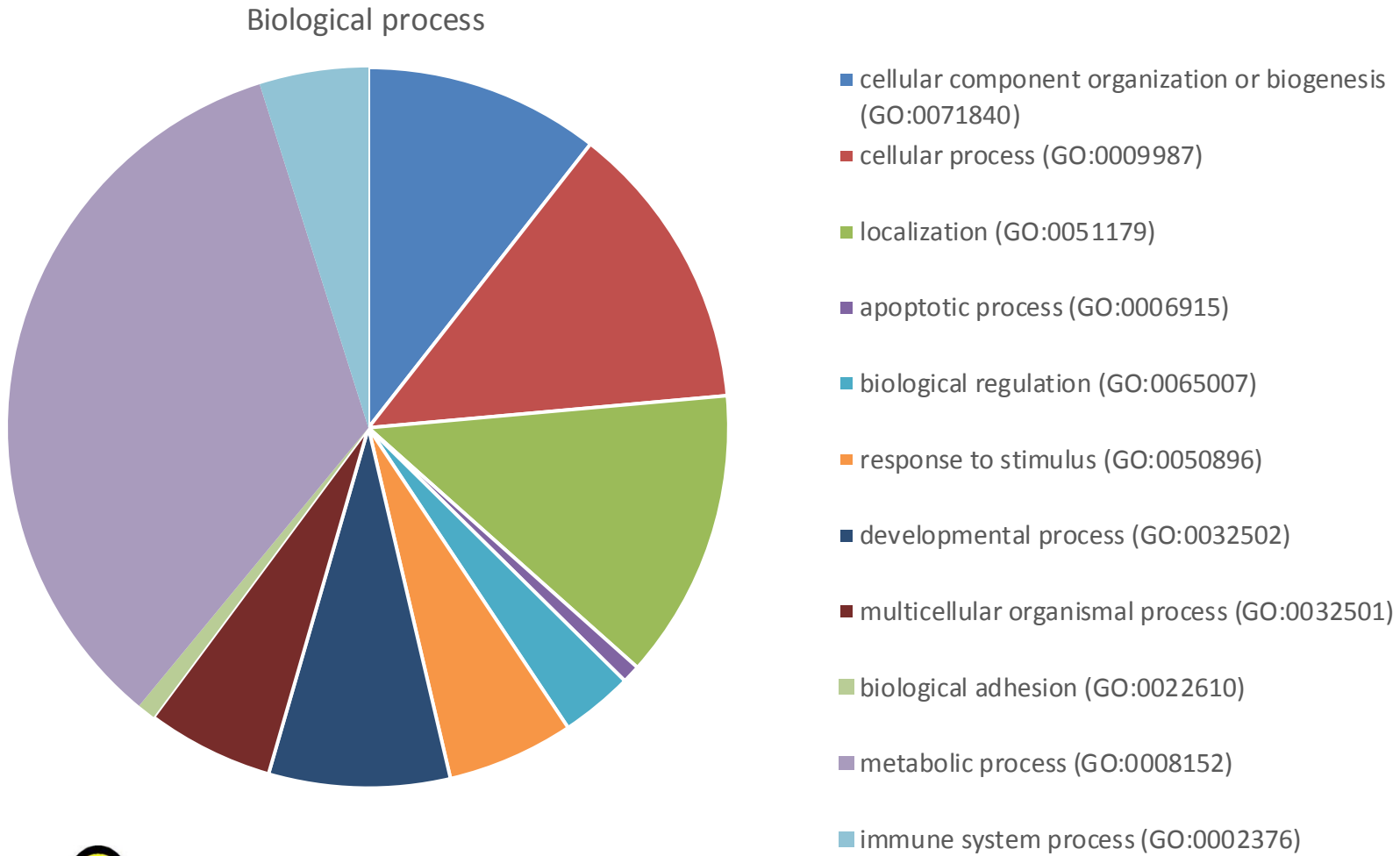


188 different spots

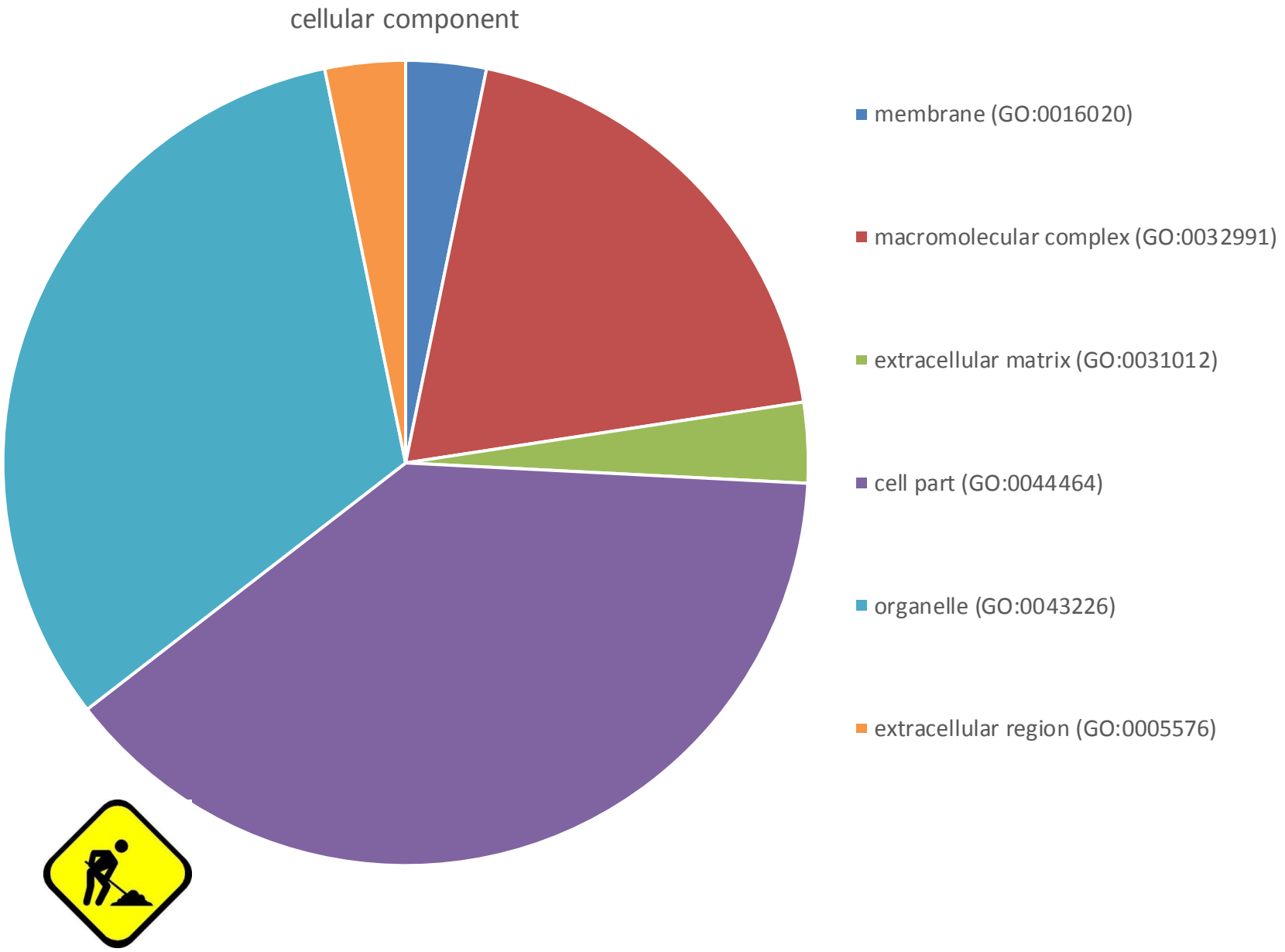
Proteome characterization

(66% ID success rate)

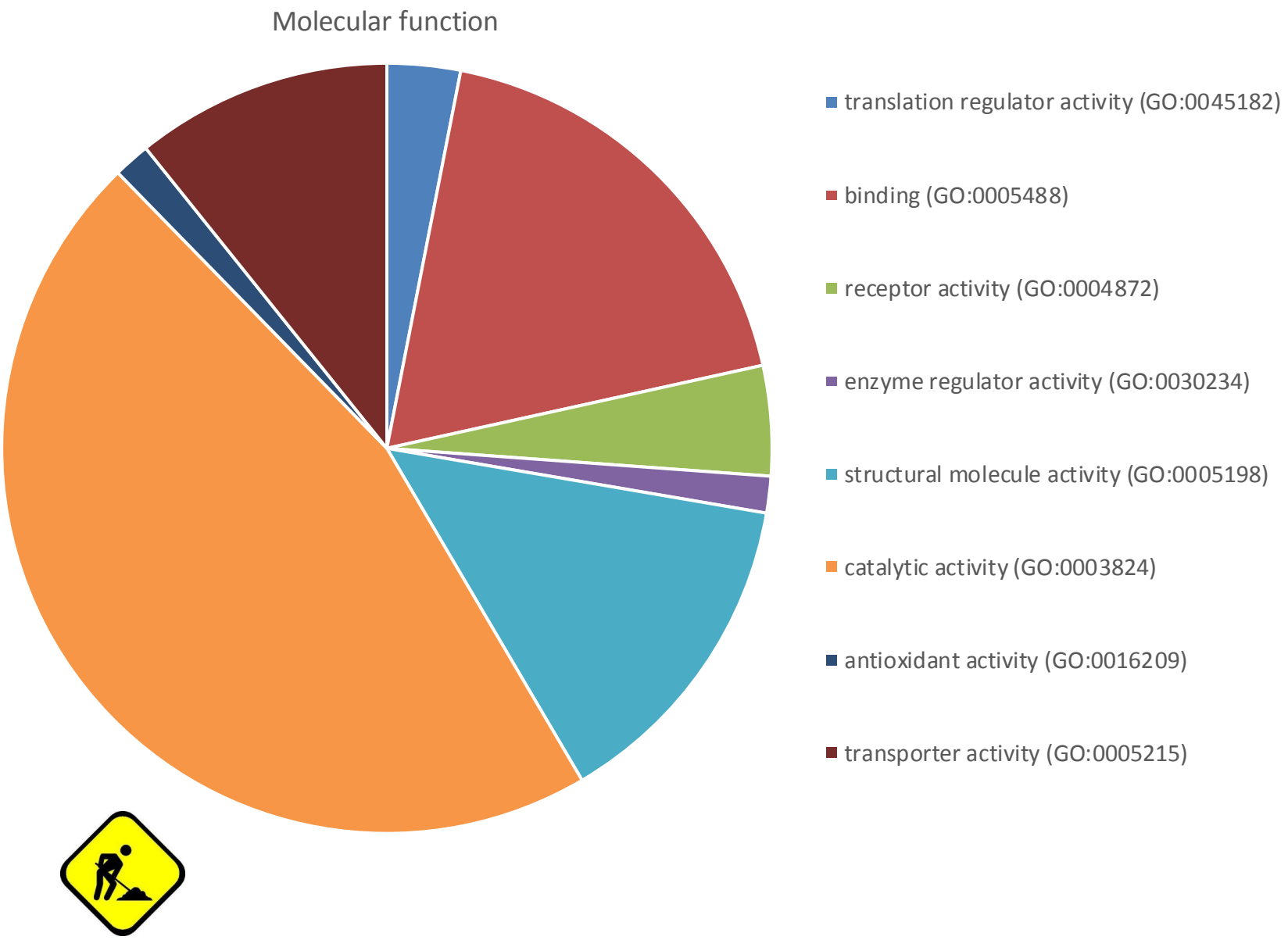
Results: Proteome Characterization



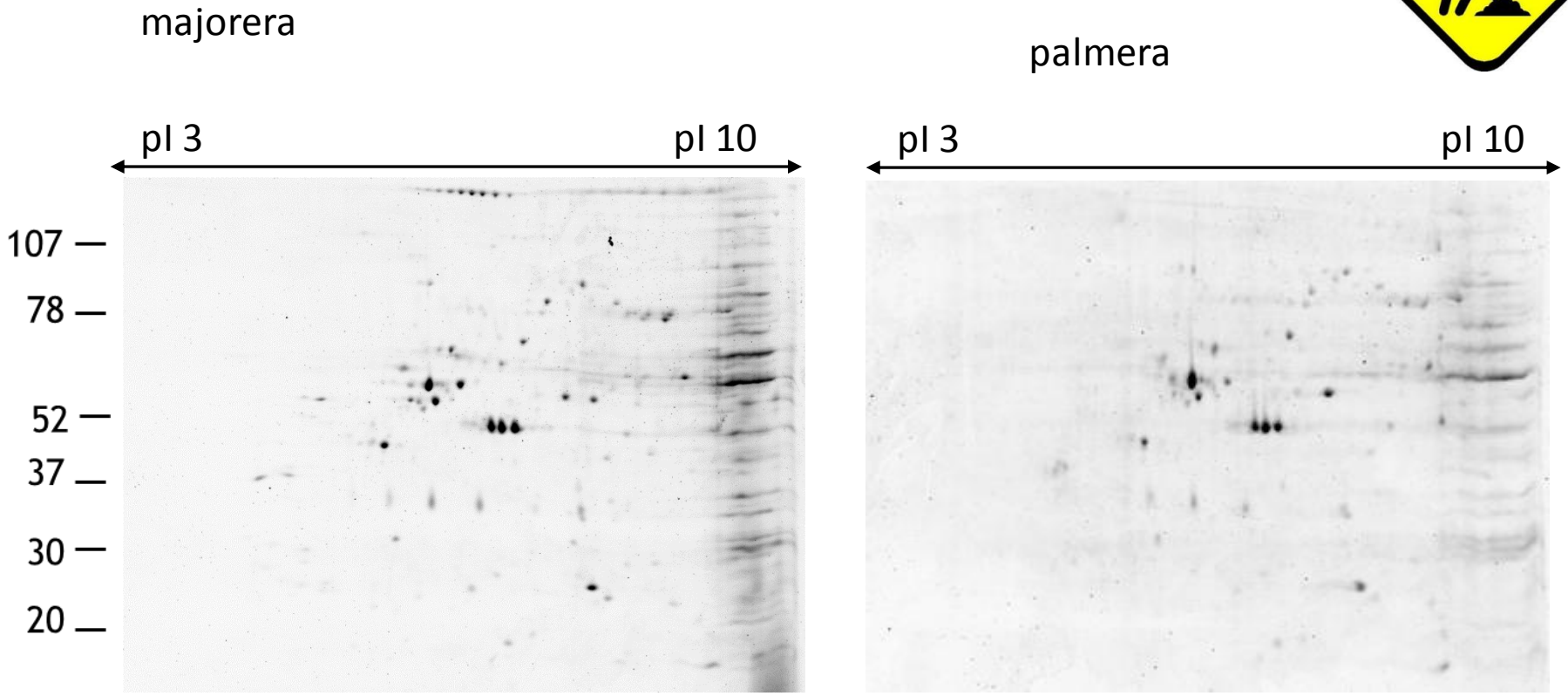
Results: Proteome Characterization



Results: Proteome Characterization



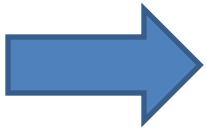
Results: Comparative study



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

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FAP Farm Animal Proteomics

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