EAAP
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SEARCHING FOR PROTEIN BIOMARKERS RELATED TO PRE-SLAUGHTER STRESS USING LIQUID ISOELECTRIC FOCUSING

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SERIDA

Background

MEAT QUALITY

"Muscle to meat" molecular events and technological transformations: The proteomics insight ☆

Gianluca Paredi[a,b], Samanta Raboni[a,b], Emøke Bendixen[d,e], André M. de Almeida[f,g], Andrea Mozzarelli[a,b,c,]*

Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved

Ahmed Ouali[a], Mohammed Gagaoua[a,b], Yasmine Boudida[b], Samira Becila[b], Abdelghani Boudjellal[b], Carlos H. Herrera-Mendez[a], Miguel A. Sentandreu[c]

What about pre-slaughter stress?

Tackling proteome changes in the *longissimus thoracis* bovine muscle in response to pre-slaughter stress

Daniel Franco[a], Ariadna Mato[b], Francisco J. Salgado[c], María López-Pedrouso[b], Mónica Carrera[d], Susana Bravo[e], María Parrado[b], José M. Gallardo[d], Carlos Zapata[b,]*

Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants?

D.M. Ferguson[a,], R.D. Warner[b]*

Background

Extrinsic

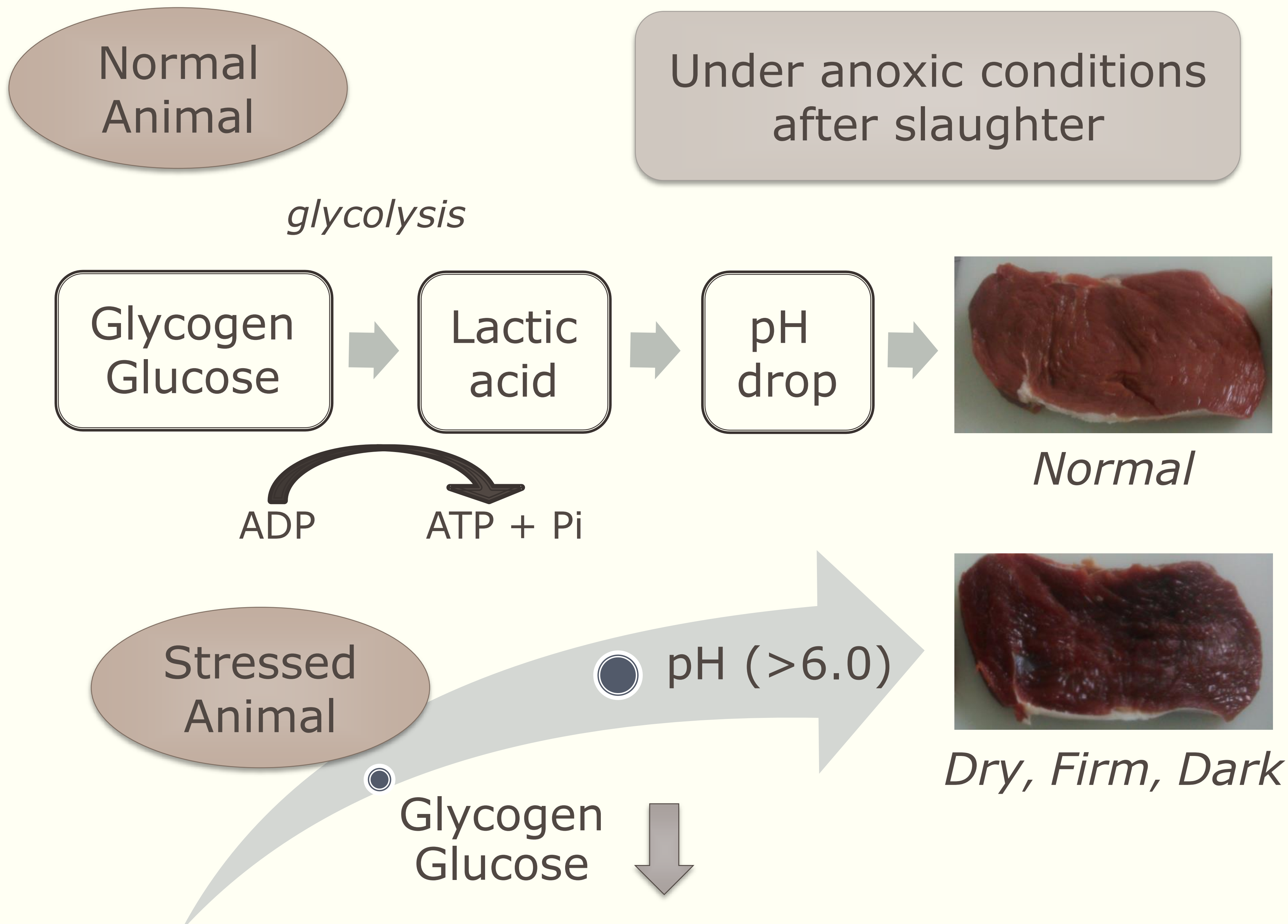
Intrinsic

Sex, age, genetics, physiology

Handling activities, human presence, water deprivation

Pre-slaughter stress

Changes in meat quality



Normal Animal
Under anoxic conditions after slaughter
glycolysis
Glycogen Glucose
Lactic acid
pH drop
Normal
ADP
ATP + Pi
Stressed Animal
pH (>6.0)
Glycogen Glucose
Dry, Firm, Dark

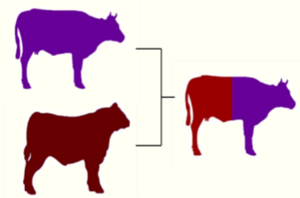
Objective

Study of sarcoplasmic sub-proteome from pre-slaughter stressed animals in comparison to normal animals using liquid isoelectric focusing (OFFGEL) in order to elucidate which proteins and biochemical pathways could be involved in pre-slaughter stress (PSS)

Materials and methods

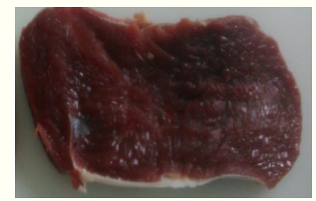
Asturiana de los Valles breed
n=8

Crossbred
n=8



pH measurement

Normal
(pH<6)
n=10



DFD
(pH>6)
n=6

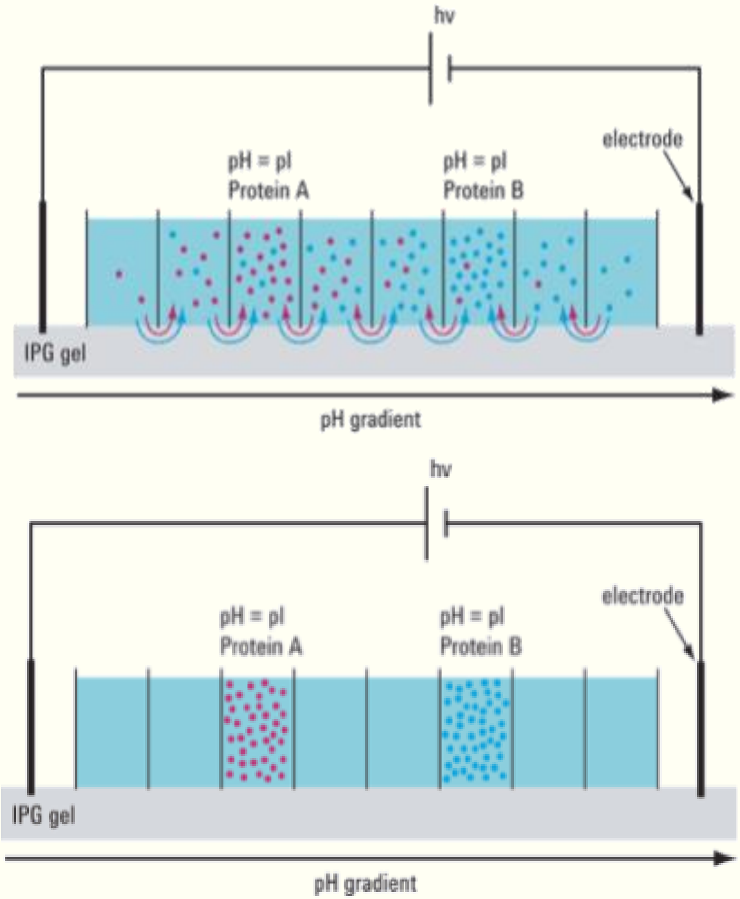
Proteome analysis

- ✓ Extraction
- ✓ Fractionation
- ✓ Quantification
- ✓ Identification

Materials and methods

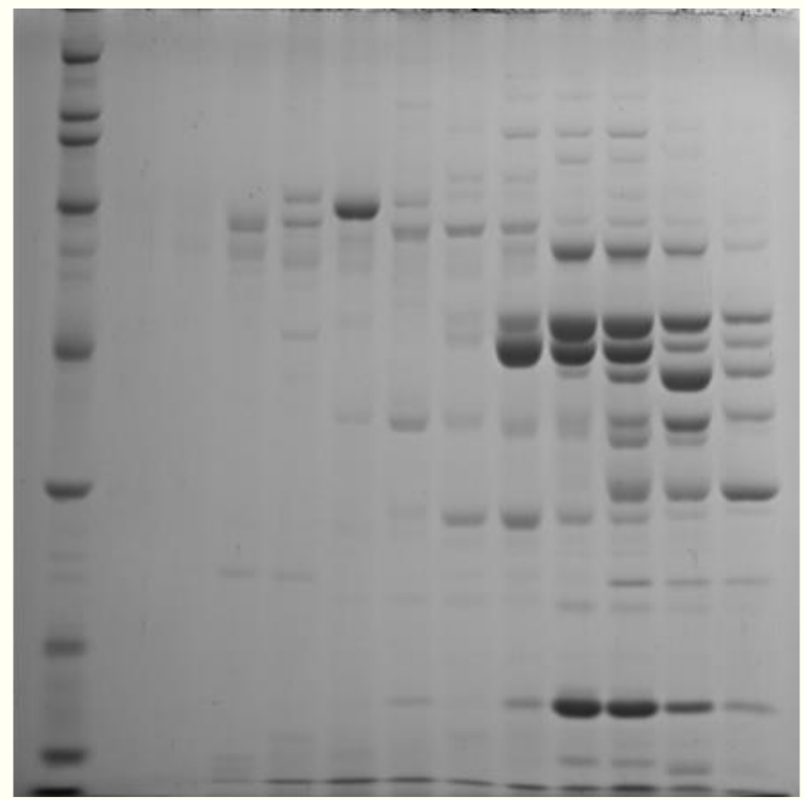
1 OFFGEL technology

✓Separation through their p*I*

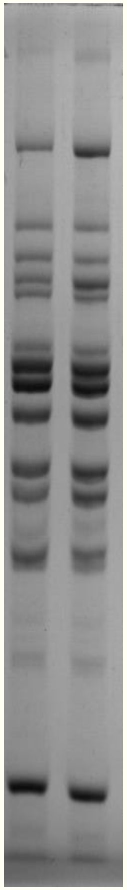


2 SDS-PAGE

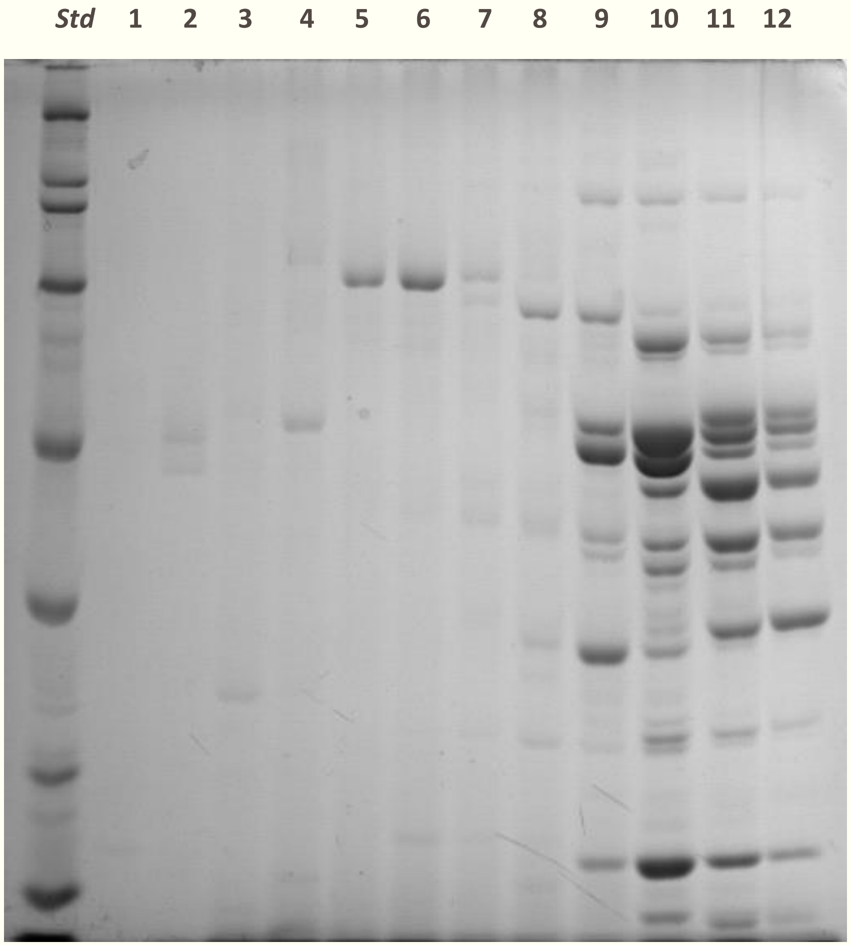
✓Separation through their Mr



Materials and methods



SDS-PAGE



OFFGEL plus SDS-PAGE

Materials and methods

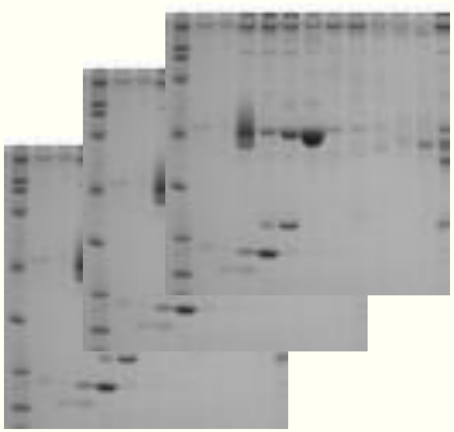
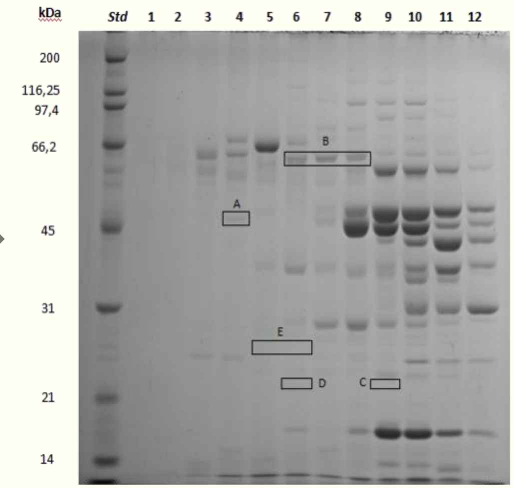


Image Analysis

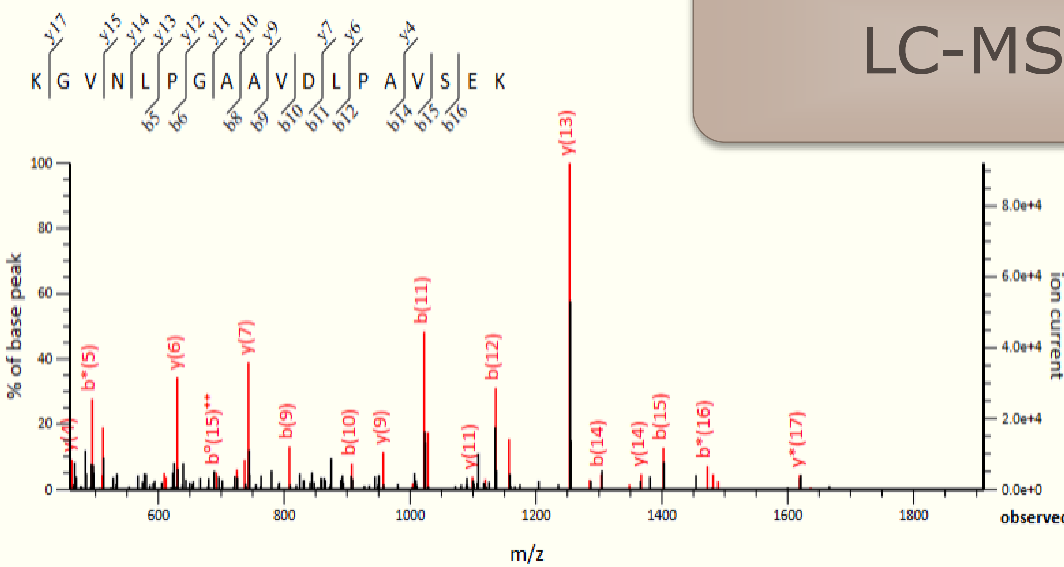
Statistical Analysis



Protein Selection

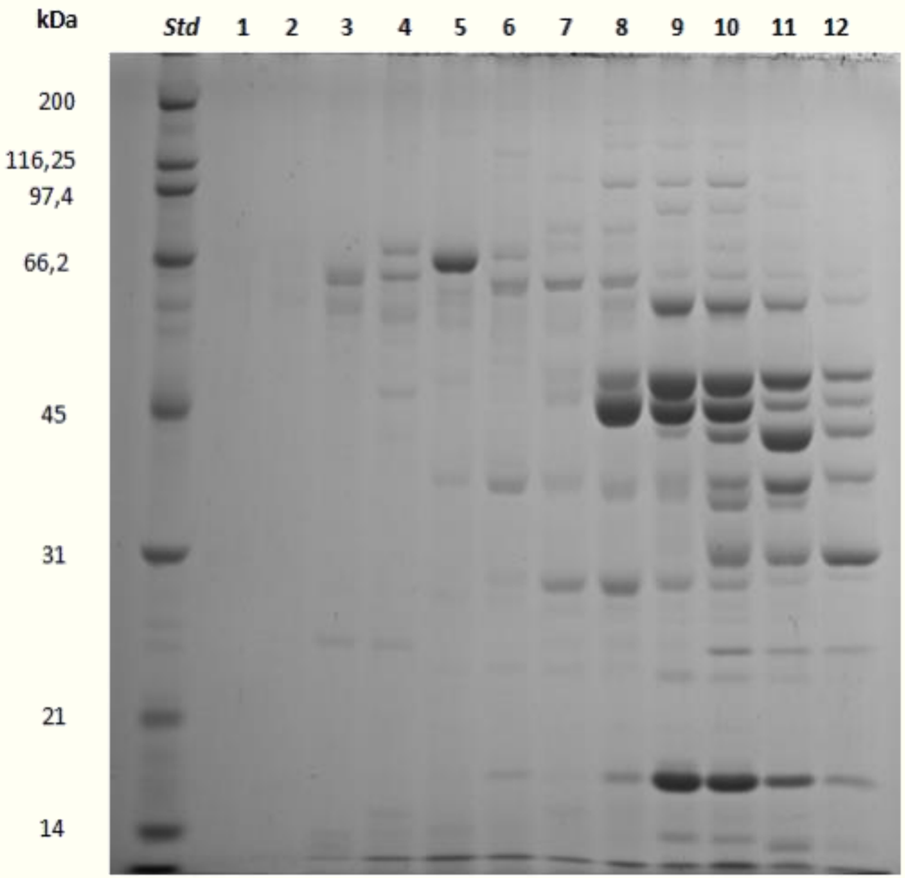
Protein Identification

LC-MS

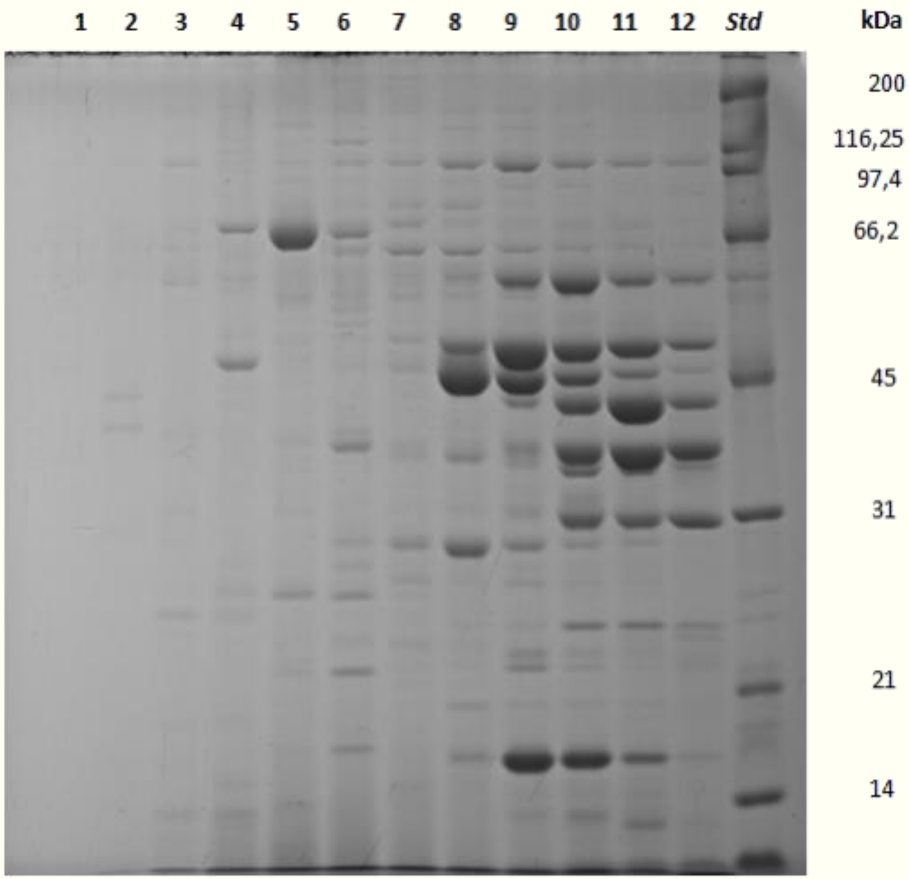


Results

Sarcoplasmic Proteome



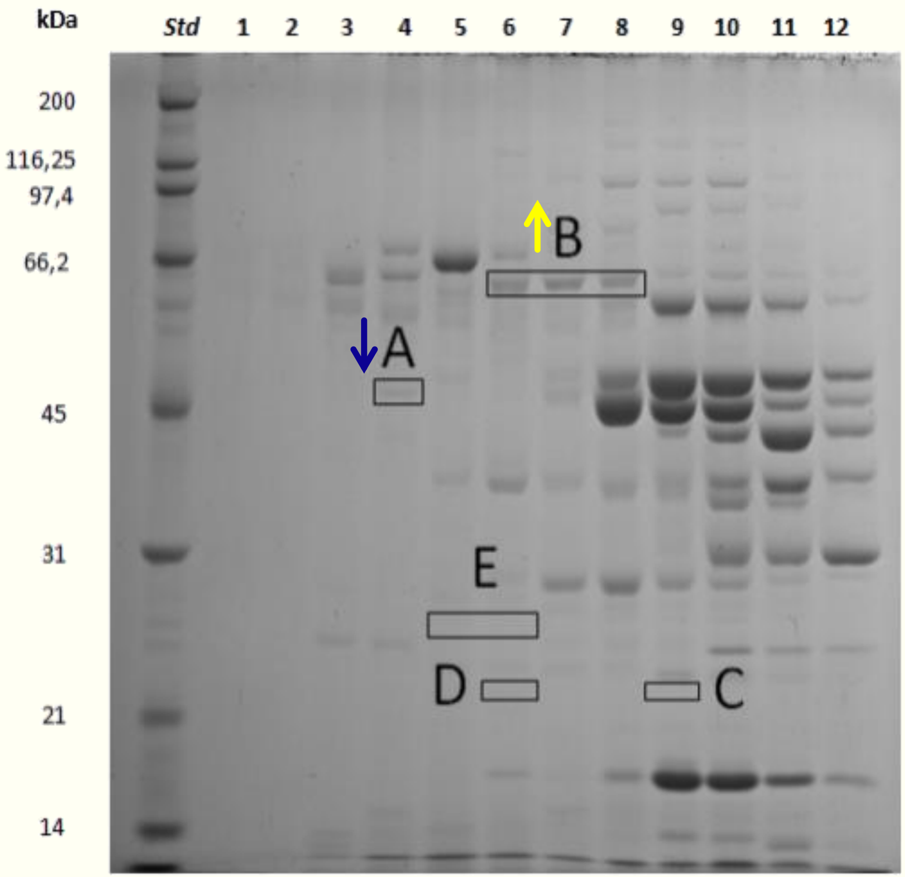
a) NORMAL



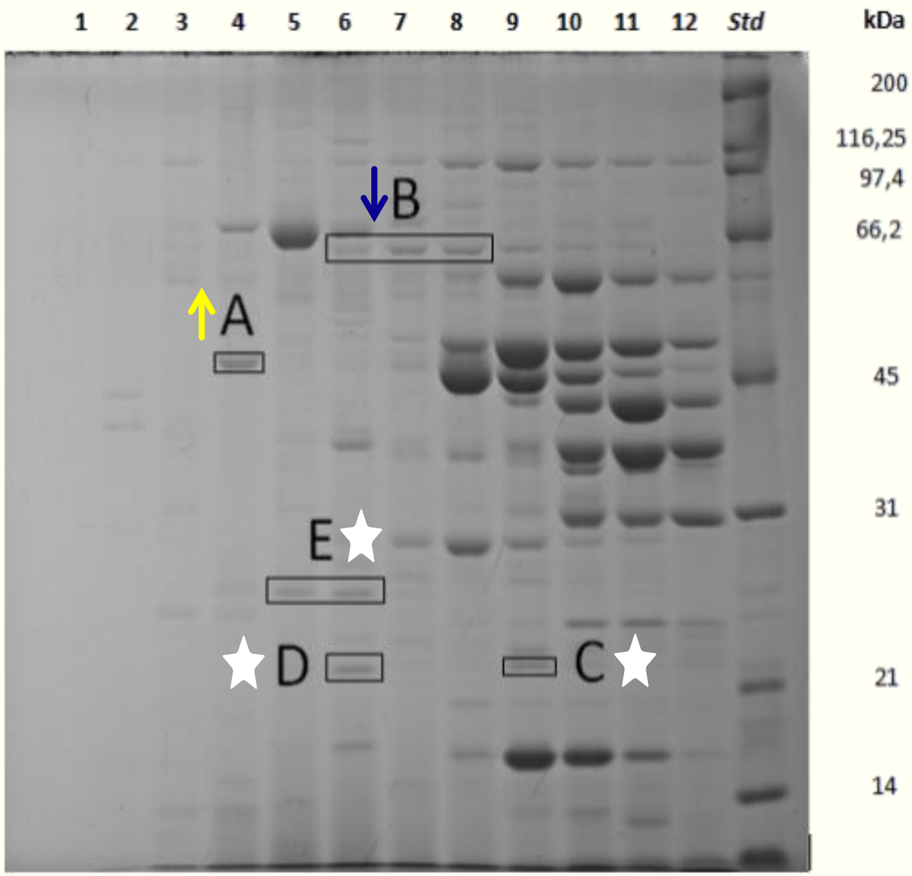
b) DFD

Results

Sarcoplasmic Proteome

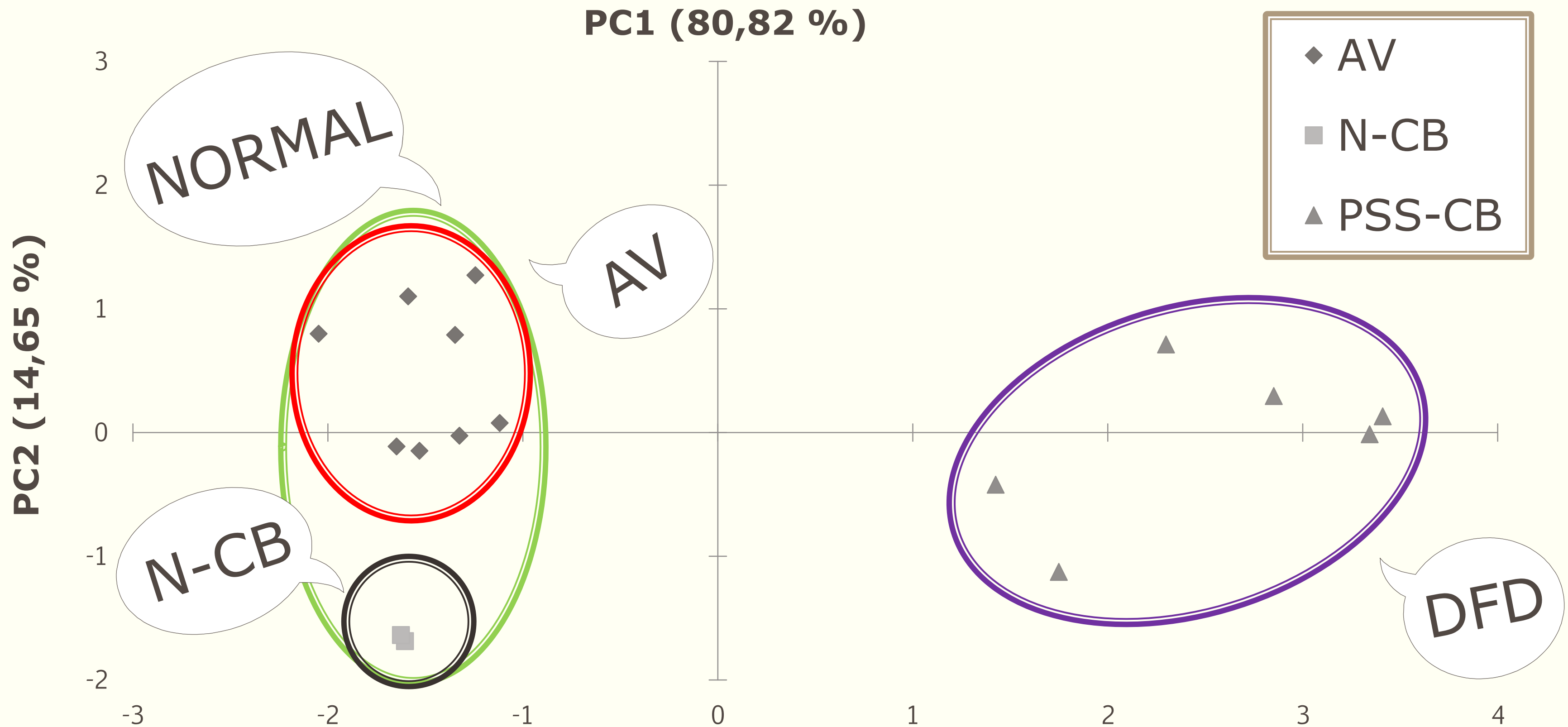


a) NORMAL



b) DFD

Principal component analysis (PCA)

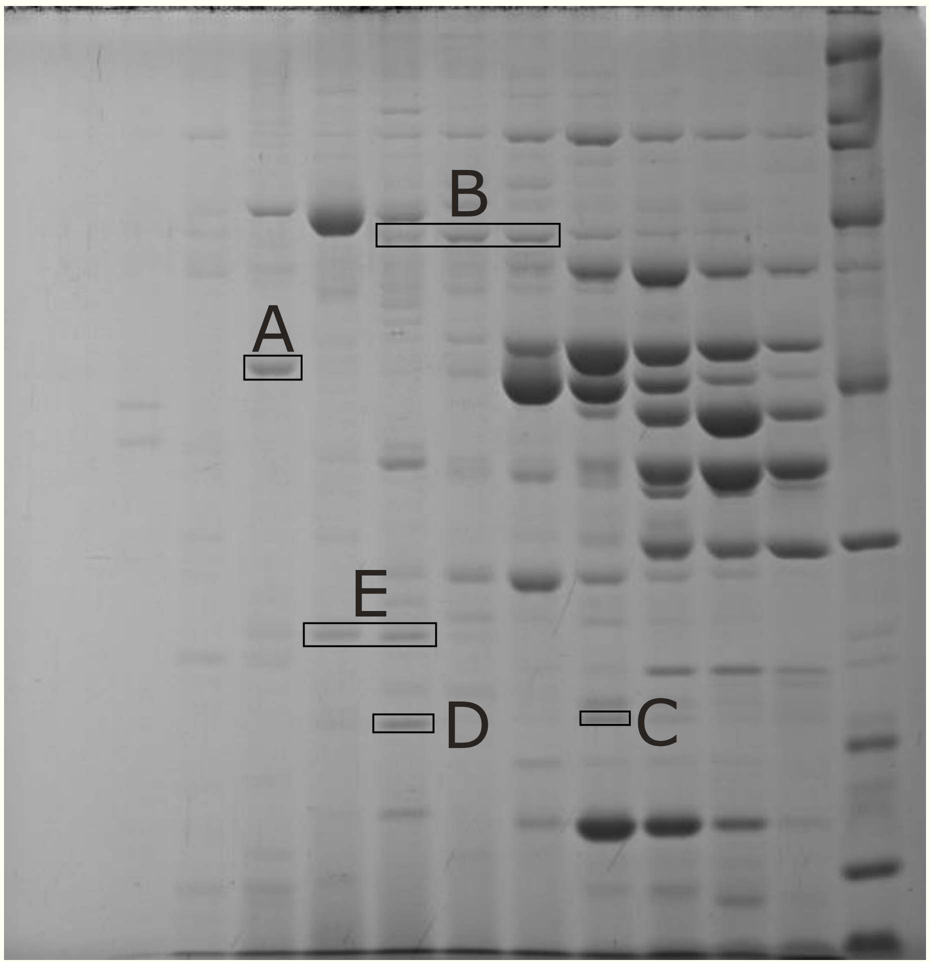


Animal sample distribution on the PCA. Each point represents an individual sample (AV: Asturiana de los Valles animals; N-CB: normal crossbred animals; PSS-CB: pre-slaughter stressed crossbred animals).

PC1: Separation between normal (AV and N-CB) and DFD (PSS-CB) groups

PC2: discriminate between AV and N-CB samples showing higher values for AV

Indentified proteins



b) DFD

Band **A**
Actin

Band **B**
Phosphoglucomutase-1

Band **C**
Alpha-crystallin B

Band **D**
Heat shock protein beta-6

Band **E**
Heat shock protein beta-1

Identified proteins

Band	Protein Identification	Biological function
A	Actin	Structural maintenance
B	Phosphoglucomutase-1	Regulation of glycogen metabolism
C	**Alpha-crystallin B**	Stress resistance
D	**Heat shock protein beta-6**	Stress resistance
E	**Heat shock protein beta-1**	Stress resistance and actin organization

Only appeared in DFD samples

$p < 0.05$

Conclusions

The comparison of sarcoplasmic sub-proteomes was successfully implemented using liquid isoelectric focusing (OFFGEL) as fractionation strategy.

The abundances of actin, alpha-crystallin B, heat shock protein B6 and heat shock protein B1 were higher in DFD samples, whereas phosphoglucomutase was over-represented in normal samples.

The identified proteins could be used as reliable biomarkers of pre-slaughter stress (PSS) through high throughput analytical approaches.

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GOBIERNO DE ESPAÑA

MINISTERIO DE AGRICULTURA Y PESCA, ALIMENTACIÓN Y MEDIO AMBIENTE

THANK YOU FOR YOUR ATTENTION

Acknowledgements:

The first author received a scholarship funded by UEECA-MAPAMA to attend the 69th EAAP annual meeting

The Department of Economic Development & Competitiveness of the Basque Government

Spanish Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (RTA2014-00034-C04) and FRIBIN S.A.T. (Binéfar, Huesca)

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INIA

FRIBIN GUSTO POR LA CARNE