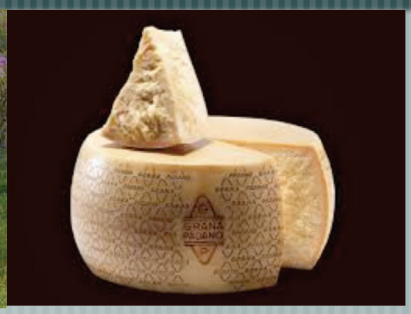
UNIVERSITÀ DEGLI STUDI DI MILANO

DIPARTIMENTO DI SCIENZE VETERINARIE E SANITÀ PUBBLICA

THE MILK PROTEOME IN DIARY SCIENCE: FROM ANIMAL WELFARE TO FOOD SAFETY





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Istituto Sperimentale Italiano L. Spallanzani- Dipartimento di Scienze Veterinarie e Sanità Pubblica- Università degli Studi di Milano, Milano, Italy

AND

Cristian Piras, Alessio Soggiu, Isabella Alloggio, Luigi Bonizzi
(Dipartimento di Scienze Veterinarie e Sanità Pubblica- Università degli Studi di Milano, Milano, Italy)

EAAP Copenhagen August 25th 2014

MILK PROTEOME

Evolution of feeding

'PARENTERAL'

'ORAL AND GI'

MILK FOR ALL MAMMALIA

HUMAN: AIM: GROWTH, CELLULAR DIFFERENTIATION

OTHER: SELECTION TO IMPROVE MILK PRODUCTION DIRECT TO HUMAN FOOD

CO-EVOLUTION!

THE VISION

ONE HEALTH CONCEPT

ONE MEDICINE

the One Health concept provides a pathway to showcase how human, environmental and animal health are interrelated. **One Health (formerly called One Medicine) is dedicated to improving the lives of all species —human and animal—through the integration of human medicine, veterinary medicine and environmental science.**

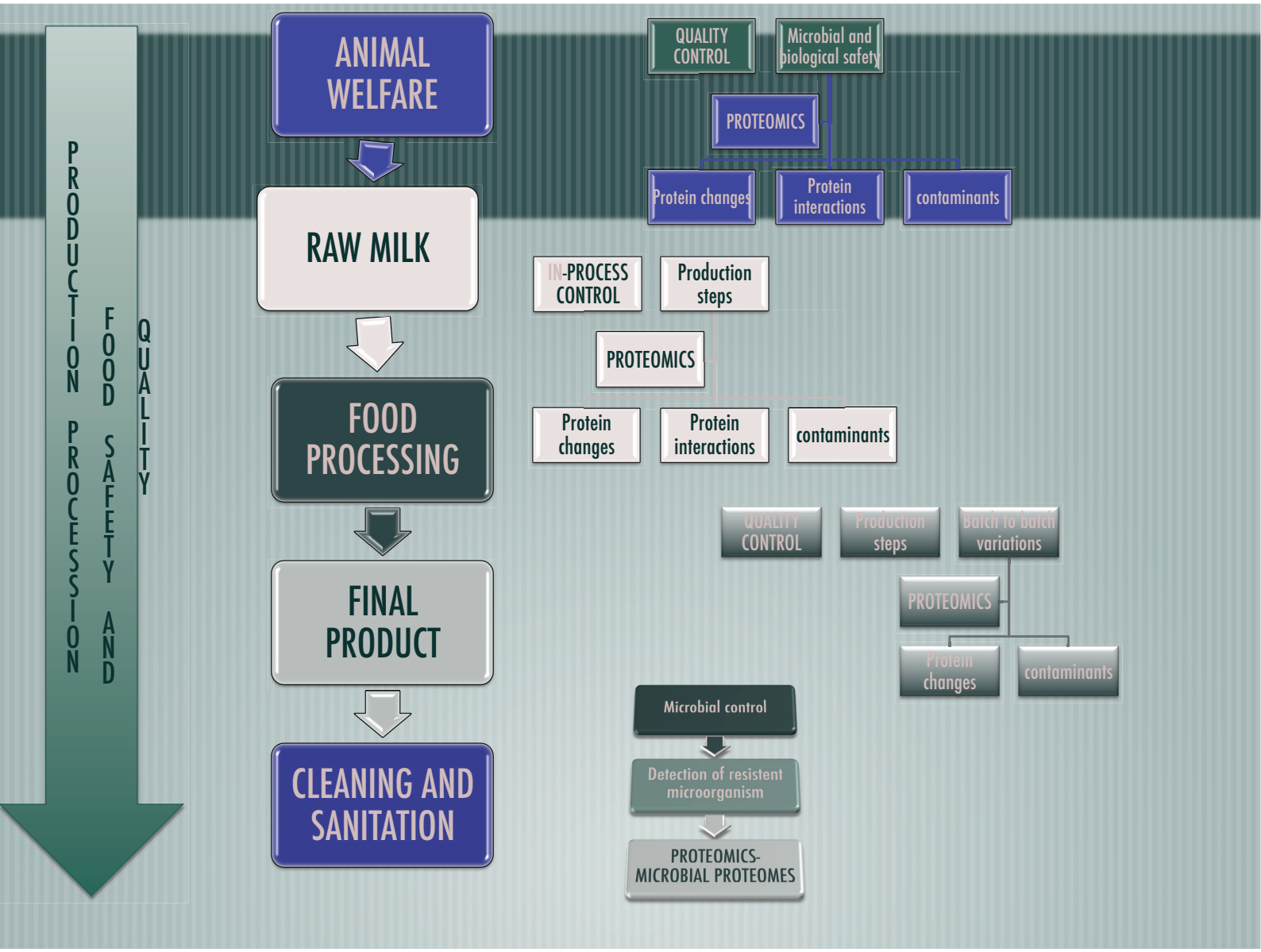
http://www.onehealthinitiative.com/

MISSION

THE LINK BETWEEN OH AND MILK

GENERAL CONCEPTS

Use of proteomics in the development pathway for milk production, and assessing of milk safety and quality


PRODUCTION PROCESSION FOOD SAFETY AND QUALITY
ANIMAL WELFARE
RAW MILK
FOOD PROCESSING
FINAL PRODUCT
CLEANING AND SANITATION
QUALITY CONTROL
Microbial and biological safety
PROTEOMICS
Protein changes
Protein interactions
contaminants
IN-PROCESS CONTROL
Production steps
PROTEOMICS
Protein changes
Protein interactions
contaminants
QUALITY CONTROL
Production steps
Batch to batch variations
PROTEOMICS
Protein changes
contaminants
Microbial control
Detection of resistent microorganism
PROTEOMICS-MICROBIAL PROTEOMES

DIAGNOSTICS

GREAT PROMISE FOR MEDICINE

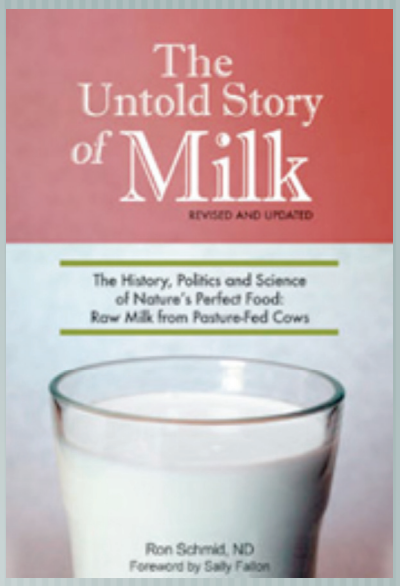
MICROBIAL PROTEOMICS: THE CHALLENGE

ANIMAL PRODUCTION

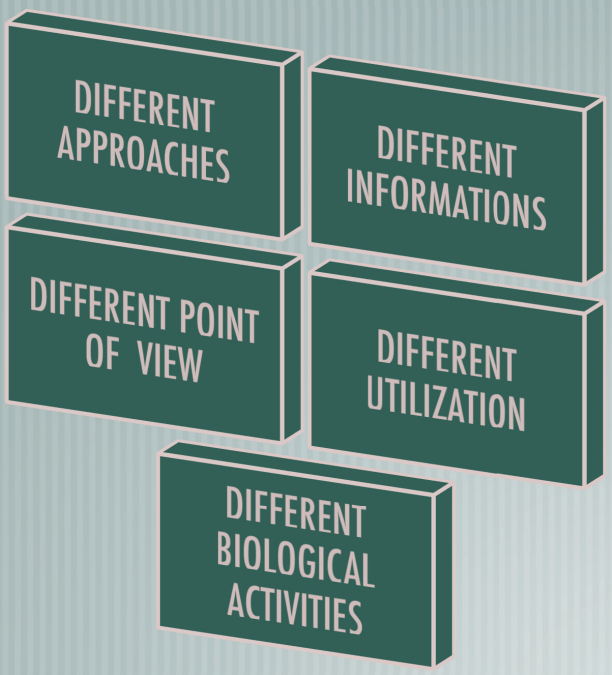
Etiology
Pathogenesis of DISEASE

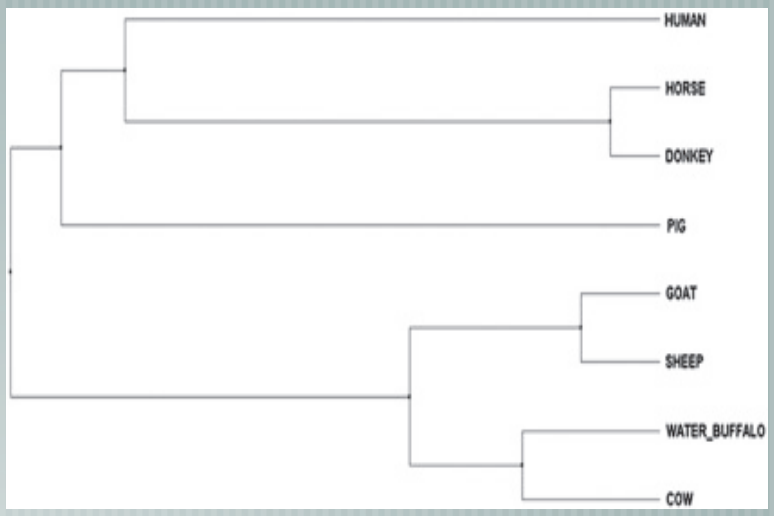
ANIMAL WELFARE
PRODUCTION
QUALITY
FOOD SAFETY

FOOD SAFETY AND THE MILK STORY



HUMAN MILK and MAMMALIAN MILK





HUMAN MILK vs OTHER MAMMALIA

NUTRITION, DEVELOPMENT OF NEWBORN

IMMUNITY

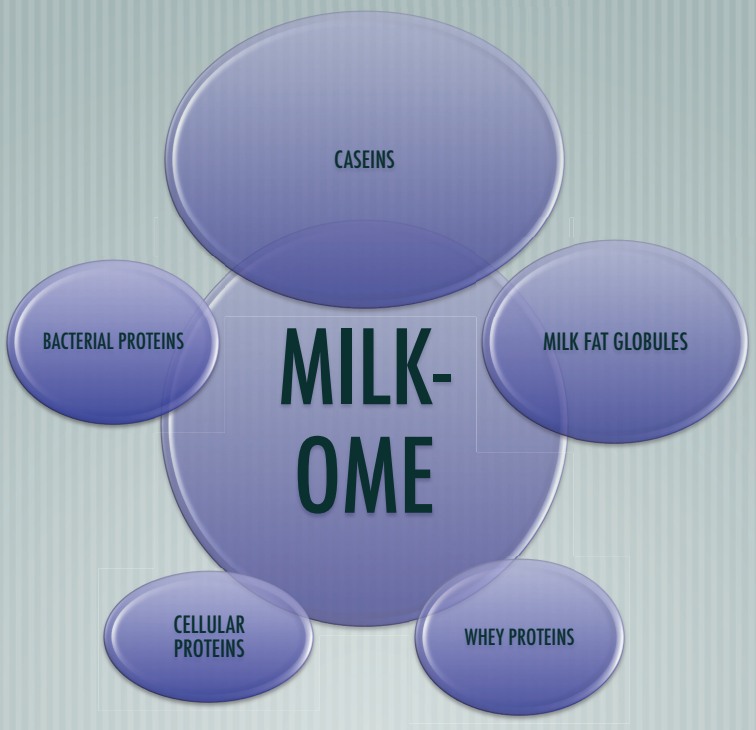
BRAIN DEVELOPMENT

ONLY FOR HUMAN NUTRITION

NUTRITION AND DEVELOPMENT OF NEWBORN.

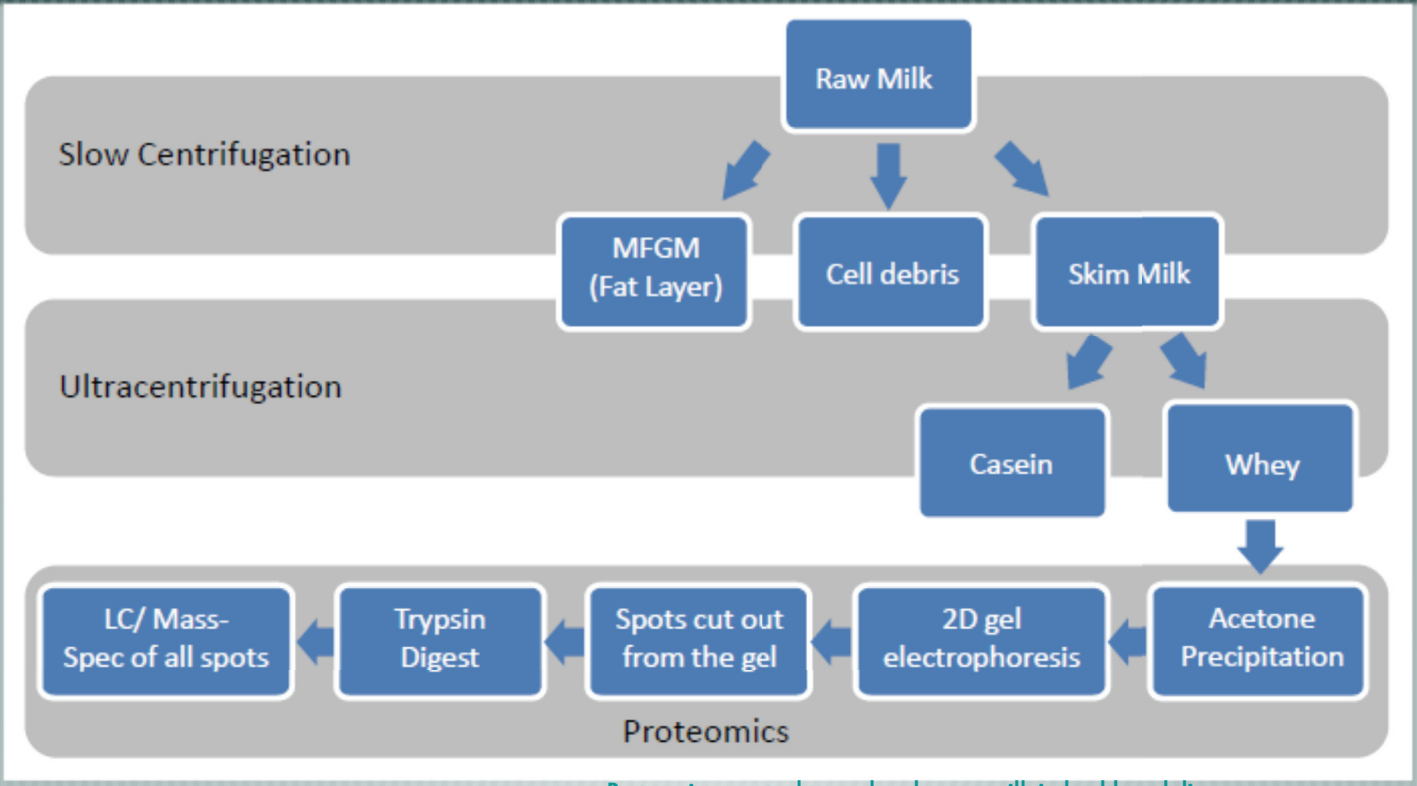
HUMAN NUTRITION!!!

DAIRY PRODUCTS!


CASEINS
BACTERIAL PROTEINS
MILK FAT GLOBULES
MILK-OME
CELLULAR PROTEINS
WHEY PROTEINS

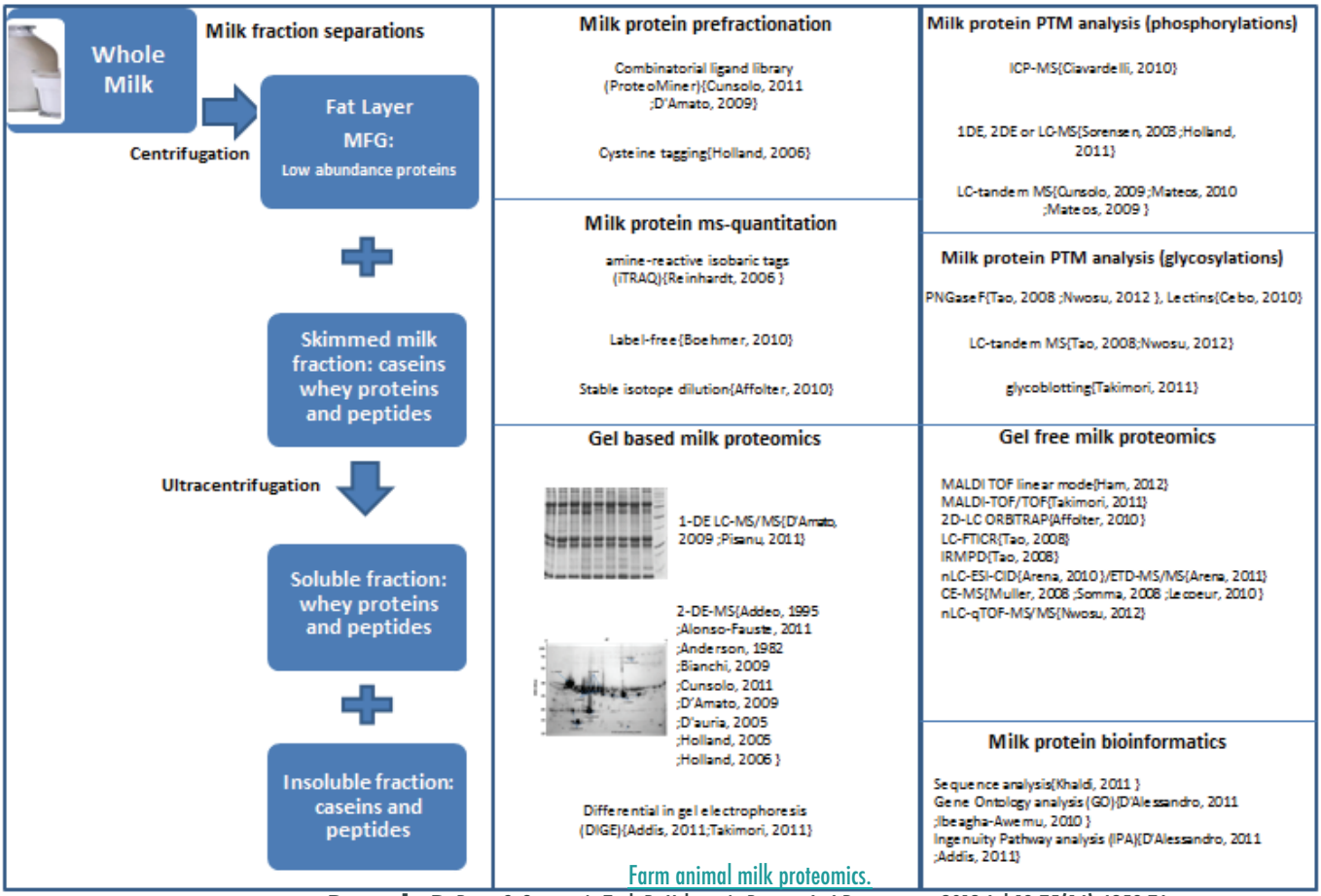
GENERAL STRATEGIES AND ANALYTICAL METHODS

PREFRACTIONATION METHODS



Proteomics as a tool to explore human milk in health and disease.
Roncada P, Stipetic LH, Bonizzi L, Burchmore RJ, Kennedy MW.
J Proteomics. 2013 Aug 2;88:47-57

MASS SPECTROMETRY

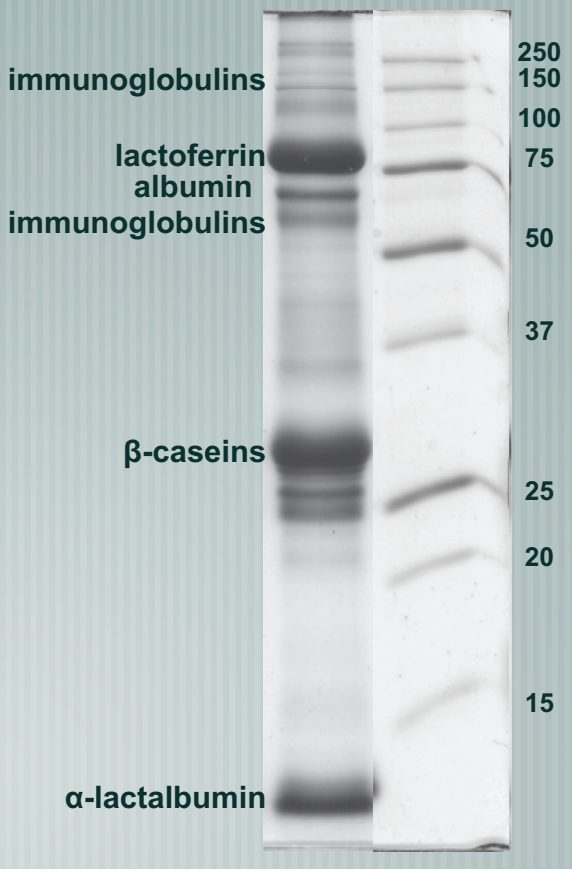


Farm animal milk proteomics.

Roncada P, Piras C, Soggiu A, Turk R, Urbani A, Bonizzi L. J Proteomics. 2012 Jul 19;75(14):4259-74.

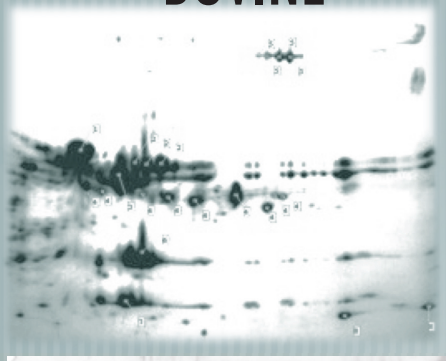
ELECTROPHORETIC SEPARATION

- ONE DIMENSIONAL



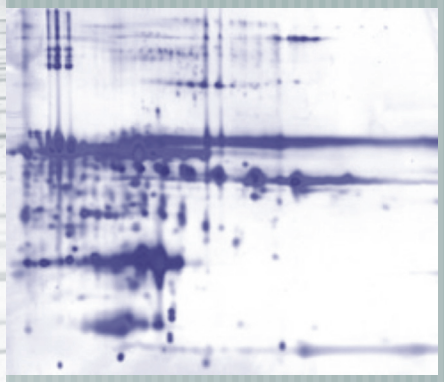
DIFFERENT SPECIES; DIFFERENT MILK....

BOVINE



DONKEY

WATER BUFFALO

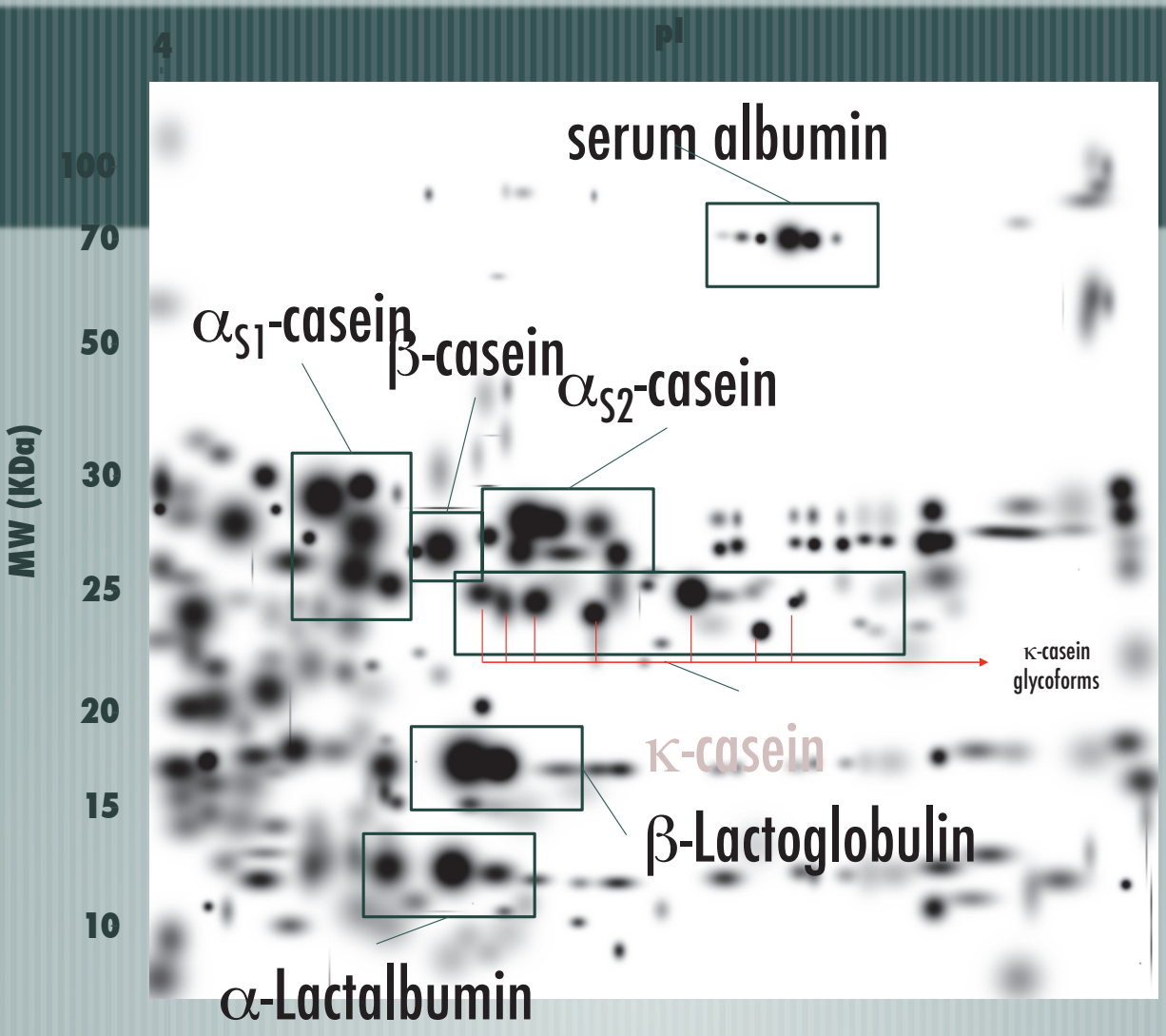


GOAT

HUMAN

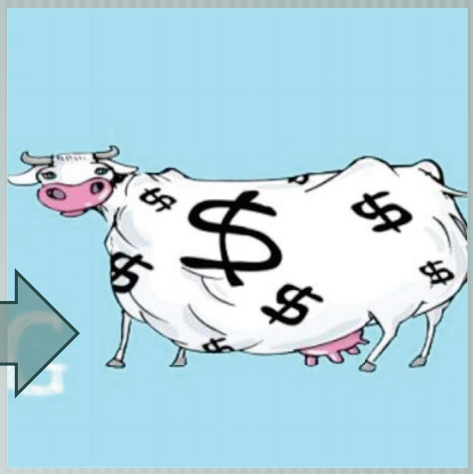
SHEEP

Proteomic evaluation of milk from different mammalian species as a substitute for breast milk.
D'Auria E, Agostoni C, Giovannini M, Riva E, Zetterström R, Fortin R, Greppi GF, Bonizzi L,**Roncada P**.
Acta Paediatr. 2005 Dec;94(12):1708-13


4
pI
100
70
50
30
25
20
15
10
MW (KDa)
serum albumin
αS1-casein
β-casein
αS2-casein
κ-casein
glycoforms
κ-casein
β-Lactoglobulin
α-Lactalbumin

FOOD SAFETY:

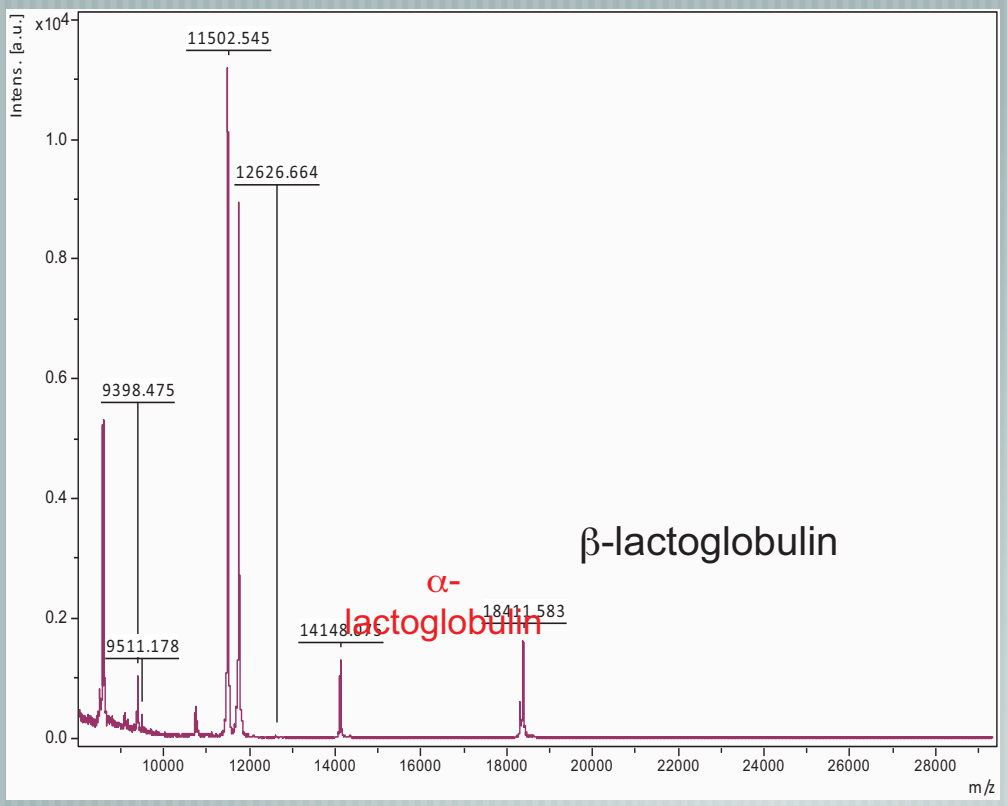
FORMULA MILK



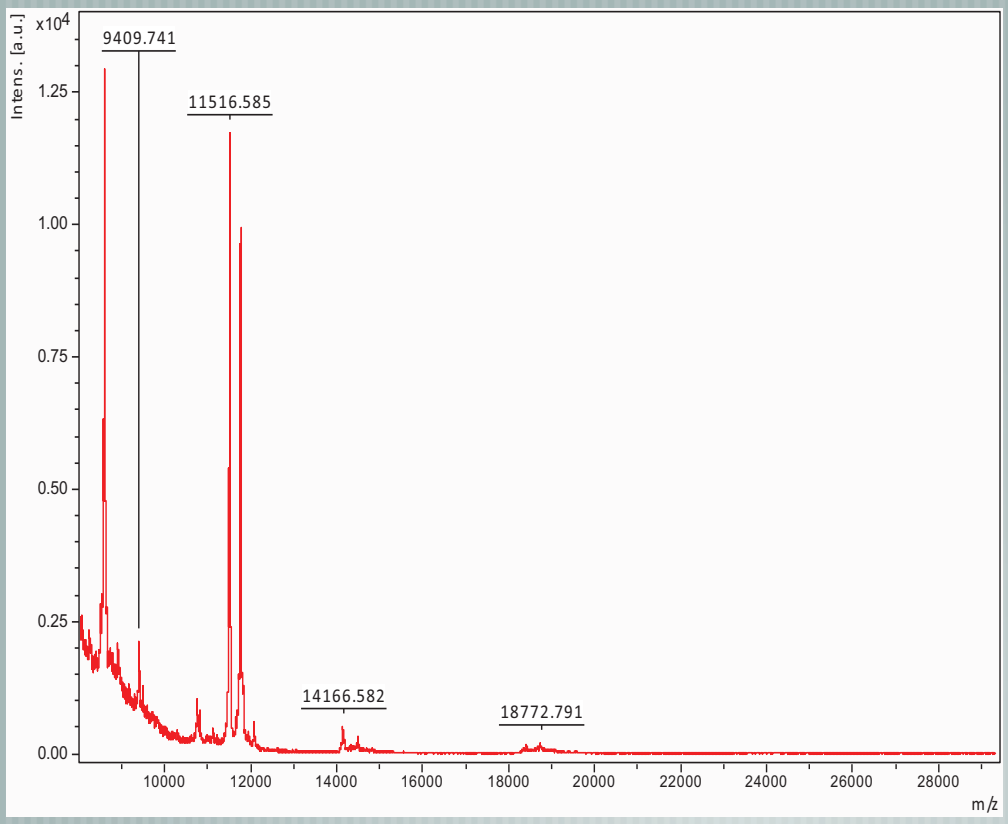
FORMULA MILK VS HUMAN MILK

- GLYCATION—MAILLARD REACTION!
- BETALACTOGLOBULIN—ALLERGENIC—LOW CONCENTRATION?

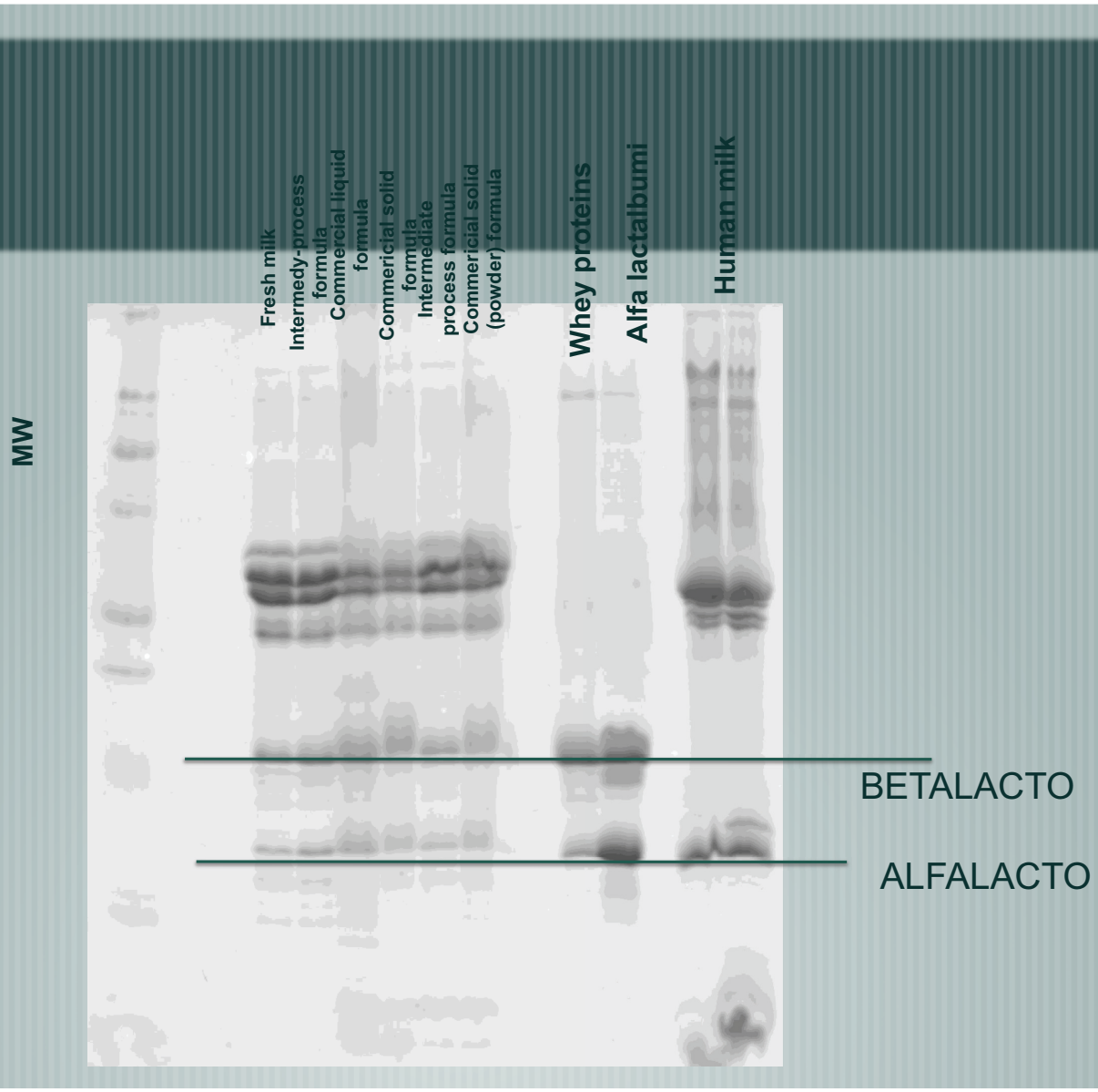
STARTING FORMULA BOVINE MILK ACQUSITION RANGE 8000-30000



END PRODUCT FORMULA MILK (POWDER)acquisition range 8000-3000 DA



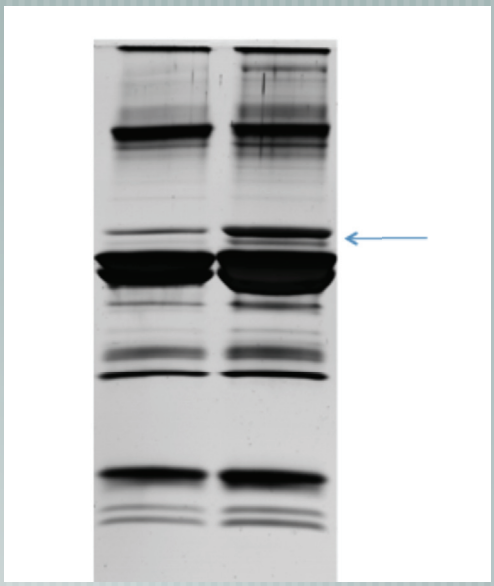
LARGE SDS-PAGE



THE UNTOLD PROBLEM IS

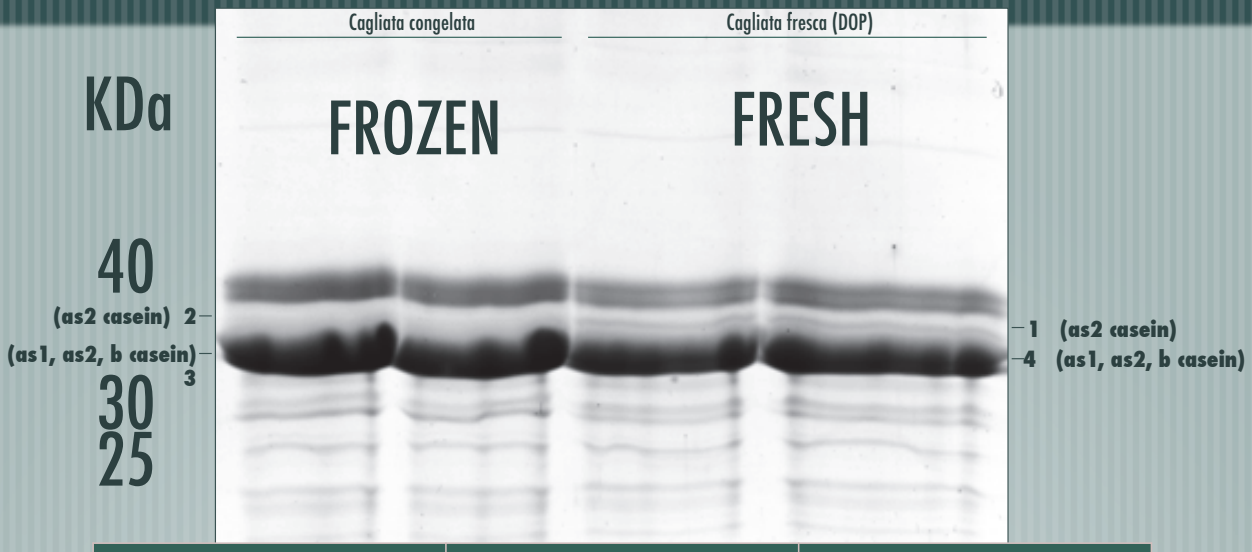
THERMIC TREATMENTS!!!

MOZZARELLA FROM FROZEN CURD



FROZEN FRESH

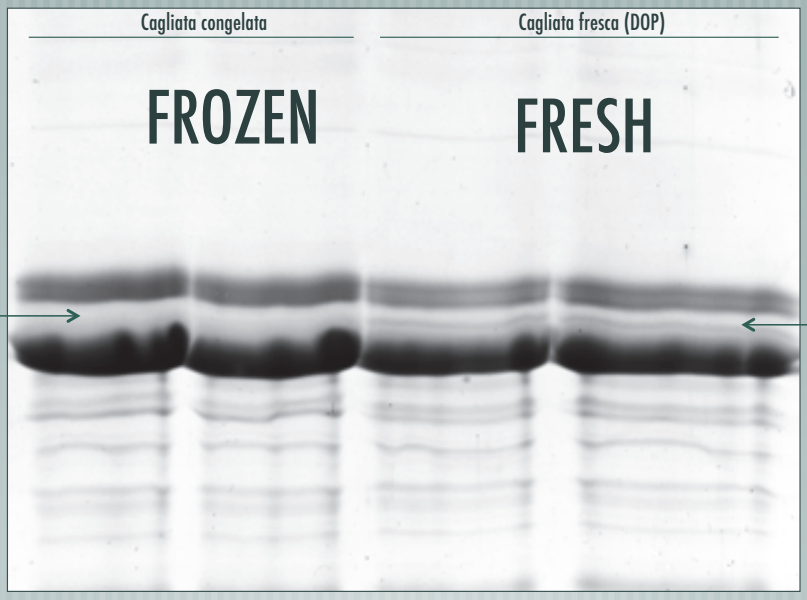
LC-MS/MS



band	FROZEN CURD	FRESH CURD
1	-	**as2 casein***
2	**as2 casein**	-
3	**as1, as2, b casein**	-
4	-	**as1, as2, b casein**

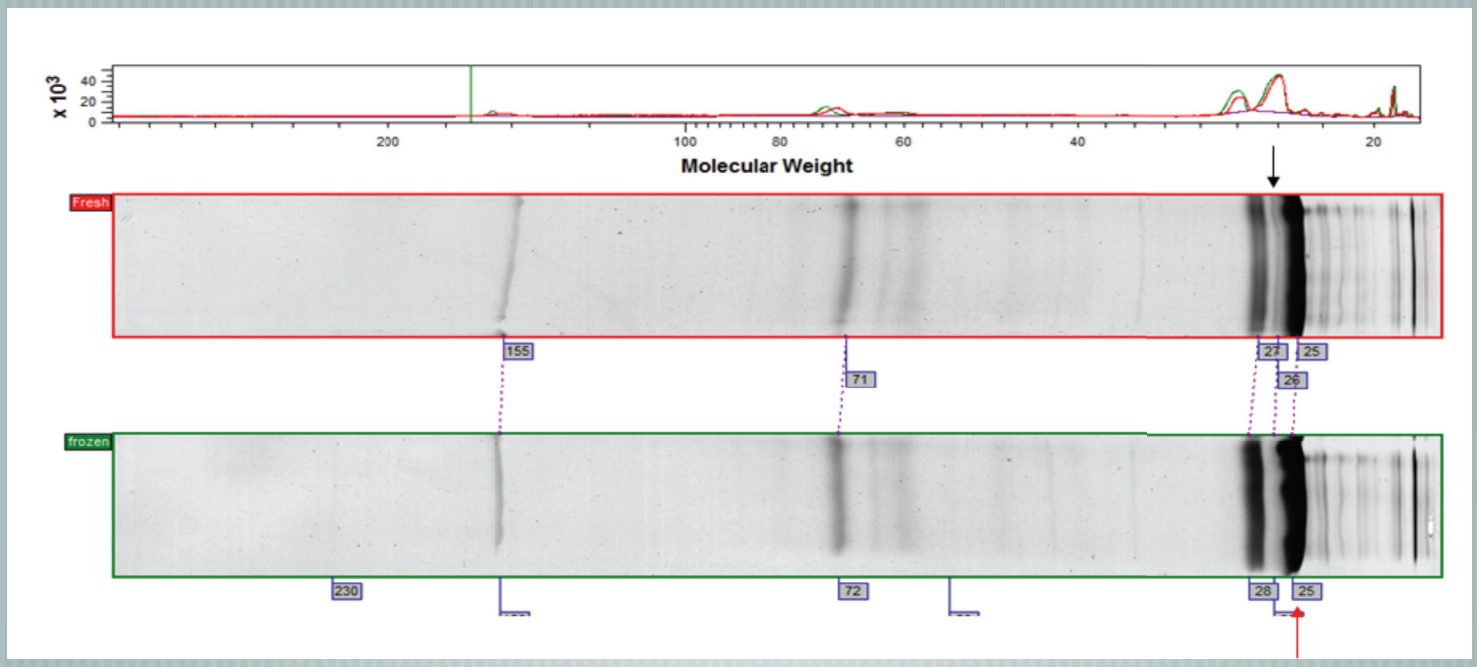
LC-MS/MS – PHOSPHORYLATION

as2-casein
K.TVDMESTEVFTK.K
p-thr posizion 7



as2-casein
K.TVDMESTEVFTK.K
P-ser position 6
P-thr position 7
Met-ox pos.4

MOZZARELLA: FRESH MILK VS POWDER MILK



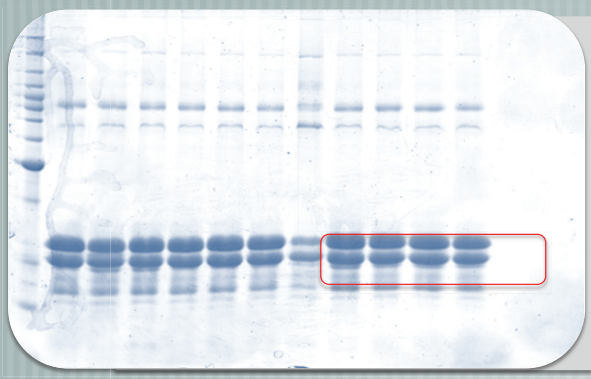
BETA CASEIN!

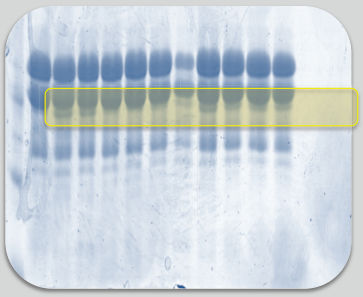
DAIRY PRODUCTS

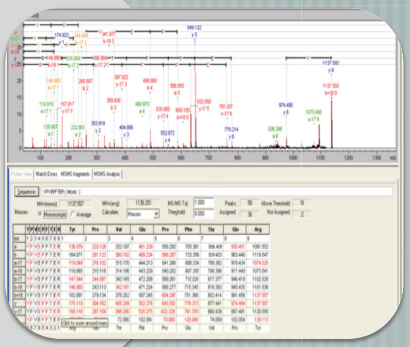
11 milk samples selected on caseification rate)

PROTEOMICS 1DE/ MS

DATA CLUSTERING SPECIFIC ISOFORMS







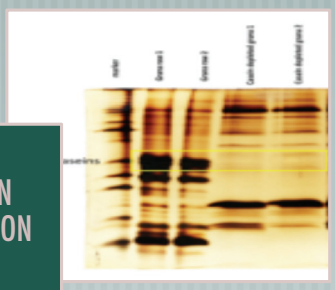
Beta casein

FOCUS ON METAPROTEOMICS (FUNCTIONAL MICROBIOME) TO COUNTERACT CLOSTRIDIA

METAPROTEOMICS: METHODS

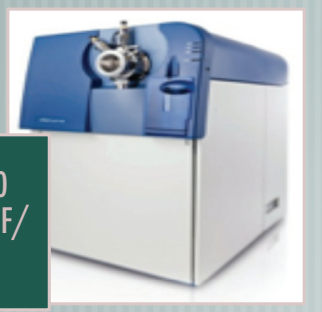
STARTING SAMPLE

CASEIN DEPLETION



PROTEIN EXTRACTION

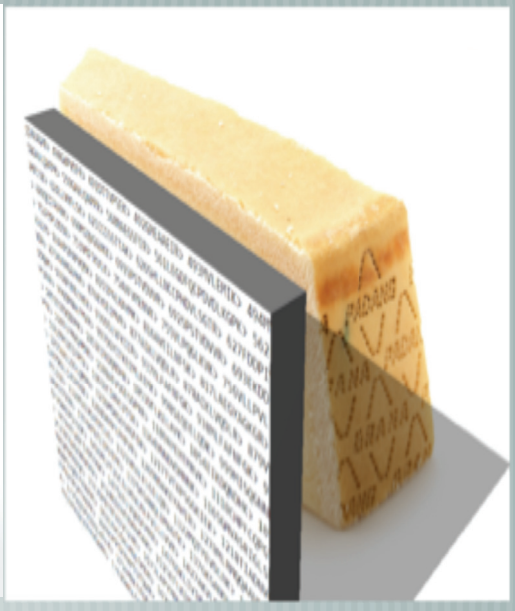
AB 5600 TRIPLE TOF/ SWATH



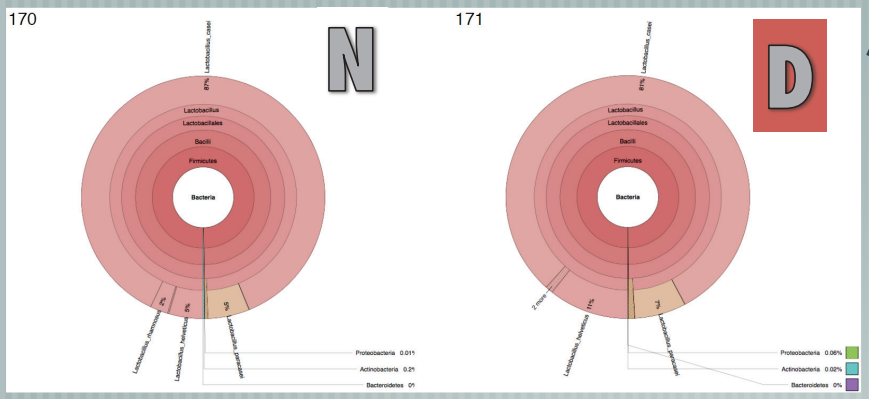
NANO 2D-LC

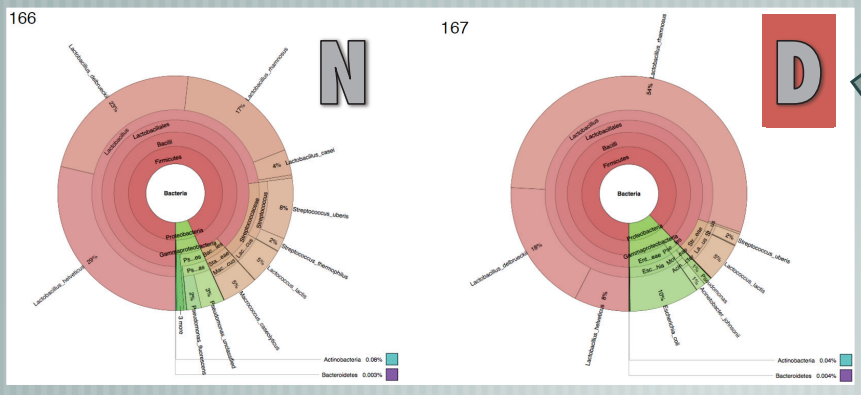
PEPTIDE PURIFICATION

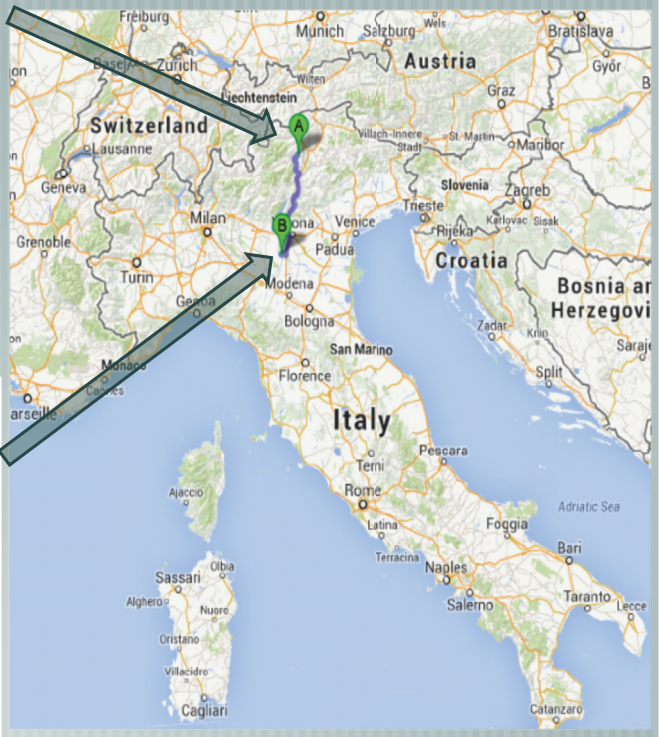
RESULTS



Evaluation of metagenome shotgun sequencing from PDO_GP



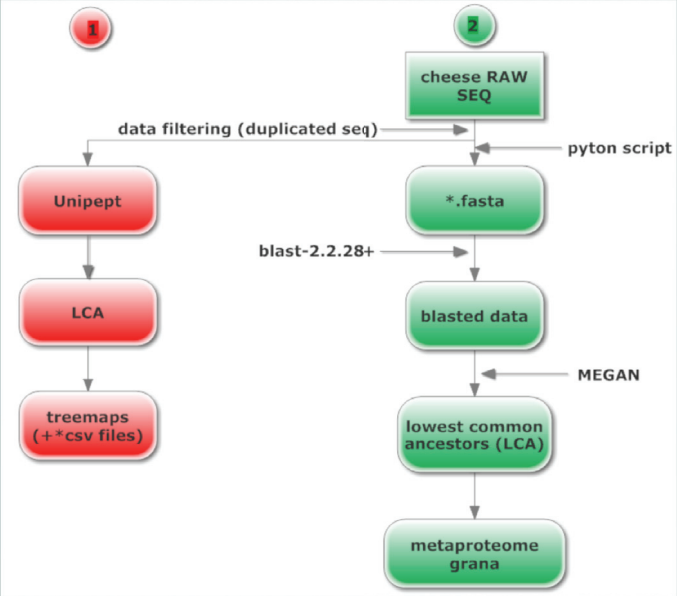




NGS-Illumina HiSeq2000

Updates on bioinformatic pipeline for metaproteomics

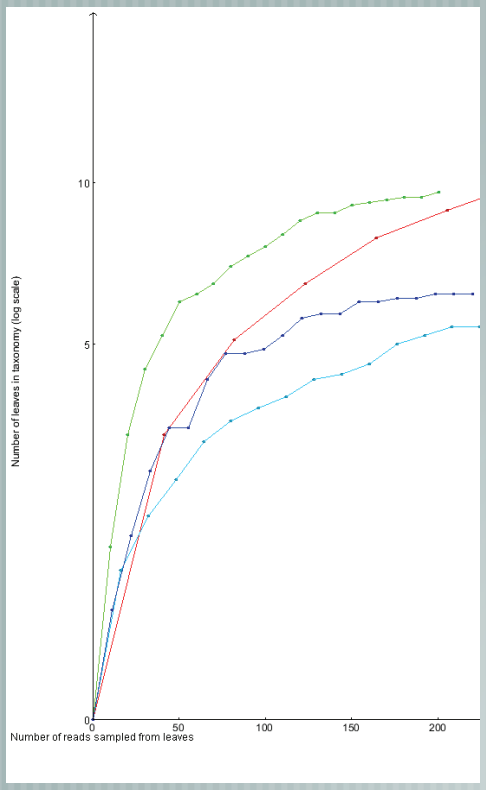
2013

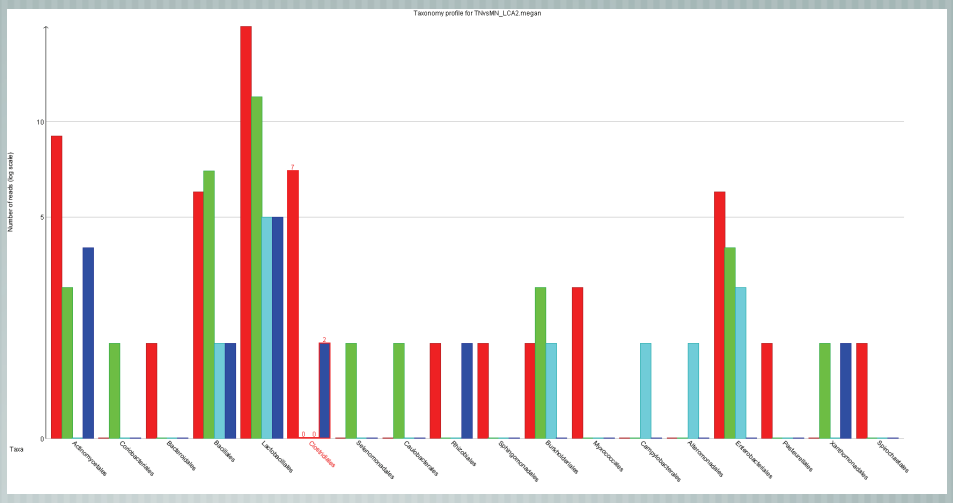


2014

- Cheese_GP spectra (FDR 1%)
- QC+Fasta file
- Offline Blast / custom DB
- taxa classification (MEGAN 5.3)
- Global taxonomic profile
- (NEW) custom functional analysis using a new bioinformatic pipeline (**see poster P-IV-01**)

Rarefaction and taxonomic analysis (TN vs MN)





SEED analysis (TN vs MN)

Legend (samples):
TN305_NB TN305_B MN427_NB MN427_B

EXPERIMENTAL CASEIFICATION

NO LYSOZYME

- LOW SPORES OF Clostridium tyrobutyricum
- HIGH SPORES OF Clostridium tyrobutyricum

SAME CONDITION OF TRENTINGRANA (TN NO LYSOZYME)

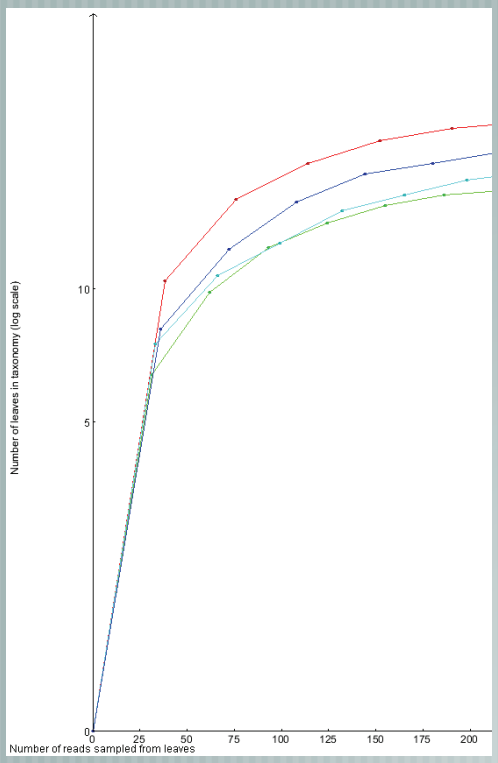
PLUS LYSOZYME

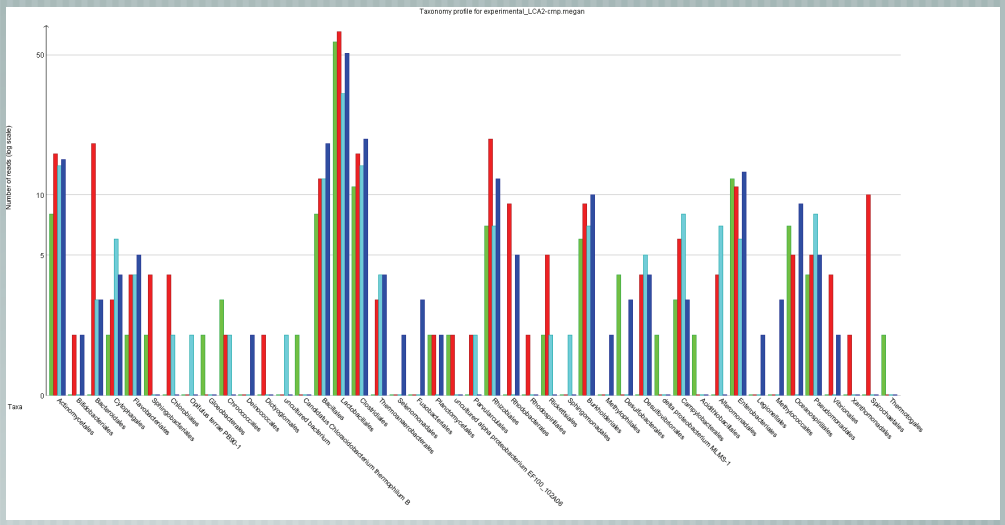
- LOW SPORES OF Clostridium tyrobutyricum
- HIGH SPORES OF Clostridium tyrobutyricum

SAME CONDITION OF MANTOVA GRANA PADANO (MN) GRANA (plus LYSOZYME)

D

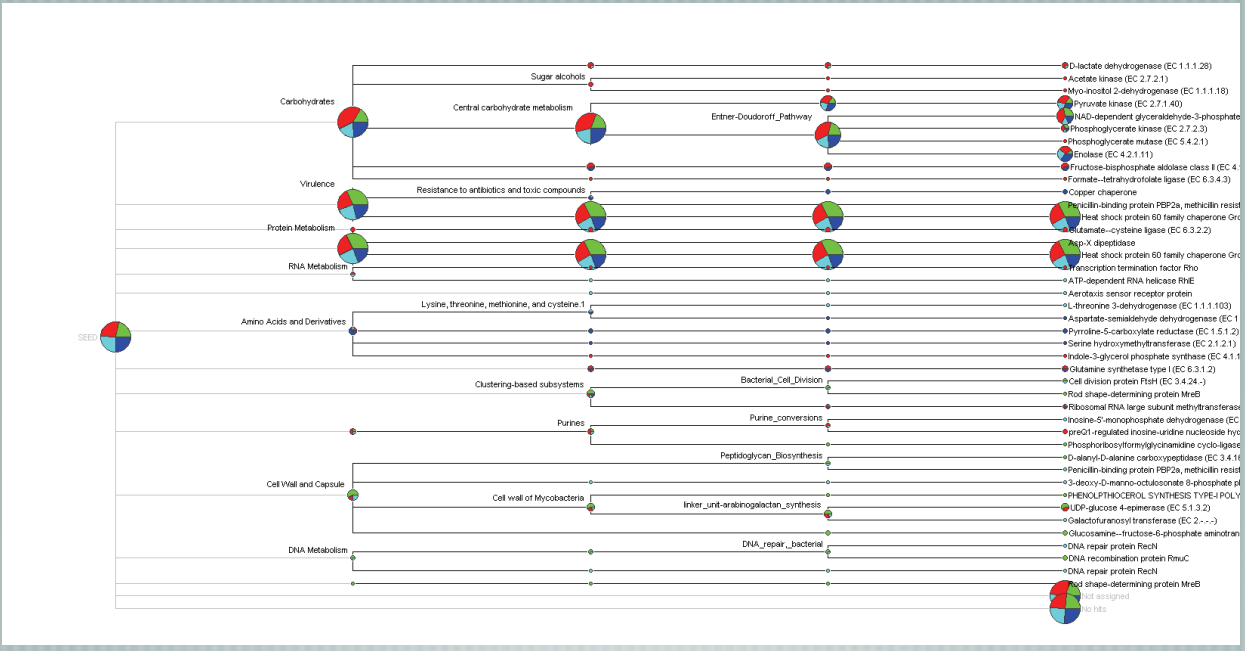
Rarefaction and taxonomic analysis (experimental)





Legend (samples):
low_ct_no_lysozyme
high_ct_no_lysozyme
low_ct_lysozyme_+
high_ct_lysozyme_+

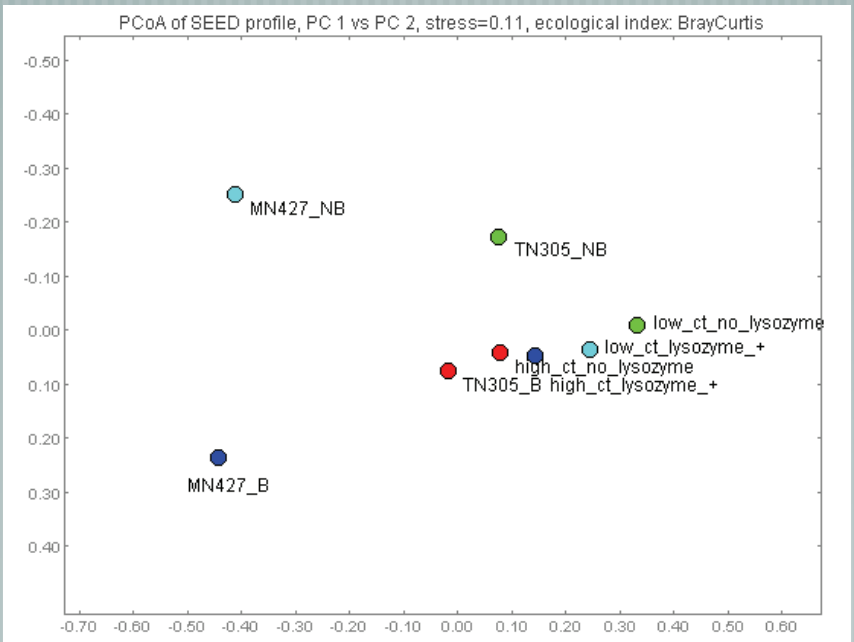
SEED analysis (experimental)



Legend (samples):
low_ct_no_lysozyme high_ct_no_lysozyme low_ct_lysozyme_+ high_ct_lysozyme_+

SEED_PCoA

To compare functional metaproteome



AND SO

- COMMERCIAL AND EXPERIMENTAL GP WITH ‘DEFECTS’ LOOK QUITE SIMILAR AT FUNCTIONAL MICROBIOME
- SELECTION OF NEW STRAINS OF LACTIC ACID BACTERIA TO COUNTERACT CLOSTRIDIA
- MORE INTEGRATION WITH METAGENOMICS IS NEEDED

MILK PROTEOMICS CAN PROVIDE TECHNOLOGIES FOR DEVELOPMENT OF FOOD PRODUCTION IN PARTICULAR FOR FOOD SAFETY AND QUALITY

*Take home message

OUR NETWORK

Human Proteome Project

Initiative on Multi Organism Proteomes

EuPA

EUROPEAN PROTEOMICS ASSOCIATION

ItPA

Italian Proteomics Association

FAP

Food Animal Proteomics

ONE HEALTH

Human Health

Animal Health

Environmental Health

Agriculture

Food Safety & Security

THANK YOU

AND HOPE TO SEE YOU IN **MILANO**

FEEDING THE PLANET
ENERGY FOR LIFE

EXPO MILANO 2015

EuPA

ITALIA
EXPO MILANO 2015

JUNE 23TH-28TH 2015

COST FARM ANIMAL PROTEOMICS FINAL MEETING
MILANO 17-18 November-2014