## The future of buffalo breeding

Mediterranean

Murrah



Michael J. D'Occhio Honorary Professor The University of Sydney michael.docchio@sydney.edu.au



Nili-Ravi

Emeritus Professor The University of São Paulo

#### Stating the obvious

The animal is fundamental.

Get the animal right for profitable market(s) and then think about other investments.





















Buffalo Milk Halloumi: vacuum-packed Buffalo Milk Fetta: vacuum-packed





Buffalo cheesecake



Buffalo salami



Buffalo geymar/kaymak



Buffalo whey sourdough with buffalo cultured butter



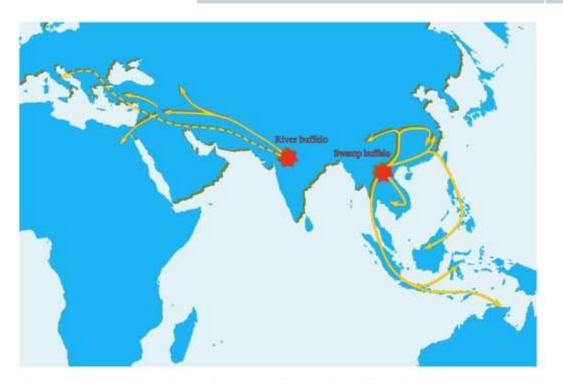
Buffalo rib fillet

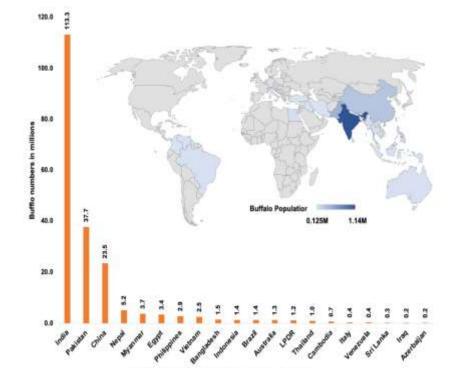


Whole-Genome Sequencing and Characterization of Buffalo Genetic Resources: Recent Advances and Future Challenges Animals 2021, 11, 904

#### Buffalo fresh whole milk

| Global          | 16% |
|-----------------|-----|
| South/Southeast | 35% |
| Asia            |     |



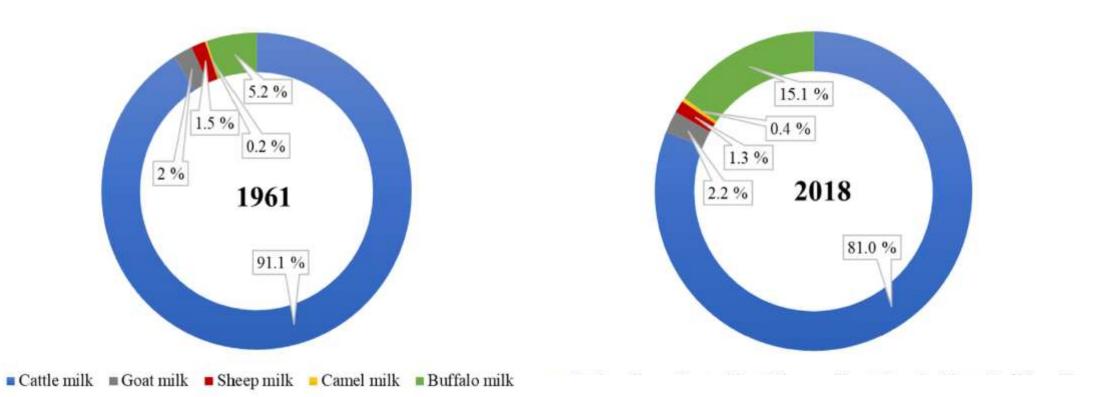


#### World buffalo and cow milk production

|      | buffa       | lo milk        | cow milk    |            |  |  |
|------|-------------|----------------|-------------|------------|--|--|
| year | tons        | % buffalo milk | tons        | % cow mill |  |  |
| 1961 | 17.858.061  | 5.19           | 313.626.619 | 91.12      |  |  |
| 1970 | 19.593.886  | 5.00           | 359.280.844 | 91.67      |  |  |
| 1980 | 27.525.084  | 5.91           | 422.351.163 | 90.67      |  |  |
| 1990 | 44.076.214  | 8.13           | 478.539.902 | 88.22      |  |  |
| 2000 | 66.650.866  | 11.50          | 489.874.522 | 84.53      |  |  |
| 2010 | 92.468.193  | 12.78          | 601.868.328 | 83.18      |  |  |
| 2018 | 127.338.184 | 15.10          | 683.217.055 | 81.04      |  |  |

Composition, Structure, and Digestive Dynamics of Milk From Different Species—A Review

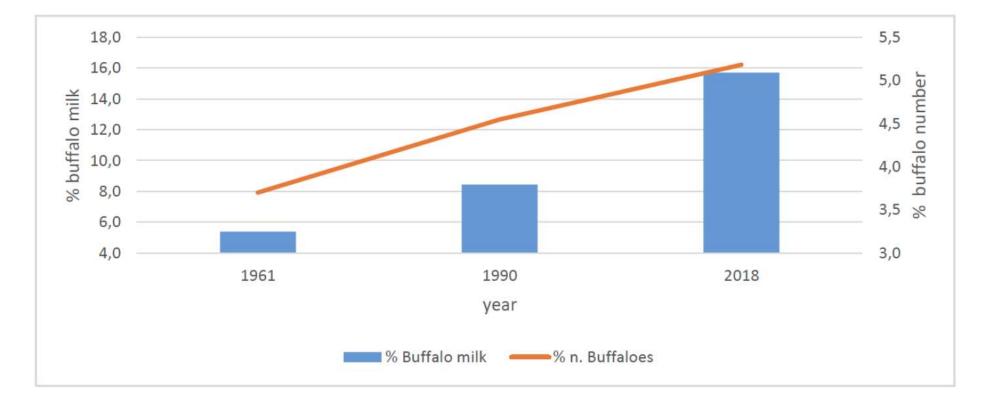
Frontiers in Nutrition October 2020 | Volume 7 | Article 577759



Current Trends in Buffalo Milk Production Journal of Buffalo Science, 2020, 9, 121-132

### Number of buffalo 1961-2018

(% of total of dairy ruminants)



A review of megatrends in the global dairy sector: what are the socioecological implications?

Agriculture and Human Values (2023) 40:373-394

#### Global milk production trends 2021-2030

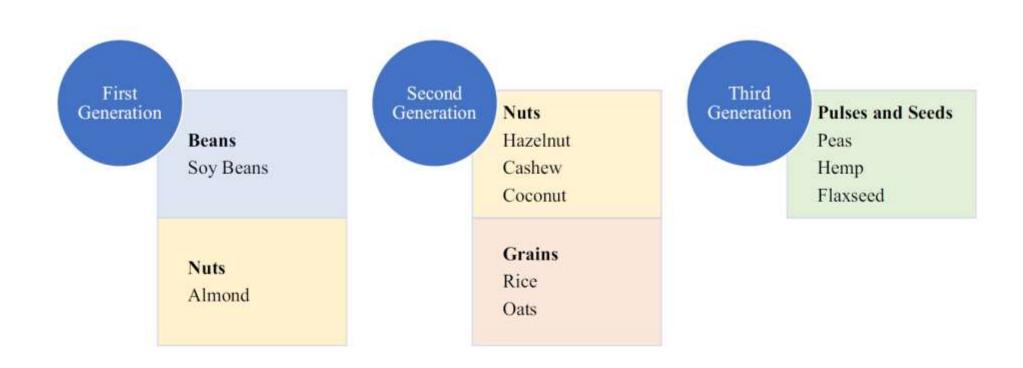
| Year | Developed Countries (kt pw) | Developing<br>Countries (kt<br>pw) |  |  |
|------|-----------------------------|------------------------------------|--|--|
| 2021 | 409,765                     | 447,115                            |  |  |
| 2030 | 435,996                     | 583,695                            |  |  |

Global milk production trends based on kilo of tonnes per week (OECD-FAO 2021)



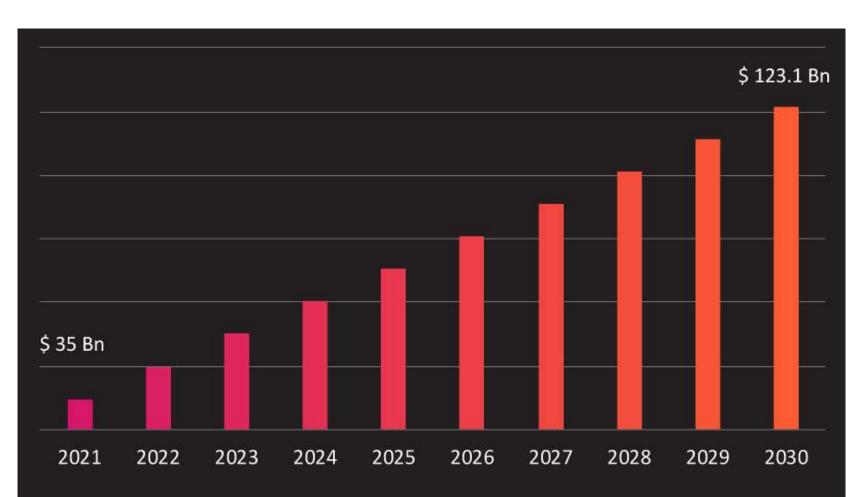
#### Competition

#### Plant-based 'milk'



#### Projected global plant-based 'milk' 2021-2030

Competition



https://www.strategicmarketresearch.com/market-report/plant-based-milk-market

#### Competition

### Laboratory-grown 'milk'



#### Company behind lab-grown milk protein eyes Canadian animal-free dairy market

Animal-free dairy is more environmentally sustainable than traditional dairy production, and it also doesn't contain lactose, cholesterol or growth hormones.

#### The Growing Consumer Opportunity for Precision Fermentation



#### Distinguishing features of buffalo milk

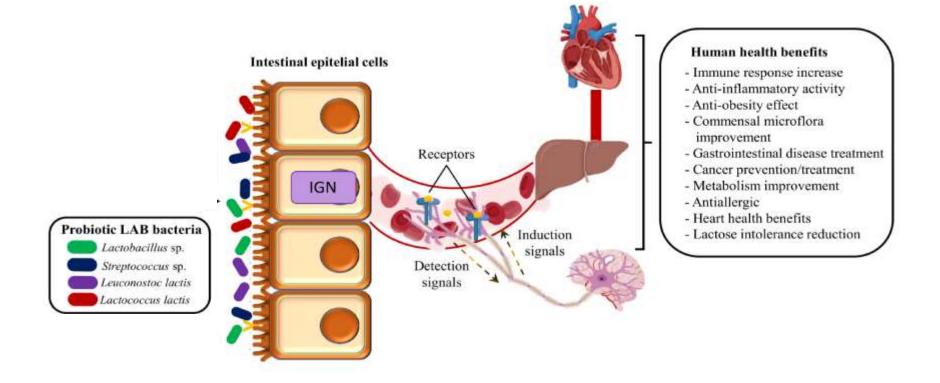
....points of product and market differentiation

Favourable fat profile Functional biomolecules Unique microbiota

#### **Buffalo Milk as a Source of Probiotic Functional Products**

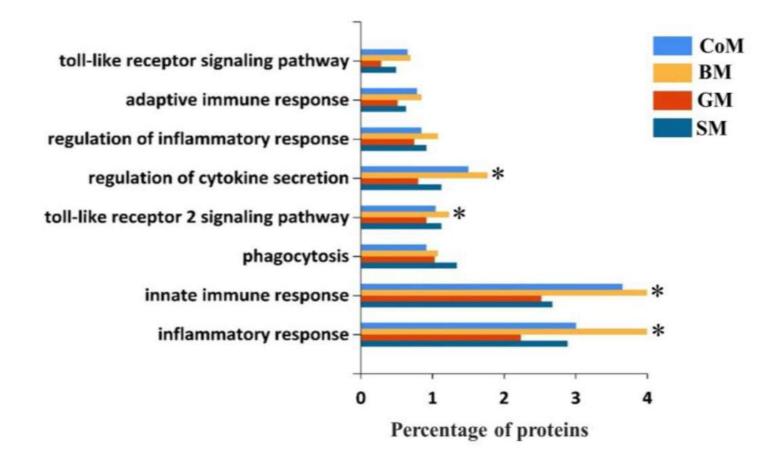
Microorganisms 2021, 9, 2303

#### Health benefits of functional molecules from buffalo milk microbiota



Isolation and Characterization of Cow-, Buffalo-, Sheep- and Goat-Milk-Derived Extracellular Vesicles *Cells* 2023, 12, 2491

Extracellular vesicles from buffalo milk are enriched with proteins implicated in immune responses



REVIEW: Potential of water buffalo in world agriculture: Challenges and opportunities

Applied Animal Science 35:255-268 2019

#### Challenges, constraints, solutions

#### \*

Genetics is permanent, additive and can undergo continuing improvement.

Solutions Education **Constraints** / •Innovative, Challenges transformative ⋇ •Genetic and influence on translational economic research traits Technology Threat of transfer global warming and climate change Delayed puberty Poor estrus signs •Longer postpartum anovulation • Lower CR with AI Cryodamages to sperm

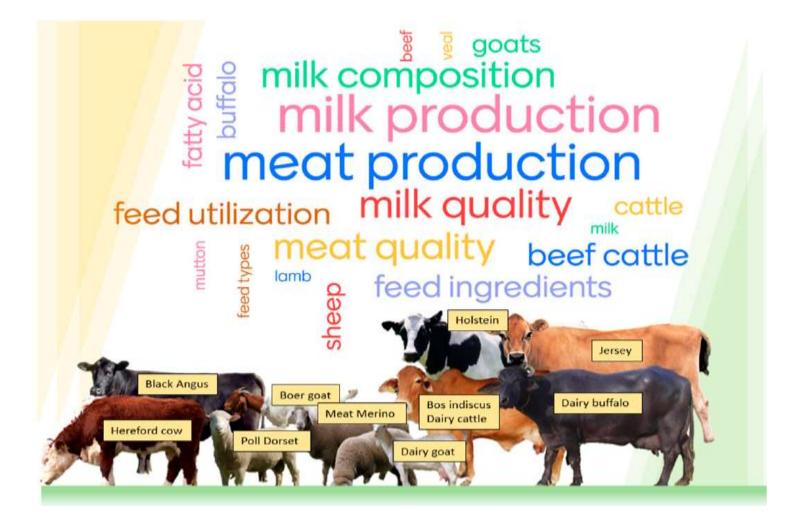
\*

Non-genetic strategies

Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview

Animals 2024, 14, 840.

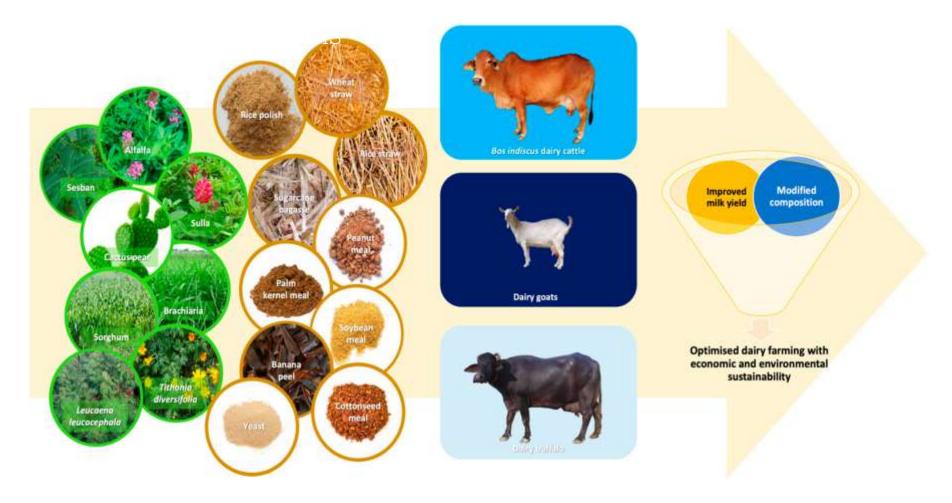
#### Nutritional factors that influence ruminant meat and milk production



Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview

Animals 2024, 14, 840.

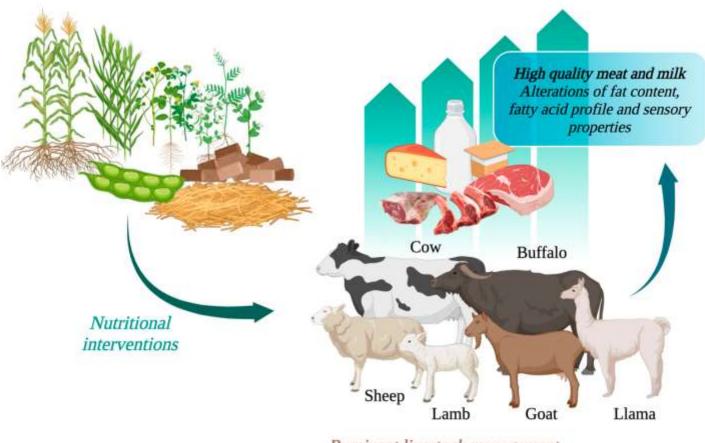
# Feed sources in tropical farming systems for ruminant milk and meat production



Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview

Animals 2024, 14, 840.

Forage, roughage and supplements to optimize the quality and nutritional value of ruminant meat and milk



Ruminant livestock management

#### Milk composition of buffalo fed TMR or TMR + green feed

#### Functional biomolecules

(mg/L)

| Fatty acids             |
|-------------------------|
| (% of total fatty acid) |

TMR

TMR + green feed

|                           | TMR                     | TMR + green feed        |  |  |  |
|---------------------------|-------------------------|-------------------------|--|--|--|
| L-carnitine               | $31.5\pm0.7~^{\rm A}$   | $42.0\pm0.5^{\text{B}}$ |  |  |  |
| acetyl-L-carnitine        | $39.1\pm0.5$ $^{\rm A}$ | $49.8\pm0.8^{\text{B}}$ |  |  |  |
| propionyl-L-<br>carnitine | $14.8\pm1.3\ ^{\rm A}$  | $21.1\pm0.4^{\text{B}}$ |  |  |  |
| γ-butyrobetaine           | $4.8 \pm 0.2$           | $4.1 \pm 0.2$           |  |  |  |
| δ-valerobetaine           | $18.2\pm0.6~^{\rm A}$   | $22.2\pm0.5^{B}$        |  |  |  |
| glycine betaine           | $7.0\pm0.02$            | $7.2\pm0.3$             |  |  |  |

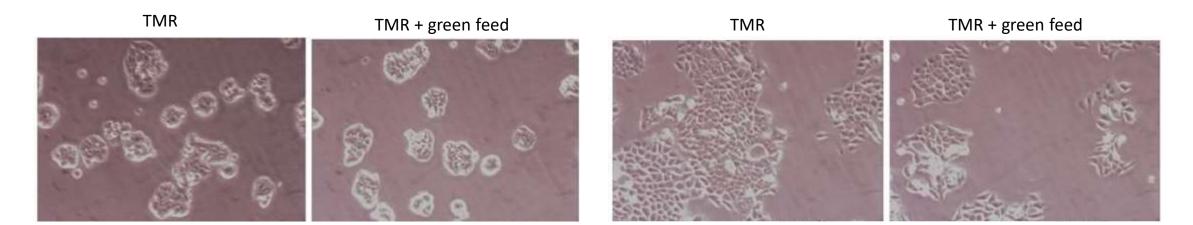
| α-Linolenic acid C18:3 n3 | $0.3\pm0.0\ ^{A}$          | $0.6\pm0.0^{\text{B}}$ |
|---------------------------|----------------------------|------------------------|
| ω-6/ω-3                   | $8.7 \pm 1.1$ <sup>a</sup> | $6.0\pm0.2^{b}$        |
| ω-6                       | $2.8\pm0.1$ a              | $3.8\pm0.3^{b}$        |
| ω-3                       | $0.3\pm0.0$ <sup>A</sup>   | $0.6\pm0.0^{\text{B}}$ |
| PUFA/SFA                  | $0.0\pm0.0$ <sup>A</sup>   | $0.1\pm0.0^{ m B}$     |
| PUFA                      | $3.1\pm0.1$ $^a$           | $4.5\pm0.4^{\text{b}}$ |
| MUFA                      | $20.9\pm0.3~^{\rm A}$      | $23.5 \pm 0.7^{B}$     |
| SFA                       | $76.0\pm0.3~^{\rm A}$      | $71.9 \pm 1.1^{B}$     |
| Fatty acids               |                            |                        |

Green feed increases antioxidant and antineoplastic activity of buffalo milk: A globally significant livestock Food Chemistry 344 (2021) 128669

### Anti-neoplastic activity of milk extract from buffalo fed TMR or TMR + green feed

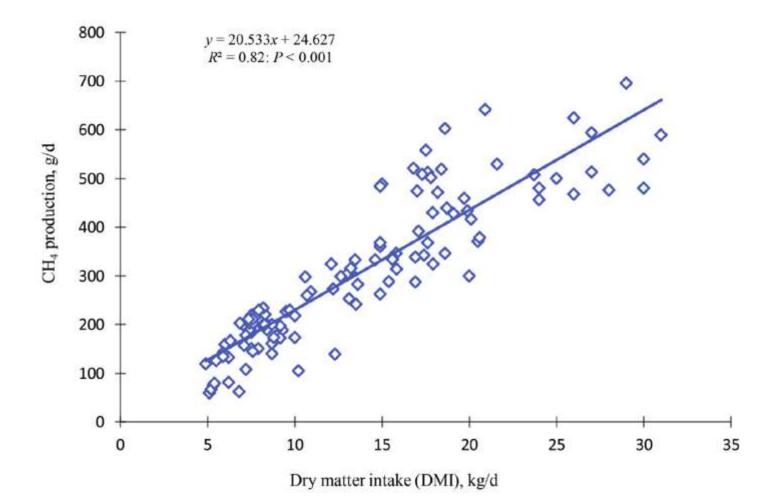
human oral squamous carcinoma cells

human colon cancer cells



Methane challenge

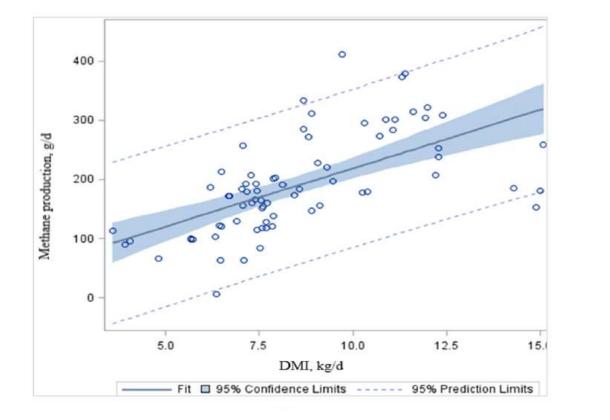
#### Dry matter intake (DMI) and methane production

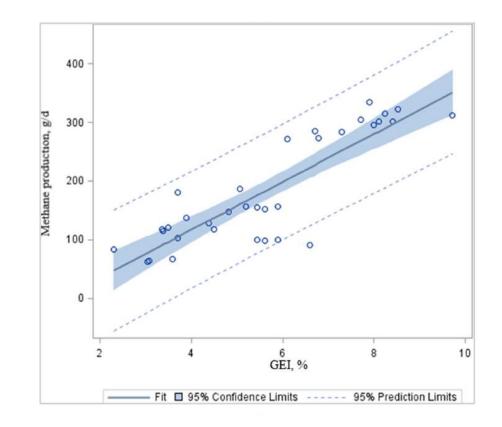


Enteric Methane Emissions and Animal Performance in Dairy and Beef Cattle Production: Strategies, Opportunities, and Impact of Reducing Emissions

Animals 2022, 12, 948

### Dry matter intake (DMI) and gross energy intake (GEI) and methane production in beef cattle





#### The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation

Trends in Genetics, April 2013, Vol. 29, No. 4

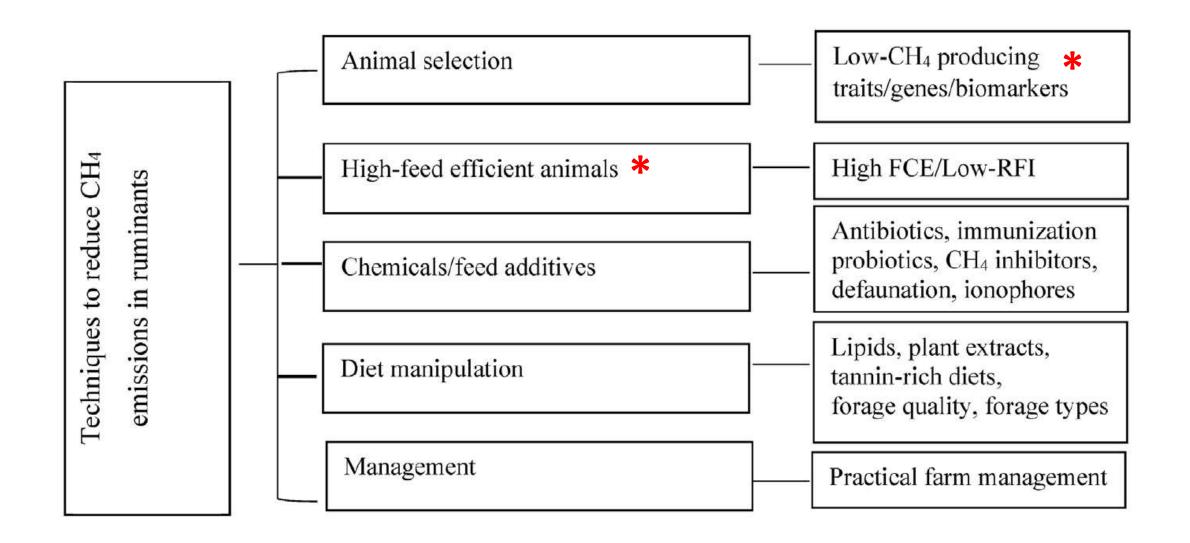
50 45 40 35 30 25 20 15 Key: • Methane (g) per liter milk 10 Methane (g) per kilogram feed eaten 5 0 0 5 10 15 20 25 30

#### Efficiency of production and methane emissions

Methane emissions are closely linked to feed intake and more efficient animals dilute intake for maintenance over more units of product.



Dietary mitigation of enteric methane emissions from ruminants: A review of plant tannin mitigation options<sup>#</sup> Animal Nutrition 6 (2020) 231-246



Relationships between enteric methane production and economically important traits in beef cattle *Livestock Science* 265 (2022) 105102

Methane production is phenotypically and genetically correlated with important production traits.

Knowledge of residual feed intake (RFI) / net feed efficiency (NFE) is highly important in future buffalo selection and breeding.

....genes linked with RFI/NFE

Genetic strategies

Reducing the cost of beef production through genetic improvement in residual feed intake: Opportunity and challenges to application<sup>1</sup>

J. Anim. Sci. 81(E. Suppl. 1):E9-E17 2003

#### Genetic correlations with residual feed intake (RFI) in Angus cattle

| Genetic correlation                        |      |
|--|------|
| Postweaning RFI with cow daily feed intake | 0.64 |
| Postweaning RFI and cow RFI                | 0.98 |

Genetics of buffalo milk production

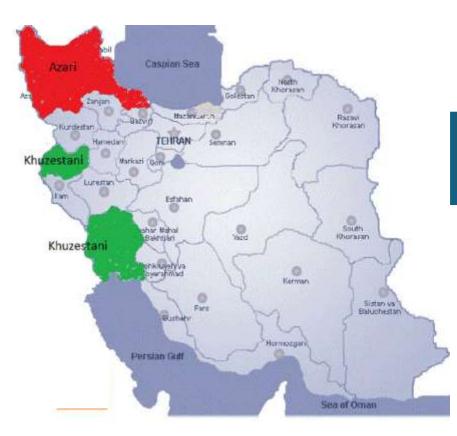
Whole-Genome Sequencing and Characterization of Buffalo Genetic Resources: Recent Advances and Future Challenges Animals 2021, 11, 904

#### Candidate genes associated with milk traits in buffalo

| Trait            | Candidate genes  |
|------------------|--|
| Milk yield       | STAT1, STATSA, LEP, MC4R, OXT INSIG2, LALBA,<br>BTN1A1, PRL, SCD, SREBF1 |
| Milk fat yield   | GHRL, A2M  |
| Milk fat (%)     | STAT1, TG, A2M, DGAT1, GHRL, LEP, MC4R, PRL, SCD, SREBF1                 |
| Milk protein (%) | CSNS1, DGT1, GHRL, ADRA1A, A2M MTNRIA, PRL,<br>SPP1, INSIG2, MC4R        |

# A genome-wide scan for signatures of selection in Azeri and Khuzestani buffalo breeds

BMC Genomics (2018) 19:449



## Candidate genes linked with milk production and composition in Azeri and Khuzestani buffalo

| Putative        | FBX09, NDFiP1, ACTR3,       |
|-----------------|-----------------------------|
| candidate genes | ARHGAP26, SERPINF2, BOLA-   |
|                 | DRB3, BOLA-DQB, CLN8, MYOM2 |

Polymorphism association and expression analysis of alpha-lactalbumin (LALBA) gene during lactation in Nili Ravi buffalo

Tropical Animal Health and Production (2020) 52:265-271

## Genotype and allelic frequency of **polymorphisms** in alpha-lactoglobulin (*LALBA*) gene in Nili-Ravi buffalo

| SNP ID | Chromosomal<br>position<br>34310913 | Change in nucleotide | Transition/<br>transversion | Allele<br>frequency |             | Genotype frequency |              |              | HWE<br><i>P</i> < 0.05 |
|--------|-------------------------------------|----------------------|-----------------------------|---------------------|-------------|--------------------|--------------|--------------|------------------------|
|        |                                     | $G \rightarrow A$    | Transition                  | A<br>0.3293         | G<br>0.6707 | AA<br>0.2683       | AG<br>0.1463 | GG<br>0.5854 | 0.0281*                |
| LALBA2 | 34310940                            | $C \rightarrow G$    | Transversion                | G<br>0.7073         | C<br>0.2927 | GG<br>0.2439       | GC<br>0.1951 | CC<br>0.5610 | 0.3262                 |
| LALBA3 | 34310958                            | $A \rightarrow G$    | Transition                  | G<br>0.7195         | A<br>0.2805 | GG<br>0.2683       | GA<br>0.2195 | AA<br>0.5122 | 0.0453*                |
| LALBA4 | 34310984                            | $A \rightarrow G$    | Transition                  | G<br>0.6829         | A<br>0.3171 | GG<br>0.2683       | GA<br>0.1951 | AA<br>0.5366 | 0.0318*                |
| LALBA5 | 34311015                            | $G \rightarrow A$    | Transition                  | A<br>0.6463         | G<br>0.3537 | AA<br>0.3871       | AG<br>0.0645 | GG<br>0.5484 | 0.0411*                |

Alpha-lactoglobulin is a bioactive milk protein

# Signal transducer and activation of transcription (*STAT1*) gene **polymorphisms** in buffalo

| No.   | Positions | Location            | Types                     |  |
|-------|-----------|---------------------|---------------------------|--|
| SNP1  | 508       | 5' UTR <sup>1</sup> | G > A                     |  |
| SNP2  | 1,079     | Intron2             | C > A                     |  |
| SNP3  | 1,901     | Intron2             | A > T                     |  |
| SNP4  | 2,338     | Intron2             | T > C                     |  |
| SNP5  | 2,965     | Intron2             | A > C                     |  |
| SNP6  | 3,173     | Intron2             | T > G                     |  |
| SNP7  | 3,328     | Intron2             | G > C                     |  |
| SNP8  | 3,666     | Intron3             | G > A                     |  |
| SNP9  | 4,007     | Intron3             | $\mathbf{T} > \mathbf{C}$ |  |
| SNP10 | 5,558     | Intron4             | G > T                     |  |
| SNP11 | 5,842     | Intron4             | T > A                     |  |
| SNP12 | 11,938    | Intron9             | A > G                     |  |
| SNP13 | 15,642    | Exon10              | $\mathbf{G} > \mathbf{T}$ |  |
| SNP14 | 29,924    | Intron21            | G > C                     |  |
| SNP15 | 30,303    | Intron21            | C > T                     |  |
| SNP16 | 31,908    | Intron22            | G > A                     |  |
| SNP17 | 33,403    | Intron23            | $\mathbf{T} > \mathbf{C}$ |  |
| SNP18 | 38,379    | 3' UTR              | C > A                     |  |

| STAT1 single nucleotide polymorphism (SNP) |                        |  |
|--|------------------------|--|
| 305-day milk yield                         | SNP4, SNP10            |  |
| Protein %                                  | SNP2, SNP5, SNP8, SNP9 |  |

STAT1 protein activates the transcription of hundreds of genes

 $^{1}$ UTR = untranslated region.

# Relationship of single nucleotide **polymorphisms** of squalene epoxidase (*SQLE*) gene to milk traits in Italian Mediterranean buffalo

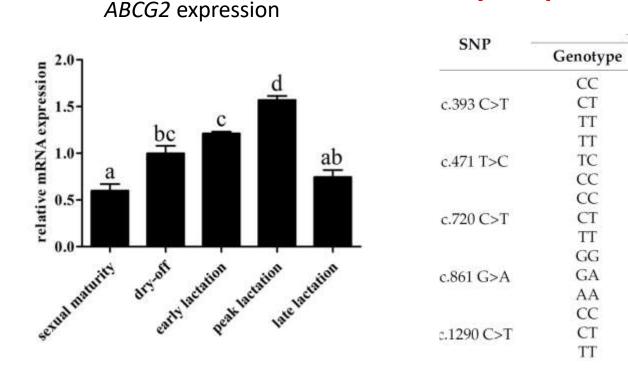
#### H1H1 H1H3 H2H3 p-Value FY (kg) $235.78 \pm 3.44$ <sup>a</sup> $226.97 \pm 5.00^{ab}$ 0.03 225.02 ± 3.09 b $7.73 \pm 0.10^{b}$ $8.03 \pm 0.07$ <sup>a</sup> $7.94 \pm 0.06$ ab FP (%) 0.04 $131.63 \pm 2.73$ ab PY (kg) 127.80 ± 1.69 b $133.33 \pm 1.88^{a}$ 0.04PP (%) $4.57 \pm 0.02$ $4.53 \pm 0.03$ $4.57 \pm 0.02$ 0.44

SQLE haplotype combinations and milk traits

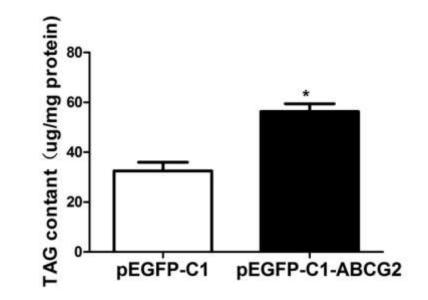
SQLE is a key enzyme in cholesterol synthesis

### ATP-binding cassette family G member 2 (*ABCG2*) gene and milk fat content in buffalo

**Polymorphisms** 



ABCG2-induced expression of liposynthesis-related genes and increased trigyceride (TAG) protein in buffalo mammary epithelial cells



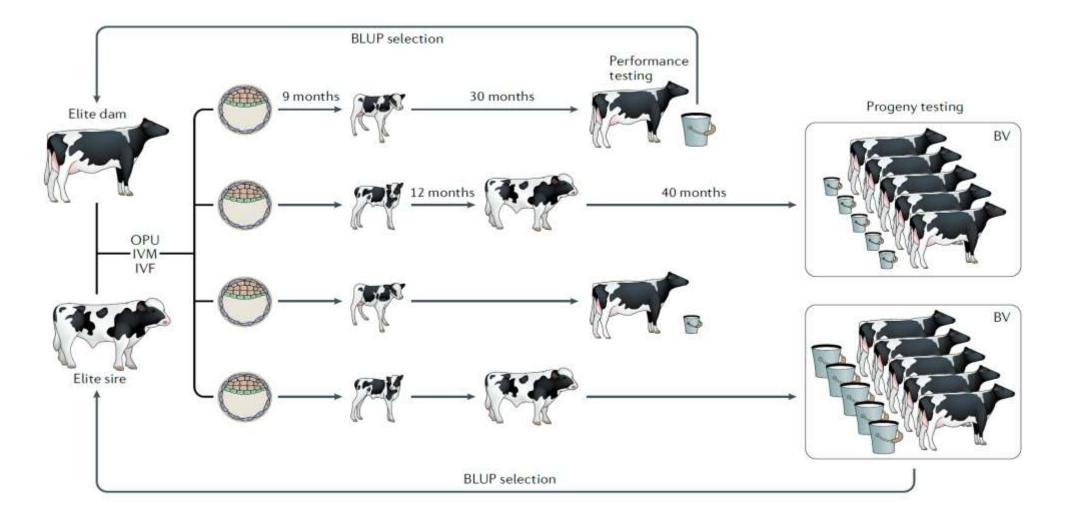
ABCG2 induces proteins involved in transport across cell membranes

Genomic breeding values

Harnessing genomic information for livestock improvement NATURE REVIEWS | GENETICS VOLUME 20 | MARCH 2019 | 135

#### Best linear unbiased prediction (BLUP) of breeding value (BV)

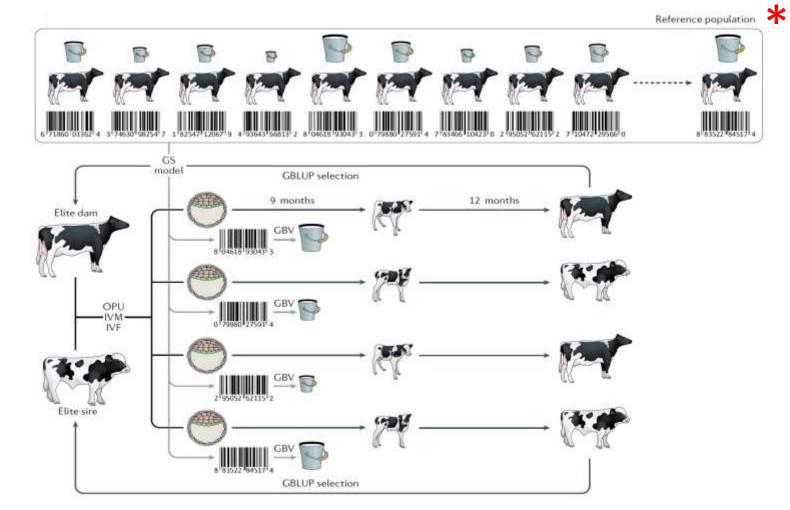
Environmental effects and pedigree information used to determine breeding value (BV)



Harnessing genomic information for livestock improvement NATURE REVIEWS | GENETICS VOLUME 20 | MARCH 2019 | 135

#### Genomic best linear unbiased prediction (GBLUP) of breeding value (GBV)

Genotyping with genome-wide single nucleotide polymorphism (SNP) arrays to determine genomic breeding value (GBV)



Statistical models for genomic selection (GS) are trained on a <u>reference population</u> of animals that have both <u>SNP genotypes</u> and <u>phenotypic</u> information



Associazione Nazionale Allevatori Specie Bufalina



<u>B</u>ufala Mediterranea <u>I</u>taliana: tecnologie innovative per il miglioramento <u>G</u>enetico

### Milk characteristics

| Characteristic | Heritability |  |
|----------------|--------------|--|
| Production     | 0.34         |  |
| Fat kg         | 0.26         |  |
| Fat %          | 0.24         |  |
| Protein kg     | 0.31         |  |
| Protein %      | 0.34         |  |



<u>B</u>ufala Mediterranea <u>I</u>taliana: tecnologie innovative per il miglioramento <u>G</u>enetico

| Records         |                                    | Years     |
|-----------------|------------------------------------|-----------|
| Milk production | 276,451 cows<br>743,904 lactations | 1984-2019 |
| Morphology      | 91,966 cows                        | 2004-2022 |

## Genomic investigation of milk production in Italian buffalo

ITALIAN JOURNAL OF ANIMAL SCIENCE 2021, VOL. 20, NO. 1, 539-547

# Prediction accuracy with best linear unbiased prediction (BLUP) and single-step genomic best linear unbiased prediction (ssGBLUP) in Italian buffalo

Report 1

|         | BLUP | ssGBLUP |
|---------|------|---------|
| Milk    | 0.60 | 0.77    |
| Fat     | 0.55 | 0.72    |
| Protein | 0.57 | 0.76    |

Including male and female genotypes improved breeding value accuracy in Italian buffalo

The present of Italian Mediterranean buffalo: precision breeding based on multi-omics data ACTA IMEKO December 2023, Volume 12, Number 4, 1 - 4

#### Prediction accuracy for milk production with BLUB and ssGBLUP in Italian buffalo

Report 2

|         | Mean | Minimum | Maximum |
|---------|------|---------|---------|
| BLUP    | 0.86 | 0.24    | 0.99    |
| ssGBLUP | 0.89 | 0.31    | 0.99    |

#### Genetic gain

$$\Delta G = \frac{r * i * \sigma_g}{N}$$

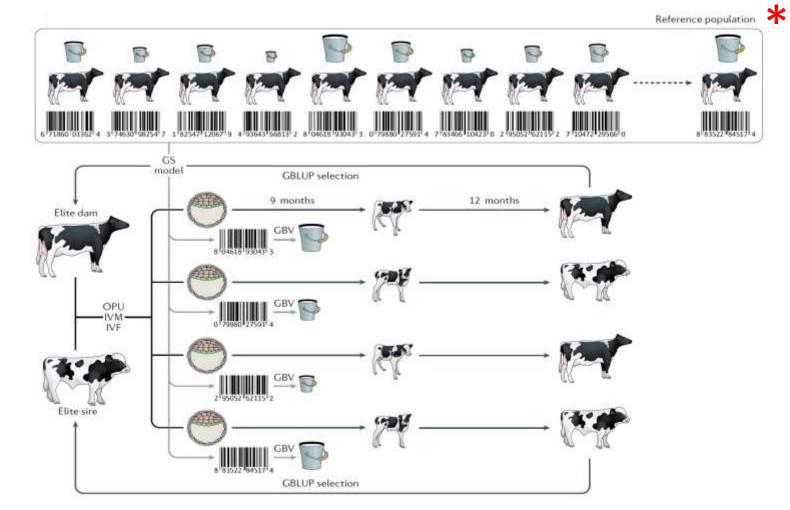
- accuracy r
- selection intensity i
- σ<sub>g</sub> genetic variationN generation interval

Harnessing reproductive technology

Harnessing genomic information for livestock improvement NATURE REVIEWS | GENETICS VOLUME 20 | MARCH 2019 | 135

#### Genomic best linear unbiased prediction (GBLUP) of breeding value (GBV)

Genotyping with genome-wide single nucleotide polymorphism (SNP) arrays to determine genomic breeding value (GBV)

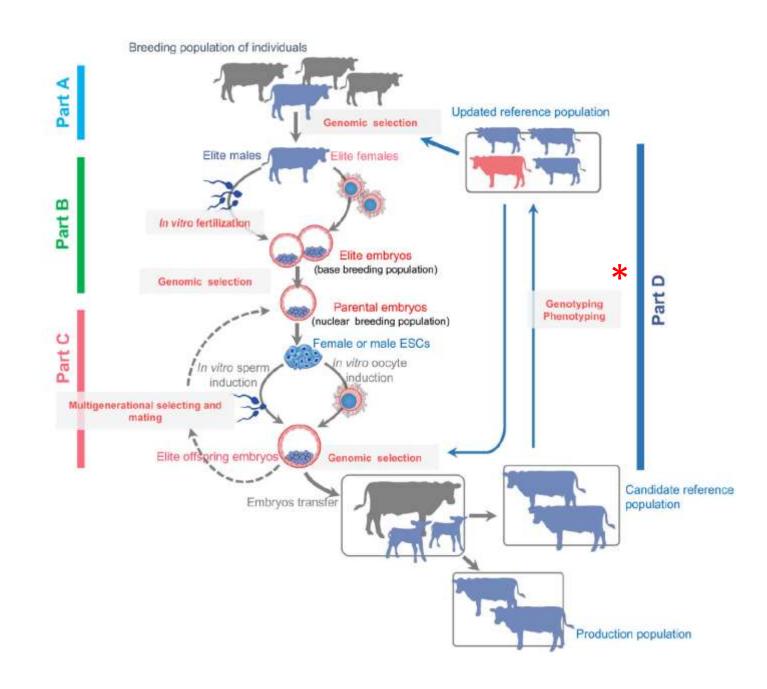


Statistical models for genomic selection (GS) are trained on a <u>reference population</u> of animals that have both <u>SNP genotypes</u> and <u>phenotypic</u> information Revolutionize livestock breeding in the future: an animal embryo-stem cell breeding system in a dish

Journal of Animal Science and Biotechnology (2018) 9:90

### Embryo-stem cell breeding system

 Part A, nuclear breeding population
 Part B, nuclear population of elite embryos
 Part C, transgenerational breeding cycle from parental embryos and offspring embryos
 Part D, reference population updating



## Revolutionize livestock breeding in the future: an animal embryo-stem cell breeding system in a dish

Journal of Animal Science and Biotechnology (2018) 9:90

#### Comparisons of different breeding systems

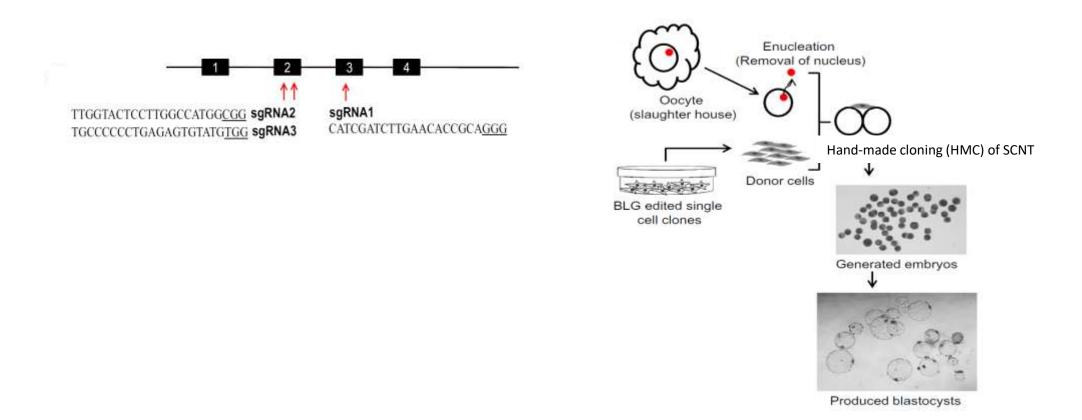
| Major breeding<br>elements | Conventional breeding  | Genomic selection  | Embryo-stem cell breeding                               |
|----------------------------|--|--|---|
| Breeding scheme            | Yes  | Yes  | Yes   |
| Pedigree record            | Yes  | Yes, can also reconstruct pedigree from genotyping data                      | Yes, can also reconstruct pedigree from genotyping data |
| Performance testing        | Breeding animals   | Only for reference population  | Only for reference population                           |
| Reference population       | No   | Yes  | Yes   |
| Candidate breeding animal  | Individual   | Individual, embryo   | Embryo  |
| Generation transfer        | Individual to individual   | Individual to individual   | Embryo to embryo  |
| Breeding value             | EBV  | GEBV   | eGEBV   |
| Gametogenesis              | In vivo gametogenesis  | In vivo gametogenesis  | In vitro induced gametogenesis                          |
| Fertilization /Embryo      | In vivo fertilization and<br>development;<br>In vitro fertilization and<br>culture | In vivo fertilization and development;<br>In vitro fertilization and culture | In vitro fertilization and culture                      |

CRISPR/Cas gene editing

#### CRISPR-mediated editing of β-lactoglobulin (*BLG*) gene in buffalo

Scientific Reports | (2024) 14:14822

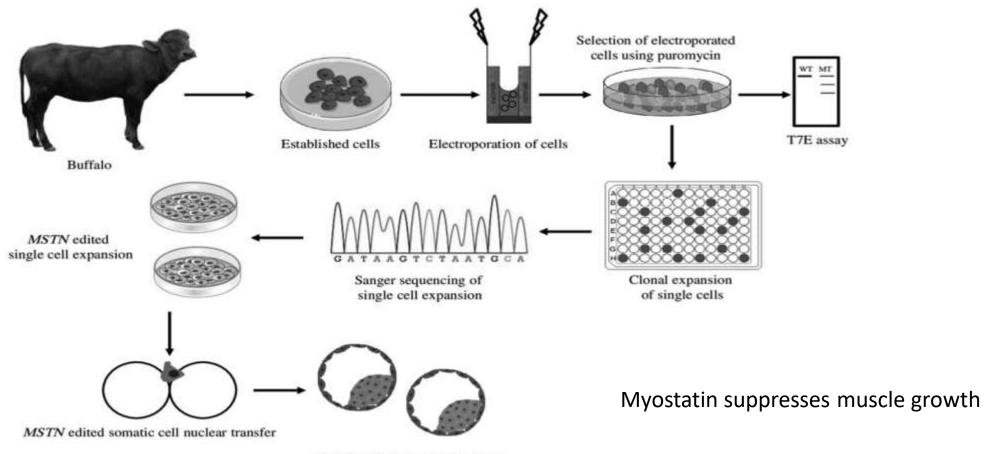
# CRISPR/Cas editing of β-lactoglobulin gene exons 2 and 3 with single guide RNAs (sgRNAs) to reduce allergenic property of buffalo milk



#### Production of MSTN Gene-Edited Embryos of Buffalo Using the CRISPR/Cas9 System and SCNT

CELLULAR REPROGRAMMING Volume 25, Number 3, 2023

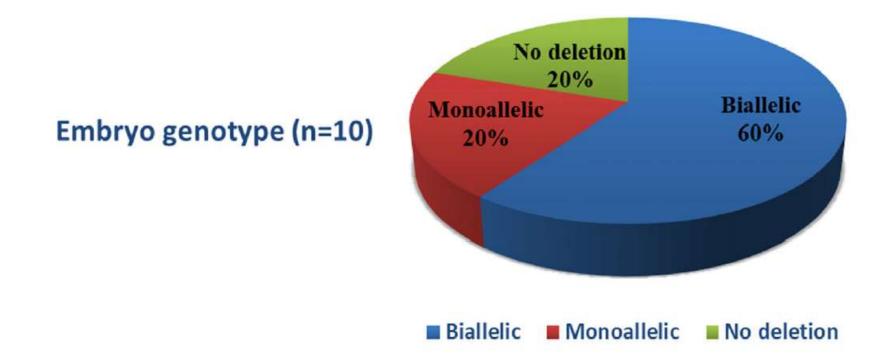
# CRISPR plasmids (pX459) containing sgRNAs targeting of myostatin (*MSTN*) gene in buffalo



MSTN edited cloned blastocysts

Animals 2024, 14, 134.

#### Efficiency of CRISPR/Cas knockout in buffalo zygotes



#### Stating the obvious

The animal is fundamental.

Get the animal right for profitable market(s) and then think about other investments.



#### Future perspective

- 1. Ongoing refinement of animal and milk traits that have local and global importance.
- 2. Global reference populations to link phenotypes with genotypes.
- 3. Validation of global reference populations within and between breeds (Mediterranean, Murrah, Nili-Ravi)
- 4. Use information from reference populations for continued development and broad adoption of genomic breeding values.
- 5. Strategic use of reproductive technology to accelerate local and global genetic improvement.
- 6. Establish global consortium to coordinate, integrate and facilitate genetic improvement.
- 7. New differentiated products for changing global demographics and wealth distribution.

Acknowledgements



### THE WHEY FACTORY

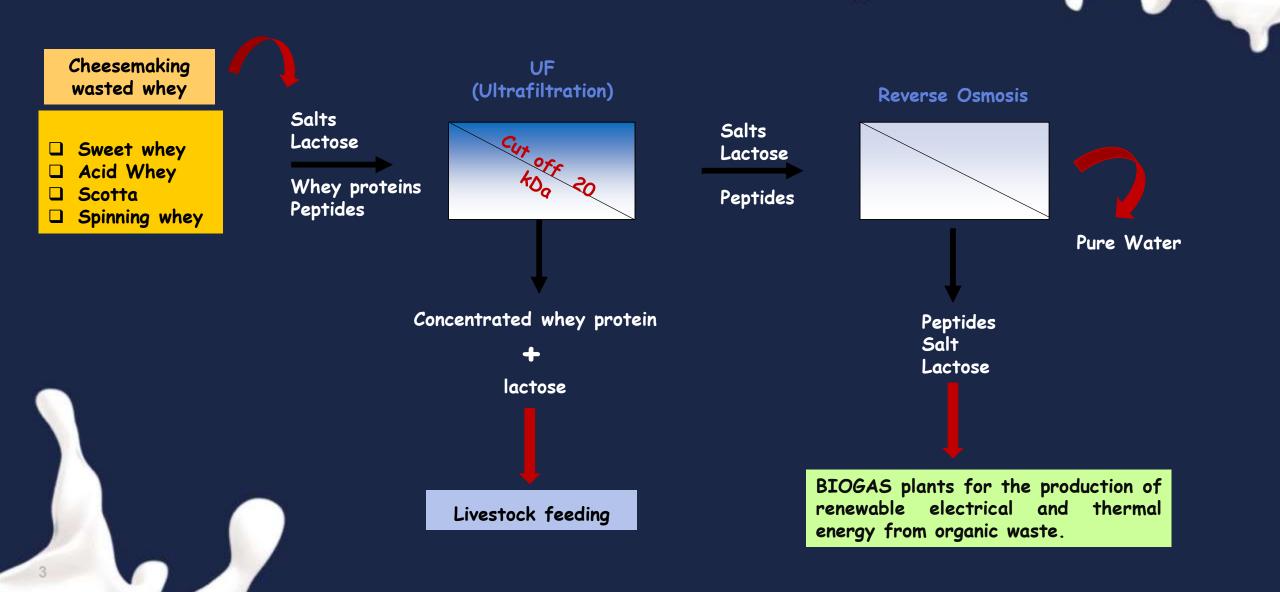
**Francesco Addeo** Department of Agriculture University of Naples Federico II – Portici addeo@unina.it COLLABORATION BETWEEN RESEARCH CENTERS AND BUFFALO DAIRIES: RESULTS

We present the results of the collaboration between buffalo dairies and our research structures for an alternative utilization of dairy effluents, which traditionally were sent to wastewater treatment plants.

### In dairies, there are four main effluents:

- sweet whey
- acidic whey
- unused whey starter
- spinning water
- deproteinized whey, known as 'scotta,' left over from Ricotta production

Whey Fractionation and Concentration Process Using Ultrafiltration and Reverse Osmosis



Buffalo Whey Supplements for Oncology Patients: A Circular Economy Opportunity



#### PROGETTO TRASFERIMENTO TECNOLOGICO E DI PRIMA INDUSTRIALIZZAZIONE PER LE IMPRESE INNOVATIVE AD ALTO POTENZIALE PER LA LOTTA ALLE PATOLOGIE ONCOLOGICHE – CAMPANIA TERRA DEL BUONO

Decreto Dirigenziale n. 357 del 12/06/2017, BURC n. 47 del 12 Giugno 2017

PROGETTO IABUPO - INTEGRATORI ALIMENTARI DA SIERO BUFALINO PER IL TRATTAMENTO DI PAZIENTI AFFETTI DA PATOLOGIE ONCOLOGICHE: UN'OPPORTUNITÀ PER LA FILIERA BUFALINA IN UN'OTTICA DI ECONOMIA CIRCOLARE



An innovative project financed by the Campania region. The objective was to develop new uses for whey proteins, going beyond their use as animal feed. The project focused on creating supplements for cancer patients, turning a by-product into a valuable resource.

#### Molecular Characterization of Buffalo Milk and Whey: Proteins and Peptides Analysis

800

700

600

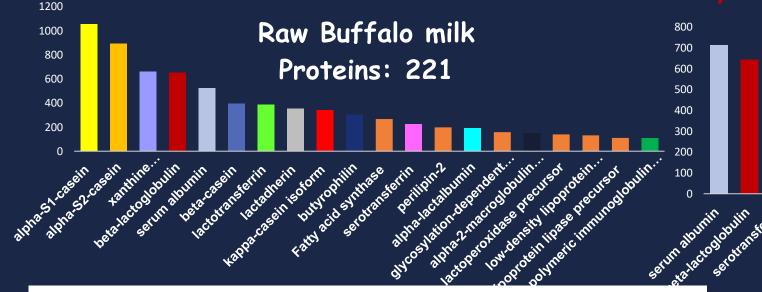
500

400

300

200

100



Sweet whey Proteins: 125

putyrophilin subfamily 1...

Kanthine...

Lactotransferrin

lactoperoridase

serotransferrin

polynoic innunoglobulin... alpha 2. macroglobulin is donn.

1200 Acid whey: 1000 Proteins: 172 800 600 400 200 0 butyrophilin subfanily 1. dhcosylaton dependent cell. polymeric immunoglooutin. LON QUALTY PROTEIN. Fatty. alpha?macroscop.unin isoform. LOW QUALITY PROTEIN. tincal hardy optober. Kanthine de hydrogenaselovidese alphash case in storm th platelet all coprotein A isoform X1 plasma protease of inhibitor serpin A3-1 isoform X1 lactoperoxidase serpin A3-T-like serpin A33 like betalactoglobulin

We analyzed buffalo milk and whey, recovering the four main types of effluents from the mozzarella production process. These fluids were fractionated, and we identified the protein and peptide compositions. In buffalo milk, we identified 221 proteins, 125 in sweet whey, and 172 in acidic whey.

alphalacalaurin

nucleopindin

glycosystion.dependent cell...

platelet all coprote in A isotorm.

serpin A3-1 isoform X1

ceruoplasnin isoform X1

#### Functionalization of Whey Proteins with Bioactive Compounds: Glutathione as a Bioavailability Enhancer

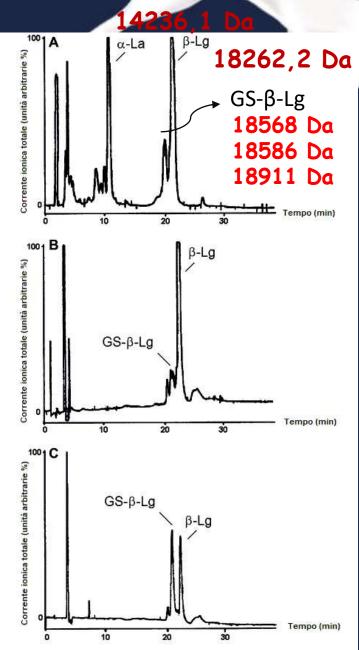
Wasted

whey

GSSG Molecular Weight 307.32

> 0 min 30' min 360' min

> > This project aimed to use beta-lactoglobulin as a carrier for glutathione, a powerful antioxidant. Glutathione exists in two forms in the body: oxidized (GSSG) and reduced (GSH). We aimed to enhance glutathione's stability and absorption by binding it to beta-lactoglobulin. After six hours of reaction, the protein had bound approximately 50% of the available glutathione.



<sup>Incubation</sup> time

### Buffalo Lactoferrin: Industrial Isolation and Health Applications

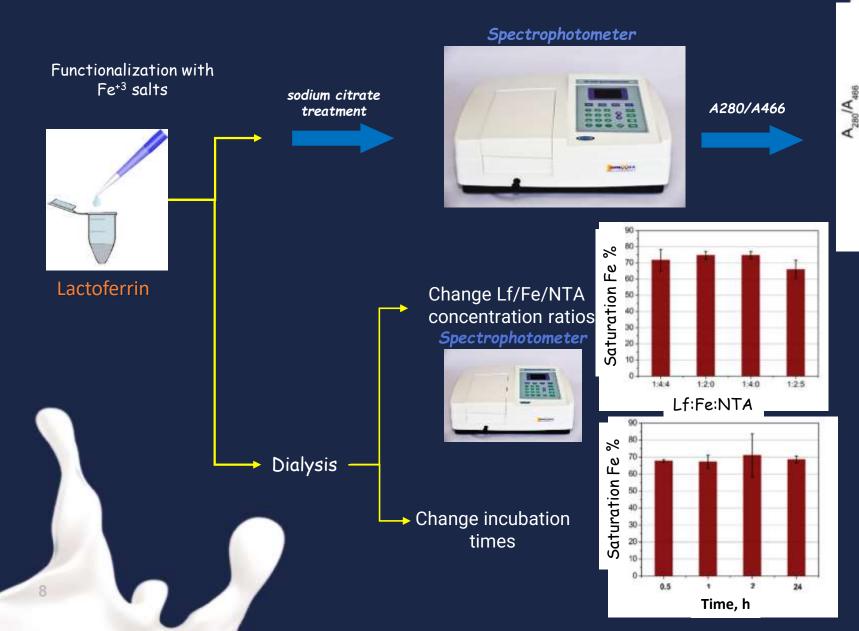


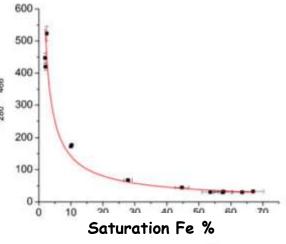
We isolated buffalo lactoferrin on an industrial scale.

Like bovine lactoferrin, buffalo lactoferrin carries two iron atoms and plays a key role in iron absorption and as a bacteriostatic agent.

The process is costly but offers promising health benefits and potential uses in nutraceuticals and cosmetics.

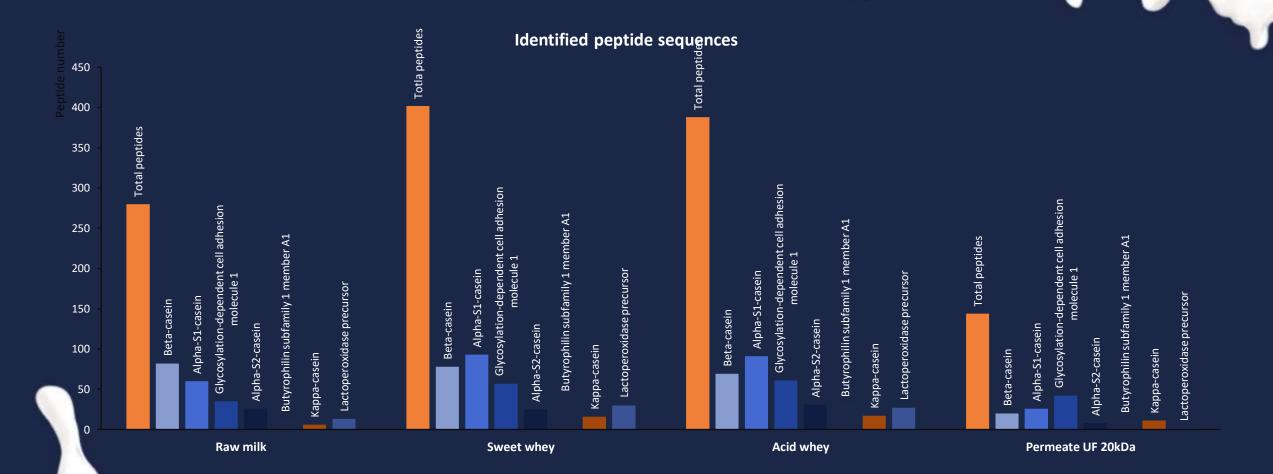
#### Functionalization of Buffalo Lactoferrin with Iron Ions: Enhancing Bioavailabilit





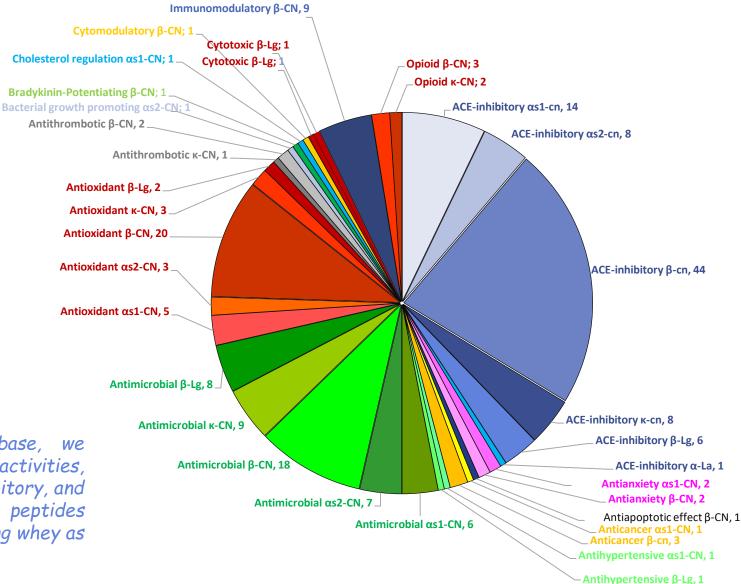
In this slide, we show the process of enriching buffalo lactoferrin with additional iron ions. This can support hemoglobin synthesis and prevent iron deficiency anemia. We optimized the conditions for iron binding, achieving a functionalization rate of over 85% at a pH of 5.0.

#### Peptide Profiles in Buffalo Milk and Whey: Identification and Origins



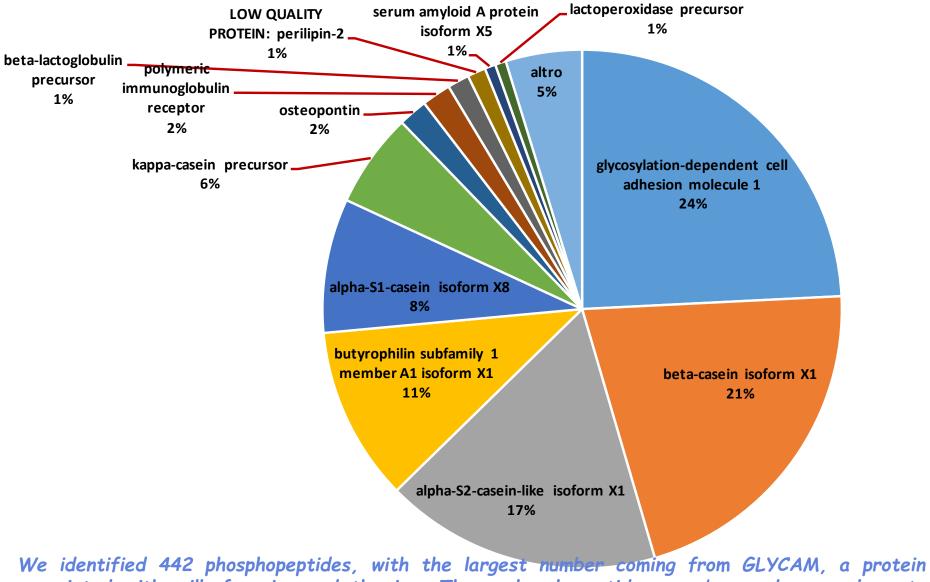
This slide provides an overview of the peptides identified in buffalo milk, sweet whey, acidic whey, and permeate. The total number of peptides was significantly higher in whey compared to milk and permeate. We also traced the origin of these peptides to understand their formation.

#### **Bioactive Peptides in Whey: Potential Health Benefits**



Using the Milk Bioactive Peptide Database, we identified peptides with various biological activities, including antimicrobial, antioxidant, ACE-inhibitory, and anticancer properties. These bioactive peptides highlight the potential health benefits of using whey as a base for functional beverages

#### Phoshopeptide Identification in Whey: Detecting Cold Storage Markers



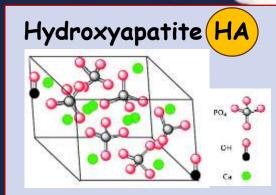
associated with milk freezing and thawing. These phosphopeptides can be used as markers to determine if frozen milk was used in mozzarella production. Phosphopeptides Applications: Nutraceuticals, Biomedical, and Food Additives

#### Patent n. 1404716

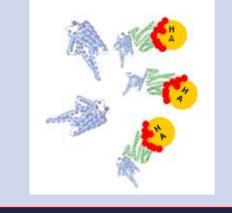
<<Preparation of a new product consisting of phosphopeptides immobilized on
hydroxyapatite for nutraceutical and (other) innovative purposes>>

#### Key Claims of the Patent

- Selective Isolation: A fast and efficient system for the selective isolation of phosphoproteins and casein phosphopeptides (CPP) in complexes with hydroxyapatite (HA).
- Nutraceutical Formulation: The HA-phosphopeptide complex forms the basis of a nutraceutical product for use as a food additive or in biomedical applications.
- High-Value Food Ingredient: The HA-phosphopeptide complex can be used to make milk a functional food, enhancing its health benefits.
- Phosphopeptide Recovery: Quantitative recovery of phosphopeptides from complex mixtures, ensuring selective recovery of phosphorylated proteins.
- Edibility and Taste: The matrix used for immobilizing phosphopeptides is edible and does not impart a bitter taste, making it suitable for the food industry.
- Dental and Bone Health: Use of the HA-phosphopeptide complex for mineralization of dental plaque, as an ingredient in toothpaste, and for increasing the bioavailability of minerals to prevent osteoporosis and other calcium-deficiency diseases.
- By-Products: The non-phosphorylated peptides can be used as bioactive peptides, such as casomorphins and antihypertensive peptides, or as a nitrogen source for microorganisms.
  - Aquaculture: Application of the HA-phosphopeptide complex as a growth initiator for eggshell formation in a solution and in the artificial breeding of bivalve mollusks and coral.



#### Protein and/or Peptide mixtures



#### Buffalo Whey Concentrate in Burrata Production: ALIFUN Project Prototype



**Codice Progetto** ARS01 0078 Fondo Europeo di Sviluppo Regionale



PON "Ricerca e Innovazione" 2014-2020 - Asse II; Area di specializzazione "Agrifood"

### PROTOTIPO DI BURRATA DI BUFALA

Ingredienti-Ripieno: Crema di latte di bufala pastorizzato (>90°C), Proteine di latte di bufala, proteine del siero concentrate e idrolizzate. Involucro: latte di bufala pastorizzato, sieroinnesto naturale, sale, caglio.

nutritional value.

**UNIONE EUROPEA** 

Prodotto da Caseificio Cirigliana s.r.l. Nello stabilimento in Via Saudina 20 81053 Riardo (Ce)





3 mg di peptidi / 100 mg di polvere



0,1 % di polvere di idrolizzato proteico This prototype burrata was developed using buffalo whey concentrate in collaboration with the Cirigliana dairy under the ALIFUN project. The product is enriched with hydrolyzed protein, enhancing its

13

From Thesis to Market: Development of a New Buffalo Whey Product

# Premio tesi di laurea

LA FILIERA AGROALIMENTARE: tra tradizione e innovazione sostenibile

# EVENTO





### ROMA, 28 marzo 2023 Hotel The Building

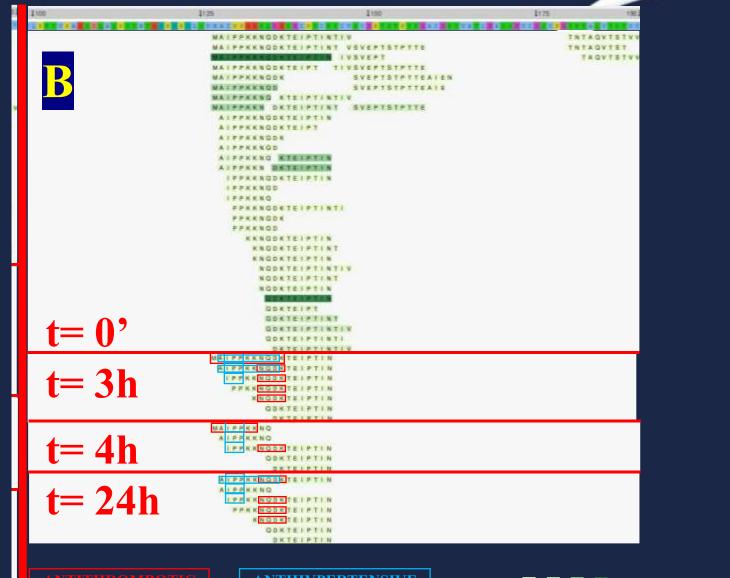
via Montebello 126 ore 14.30 - 17.00

**Premio Granarolo**, vincitore Ferdinando Cropano, consegna Gianpiero Calzolari, *Presidente Granarolo* 



This innovative product originates from a university thesis by a student who developed a new product based on concentrated buffalo whey. The product was created by hydrolyzing whey proteins with nonhuman digestive enzymes, producing bioactive peptides.

## PEPTIDES FROM ENZYMATIC HYDROLYSIS OF 12% WHEY PROTEINS



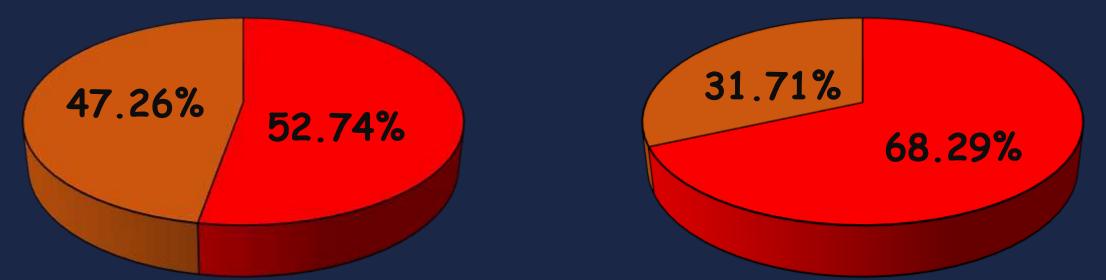
ANTIHYPERTENSIVE



PEPTIDES IDENTIFIED IN <u>SPRAY-DRIED</u> AND 12% <u>CONCENTRATED WHEY</u> AFTER HYDROLYSIS WITH ALCALASE® Food grade

# ACE-INHIBITORY

# ANTITROMBOTIC



Whey proteins undergo progressive digestion during incubation with alcalase<sup>®</sup>.

The process leads to the formation of shorter peptides that include amino acid sequences recognized for their *ACE-Inhibitory*, antithrombotic and antihypertensive activities. The peptide content varies between spray-dried whey and concentrated whey.

6

### Absorptomics: A New Frontier in Nutrition Science





Trends in Food Science & Technology Volume 126, August 2022, Pages 41-60

## *In vivo* absorptomics: Identification of bovine milk-derived peptides in human plasma after milk intake

Simonetta Caira <sup>a</sup> A 🖾 , Gabriella Pinto <sup>b</sup>, Gianluca Picariello <sup>c</sup>, Paola Vitaglione <sup>d</sup>, Sabrina De Pascale <sup>a</sup>, Andrea Scaloni <sup>a</sup>, Francesco Addeo <sup>d</sup>



Food Chemistry



Go to Food Chemistry on ScienceDirect

### Beyond the gut: Investigating the mechanism of formation of β-casomorphins in human blood

Simonetta Caira <sup>a</sup> <sup>A</sup> <sup>⊠</sup>, Antonio Dario Troise <sup>a</sup>, Gianluca Picariello <sup>b</sup>, Sabrina De Pascale <sup>a</sup>, Gabriella Pinto <sup>c</sup>, Marcella Pesce <sup>d</sup>, Francesca Marino <sup>e</sup>, Giovanni Sarnelli <sup>d</sup>, Andrea Scaloni <sup>a</sup> <sup>A</sup> <sup>⊠</sup>, Francesco Addeo <sup>f</sup> Recent developments in peptidomics for the quali-quantitative analysis of food-derived peptides in human body fluids and tissues

Simonetta Caira <sup>a</sup>, Gianluca Picariello <sup>b</sup>, Giovanni Renzone <sup>a</sup>, Simona Arena <sup>a</sup>, Antonio Dario Troise <sup>a</sup>, Sabrina De Pascale <sup>a</sup>, Valentina Ciaravolo <sup>a</sup>, Gabriella Pinto <sup>c</sup>, Francesco Addeo <sup>a d</sup>, Andrea Scaloni <sup>a</sup> **A** 🖾



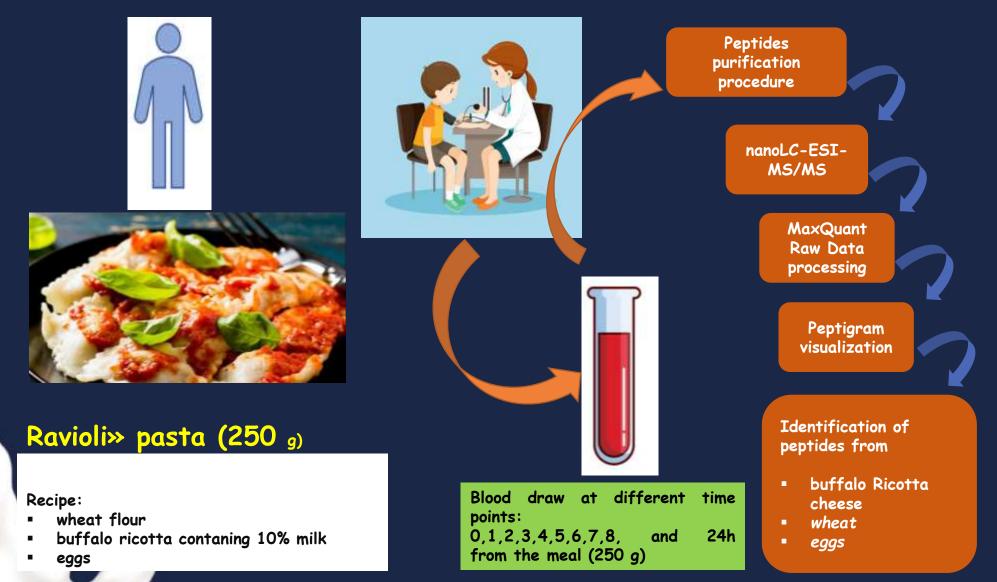
Journal of Functional Foods Volume 113, February 2024, 106004

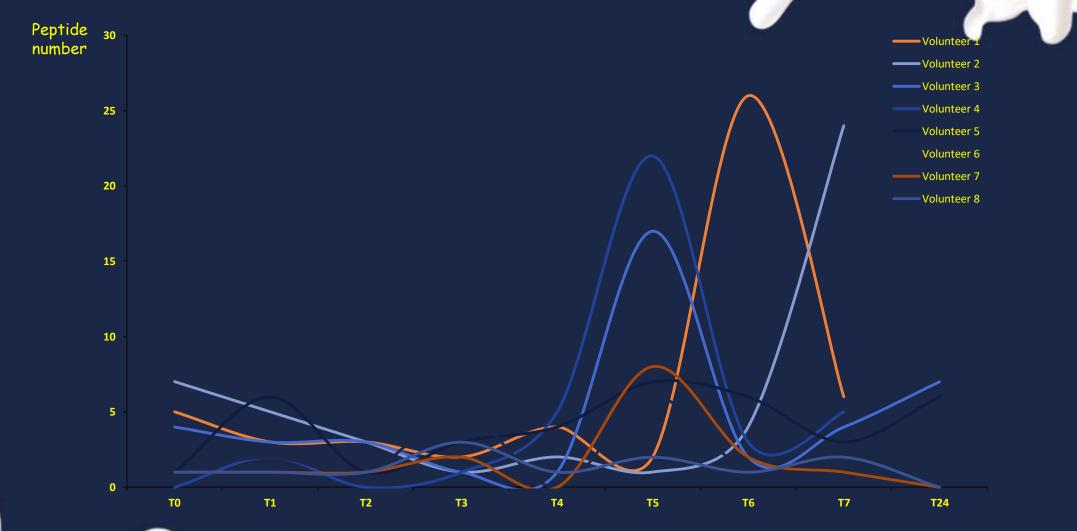


*Ex vivo* degradation of β-Casomorphin-7 by human plasma peptidases: Potential implications for peptide systemic effects

Sabrina De Pascale ª, Gianluca Picariello <sup>b</sup>, Antonio Dario Troise ª, Simonetta Caira ª 🗙 ⊠, Gabriella Pinto <sup>c</sup>, Francesca Marino <sup>d</sup>, Andrea Scaloni ª 🎗 ⊠, Francesco Addeo <sup>e</sup> Monitoring the absorption of peptides into the bloodstream of 10 volunteers who consumed "ravioli" pasta filled with high-protein buffalo ricotta,

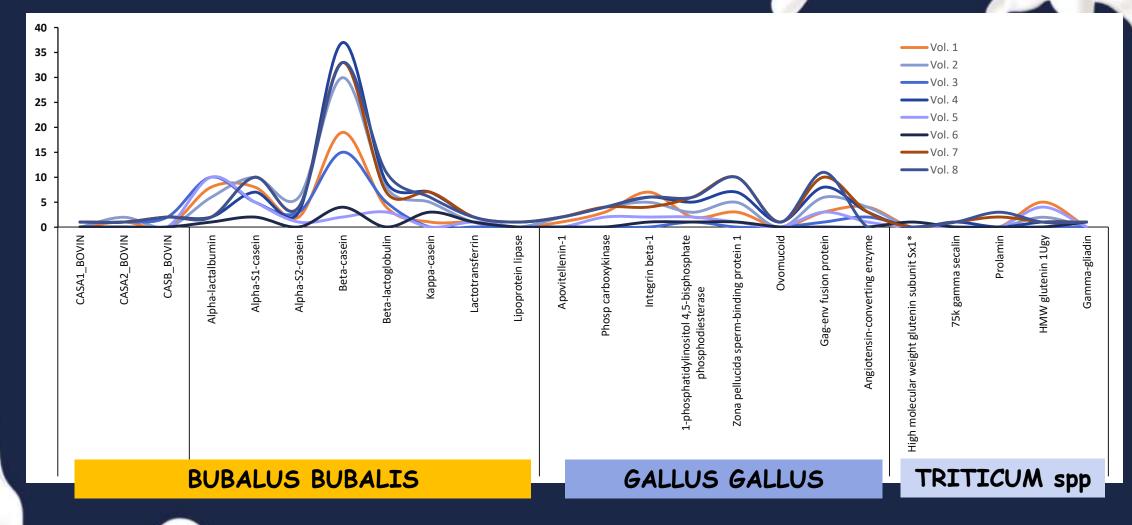
Volunteers: 1,2 .....10





### Peptide Absorption Kinetics: Variability Among Volunteers

The results show significant variation in the number of circulating peptides between subjects, with the pe occurring between 5 and 7 hours after the meal. We also tracked peptide elimination through urine.



## Origin and Circulation Time of Identified Peptides: Buffalo, Chicken, and Wheat Proteins

### Comparison of Beta-Lactoglobulin and Alpha-Lactalbumin Peptides in Blood and Enzymatic Hydrolysates

| Sequence    | Mass   | Description        | species | Start | End |
|-------------|--------|--------------------|---------|-------|-----|
| AIVQNNDSTE  | 1089,5 | Alpha-lactalbumin  | Buffalo | 40    | 49  |
| GYDTQ       | 582,23 | Alpha-lactalbumin  | Buffalo | 35    | 39  |
| KDLKDY      | 780,4  | Alpha-lactalbumin  | Buffalo | 13    | 18  |
|             |        |                    |         |       |     |
| LACAAQAIIVT | 1072,6 | Beta-lactoglobulin | Buffalo | -6    | 4   |
| DAQSAPLRVY  | 1118,6 | Beta-lactoglobulin | Buffalo | 33    | 42  |
| LKPTPEGDLE  | 1097,6 | Beta-lactoglobulin | Buffalo | 46    | 55  |
| KKIIAEKTKIP | 1267,8 | Beta-lactoglobulin | Buffalo | 69    | 79  |
| ALNENKVLVL  | 1111,7 | Beta-lactoglobulin | Buffalo | 86    | 95  |
| DTDYKKY     | 931,43 | Beta-lactoglobulin | Buffalo | 96    | 102 |
| LVRTPEVDDE  | 1171,6 | Beta-lactoglobulin | Buffalo | 122   | 131 |
| LKALPMHIRLS | 1277,8 | Beta-lactoglobulin | Buffalo | 140   | 150 |

We compared the beta-lactoglobulin and alpha-lactalbumin peptides identified in volunteers' blood with those generated through enzymatic hydrolysis. None of the peptides in the blood were identical to those produced by hydrolysis, demonstrating the unique activity of enzymatically derived peptides.

21

## PROSALAB Project: Health-Promoting Products from Buffalo Milk



### POR CAMPANIA FESR 2014/2020

#### AVVISO PUBBLICO PER IL SOSTEGNO ALLE MPMI CAMPANE NELLA REALIZZAZIONE DI PROGETTI DI SVILUPPO SPERIMENTALE, TRASFERIMENTO TECNOLOGICO E INDUSTRIALIZZAZIONE

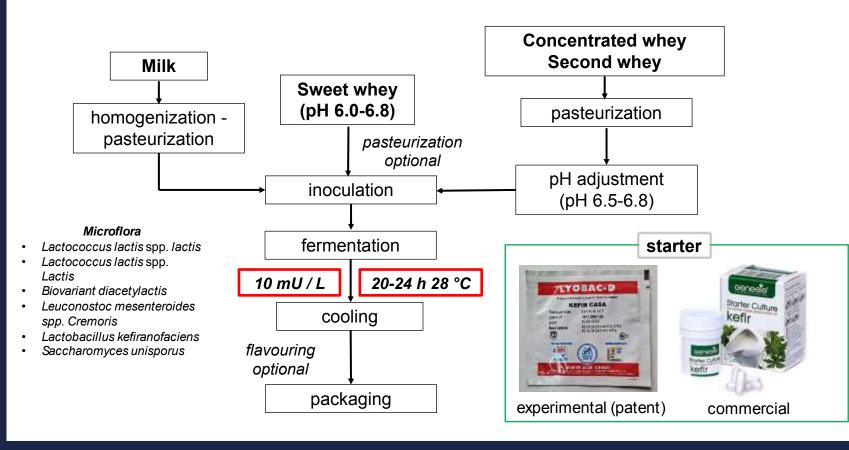
Progetto PROSALAB: Nuovi PROdotti SAlutistici dal LAtte di Bufala sano

Soggetto proponente: Aziende Agricole Associate (Cancello Arnone, Caserta)

As part of the PROSALAB project, developed with Azienda Agricole Associate, we created new health-promoting products from buffalo milk. One of the key innovations was the development of buffalo kefir, a fermented beverage with numerous health benefits.

### Technology for Kefir Production from Buffalo Milk and Whey By-products

Kefir from buffalo milk and from byproducts of Mozzarella cheese manufacturing



This slide outlines the technological process for producing kefir from buffalo milk and whey by-products. The process involves pasteurization, inoculation, fermentation, and optional flavoring before packaging, with the final product offering numerous health benefits.

### Kefir Varieties and Health Benefits from Whey and Concentrated Whey







We expanded kefir production to include versions made from whey and concentrated whey. Buffalo whey kefir contains about 1% protein, making it a refreshing beverage, while kefir from concentrated whey provides a high-protein option, ideal for athletes.

### Kefiran: The Key Difference Between Kefir and Yogurt

The key difference between kefir and yogurt is the presence of kefiran, a polysaccharide produced during kefir fermentation.

Kefiran has antioxidant, anti-inflammatory, and antimicrobial properties, and has attracted commercial interest for its potential health benefits.

# Kefiran Production from Ricotta Whey: A New Functional Ingredient

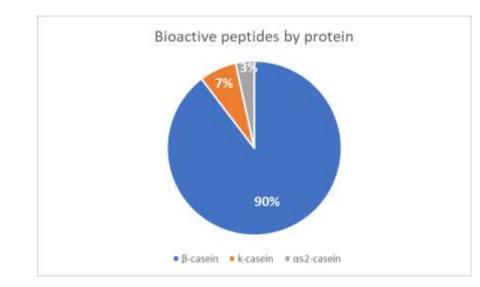
Obtained by fermenting the deproteinized whey with a starter rich in Lactobacillus kefiranofaciens, we produced kefiran, which increased the viscosity of the liquid. This innovation has already attracted interest from the Probiotical company for use as a supplement and in cosmetics.

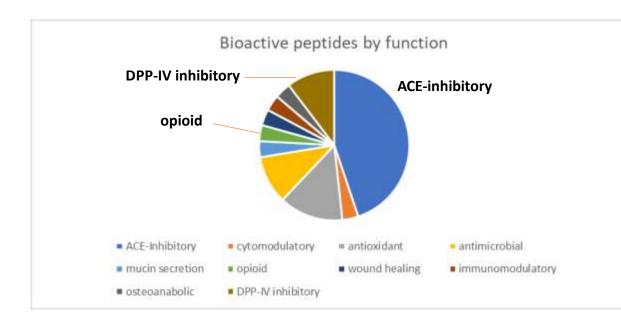
# Kefirani



#### Classification of bioactive peptides released in buffalo milk kefir

|                  | Peptides            | Species Homology            | Functions                          | Protein IDs   |  |  |
|------------------|---------------------|-----------------------------|------------------------------------|---------------|--|--|
| Grouped Results: |                     | Homo sapiens: 2             | ACE-inhibitory: 13                 | P05814: 2     |  |  |
|                  |                     | Bos taurus: 26              | Cytomodulatory: 1                  | P02666: 25    |  |  |
|                  |                     | Bubalus bubalis: 1          | Antioxidant: 4                     | P02668: 1     |  |  |
|                  |                     |                             | Antimicrobial: 3                   | A0A1L6KYI1: 1 |  |  |
|                  |                     | Increase mucin secretion: 1 |                                    |               |  |  |
|                  | Opioid: 1           |                             |                                    |               |  |  |
|                  | Wound healing: 1    |                             |                                    |               |  |  |
|                  | Immunomodulatory: 1 |                             |                                    |               |  |  |
|                  |                     | Osteoanabolic: 1            |                                    |               |  |  |
|                  |                     |                             | DPP-IV Inhibitory: 2               |               |  |  |
|                  |                     |                             | Prolyl endopeptidase-inhibitory: 1 |               |  |  |
| Total Counts:    | 26                  | 3                           | 11                                 | 4             |  |  |



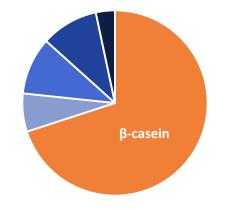


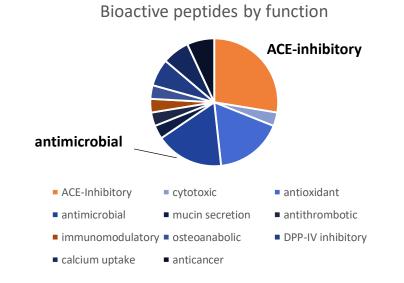
Bioinformatic search in: Milk Bioactive Peptide Database (https://mbpdb.nws.oregonstate.edu/)

#### Classification of bioactive peptides released in buffalo whey kefir

|                  | Peptides | Species Homology   | Functions                          | Protein IDs   |
|------------------|----------|--------------------|------------------------------------|---------------|
| Grouped Results: |          | Bos taurus: 26     | Antioxidant: 5                     | P02662: 3     |
|                  |          | Bubalus bubalis: 1 | Increase calcium uptake: 2         | P02666: 20    |
|                  |          | Bos spp : 1        | ACE-inhibitory: 8                  | P02754: 1     |
|                  |          | Capra hircus: 1    | Antimicrobial: 5                   | P02668: 2     |
|                  |          |                    | Prolyl endopeptidase-inhibitory: 1 | A0A1L6KYI1: 1 |
|                  |          |                    | Cytotoxic: 1                       | A0A344X7B9: 1 |
|                  |          |                    | Antithrombitic: 1                  | P33048: 1     |
|                  |          |                    | Osteoanabolic: 1                   |               |
|                  |          |                    | DPP-IV Inhibitory: 1               |               |
|                  |          |                    | Anticancer: 2                      |               |
|                  |          |                    | Antithrombotic: 1                  |               |
|                  |          |                    | Immunomodulatory: 1                |               |
| Total Counts:    | 25       | 4                  | 12                                 | 7             |

Bioactive peptides by protein





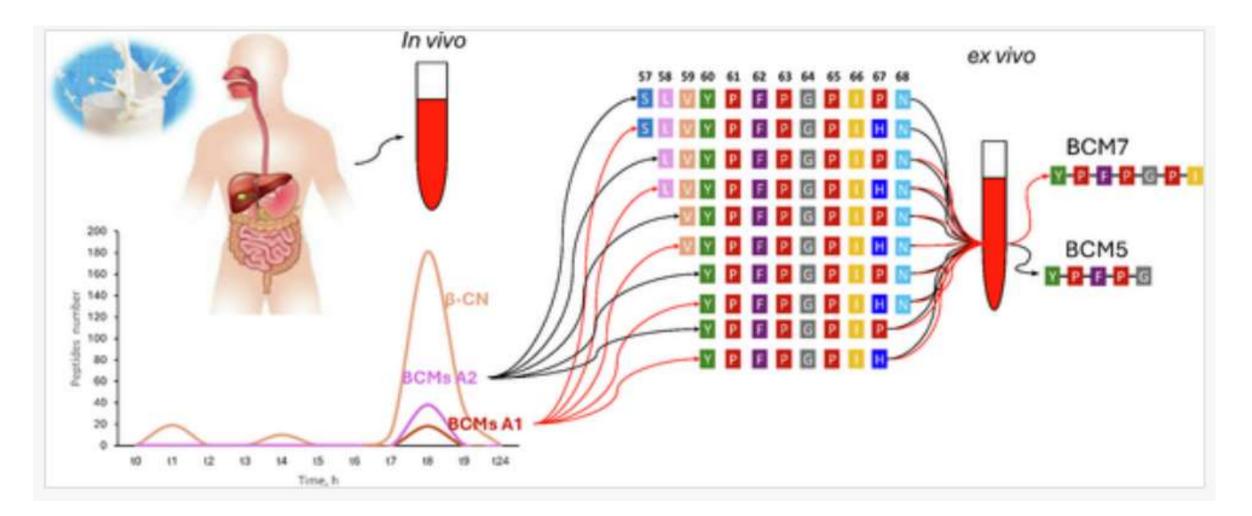
Bioinformatic search in: Milk Bioactive Peptide Database (https://mbpdb.nws.oregonstate.edu/)

# CONCLUSIONS

- Buffalo dairy can make significant progress by closely collaborating with research institutes and universities.
- Scientific innovation combined with dairy tradition offers unprecedented opportunities to improve production processes and create new products with high added value.
- Scientific research can provide tools for optimizing resources, such as the use of whey and other by-products, while reducing waste and increasing sustainability.
- Furthermore, collaboration with researchers can pave the way for innovative products, such as buffalo kefir, phosphopeptides and kefirans, with applications not only in food but also in nutraceuticals and cosmetics.

By working together, dairies and research institutes can not only respond to market needs, but also anticipate new trends, ensuring a future of growth and success for the buffalo sector.

### Monitoring Peptide Absorption from Buffalo Ricotta in Human Blood





Food Chemistry Volume 141, Issue 1, 1 November 2013, Pages 597-603



CrossMark

Analytical Methods

#### A signature protein-based method to distinguish Mediterranean water buffalo and foreign breed milk

Simonetta Caira <sup>a</sup> , Gobriella Pinto <sup>a</sup>, Valentin A. Balteanu <sup>b</sup>, Lina Chianese <sup>a</sup>, Francesco Addeo <sup>a</sup>

Anal Bioanal Chem (2016) 408:5609–5621 DOI 10.1007/s00216-016-9663-0

RESEARCH PAPER

Simultaneously tracing the geographical origin and presence of bovine milk in Italian water buffalo Mozzarella cheese using MALDI-TOF data of casein signature peptides

Simonetta Caira<sup>1</sup> · Gabriella Pinto<sup>2</sup> · Maria Adalgisa Nicolai<sup>2</sup> · Lina Chianese<sup>2</sup> · Francesco Addeo<sup>2</sup>

Received: 12 April 2016/Revised: 18 May 2016/Accepted: 20 May 2016/Published online: 14 June 2016 
© Springer-Verlag Berlin Heidelberg 2016



Eventual limits of the current EU official method for evaluating milk adulteration of water buffalo dairy products and potential proteomic solutions

Simonetta Cairo <sup>b</sup> A 🖾 , Maria Adalgisa Nicolal <sup>a</sup>, Sergio Lilla <sup>a</sup>, Maria Grazia Calabrese <sup>a</sup>, Gabriella Pinto <sup>a</sup>, Andrea Scaloni <sup>b</sup>, Lina Chianese <sup>a</sup>, Francesco Addeo <sup>a</sup>



Food Chemistry Volume 277, 30 Morch 2019, Poges 195-204



A non-canonical phosphorylation site in βcasein A from non-Mediterranean water buffalo makes quantifiable the adulteration of Italian milk with foreign material by combined isoelectrofocusingimmunoblotting procedures

Simonetta Caira ° 🎗 🖾, Gabriella Pinto <sup>5</sup>, Maria Adalgisa Nicolai <sup>6</sup>, Gianfranco Novi °, Francesco Addeo <sup>6</sup>, Andrea Scoloni ° 🎗 🔤

#### A Genotyping Method for Detecting Foreign Buffalo Material in Mozzarella di Bufala Campana Cheese Using Allele-Specific- and Single-Tube Heminested-Polymerase Chain Reaction

by Rosario Rullo <sup>1,\*</sup> <sup>∞</sup>, Simonetta Caira <sup>1</sup> <sup>∞</sup>, Ioana Nicolae <sup>2</sup> <sup>∞</sup>, Francesca Marino <sup>3</sup> <sup>∞</sup>, Francesco Addeo <sup>4</sup> <sup>∞</sup> and Andrea Scaloni <sup>1</sup> <sup>∞</sup>

- <sup>1</sup> Institute for the Animal Production System in the Mediterranean Environment, National Research Council, 80055 Portici, Italy
- <sup>2</sup> Research and Development Institute for Bovine, 077015 Balotesti, Romania
- <sup>3</sup> Department of Clinical Medicine and Surgery, Endocrinology Unit, University Federico II, 80131 Naples, Italy
- <sup>4</sup> Dipartimento di Agraria, Università degli Studi di Napoli "Federico II", 80055 Portici, Italy
- \* Author to whom correspondence should be addressed.

Foods 2023, 12(12), 2399; https://doi.org/10.3390/foods12122399

Submission received: 5 May 2023 / Revised: 31 May 2023 / Accepted: 12 June 2023 / Published: 16 June 2023

# PRECURSORS OF BIOACTIVE PEPTIDES AFTER ALCALASE ACTION

| PEPTIDE                 | MW (Da)                 | SOURCE                 | ACTIVITY          | REFERENCE   |
|-------------------------|-------------------------|------------------------|-------------------|---|
| QTPVV <b>VPP</b> F      | 982,54877               | <b>β-CN</b> (f84-86)   | ACE-inhibitory    | Nakamura et al., (1995)   |
| SQSKVLPVPQ              | 1081,6132               | <b>β-CN</b> (f169-174) | ACE-inhibitory    | Maeno et al., (1996)  |
| SQSKVLPVPQ              | 1081,6132               | <b>β-CN</b> (f169-175) | ACE-inhibitory    | Maeno et al., (1996)  |
| LQDKIHPF                | 996,53927               | <b>β-CN</b> (f47-52)   | ACE-inhibitory    | Gobbetti et al., (2000)   |
| HK <b>EMPFPK</b> YPVEPF | 1744,8647               | <b>β-CN</b> (f108-113) | Antihypertensive  | Pihlanto-Leppala et al., (1998)   |
| MAIPPKKNQDKTEIPTIN      | 2037,0929               | <b>к-СN</b> (f106-116) | Antithrombotic    | Fiat and Jollèe (1989); Jollès et al., (1986); Schlimme and Meisel (1995) |
| MAIPPKKN                | 897,51061               | <b>к-СN</b> (f106-112) | Antithrombotic    | Fiat and Jollèe (1989); Jollès et al., (1986); Schlimme and Meisel (1995) |
| <b>NQDK</b> TEIPTIN     | 1271,6357               | <b>к-СN</b> (f113-116) | Antithrombotic    | Fiat and Jollèe (1989); Jollès et al., (1986); Schlimme and Meisel (1995) |
| AIPPKKN                 | 766,47012               | <b>к-СN</b> (f108-110) | Antihypertensive  | Nakamura et al., (1995)   |
| AIPPKKNQD               | 1009,5556               | <b>к-СN</b> (f128-136) | ACE-inhibitory    | Shuang et al., (2008)   |
| DTDYKKYLLF              | 1304,6653               | <b>β-Lg</b> (f103-105) | ACE-inhibitory    | Hernandez-Ledesma et al., (2002)  |
| ALPMHIR                 | 836,46908               | <b>β-Lg</b> (f147-148) | ACE-inhibitory    | Mullally et al., (1996)   |
| HIRLSFNPT               | 1083,5825               | <b>β-Lg</b> (f148-149) | ACE-inhibitory    | Mullally et al., (1996)   |
| ALPMHIR                 | 836,46908               | <b>β-Lg</b> (f142-148) | ACE-inhibitory    | Mullally et al., (1997)   |
| DKALKALPM               | 985,56304               | <b>β-Lg</b> (f142-145) | ACE-inhibitory    | Murakami et al., (2004)   |
| DAQSAPLRVY              | 1118.572 <mark>.</mark> | <b>β-Lg</b> (f49-58)   | ACE-inhibitory    | Tavares et al., (2011)  |
| LVRTPEVDDEALEK          | 1612,8308               | <b>β-Lg</b> (f141-151) | DPP-IV Inhibitory | Silveira et al., (2013)   |
| LVR <b>TPEVDDEALEK</b>  | 1612,8308               | <b>β-Lg</b> (f141-151) | Antimicrobial     | Demers-Mathieu et al., (2013)   |

# BUFFALO DAIRY PRODUCTS IN THE WORLD

# **Antonio BORGHESE**



General Secretary International Buffalo Federation, Former Director Animal Production Research Institute, Monterotondo – Rome, Italy

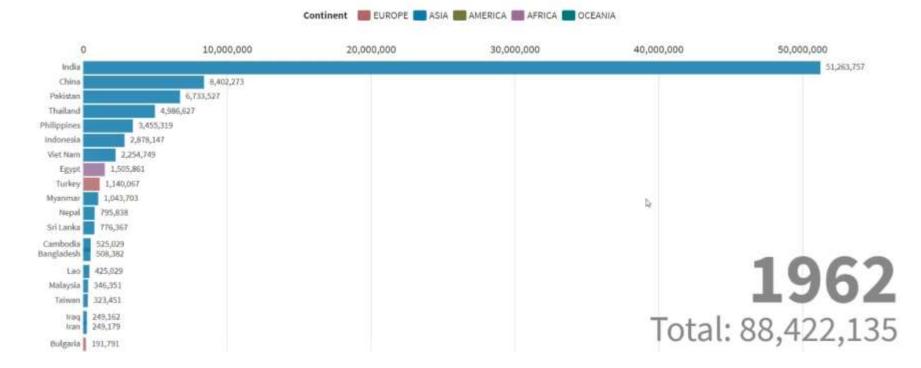
> Corresponding e-mail: <u>antonio.borghese@email.it</u> – <u>antonioborghese32@gmail.com</u>



# TARGET

Bubalus bubalis has a pivotal role in protein supplying and in the global economy, respecting sustainability

#### World Buffalo population



Buffalo population and production in the most representative countries (FAO, 2022)

|             | Head (mil)  | Milk (T)    |
|-------------|-------------|-------------|
| Asia        |             |             |
| India       | 111.856.246 | 94.383.692  |
| Pakistan    | 43.676.000  | 36.445.000  |
| China       | 26.876.707  | 2.905.807   |
| Nepal       | 5.132.931   | 1.419.412   |
| Philippines | 2.774.471   | 36.444      |
| Viet Nam    | 2.231.600   | 26.622      |
| Myanmar     | 2.000.000   | 176.137     |
| Bangladesh  | 1.508.000   | 35.714      |
| Laos        | 1.208.634   |             |
| Indonesia   | 1.170.209   | 91.426      |
| Thailand    | 735.248     | 6.674       |
| Africa      |             |             |
| Egypt       | 3.445.172   | 1.508.000   |
| Americas    |             |             |
| Brazil      | 3.000.000   |             |
| Venezuela   | 2.800.000   | A           |
| Colombia    | 485141      | 23          |
| Europe      |             |             |
| Italy       | 416000      | 257.460     |
| Bulgaria    | 20320       |             |
| Romania     | 17900       |             |
| Germany     | 11680       |             |
| Oceania     |             |             |
| Australia   | 180.000     |             |
| World       | 205141830   | 137.761.643 |

- Buffalo milk is utilized mostly as a drink following defattening, except in Italy.
- Different types of cheese are produced according to local traditions and processing techniques.

Classification per water content 1. **Soft cheese** (water content > 45 percent): Karish, Mish and Domiati in Egypt; Madhfor in Iraq; Mozzarella in Italy; Alghab in Syria; Vladeasa in Romania.

2. **Semi-hard cheese** (water content 40-45 percent): Beyaz peyneri in Turkey.

3. Hard cheese (water content < 40 percent): Braila in Romania; Rahss in Egypt; White brine in Bulgaria; Akkawi in Syria.

# Types of coagulation

**Spontaneous acidification** (Domiati, Karish, Mish, in Egypt; Madhfor in Iraq; Alghab in Syria).

Adding starters, i.e. lactic bacteria cultures (Vladeasa, Beyaz peyneri) or natural whey cultures (Mozzarella).

Starters are also used in cheeses where enzymatic coagulation prevails, to favour rennet activity and following the processing stages (White brine cheese, Fresh cheese of Iraq, Braila).

# Type of preserving

Some cheeses are consumed **FRESH**, i.e. only a few days after processing (Karish, Fresh cheese of Iraq, Mozzarella and Ricotta in Italy, Alghab )

Brine, to guarantee excellent conservation without expensive investments, such as refrigerators.

In Mish, acid buttermilk, skimmed milk and whey are added to the brine. In Domiati and Akkawi cheese, salt is added to the milk before processing

Ripening

# dairy products processing

**FAT-RICH MILK PRODUCTS:** butter from buffalo cream displayed more stability than cow cream, due to the more solid fat. The texture of buffalo **ghee** is better than cow ghee due to its bigger grain size

HEAT-DESICCATED MILK PRODUCTS: Buffalo milk is preferred in India for heatconcentrated milk products like khoa, rabri, kheer and basundi.

**HEAT-ACID COAGULATED MILK PRODUCTS**: the quality of buffalo **paneer** is superior to the cow milk one.

**FERMENTED MILK PRODUCTS**: the buffalo superior body and texture of **dahi** could be attributed to the higher total solids content, especially fat and protein; **yoghurt** from buffalo milk is produced in many countries of Asia and in Europe.



# dairy products processing

**CREAMS:** as **queshta mosakhana** in Egypt, **Gaymar** in Iraq, and **quishada** in Syria are obtained through different processing.

FROZEN MILK PRODUCTS: as ice creams.

**DEHYDRATED MILK PRODUCTS**: buffalo milk is more appropriate for **tea and coffee whitener powders** production because milk and cream are intrinsically whiter and more viscous

**RICOTTA and ALKARISH:** produced from cheese whey and exploit the proteins lost in the whey (very rich in sulphurate amino-acids), are not produced in other countries



Gaymar

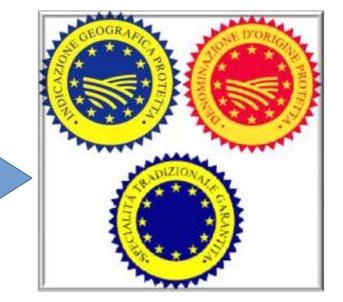


queshta mosakhana



Ice creams

## **QUALITY LABELS**



Italy is the European country with the largest number of food products with designation of origin and geographical indication recognized by the European Union.

The EU's system of geographical indications favors the production system and the local economy protecting the environment, because the indissoluble bond with the land of origin requires the preservation of ecosystems and biodiversity.

It supports social cohesion of the entire community.

325 DOP, IGP, STG products 527 DOCG, DOC, IGT wines





# .....more than one product, is a cultural value



Bocconcini



Mozzarella



Aversana



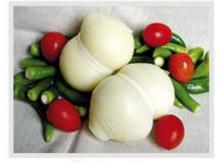
Treccia



Nodini



Provola Affumicata



Caciocavallo



Ricotta

# Other European countries



Germany: good quality dairy products together with cosmetics



Bulgaria Istituto SHUMEN: white brine cheese, **yoghurt, kiselo miyako,** where lactic acid is fermented by *Lactobacillus bulgaricus* and by *Streptococcus thermophilous* 



Macedonia: simple cheese in rural villages

# Other European countries



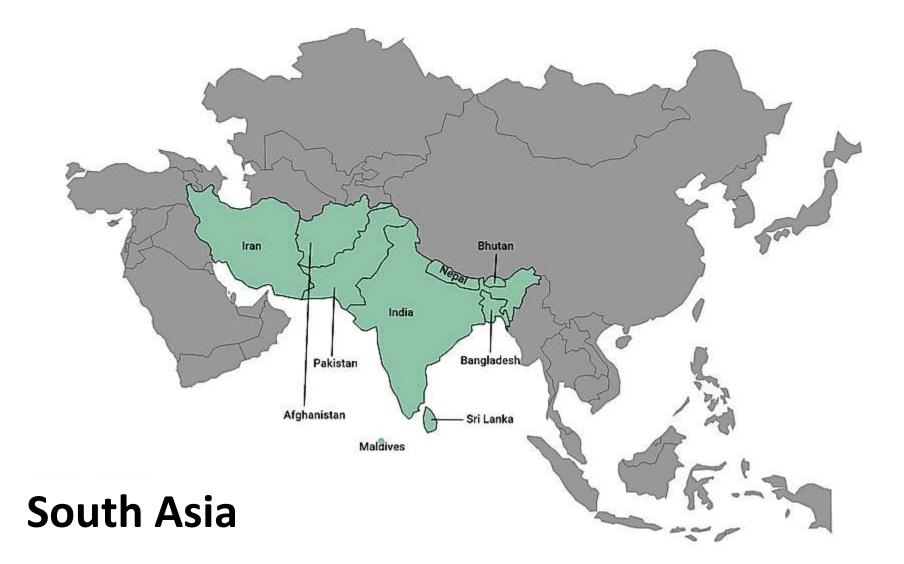
Greece: good quality yoghurt like Bulgaria



Hungary: Hungarian buffalo live free in National Park, a farm was founded in Mezotur with Mediterranean Italian to provide cheeses like Italy to the rich market in Budapest



Romania: Transilvania buffaloes live on pastures, a farm producing cheeses like Italy was founded by Transilvania Lactate to serve the market in Brasov



**India** is the first country in the world for the number of buffalo (**111 millions**, 54% of total population) and milk production (**94 millions tonnes**).

India possesses the best River Dairy Breeds of Asia as Murrah, Nili-Ravi, Surti, Jaffarabadi

Pakistan is the second most important country with **42** millions head and **36 million tons of** milk production South asian products

The most quantity of milk is used for **direct consumption after skimming**, fat is used to produce **butter**, **ghee and cream**. **Dry milk**, **condensed milk**, **milk replacers** are very used in national market and for export, as different industrial utilizations.

**Paneer** is a cottage cheese, used in several vegetarian curry dishes in India and other countries.

The **National Dairy Research Institute of India** formulated different new functional dairy products such as probiotic cheese, sports drinks, low-cholesterol ghee, ice cream, and burfi for diabetics.



#### China and Southeast Asia

The buffalo in this area is **Swamp type**, with a total of 18 local breeds.

Guangxi Buffalo Research Institute in China, the Philippines Carabao Center has successfully developed buffalo milk cheeses and created a food market.

In Indonesia, The Philippines, Vietnam and Thailand in the past years, there was a decreasing trend in the swamp buffalo population.



#### Papangan buffalo in the marshes (Borghese photo)







Milk putting in bamboo-cane





PASEU



Dairy buffalo is important also in the Near East Asian Countries, as Turkey, Iraq and Iran.

The most appreciated products in Iran and Iraq are: yoghurt, Ayran, fresh cream, fresh cheese, butter, ice cream, rice pudding, churned yoghurt, dried whey, ghee, sweet and cake.

Skimmed milk is used also for direct consumption.

#### WorldAtlas



#### Mesopotamian buffalo in Tigris river (Jabbar Al Saedy photo)



Sweet, curd and fresh cheeses

(Khalid Al-Fartosi photo)

#### Buffalo Dairy products in the Americas



Today there is great enthusiasm about buffalo in America, particularly among buffalo breeders and livestock associations.



Buffalo numbers have significantly increased to about 6.7 million head (Patino, 2023), as buffalo is not bred only for meat purposes as in the past in extensive system.



In the recent years the emerging request of cheese market produced a developing interesting for milk purposes, similarly to Italian feeding style.



in South America, the buffalo dairy market is similar to the Italian one, with mozzarella, yogurt, cream, butter, and other cheeses.



#### Buffalo management in the humid savannah

#### Conclusions



Buffalo Mozzarella according to the Italian style is wellrenowned and spread in all the countries



Buffalo milk is prevalently used fresh directly after being defattened. The fat is used to produce ghee for cooking. Often the milk is dehydrated to be conserved as milk powder.



Curd is used mixed to vegetables, cheese is not commonly used. The problem is the preservation in hot climate, particularly in the villages with no electricity.



# Buffalo dairy supply chain, present and future scenarios linked to new sustainability challenges

#### Antonio Limone





DATI ECONOMICI 2022 Campania DOP IGP

*Ismea* Qualivita rapporto *2023* 





#### 

14% peso DOP IGP su agroalimentare

58 prodotti

+9,4% su 2021

8° regione per impatto

9.082 operatori

TERRITORI

FILIERE

Caserta 321 milioni €

Napoli 296 milioni €

Salerno 188 milioni€

Benevento 58 milioni € Formaggi 54% Paste alimentari

30%

Vino 12%

Ortofrutticoli 4%

### IsmeaQualivita rapporto 2023

co Sperim

Ż

TAB.

07

Toopt

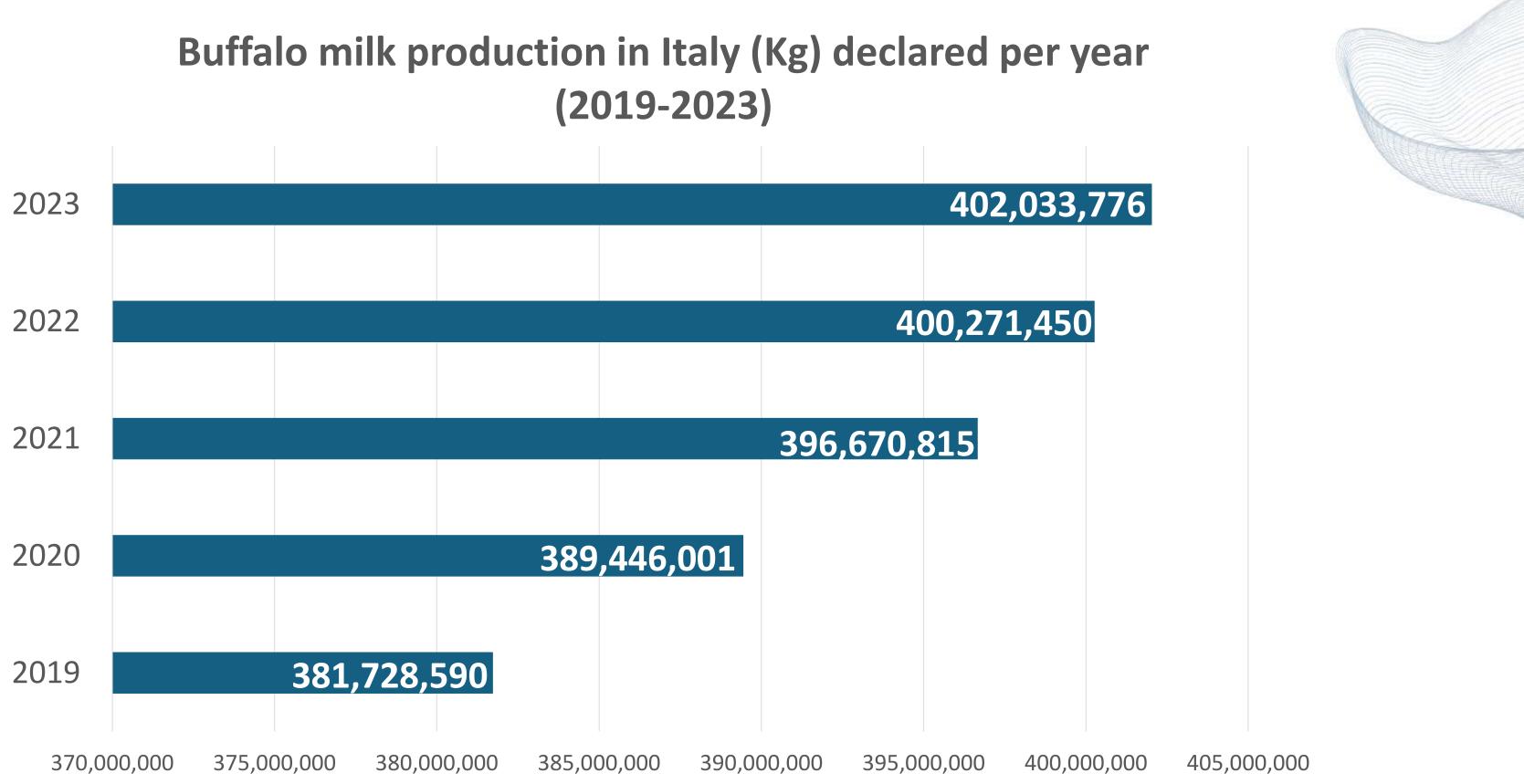
## Formaggi DOP IGP STG

| PRODUZIONE CERTIFIC<br>(tonneliste) |         | TIFICATA | VALORE ALLA PRODUZIONE<br>(millioni di euro) |       |       | VALORE AL CONSUMO<br>(milioni di euro)   |       |       | VALORE ALL'EXPORT<br>(millioni di euro)   |      |       |           |
|-------------------------------------|---------|----------|--|-------|-------|--|-------|-------|---|------|-------|-----------|
| Prodotto                            | 2021    | 2022     | Vier 22/21                                   | 2021  | 2022  | Var 22/21  | 2021  | 2022  | Var 22/21   | 2021 | 2022  | Var 22/21 |
| Grana Padano DOP                    | 203.290 | 202.051  | -0,6%  | 1.460 | 1.734 | +18,8%   | 2.517 | 2.722 | *B,3%   | 922  | 1.006 | +9,2%     |
| Parmigiano Reggiano DOP             | 155.277 | 161.520  | +4,0%  | 1.607 | 1.720 | +7,0%  | 2.756 | 2.949 | +7,0%   | 845  | 905   | +7,0%     |
| Mozzarella di Bufala Campana DOP    | 54.039  | 55.815   | +3,3%  | 459   | 502   | +9,4%  | 838   | 893   | +6,6%   | 163  | 217   | +33,7%    |
| Pecorino Romano DOP                 | 34.303  | 32.602   | -5,0%  | 302   | 378   | +25,1%   | 465   | 595   | +27,8%  | 216  | 271   | +25,8%    |
|                                     |         |          |  |       |       | and the second design of the s |       |       | the second se |      |       |           |

|                                     | Produzione<br>Certificata<br>(tonnellate) |        | Valore Alla<br>Produzione<br>(milioni di euro) |      | Valore al<br>consumo<br>(milioni di euro) |              | Valore<br>all' Export<br>(milioni di euro) |      |              |      |      |              |
|-------------------------------------|---|--------|--|------|---|--------------|--|------|--------------|------|------|--------------|
|                                     | 2021                                      | 2022   | Var<br>22/21                                   | 2021 | 2022                                      | Var<br>22/21 | 2021                                       | 2022 | Var<br>22/21 | 2021 | 2022 | Var<br>22/21 |
| Mozzarella di Bufala<br>Campana DOP | 54.039                                    | 55.815 | +3,3%  | 459  | 502                                       | +9,4%        | 838  | 893  | +6,6%        | 163  | 217  | +33,7%       |
|                                     | ALIVITA                                   |        |  |      | 24  |              |  |      | /smen        |      | Ā    |              |



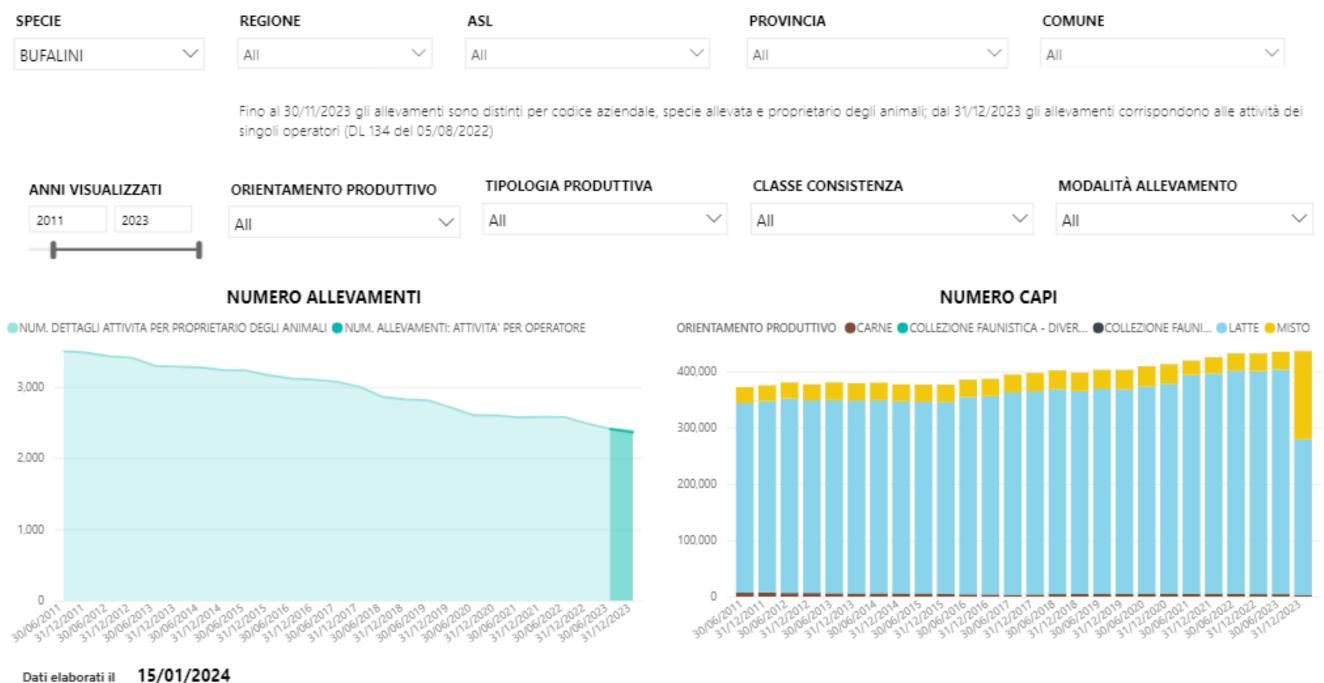
## (2019-2023)





器 Sistema Informativo Veterinario - Statistiche Variazioni patrimonio nel tempo Densità allevamenti e capi bovini e bufalini Consistenza allevamenti e capi per orientamento produtt

#### VARIAZIONE PATRIMONIO BOVINO E BUFALINO NEL TEMPO



| ivo | Consistenza alle | evamenti e capi per cla | sse di consistenza | Consis |
|-----|------------------|-------------------------|--------------------|--------|
|     |                  |                         |                    |        |
|     |                  | COMUNE                  |                    |        |
|     | $\sim$           | All                     | $\sim$             |        |
|     |                  |                         |                    |        |



### Variazione patrimonio Bufalino Campo dal 2013 al 2023

| Ø  |    |   | À |
|----|----|---|---|
| S. | んら | 2 |   |
| -  | S. | 0 |   |

Sistema Informativo Veterinario - Statistiche

| lini | Variazioni patrimonio nel tempo | Densità allevamenti e capi bovini e bufalini | Consistenza allevamenti e capi per orientamento produttivo | Consistenza allevamenti e capi per classe di consistenza | Cor |
|------|---------------------------------|--|--|--|-----|
|------|---------------------------------|--|--|--|-----|

#### VARIAZIONE PATRIMONIO BOVINO E BUFALINO NEL TEMPO

| SPECIE<br>BUFALINI ~  | REGIONE<br>CAMPANIA $\checkmark$   | ASL                                    | $\sim$   | All   | All  | $\sim$              | Numer |
|---|--|--|--|---|--|---------------------|-------|
|   | Fino al 30/06/2023 gli allevamenti<br>singoli operatori (DL 134 del 05/08  |  | specie alle  | vata e proprietario degli animali; dal 31/12/2023 ;   | gli allevamenti corrispondono alle a   | attività dei        |       |
| ANNI VISUALIZZATI   | ORIENTAMENTO PRODUTTIVO  | TIPOLOGIA PRODUTTIVA                   |  | CLASSE CONSISTENZA  | MODALITÀ ALLEVAMENTO   |                     |       |
| 2013 2023   | All 🗸  | All                                    | $\sim$   | All   | All  | $\sim$              |       |
| NUM. DETTAGLI ATTIVITA PER PROPRI      1,500      1,000   | NUMERO ALLEVAMENTI   | ATTIVITA' PER OPERATORE                | ORIENTAM<br>300,000 -<br>200,000 -   |   |  |                     | Latt  |
| 500<br>0<br>30/06/01 <sup>3</sup> 201 <sup>3</sup> 201 <sup>4</sup> 201 <sup>4</sup> 201 <sup>4</sup> 201 <sup>5</sup> 201 <sup>5</sup><br>30/06/01 <sup>3</sup> 201 <sup>6</sup> 211 <sup>12</sup> 201 <sup>6</sup> 211 <sup>12</sup> 201 <sup>6</sup> 2 | 016/2016/2011/2011/2018/2018/2018/2018<br>11-2016/311-2016/311-2016/2018/2018/2018<br>2016/311-2016/311-2016/2018/2018/2018/2018/2018/2018/2018/2018 | 200 1022 1022 1022 1022 1022 1022 1022 | 100,000 -<br>- 0<br>-<br>- 0<br>-<br>- 0<br>-<br>- 0<br>- 0<br>-<br>- 0<br>- 0 | 1 <sup>3</sup> 20 <sup>13</sup> 20 <sup>14</sup> 20 <sup>14</sup> 20 <sup>15</sup> 20 <sup>15</sup> 20 <sup>16</sup> 20 <sup>16</sup> 20 <sup>16</sup> 20 <sup>11</sup> 20 <sup>17</sup> 20 <sup>16</sup> 20 <sup>18</sup> 20 <sup>17</sup> 20 <sup>16</sup> 20 <sup>18</sup> 20 <sup>17</sup> 20 <sup>16</sup> 20 <sup>18</sup> 20 | 6/20 <sup>19</sup> /201 <sup>9</sup> /2020/2020<br>6/20172/2019/2020/2020/2020/2020/2020<br>31/12/2019/31/12/2019/2012/2019/2019/2019/2019/2019/ | 18013<br>31 N2/2023 |       |
| Dati elaborati il 15/01/20  | 24 I dati al 31/12/2023 sono stat  | i aggiornati il 19/02/2023, per        | corregge   | re gli orientamenti   |  |                     |       |

| ano                       |                        |  |
|---------------------------|------------------------|--|
| otale Allevamenti         | • <mark>-21,62%</mark> |  |
| o totale capi<br>Sufalini | • <mark>+8,37%</mark>  |  |
| e +Misti                  | • <mark>+8,33%</mark>  |  |
| Carne                     | • <mark>+17,16%</mark> |  |
|                           |                        |  |

Numero to





Variazioni patrimonio nel tempo

Densità allevamenti e capi bovini e bufalini

Consistenza allevamenti e capi per orientamento produttivo

#### DENSITÀ ALLEVAMENTI E CAPI BOVINI E BUFALINI

| DATA RIFERIMENTO |        | SPECIE            |             | ORIENTAMENTO PRODUTTIVO |                        | TIPOLOGIA PRODUTTIVA |                    | CLASSE      |  |
|------------------|--------|-------------------|-------------|-------------------------|------------------------|----------------------|--------------------|-------------|--|
| 30/06/2024       | $\sim$ | BUFALINI          | $\sim$      | All                     | $\sim$                 | All                  | $\sim$             | All         |  |
| REGIONE          |        | Fino al 30/06/202 | 3 gli allev | amenti sono distinti p  | er codice aziendale, s | pecie allevata e p   | proprietario degli | animali; da |  |

CAMPANIA

Fino al 30/06/2023 gli allevamenti sono distinti per codice aziendale, specie allevata e proprietario degli animali; dal 31/12/2023 gli allevamenti corrispondono alle attività dei singoli operatori (DL 134 del 05/08/2022)

| REGIONE   | NUMERO<br>ALLEVAMENTI | NUMERO CAPI |  |
|-----------|-----------------------|-------------|--|
| CAMPANIA  | 1,182                 | 307,297     |  |
| AVELLINO  | 8                     | 345         |  |
| BENEVENTO | 19                    | 2,429       |  |
| CASERTA   | 740                   | 186,109     |  |
| NAPOLI    | 16                    | 4,342       |  |
| SALERNO   | 399                   | 114,072     |  |
| Total     | 1,182                 | 307,297     |  |
|           |                       |             |  |

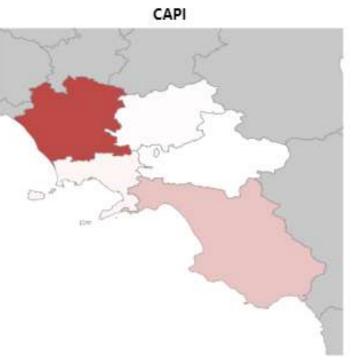


Densità allevamenti CE 0,2804(n.allev per kmq N all 740 SA 0,0811(n.allev per kmq N all 399 Densità Capi CE 70,5124 (capi per kmq N capi 186,109 SA 23,1734 (capi per kmq N capi 114,072











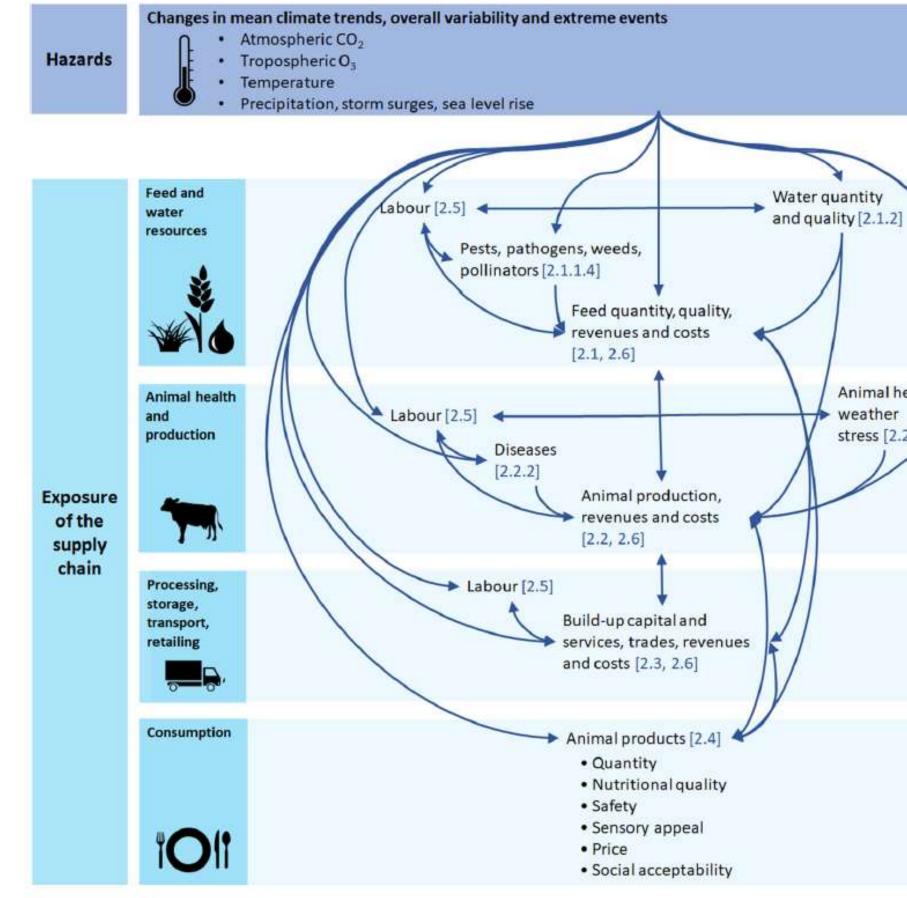


#### **Mediterranean Region**

Decrease in crop yields

- Temperature rise larger than european average
- Decrease in annual precipitation
- Decrease in annual river flow
- Increasing risk of biodiversity losse
- Increasing risk of desertification
- Increasing Water demand for agricolture
- Increasing risk of forest fire
- Increase in mortality from heatwaves
- Expansion of habitats for disease-carrying insects Decrease in hydropower potential





Godde et al 2021

Animal heat, weather stress [2.2.1]

Potential impacts on human, social, natural, physical and financial capitals



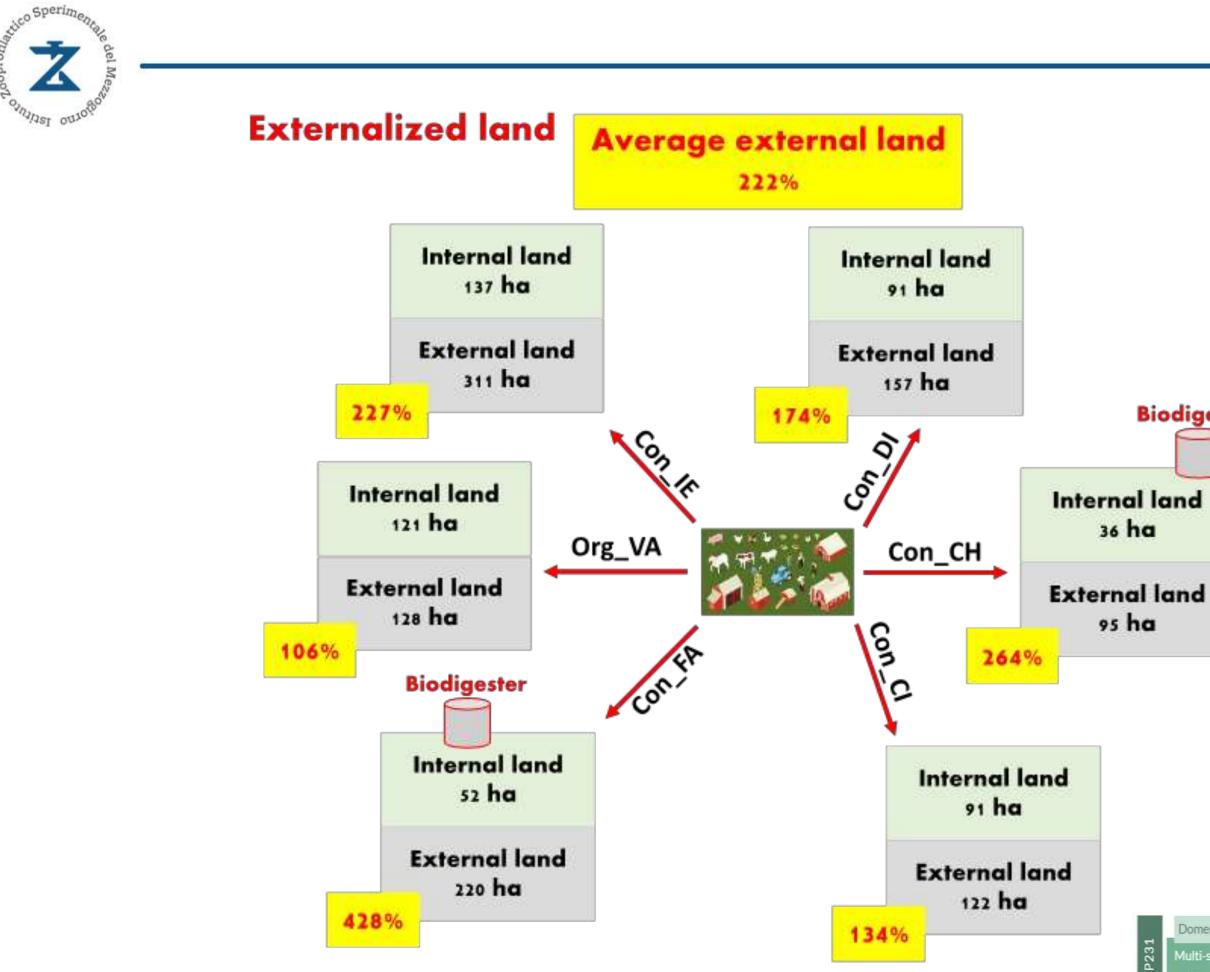


Figura 37 Contributo di utilizzo di terre intra-aziendali ed extra-aziendali per garantire il flusso di alimenti (foraggi e concentrati) alla mandria.

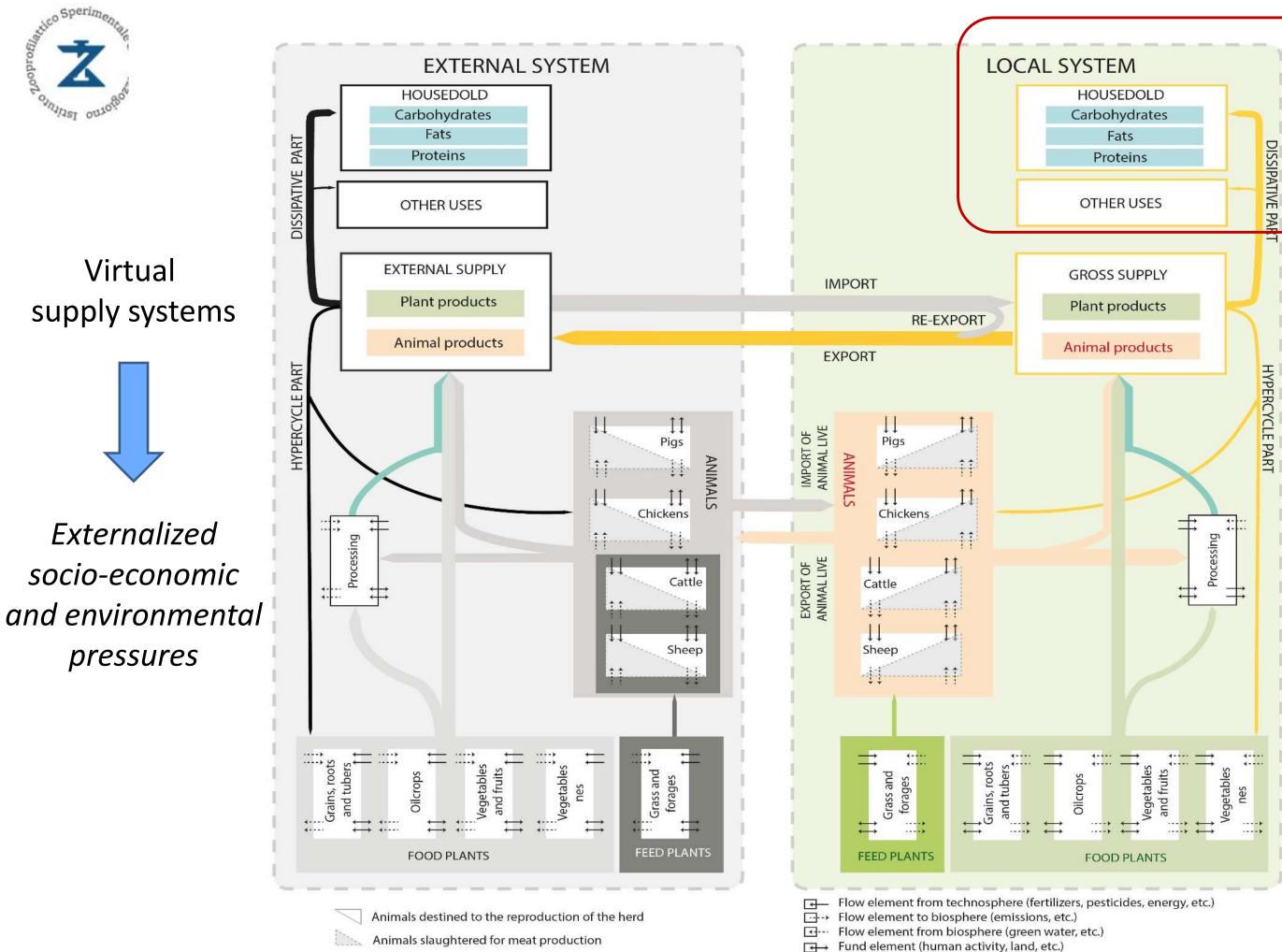


## **Biodigester**

Domenico Vecchio

**IZSME 08/18 RC** 

ulti-scale integrated accounting of buffalo farms' metabolism



#### The MAGIC tool-kit

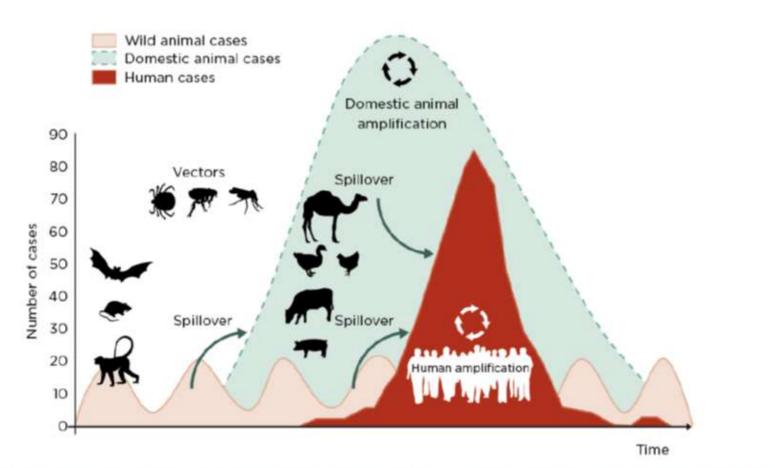
**Required supply** in the diet

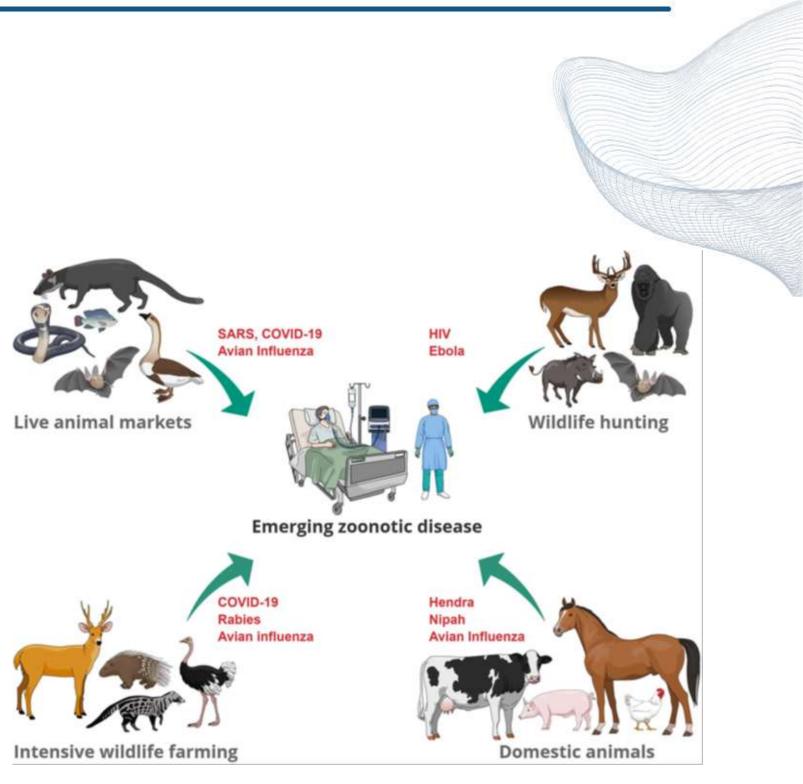
> Actual supply systems





#### The Drivers of Wildlife Interactions with Livestock Global drivers of wildlife–livestock interactions





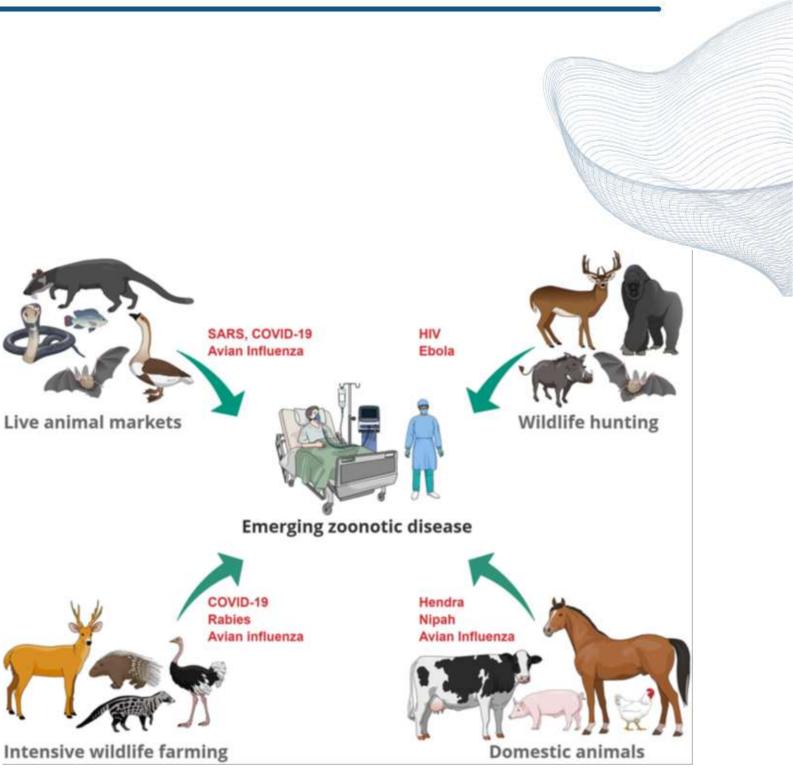
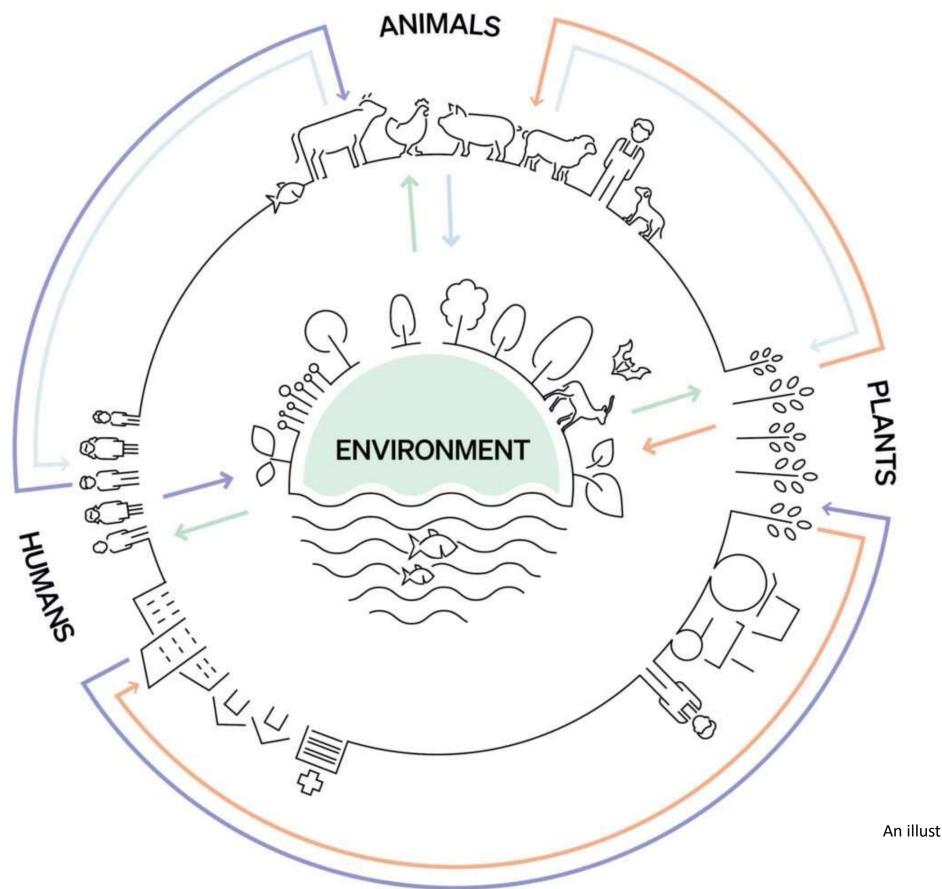


Figure 12: Transmission and amplification of zoonotic diseases. Transmission of a pathogen to people can occur directly from a wild animal or following an outbreak in livestock that amplifies the likelihood of transmissions to humans.

Source: Redrawn from Karesh, et al. (2012).

Magouras et al.2020. doi:10.3389/fvets.2020.582743

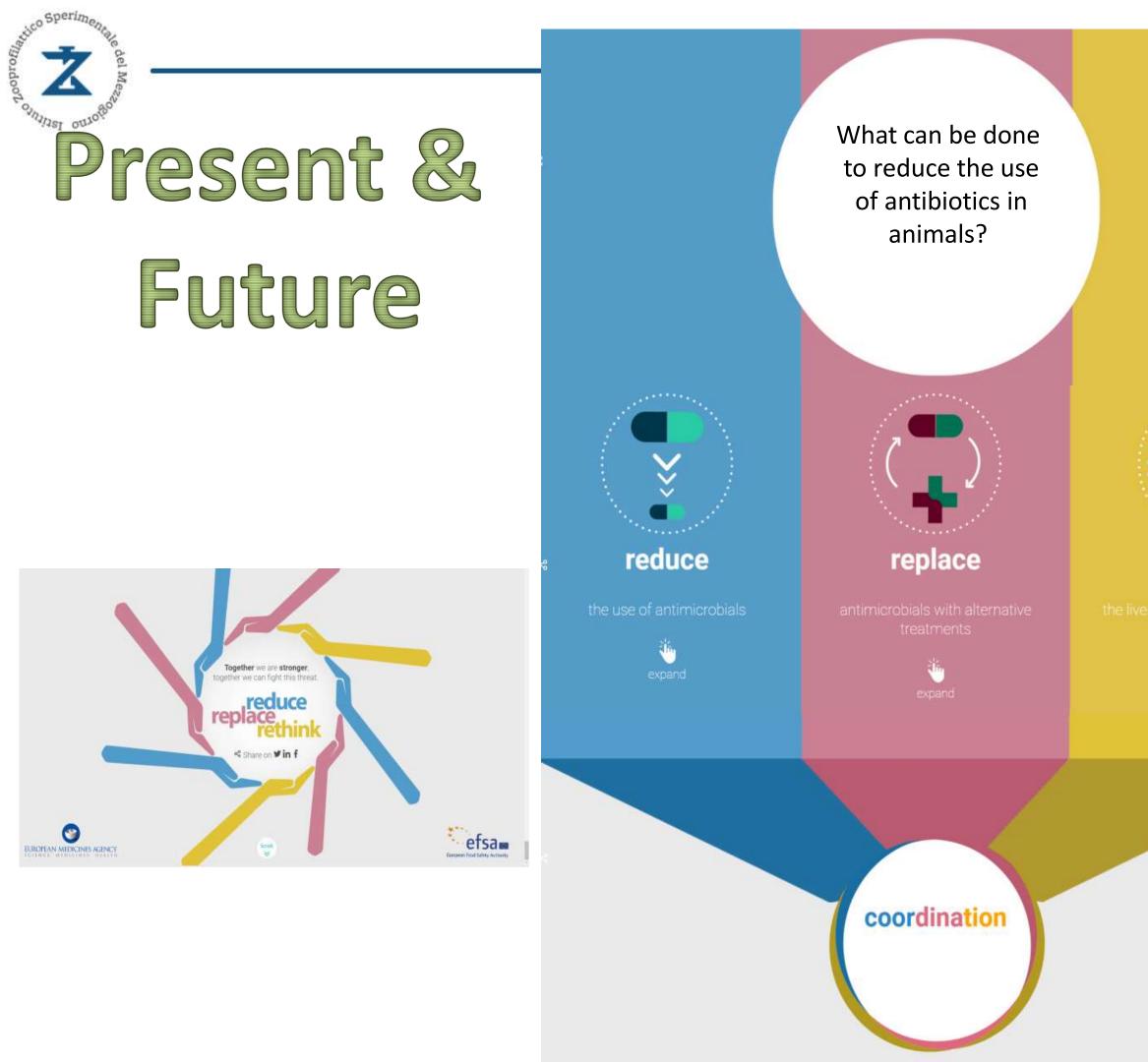




#### **One Health and Zoonoses**



An illustration of the ecology of vector-borne diseases like the 2018 ebolavirus and 2019 coronavirus (Covid-19) by the artist Olaf Hajek.



#### rethink



Improve prevention and control of diseases in animals

more info



Consider alternative farming system

⊕ more info



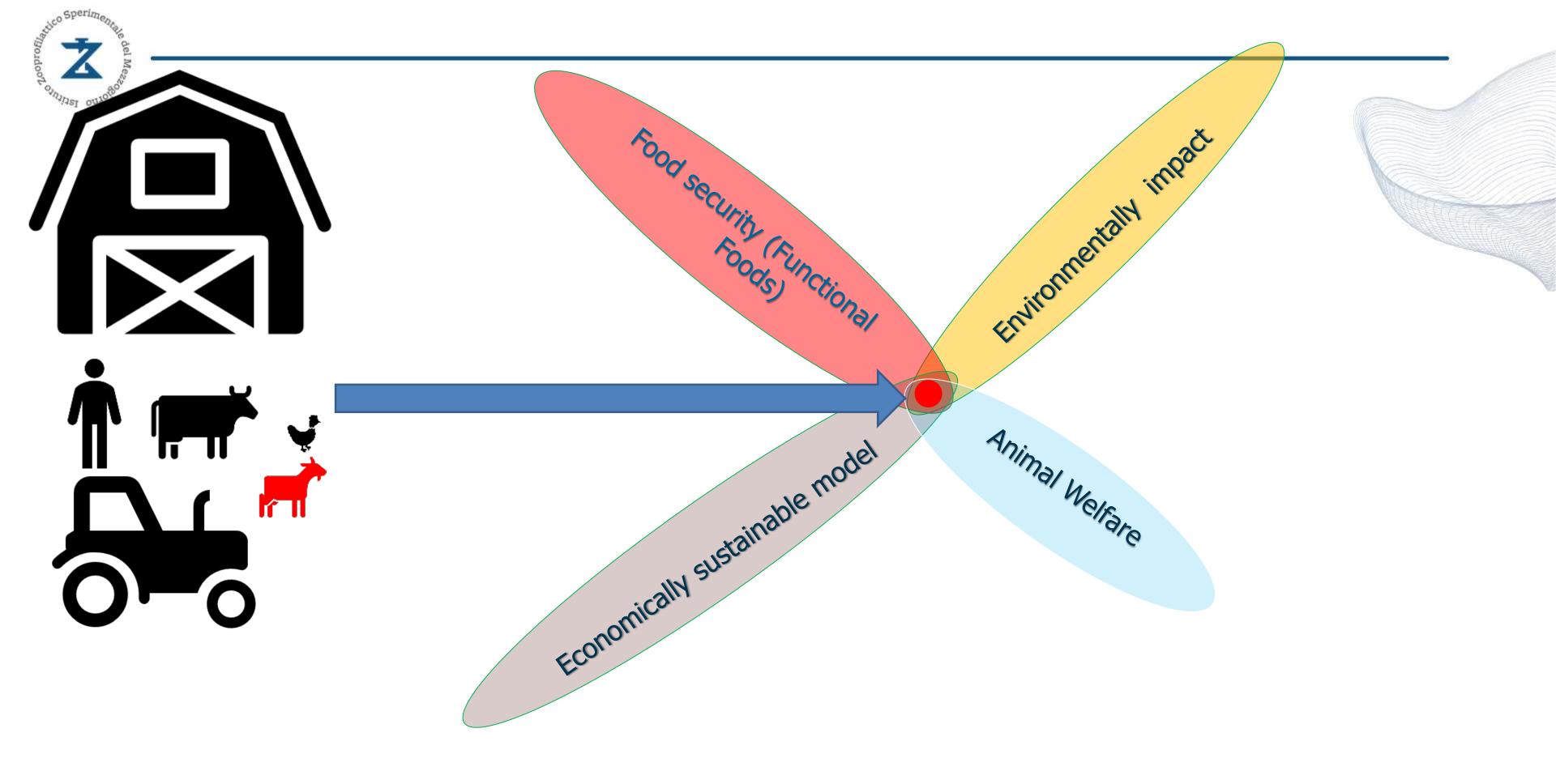
Offer education

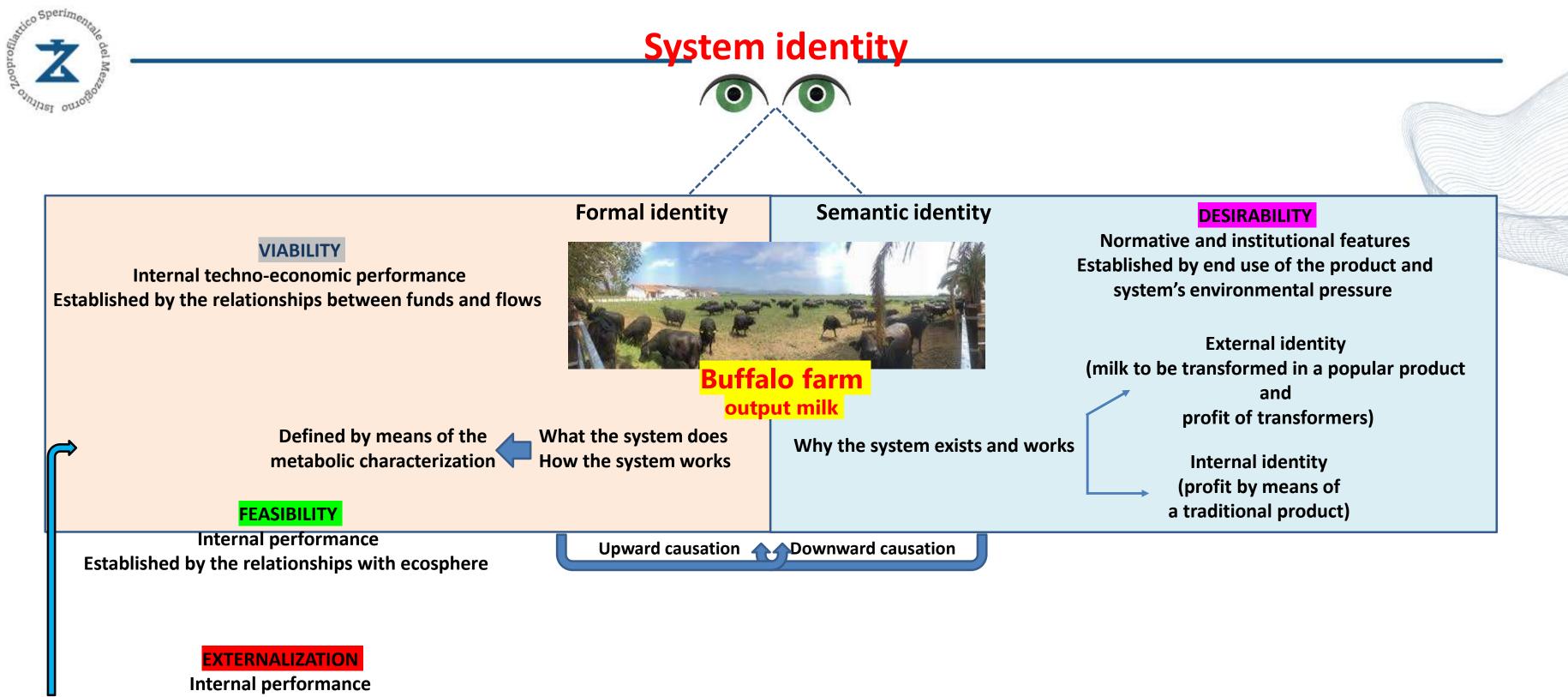
Education and awareness of AMR should be addressed to all levels of society but in particular to veterinariam and farmers.



tock production system







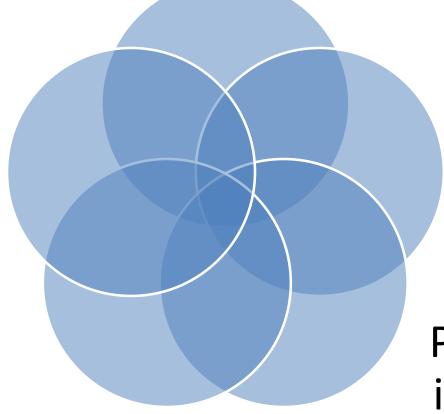
Established by the relationships with external funds and flows



| DEFCON 1 |
|----------|
| DEFCON 2 |
| DEFCON 3 |
| DEFCON 4 |
| DEFCON 5 |

Vulnerability of the system

REACTIVITY AND READINESS



Mitigating the risks



#### Monitoring the critical point

Preparing an intervention plan

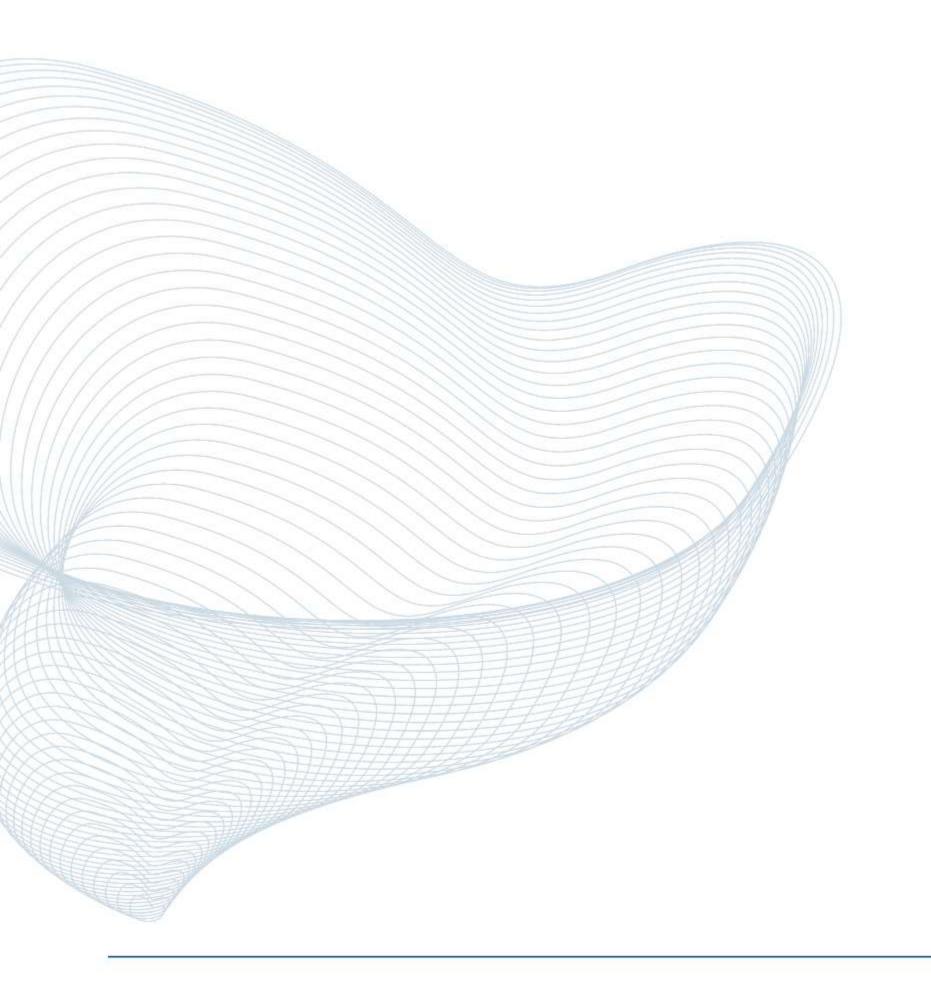


## Dotheywantthis? Desirability

Should we do this? Viability

### Can we do this? Feasibility

# `` The most valuable design



## THANKS

#### **Antonio Limone**



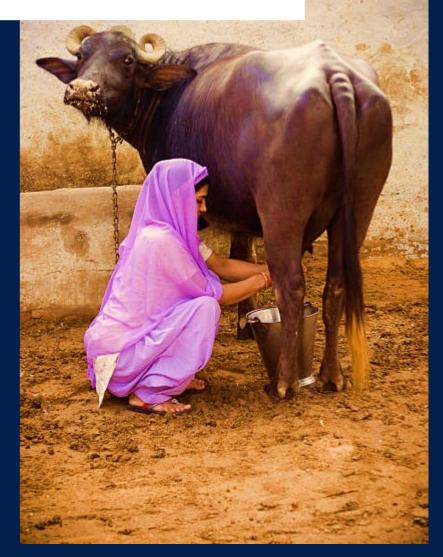
Istituto Zooprofilattico Sperimentale del Mezzogiorno <sub>Campania | Calabria</sub>



## First International Conference on Buffalo Mozzarella & Milk Products (BMMP)

**BUFFALO MILK – BLACK GOLD** Opportunities and Challenges in Production and Marketing

> Dr. R S Sodhi President, Indian Dairy Association (IDA) Chairperson, NIFTEM –T 25<sup>th</sup> SEPT. 2024, NAPLES,ITALY



#### Contents

- World Dairy Scenario and India
- India Building World Dairy Leadership
- India's Reasons for Success in Milk Production
- > Buffalo Milk: World and India
- Challenges of Buffalo Farming
- Buffalo Milk and Milk Products
- Growth in Buffalo Milk Production
- Sustainability
- > Opportunities & Challenges

#### World Dairy Scenario & India





#### World Milk Production (Year 2023)

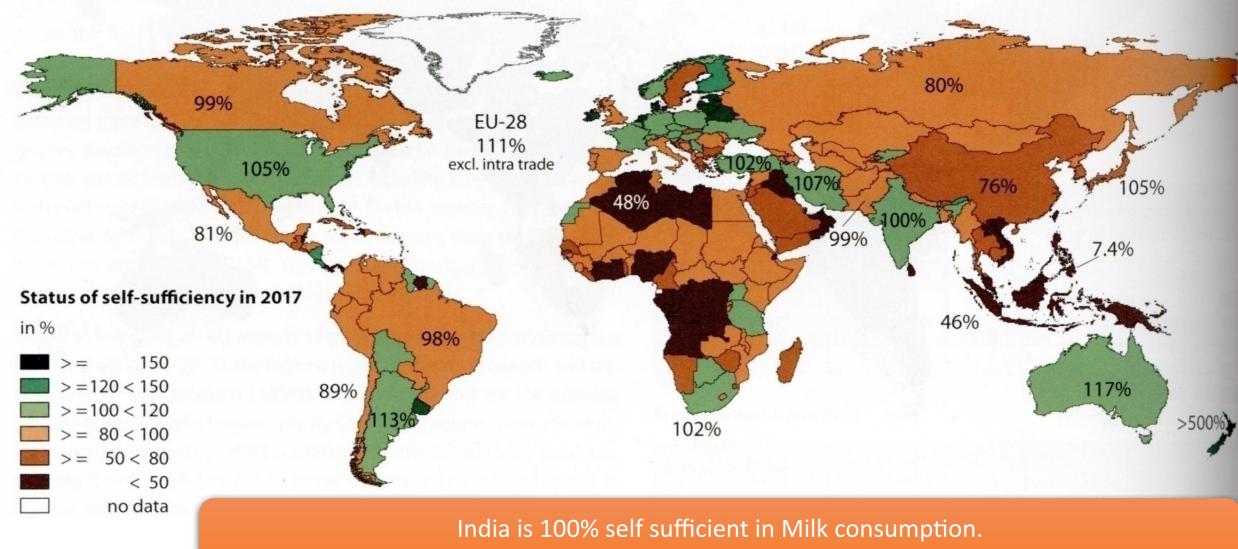


World milk production: **944 MMT** World milk production growth: **1.8% of last 20 years**  INDIA No. 1 Milk Producer: 231 MMT (24% of world)

#### **MMT: Million Metric Tonne**

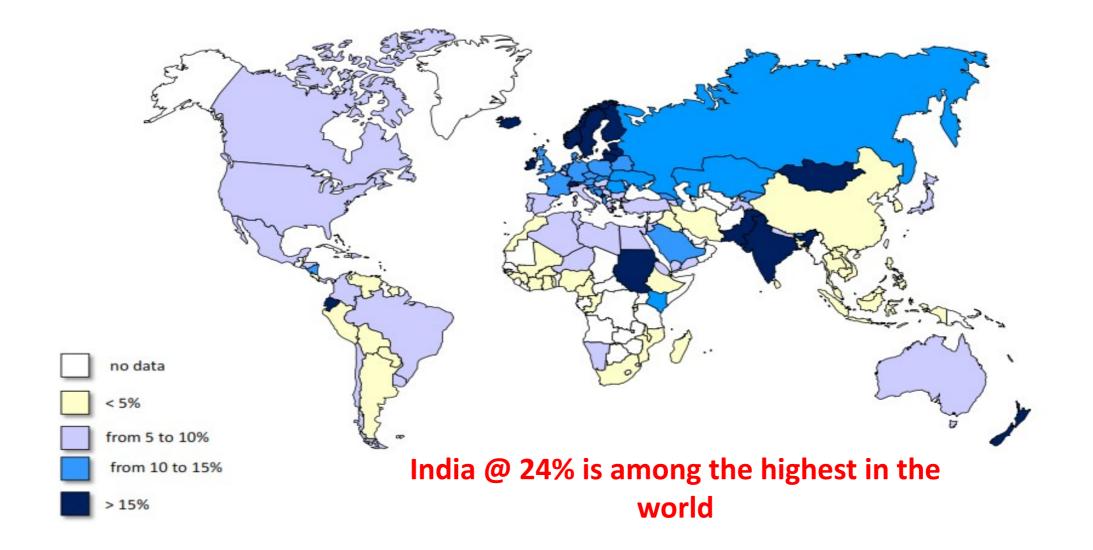
Source: FAO

#### **Status of Self Sufficiency for Milk**



• Huge potential for export in neighboring milk deficit nations

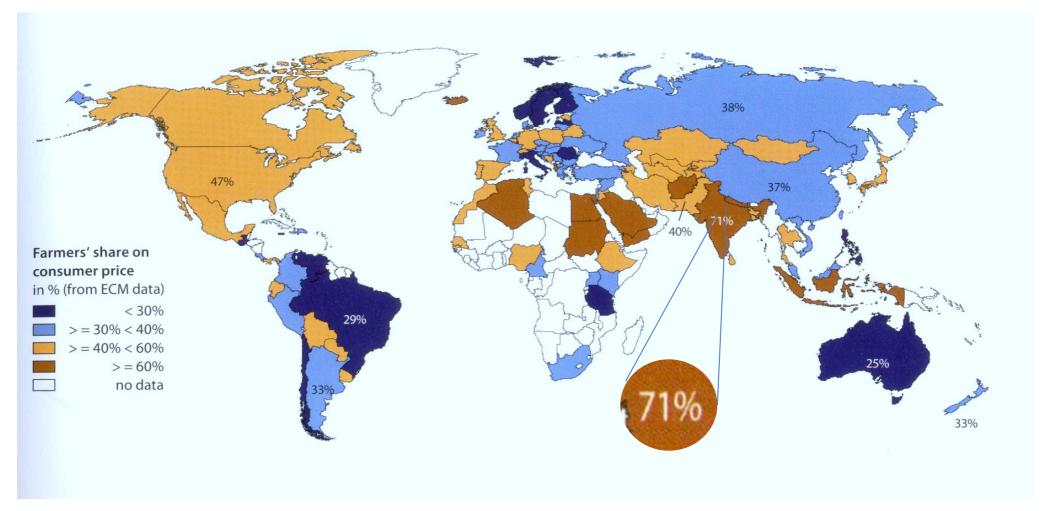
#### % Share of Dairy Sector in Total Agricultural Production



# India: Building World Dairy Leadership

# **Competitiveness of Indian dairy products**

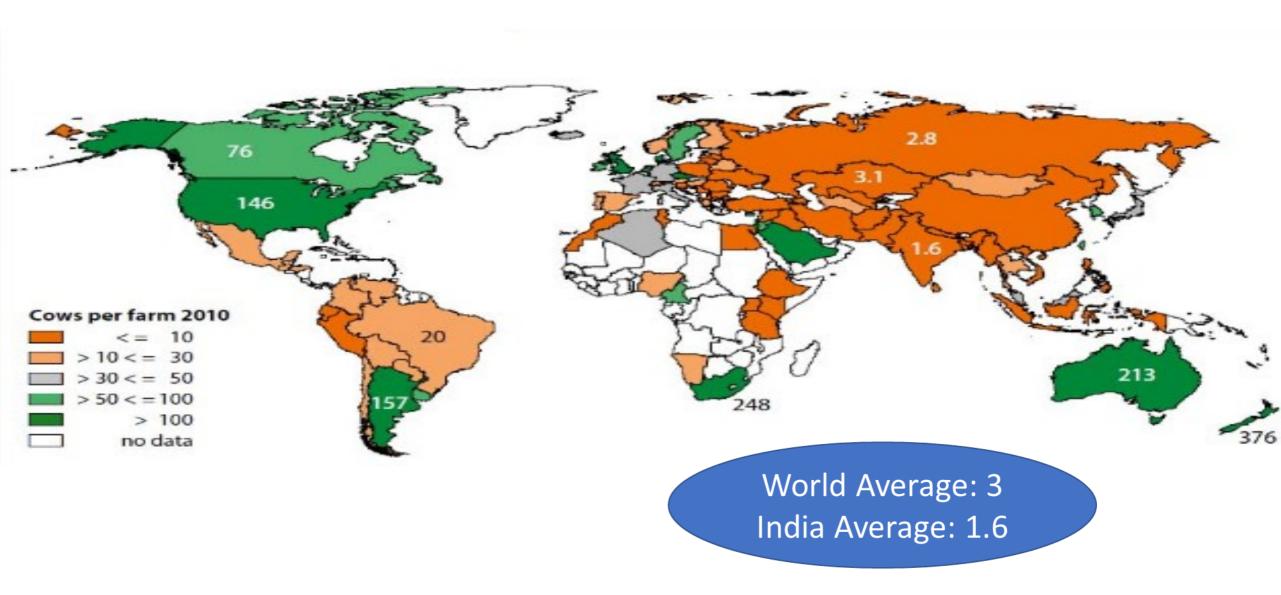
Farmer's share in consumer price



Indian Farmer has the highest share of consumer price

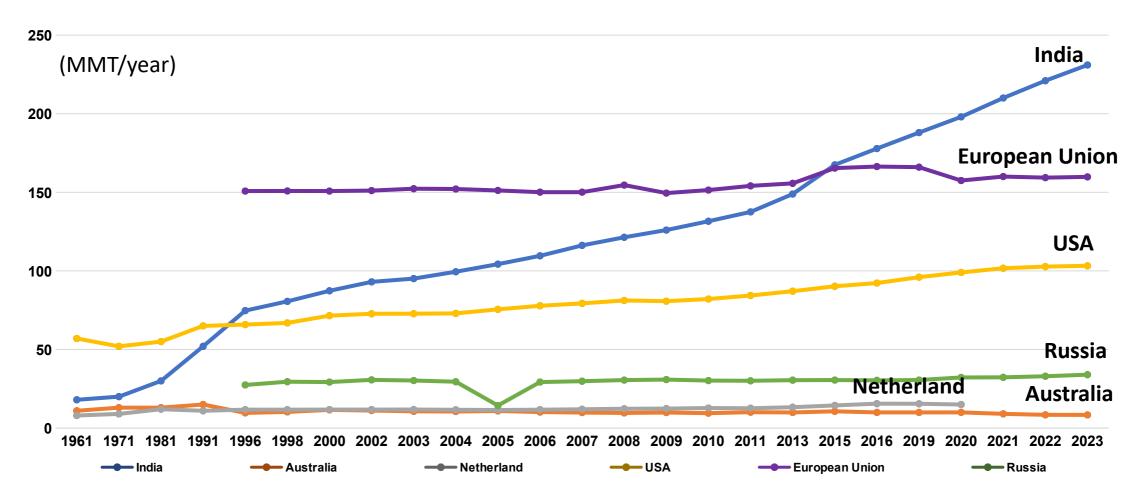
Source: IFCN

### World Average Farm Size



### **Comparative Milk Production Growth**

CAGR of last 15 years: India: 4.9% | USA: 2.3% | EU : 1.3% | AUS: 0.5% | Netherland: 2.7% | World: 1.3%



Milk production of India, Australia and Netherlands was almost similar in 1971

Source: FAO

Growth in Different Sectors and Major Products of Agriculture and their Share in Sectoral Growth between 1970-71 and 2020-21

| Item                            | Compound<br>Growth Rate % | Increase:<br>times | Share in total increase in agri & allied output |
|---------------------------------|---------------------------|--------------------|---|
| 1. All crops                    | 2.46                      | 3.38               | 52.59   |
| Cereals                         | 2.10                      | 2.83               | 13.23   |
| Paddy                           | 2.08                      | 2.80               | 6.66  |
| Wheat                           | 3.03                      | 4.46               | 5.54  |
| Maize                           | 2.81                      | 4.00               | 1.32  |
| Pulses                          | 1.68                      | 2.30               | 2.36  |
| Oilseeds                        | 2.42                      | 3.30               | 4.58  |
| Condiment & spices              | 4.20                      | 7.83               | 3.61  |
| Fruits total                    | 3.53                      | 5.66               | 10.64   |
| Vegetable total                 | 3.43                      | 5.41               | 6.08  |
| 2. Livestock                    | 4.37                      | 8.50               | 37.11   |
| Milk                            | 4.71                      | 9.98               | 25.26   |
| Poultry                         | 6.42                      | 22.45              | 6.30  |
| 3. Fishery                      | 5.02                      | 11.57              | 7.81  |
| 4. Forestry                     | 0.55                      | 1.31               | 2.49  |
| 5. Total Agriculture and allied | 2.70                      | 3.80               | 100.0   |
| 6. Human Population             | 1.84                      | 2.50               |   |
| 7. Female bovine population     | 1.50                      | 2.0                |   |

#### **Consumption Trends Dairy V/s Bread, Pulses & Sugar**

#### MILK > PULSES+BREAD+SUGAR

| Item Groups             | MPC   | E (Rs) | % Share in Total |       |  |
|-------------------------|-------|--------|------------------|-------|--|
| item droups             | Rural | Urban  | Rural            | Urban |  |
| Cereals & Substitute    | 185   | 235    | 4.91             | 3.64  |  |
| Pulses & Their Products | 76    | 90     | 2.01             | 1.39  |  |
| Sugar & Salt            | 35    | 39     | 0.93             | 0.6   |  |
| Roti+Dal+Chini          | 296   | 364    | 7.85             | 5.63  |  |
| Milk & Milk Products    | 314   | 466    | 8.33             | 7.22  |  |

## **Growth of Indian Dairy Industry**

| Year  | 1972 | 1997 | 2023 | 2030 | 2048 |
|---|------|------|------|------|------|
| Milk Production in MMT                            | 24   | 71   | 231  | 330  | 628  |
| % of World Milk Production                        | 6    | 14   | 24   | 30   | 45   |
| Per Capita Consumption<br>Gram Per Person Per Day | 110  | 214  | 466  | 550  | 852  |

700

600

500

**400** 

300

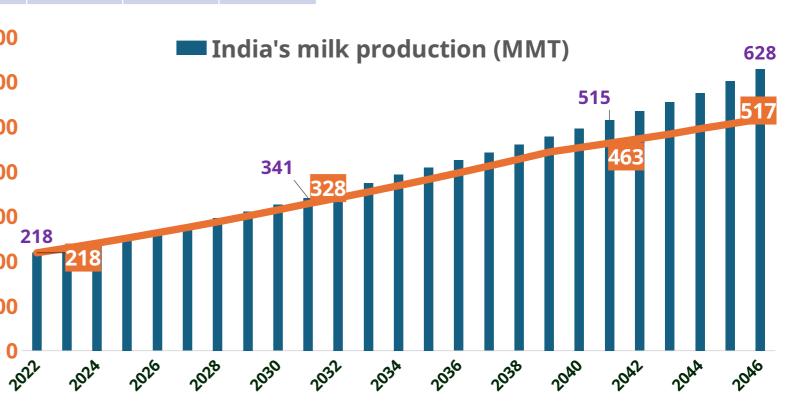
200

100

MMTs

#### By 2047, India will have export surplus of around 111 MMTs

In next 25 years, Milk Production of India will reach 628 MMTs, while demand for Milk and Dairy products will increase to 517 MTs



#### How did India succeed so spectacularly in Dairy Industry ?

## The Amul Model

- Establishment of a direct linkage between milk producers and consumers by eliminating middlemen
- Milk Producers (farmers) control procurement, processing and marketing
- Professional management

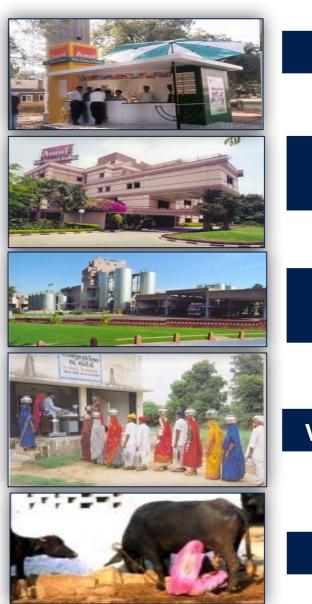
#### **INDIA**

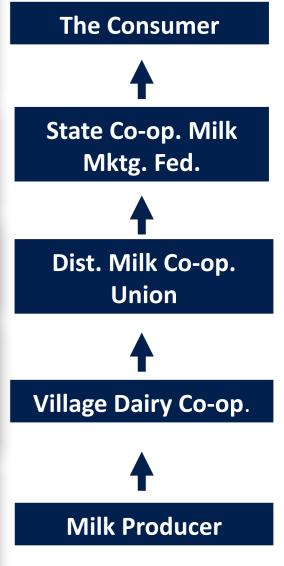
Indian dairy cooperatives ensure that their farmers get **70%-86%** of Consumers Rupee spent on Milk & Milk Products

#### **OTHER COUNTRIES**

In USA, producers get **38%** of consumers' money spent on milk

In UK, producers get only **36%** 

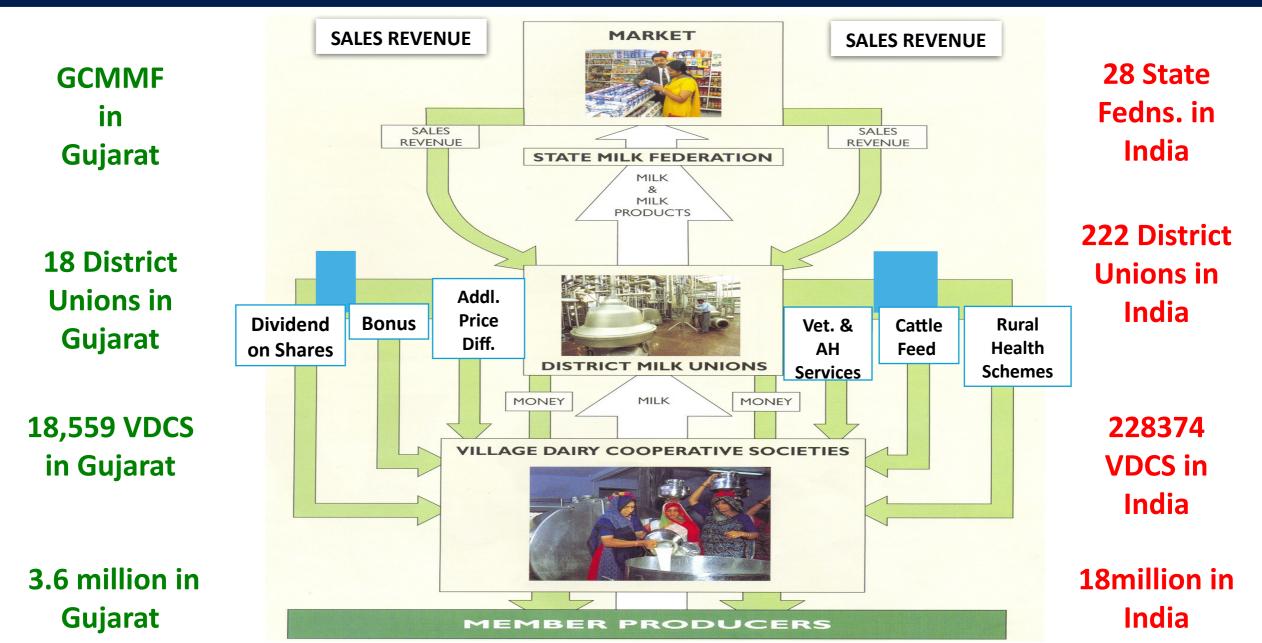




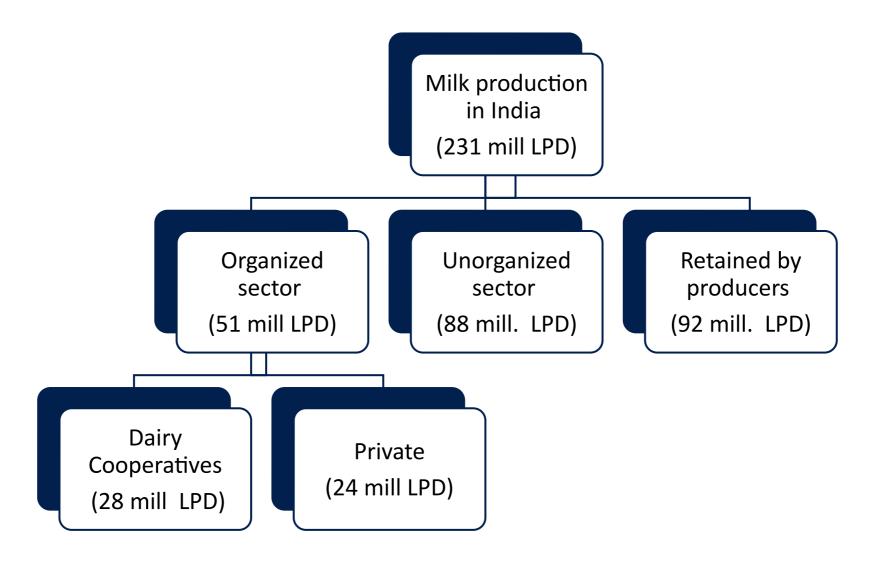
#### By harnessing the collective power of our 80 million Smallholder Dairy Ecosystem



# The Amul Model



#### **Milk Production in India**



### **Reasons for Success of White Revolution**

- Vision -1970 self-sufficiency or "Atma Nirbharta"
- > Farmer's owned very efficient supply chain
- Selfless dedicated leadership
- > Livelihood partial or major for 80 million families
- > Very detailed planning, meticulous execution and monitoring OF
- > Engaging best of the professionals
- > Long-term investment in infrastructure rather than subsidies
- > Emphasis on marketing and branding
- > Technology integration and innovation and digitalization

**BUFFALO MILK** 

# **World Buffalo Milk Production**

Ranking of countries with the highest buffalo milk production

| Country     |   | Pound            | % of Top 20 |
|-------------|---|------------------|-------------|
| India       | £ | 202,82,50,40,000 | 68.78       |
| Pakistan    | £ | 75,77,49,94,020  | 25.70       |
| China       | £ | 6,45,59,40,865   | 2.19        |
| Egypt       | £ | 5,49,94,24,590   | 1.19        |
| Nepal       | £ | 3,02,67,33,821   | 1.03        |
| Myanmar     | £ | 45,13,95,945     | 0.15        |
| Iran        | £ | 28,21,91,360     | 0.10        |
| Sri Lanka   | £ | 16,21,85,075     | 0.06        |
| Turkey      | £ | 17,49,16,755     | 0.06        |
| Indonesia   | £ | 18,84,37,690     | 0.06        |
| Bangladesh  | £ | 7,89,03,350      | 0.03        |
| Iraq        | £ | 7,93,24,432      | 0.03        |
| Vietman     | £ | 5,99,89,913      | 0.02        |
| Bulgaria    | £ | 2,91,00,984      | 0.01        |
| Romania     | £ | 3,19,66,900      | 0.01        |
| Netherlands | £ | 61,72,936        | 0.00        |
| Syria       | £ | 1,36,24,552      | 0.00        |
| Georgia     | £ | 1,40,61,066      | 0.00        |
| Malaysia    | £ | 1,69,55,732      | 0.00        |

India produces ~70% of the world's buffalo milk

Source: Discover Food

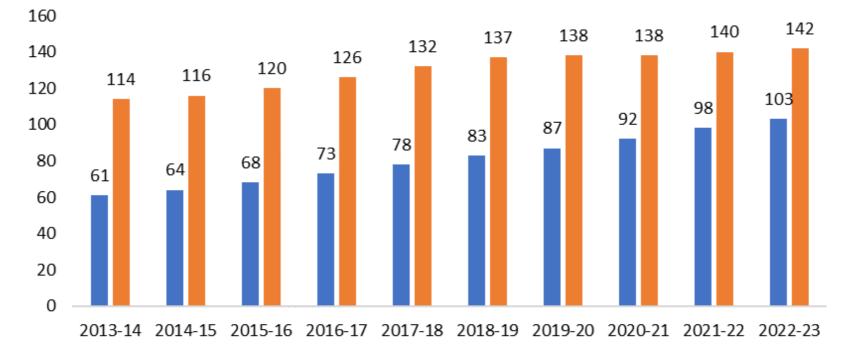
# **Buffalo Milk Production: World vs India**

| Country | 2013-14 | 2014-15 | 2015-16 | 2016-17 | 2017-18 | 2018-19 | 2019-20 | 2020-21 | 2021-22 | 2022-23 |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| India   | 61      | 64      | 68      | 73      | 78      | 83      | 87      | 92      | 98      | 103     |
| World   | 114     | 116     | 120     | 126     | 132     | 137     | 138     | 138     | 140     | 142     |
|         | 53%     | 55%     | 57%     | 58%     | 59%     | 60%     | 63%     | 67%     | 70%     | 73%     |

**Buffalo Milk Production** 

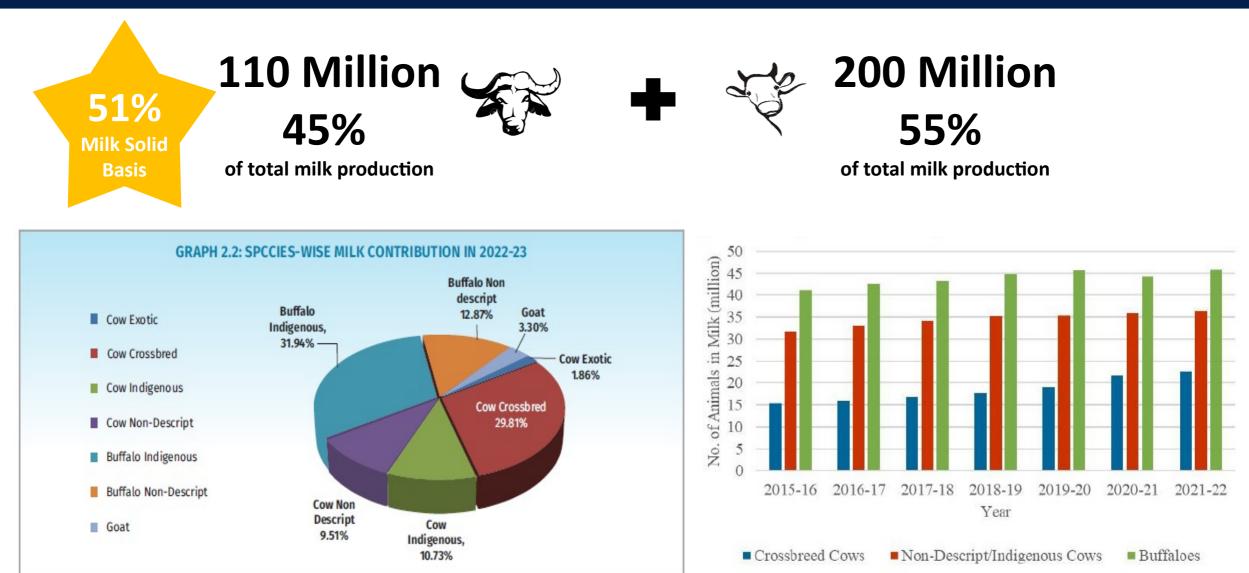
India World

Annual buffalo milk production of over 110 MMT with a consistent CAGR of 4-5% in India



Source: FAOSTAT

#### Largest Bovine Population In The World : 310 Million



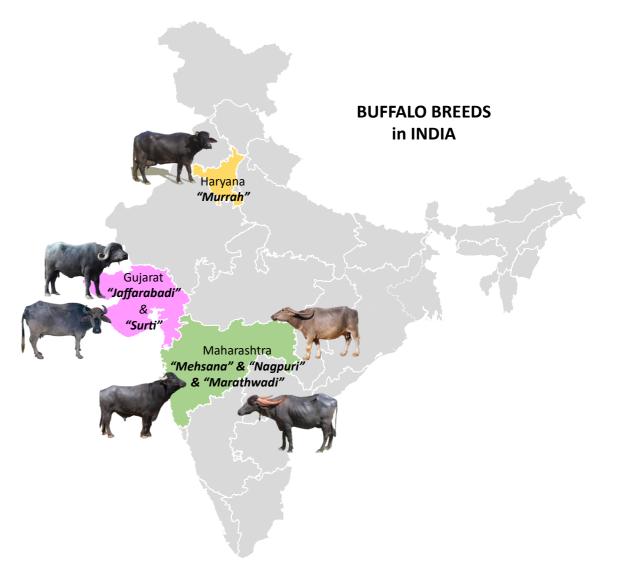
#### Source: BAHS 2023

# **Buffalo Milk: An Essential Natural Adjuvant**

Average Lactation Yield Per Animal (kg) 2023

| Breed                | Yield in Kg |
|----------------------|-------------|
| Murrah               | 1752        |
| Jaffarbadi           | 2239        |
| Surti                | 1300        |
| Mehsana              | 1988        |
| Nagpuri/ Pandharpuri | 1790        |
| Marathwadi           | 1120        |

Density of milk: 1.03 g/mL



#### Source: Discover Food

### **Comparison of Cow and Buffalo Milk**





| 1 kg of <b>Cheese</b>  | = 8                  | kg of Cow Milk  | 5 kg of Buffalo Milk   | 1 |
|------------------------|----------------------|---|--|---|
| kg of <b>Butter</b> =  | 14 k                 | g of Cow Milk   | 10 kg of Buffalo Milk  |   |
| MILK PROPERTIES        |                      | COW   | BUFFALO  |   |
| Fat                    |                      | 3-4%  | 6-8%   |   |
| Protein                |                      | 3-4%  | 4-5%   |   |
| Shelf Life             |                      | 6 days  | 7-10 days  |   |
| PH                     |                      | Low   | High   |   |
| Production             | 1                    | 5-20 litres/ day  | 7-11 litres/ day   |   |
| Lactose                |                      | 4.7%  | 4.86%  |   |
| Minerals (Daily Value) | 2 <sup>-</sup><br>6% | of Phosphorous<br>1% of Calcium<br>of Magnesium<br>% of Vitamin A | 41% of Phosphorous<br>32% of Calcium<br>19% of Magnesium<br>14% of Vitamin A |   |

#### Source: Discover Food

### **Superiority of Buffalo Milk**

#### **PROPERTIES**

- Rich composition
- More protein and fat content
- High in vitamin B12 (Decreased risk of heart disease, stroke and other cardiovascular conditions)
- Exceptionally smooth, thicker consistency and white due to lack of carotene
- High richness makes it very ideal for processing- cost effective

- Fatter globule size
- Higher nutritious grade
- Resilient whey proteins
- Lactose in raw buffalo milk is 4.86%
- Great source: Ca, Mg, P, Zn and Vitamins A, B,
   D, E and K
- Therapeutic benefits and acts as an adjuvant
- 30% more total solids than cow milk
- Greater viscosity and antioxidant capacity than cow milk

#### Source: Discover Food, IDA

# **Traditional Buffalo Farming**







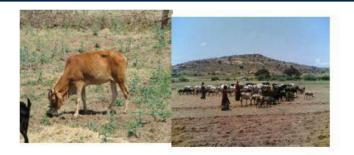








# **Buffalo Farms: Challenges**













- 2-200 Buffalo herd size Tied up Buffalo
- Hand Milking Labour Intensive
- Buffalo Temperament
- Poor Animal Comfort & Hygiene
- Frequent Breeding Failure
- Reducing availability of Skilled Labour / Milker
- Different udder shapes Different teat size
- Special physiology of Milk Ejection Stimulation/ Calf







**Conical shaped** 

Cylindrical shaped

**Bottle shaped** 

# **Evolution of Organized Buffalo Farms in India**

#### Adoption of Milking Solution for Different segment of Farms



Buffalo Bucket Milking Machine

Pipeline Milking System - Speedline



Fully Automatic Buffalo Milking Parlour



### **Buffalo Farm: New Generation Adopting Technology**

#### **NOBLE DAIRY FARM**

- Traditional third generation farmer with 2000 + Buffaloes at Mumbai
- New generation in family decided for Green field farm for 800 Buffaloes
- Location: Ghoti, Igatpuri, Nasik (Maharashtra)
- Complete mechanized Commercial Buffalo Dairy Farm
- Data collection and analysis automated with Delpro system from Delaval





Loose Housing Shed for comfort

DeLaval Herring Bone 2\*24 Parlour

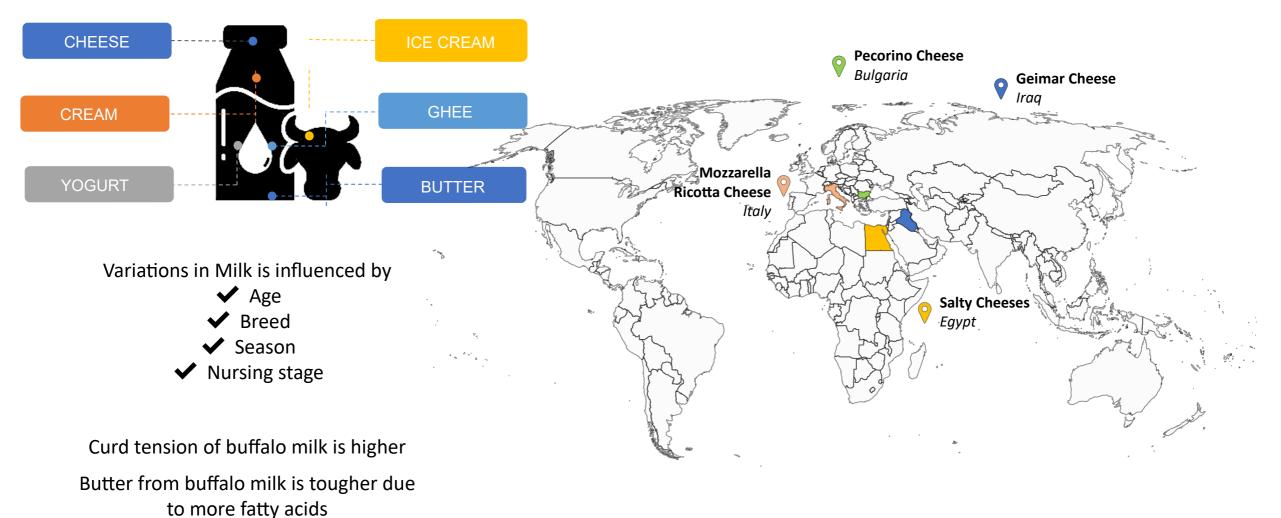


Cow Shower to address body heat and cleaning



Spacious Alley for TMR Feeding

### **Buffalo Milk & Products**



Source: Discover Food

### **Indian Buffalo Milk & Products**



### **Indian Buffalo Milk & Products**



#### GHEE

Buffalo ghee

SID'S

FARM

PURE CULTURED BUFFALO GHEE



# = PARSI BAIRY FARM = aut to







#### CHEESE







#### LIES OF PLANT BASED





#### **Plant Milk & Products**

# **Growth in Buffalo Milk Production & Consumption**

#### GEOGRAPHIC REGION

#### DAIRY INDUSTRY

### • Some northern states prefer buffalo milk over cow milk

- Often used in traditional Indian cuisine and sweets
- Commands a premium price of C84/ ltr vis-à-vis cow milk at C67/ ltr

- Heavy dependence on buffalo milk for various dairy products
- Cheese, yogurt, butter, ghee, condensed milk, ice cream

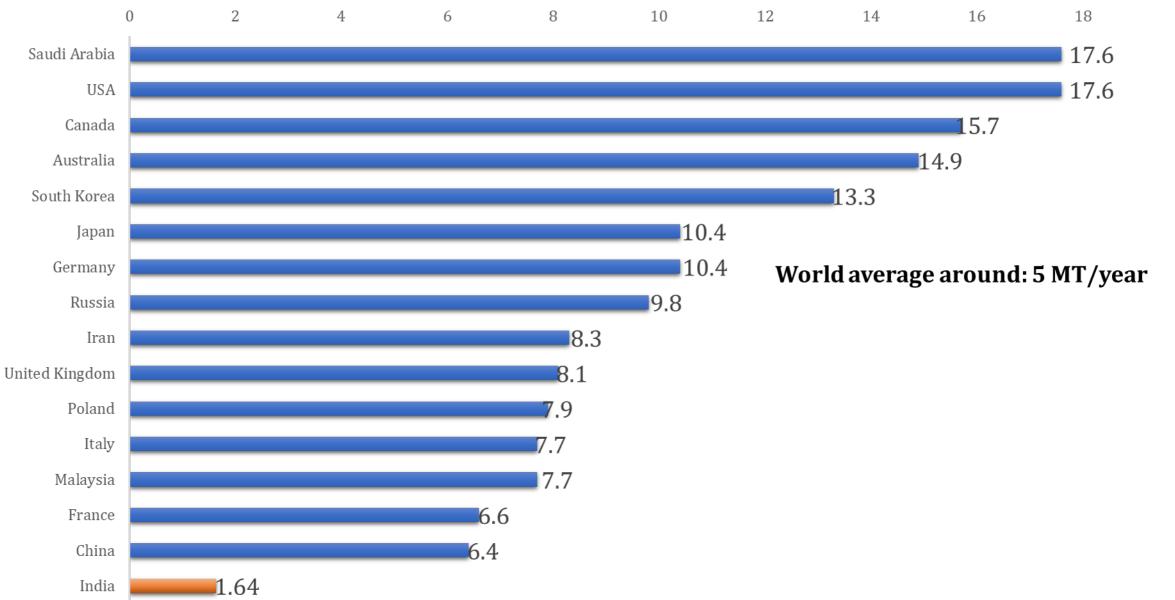
#### ECONOMIC IMPORTANCE

• Provides livelihood opportunities for almost 80 million households in India

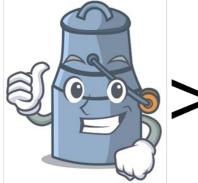
# Sustainability



### Per capita emission (MT/year)



#### Indian Dairy Industry: Small Holder System (Economical Aspect)











Number of families depends on dairy ~ 80 million.



**75 – 80%** consumer money goes back to milk producers. Twice than developed nations.

Value: USD 110 Billion 25% of Indian agriculture 231 MMT/year, CAGR: 4.5% of India & < 2% of world



Dairy plants capacity: 120 million liter/day, Cattle feed plants: 80,000 MT/day and Semen station capacity: 90 million/year Symbiotic relationship between rural producers & urban consumers



1 million retailers selling milk and milk products

#### Indian Dairy Industry: Small Holder System (Environmental Aspect)



Largest producer of rice & fruits and second largest producer of wheat, sugarcane, groundnut, vegetables, fruit and cotton



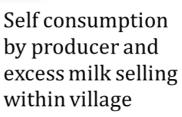
Largest producer of roughages.



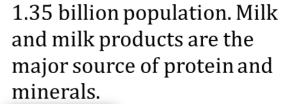
Grains and pulses: Major source of nutrients



75% of bovine diet is not consumed by humans.









#### Indian Dairy Industry: Small Holder System (Environmental Aspect)



Manure for agriculture

Gas supply @ producer house

Dung cake as fuel

Used as bio fertilizer

#### Flexi Dome Gobar Gas Plant Installation at Farmers Doorstep





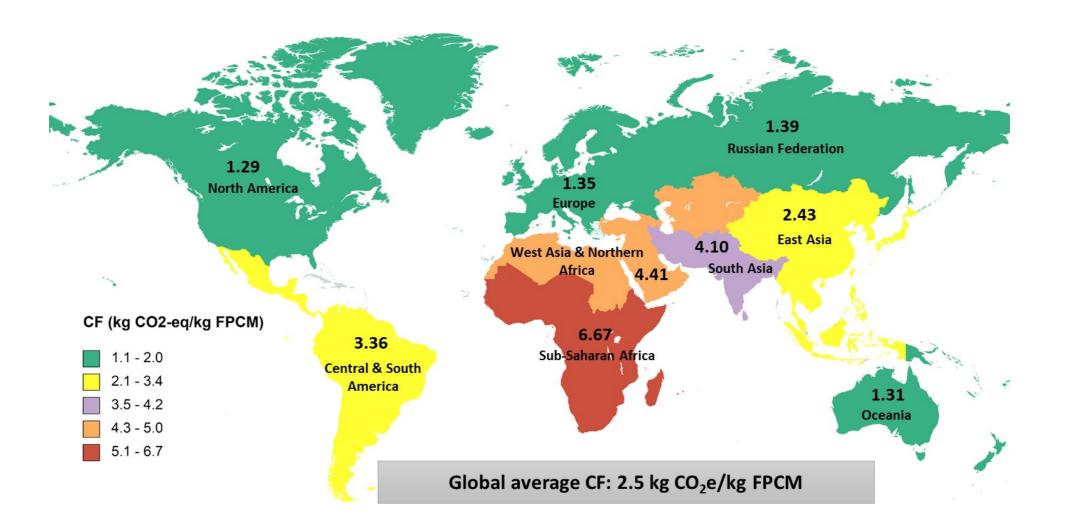






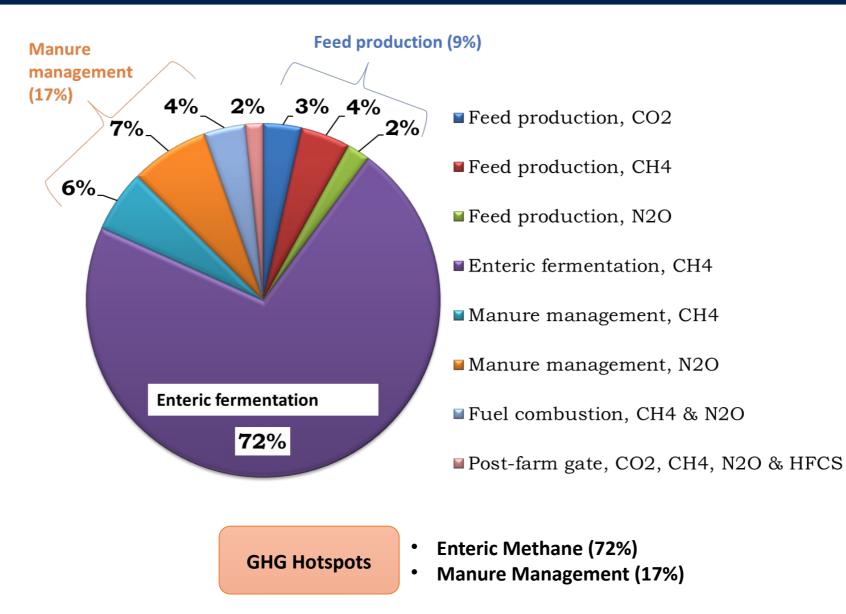


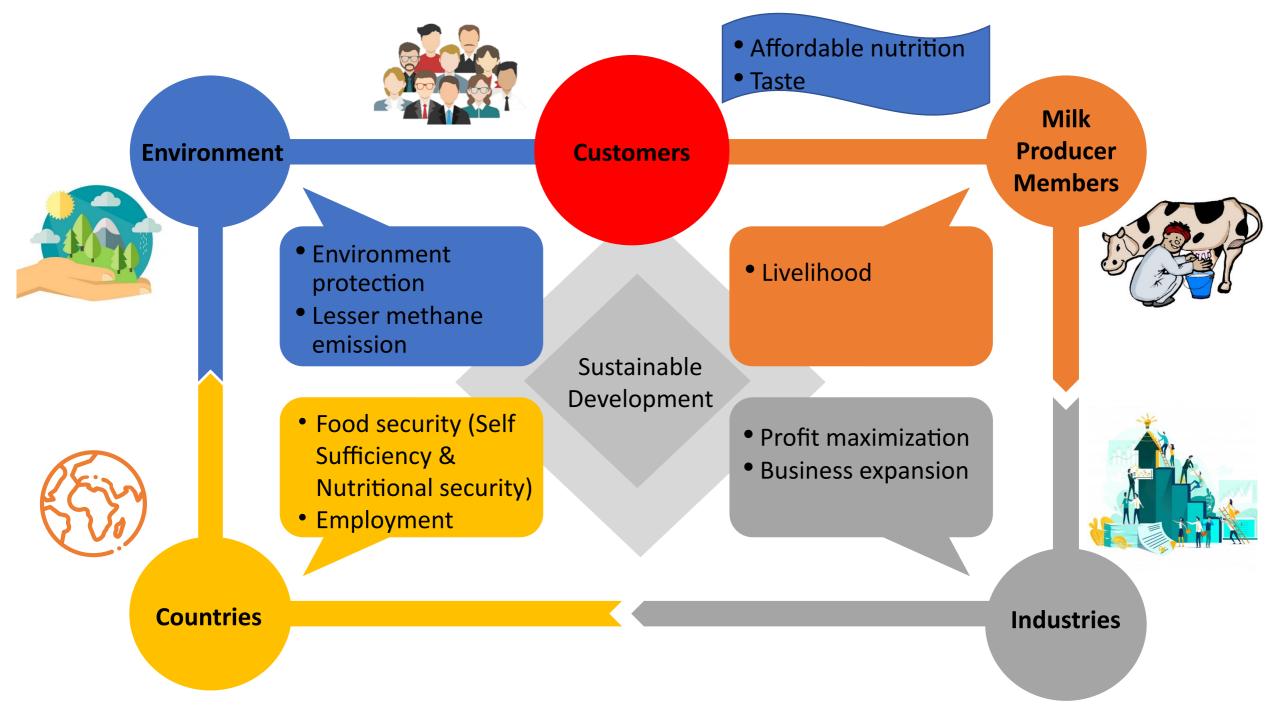
## Carbon Footprint (CF) of Milk by Region



Source: FAO & GDP

## **GHG Emissions by the Indian Dairy Sector**





## **Sustainability Starts When Stomach Is Full**



## **Emission for Livelihood vs Emission for luxury**

## **Challenges to Dairy Industry**

- Milk Production
- > Animal productivity and Breeding
- > Animal health and prevention
- Animal feeding
- ➤ Policy level
- Adulteration
- > Analogue products

- ➢ Free Trade Agreement (FTA)
- Price Volatility
- Next Gen: Not ready to work in dairying
- Shortage of professionals
- ➤ A1 versus A2
- Cow versus Buffalo Milk
- ➤ Leadership

## And Finally....



"We have traversed a path few have dared to.

We are continuing on a path still fewer have the courage to follow.

We must pursue the path that even fewer can dream to pursue.

Yet, we must, because we hold in trust the aims and aspirations of millions of our countrymen."

- Dr V. Kurien

THANK YOU





#### CONSORZIO DI MOZZARELLA DI BUFALA CAMPANA

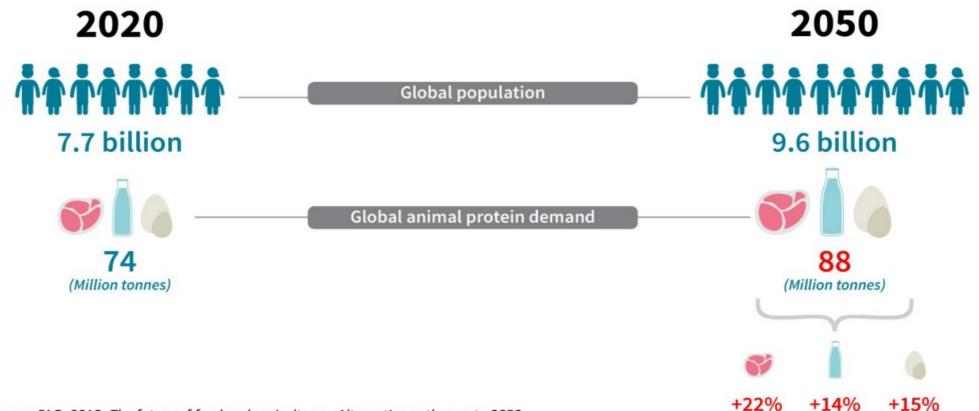
25 Settembre 2024

#### PIERCRISTIANO BRAZZALE, FIL-IDF PRESIDENT



## How to feed people in this planet?

Population growth and global demands of animal-source foods

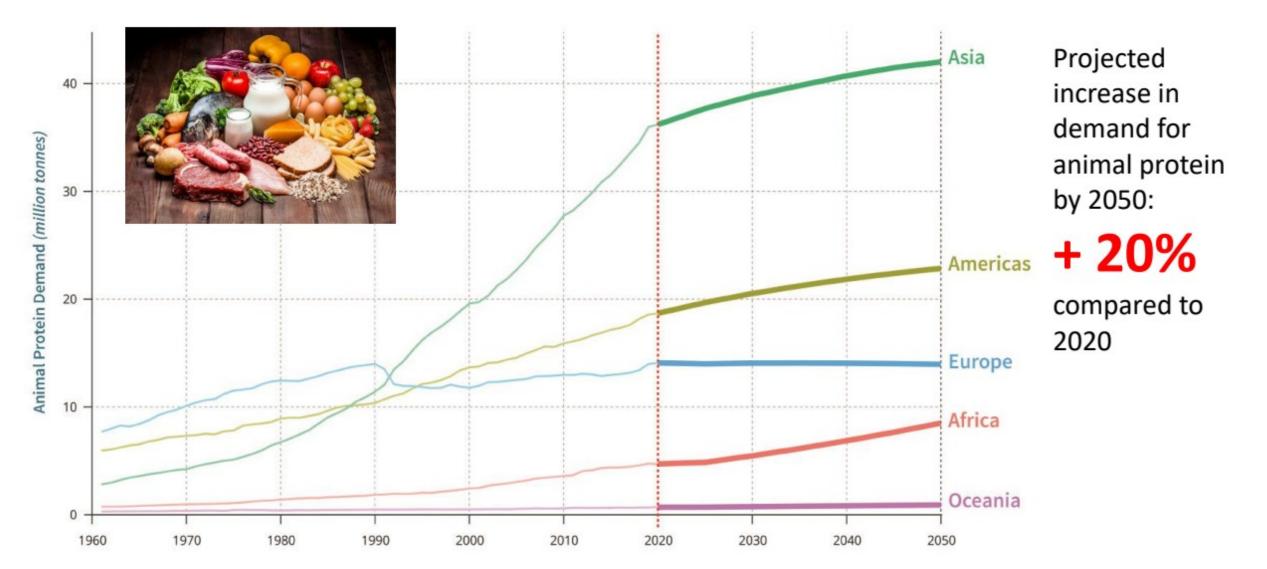




Source: FAO. 2018. The future of food and agriculture – Alternative pathways to 2050.

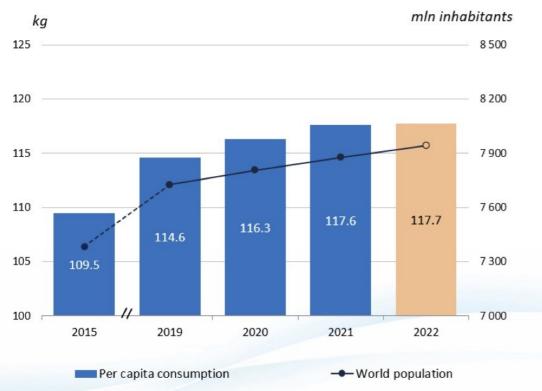


## **Demands of animal-source foods by Region**



## DEMAND

## Economic outlook



#### World: per capita consumption and population

#### Global dairy supply and demand outlook Updated 2023





Source: Rabobank, 2023



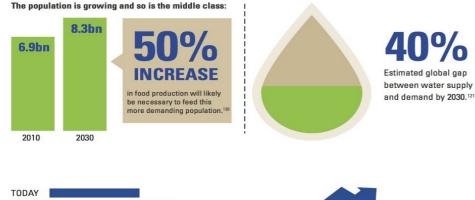
IDF World Dairy Situation - 2023

#### Global megatrend #8 Resource stress

he combined pressures of population growth, economic growth and climate change will place increased stress on essential natural resources including water, food, arable land and energy. These issues will place sustainable resource management at the center of government agendas.

By 2030, significant changes in global production and consumption, along with the cumulative effects of climate change, are expected to create further stress on already limited global resources. Stress on the supply of these resources directly impacts the ability of governments to deliver on their core policy pillars of economic prosperity, security, social cohesion and environmental sustainability.

#### The evidence of change



The International Energy Agency projects an approximate **40%** increase in global energy demand by 2030.<sup>122</sup>

DRIVEN BY

2030

Economic growth





Technological advancements



About More people will live in areas of water stress by 2030 in a business-as-usual scenario.<sup>124</sup> = 200 million Both growing demands and unstable production patterns due to climate change will cause global food prices to double between 2010 and 2030.  $^{\rm 125}$ 

The consequences of resource stress

2010

2030





## **Agri-food systems**

#### uses 70% of fresh water



#### uses 33% of land area





## uses 30% of global energy





## ... But feed 100% of us





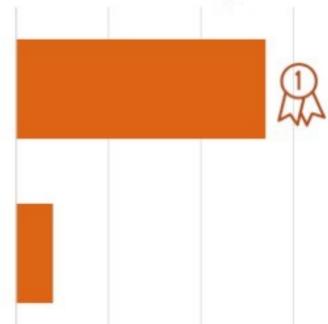


## **Metrics Matter**

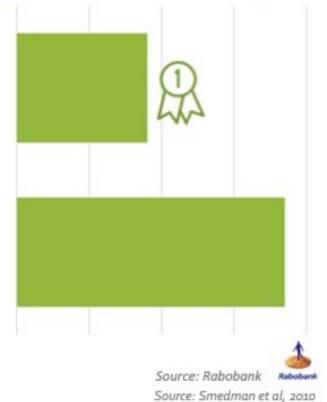
## CO2eq emissions per 100 GRAM



## Nutrient Density Index

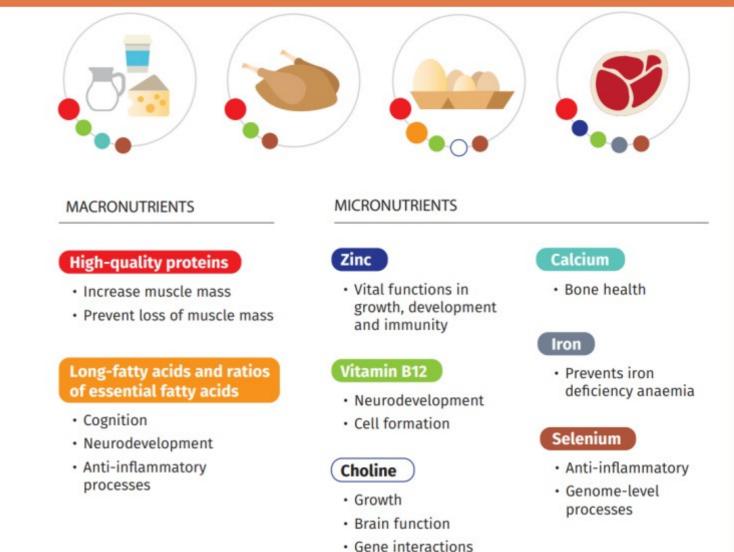


## CO2eq emissions per Nutrient Density value



## Value of terrestrial animal source food in human health

Nutrients found concentrated and bioavailable in terrestrial animal source food play important roles in human health



# IDF Dairy Matrix - the Factsheets Series pubblished on November 2023

## The (2023) series of IDF factsheets consists of 4 factsheets: general, milk, yogurt, cheese.



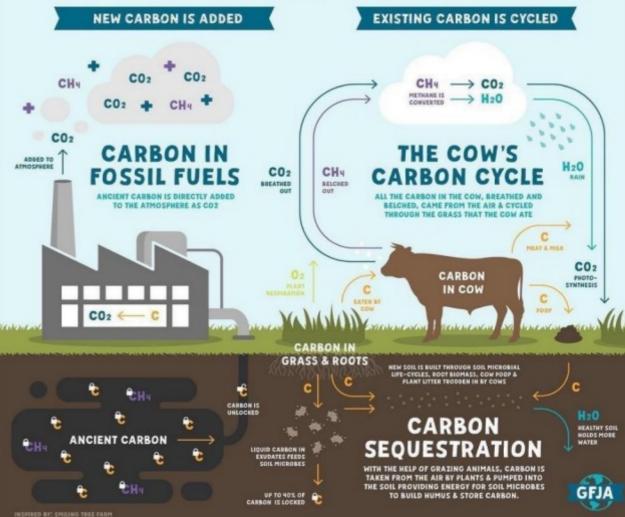
The factsheets report the synthesis of the most recent scientific evidence on the dairy matrix.

Fundamental concept: "the combination effect": the action of a single nutrient is probably also dependent on the others and on how they are "combined" in the food itself.





## CATTLE EMISSIONS ARE CYCLED, FOSSIL FUELS ARE ADDED



Net zero means we are not adding new emissions to the atmosphere Emissions will continue, but will be balanced by absorbing and cyclying.





**IDF WDS DAEJEON 2018** Dairy for the Next Generation! 00= IDF WDS DAFEON 2018

## Milk is Perfection

Milk is critical and essential for international efforts to combat poverty and hunger.

OF WEST DALED AND ADDR

1111

## 6 AND SANTUTION 3 AND WELL-SERVE 0 THE GLOBAL GOALS

8 DECENT WORK AND ECONOMIC GROWTH

13 LETION

**IDF WDS DAEJEON 20** Dairy for the Next Generation



IDF position to CL 2022/06/OCS - CCEXEC Request for Information on New Food Sources and production systems; Need for Codex guidance and attention to inform the CCEXEC sub-committee on working on this topic (5 April '22)

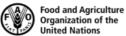
The International Dairy Federation (IDF) is grateful for the opportunity to provide a response to the Request for Information on New Food Sources and production systems; Need for Codex guidance and attention to inform the CCEXEC sub-committee on working on this topic.

In the context of this work on new food sources and production systems, we consider it essential to focus on and ground the work within the mandate of Codex of protecting public health via food safety and quality and ensuring fair practices in food trade. These new food technologies may raise significant challenges in terms of food safety, labelling, fair trade practices, and could have unknown impacts on human health over time. Currently, there is still the need to understand and learn about the impact of these "new foods" and innovative technologies at the different levels. Any work within Codex should be aligned with its mandate and its procedure manual<sup>1</sup>.

In addition, Codex must carefully avoid conflicts with any existing Codex Standards, Guidelines, or Codes of Practice when initiating new work. We would, therefore, specifically note the relevancy of the Codex General Standard for the Use of Dairy Terms (GSUDT) (CXS 206-1999) to many issues associated with some of the new food sources identified in the CL. Established in 1999, the GSUDT provides guidance on the correct use of terms which are universally identified with dairy products. The GSUDT defines milk as "the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or further processing." If new work is initiated on these new food sources, this definition and other core principles of CXS 206-1999 must be considered and not undermined.

Consistent with the Codex General Standard for the Labelling of Prepackaged Foods, a core principle of the GSUDT is that foods shall be described and presented in a manner that ensures consumers are not misled or confused<sup>2</sup>. Labelling of dairy products or nondairy products using dairy terms shall not be false, misleading, deceptive, or create an erroneous impression regarding its character in any respect, including being suggestive of any other product with which the food might be confused.

#### CODEX ALIMENTARIUS COMMISSION







Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org Agenda Item 4 CX/EXEC 22/83/4

October 2022

E

JOINT FAO/WHO FOOD STANDARDS PROGRAMME EXECUTIVE COMMITTEE OF THE CODEX ALIMENTARIUS COMMISSION

**Eighty-third Session** 

14 - 18 November 2022

#### CCEXEC SUB-COMMITTEE ON NEW FOOD SOURCES AND PRODUCTION SYSTEMS - REPORT

(Prepared by the Chairperson of the sub-committee)

#### Introduction

Since FAO and WHO first introduced new food sources and production systems (NFPS) as an issue that needed attention, Codex has held discussions and collected information on several occasions. Discussions began at 81st session of the Executive Committee of the Codex Alimentarius Commission (CCEXEC81), which established a CCEXEC sub-committee to consider this issue further<sup>1</sup>. CAC44 subsequently considered the issue, supported the need for Codex to be prepared to address cross-cutting, overarching and emerging issues, and requested the Codex Secretariat to issue a Circular Letter<sup>12</sup> (CL) to collect information from Members and observers on ongoing developments related to NFPS. The CCEXEC sub-committee supported the development of the CL and in addition a letter was sent to all Codex Members and observers inviting informal conversations with the Chairperson and Vice-Chairpersons of the Commission to share views on this issue. A detailed overview of this first phase of the work was presented to CCEXEC82as an interim report of the sub-committee<sup>3</sup>.

#### Overview of discussions at CCEXEC82 and ongoing work of the sub-committee<sup>4</sup>

2. CCEXEC82 considered the interim report of the sub-committee and underlined the complexity of this area. CCEXEC Members expressed different views about the pathway forward, including the need for sufficient time to consider the issues, and the need for specific expertise, or other working mechanisms to engage with the wider Codex membership (e.g. the establishment of an electronic working group (EWG) of the Commission). CCEXEC82 recognised that this ongoing CCEXEC work on NFPS did not preclude Codex committees from undertaking new work on such emerging issues falling within their respective mandates, using existing Codex working mechanisms. In noting the interim report of the sub-committee, the comments put forth during the debate and the extensive amount of data received in response to the CL, CCEXEC82 agreed that the subcommittee should continue its stepwise consideration of the issues, informed by an analysis of the information collected through the CL, CRDs and report of CCEXEC82.

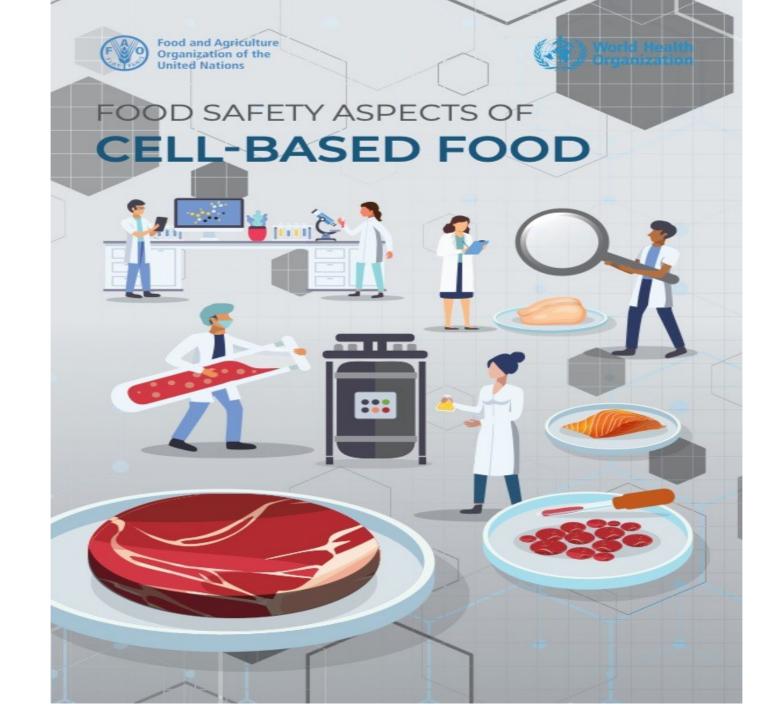
 With the support of FAO, a summary of the replies to the CLs was subsequently commissioned and is included as Appendix 2 to this report. This was considered further by an informal virtual meeting of the subcommittee.

4. Based on the summary of the replies from the CL, which allowed for a better understanding of the breadth of information about NFPS that was collected through the CL and the informal discussions, a virtual meeting of the sub-committee considered the potential way forward. Members recognized that NFPS presented both a

<sup>&</sup>lt;sup>1</sup> Codex procedure manual. Twenty-seventh edition. 2019. Criteria for the establishment of work priorities p43 <sup>2</sup> IDF Bulletin 507/2020 – The Codex General Standard for the Use of Dairy Terms. Its nature, intent and implications (accessed on 22 March '22 <u>Bulletin-of-the-IDF-507\_2020 The-Codex-General-Standard-for-the-Use-of-Dairy-Terms.CAT\_snusw3.pdf (fil-idf.org)</u>)

<sup>1</sup> REP21/EXEC 22/82/4, para 110

<sup>&</sup>lt;sup>2</sup> The CL received replies from 25 Members and 10 Observers. Informal conversations were held with the six Regional Coordinators, CCAFRICA (Uganda), CCASIA (China), CCEURO (Kazakhstan), CCLAC (Ecuador), CCNASWP (Fiji) and CCNE (Saudi Arabia) and with the European Union, FAO, the Good Food Institute (GFI), International Dairy Federation (IDF), Germany and the United States of America <sup>3</sup> CX/EXEC 22/82/4 4 REP22/EXEC1, paragraphs 70-85





Environmental impacts of cultured meat: A cradle-to-gate life cycle assessment Derrick Risner<sup>1</sup>, Yoonbin Kim<sup>1</sup>, Cuong Nguyen<sup>2</sup>, Justin B. Siegel <sup>3,4,5,6</sup>, Edward S. Spang<sup>1,6</sup> <sup>1</sup> Department of Food Science and Technology, University of California, Davis, CA 95616, USA <sup>2</sup> Division of Agriculture and Natural Resources, University of California, Holtville, CA 92250, USA <sup>3</sup> Genome Center, University of California, Davis, CA 95616, USA <sup>4</sup> Departments of Chemistry, Biochemistry and Molecular Medicine, University of California, Davis, CA 95616, USA <sup>5</sup> Innovation Institute for Food and Health, University of California, Davis, CA 95616, USA <sup>6</sup> USDA, AI Institute for Next Generation Food Systems (AIFS), University of California, Davis, CA 95616, USA \*Correspondence: esspang@ucdavis.edu; Tel.:+1-530-754-544

#### Abstract

Interest in animal cell-based meat (ACBM) or cultured meat as a viable environmentally conscious replacement for livestock production has been increasing, however a life cycle assessment for the current production methods of ACBM has not been conducted. Currently, ACBM products are being produced at a small scale and at an economic loss, however ACBM companies are intending to industrialize and scale-up production. This study assesses the potential environmental impact of near term ACBM production. Updated findings from recent technoeconomic assessments (TEAs) of ACBM and a life cycle assessment of Essential 8<sup>TM</sup> were utilized to perform a life cycle assessment of near-term ACBM production. A scenario analysis was conducted utilizing the metabolic requirements examined in the TEAs of ACBM and a purification factor from the Essential 8<sup>TM</sup> life cycle assessment was utilized to account for growth medium component processing. The results indicate that the environmental impact of near-term ACBM production is likely to be orders of magnitude higher than median beef production if a highly refined growth medium is utilized for ACBM production.

#### Introduction

Livestock production is an integral component of the global food system, providing staple proteins (milk, eggs, and meat) consumed worldwide, contributing to crop productivity via utilization of manure as fertilizer, and providing critical nutrition and income to underprivilege households in low to middle income countries (Gilbert et al., 2018; Robinson et al., 2011). Global meat production has increased from 70.57 million tonnes in 1961 to 337.18 million tonnes in 2020, though the consumption of different meat sources is highly regionalized (FOA, 2022; Ritchie et al., 2019). In 2020, beef and buffalo meat accounted for ~22% of global meat production, and poultry and pork accounted for ~39% and ~32% of worldwide meat production respectively (FOA, 2022; Ritchie et al., 2019).



## Cell-based meat could emit 25 times more than retail beef, study

#### Beef Central, 15/05/2023



RESEARCHERS from a renowned university in the United States say traditional meat production is likely to have less of an environmental footprint than producing meat in a lab.

The group from University of California Davis recently did life-cycle assessments on lab-grown meat and compared it to the warming potential of conventional meat. It has released the findings of the report, with the peer-review still to come.

Lab-grown meat, or animal cell-based meat, has been tipped as a more environmentally friendly alternative way of producing protein. But previous Beef Central articles have raised doubts about its viability.

The results have shown that its environmental impact could be four-to-25 times greater than retail beef. The study focused on energy used to grow the animal cells and says it was likely to show the minimal impact of lab-grown meat.

"We also acknowledge that our analysis may be viewed as minimum environmental impacts due to several factors including incomplete datasets," the study says.

"The exclusion of energy and materials required to scale the ACBM industry and exclusion of the energy and materials needed to scale industries which would support ACBM production."

#### Measuring a burgeoning industry

One of the main limiting factors to the study was the small-scale nature of the cellbased meat industry. The study was based on impact of a scaled-up cell-based meat industry.

"Currently, ACBM products are being produced at a small scale and at an economic loss, however ACBM companies are intending to industrialise and scale-up production," it says.

Home > The International Journal of Life Cycle Assessment > Article

#### CARBON FOOTPRINTING Open Access Published: 26 August 2022

Comparison of carbon footprint and water scarcity footprint of milk protein produced by cellular agriculture and the dairy industry

<u>Katri Behm</u> ⊠, <u>Marja Nappa</u>, <u>Nina Aro</u>, <u>Alan Welman</u>, <u>Stewart Ledgard</u>, <u>Marjut Suomalainen</u> & <u>Jeremy</u> <u>Hill</u>

The International Journal of Life Cycle Assessment 27, 1017–1034 (2022) Cite this article

5253 Accesses | 2 Citations | 63 Altmetric | Metrics

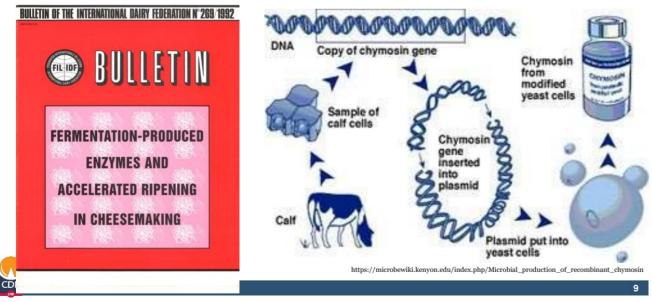
#### Abstract

#### Purpose

This paper studies the carbon footprint and water scarcity footprint (WSF) of a milk protein, beta-lactoglobulin, produced by cellular agriculture and compares this to extracted dairy protein from milk. The calculations of the microbially produced proteins were based on a model of a hypothetical industrial-scale facility. The purpose of the study is to examine the role relative to dairy of microbially produced milk proteins in meeting future demand for more sustainably produced protein of high nutritional quality.

#### Mathada

#### Is Precision Fermentation New to the Dairy Industry? No!



## Questions

• Can precision fermentation replicate the full structure and functionality of caseins/micelles (including the ability to make cheese using the traditional clotting approach, etc)?

No? The formation of casein micelles requires two key modifications of the primary casein protein sequence which is done by **enzymes** in the mammary cells

- Phosphorylation (adding phosphate), allows the caseins to bind calcium
- Glycosylation (adding sugars) stabilizes the micelles

The industrial bacteria/yeast do not have these specific enzymes and cannot recreate the environmental conditions, (pH, salt, temperature) needed to carefully bioassemble these various components. Bacteria/yeast could be engineered to do these modification but where/how to do the bioassembly?

Micelles produced by cows are made up of **4 different types of caseins**, each would need to be generated separately and then carefully mixed with various salts!!

## Scientific Status of Cell-based Processes to Make Milk Proteins

- Lack of peer reviewed studies on the structural, functional and allergenicity of any proposed Startup's recombinant milk protein (some studies on recombinant β-lactoglobulin produced by academic researchers, could be different folding, altered binding sites, other modifications, etc)
- No published studies on the properties of any proposed Startup's recombinant caseins or casein micelles
- Lack of published studies on the carbon footprint of these non-cow milk proteins (just websites and marketing info)

## Questions

• Can milk be recreated without the cow (in the lab, by plants)?

Not by precision fermentation or transgenic plants (possibly by mammary cell-lines but that's not scalable for now, very expensive)

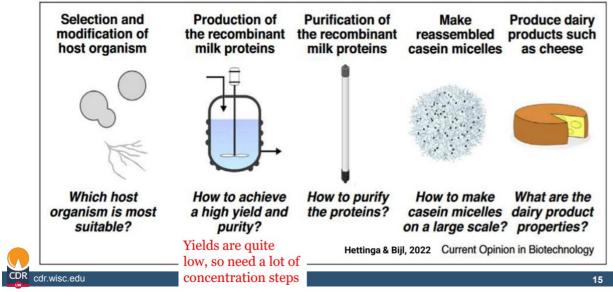
• Too complex, thousands of molecules, highly diverse,

CDR cdr.wisc.ed

- Designed for nutrition and health, not just a few basic components.
- **Requires bioassembly** into complex structures (casein micelles, fat globules) is critical for the functionality and nutritional aspects.

Startups will make a simple mixture of a couple of components and declare they have "replicated" milk!! Success!!

Some challenges for producing casein micelles by a precision approach, need to include how to do the post-translational modification?



## Challenges

- Scalability (available fermentation capacity)
- Low yields (both precision fermentation and plant approaches)
- Cost

CDR

cdr.wisc.edu

- Still a milk allergen
- Beyond the marketing hype of startups, the real LCA comparison to dairy components?
  - Environmental impact of a "milk" protein produced by precision fermentation had a similar footprint to proteins derived from the cow (Behm et al. 2022, Int. J. of Lifecycle Assessment, 27:1017)
- Sterility (for cell-cultured products)
- Consumer acceptance
- Regulatory acceptance

## **Opportunities for Precision Fermentation in the Dairy Industry?**

- Focus on high value components
  - e.g., rennet is the most expensive ingredient in cheese
- Focus on components present at low concentrations in milk – precision fermentation can make more of it cost effectively
- Focus on simple proteins

CDR cdr.wisc.edu

- where post-translation modification or further bio-assembly is not required
- *Examples:* lactoferrin, lactoperoxidase, immunoglobulins, oligosaccharides, etc
- Use of lactose-rich dairy feedstocks (e.g., permeate, acid whey) for the fermentation process improves the sustainability of dairy processing [*under one roof!*]

## Conclusions

- Simple proteins or enzymes can be successfully made using the precision fermentation approach, e.g., rennet and  $\beta$ -LG
- Complex proteins, like casein, require critical post-translational modifications, unclear how these modifications can be done outside the mammary cells? Or if modified by a m/o then it's a GMO
- Unclear how bio-assembly of complex proteins structures like casein micelles can be done without the post-translation modification
- What is the functionality of these non-cow derived casein proteins? [better than plant proteins but not identical to cow-derived]
- Suggest precision fermentation focus should be on high-value proteins present in milk at low concentrations (e.g., lactoferrin)

CDR cdr.wisc.edu

23

Antibiotic resistance in potential probiotic and pro-technological lactic acid bacteria isolated from buffalo milk FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

R.L. Ambrosio<sup>1</sup>, M. Di Paolo<sup>1</sup>, V. Vuoso<sup>1</sup>, F. Troise<sup>1</sup>, A. Anastasio<sup>1</sup>

<sup>1</sup>University of Napoli Federico II - Department of Veterinary Medicine and Animal Production, Italy







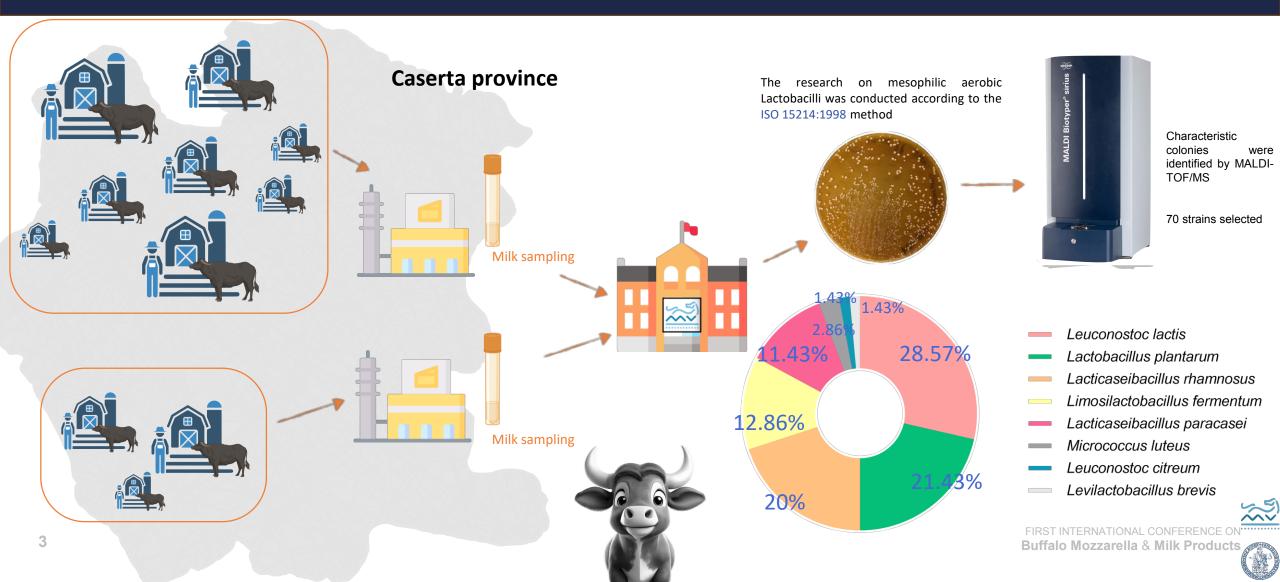
Lactic acid bacteria (LAB) are safely used as probiotics and normally used technologically in dairy production. However, these bacteria could spread antibiotic resistance genes.

In this context, this study aimed to contribute to expanding knowledge on probiotic and protechnological bacteria of buffalo milk.



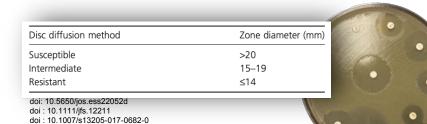


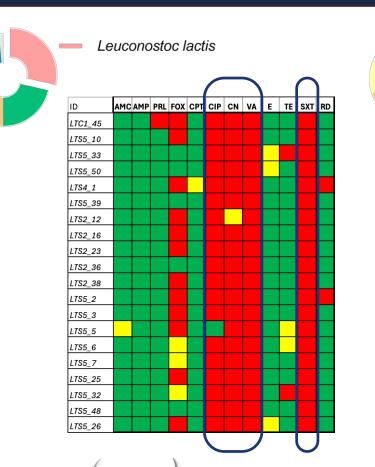
## M&M and Results

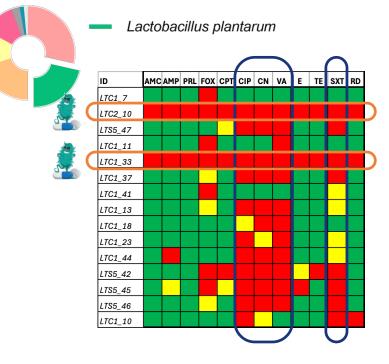


## **M&M and Results**

| code | antibiotics                   | disk content           |
|------|-------------------------------|------------------------|
|      | penicillins                   |                        |
| AMC  | Amoxicillin - clavulanic acid | 20 <b>-</b> 10 µg      |
| AMP  | Ampicillin                    | 2 µg                   |
| PRL  | Piperacillin                  | 30 µg                  |
|      | cephalosporins                |                        |
| FOX  | Cefoxitin                     | 30 µg                  |
| CPT  | Ceftaroline                   | 30 µg                  |
|      | fluoroquinolones              |                        |
| CIP  | Ciprofloxacin                 | 5 µg                   |
|      | aminoglycosides               |                        |
| CN   | Gentamicin                    | 10 µg                  |
|      | glycopeptides                 |                        |
| VA   | Vancomycin                    | 30 µg                  |
|      | macrolides                    |                        |
| E    | Erythromycin                  | 15 µg                  |
|      | tetracyclines                 |                        |
| TE   | Tetracycline                  | 30 µg                  |
|      | miscellaneous agents          |                        |
| SXT  | Trimethoprim-sulfamethoxazole | 1.25 <b>-</b> 23.75 μg |
| RD   | Rifampicin                    | 5 µg                   |

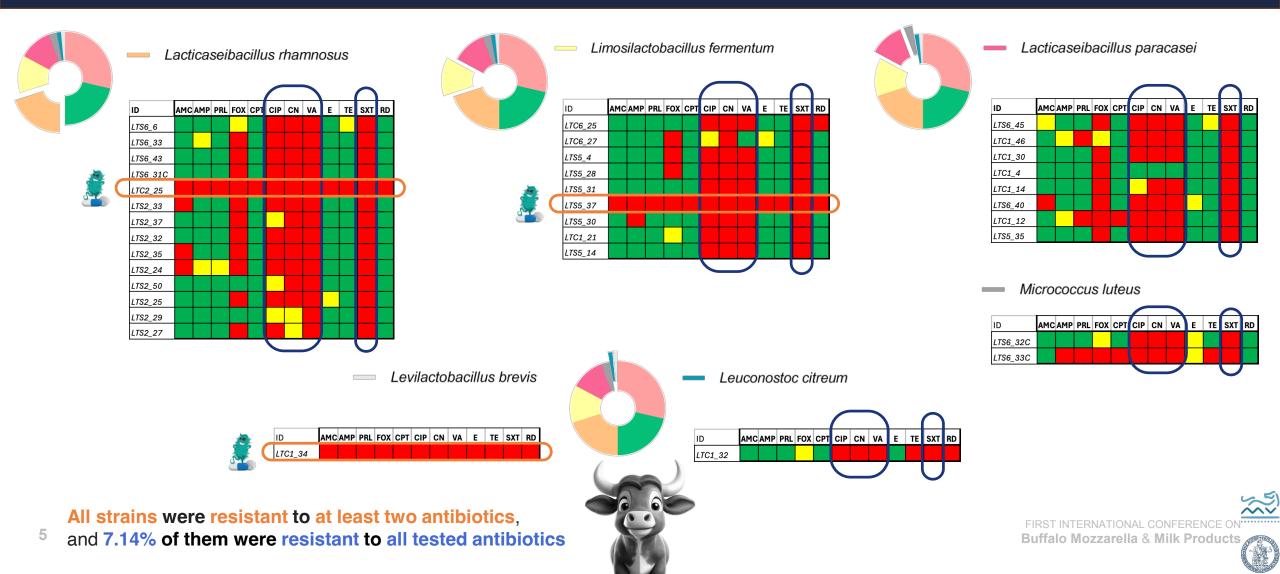






FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

## **Results**

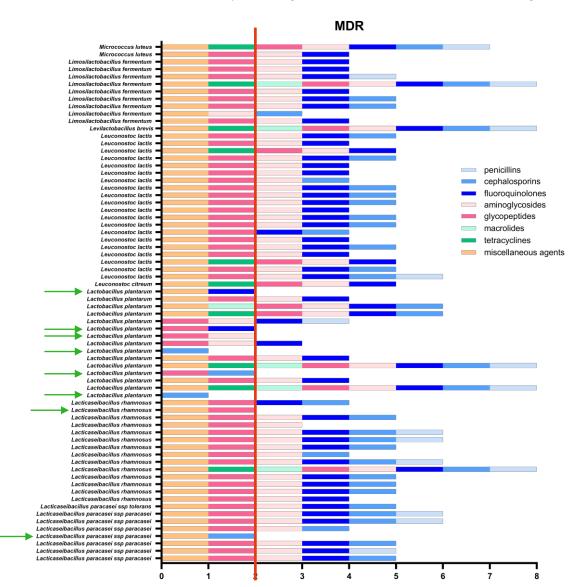


# Results & Discussion

Percentage of resistance (R), intermediate sensitivity (I) and sensitivity (S) of the strains with respect to the tested antibiotic

#### RD-SXT-TE-E٠ VA-S CN-CIP CPT R FOX-PRL AMP AMC 20 30 40 50 60 70 80 90 100 10 0 %

The highest resistances were found for vancomycin (92.86%), trimethoprim-sulfamethoxazole (90%), gentamicin (87.14%) and ciprofloxacin (84.29%)

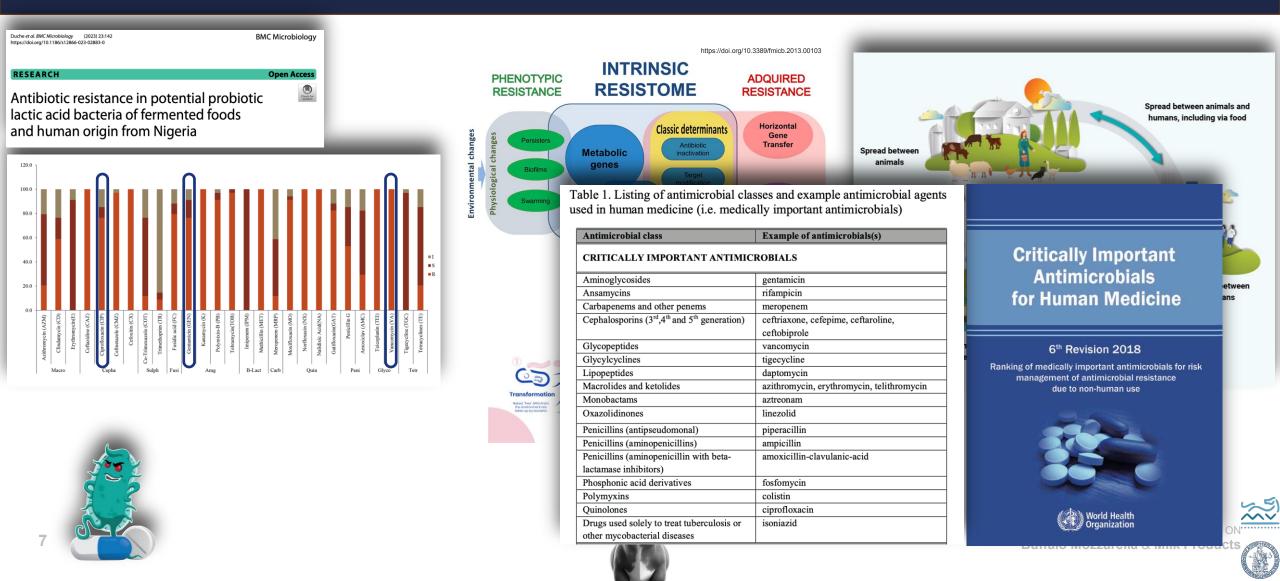


#### 88.57% of strains were multi-resistant



#### Overall AMR picture

## Discussion



## New ideas, future prospects and Conclusions



# ARGs analysis

The awareness that LAB can present AMR genes transferable to other microorganisms, including pathogenic ones, must move the scientific/managerial communities towards monitoring plans that better allow the surveillance and containment of the spread of AMR.

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

# Grazie per l'attenzione!

### Rosa Luisa Ambrosio

DVM, Ph.D., Post Doc fellow

rosaluisa.ambrosio@unina.it



Differential SCC and MPO evaluation as buffaloes milk indicators of udder status under heat stress FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

M. G. Ciliberti, A. Santillo, M. Caroprese, R. Marino, A. Sevi, M. di Corcia, M. Albenzio

Department of Agriculture, Food, Natural Resources, and Engineering (DAFNE), University of Foggia, 71122, Foggia, Italy

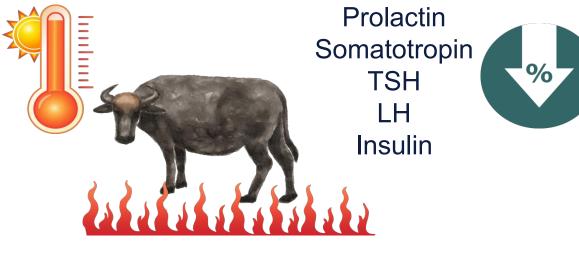






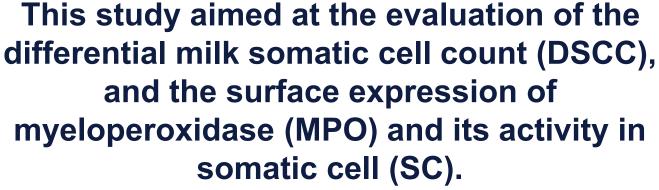
## Context

When addressing GW and livestock species, heat stress is the most important outcome that negatively impacts animals' health, welfare, and productive performance (Bernabucci, 2019).



%

Milk yield and composition Growth Disease resistance Reproduction efficiency





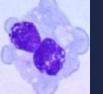
# Methods

### SUMMER

3 THI classes THI < 72 (no HS) THI>72<76 (moderate HS) and THI > 76 (severe HS)



**SPRING** 



Macrophages

Abs panel crossreactivity with buffalo



PNMs



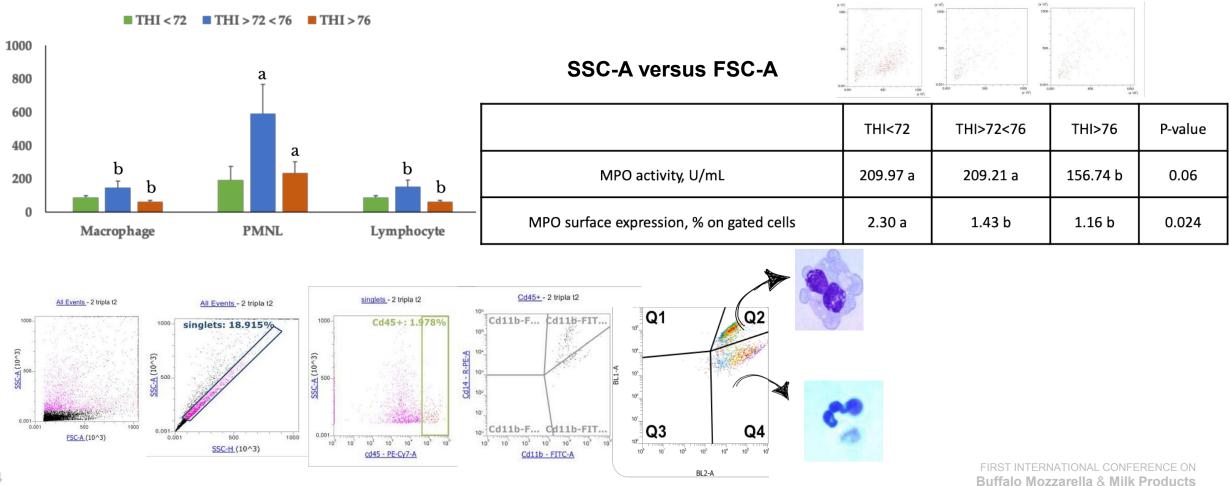


Lymphocytes

MPO surface expression MPO activity by ELISA

### **Results**

Morphological dot plot of positive somatic cell to MPO-FITC antibody



4

# Grazie per l'attenzione!

Maria Giovanna Ciliberti

☑ maria.ciliberti@unifg.it

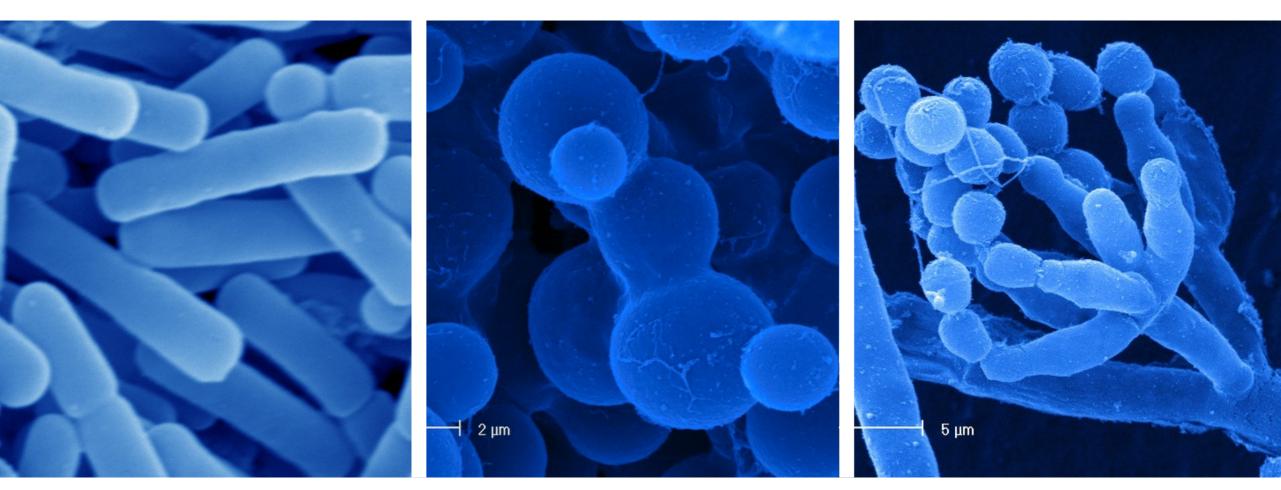


## To be or not to be safe and sustainable? Comparing different scenario in labgrown food and dairy trades

Pier Sandro Cocconcelli Università Cattolica del Sacro Cuore Piacenza-Cremona piercocconcelli@unicattit

## Cell Factories for protein production

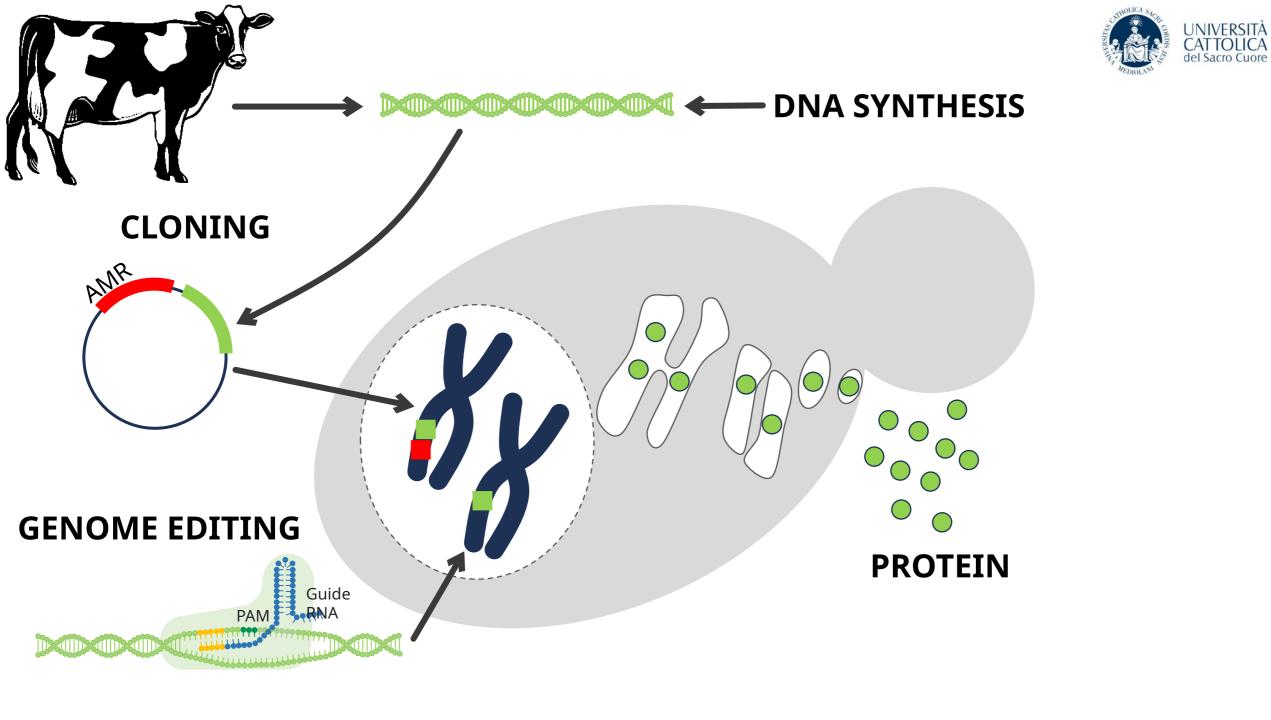




### BACTERIA



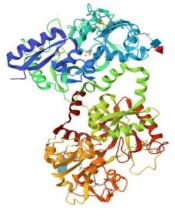
### FUNGI



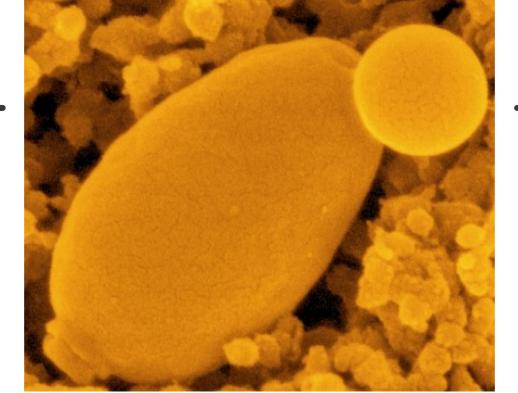
### **ENZYMES**+



**CHYMOSIN** 



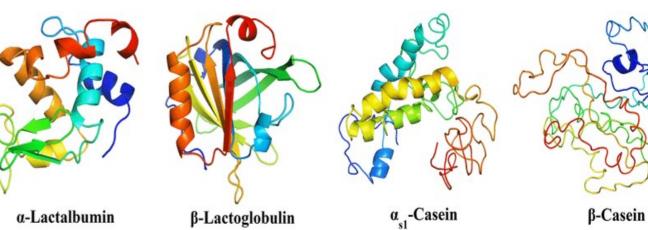
**LACTOFERRIN** 











a-Lactalbumin

β-Lactoglobulin

β-Casein

# Milk Components



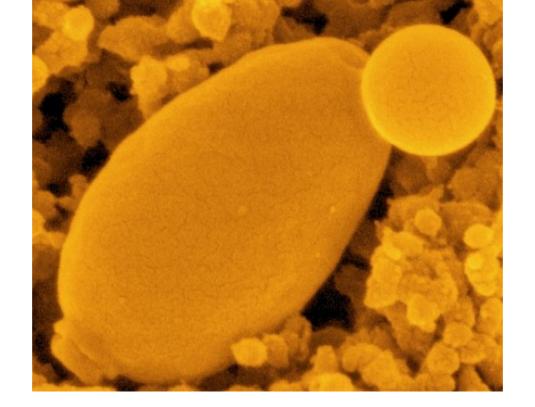
|   |  |   |   | The DIOLANS   |  |
|---|--|---|---|---|--|
| Proteins<br>Casein Proteins (80% of total mi<br>αS1-casein (Alpha S1-casein): 32-3<br>αS2-casein (Alpha S2-casein): 8-10<br>β-casein (Beta-casein): 25-28%<br>κ-casein (Kappa-casein): 8-10%<br>Whey Proteins (20% of total mil<br>β-lactoglobulin (Beta-lactoglobulin<br>α-lactalbumin (Alpha-lactalbumin)<br>Immunoglobulins (IgG, IgA, IgM):<br>Bovine Serum Albumin (BSA): 5-10<br>Lactoferrin: < 1%<br>Lysozyme: Trace amounts<br>Minerals<br>Calcium (Ca):<br>Phosphorus (P): | 35% of total milk prote<br>0%<br>( <b>k protein)</b><br>n): 50% of whey prote<br>): 20-25%<br>: 10-15%<br>0%<br><b>Vitamin</b> B1 (Thiamin<br>Vitamin B2 (Riboflay<br>Vitamin B3 (Niacin)<br>Vitamin B5 (Pantoth | Glutami<br>eineucine<br>Lysine<br>Aspartic<br>Arginine<br>Isoleuci<br>eineroline<br>Valine<br>Phenyla<br>Histidin<br>Methior<br>Tryptop | ic acid<br>c acid<br>e l<br>ne<br>alanine<br>e<br>nine<br>han                             | Triglycerides:<br>component, 98% of total milk fat<br>Phospholipids: ~1%, essential for cell me<br>Cholesterol: ~0.3%<br>Fatty Acids: Includes both saturated (65-7)<br>unsaturated fatty acids (30-35%)<br>Saturated:<br>Palmitic acid,<br>Myristic acid<br>Stearic acid<br>Unsaturated:<br>Oleic acid,<br>Linoleic acid<br>α-linolenic acid |  |
| Magnesium (Mg):<br>Potassium (K)<br>Sodium (Na):<br>Chloride (Cl):<br>Trace minerals:<br>Zinc (Zn), Copper (Cu),<br>Iron (Fe) Iodine (I)<br>Selenium (Se)   | acid)<br>Vitamin B6 (Pyridox<br>Vitamin B7 (Biotin)<br>Vitamin B9 (Folate)<br>Vitamin B12 (Cobala<br>Vitamin C (Ascorbic<br>Vitamin A (Retinol)<br>Vitamin D<br>Vitamin E<br>Vitamin K                           | ine)<br>amin)   | <b>Enzymes</b><br>Lactoperoxida<br>Xanthine oxida<br>Lipase<br>Alkaline phosp<br>Catalase | ase   |  |

# Milk Components



**Proteins Free Amino Acids Triglycerides: Casein Proteins (80% of total milk protein)** Glutamic acid component, 98% of total milk fat αS1-casein (Alpha S1-casein): 32-35% of total milk proteine Phospholipids: ~1%, essential for cell membran αS2-casein (Alpha S2-casein): 8-10% Lysine Cholesterol: ~0.3% β-casein (Beta-casein): 25-28% Aspartic acid к-casein (Kappa-casein): 8-10% Arginine Fatty Acids: Includes both saturated (65-70%) ar Whey Proteins (20% of total milk protein) Isoleucine unsaturated fatty acids (30-35%) β-lactoglobulin (Beta-lactoglobulin) Proline Saturated: α-lactalbumin (Alpha-lactalbumin): 20-25% Valine Palmitic acid, Immunoglobulins (IgG, IgA, IgM): 10-15% Phenylalanine Bovine Serum Albumin (BSA): 5-10% Myristic acid Histidine Lactoferrin: < 1% **Methionine** Stearic acid Lysozyme: Trace amounts Tryptophan Vitamins Unsaturated: Vitamin B1 (Thiamine) Oleic acid, **Minerals** Vitamin B2 (Riboflavin) Linoleic acid Calcium (Ca): Vitamin B3 (Niacin) α-linolenic acid Phosphorus (P): Vitamin B5 (Pantothenic Magnesium (Mg): Short-chain fatty acids: acid) Potassium (K) Vitamin B6 (Pyridoxine) Butyric acid Sodium (Na): Vitamin B7 (Biotin) Caproic acid Chloride (Cl): Enzymes Vitamin B9 (Folate) Caprylic acid Lactoperoxidase Trace minerals: Vitamin B12 (Cobalamin) Xanthine oxidase Zinc (Zn), Copper (Cu), Vitamin C (Ascorbic acid) Iron (Fe) Iodine (I) Lipase Vitamin A (Retinol) Selenium (Se) Alkaline phosphatase Vitamin D Catalase Vitamin E

Vitamin K



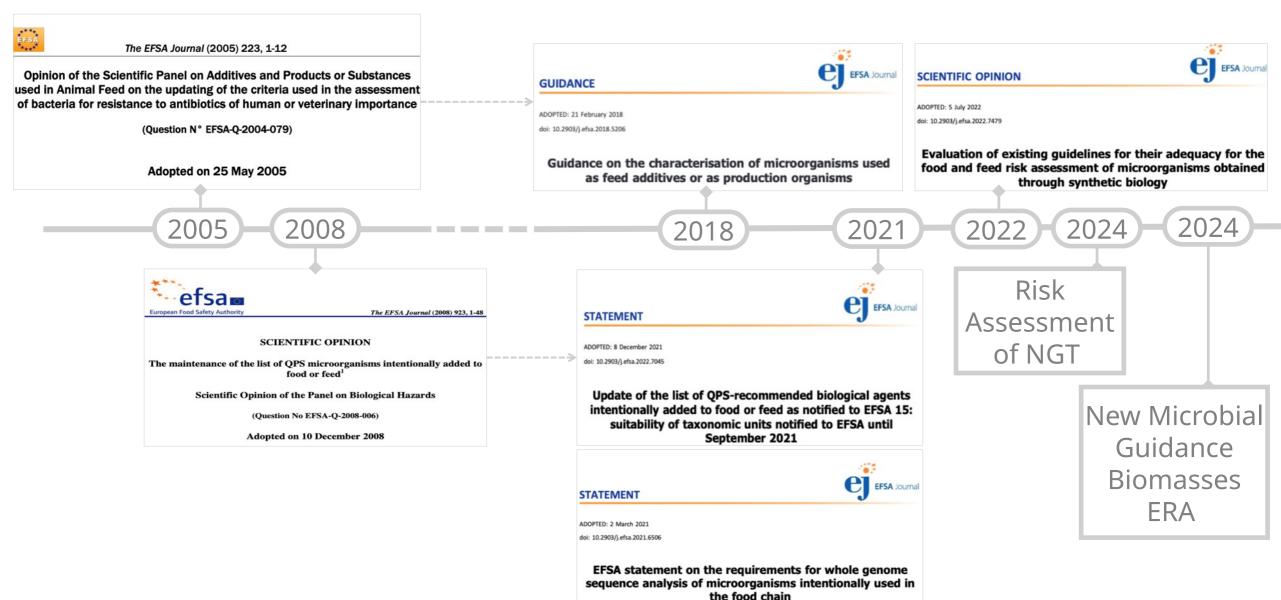


### SAFETY

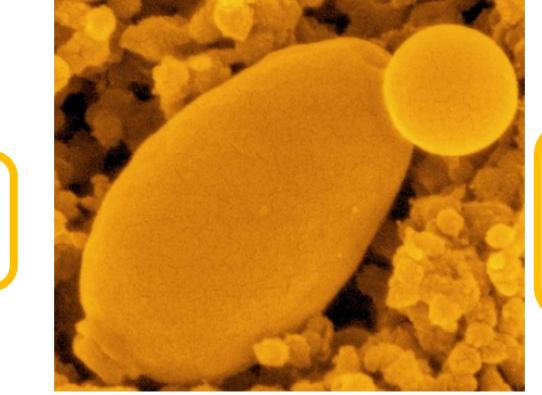
# SUSTAINABILITY

# Risk Assessment of microorganisms and their products non-GM - GM - NGT (genome editing)





European Food Safety Authority (EFSA)



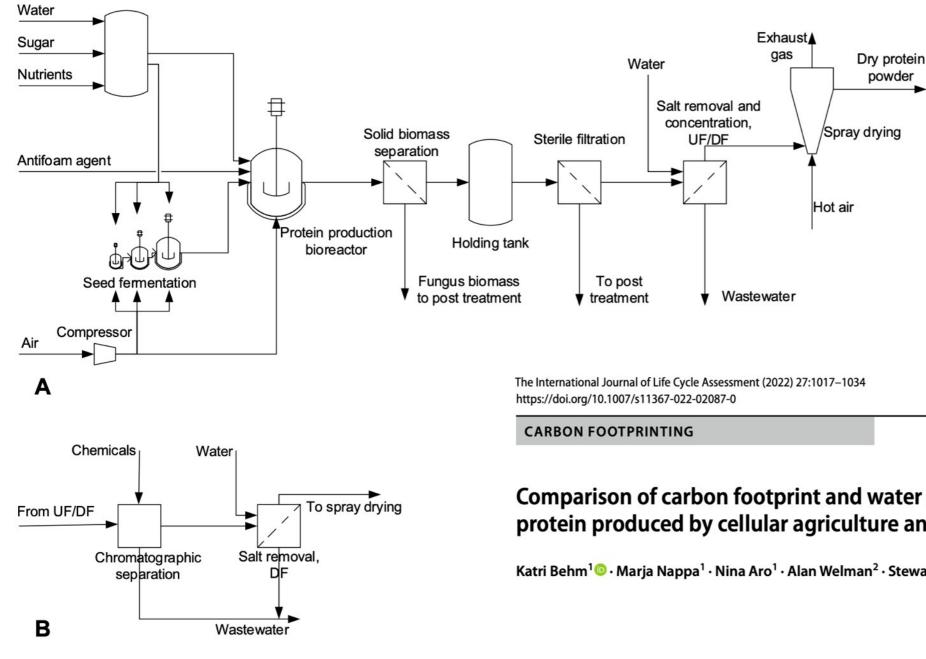


Food Enzymes Processing Aids Food/Feed additives Novel Foods

- Whole genome Sequence and GMM assessment of the strains
- Presence of Antimicrobial Resistance Gene(s)
- Toxicology

**SAFETY** 

- Manufacturing process
- Presence/Absence of viable cells and DNA of the production host







#### Comparison of carbon footprint and water scarcity footprint of milk protein produced by cellular agriculture and the dairy industry

Katri Behm<sup>1</sup> · Marja Nappa<sup>1</sup> · Nina Aro<sup>1</sup> · Alan Welman<sup>2</sup> · Stewart Ledgard<sup>3</sup> · Marjut Suomalainen<sup>1</sup> · Jeremy Hill<sup>2,4</sup>



### **SUSTAINABILITY - efficiency - LCA**

| V |
|---|
|   |
| 1 |
|   |

| volume | protein<br>yield/d | protein<br>kg/d | hard<br>cheese |
|--------|--------------------|-----------------|----------------|
| 45 L   | 3.3%               | 1.44            | 4.5            |
| 1000 L | 0.13%              | 1.33            | ?              |



FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Breeding techniques and nutritional quality of milk productions

Dr. Angela Salzano

Dipartimento di Medicina Veterinaria e Produzioni Anima Università degli Studi di Napoli – Federico II



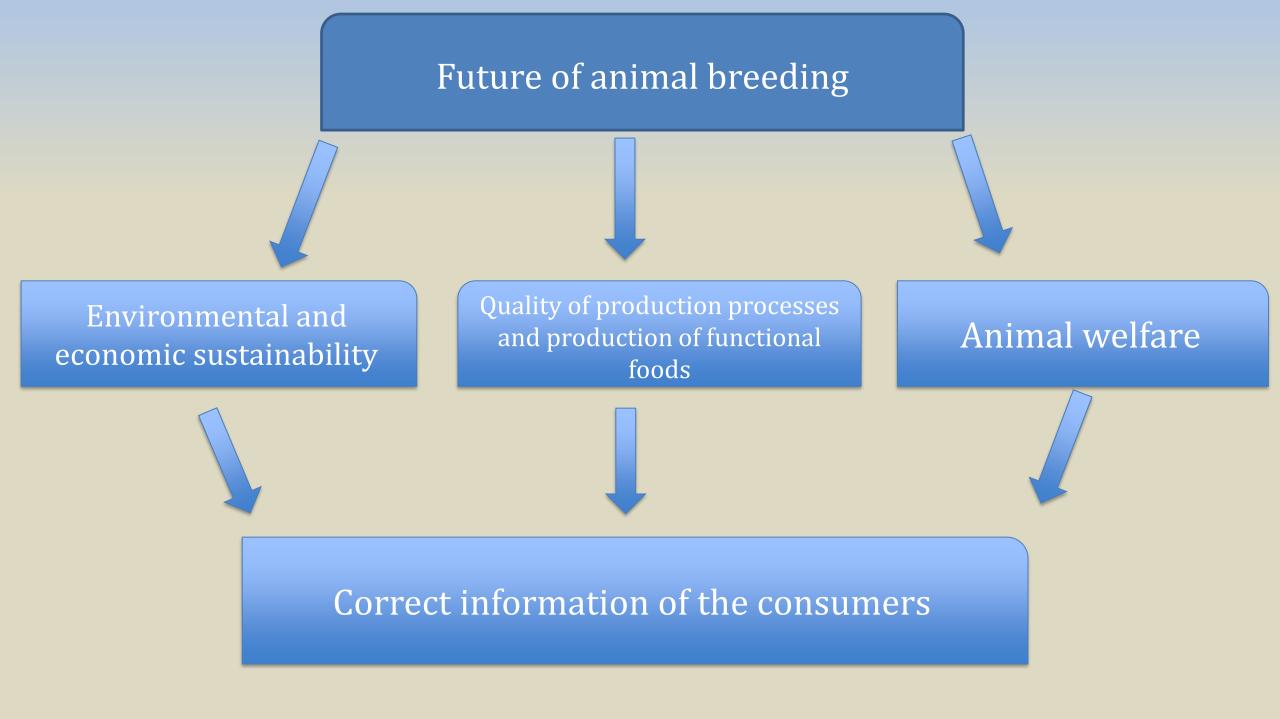
Describer of a second and a second a seco

Powered by

# Problems related to animalderived products

- ✓ "Intensification" of livestock systems
- ✓ Excessive exploitation of farm animals
- ✓ Increased exploitation of environmental resources
- Excessive use of antibiotics and synthetic products (residues)
- ✓ Use of GMOs

### Lack of trust in animal-derived products



# Functional properties of buffalo products

Formazione dei Radicali Liberi

Danneggiamento del DNA

100

Globuli bianchi

Inquinamento

Metabolismo

02'----- H2O2 --- OH

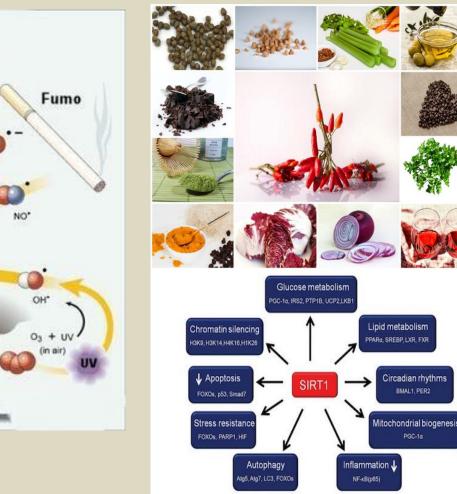
Infiammaz joni

OH.

Radiazioni



SIRT foods: foods for longevity



### **ACTIVITY OF MILK BIOPEPTIDES**

Opioid-like and anti-opioid activity
 Caseomorphins – Lactorphines;
 Casoxin;

• Antihypertensive activity Casochinine – Lactochinine;

• Antithrombotic activity **Caseoplateline**;

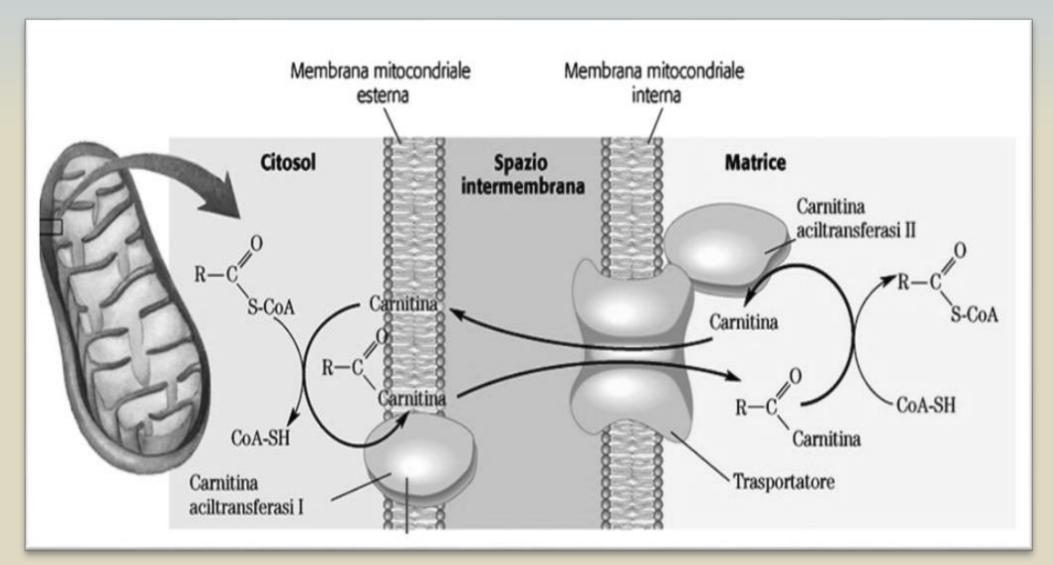
• Fixing activity of minerals \_\_\_\_\_\_ Phosphopeptides

### MILK AND NUTRITIONAL PROPERTIES: CARNITINE

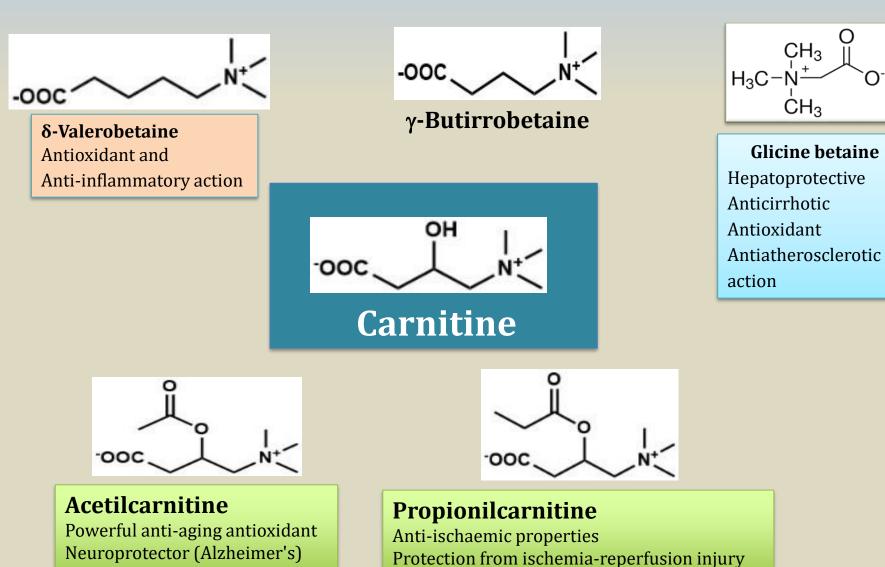
Milk and dairy products, together with fish and meat, are the main source of **carnitine**. Chemically, carnitine belongs to the methylamine family and, despite being structurally similar to an amino acid, it does not form proteins, and is more similar to acetylcholine. It is a short-chain carboxylic acid and amino acid.

$$CH_{3} \xrightarrow[]{} CH_{3} \\ H_{3} \xrightarrow[]{} CH_{2} \xrightarrow[]{} CH_{2} - CH - CH_{2} - COO^{-} \\ H_{3} \\ CH_{3} \\ OH$$

### CARNITINE MEDIATED THE TRANSPORT OF FATTY ACIDS INTO THE MITOCHONDRIA



### **Bio-molecules of buffalo milk**



Improve endothelial function



### $\delta$ -Valerobetaine in different milk sources

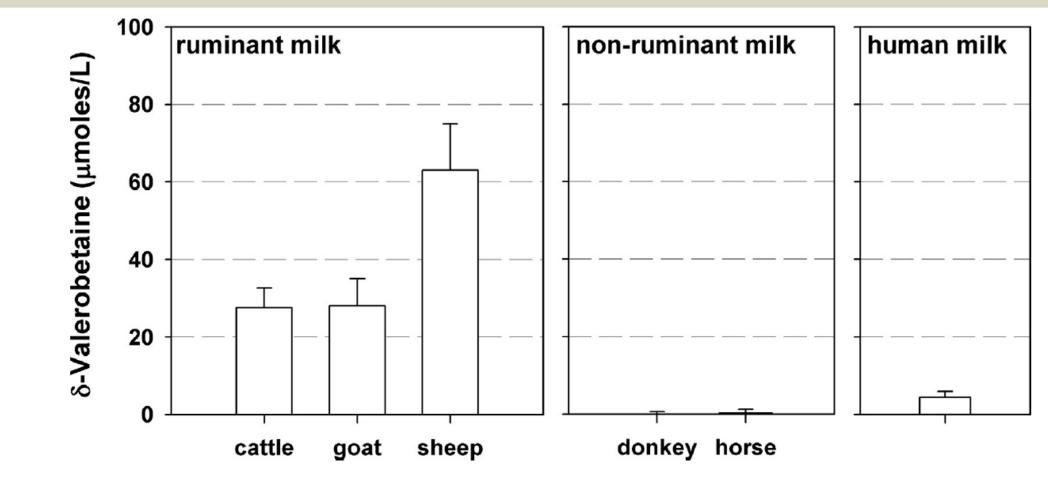
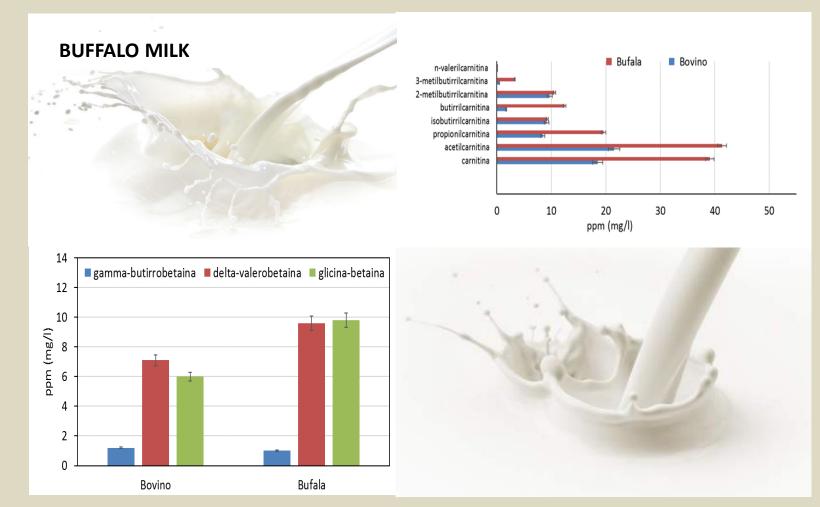


Fig. 3. Distribution of  $\delta$ -valerobetaine content in ruminant and non-ruminant milk.

### **Biomolecules of buffalo milk**



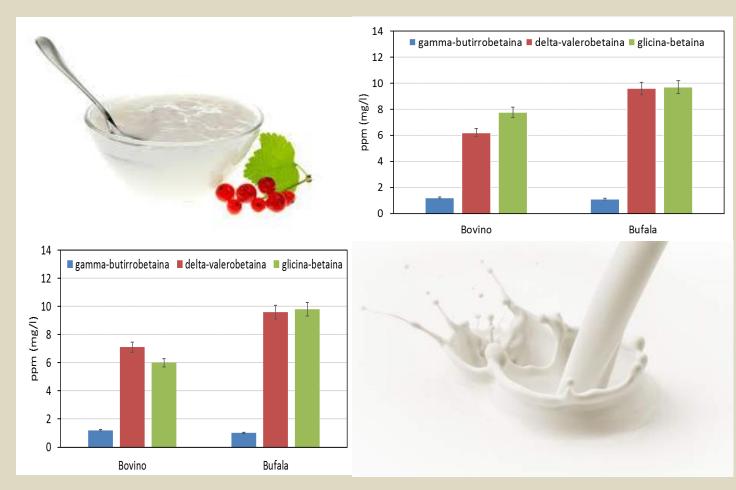
Buffalo milk is rich in carnitine, acetyl carnitine, propionyl carnitine, d-valerobetaine and glycine-betaine compared to bovine milk

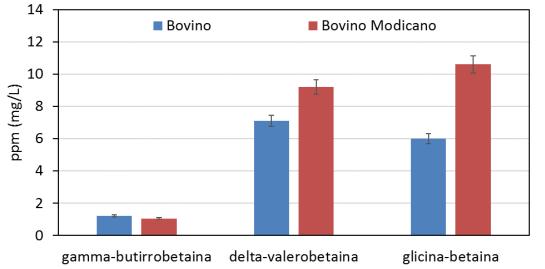




### **Biomolecules of buffalo milk**

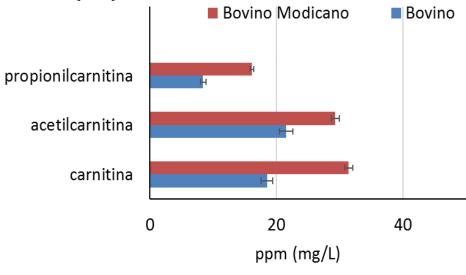
...as well as yogurt and whey...



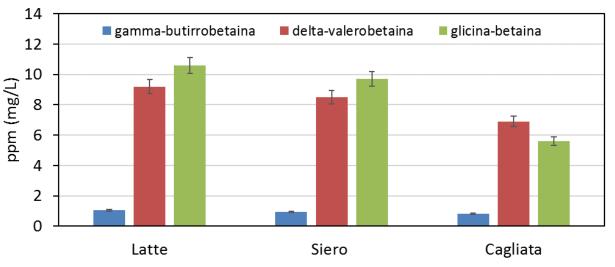


#### Betaine levels in Holstein (blue) and Modicana (red) milk

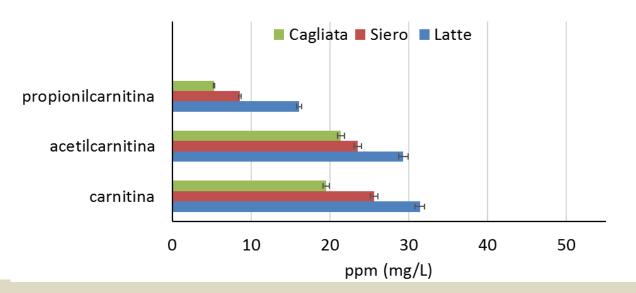
#### Carnitine and acil-carnitine levels in Holstein (blue) and Modicana (red) milk



#### Betaine levels in Modicana milk and dairy products



#### Carnitine levels in Modicana milk and dairy products



### Use of breeding techniques

### **Availability of space**

A greater availability of per capita space increases the nutraceutical power of milk (Salzano et al., 2019)



Using green forage, especially alfalfa, in the diet increases the health-promoting power of the milk (Salzano et al., 2021)







# **Biomolecules of buffalo milk**

|            |                    | Biomolecules <sup>1</sup> expressed in mg/l |                       |                       |                     |                     |         |                       |                       |  |
|------------|--------------------|---|-----------------------|-----------------------|---------------------|---------------------|---------|-----------------------|-----------------------|--|
| Product    | Group <sup>2</sup> | Car   | C <sub>2</sub> Car    | C <sub>3</sub> Car    | IC <sub>4</sub> Car | nC <sub>4</sub> car | γ-BB    | δ-VB                  | glyBet                |  |
| Milk       | S15                | 56,7±1,1 <sup>A</sup>                       | 51,9±0,3 <sup>A</sup> | 34,8±1,0 <sup>A</sup> | 10,9±0,6            | 14,2±1,2            | 6,6±0,2 | 24,2±0,5 <sup>A</sup> | 23,1±2,0 <sup>A</sup> |  |
|            | S10                | 39,8±0,7 <sup>B</sup>                       | 39,7±0,7 <sup>B</sup> | 21,0±0,9 <sup>B</sup> | 11,3±0,9            | 12,6±0,9            | 6,1±0,2 | 16,7±0,5 <sup>B</sup> | 13,5±1,6 <sup>B</sup> |  |
| Whey       | S15                | 40,9±0,8 <sup>A</sup>                       | 41,1±1,7 <sup>A</sup> | 26,9±0,8 <sup>A</sup> | 10,4±1,2            | 14,3±1,2            | 6,0±0,4 | 22,0±0,9 <sup>A</sup> | $10,7\pm0,4^{A}$      |  |
|            | S10                | 31,7±0,7 <sup>B</sup>                       | 28,7±2,6 <sup>B</sup> | 17,6±1,2 <sup>B</sup> | 11,3±1,3            | 13,6±1,3            | 5.9±0,3 | 15,5±0,7 <sup>B</sup> | 7,9±0,5 <sup>B</sup>  |  |
| Mozzarella | S15                | 27,0±0,7                                    | 12,2±1,0              | 6,0±0,3               | 2,4±0,2             | 2,6±0,1             | 2,3±0,2 | 6,1±0,1               | 4.3±0.5               |  |
|            | S10                | 28,3±1,3                                    | 12,0±1,0              | 6,3±0,3               | 2,1±0,2             | 2,5±0,2             | 2,6±0,3 | 5,9±0,1               | 3,8±0,4               |  |
| Ricotta    | S15                | 44,2±2,2                                    | 39,3±1,4              | 22,5±0,9              | 9,8±0,7             | 11,1±0,5            | 6,5±0,3 | 15,7±0,3              | 14,9±0,8              |  |
|            | S10                | 41,4±0,9                                    | 36,8±0,8              | 23,9±0,7              | 10,7±0,5            | 11,7±0,7            | 6,1±0,2 | 16,0±0,4              | 15,6±0,7              |  |

<sup>1</sup>Car = l-carnitine; C2Car = acetilcarnitine; C3Car = propionilcarnitine;  $\delta$ -VB =  $\delta$ -valerobetaine;  $\gamma$ -BB =  $\gamma$ -butirrobetaine; iC4Car = isobutirrilcarnitine; nC4Car = butirrilcarnitine; glyBet = glicine betaine.

 $^{2}$ S15= pro capita space of 15m<sup>2</sup> S10= pro capita space of 10m<sup>2</sup>

 $^{A, B}$ , Values whitin columns with different quotes differ; P <0.01

### Influence of animal welfare (space availability: 10 vs. 15 m<sup>2</sup>)



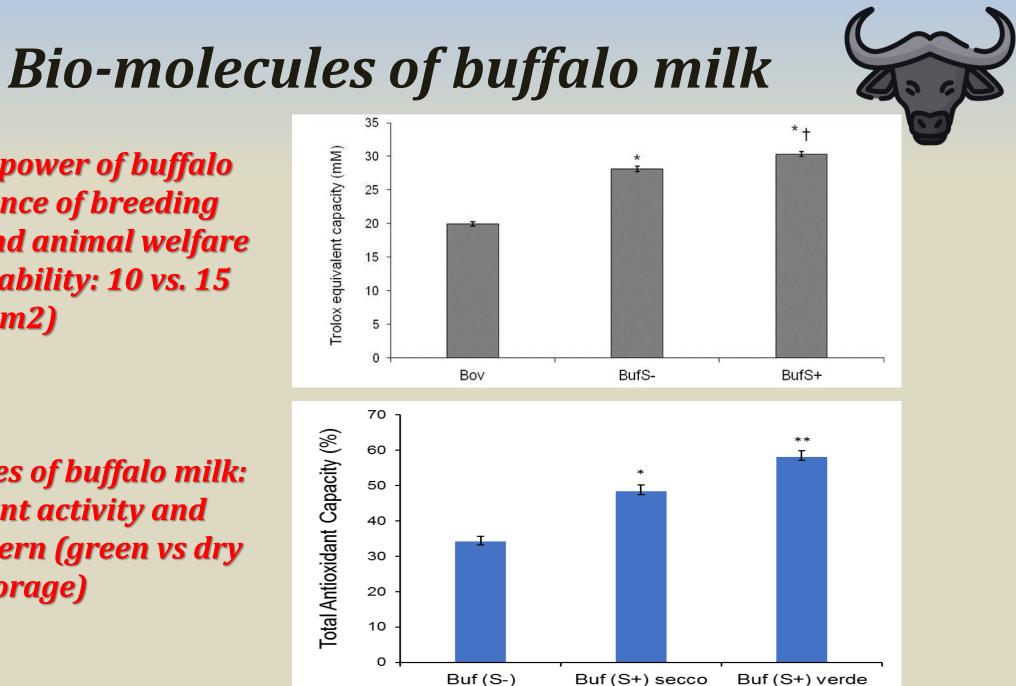
# Biomolecules of buffalo milk

|              |                    | Biomole               | Biomolecules <sup>1</sup> expressed in mg/l |                                 |                     |                     |                       |             |                           |  |  |
|--------------|--------------------|-----------------------|---|---------------------------------|---------------------|---------------------|-----------------------|-------------|---------------------------|--|--|
| Product      | Group <sup>2</sup> | Car                   | C <sub>2</sub> Car                          | C <sub>3</sub> Car              | IC <sub>4</sub> Car | nC <sub>4</sub> car | δ-VB                  | γ-BB        | glyBet                    |  |  |
| Milk         | dry                | 31.5±0.8 <sup>a</sup> | 39.1±1.3 <sup>a</sup>                       | 13.5±1.3 <sup>a</sup>           |                     |                     | 18.3±0.4ª             | 4.8±0.3     | 7.01±1.7                  |  |  |
|              | green              | 41.6±0.5 <sup>b</sup> | 49.7±0.8 <sup>b</sup>                       | 20.5±1.7 <sup>b</sup>           |                     |                     | 21.8±0.4 <sup>b</sup> | 3.3±1.1     | 7.11±0.7                  |  |  |
| Yogurt       | dry                | 35.7±1.7              | 24.8±5.2                                    | 25.5±5.6                        | 10.9±0.5            | 11.6±0.8            | 17.2±0.5              | 5.6±0.2     | 5.2±0.5                   |  |  |
|              | green              | 31.1±3.6              | 20.6±2.0                                    | 23.0±1.6                        | 8.6±1.2             | 8.4±0.6             | 14.0±3.0              | 3.4±0.6     | 4.9±0.2                   |  |  |
| Mozzarella   | dry                | 15.1±1.2              | 8.8±0.3                                     | 1.4±0.1                         | 0.6±0.1             | 0.4±0.1             | 1.6±0.3               | 6.3±0.1     | 2.0±0.2                   |  |  |
|              | green              | 13.6±0.1              | 7.3±1.2                                     | 1.1±0.2                         | 0.6±0.1             | 0.4±0.1             | 1.4±0.2               | 5.0±0.8     | 2.0±0.7                   |  |  |
| Ricotta      | dry                | 23.8±0.3              | 15.9±0.4                                    | 7.2±1.1                         | 3.3±0.1             | 2.2±0.2             | 1.2±0.2               | 13.2±0.8    | 6.6±0.7                   |  |  |
|              | green              | 21.1±0.5              | 13.8±0.6                                    | 5.9±1.0                         | 3.0±0.5             | 1.9±0.3             | 1.4±0.4               | 11.0±2.3    | 14.9±1.3                  |  |  |
| Whey         | dry                | 39.2±0.1              | 24.1±2.5                                    | 27.1±2.5                        | 11.4±0.5            | 15.8±0.5            | 2.2±0.3               | 23.3±0.8    | 9.3±0.3                   |  |  |
|              | green              | 31.2±0.5              | 24.4±2.5                                    | 25.1±0.9                        | 10.6±0.0            | 14.7±0.5            | 2.7±0.3               | 20.9±0.5    | 8.5±0.7                   |  |  |
| 1Cor = 1 cor | rnitina, C2C       | or – ocotila          | annitina. C                                 | $C_{\alpha n} = n_{n \alpha n}$ | ioniloonni          | ting & VD -         | - S valarah           | ataina. v D | $\mathbf{D} = \mathbf{v}$ |  |  |

<sup>1</sup>Car = l-carnitine; C2Car = acetilcarnitine; C3Car = propionilcarnitine; δ-VB = δ-valerobetaine; γ-BB = γbutirrobetaine; iC4Car = isobutirrilcarnitine; nC4Car = butirrilcarnitine; glyBet = glicine betaine.

 $^{a, b}$ , Values whitin columns with different quotes differ; P <0.05

### Influence of feeding regimen (green vs. dry forage)



Antioxidant power of buffalo milk: influence of breeding techniques and animal welfare (space availability: 10 vs. 15 m2)

**Bio-molecules of buffalo milk:** antioxidant activity and dietary pattern (green vs dry forage)

### What happens inside the rumen?

The study was carried out over 60 days by using Italian Mediterranean dairy buffaloes (n=16; 8 per group). Animals were randomly assigned to two homogeneous groups (Control and Treated) according to parity, age, days in milk and average milk production.

Control buffaloes received a total mixed ration (TMR) whilst treated buffaloes received TMR + green forage which comprised ryegrass (30% of the diet).

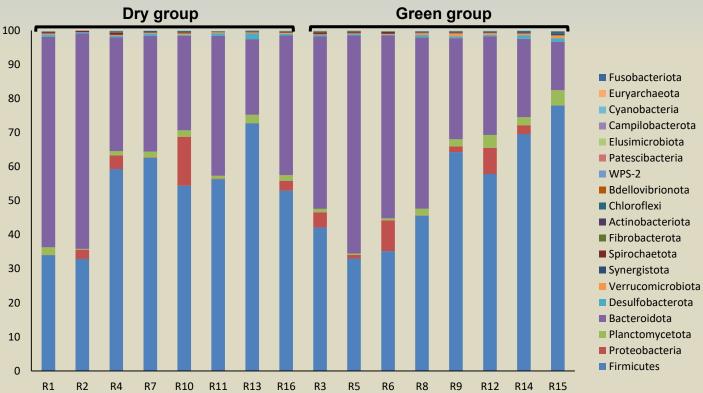
The two diets were created isonitrogenous and isoenergetic and differed only in the inclusion of ryegrass in treated animals.

At the end of the trial, samples of rumen and rumen liquid were collected for molecular studies.

| Item                  | Control<br>buffaloes | Treated<br>buffaloes |
|-----------------------|----------------------|----------------------|
| Component             | Amount (l            | kg of feed)          |
| Ryegrass              | -                    | 25                   |
| Corn silage           | 18                   | 13                   |
| Alfalfa hay           | 5                    | 1                    |
| Soybean meal<br>(48%) | 1.6                  | -                    |
| Concentrate           | 4.4                  | 4                    |
| Corn meal             | 1                    | 1.8                  |
| Hydrogenated fats     | 0.3                  | 0.3                  |
| Calcium Carbonate     | 0.1                  | -                    |
| Salt 1:3              | 0.1                  | 0.1                  |
| Vitamins              | 0.1                  | -                    |
| Composition           | n (% on dry matte    | er intake)           |
| Dry matter            | 16.5                 | 16.6                 |
| СР                    | 14.7                 | 14.7                 |
| Fat                   | 6.0                  | 7.0                  |
| NDF                   | 36.8                 | 36.8                 |
| ADF                   | 21.2                 | 19.5                 |
| NSC                   | 33.8                 | 34.7                 |
| Starch                | 18.8                 | 18.8                 |
| Ash                   | 8.8                  | 6.8                  |
| Calcium               | 0.9                  | 1                    |
| Phosphorus            | 0.4                  | 0.4                  |
| MFU                   | 0.93                 | 0.93                 |

**Table.** Feed and chemical composition of the buffalo diets without (Control) or with (Treated) 30% green ryegrass. NDF, neutral detergent fiber; ADF, acid detergent fiber; NSC, non-structural carbohydrates; MFU, milk forage units.

### Green feed increases rumen bacterial diversity in dairy buffaloes



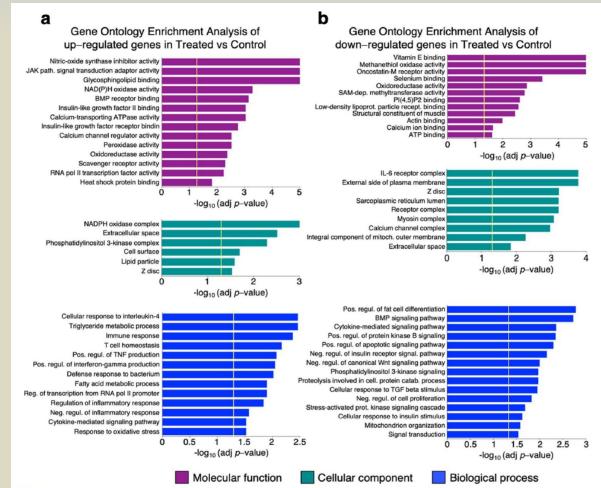
Green diet regimen slightly affects the microbial compostion of the rumen of water buffaloes. Rumens of animals fed green diet have an increased microbial biodiversity, mainly due to low (below 1%) represented bacterial genera. The metagenomic analysis indicated an increased number of metabolic functions associated to the rumen microbiota of the animals fed Green diet and different Carbohydrate-active enzymes that support the synthesis of functional biomolecules in milk.

Salzano et al., 2024 under review

### Transcriptomic profiles of buffaloes ruminal wall fed green forage

Green feed diet in ruminants exerts a beneficial effect on rumen metabolism and enhances the content of milk nutraceutical quality. We performed RNA-sequencing in the rumen of buffaloes fed green feed (treated) or TMR (control), and identified differentially expressed genes.

Green feed modulates biological processes relevant for the rumen physiology and, then, health and well-being of buffaloes, such as lipid and protein metabolism, response to the oxidative stress, immune response, and muscle structure and function.



**Fig. 4** Gene ontology enrichment analysis of differentially expressed genes. Selected GO-terms, enriched in genes up-regulated (**a**) and down-regulated (**b**) in rumen of buffaloes fed a TMR + green feed (Treated) in comparison with those fed the TMR diet (Control), are shown. GO terms were classified as molecular function (purple bars), cellular component (green bars) and biological process (blue bars). Bar graphs indicate the statistical significance of the enrichment, as -log<sub>10</sub> (adj *p*-value). Vertical yellow bars indicate the cut-off level for significance (*p* < 0.05, adjusted by Benjamini-Hochberg correction)

#### **GENES RELATED TO FACTORS INVOLVED IN THE REGULATION OF BIOLOGICAL PROCESSES**

#### ENERGY, AMINO ACID, AND LIPID METABOLISM

ARFGEF3: Codes for the BIG3 protein, which participates in the regulation of systemic glucose homeostasis, through the regulation of insulin and glucagon secretion.

HSD17B13: Associated with an increase in lipogenesis.

CDO1 and SELENBP1: Related to the metabolism of cysteine/methionine, serine, and lysine.

#### **CELLULAR RESPONSE TO STRESS**

**1** VNN1: Codes for the pantetheinase enzyme, involved in the production of pantothenic acid (vitamin B5) and coenzyme A (CoA).

#### **IMMUNE SYSTEM AND INFLAMMATION**

TRIM14: Codes for a protein belonging to the TRIM family, also known to promote the body's defense against viral infections.

IGFBP6: Involved in the immune response.

#### GENES RELATED TO FACTORS INVOLVED IN THE FUNCTIONALITY OF THE RUMEN

#### **ORGANIZATION OF THE EXTRACELLULAR MATRIX**

LAMA1 and COL4A6: Main constituents of the extracellular matrix.

COL1A1 and UGDH: Main constituents of the extracellular matrix.

#### **MUSCLE STRUCTURE/FUNCTION**

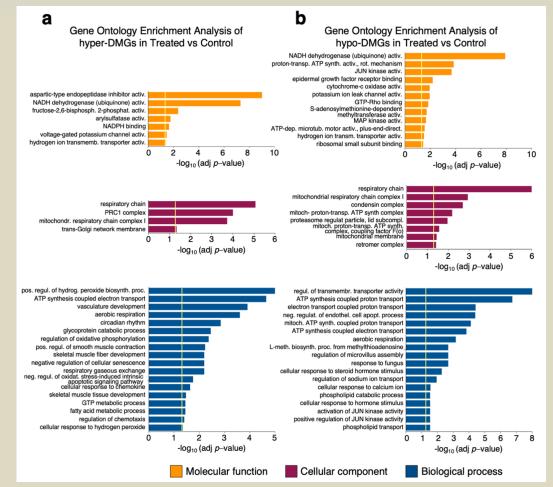
KCNK10, CACNG4 and ATP2B4: Codes for factors that modulate Ca2+ homeostasis.

### Green forage impacts on the DNA methylation in the ruminal wall of IMB

The aim of this study was to identify genomic regions differentially methylated in the ruminal wall of IMB fed green forage compared to a standard TMR diet, through the RRBS technique.

We highlighted 4648 genes associated with differentially methylated regions, the majority of which (82.4%) were protein-coding genes. Gene Ontology found categories related to response to oxidative stress, circadian rhythms, fungal infections and inflammation, rumen functionality, energy, lipid, and amino acid metabolism.

The integration of differential expression data with methylation data allowed to identify a discrete number of genes for which their expression varies as a function of DNA methylation.



Gene ontology enrichment analysis of genes associated with DMRs. Selected GO-terms, enriched in genes associated with hyper-DMRs (a) and hypo-DMRs (b) in the ruminal wall of BMI fed green forage in comparison with animals fed standard diet, are shown.

#### Fioriniello et al., 2024 under review

# Buffalo milk and rumen fluid metabolome are significantly affected by green feed

The study of milk metabolomic profile revealed a compositional differences between buffaloes fed TMR only (DD group) or green feed (ZG group). As resulted from the multi- and univariate analysis of the LC–MS dataset, DD and ZG groups cluster separately from each other when comparing samples collected at the same time point, differing in the abundance of specific metabolites (Table 2). In rumen fluid, among the molecules differentially accumulated, four have been putatively identified (Table 5). All these compounds are down regulated in control vs green feeding.

#### Milk

|                          |                        | Regulation DD vs ZG |      |        |             |             |               |  |
|--------------------------|------------------------|---------------------|------|--------|-------------|-------------|---------------|--|
| Compound                 | Monoisotopic mass (Da) | June                | July | August | LnFC (June) | LnFC (July) | LnFC (August) |  |
| γ-Butyrobetaine          | 145.1098               | Down                | Down | Down   | - 18.1088   | - 7.8222    | - 16.2603     |  |
| δ-Valerobetaine          | 159.1254               | n.d.                | Down | Down   | n.d.        | - 2.98936   | - 14.3323     |  |
| L-Carnitine              | 161.1048               | n.d.                | Down | Down   | n.d.        | - 3.20882   | - 14.6969     |  |
| Acetylcarnitine          | 203.1163               | Up                  | Down | Down   | 21.28232    | - 2.96594   | - 14.6381     |  |
| Propionylcarnitine       | 217.1312               | n.d.                | Down | Down   | n.d.        | - 14.4192   | - 10.7945     |  |
| Butyrylcarnitine         | 231.1485               | Up                  | Down | Up     | 22.13542    | - 2.93003   | 7.460587      |  |
| 2-Methylbutyrylcarnitine | 245.1644               | Up                  | Down | Up     | 19.65461    | - 3.0367    | 5.092634      |  |
| Glycerophosphocholine    | 257.1036               | Down                | Down | n.d.   | - 12.3487   | - 3.10791   | n.d.          |  |
| 2-Hexenoylcarnitine      | 257.1664               | Up                  | n.d. | n.d.   | 15.89597    | n.d.        | n.d.          |  |
| Hexanoylcarnitine        | 259.1796               | Down                | n.d. | n.d.   | - 15.6068   | n.d.        | n.d.          |  |

Table 2. Metabolites obtained from LC–MS data (positive mode), that are differentially accumulated in milk samples from buffaloes of DD Group (fed a total mixed ratio (TMR)) and ZG Group (fed TMR + 30% of green forage) analyzed for 3 months (June, July and August). Identifications were performed by comparing results with known compounds present in freely available electronic databases. Milk Composition Database (MCDB) and Bovine Metabolome Database (BMDB) were used for the identification. Up, up regulated; Down, down regulated; n.d., compounds not detected after statistical analysis. LnFC, Natural logarithm of Fold Change.

#### Rumen fluid

| Compound                       | Monoisotopic mass (Da) | Regulation DD vs ZG | LnFC (DD vs. ZG) |
|--------------------------------|------------------------|---------------------|------------------|
| 3-(2-hydroxyphenyl)-propanoate | 165.0338               | Down                | - 19.4548        |
| Indole-3-acrylic acid          | 187.0635               | Down                | - 4.18757        |
| Oleamide                       | 281.2724               | Down                | - 3.20791        |
| 20-Carboxy-leukotriene B4      | 366.2024               | Down                | - 6.38134        |

Table 5. Metabolites obtained from LC–MS data (positive mode), that are differentially accumulated in ruminal fluid samples from buffaloes of DD Group (fed a total mixed ratio (TMR)) and ZG Group (fed TMR + 30% of green forage). Identifications were performed by comparing results with known compounds present in a freely available electronic database. Bovine Metabolome Database (BMDB) was used for the identification. Up, up regulated; Down, down regulated. LnFC, Natural logarithm of Fold Change.

# Former food products (FFPs) and biomolecules in milk

Our hypothesis was to test if FFPs could be used as an alternative to green forages to improve rumen metabolism and ensures higher levels of carnitine precursors during all year round. The inclusion of FFPs containing 87% biscuit meal in the diets of dairy buffaloes reached similar values to green feed for  $\delta$ -valerobetaine and acetyl-L-carnitine while the antioxidant activity in milk and plasma is still higher in green feed compared to FFPs.

| Group                 | Green               | FFPs                |
|-----------------------|---------------------|---------------------|
| y-butyrobetaine       | $6.24 \pm 0.10^{A}$ | $5.87 \pm 0.10^{B}$ |
| δ-valerobetaine       | $17.4 \pm 0.14$     | $17.2 \pm 0.21$     |
| glycine betaine       | $15.5 \pm 0.14^{A}$ | $15.0 \pm 0.12^{B}$ |
| L-carnitine           | $39.6 \pm 0.36^{A}$ | $37.3 \pm 0.17^{B}$ |
| acetyl-L-carnitine    | $45.4 \pm 0.59$     | $43.8 \pm 0.71$     |
| propionyl-L-carnitine | $25.0 \pm 0.36^{A}$ | $22.3 \pm 0.32^{B}$ |

Note: Different letters along the row indicate statistically significant differences (<sup>A, B</sup> p<0.01). Values are expressed as mean ± SEM. (standard error of the mean). Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

TABLE 8 Total antioxidant activity (TAC) and Ferric Reducing Antioxidant Power (FRAP) in blood and milk

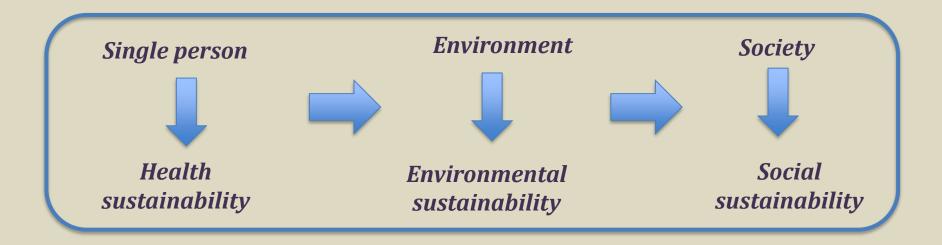
|      |        | Green                     | FFPs                        |
|------|--------|---------------------------|-----------------------------|
| TAC  | Milk   | 276.51 ± 9.31 b           | 252.45 ± 7.66 ª             |
| FRAP | MUK    | 240.62 ± 6.00 B           | 213.86 ± 4.89 A             |
| TAC  | Plasma | 77.52 ± 1.43 <sup>B</sup> | $71.66 \pm 1.74^{\text{A}}$ |
| FRAP | Plasma | 58.53 ± 2.22 b            | 51.10 ± 1.89 ª              |

Note: Different letters along the row indicate statistically significant differences (a,b p<0.05, A,B p<0.01). Values are expressed as mean ± SEM. (standard error of mean).

Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®). Abbreviations: TAC, total antioxidant capacity, FRAP, ferric reducing antioxidant power assay.

# Bio-molecules of buffalo products: quality nutrition

Offer the consumer food able to maintain health without negative effects on the environment



# Thanks for the attention

# BUFFALO MILK: A HEALTHY FOOD IN THE PREVENTION OF AGING DISEASES

FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Maria Luisa Balestrieri

Powered by

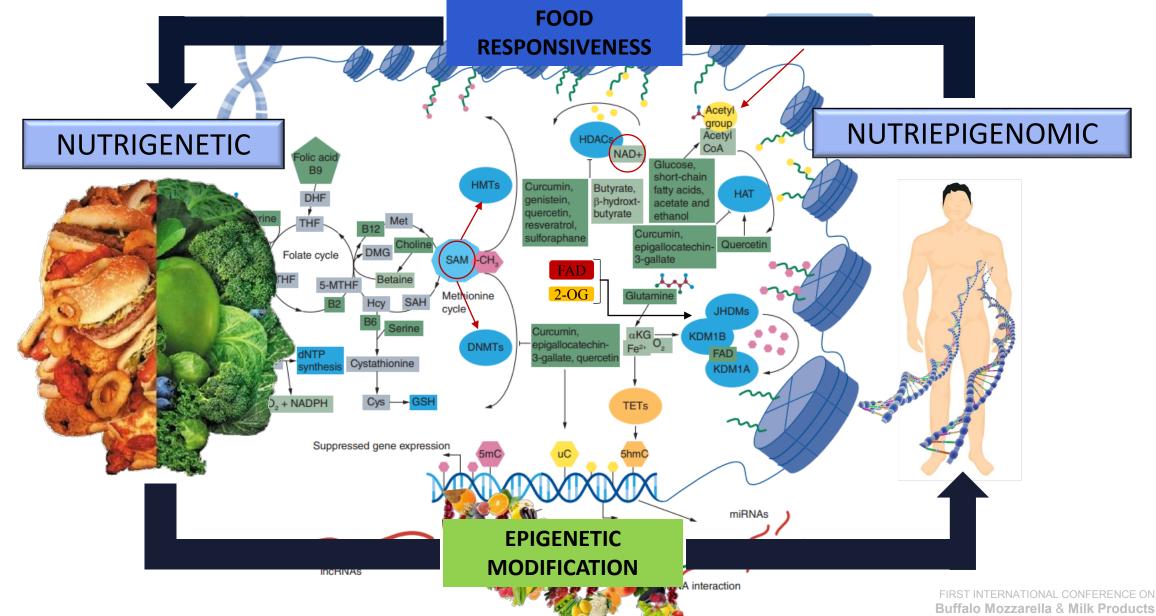


EDERICO II



Università Scuola di Medicina e Chirugia degli Studi Dipartimento di Medicina della Campania di Precisione Luigi Vanvitelli

#### Interplay among nutrients, metabolites and epigenetic pathways

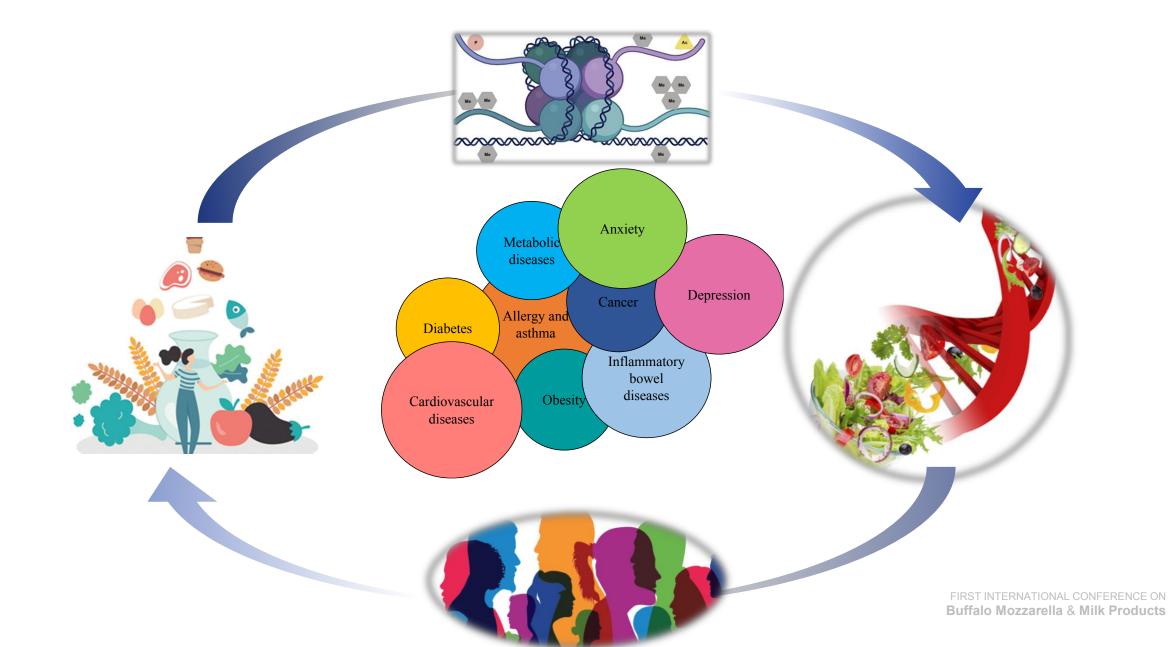


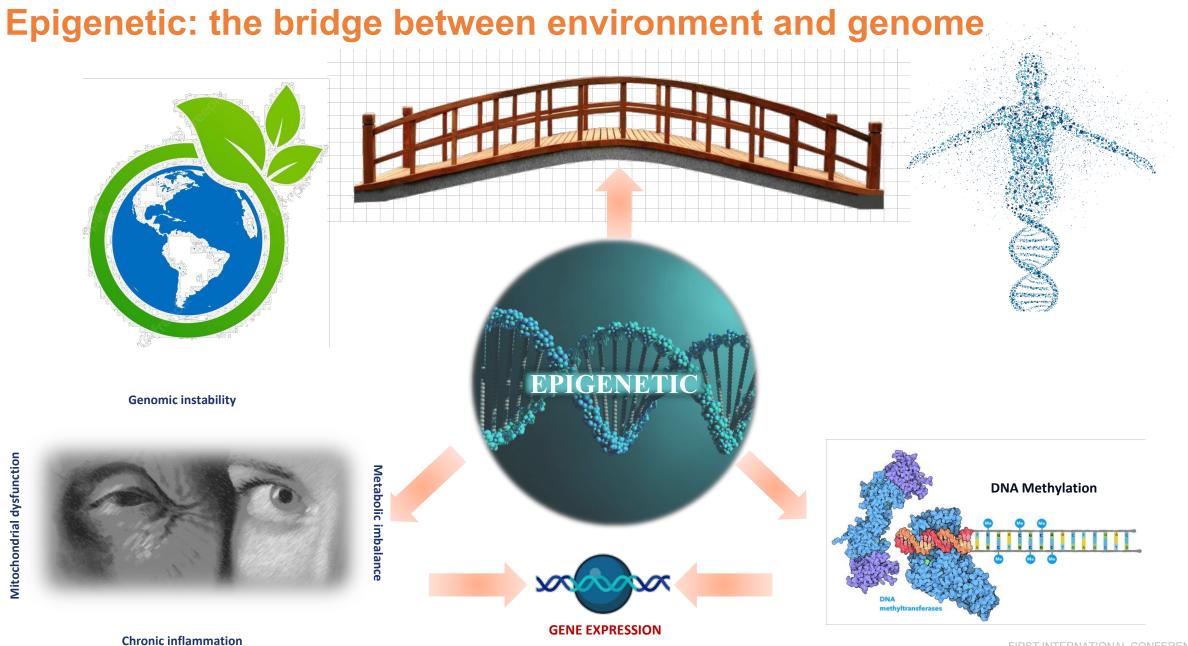
Coppedè F, et al. Epigenomics, 14(20), 1281–1304, 2022

3

#### Nutritional factors influence the epigenome

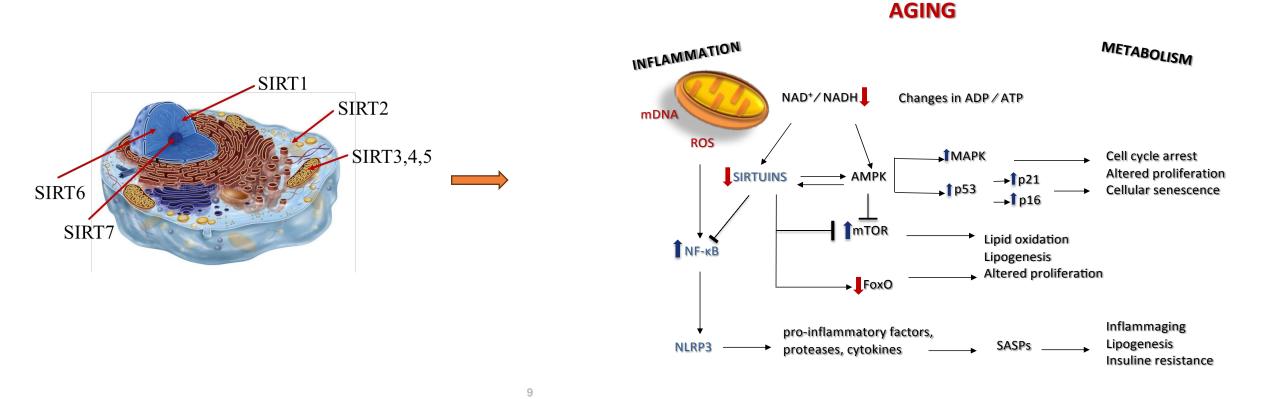
4





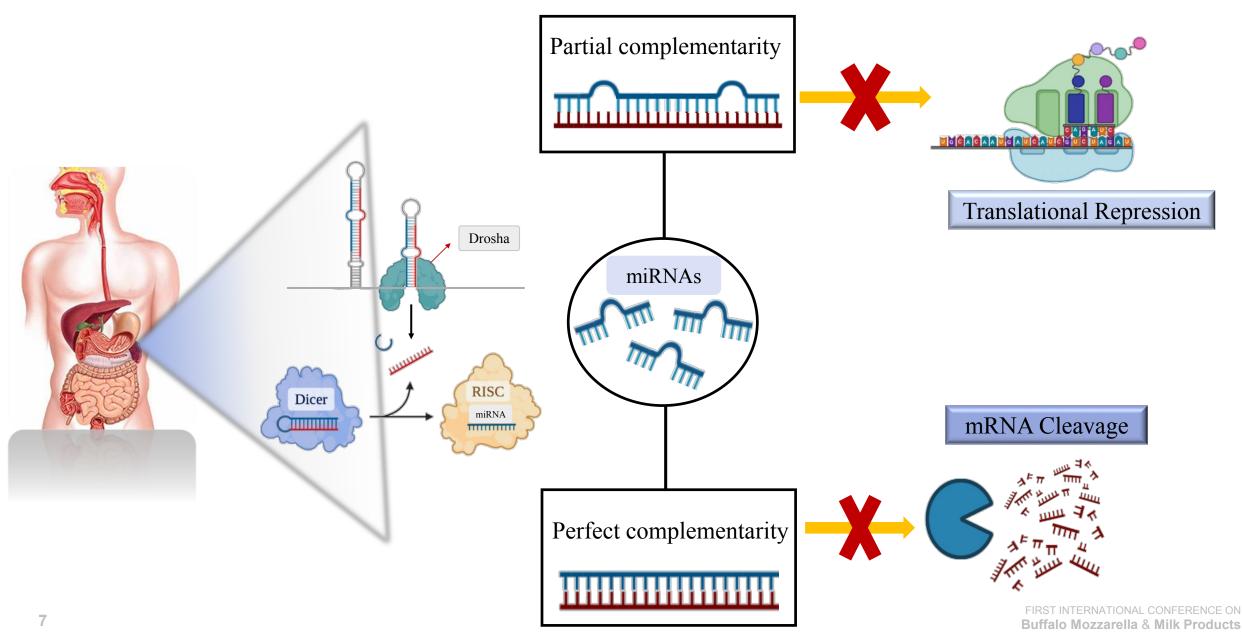
FIRST INTERNATIONAL CONFERENCE ON **Buffalo Mozzarella & Milk Products** 

## Sirtuin: the epigenetic stressor modulator



#### FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

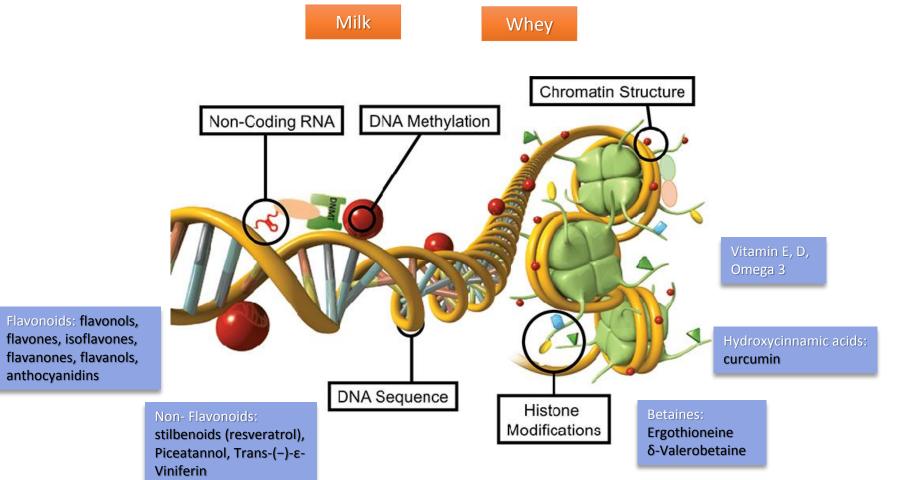
## **MicroRNAs in epigenetic regulation**



Molecular mechanism through which buffalo milk targets aging pathways

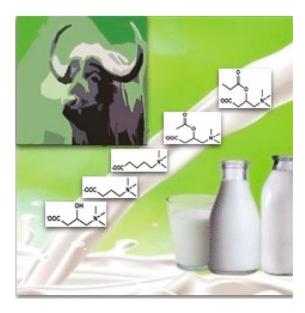


## **Dietary Epigenetic Modulators**



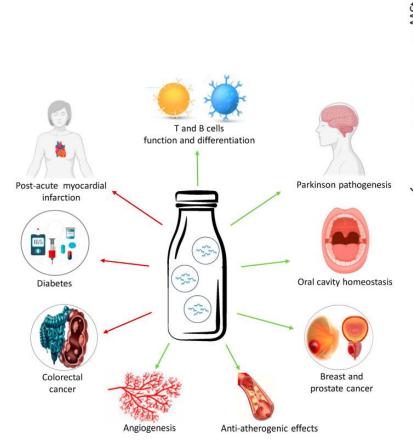
FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

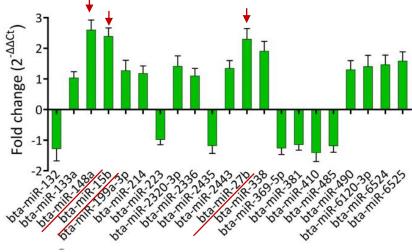
### **Epigenetic modulators of buffalo milk**



#### Betaine and short chain acyl-carnitines

Servillo L, et al. JAFC, 1;66(30):8142-8149, 2018

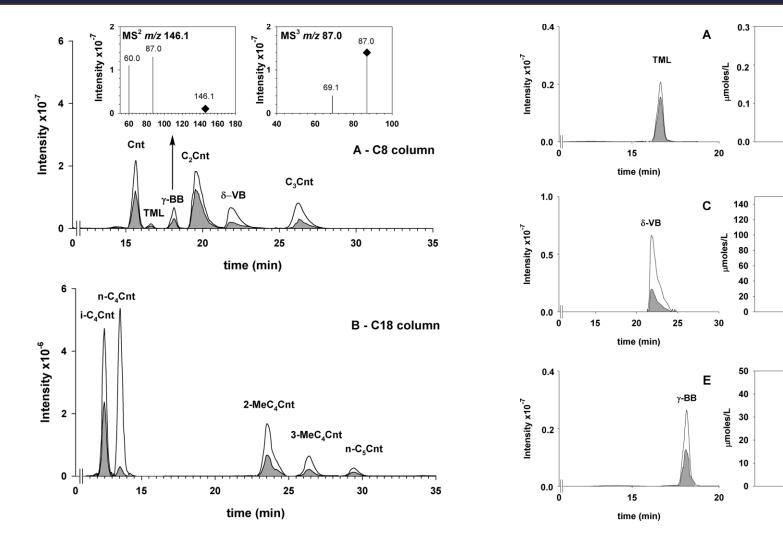




#### **Exosome containing microRNA**

Martino E, et al. Nutrients. 29;14(23):5081, 2022

#### Betaine profile of buffalo milk



Buffalo Mozzarella & Milk Products

в

D

F

Cow

Cow

Cow

Buffalo

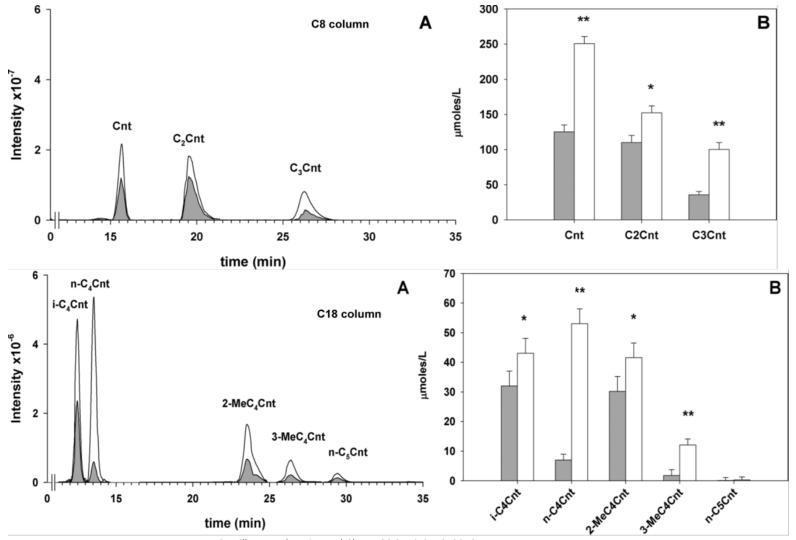
\*\*

Buffalo

Buffalo

Servillo L, et al. J Agric Food Chem, Aug 1;66(30):8142-8149, 2018

#### Carnitine and short-chain acyl carnitine profile of buffalo milk



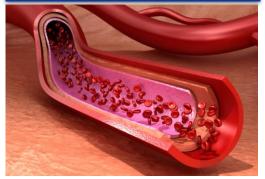
FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

Servillo L, et al. Agric Food Chem, 66:8142-8149, 2018

#### The experimental approach



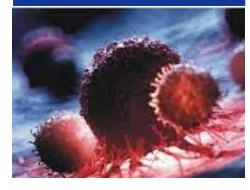
#### Insuline resistance - Type 2 diabetes



In vitro models of human endothelial cells

**Colorectal cancer** 

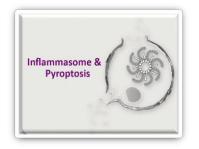
#### Oral Squamous Cell Carcinoma



In vitro model of human oral squamous cell carcinoma











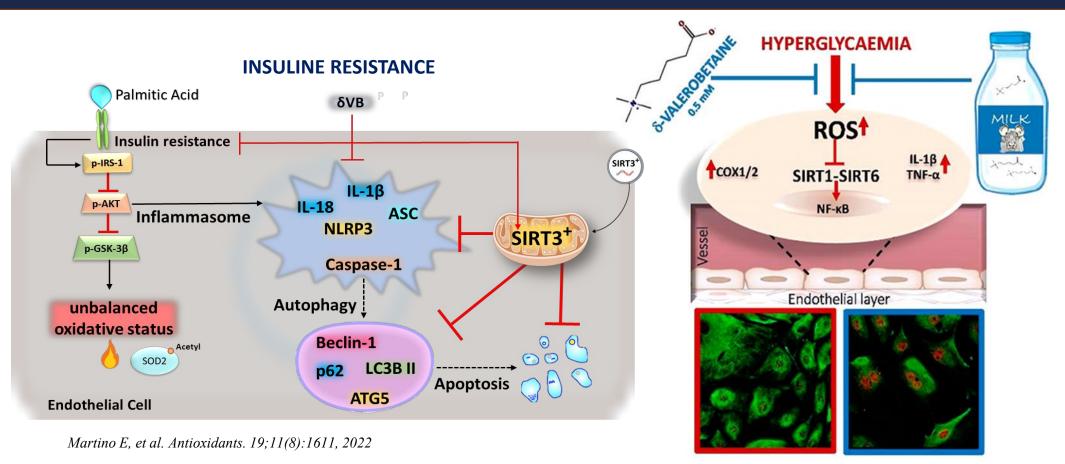
In vitro model of human colorectal cancer cells



Mouse model

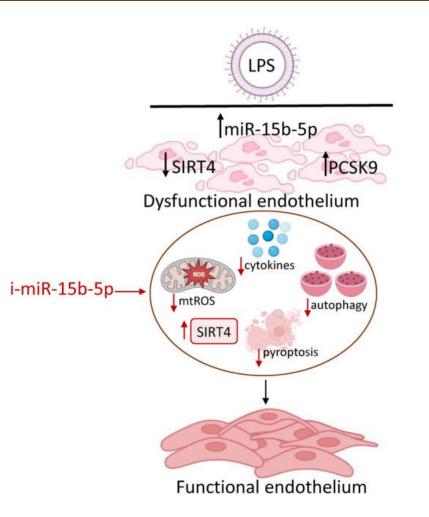
FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

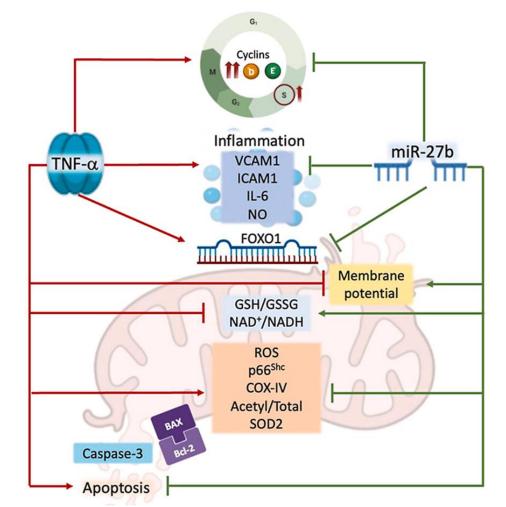
# Epigenetic signature of buffalo milk-δ-valerobetaine in altered glucose homeostasis



D'Onofrio N, et al. JAFC, 67(6), 1702–1710, 2019

## Health effects of buffalo milk-derived microRNAs

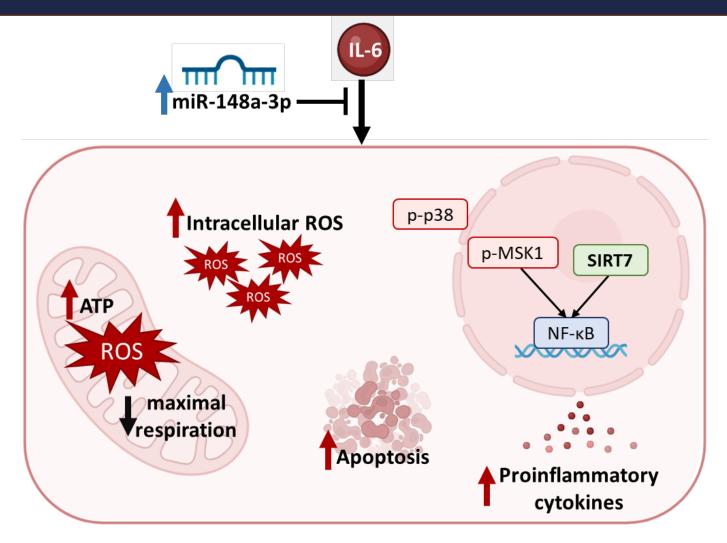




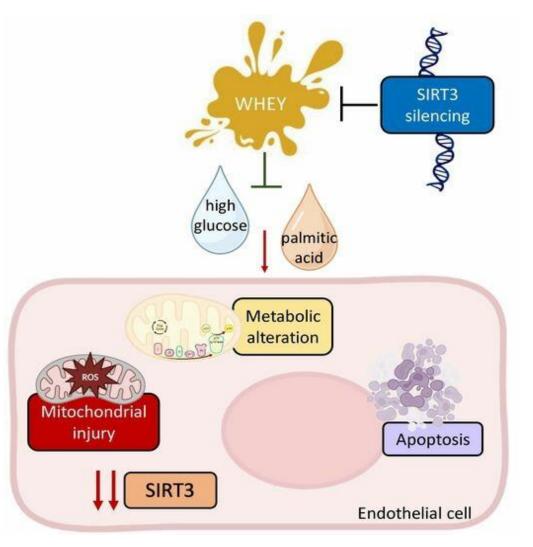
D'Onofrio N, et al . Redox Biol, 62, 102681, 2023

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

#### MiR-148a-3p/SIRT7 Axis Relieves Inflammatory-Induced Endothelial Dysfunction

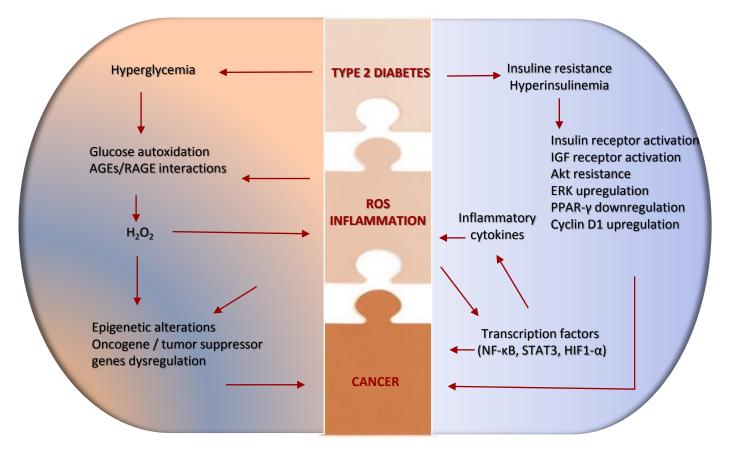


## The health benefits of whey in endothelial cells occur via SIRT3

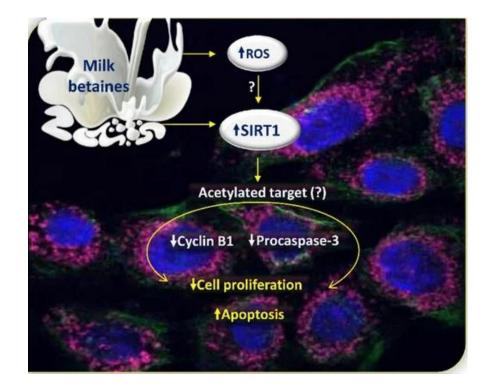


*Martino E., et al. Antioxidants. 12(6), 1311, 2022* 

#### Pathways common to cancer and diabetes

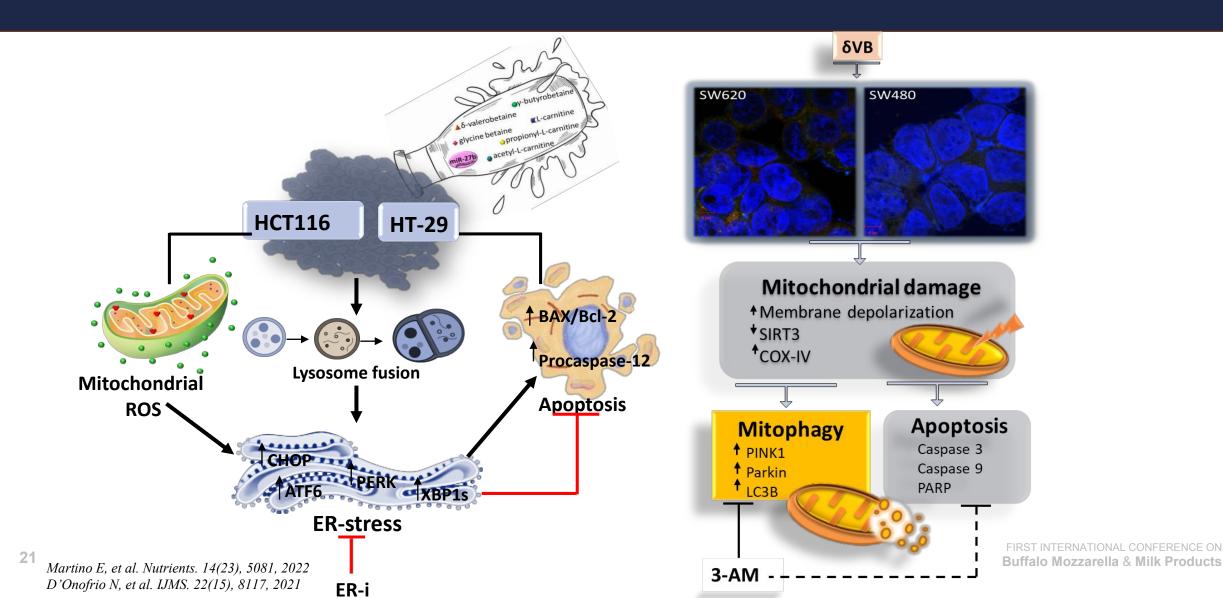


# Synergistic Effect of Dietary Betaines in Human Oral Squamous Cell Carcinoma

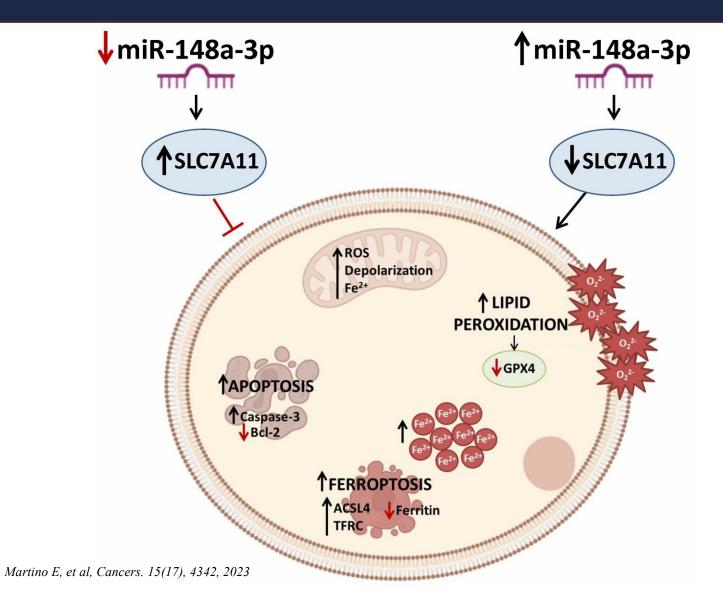


D'Onofrio N et al. Cancers (Basel). 31;12(9):2468, 2020

#### Epigenetic signature of buffalo milk in colorectal cancer

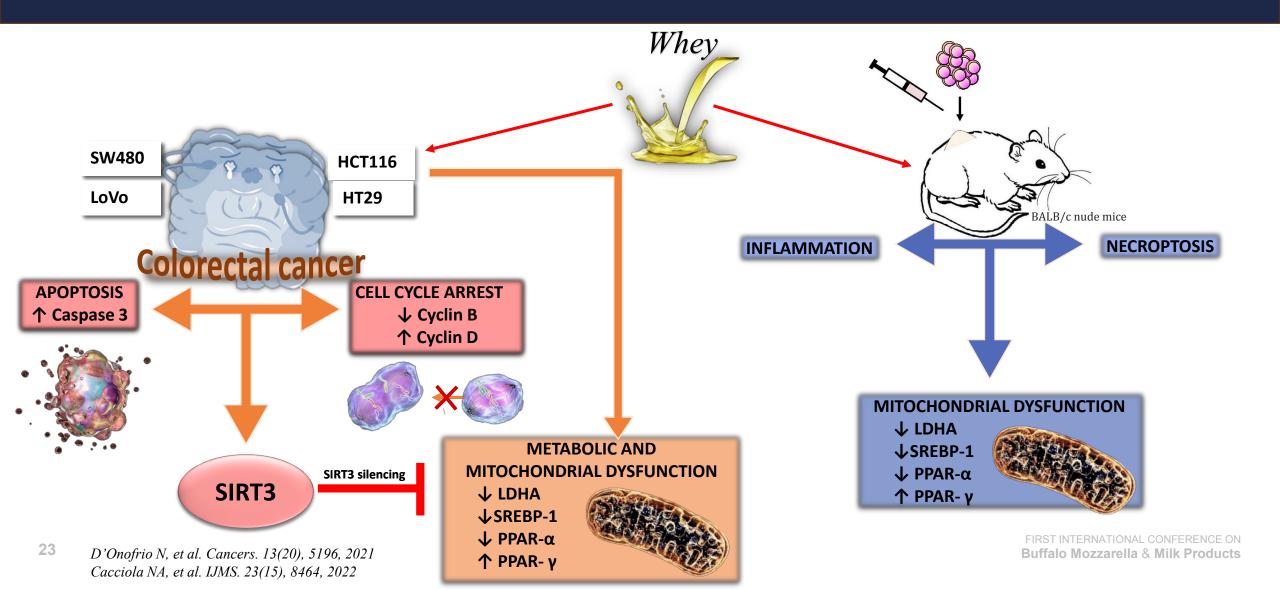


#### MiR-148a-3p Promotes Colorectal Cancer Cell Ferroptosis by Targeting SLC7A11

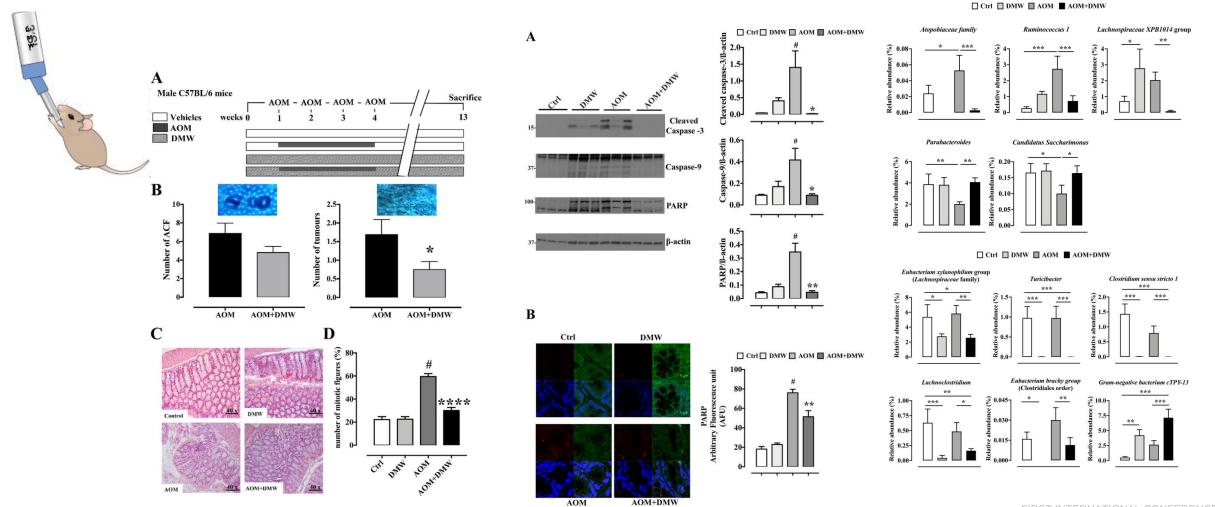


FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

## Chemopreventive effect of milk whey



## **Chemopreventive effect of milk whey**

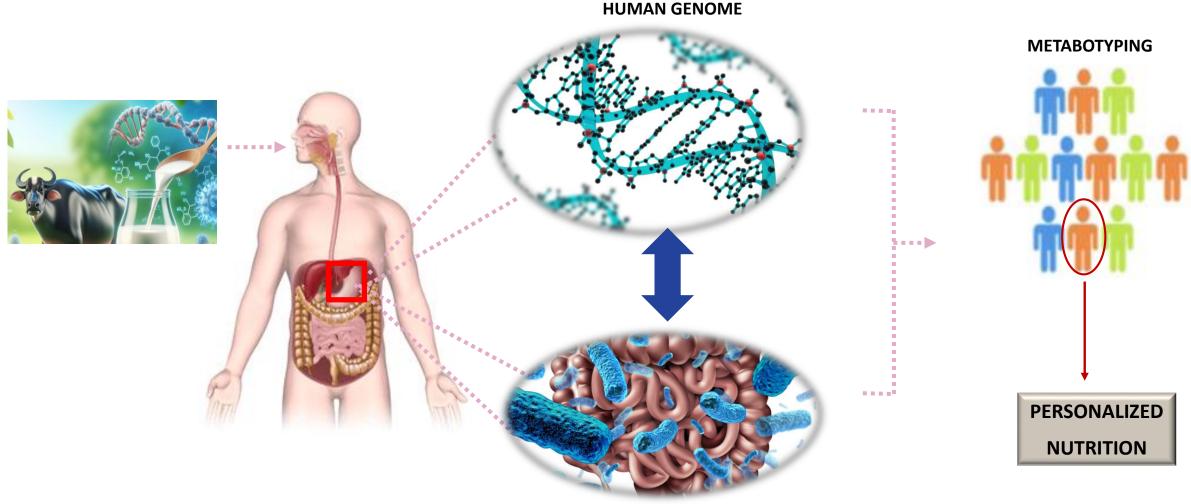


FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

Cacciola NA, et al. Cell Commun Signal Sep 20;21(1):245,. 2023

24

## Buffalo milk and whey: epigenetic modifiers for precision medicine



FIRST INTERNATIONAL CONFERENCE ON **Buffalo Mozzarella & Milk Products** 

# Grazie per l'attenzione!

Maria Luisa Balestrieri

🖂 marialuisa.balestrieri@unicampania.it

First International Conference on Buffalo Mozzarella & Milk Products 24-25 September 2024

**BMMP**2024

Naples, Italy

# EXPLORING WINE AND CHEESE PAIRING



## Angelita Gambuti

Department of Agricultural Sciences, Section of Vine and Wine Sciences, University of Napoli "Federico II",

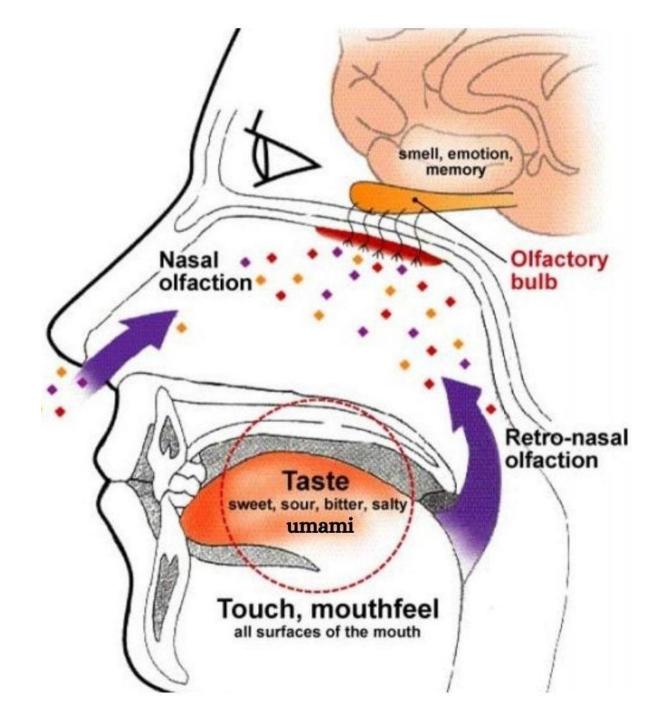
# Which wines pair best with what cheeses?



Wine Racks America

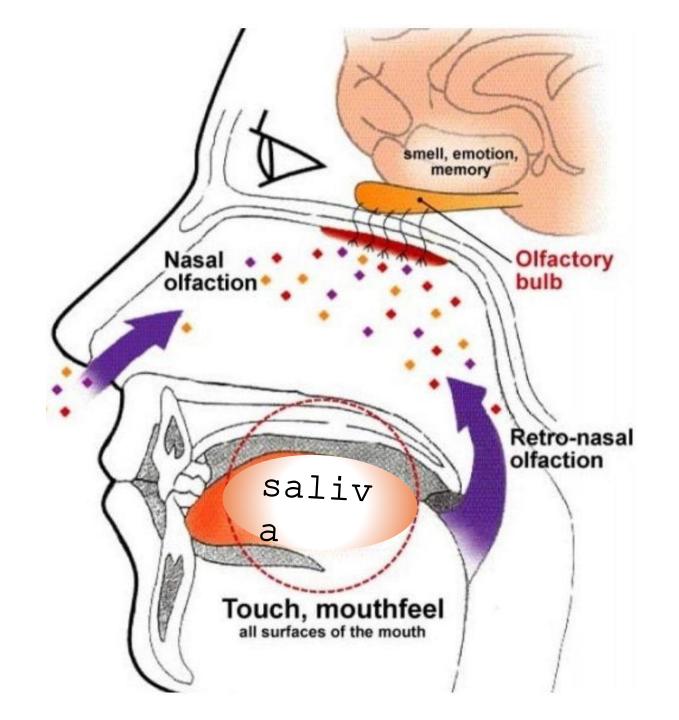
......a question of interaction

## NUTRITIONAL or SENSORY POINT OF VIEW?



#### Stimula:

- Odour active volatile compounds
- Tasty molecules
- «Touch molecules»



Stimula:

- Odour active volatile compounds
- Tasty molecules
- Polyphenols, proteins, fats

#### Mouthfeel active compounds



#### Mouthfeel active compounds



ethanol, sugars, acids, volatile compounds, phenolic compounds

#### MOUTHFEEL SENSATION



A complex system:

heese compounds (fats and proteins) wine compounds (polyphenols and acids) saliva



Food and beverages pairing by experts was described to depend on three perceptual principles: 1) rinsing for maintaining the qualities of each product;

- 2) masking for suppressing off-flavor in one product;
- 3) synergy for enhancing a positive attribute in a product.

Current Research in Food Science 9 (2024) 100792



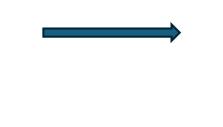
Exploring cheese and red wine pairing by an in vitro simulation of tasting

Alessandra Rinaldi <sup>a,b,d</sup>, Giovanna Bifulco <sup>c</sup>, Alessandra Luciano <sup>a</sup>, Luigi Picariello <sup>a</sup>, Luigi Moio <sup>a</sup>, Raffaele Marrone <sup>c</sup>, Giuseppe Campanile <sup>c</sup>, Angelita Gambuti <sup>a,\*</sup>

<sup>a</sup> Department of Agricultural Sciences, Section of Vine and Wine Sciences, University of Napoli Federico II, Viale Italia, Avellino 83100, Italy <sup>b</sup> Better Sensing, TERINOV, Parque de Ciencia e Tecnologia da Ilha Terceira, 9700-702, Terra Chà, Angra do Heroísmo, Azores, Portugal <sup>c</sup> Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137, Naples, Italy <sup>d</sup> Fondazione Italiana per gli Studi sul Vino (FISSV) Avellino, Italy

Two pivotal factors may contribute to a positive evaluation of the cheese and red wine pairing:

suppression of **astringency** of red wine



By determining the precipitation of astringent tannins during tasting due to the cheese coating of mouth and saliva

oral cleansing: effect of red wine cleansing the in oral cavity from mouth-coating and lingering aftertaste due to fat and proteins of cheese

By determining the decrease in residual proteins in mouth occurring during wine tasting



#### WINES

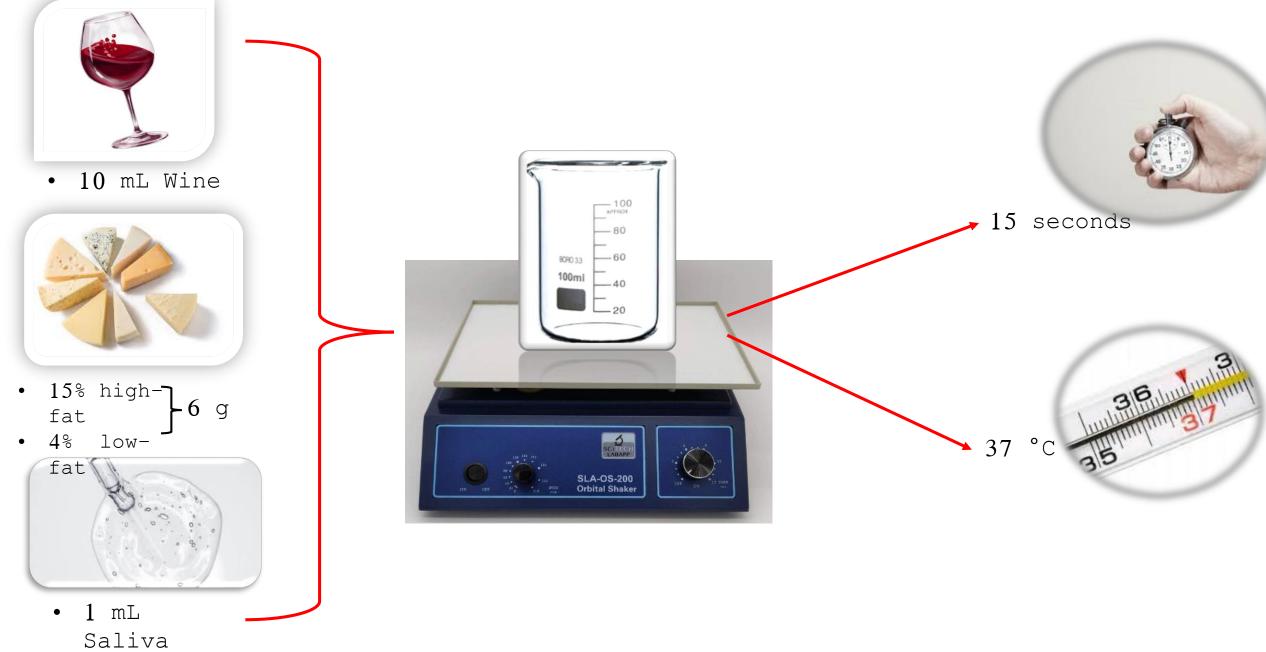


CHEESE

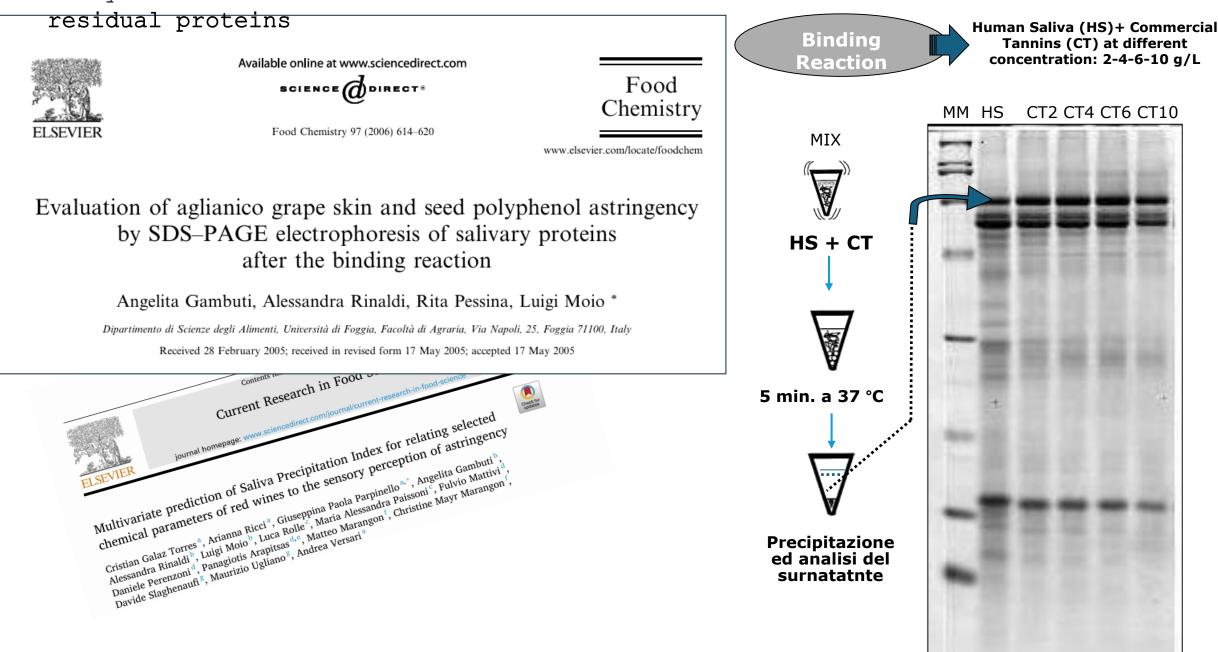
|                       | % Fat |
|-----------------------|-------|
| Primo sale cheese (P) | 17    |
| Semi-hard cheese (M)  | 50    |
| Hard cheese (D)       | 45    |
| Dry ricotta (R)       | 23    |

|      | Ethan | Titratab | рH             | Glucose        | Malic          | Lactic        | Free           | Total          |
|------|-------|----------|----------------|----------------|----------------|---------------|----------------|----------------|
|      | ol    | le       |                | +              | acid           | acid          | sulphur        | sulphur        |
|      | (%v/V | acidity  |                | fructose       | (g/r)          | (g/L)         | dioxide        | dioxide        |
|      |       | (g/L)    |                | (g/L)          |                |               | (mg/L)         | (mg/L)         |
| Wine | 12.03 | 4.31±0.0 | 3.96±0.0       | 0.13±0.0       | $0.02 \pm 0.0$ | $1.7 \pm 0.1$ | $21.94 \pm 1.$ | 36.00±1.       |
| 1    | ±0.1  | 2        | 4              | 2              | 1              |               | 2              | 22             |
| Wine | 12.11 | 4.27±0.0 | $3.97 \pm 0.0$ | $0.10 \pm 0.0$ | $0.57 \pm 0.0$ | 1.6±0.1       | 6.53±0.9       | $28.23 \pm 1.$ |
| 2    | ±0.03 | 5        | 3              | 4              | 3              |               | 4              | 35             |

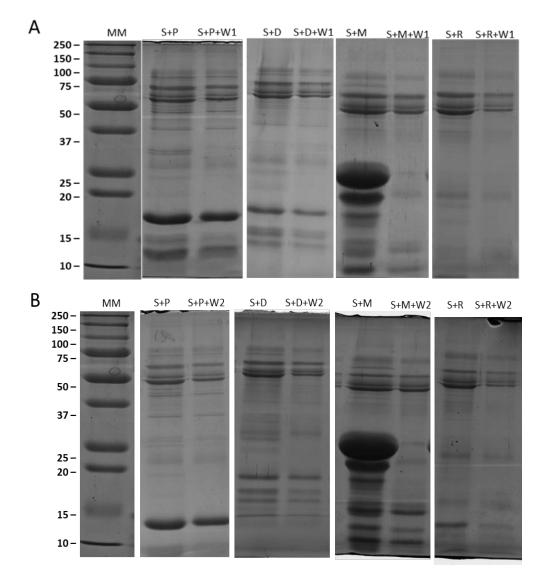
#### SIMULATION OF TASTING



#### Analytical tools: SPI and

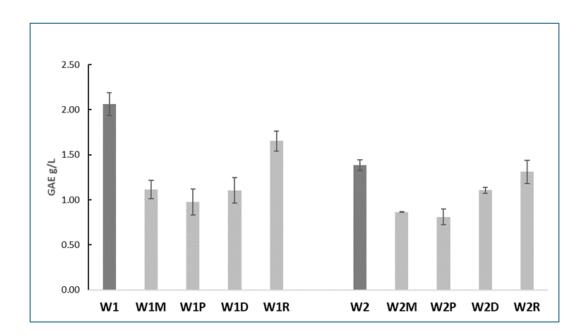


#### cleansing effect



The SDS-PAGE electrophoretic pattern of salivary proteins (S) interacting with cheeses (P, D, M, R), before (S+P, S+D, S+M, S+R) and after (S+P+W1, S+D+W1, S+M+W1, S+R+W1) the interaction with wine W1 (Figure 5A) and W2 (Figure 5B). MM: molecular marker (kDa).

SPI



The Saliva Precipitation Index (SPI) of wines before (W1 and W2) and after the interaction (W1M, W1P, W1D, W1R; W2M, W2P, W2D, W2R) with cheeses (M=semi-hard cheese; P= Primo sale cheese; D=Hard cheese; R=dry ricotta) expressed in g/L of gallic acid equivalent (GAE). Error bars represent standard deviation over three replications.

# OP Optimal Pairing Index

In equation 1 (Eq.1), let:

OP be the optimal pairing score between cheese and wine.

 $\Delta_{\text{cleansing effect}}$  be the percentage decrease of total proteins (cheese and saliva) by wine.

 $\Delta_{\rm SPI}$  be the **decrease of astringency by cheese**, measured by SPI.

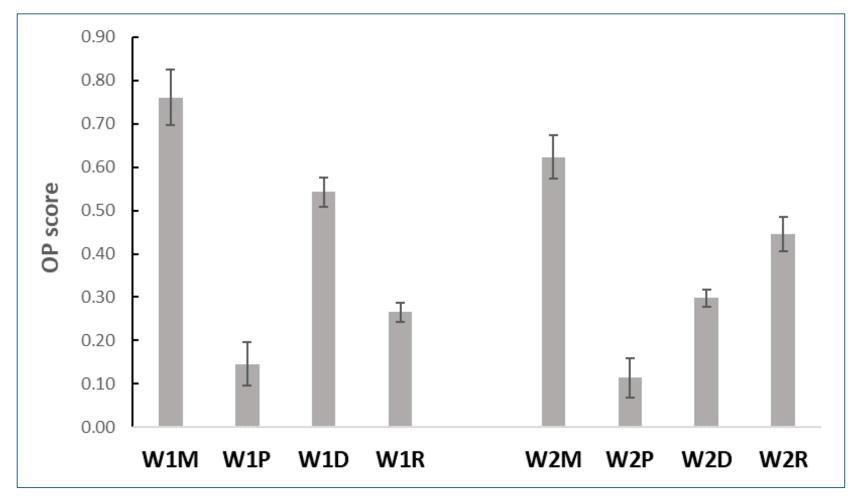
Residual fat be the content in fat (g) that would remain to coating mouth after eating a piece of cheese of 6 g (Repoux et al., 2012).

The OP is a function of these factors:

Eq.1: 
$$OP = [1 - (1 - \Delta_{cleansing effect}) \cdot (1 - \Delta_{SPI})] * residual fat (g)$$

The **Eq.1** assumes that a higher percentage decrease in cheese protein by wine and a higher decrease in astringency (SPI) by cheese in presence of saliva contribute positively to the pairing score.

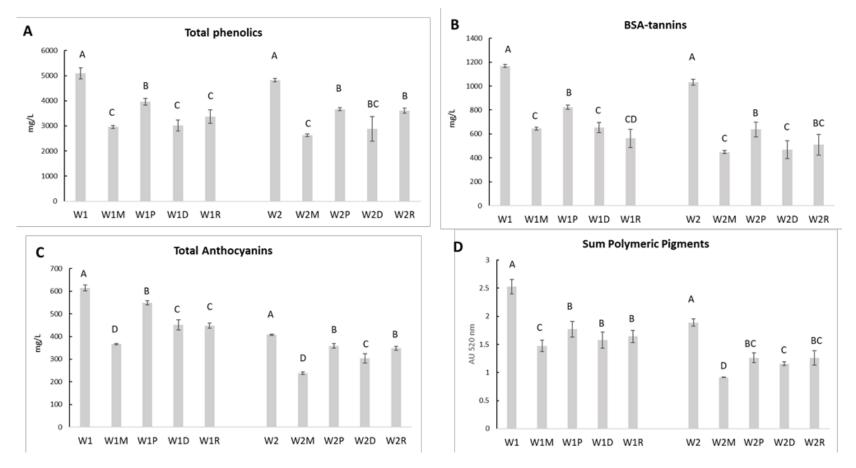
### OP Optimal Pairing



Cheese wine optimal pairing (OP) between wines (W1 and W2) and cheeses (M=semi-hard cheese; P= Primo sale cheese; D=hard cheese; R=dry ricotta). Error bars represent standard deviation over three replications.

# NUTRITIONAL POINT OF VIEW?

Content of residual phenolic compounds in supernatant



Concentration of total phenolics (A), BSA-reactive tannins (B), total anthocyanins (C), and polymeric pigments (D) in wines before (W1 and W2), and after the interaction 1 with saliva and cheese (M=semi-hard cheese; P= Primo sale cheese; D=hard cheese; R=dry ricotta). Error bars represent standard deviation over three replications.

What is the effect on bioaccessibility and bioavailability?

# CONCLUSION

The analysis of the proteins and phenolic compounds after the binding reactions represented only one side of the chemical interactions in food and wine pairing.

The study of the aroma and texture of both wine and cheese should also be considered from a sensory point of view.

Several variables affect the entity of the reciprocal interactions between polyphenols and other cheese compounds. Deeper in vitro and in vivo studies are urgently needed to understand all the variables that can influence the entity of these reciprocal interactions, from the cheese matrices (i.e., types, structures, concentrations, to the environmental conditions (pH, temperature, processing methods), until to



# Thank you for your kind attention !



ANY QUESTIONS?

Utilization of molasses-derived feeds in Italian Mediterranean buffaloes FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Alfio Calanni Macchio<sup>1</sup>, Roberta Matera<sup>1</sup>, Gabriele di Vuolo<sup>2</sup>, Giuseppina Pedota<sup>3</sup>, Valentina Longobardi<sup>1</sup>, Federica Piscopo<sup>1</sup>, Francesca Aragona<sup>1</sup>, Gianluca Neglia<sup>1</sup>

<sup>1</sup> Department of Veterinary Medicine and Animal Production, University of Naples Federico

II, Naples, Italy

<sup>2</sup> Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy

<sup>3</sup> Associazione Regionale Allevatori della Basilicata, Potenza, Italy



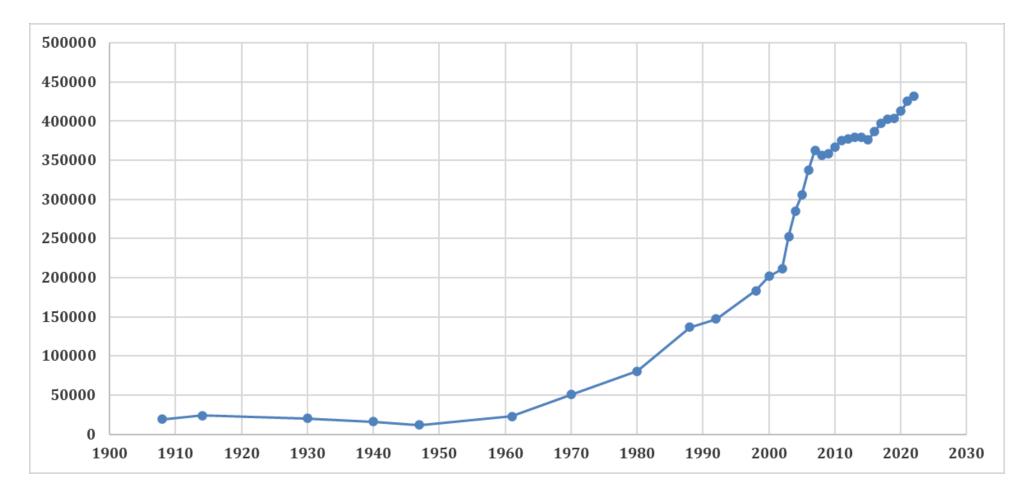




Powered by



# Background



Buffalo population in Italy has grown significantly - from just 12,500 animals in 1947 to nearly 432,000 in 2022 (National Zootechnical Database).

# Materials and Methods



64 Italian Mediterranean buffaloes



Cancello ed Arnone, Italy



134 days from February to June 2023

| Two homogeneous g<br>ead          | groups (32 buffaloes<br>ch)                      |
|-----------------------------------|--|
| Group C (control)                 | Group T (treatment)                              |
| Standard total mixed ration (TMR) | TMR + molasses-based liquid<br>feed (SUGARPLUS®) |



- Buffaloes' milk production (daily)
- Milk samples (every 15 days) fat, protein, casein levels
- Blood samples metabolic profiles
- In vitro fermentation tests impact of molasses on rumen efficiency

# Diet and Production Efficiency





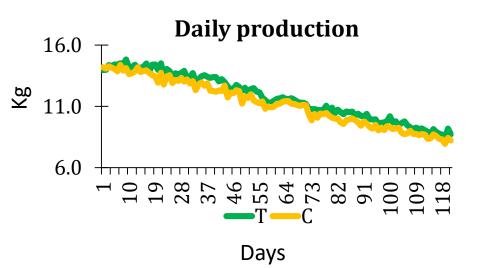




Molasses-based liquid feeds: **ADVANTAGES** 

- Highly palatable
- Readily fermentable sugars, which increase feed intake
- Rumen efficiency
- Better digestion, nutrient absorption, and overall productivity

# **Experimental results**



Buffaloes in the molasses-fed group produced more milk daily, averaging 12.2 kg per day, compared to 11.7 kg in the control group.

| Gruppo    | Grasso<br>(%)          | Proteine<br>(%) | Lattosio<br>(%) | Caseina (%) | SCC<br>(n)  | DSCC<br>(%) | Urea<br>(%)             | Acetone<br>(%) | BHB<br>(%) |
|-----------|------------------------|-----------------|-----------------|-------------|-------------|-------------|-------------------------|----------------|------------|
| Controllo | 8.34±0.06 <sup>A</sup> | 4.42±0.03       | 4.68±0.02       | 3.54±0.03   | 114.5±11.53 | 46.23±1.25  | 43.07±0.83 <sup>A</sup> | 0.32±0.02      | 0.22±0.0   |
| Trattato  | 8.59±0.06 <sup>B</sup> | 4.43±0.03       | 4.63±0.02       | 3.57±0.02   | 150.4±39.48 | 44.20±1.13  | 35.68±0.71 <sup>B</sup> | 0.36±0.03      | 0.28±0.0   |

Treated group's milk had a higher fat content—8.58% compared to 8.33% in the control group.

| Resa alla ca | aseificazione | registrata dur           | ante la prova.            |                |                             |                              |                                 |                    |
|--------------|---------------|--------------------------|---------------------------|----------------|-----------------------------|------------------------------|---------------------------------|--------------------|
| Gruppo       | pH<br>inizio  | pH<br>aggiunta<br>caglio | pH<br>rottura<br>cagliata | pH<br>filatura | t<br>inizio-caglio<br>(min) | t<br>caglio-rottura<br>(min) | t<br>rottura-<br>filatura (min) | Mozzarella<br>(kg) |
| Controllo    | 6,6975±0,0    | 6,635±0,0                | 6,4325±0,1                | 4,87±0,3       | 9,25±2,1                    | 91,25±11,5                   | 220,75±47                       | 27,25±1,2          |
| Trattato     | 6,7025±0,0    | 6,6225±0,0               | 6,4125±0,1                | 4,97±0,2       | 7,75±2,2                    | 93,75±11,8                   | 219±49                          | 28,25±1,3          |

Cheese yield was higher in the treated group, increasing from 27.3% in the control group to 28.3%.

### **Experimental results**

#### Impact on Milk Quality

| Profilo acidi | i <u>co</u> del latte regi | istrato nel cors        | o dello studio       |                       |                      |                     |  |                     |  |
|---------------|----------------------------|-------------------------|----------------------|-----------------------|----------------------|---------------------|--|---------------------|--|
| C             | SCFA MCFA                  |                         | LCFA                 | LCFA MUFA PUFA        |                      |                     | SFA                                      | TFA                 |  |
| Gruppo        | (mg/100 g)                 | (mg/100 g)              | (mg/100 g)           | (mg/100 ml)           | (mg/100 ml)          | (mg/100 ml)         | (mg/100 ml)                              | (mg/100 ml)         |  |
| Controllo     | 768,9±53,13°               | 3135±77,99ª             | 3242±79,59           | 2171±59,88            | 0,20±0,01            | 2076±54,58          | 5706±144,1 <sup>b</sup>                  | 0,14±0.01           |  |
| Trattato      | 913,2±54,02 <sup>d</sup>   | 3379±79,63 <sup>b</sup> | 3330±82.79           | 2226±61,04            | 0,21±0,01            | 2124±54,75          | 6140±150,4ª                              | 0.13±0.01           |  |
|               |                            | Val                     | ori con lettere diff | erenti nella stessa c | olonna sono signific | ativamente differen | ti ( <u>c,d,</u> P<0,10; <u>a,b</u> , Ρ· | <0,05; A,B, P<0,01) |  |

| Parametri di coagulazio | ne registrati nel corso de  | ello studio.                           |                                     |
|-------------------------|-----------------------------|--|-------------------------------------|
| Gruppo                  | RCT (min)                   | K20 (min)                              | A30 (mm)                            |
| Controllo               | 12,0 <sup>a</sup>           | 1,43                                   | 43,26 <sup>a</sup>                  |
| Trattato                | <b>11,0</b> <sup>b</sup>    | 0,98                                   | 48,63 <sup>b</sup>                  |
|                         | Valori con lettere differei | nti nella stessa colonna sono signific | ativamente differenti (a.h. P<0.05) |

Improvements in fat and protein content

Milk from the treated buffaloes had a shorter coagulation time and firmer curd

Risultati ottenuti in seguito al setacciamento delle feci nel corso dello studio. Setaccio superiore (0,47 mm) Setaccio intermedio (0,32 mm) Setaccio inferiore (0,16 mm) Gruppo (%) (%) (%) < 10% < 20% < 50% 7,45<sup>^</sup> Controllo 11,5 20,28 2,14<sup>B</sup> Trattato 11,8 21,27

Milk from the treated group had higher levels of short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs)

## Conclusions



Using molasses-based liquid feeds can improve both the quantity and quality of milk, boost milk yield, enhance milk's suitability for cheese-making, particularly for mozzarella production.

Sustainable and efficient way to improve production outcomes.

# Grazie per l'attenzione!

Alfio Calanni Macchio

🖾 alfio.calannimacchio@unina.it

# Effect of feeding and ripening system on nutritional and functional profile of buffalo cheeses

**Marika Di Paolo<sup>1</sup>**, Nunzia D'Onofrio<sup>2</sup>, Angela Salzano<sup>1</sup>, Alfio Calanni Macchio<sup>1</sup>, Alessandro Cuomo<sup>3</sup>, Maria Luisa Balestrieri<sup>2</sup>, Raffaele Marrone<sup>1</sup>

<sup>1</sup> University of Napoli Federico II - Department of Veterinary Medicine and Animal Production, Italy; <sup>2</sup> University of Napoli Federico II - Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Italy; <sup>3</sup> Arredo Inox S.r.l., Crotone, Italy. FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Powered by





 Università
 degli Studi
 della Campan Luigi Vanvitelli





Dipartimento Medicina Veterinaria Produzioni Animeli



# **INTRODUCTION AND AIM**



**CAPSULE** - Ottimizzazione delle te<u>C</u>niche di <u>A</u>llevamento e dei <u>P</u>rocessi produttivi del <u>S</u>ettore lattiero-caseario b<u>U</u>falino e del vino per la produzione di a<u>L</u>im<u>E</u>nti funzionali



The chemical characteristics of buffalo milk interfere and extend the ripening process of buffalo cheeses

#### Ripened buffalo cheeses?

#### **Accelerated Ripening**

Biochemical changes that occur during ripening are relatively slow in buffalo milk cheeses (El Soda, 1993; Kanawjia and Singh, 1991). As a consequence, flavor development in buffalo milk Cheddar cheese is considerably slower than in regular Cheddar. Therefore, there is a need to accelerate ripening of cheeses made from buffalo milk. Several approaches, such as an elevated ripening temperature, stimulation of starter culture, use of an adjunct culture, addition of enzymes, and supplementation with goats' milk, among

SOURCE: Batool et al., 2018



#### Inclusion of green feed

#### The present study proposes to evaluate the effect of

provide solutions that enable the production of **nutritionally competitive and sustainable products** 

#### **Innovative Ripening System**

a cost for a company that wants to

invest in buffalo ripened cheeses

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

# **MATERIALS AND METHODS** Animals, Diet, Cheese Making and Ripening process

#### Animals and Diet

| Feed (Kg)         | Di    | iet   | - |
|-------------------|-------|-------|---|
| -                 | CTL   | FRS   | - |
| Concentrate       | 5.50  | 2.50  | - |
| Alfalfa           | 4.50  | 2.60  |   |
| Straw             | 1.20  | 1.20  |   |
| Silomais          | 23.00 | 18.00 |   |
| Green ryegrass    | -     | 25.00 |   |
| Hydrogenated fats | -     | 0.30  |   |
| Calcium carbonate | -     | 0.05  |   |
| TOTAL             | 34.20 | 49.65 |   |

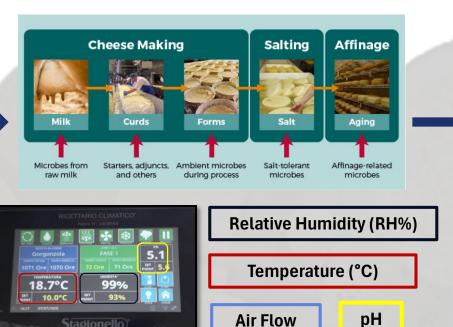
Table 1. Diets composition (kg) of buffaloes fed with (FRS) and without (CTR) green feed inclusion.

Buffalo were randomly divided in two group:

- Control group, **CTR**; *n* = 25
- Fresh group, **FRS**; *n* = 25

#### **Cheese Making**

Stagionello



#### *Ripening system*

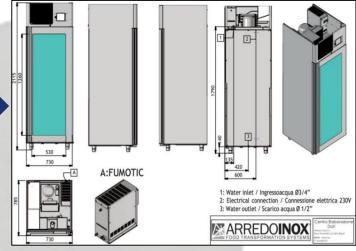
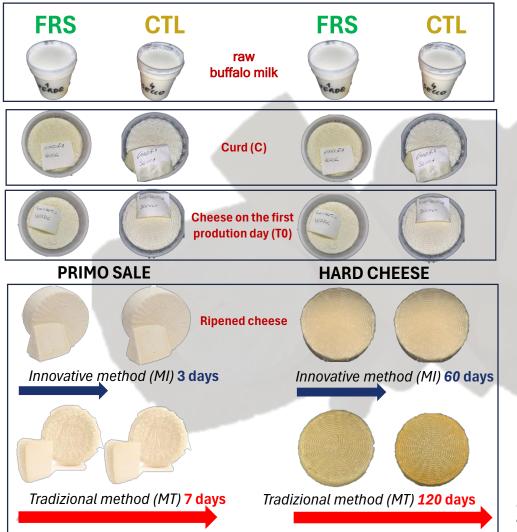


Figure 1. Industrial ripening plant (Stagionello® European Patented Device and controlled pH - n. EP 2769276B1) to ripen cheeses.

The cheeses were ripened using a system called Stagionello<sup>®</sup>, equipped with an **internal** control device that allow the monitoring of the physical and chemical state of cheese during the ripening by continuously measuring of pH and climatic parameters.

# MATERIALS AND METHODS

Sampling procedure



#### **Climatic recipes**

The cheeses obtained were ripened using two different methods: a **longer**, **traditional method** and a **shorter**, **experimental method**.

| Cheese      | <b>Ripening time</b>         | Ripening steps         | pН  | Air temperature (°C) | RH (%) | Airflow |
|-------------|------------------------------|------------------------|-----|----------------------|--------|---------|
|             | inpening time                | Stewing (1 h)          | 5.5 | 26                   | 85     | 0       |
|             |                              | Dripping (8 h)         | 4.8 | 24                   | 80     | 1       |
|             |                              | Drying 1 (24 h)        | 5.0 | 22                   | 75     | 0       |
|             |                              | Drying 2 (24 h)        | 5.0 | 21                   | 76     | 0       |
|             |                              | Drying 3 (24 h)        | 5.1 | 19                   | 78     | 0       |
|             | INNOVATIVE<br>60 days        | Drying 4 (24 h)        | 5.1 | 17                   | 80     | 0       |
| Hard cheese | 00 uays                      | Pre- ripening 1 (48 h) | 5.1 | 15                   | 82     | 0       |
|             |                              | Pre-ripening 2 (48 h)  | 5.2 | 14                   | 82     | 0       |
|             |                              | Pre- ripening 3 (48 h) | 5.3 | 13                   | 84     | 0       |
|             |                              | Pre- ripening 4 (48 h) | 5.4 | 12                   | 85     | 0       |
|             |                              | Ripening (48 days)     | 5.5 | 11                   | 73     | 1       |
|             | TRADITIONAL<br>120 days      |                        | -   | +14/15               | 90-96  | -       |
|             | INNOVATIVE<br>3 days         | Ripening (72 days)     | 5.6 | 10                   | 35     | 0       |
| Primo sale  | <b>TRADITIONAL</b><br>7 days | _                      | -   | 13                   | 85     | -       |

**Table 2.** Experimental ripening design for hard cheese and primo sale cheese.

For each interval, 2 samples were collected and classified based on the type of feed administered to the animals (CTL = without green feed; FRS = green feed).

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

## MATERIALS AND METHODS Analysis

The effects of the feeding and accelerated ripening were evaluated through **physicochemical analysis**, examination of **health-promoting biomolecules** and **antioxidant power**.

#### Study of the physical and chemical characteristics

- Chemical composition (AOAC International, 2002)
- Color (CIE L\*a\*b\*) (Serrapica et al., 2020)
- Texture profile Analysis TPA (Di Paolo et al., 2023)

#### Study of the lipolysis and oxidation indexes

- Free Fatty Acids FFA (Manzo et al., 2019)
- > Thiobarbituric Acid TBARs (Ambrosio et al., 2014)
  - Peroxide Value PV (De Luca et al., 2019)



#### Study of health-promoting biomolecules and antioxidant power

- Health-promoting biomolecules: γ-butyrobetaine, glycine betaine, δ-valerobetaine, lcarnitine, acetyl-l-carnitine and propionyl-l-carnitine (Salzano et al., 2021)
- Antioxidant power FRAP; Assay Kit (MBS169262) and total antioxidant capacity (TAC); Assay Kit (#K274-100) (Salzano et al., 2021)

# **RESULTS AND DISCUSSION**

#### Study of the chemical composition and the lipolysis and oxidation indexes

|           |     |              | Primo sale  |            |            |             | Hard cl     | neese      |            |
|-----------|-----|--------------|-------------|------------|------------|-------------|-------------|------------|------------|
|           |     | Semi-finishe | ed products | Ripened    | cheeses    | Semi-finish | ed products | Ripened    | cheeses    |
| Item      |     | С            | T0          | MI         | MT         | С           | T0          | MI         | MT         |
| Fat,      | CTL | 17.56±0.65   | 18.22±1.23  | 16.85±0.81 | 16.56±1.77 | 23.35±1.07  | 34.52±1.24  | 33.58±1.25 | 44.43±0.81 |
| %         | FRS | 21.66±0.38   | 19.23±1.61  | 13.06±0.41 | 15.98±0.35 | 24.83±1.18  | 33.95±0.80  | 44.86±2.40 | 45.61±0.87 |
| Protein,  | CTL | 12.56±0.77   | 11.61±0.49  | 16.85±0.81 | 16.56±1.77 | 15.37±0.50  | 16.69±0.74  | 24.83±1.12 | 19.26±0.78 |
| %         | FRS | 11.45±0.77   | 12.54±0.87  | 13.06±0.41 | 15.98±0.35 | 14.97±0.65  | 15.34±0.74  | 25.51±2.46 | 19.41±1.04 |
| Moisture, | CTL | 56.63±1.43   | 57.74±0.57  | 39.60±1.94 | 32.77±1.31 | 49.16±0.86  | 49.30±0.80  | 18.23±0.78 | 13.55±0.76 |
| %         | FRS | 58.86±1.66   | 56.23±1.44  | 42.20±0.74 | 37.91±1.93 | 48.69±1.52  | 49.00±2.54  | 17.56±0.81 | 13.35±0.76 |
| NaCl,     | CTL | 0.20±0.06    | 2.53±0.09   | 4.19±0.31  | 6.13±0.45  | 0.19±0.03   | 0.41±0.03   | 1.76±0.38  | 1.67±0.22  |
| %         | FRS | 0.15±0.01    | 2.49±0.14   | 4.33±0.29  | 5.44±0.55  | 0.09±0.01   | 0.37±0.05   | 1.70±0.18  | 2.11±0.12  |

**Table 3.** Effects of feeding system and ripening time on **chemical composition** of primo sale cheesesand hard cheeses.

|           |     |              | Primo sale  |           |        | Hard cheese |             |         |         |  |  |
|-----------|-----|--------------|-------------|-----------|--------|-------------|-------------|---------|---------|--|--|
|           |     | Semi-finishe | ed products | Ripened c | heeses | Semi-finish | ed products | Ripened | cheeses |  |  |
| -<br>Item |     | С            | TO          | MI        | MT     | С           | TO          | MI      | MT      |  |  |
| PV,       | CTL | 1.346        | 1.965       | 2.964     | 5.028  | 1.538       | 1.550       | 1.395   | 1.257   |  |  |
| meqO2/kg  | FRS | 1.683        | 2.348       | 2.475*    | 4.582* | 1.328       | 1.423       | 1.741   | 1.431   |  |  |
| FA,       | CTL | 0.047        | 0.037       | 0.065     | 0.052  | 0.148       | 0.112       | 0.693   | 0.946   |  |  |
| %         | FRS | 0.056        | 0.037       | 0.047     | 0.054  | 0.076       | 0.114       | 0.630   | 1.080   |  |  |
| TBARs,    | CTL | 0.028        | 0.012       | 0.064     | 0.025  | 0.054       | 0.076       | 0.061   | 0.538   |  |  |
| mg/kg     | FRS | 0.012        | 0.125       | 0.055     | 0.010  | 0.086       | 0.132       | 0.129   | 0.429*  |  |  |

**Table 4.** Effects of feeding system and ripening time on the **lipolysis and oxidation indexes** of primo sale cheeses and hard cheeses.

#### Effect of feeding

The inclusion of green feed in the buffalo diet did **not significantly affect** the chemical composition of buffalo cheeses.

The **lowest peroxide values** were observed in **cheeses** of fresh group (FRS), pointed out the influence of the feeding system on the levels of antioxidant compounds during ripening.

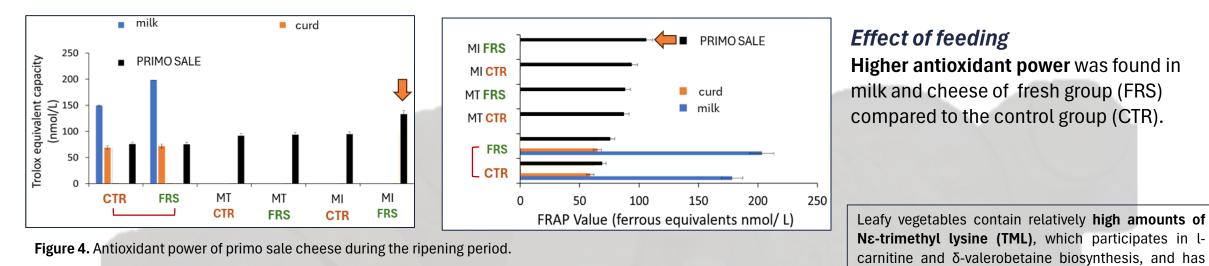
#### Effect of ripening method

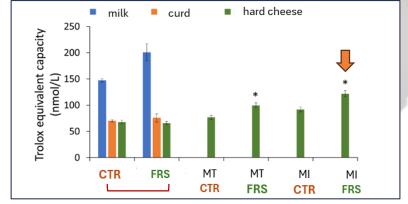
The ripening method did **not significantly affect** the chemical composition of buffalo cheeses.

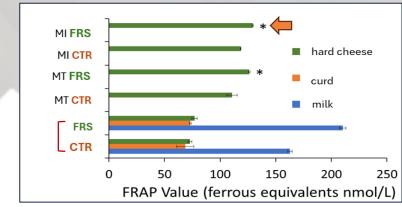
# Lower levels of lipid oxidation in cheeses subjected to a fast innovative ripening method (MI).

# **RESULTS AND DISCUSSION**

#### Study of antioxidant power







#### Effect of ripening method

The innovative ripening method (MI) **improved the antioxidant properties** of both buffalo cheeses.

recognized antioxidant properties (Salzano et al 2021).

Figure 5. Antioxidant power of hard cheese during the ripening period. \*=p<0.05 vs CTR group.

## **RESULTS AND DISCUSSION** *Study of health-promoting biomolecules*

|                    |     |              | Primo sa    | ale        |             | Hard che   |  |              |            |             | ese         |            |  |
|--------------------|-----|--------------|-------------|------------|-------------|------------|--|--------------|------------|-------------|-------------|------------|--|
| ppm                |     | milk         | С           | Т0         | MI          | MT         |  | milk         | С          | T0          | MI          | MT         |  |
| v hutime heteine   | CTL | 5.09±0.15    | 2.12±0.05   | 5.76±0.35  | 6.84±0.02   | 6.16±0.29  |  | 4.74±0.44    | 2.38±0.38  | 2.81±0.15   | 3.02±0.06   | 3.09±0.14  |  |
| γ-butirrobetaina   | FRS | 5.96±0.5*    | 2.54±0.03   | 6.41±0.18  | 7.14±0.10*  | 6.42±0.07  |  | 5.00±0.51*   | 2.73±0.09  | 3.82±0.17*  | 4.03±0.06   | 3.87±0.61  |  |
| T volovokotojno    | CTL | 17.57±0.46   | 6.25±0.07   | 14.56±0.47 | 15.15±0.04  | 14.97±0.24 |  | 18.58±0.22   | 6.11±0.47  | 6.04±0.03   | 6.08±0.10   | 5.98±0.19  |  |
| δ-valerobetaina    | FRS | 26.22±0.62** | 6.43±0.19   | 16.29±0.42 | 16.85±0.14* | 15.36±0.17 |  | 27.10±1.24** | 7.05±0.46* | 6.77±0.32*  | 7.03±.04*   | 6.95±0.39  |  |
| glicinabetaina     | CTL | 16.87±0.11   | 4.19±0.3    | 14.23±0.05 | 14.47±0.07  | 14.16±0.07 |  | 16.57±0.88   | 3.90±0.09  | 4.93±0.13   | 5.05±0.06   | 5.01±0.09  |  |
|                    | FRS | 18.53±1.75*  | 5.11±0.06   | 14.88±0.22 | 15.61±0.24* | 14.96±0.13 |  | 18.60±0.30*  | 4.95±0.12* | 5.09±0.20   | 5.35±0.17   | 5.12±0.11  |  |
| carnitina          | CTL | 38.45±1.73   | 21.99±2.30  | 30.10±1.64 | 37.69±0.55  | 34.62±0.26 |  | 39.8±0.86    | 23.54±0.35 | 30.37±0.44  | 30.58±0.60  | 29.96±0.21 |  |
|                    | FRS | 45.26±1.75** | 25.50±1.10  | 30.15±0.47 | 33.2±0.7*   | 29.99±0.71 |  | 44.3±1.91*   | 24.25±0.39 | 32.04±0.80  | 32.32±0.70* | 31.96±0.09 |  |
| acetilcarnitina    | CTL | 40.20±0.6    | 11.62±1.78  | 23.02±0.08 | 23.99±0.40  | 23.370.50  |  | 40.38±0.70   | 12.12±0.40 | 13.76±0.15  | 14.15±0.04  | 19.96±0.08 |  |
|                    | FRS | 49.94±0.96** | 13.55±0.98* | 22.62±0.47 | 24.75±0.16* | 22.26±0.31 |  | 49.08±0.56** | 13.37±0.60 | 14.79±0.93* | 15.2±0.12*  | 14.91±0.09 |  |
| propionilcarnitina | CTL | 16.72±1.52   | 5.54±0.12   | 20.54±0.41 | 21.63±0.21  | 20.49±0.29 |  | 16.80±1.61   | 5.44±0.17  | 5.93±0.13   | 6.06±0.04   | 5.98±0.07  |  |
|                    | FRS | 27.01±1.88** | 6.02±0.18   | 23.27±0.24 | 23.61±0.29  | 23.71±0.50 |  | 28.83±0.95** | 6.26±0.25* | 6.21±0.04*  | 6.35±0.16   | 6.19±0.11  |  |

#### Effect of feeding

Higher concentration of the health-promoting biomolecules in milk and cheeses of fresh group (FRS) compared to those fed without green feed (CTR).

The greater  $\delta$ -valerobetaine content of milk in buffaloes that received green feed provides one explanation for the greater total antioxidant capacity compared with buffaloes of the CTR group (**D'Onofrio et al., 2019**).

Table 5. Effects of feeding system and ripening time on health-promoting biomolecules of primo sale cheeses and hard cheeses.

#### Effect of ripening method

The innovative method, compared to the traditional one, did not negatively affect the content of betaine and carnitine but **positively contributed to their accumulation** in the cheeses.

# CONCLUSION



**Inclusion of green feed** 

#### ||O| *foods*

#### Article

Role of Feeding and Novel Ripening System to Enhance the Quality and Production Sustainability of Curd Buffalo Cheeses

Marika Di Paolo <sup>1</sup>, Valeria Vuoso <sup>1</sup>, Rosa Luisa Ambrosio <sup>1,\*</sup>, Anna Balestrieri <sup>2</sup>, Giovanna Bifulco <sup>1</sup>, Aniello Anastasio <sup>1</sup> and Raffaele Marrone <sup>1</sup>



MDPI

#### **Innovative Ripening System**

the role of a diet with green forage on buffalo productions

the importance of choice suitable climatic parameters

Therefore, **accelerated ripening methods** and **functional diets** could be candidates as promising systems for the valorization of buffalo cheeses in the dairy industry.

# Grazie per l'attenzione!

Marika Di Paolo

PhD student in Veterinary Science Department of Veterinary Medicine and Animal Production University of Naples "Federico II" Via Federico Delpino n°1 80137 Naples - Italy

🖾 marika.dipaolo@unina.it

Effect of dietary administration of green forage on carbohydrate-active enzymes (CAZymes) and milk quality in Italian Mediterranean buffaloes

<u>Roberta Matera</u><sup>1</sup>, Angela Salzano<sup>1</sup>, Anella Saggese<sup>2</sup>, Elisa Martino<sup>3</sup>, Martina Cascone<sup>2</sup>, Matteo Selci<sup>2</sup>, Gianmaria Pacelli<sup>1</sup>, Giovanna Bifulco<sup>1</sup>, Giuseppe Campanile<sup>1</sup>

1 Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy 2 Department of Biology, University of Naples "Federico II", 80126, Naples, Italy 3 Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80128, Naples, Italy



Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

Dipartimento Medicina Veterinari Produzioni Animali  Università degli Studi
 della Campania Luigi Vanvitelli





AGRARIA

# **Buffalo Farming**

### Buffaloes are a major global livestock of economic and social importance in both developing and developed economies



# **Functional products**

"Diet is the "medicine" we take daily to improve our health!!!"

In recent years, consumer attention has focused on functional foods of animal origin, especially if produced with respect for the environment and animal welfare.



Green feed increases antioxidant and antineoplastic activity of buffalo milk: A globally significant livestock Angela Salzano<sup>a</sup>, Gianluca Neglia<sup>a</sup>, Nunzia D'Onofrio<sup>b,\*</sup>, Maria Luisa Balestrieri<sup>b</sup>, Antonio Limone<sup>c</sup>, Alessio Cotticelli<sup>a</sup>, Raffaele Marrone<sup>a</sup>, Aniello Anastasio<sup>a</sup>, Michael J. D'Occhio<sup>d</sup>, Giuseppe Campanile<sup>a</sup> <sup>a</sup> Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy <sup>b</sup> Department of Procision Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy <sup>c</sup> Istinuto Zooprofilatico Sperimentale del Mezzogiorno, 80055 Portici, Italy <sup>d</sup> School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, New South Wales 2000 Australia

#### scientific reports

OPEN Buffalo milk and rumen fluid metabolome are significantly affected by green feed

G. Neglia<sup>1</sup>, A. Cotticelli<sup>1</sup>, A. Vassetti<sup>2</sup>, R. Matera<sup>1</sup>, A. Staropoli<sup>2,3</sup>, F. Vinale<sup>1,270</sup>, A. Salzano<sup>1</sup> & G. Campanile<sup>1</sup>

 Salarno et al BMC Genomica
 2023/26133
 BMC Genomics

 RESEARCH
 Open Access

 Transcriptomic profiles of the ruminal wall
 Image: Comparison of the ruminal wall

 in Italian Mediterranean dairy buffaloes fed
 green forage

Angela Salzano<sup>1†</sup>, Salvatore Fioriniello<sup>2†</sup>, Nunzia D'Onofrio<sup>3</sup>, Maria Luisa Balestrieri<sup>3</sup>, Riccardo Aiese Cigliano<sup>4</sup>, Gianluca Neglia<sup>1</sup>, Fioriana Della Ragione<sup>25\*</sup> and Giuseppe Campanile<sup>1</sup>

The ruminal microbiota produces carbohydrate-active enzymes (**CAZymes**) which include lignocellulolytic enzymes that fundamentally determine the degradation and debranching of plant polysaccharides.

**Glycoside hydrolases** (GH, hydrolyze and/or rearrange glycosidic bonds) Polysaccharide lyases (PL, non-hydrolytic cleavage of glycosidic bonds) Auxiliary activities (AA, redox enzymes that act in conjunction with CAZymes)

**Glycosyl transferases** (GT, form glycosidic bonds) Carbohydrate esterases (CE, hydrolyze carbohydrate esters)



# AIM

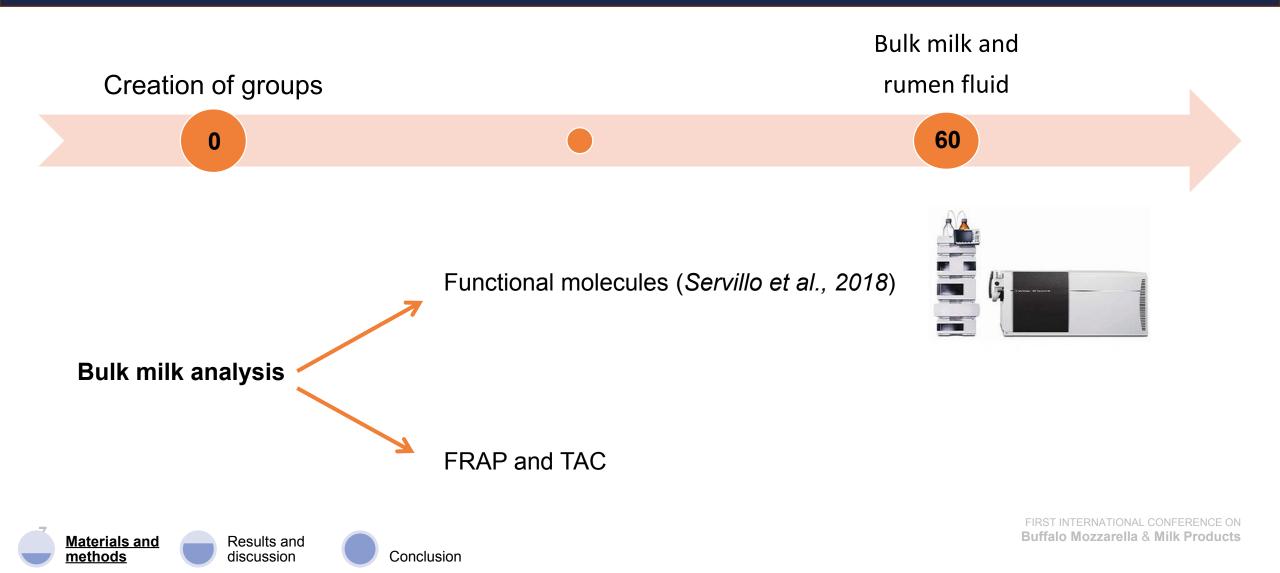
To determine the effect of including green forage (GF) in the diet of Italian Mediterranean buffaloes (IMB) on the CAZymes profile, functional biomolecules and total antioxidant activity in milk.

# **Animals and Diets**

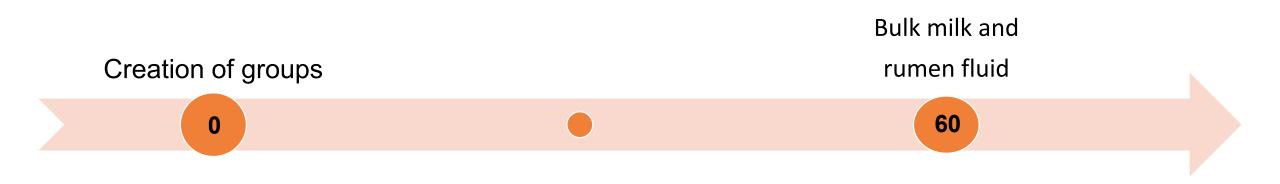
| Commercial buffalo farm located in   | Item                            | TMR    | TMR + green fee |
|--|---------------------------------|--------|-----------------|
| Southern Italy   | Component (kg of feed)          | Amount |                 |
|  | Ryegrass                        | -      | 24              |
| 60 days  | Corn silage                     | 22     | 17              |
|  | Alfalfa hay                     | 4      | 2.5             |
|  | Soybeanmeal (48)                | 0.6    | -               |
| 16 Italian Mediterranean buffaloes   | Concentrate                     | 3.8    | 2.4             |
|  | Wheat straw                     | 1      | 1.1             |
|  | Hydrogenated fats               | 0.3    | 0.3             |
|  | Calcium Carbonate               | 0.1    | 0.2             |
| Two diets iso-nitrogenous and iso-energetic<br>Two homogeneous groups by live weight, parity | Salt 1:3                        | 0.2    | 0.2             |
|  | Vitamins                        | 0.1    | -               |
|  | Composition (dry matter intake) |        |                 |
|  | Dry matter                      | 16.0   | 16.1            |
|  | СР                              | 14.5   | 14.4            |
|  | Fat                             | 4.8    | 5.0             |
|  | NDF                             | 38.0   | 38.5            |
| ODP), days in milk (DIM), production (MY)  | ADF                             | 24.0   | 23.4            |
| Gruppo C – TMR standard  | NSC                             | 34.0   | 33.1            |
|  | Starch                          | 21.0   | 15.5            |
| Gruppo T – TMR + Green forage  | Ash                             | 8.1    | 9.0             |
|  | Calcium                         | 0.9    | 1               |
|  | Phosphorus                      | 0.4    | 0.4             |
|  | MFU                             | 0.93   | 0.91            |

FIRST INTERNATIONAL CONFERENCE ON **Buffalo Mozzarella & Milk Products** 

# Experimental Design



# **Experimental Design**



#### Rumen fluid

Microbiomes were investigated through metagenomic analysis and CAZymes were identified using the HMMER 3.0 package with the dbCAN CAZyme database.

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

### Results

|                           | TMR                    | TMR + green feed       |
|---------------------------|------------------------|------------------------|
| L-carnitine               | 30.4±0.7 <sup>A</sup>  | 41.0±0.6 <sup>B</sup>  |
| acetyl-L-carnitine        | 38.6±0.5 <sup>A</sup>  | 48.4±0.6 <sup>B</sup>  |
| propionyl-L-<br>carnitine | 15.7±1.0 <sup>A</sup>  | 22.1±0.5 <sup>B</sup>  |
| γ-butyrobetaine           | 4.4±0.3                | 3.8±0.2                |
| δ-valerobetaine           | 19.1±0.6 <sup>A</sup>  | 23.4±0.6 <sup>B</sup>  |
| glycine betaine           | 7.3±0.1                | 7.4±0.3                |
| TAC                       | 220.3±5.5 <sup>A</sup> | 249.5±7.7 <sup>B</sup> |
| FRAP                      | 194.3±4.2 <sup>A</sup> | 225.8±4.8 <sup>B</sup> |

Conclusion

### **Results**

#### GT Genus

- GT4 Selenomonas, Prevotella, Oscillospiraceae
- GT14 Prevotella
- GT20 Prevotella
- GT26 Selenomonas, Prevotella, Oscillospiraceae
- GT30 Selenomonas, uncultured Prevotella
- GT39 Oscillospiraceae

GT56

*Group T* had a greater (p<0.01) abundance of CAZymes of the **GT** class (GT4, GT14, GT20, GT26, GT39) and **AA** class (AA1, AA3, AA6)



The inclusion of green feed in the diet of dairy buffaloes favors ruminal microbiota that produce **CAZymes** that support the synthesis of amino acids and *functional biomolecules*.

The findings provide incentive to further refine feeding strategies that meet consumer preference for food products sourced from animals fed natural diets.

The approach adopted in the present study for buffaloes should also be applicable to dairy cattle.





# Grazie per l'attenzione!

R. Matera

ACKNOWLEDGEMENTS:

This research was supported by:

- Project "**CAPSULE**: Ottimizzazione delle teCniche di Allevamento e dei Processi produttivi del Settore lattierocaseario bUfalino e del vino per la produzione di aLimEnti funzionali", PON I&C 2014-2020, Project n. F/200016/01-03/X45. CUP: B61B20000170005.

- PSR REGIONE CAMPANIA 2014-2020 measure 16.1.2. Project" **STRABUF**: Strategie per il miglioramento della redditività dell'allevamento bufalino (Strategies for improving the profitability of buffalo breeding)". CUP: B68H19005200009.

Ľ

Milk and dairy buffalo supply chain in the Americas

**Otavio Bernardes** 

Estimative herd 4 - 5 million Source: local breeders estimatives

# Pastures: the most common model of exploitation in tropical and equatorial areas of Latin America





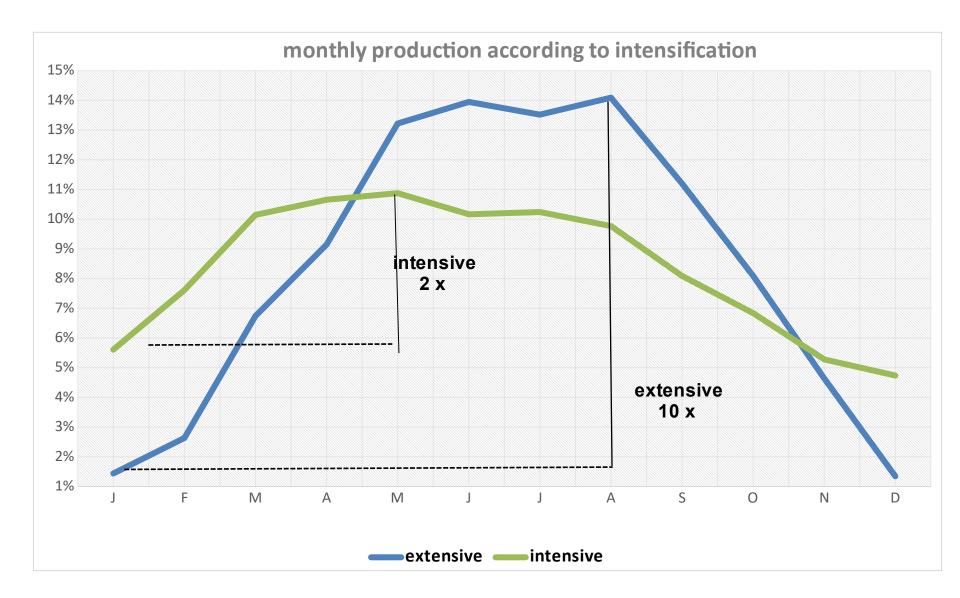
|                        | supplementation<br>and irrigation | without<br>supplements |
|------------------------|-----------------------------------|------------------------|
| Kg of milk/animal/year | 3.552 kg                          | 1.460 kg               |
| Kg of milk/ ha /year   | 11.668 kg                         | 1.168 kg               |

Fat (%):6,6 - 7,5 %Protein (%):4,1 a 4,3 %Lactose(%):4,4 a 5,5 %

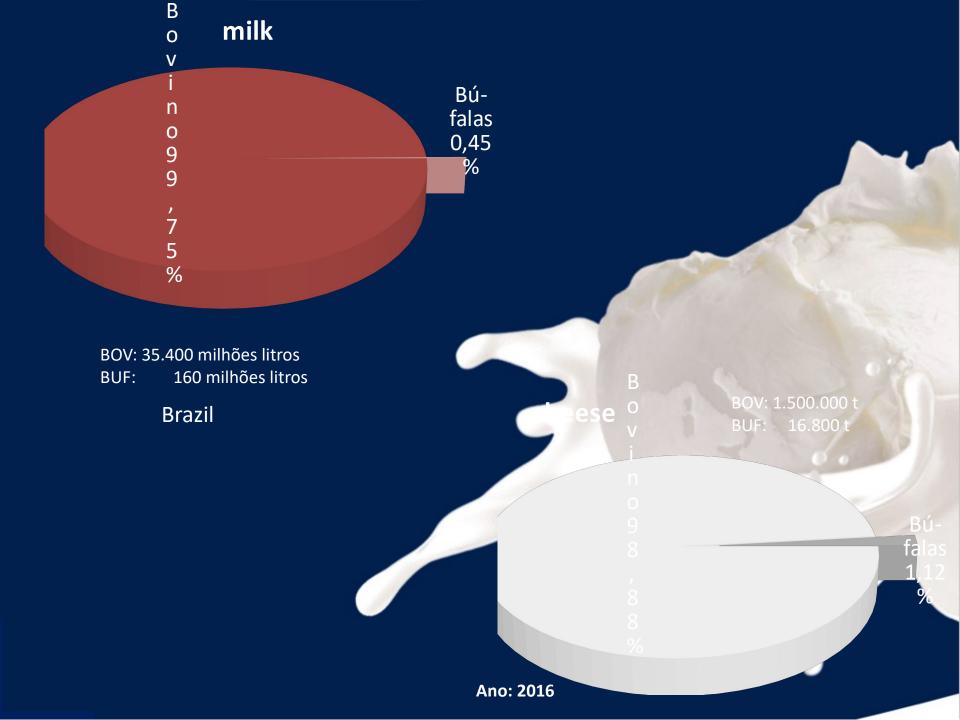
#### Industrial yield: 16-18 %

Tabela 6 - Teores de gordura, proteína e nitrogênio uréico no leite de búfalas ao longo da lactação

| Variáveis | Faz. |                       |                       |                       |                        | Colheitas               |                        |                        |                         |          |
|-----------|------|-----------------------|-----------------------|-----------------------|------------------------|-------------------------|------------------------|------------------------|-------------------------|----------|
|           |      | Abril                 | maio                  | junho                 | julho                  | agosto                  | setembro               | outubro                | novembro                | Média    |
| Gordura   |      |                       |                       |                       |                        |                         |                        |                        |                         |          |
|           | 1    | 5,8±0,381c            | 6,7±0,34 <sup>b</sup> | 7,7±0,64 <sup>a</sup> | 7,5±0,34 <sup>ab</sup> | 7,6±0,34ª               | 7,5±0,34 <sup>ab</sup> | 8,4±0,34 <sup>a</sup>  | 8,6±0,68 <sup>a</sup>   | 7,5±0,16 |
|           | 2    | 5,4±0,34 <sup>b</sup> | 7,4±0,34 <sup>a</sup> | 7,3±0,34 <sup>a</sup> | 7,4±0,34ª              | 7,3±0,53 <sup>a</sup>   | 7,6±0,34 <sup>a</sup>  | 7,6±0,34 <sup>a</sup>  | 7,7±0,48 <sup>a</sup>   | 7,2±0,15 |
|           | 3    | 5,9±0,40°             | 6,6±0,34 abc          | 6,8±0,34 abc          | 6,5±0,34 <sup>bc</sup> | 6,6±0,37 <sup>abc</sup> | 7,4±0,39 <sup>ab</sup> | 7,6±0,40 <sup>a</sup>  | 7,1±0,56 <sup>abc</sup> | 6,8±0,16 |
|           | 4    | 6,4±0,37 <sup>b</sup> | 6,3±0,36 <sup>b</sup> | 6,3±0,34 <sup>b</sup> | 6,1±0,34 <sup>b</sup>  | 6,1±0,34 <sup>b</sup>   | 6,3±0,34 <sup>b</sup>  | 7,0±0,55 <sup>ab</sup> | 8,3±0,56 <sup>a</sup>   | 6,6±0,17 |
|           | 5    | 5,7±0,34 <sup>b</sup> | 6,5±034 <sup>a</sup>  | 7,3±0,34ª             | 6,9±0,36ª              | 6,7±0,34ª               | 6,4±0,34 <sup>ab</sup> | 7,0±0,34ª              | 6,4±0,48 <sup>ab</sup>  | 6,6±0,15 |
| Proteína  |      |                       | -,                    |                       |                        | -1                      |                        |                        |                         |          |
|           | 1    | 4,2±0,13 <sup>b</sup> | 4,1±0,12°             | 4,1±0,12°             | 4,0±0,12°              | 4,0±0,12°               | 4,4±0,12 <sup>b</sup>  | 4,5±0,12 <sup>a</sup>  | 4,5±0,24 <sup>a</sup>   | 4,2±0,06 |
|           | 2    | 4,3±0,12b             | 4,1±0,12°             | 4,1±0,12°             | 4,2±0,12°              | 4,2±0,14°               | 4,3±0,12 <sup>b</sup>  | 4,8±0,12 <sup>a</sup>  | 4,6±0,17 <sup>a</sup>   | 4,3±0,06 |
|           | 3    | 4,0±0,14 <sup>b</sup> | 4,0±0,12°             | 4,0±0,12°             | 3,9±0,12°              | 4,1±0,13°               | 4,4±0,14 <sup>b</sup>  | 4,7±0,14 <sup>a</sup>  | 4,6±0,19 <sup>a</sup>   | 4,2±0,06 |
|           | 4    | 4,3±0,13 <sup>b</sup> | 3,8±0,13°             | 3,9±0,12°             | 3,9±0,12°              | 3,7±0,12°               | 3,9±0,12 <sup>b</sup>  | 4,3±0,19 <sup>a</sup>  | 4,9±0,20 <sup>a</sup>   | 4,1±0,06 |
|           | 5    | 4,5±0,12 <sup>b</sup> | 4,1±0,12°             | 4,1±0,12°             | 3,8±0,13°              | 4,0±0,12°               | 4,0±0,12 <sup>b</sup>  | 4,2±0,12 <sup>a</sup>  | 4,5±0,17 <sup>a</sup>   | 4,2±0,06 |



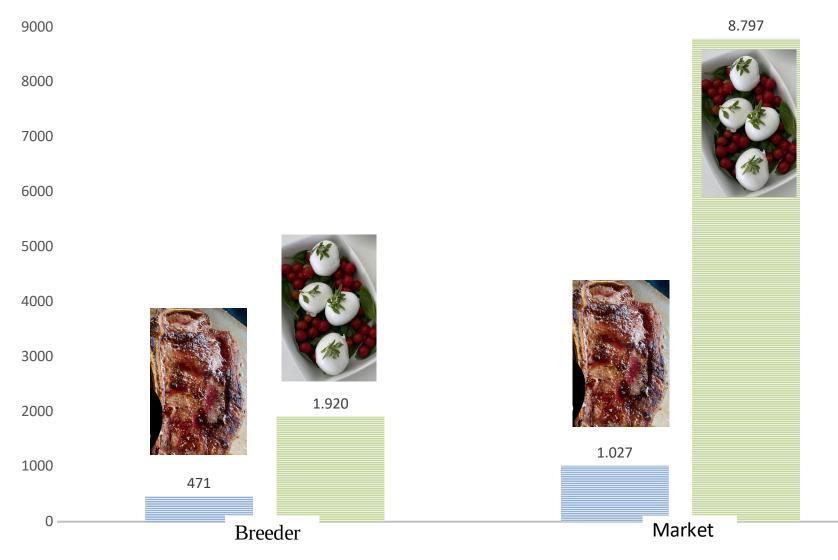


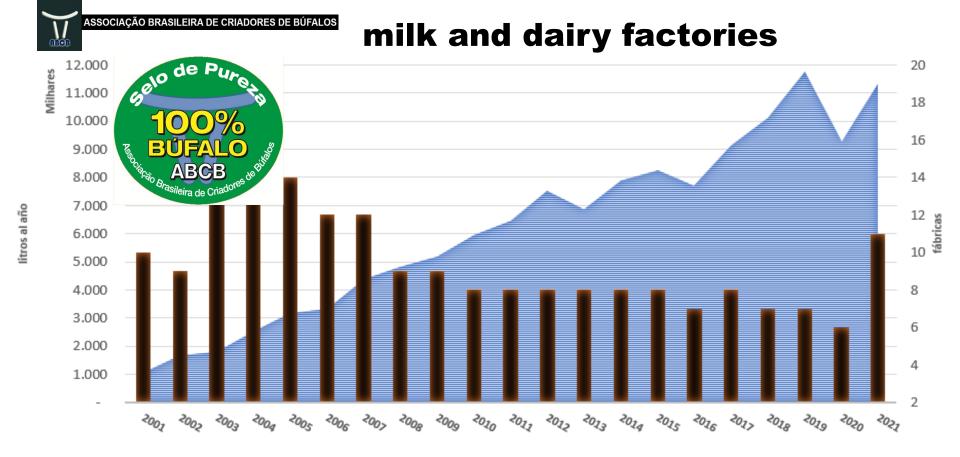


# Colombia64 milhões de litrosVenezuela40% processed milk

## Revenue/value (R\$) in Brazil per buffala in the herd according to type of farm

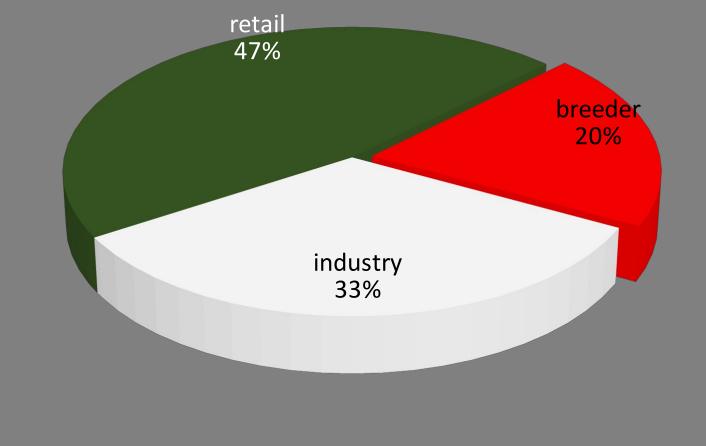
🔳 Corte 🛛 🔳 Leite





The milk processed under the program maintains an average annual growth of **12.7%** between 2001 and 2021, higher than that of bovine milk (4,5%)

# Added value per liter in the buffalo milk mozzarella "chain"



milk price (breeder):€ 0,60 - 0,70 / litrowholesale value:£ 10 - 12 / kg bocconciniRetail price:£ 18 - 20 / kg

## Traditional cheeses made with buffalo milk





### Suero costeño



### **Queso campesino**



### Queso llanero venezuelano



### Queso de Mano





### Queso Criollo



### **Queso maduro**











# Requeijão cremoso de búfala











# Manteiga de leite de búfala



MANTEIGA DE BÚFALA





# Doce de leite de búfala







# Coalhada de búfala



# Requeijão marajoara



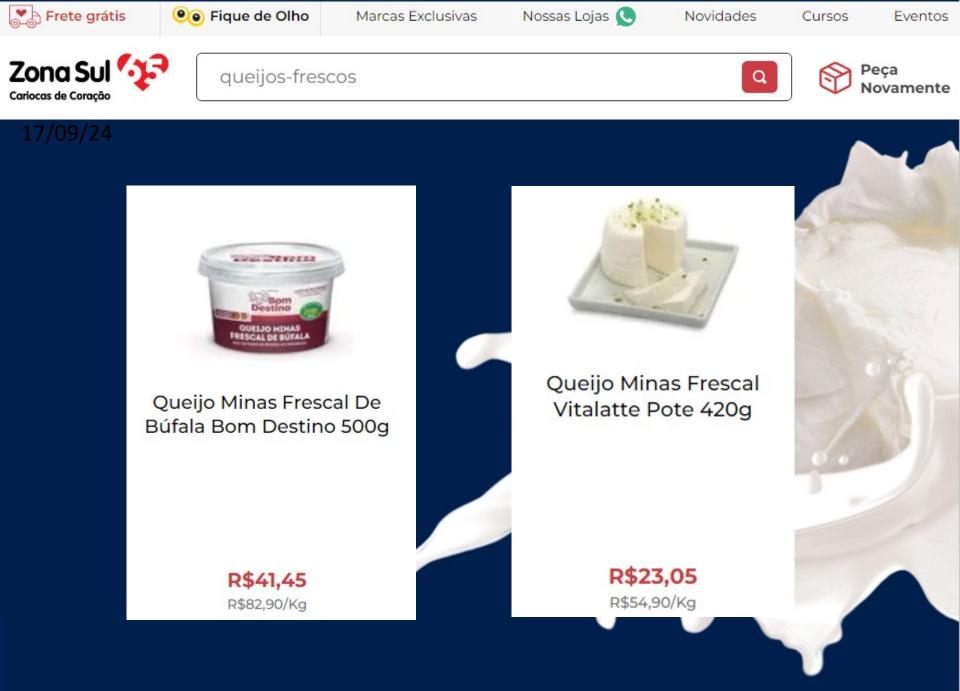
## Queijo coalho de búfala







Consumers attribute value to dairys produced with buffalo milk



🕐 Entrega en:Medellín

#### Q. ¿Qué producto buscas?

acesso em 14/07/2021

-20 %

Leche búfala

STERO COSTER

### Leche vaca

the state of the s

Conull



#### Leche búfala



#### \$10.100 \$8080 VISA (1)

Gramo a \$ 40,56 Queso Mozzarella Bien Star 15 Tajadas x 249 gr



+ 42%

#### Leche vaca



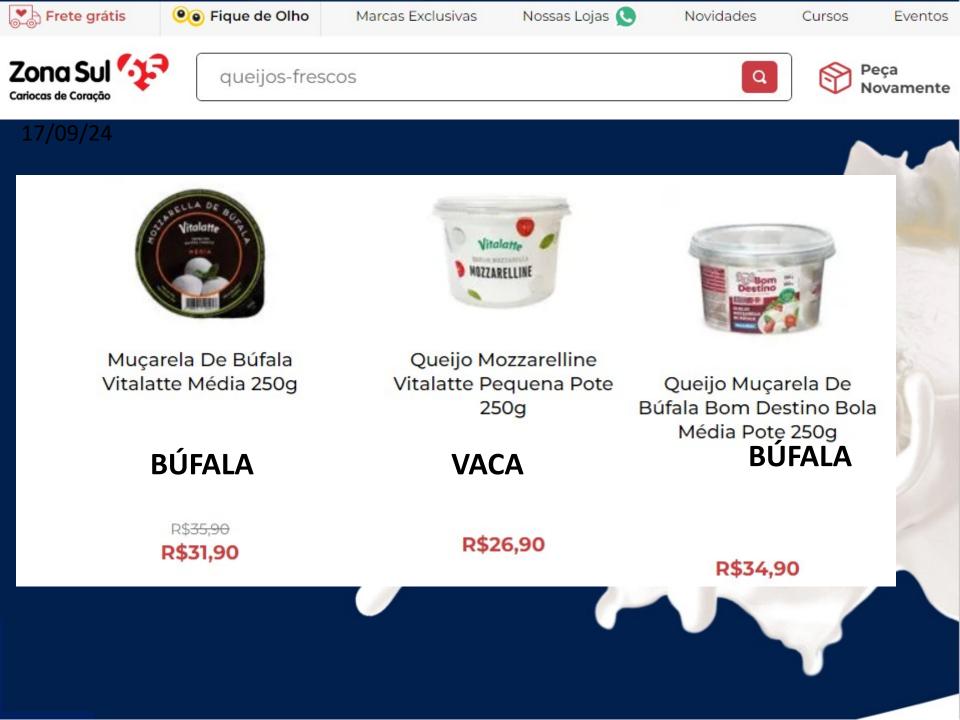
\$ 3500 \$ 2800 VISA (i) Gramo a \$ 17,5 Suero costeño ALQUERIA

200 gr

a <del>\$ 4600</del> **\$ 3680 VISA** ()

Gramo a \$ 23 Suero costeño bufala PLANETARICA 200 gr

+ 31%





#### VACA



#### MOZZARELLA BOCCONCINO

#### \$16,852.00



#### BÚFALA

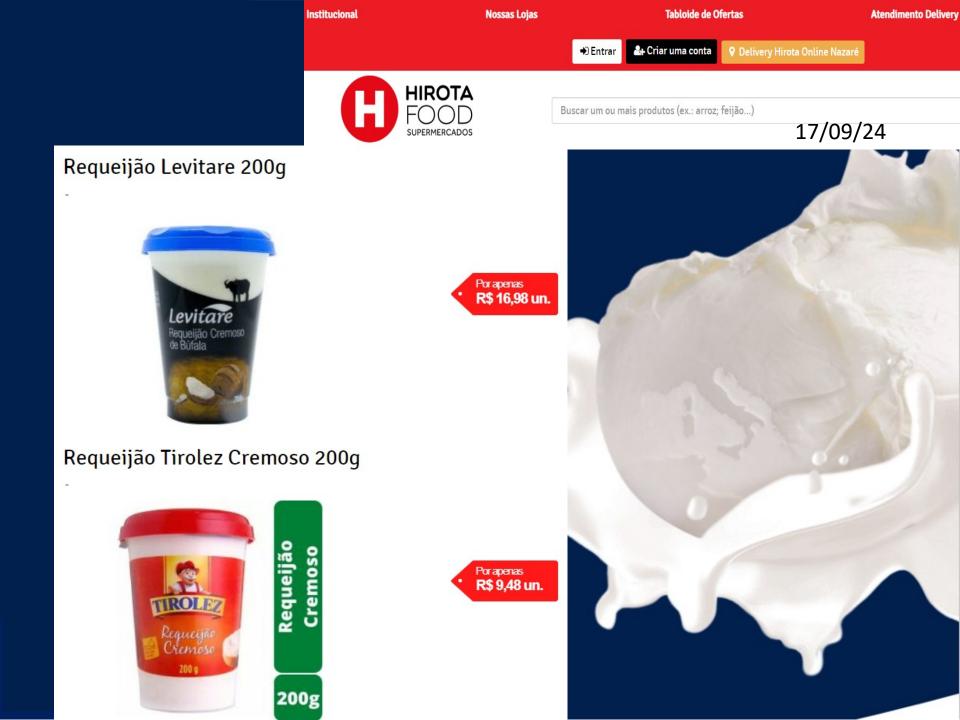


### Mozzarella

### \$6.614,00 ARS \$44.093 / 1000 g

#### Presentación

Mozzarella - Pulpeta x 150 g



alternative products are beginning to be made with buffalo milk

# Leite e creme de leite pasteurizados de búfala (A2A2)



# Embalagem para consumo (sem refrigeração)





### Embalado à vacuo









Mixed products (bovine and buffalo)

Yema Queijo Mozzarella Bocconcino

#### Produtos orgânicos certificados







# Queijos orgânicos de leite de búfala





Manufacturers process multiple types of derivatives seeking to reach a greater number of consumers and increase the sales ticket.



#### Maior diversidade aumento do "cupom"











Maior diversidade aumento do "cupom"



Inclusion of bovine line to expand portfolio and overcome seasonality



## Canada



#### Our Cheeses

#### Soft Cheeses

#### Fresh Cheeses

Semi-Soft, Semi-Firm & Firm Cheese

heeses



Courtenay, Vancouver Island British Columbia, Canada

#### Water Buffalo Cheese

Cheese made with water buffalo milk is graceful, porcelain white freshness. Water buffalo milk is

thicker, richer and nutritionally superior to cow's mi is sourced from Island farms where the gentle wate

Surprise your palate with a simple but profound taste by brie with our tender, moist Mozzarella di Bufala, Buffalo Or, for a truly exotic indulgence, add our handcrafted Bu



#### Mozzarella di Bufala

Authentic Italian mozzarella made with 100% free range water buffalo milk from Vancouver Island. Pure white, firm on the outside and moist creaminess on the inside. Delivers a simple yet profound taste that unfolds in layers of flavour and texture.

2007 British Empire Cheese Competition, Third Place, Pasta Filata Type



#### **Buffalo Brie**

An exotic twist on our world champion Comox Brie, our new Buffalo Brie is the very essence of traditional and inspired cheesemaking. Rich water buffalo milk from the Island transforms this cheese's texture and allows your taste buds to explore new pathways.





## **USA**

Fading D Farm

Water Buffalo

Ricotta di Bufala

Keep Refrigerated ~ Lot #

LUT ALLY HEST BETVETV2

Fading

lassic

Mozzarella

Bufala

100%

Shop Events Caterina

About





🝘 ANNABELLA'

De

Why Water Buffalo? Shop Online Products Find in Stores Blog FAQ More

#### Spoon Up the Goodness!

With Our Rich, Delicious & Super Healthy Water Buffalo Milk Products

Our multiple award-winning yogurts are distinctly different and made with love





FREE RANGE W GRASS FED

MOZZAVELLA di bufala

WATER BUFFALO MOZZARELLA

NET WT. 10 OZ (280G)



Fading D Farp FullCine

> Vater Buil Milk Pet Food



Mozzarella Di Buffala PDO (Italy)

#### \$7.99 Only I available

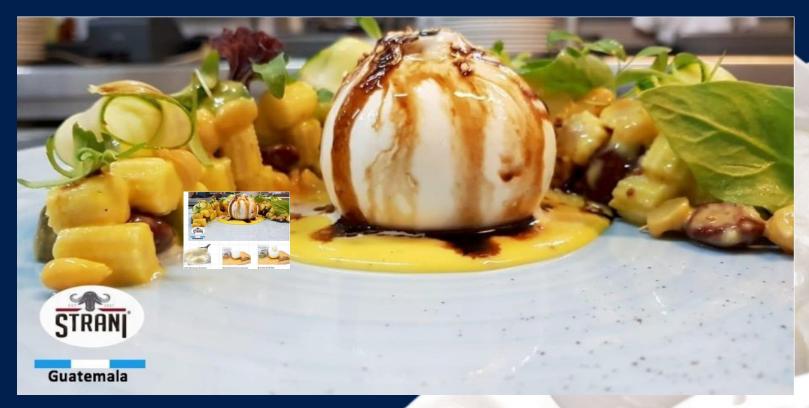
Mozzarella di Bufala Campana PDO (buffalo mozzarella) is a traditional soft cheese, with a white porcelain colour. Original and guaranteed products are packaged with the label "Mozzarella di Bufala

Type of Milk: Italian Mediterranean Buffale Production Area: Mozzarella di Bufala Campana PDO is mainly produced in the Campania region, in the provinces of Caserta Salerno and in several municipalities in the





### Guatemala





GHEE Mantequilla de Bufala



Bufala Mozzarella en salmuera



Burrata de Búfala



## **Costa Rica**



# The second secon

**Buffalo Republic CR** 

176 curtidas • 187 seguidores



CR Cheese Factory @CRCheeseFactory



# Cuba







### Queso de Búfala

# **República Dominicana**



Aperturan en Punta Cana primera quesería que fabrica quesos con leche 100% italiana





# Equador



### Peru



## Bolívia



### Quesos BAYRO

1,1 mil curtidas • 1,2 mil seguidores





# Paraguai







# Equador





## Chile

8



## Argentina









# Colômbia











## Venezuela









#### ASSOCIAÇÃO BRASILEIRA DE CRIADORES DE BÚFALOS

### Selo de pureza



**Otavio Bernardes** 



### **CREA** – Council for Agricultural Research and Economics

FIRST INTERNATIONAL CONFERENCE ON Buffalo

Mozzarella & Milk Products

24/25 Sept. 2024

#### Buffalo mozzarella cheese's: the contribution of the CREA cheesemaker school

#### S. Claps<sup>1</sup>, M.A. D'Oronzio<sup>2</sup>

CREA<sup>1</sup> Council for Agricultural Research and Economics)- Research Centre for Animal Production and Aquaculture), Italy

CREA<sup>2</sup> Council for Agricultural Research and Economics)- Research Centre of Policy and Bioeconomy, Italy



### **CREA** – Council for Agricultural Research and Economics

The Center deals with animal husbandry and aquaculture, carrying out genetic improvement programs and developing innovations in the field of products of animal origin and the control of their sophistication, as well as systems and technologies for the optimization of farms. The center carries out conservation activities of zootechnical biodiversity, as well as genetic improvement of forage and protein species for zootechnical nutrition.







Research Center for Animal Production and Aquaculture (CREA- ZA)

**Structures:** Monterotondo (RM), **Lodi**, Modena, Bella Muro (PZ) Director: Salvatore CLAPS salvatore.claps@crea.gov.it <u>https://www.researchgate.ne</u>



MODENA

MONTEROTONDO

#### **Research unit of CREA-ZA:**

Lodi: Viale Piacenza, 29 e Via Antonio Lombardo, 11



Monterotondo (RM): Via Salaria, 31



Bella Muro (PZ): S.S. 7 Via Appia



Modena: Via Beccastecca, 345 San Cesario sul Panaro





### **CREA** – Council for Agricultural Research and Economics



#### **CREA** cheesemaker school

#### Bella Muro (PZ): S.S. 7 Via Appia

CREA School was set up, at the Bella (PZ) site of CREA research Centre for Animal Production and Aquaculture, in collaboration with Research Centre of Policy and Bioeconomy Basilicata (CREA-PB)







### **Target - To train young people in the dairy sector**

# Specialised profiles capable of preserving the typicality of traditional cheeses and innovation

*This cheesemaker's school develops joint actions with public and private objects for the development of the dairy sector and the Italian inner areas* 

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

24/25 Sept. 2024





CREA-CENTRO DI POLITICHE E BIOECONOMIA DI POTENZA | CREA-CENTRO DI ZOOTECNIA E ACQUACOLTURA DI BELLA (PZ)

CORSO PER TECNICI SPECIALIZZATI NELLE PRODUZIONI LATTIERO-CASEARIE TRADIZIONALI SOSTENIBILI

#### PROGRAMMA

MODULO INTRODUTTIVO 14 novembre 2022 – 16 novembre 2022

SOSTENIBILITÀ/BIODIVERSITÀ 17 novembre 2022 – 18 novembre 2022

QUALITÀ DELLE PRODUZIONI PRIMARIE: IL LATTE 21 novembre 2022–25 novembre 2022

IMPIANTI, METODI E CERTIFICAZIONI PER LA QUALITÀ 28 novembre 2022 – 2 dicembre 2022

PASTE FILATE 12 dicembre 2022–16 dicembre 2022

I FORMAGGI A PASTA DURA TRADIZIONALI E INNOVATIVI 19 dicembre 2022–23 dicembre 2022

GESTIONE AMMINISTRATIVA DEI CASEIFICI TRADIZIONALI E INNOVATIV 16 gennaio 2023 – 20 gennaio 2023

I FORMAGGI TRADIZIONALI E INNOVATIVI OVINI E CAPRINI 23 gennaio 2023–27 gennaio 2023

SOSTENIBILITÀ E INNOVAZIONE 30 gennaio 2023 – 1° febbraio 2023

LA STAGIONATURA E I DIFETTI DEI FORMAGGI 2 febbraio 2023 – 8 febbraio 2023

CASEIFICAZIONI CON DIVERSE TIPOLOGIE CASEARIE E ANALISI SENSORIALE 9 febbraio 2023 – 14 febbraio 2023

I FORMAGGI A PASTA FRESCA E MOLLI E "MANI IN PASTA" 15 febbraio 2023–21 febbraio 2023

GESTIONE DEI PROCESSI PRODUTTIVI E CONTROLLO DELLA QUALITÀ AGRO-ALIMENTARE 22 febbraio 2023 – 24 febbraio 2023

VALORIZZAZIONE E MARKETING 27 febbraio 2023 – 28 febbraio 2023

STAGE 1° marzo 2023 - 7 aprile 2023

VERIFICHE E CHIUSURE CORSO 13 aprile 2023 – 14 aprile 2023

Il corso prevede una forte integrazione tra le attività in aula e le attività di caseificazione.

SEGRETERIA E INFO: SALVATORE.CARICATI@CREA.GOV.IT

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

### Technician of traditional sustainable dairy production

This course aimed at young people under 40 years of age with a technical or professional diploma or with skills acquired in the dairy sector. This is a 540-hour course, including 240 hours of internship in affiliated companies, to acquire skills in different subject multiple areas: sustainability and biodiversity, quality of primary productions, plants, methods and certifications for quality, administrative management of traditional and innovative dairies, pasta filata, fresh and soft cheeses, hard cheeses, sheep and goat cheeses, cheese-making with different types of cheese with sensory analysis



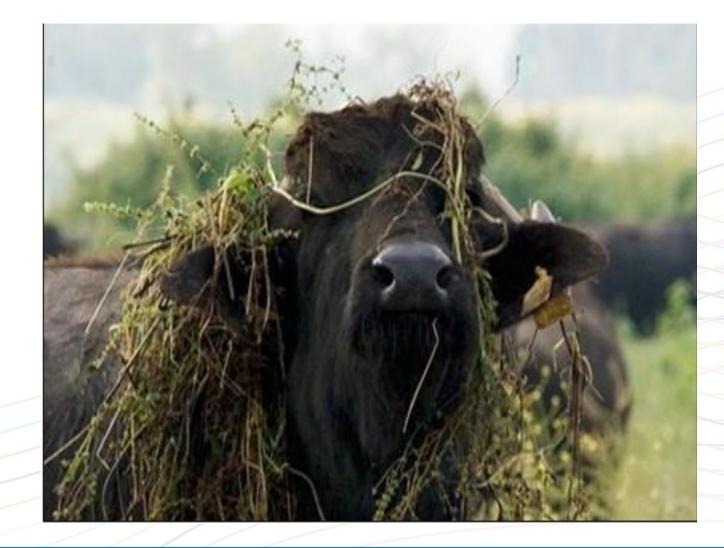
#### CONCLUSIONS

Training is one of the fundamental factors in meeting the challenges of competitiveness and innovation by guaranteeing the quality of our Italian excellence





### **Thank you for your attention !**



FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella

& Milk Products

24/25 Sept. 2024

### **EVALUTATION OF THE NON-COMPLIANCES IN BUFFALO MILK AND BUFFALO DAIRY PRODUCTS IN THE PROVINCE OF CASERTA**

FIRST INTERNATIONAL CONFERENCE ON

### Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

#### M.F. Peruzy<sup>1</sup>, G. Smaldone<sup>2</sup>, N. Gammarano<sup>2</sup>, R. Taglialatela<sup>2</sup>, N. Murru<sup>1</sup>

<sup>1</sup> Department of Veterinary Medicine and Animal Production, University of Naples "Federico II",80137 Naples, Italy;

<sup>2</sup> Prevention Department ASL Caserta, UOC Food Hygiene of A.O.. Via Feudo di San Martino 10, 81100 Caserta (CE)

Powered by







Dipartimento Medicina Veterinaria Produzioni Animali





### The Buffalo (Bubalus bubalis)





## The Buffalo (Bubalus bubalis)

#### **Official controls**

The activity is carried out through official control over the entire milk production chain, from the farm to the final consumer, and is implemented across all stages: raw material production, thermal treatment, processing, storage, and distribution.

Why?

 $\clubsuit$  check the health of animals,

monitoring the cleanliness and hygiene of the milking process,

\* Test the milk and dairy products for contaminants or diseases.



# Aim





# Analysis of the prevalence of non-compliance observed in buffalo milk and buffalo dairy products in the province of Caserta during the years 2022 and 2023.







# **Materials and Methods**



#### **Materials and Methods**

 $\leftarrow \rightarrow C$ 

○ A ब https://www.gisacampania.it



ATTENZIONE GISA e gli altri sistemi sono accessibili esclusivamente tramite SPID/CIE al link <u>sca.gisacampania.it</u>. Solo i NUOVI utenti dovranno prima registrarsi seguendo le istruzioni indicate al medesimo link.



☆













Catalogo Riuso Agid

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products







#### n. 1330 samples (2022= 829, 2023= 501)

# 837 Milk samples and 483 Dairy products (35 Food matrices )





#### 813 livestock companies



#### **276** approved companies



#### 240 registered companies



FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products



|                   | Chemicals                              |          |                                |   |                  |                          |
|-------------------|--|----------|--------------------------------|---|------------------|--------------------------|
| ADDITIVES         | Polyphosphates                         | 2        | Bacteriological                |   |                  |                          |
| ALLERGENS         | Lactose                                | 2        |                                |   |                  |                          |
| CONTAMINANTS      | Arsenic                                | 1        | BACTERIA                       | B. Cereus                                       | 3                |                          |
|                   | Dioxin<br>Dioxin And Pcb Dioxin Like   | 3        |                                | Sulfite-Reducing Bacteria                       | 1                |                          |
|                   | Phytosanitary                          | 13       |                                | <i>Brucella</i> Spp.                            | <mark>557</mark> |                          |
|                   | Polycyclic Aromatic Hydrocarbons (Pah) | 1        |                                | Brucella Abortus                                | 44               |                          |
|                   | Pcb Dioxin Like                        | 4        |                                |   |                  |                          |
|                   | Pcb-Ndl                                | 8        |                                | Mesophilic Bacteria                             | 87               |                          |
| CHEMICAL ELEMENTS | Nickel                                 | 1        |                                | Clostridium Perfrigens                          | 1                |                          |
| INHIBITING        | Inhibiting                             | 5        |                                | Coliforms                                       | 1                |                          |
| HEAVY METALS      | Lead                                   | 15       |                                | Enterobatteriacee                               | 24               |                          |
|                   | Cadmium                                | 2        |                                |   |                  | -                        |
| MYCOTOXINS        | Mercury<br>Aflatoxin M1                | 1        |                                | Staphylococcal Enterotoxin                      | 74               | (a)                      |
| MICOIOAINS        | Aflatoxin B1                           | +8       |                                | Escherichia Coli O157:H7                        | 3                |                          |
| FOOD QUALITY      | Caseinate And Cow's Milk Revelation    | 2<br>114 |                                | Detection Of Escherichia Coli                   | 14               |                          |
|                   | Species Identification 2               |          | Verocitotoxic Escherichia Coli | 15  |                  |                          |
|                   | Species Proteins                       | 2        |                                |   | 5                | <b>N</b> 11              |
|                   | Lactose Quantification                 | 4        |                                | Listeria Spp                                    | 3                |                          |
|                   | Cow Milk Research                      | 1        |                                | Listeria Monocytogenes                          | 101              |                          |
| DRUG DEGIDUEG     | Cow's Milk Whey Protein                | 6        |                                | Enumeration Of Escherichia Coli                 | 11               |                          |
| DRUG RESIDUES     | Polypeptide Antibiotics<br>Avermectin  | 1        |                                | Enumeration Of Pseudomonas                      | 4                |                          |
|                   | Avermectins<br>Avermectins/Milbemicins | 4        |                                | Enumeration Of Stafiloccoccus Aureus            | 1                |                          |
|                   | Benzimidazoles                         | 9        |                                |   | 4                |                          |
|                   | Caf                                    | 1        |                                | Enumeration OF Coagulase-Positive Staphylococci | 29               |                          |
|                   | Quinolonics                            | 10       |                                | Salmonella Spp                                  | 37               |                          |
|                   | Chloramphenicol                        | 10       |                                | Salmonella Thyphimurium                         | 2                |                          |
|                   | Macrolides                             | 4        |                                | Staphylococci                                   | 2                |                          |
|                   | Penicillins                            | 13       |                                |   | ے<br>11          |                          |
|                   | Sulphamides                            | 8        |                                | Streptococci                                    |                  |                          |
|                   | Tetracyclines                          | 12       |                                |   |                  |                          |
|                   |  |          |                                | Gene Sequencing                                 | 1<br>FIRST INTER | RNATIONAL CONFERENCE ON  |
| 10                |  |          |                                | Radionuclides                                   |                  | zzarella & Milk Products |
|                   |  |          |                                | Mould And Yeast                                 | 11               |                          |

#### 8.38 %, (n= 71/847)





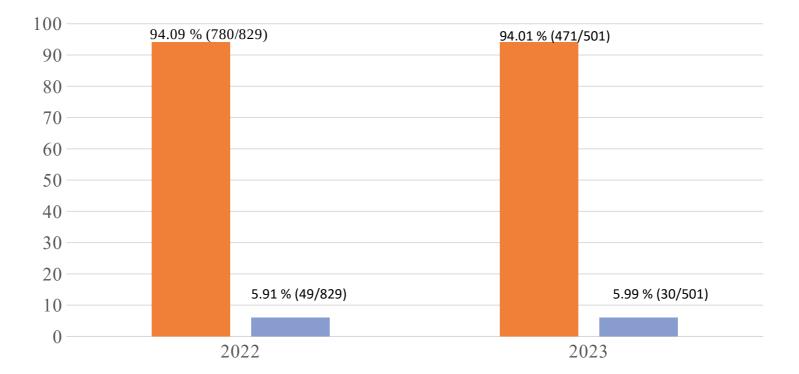
#### Caseinate and Cow's Milk Revelation



#### Enterobacteriaceae



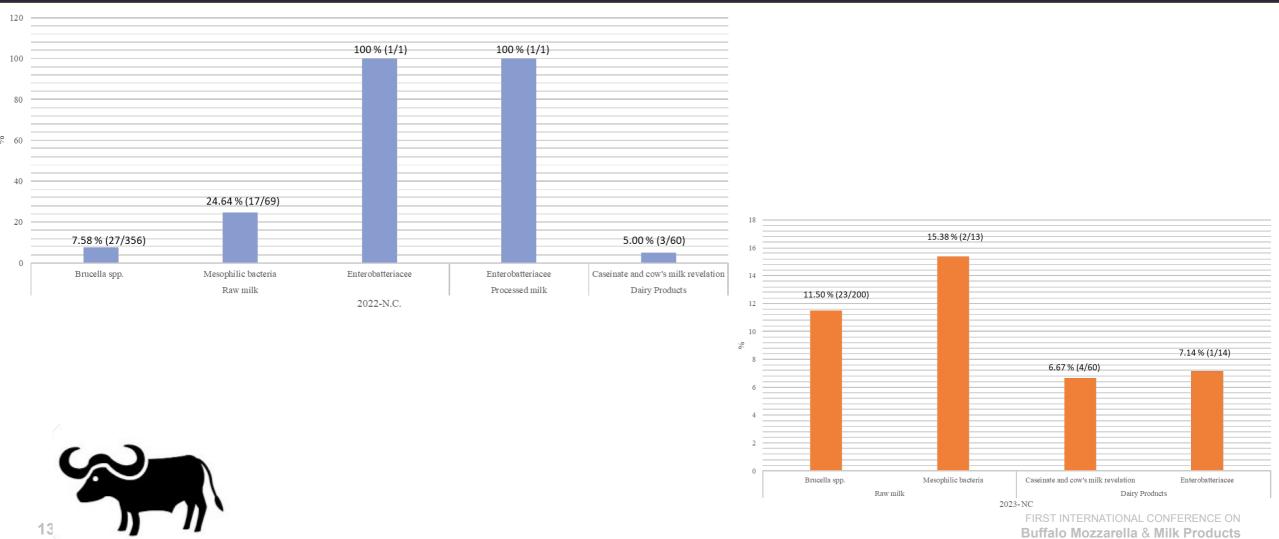
FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products





■C. ■N.C.

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products







#### 23.17 % of samples exceed the limit for mesophilic bacteria in milk



02004R0853 - EN - 01.01.2021 - 020.001 - 1

This text is meant purely as a documentation tool and has no legal effect. The Union's institutions do not assume any liability for its contents. The authentic versions of the relevant acts, including their preambles, are those published in the Official Journal of the European Union and available in EUR-Lex. Those official texts are directly accessible through the links embedded in this document

▶<u>E</u> ▶<u>C1</u> REGULATION (EC) № 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004

laying down specific hygiene rules for food of animal origin ◀

(OJ L 139, 30.4.2004, p. 55)

- (a) Food business operators must initiate procedures to ensure that raw milk meets the following criteria:
  - (i) for raw cows' milk:

| Plate count at 30 °C (per ml) | ≤ 100 000 (*)       |
|-------------------------------|---------------------|
| Somatic cell count (per ml)   | $\leq$ 400 000 (**) |

(\*) Rolling geometric average over a two-month period, with at least two samples per month.

(\*\*) Rolling geometric average over a firse-month period, with at least one sample per month, unless the competent authority specifies another methodology to take account of seasonal variations in production levels.

(ii) for raw milk from other species:

| Plate count at 30 °C (per ml)                                      | $\leq 1 \ 500 \ 000 \ (*)$    |
|--|-------------------------------|
| (*) Rolling geometric average over a two<br>two samples per month. | o-month period, with at least |

(b) However, if raw milk from species other than cows is intended for the manufacture of products made with raw milk by a process that does not involve any heat treatment, food business operators must take steps to ensure that the raw milk used meets the following criterion:

Plate count at 30 °C (per ml)

& Milk Products

(\*) Rolling geometric average over a two-month period, with at least two samples per month.

< 500 000 (\*)

#### 9 % of samples were positive to the milk-ELISA test

- milk-ELISA test is a screening test
- No information on the outcome of the serological tests.

#### 5.83 % of samples were positive to caseinate and cow's milk revelation

The addition of cow milk is the most common fraud in the production of Mozzarella di Bufala Campana PDO (buffalo mozzarella)







# Conclusions



A low number of non-compliances was detected; however, while the results are encouraging, further and continued monitoring would be necessary to ensure long-term safety of buffalo dairy products



# Grazie per l'attenzione!

Maria Francesca Peruzy



DVM, Ph.D., Dipl. ECVPH Dep. Of Veterinary medicine and A.P., UNINA

mariafrancesca.peruzy@unina.it

Index of freshness: a tool to assess the quality of the Buffalo Mozzarella cheese production

B. la Gatta, M. Rutigliano, F. Dilucia, M.T Liberatore, A. Di Luccia

Department of Sciences of Agriculture, Food, Natural Resources, and Engineering (DAFNE), University of Foggia, Via Napoli, 25, 71122, Foggia, Italy



Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Powered by







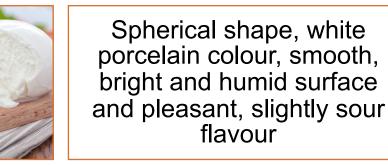


### Freshness of Buffalo Mozzarella Cheese (BMC)

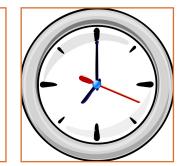
Freshness is a complex attribute to define, since it is the expression of technological, chemical and sensory elements and it is one of the attributes expected by the

consumers.





The use of fresh milk and the preservation of technological properties (i.e., texture and aroma)



The use of milk within 60 hours from milking, allow to expect very specific characteristics of the raw material Regulation (EC) no. 1107/1996

#### **Plasmin Activity**

- Lys<sup>28</sup>-Lys<sup>29</sup>
- Lys<sup>68</sup>-Ser<sup>69</sup>
- Lys<sup>105</sup>-His<sup>106</sup>
- Lys<sup>107</sup>-Glu<sup>108</sup>



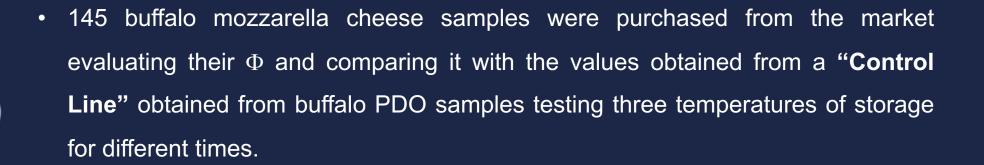
The occurrence of primary proteolysis

# Index of Freshness Chemical assessment

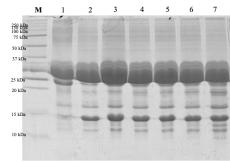
The study of primary proteolysis on  $\beta$ -CN, with the formation of  $\gamma$ -CN fragments, through an electrophoretic approach was applied as method to assess the freshness of PDO Buffalo Mozzarella cheese.

$$\Phi = \frac{\beta - CN}{\beta - CN + \gamma 1 + (\gamma 2 + \gamma 3) + \gamma 4} \times 100$$

Trace quantiity of the electrophoretic bands, obtained by the image analysis



### **Outcomes of the investigation**



SDS-PAGE of control line samples. Electrophoretic profiles of the samples stored at room temperature

|  | 14       |
|--|----------|
|  | 15       |
|  | 16       |
| <sup>20</sup> - At room temperature:   | 17       |
| 75   | 18       |
| freshness ranged from 75.10%   | 19       |
|  | 20       |
| <sup>1</sup> / <sub>a</sub> <sup>1</sup> / <sub>x<sup>-1,4854+7,477</sup></sub> <sup>409</sup> / <sub>z</sub> to 69.84 % (3 days). Then,   | 21<br>22 |
| " freshness decreased with a   | 23       |
| in treshness decreased with a  | 23       |
| concomitant increase of pH (pH   | 25       |
| · · · ·  | 26       |
| <sup>10</sup> <sup>2</sup> <sup>4</sup> <sup>6</sup> <sup>10</sup> <sup>12</sup> <sup>14</sup> <sup>16</sup> <sup>10</sup> <sup>12</sup> <sup>14</sup> <sup>16</sup> <sup>10</sup> <sup>10</sup> <sup>12</sup> <sup>14</sup> <sup>16</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> <sup>10</sup> | 27       |
|  | 28       |
| Lines of the structure (pH 7.05);  | 29       |
| 10 , p r 8,00  | 30       |
| <sup>80</sup><br><sup>15</sup> B [ <sup>8,00</sup><br><sub>7,50</sub>  | 31 32    |
| y = d 3979 + 72 1 = 20   | 32       |
|  | 34       |
| At 4°C: from 75.10% up to  | 35       |
|  | 36       |
| 69.75 % (5 days).  | 37       |
| 50   | 38       |
| 45 - 459   | 39       |
| 40 L 40 L 409<br>0 5 10 15 20 25 50  | 40       |
| Days of Storage  | 41<br>42 |
| Anders of Protinces epil   | 42       |
|  | 43       |
| <sup>30</sup> c  | 45       |
| <sup>1</sup> At 20°C, freebace could be  | 46       |
|  | 47       |
| considered prolonged until 14  | 48       |
|  | 49       |
| days (from 75.10% up to 68.84  | 50       |
| <sup>50</sup><br>s   | 51       |
|  | 52       |

#### Index of freshness of the samples from the Market

| Batch 1  |                |                  | Batch 3            |         |       |         |       |
|----------|----------------|------------------|--------------------|---------|-------|---------|-------|
| Samples  | Ф (%)          | Samples          | Batc<br>\$\Phi (%) | Samples | Ф (%) | Samples | Ф (%) |
| 1        | 66,03          | 1(53)            | 56,88              | 53(105) | 72,5  | 1(127)  | 68,02 |
| 2        | 70,39          | 2(54)            | 54,53              | 54(106) | 70,13 | 2(128)  | 66,16 |
| 3        | 74,00          | 3(55)            | 59,7               | 55(107) | 63,92 | 3(129)  | 66,63 |
| 4        | 72,62          | 4(56)            | 50,7               | 56(108) | 68,14 | 4(130)  | 64,03 |
| 5        | 71,09          | 5(57)            | 58,86              | 57(109) | 76,07 | 5(131)  | 60,55 |
| 6        | 72,31          | 6(58)            | 62,24              | 58(110) | 57,33 | 6(132)  | 60,37 |
| 7        | 74,67          | 7(59)            | 67,57              | 59(111) | 79,17 | 7(133)  | 59,73 |
| 8        | 70,51          | 8(60)            | 59,42              | 60(112) | 67,19 | 8(134)  | 62,13 |
| 9        | 74,32          | 9(61)            | 60,92              | 61(113) | 74,06 | 9(135)  | 63,04 |
| 10       | 71,1           | 10(62)           | 65,54              | 62(114) | 75,48 | 10(136) | 63,67 |
| 11       | 70,77          | 11(63)           | 59,5               | 63(115) | 74,02 | 11(137) | 65,19 |
| 12       | 75,56          | 12(64)           | 64,65              | 64(116) | 71,91 | 12(138) | 61,38 |
| 13       | 74,67          | 13(65)           | 58,91              | 65(117) | 79,59 | 13(139) | 62,89 |
| 14       | 56,1           | 14(66)           | 64,3               | 66(118) | 76,07 | 14(140) | 67,43 |
| 15       | 59,74          | 15(67)           | 63,65              | 67(119) | 75,68 | 15(141) | 65,43 |
| 16       | 65,1           | 16(68)           | 63,66              | 68(120) | 79,04 | 16(142) | 63,77 |
| 17       | 71,76          | 17(69)           | 53,76              | 69(121) | 62,73 | 17(143) | 64,89 |
| 18       | 62,1           | 18(70)           | 58,3               | 70(122) | 70,59 | 18(144) | 62,09 |
| 19       | 75,51          | 19(71)           | 66,69              | 71(123) | 66,22 | 19(145) | 62,59 |
| 20       | 67,34          | 20(72)           | 67,89              | 72(124) | 78,52 |         |       |
| 21       | 67,22          | 21(73)           | 64,24              | 73(125) | 74,06 |         |       |
| 22       | 65,99          | 22(74)           | 62,25              | 74(126) | 74,34 |         |       |
| 23       | 68,25          | 23(75)           | 61,06              |         |       |         |       |
| 24       | 71,82          | 24(76)           | 66,78              |         |       |         |       |
| 25       | 64,75          | 25(77)           | 65,78              |         |       |         |       |
| 26       | 54,00          | 26(78)           | 59,2               |         |       |         |       |
| 27       | 64,23          | 27(79)           | 59,03              |         |       |         |       |
| 28       | 68,98          | 28(80)           | 74,69              |         |       |         |       |
| 29       | 60,36          | 29(81)           | 69,41              |         |       |         |       |
| 30       | 66,31          | 30(82)           | 66,6               |         |       |         |       |
| 31       | 70,98          | 31(83)           | 70,67              |         |       |         |       |
| 32<br>33 | 60,07          | 32(84)           | 59,05              |         |       |         |       |
| 33       | 64,39<br>63,02 | 33(85)           | 73,4<br>67,96      |         |       |         |       |
| 34       | 61,31          | 34(86)<br>35(87) | 72,92              |         |       |         |       |
| 36       | 61,7           | 36(88)           | 75,32              |         |       |         |       |
| 37       | 65,38          | 37(89)           | 72,81              |         |       |         |       |
| 38       | 51,48          | 38(90)           | 70.79              |         |       |         |       |
| 39       | 65,61          | 39(91)           | 74,91              |         |       |         |       |
| 40       | 59,37          | 40(92)           | 69,52              |         |       |         |       |
| 41       | 64,43          | 41(93)           | 69,48              |         |       |         |       |
| 42       | 65,49          | 42(94)           | 72,49              |         |       |         |       |
| 43       | 60,23          | 43(95)           | 69,21              |         |       |         |       |
| 44       | 63,65          | 44(96)           | 70,96              |         |       |         |       |
| 45       | 62,36          | 45(97)           | 73,2               |         |       |         |       |
| 46       | 67,82          | 46(98)           | 71,74              |         |       |         |       |
| 47       | 60,32          | 47(99)           | 73,28              |         |       |         |       |
| 48       | 62,61          | 48(100)          | 72,86              |         |       |         |       |
| 49       | 63,81          | 49(101)          | 73,22              |         |       |         |       |
| 50       | 66,74          | 50(102)          | 70,51              |         |       |         |       |
| 51       | 61,44          | 51(103)          | 73,87              |         |       |         |       |
| 52       | 56,55          | 52(104)          | 79,11              |         |       |         |       |
|          |                |                  |                    |         |       | ı       |       |

The values of the index of freshness ranged from a maximum of 79.59% to a minimum of 50.7%. Overall, 34.5% of the samples had an index of freshness > 70.00% and 65.5% had an index of freshness < 70.00%, but from these latest, only 21 samples (15%) had an index of freshness <60%.

A value higher than 70% is maintained up to 3 days at RT, 5 days at 4°C and about 10 days at 20°C. Comparing results of the samples from the market with those of the "Control Line" samples, the data of the index of freshness of the unknown samples were consistent with them, considering that the manufacturing and the quality of the raw matter can influence the rate of proteolysis and, consequently, the index.

Our results allowed to verify the feasibility of the *Index of freshness* as a helpful tool to assess the quality of the Buffalo mozzarella samples. Therefore, the Index of freshness can be considered an important contribution to the enhancement of an excellence of the *Made in Italy* production.

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

Results from Rutigliano et al., (2022).

Trends of the index of freshness and pH of control line samples.

# Grazie per l'attenzione!

Mariacinzia Rutigliano

mariacinzia.rutigliano@unifg.it



# Dairy under attack? Let's safeguard the essentials at EU level! Alexander Anton

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

24/25 Sept. 2024







#### Giuseppe Ambrosi, Ambrosi spa (IT)



**Peter Giørtz-Carlsen**, Arla (DK)



**Ingo Müller,** DMK (DE)



**Albert de Groot**, Vreugdenhil (NL)



**Jan Teplý,** Madeta (CZ)



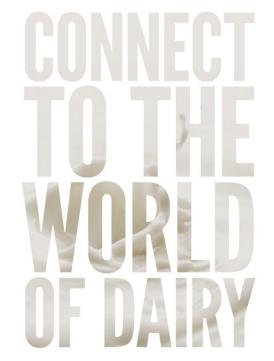


# European Dairy Association



- Association of national dairy industry associations of 20 EU Member States, the UK and Georgia
- Cooperative and private milk processors
- More than 90% of the milk volumes processed in the EU





## Squadra lactosferica: EDA team in Brussels







"The role of the European Dairy Association in ensuring that the voice of your stakeholders is heard in Europe is key. I look forward to continuing our close cooperation to ensure that dairy has a promising future in Europe."

> Ursula von der Leyen President of the European Commission

## **Dairy Policy Conference**

European Dairy & Next Generation EU 2024 - 2029 IO April 2024

## MEP **Christophe Hansen** (EPP, LUX)

Nominated EU Commissioner for Agriculture & Food 2024 - 2029



# High Level Mission of EU Commissioner Janusz Wojciechowski to China 2018





Connect to the world of dalry\_

## Mozzarella at the EDA offices!

This week, EDA was surprised with a truly wonderful gift - none other than the exquisite fine cheese **Mozzarella di Bufala Campana** from the Consorzio in Caserta. The Mozzarella di Bufala Campana, is a Protected Designation of Origin (PDO), meaning that it has the strongest link to the place where it's made the Campania region of Italy - and stands as a testament to the rich heritage and unparalleled craftsmanship of the region – get inspired: https://www.mozzarelladop.it/territory



Mille grazie, dott Pier Maria Saccani, merci beaucoup, cher Pier Maria!



#### Welcome to our new trainees:

#### Marcella and Florian!



I love all varieties of cheese but my favourite one is for sure Stracciatella di bufala Florian Spiegelhalter Trade and Economics Trainee



That is like asking who your favourite child is but let's go with Mozzarella di bufala, PDO from Campania!!

Marcella Rosato

Food, Environment and Health Trainee

# **EDA Vision for Agriculture**

**15 November - Rome Italy** 

Strategic Autonomy in Food Production

#### The Importance of the Dairy Sector

- Dairy as a Cultural and Economic Pillar
- Moving Beyond Commoditization

Single Market – The Fundamentals of Europe

Harmonized Legal Framework

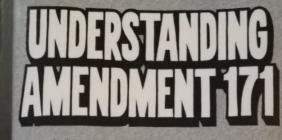
#### Global Dairy Trade Leadership

- Reshoring and Diversification
- Prioritizing Trade Policy
- Fostering Competitiveness, Transition, and Resilience – CAP 2027

#### Protection of Dairy Terms

#### **Promotion Policy**

### **School Milk Scheme**



In October, the European Parliament voted YES on Amendment 171 which could make it illegal for plant-based foods to reference dairy in any way, shape, or form. "Dairy-free alternatives"? Banned. "Creamy texture"? Banned. The amendment could even make our current packaging illegal simply because it's the same type of packaging dairy products use. And even crazier, the amendment might prohibit climate footprint comparisons which awkwardly goes directly against the EU's own climate goals.

So who's behind such a silly law? Well, when AM 171 passed in the Parliament, the European Dairy Association said:

#### THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!

We're confident the European Council of Ministers will strike down this amendment. They're smart enough to see through the dairy lobby's tricks and they know that the people of Europe are not actually stupid. But just to be safe, join us in signing the petition:

# UNDERSTANDING AMENDMENT 171

In October, the European Parliament voted YES on Amendment 171 which could make it illegal for plant-based foods to reference dairy in any way, shape, or form. "Dairy-free alternatives" Banned. "Creamy texture"? Banned. The amendment could even make our current Jackaging illegal simply because it the same type of packaging dairy products use. And even crazier, the amendment might prohibit climate footprint comparisons which auko goes directly against the EU's out So who's behind such a silly l climate goals. when AM 171 passed in the Pa





So who's behind such a silly law? Well, when AM 171 passed in the Parliament, the European Dairy Association said:

### THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!



71 which could 11-based foods 14-based foods 14-based foods 15 way, shape, 17 anned. The 18 ke our current 19 because it's 19 dairy 19 razier, the 1 climate 11 hich awkwardly 19 EU's own

illy law? Well, e Parliament, ciation said:

#### THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!

We're confident the European Council of Ministers will strike down this amendment. They're smart enough to see through the dairy lobby's tricks and they know that the people of Europe are not actually stupid. But just to be safe, join us in signing the petition:





# whey. for living. for life.





**Giuseppe Ambrosi** 



**Emmanuel Besnier** 



Rose O'Donovan



Paolo Zanetti



Jan Vreugdenhil



**James Neville** 



Gianpiero Calzolari



**Pat Murphy** 



14 - 15 November - Rome Italy



Associazione Italiana LATTIERO CASEARIA

www.eda2024.eu



# Dairy under attack? Let's safeguard the essentials at EU level! Alexander Anton

FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

24/25 Sept. 2024







### Giuseppe Ambrosi, Ambrosi spa (IT)



**Peter Giørtz-Carlsen**, Arla (DK)



**Ingo Müller,** DMK (DE)



**Albert de Groot**, Vreugdenhil (NL)



**Jan Teplý,** Madeta (CZ)



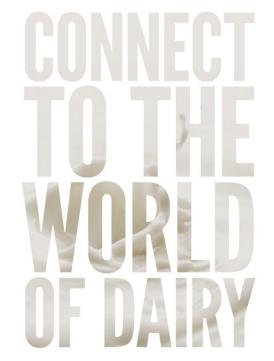


# European Dairy Association



- Association of national dairy industry associations of 20 EU Member States, the UK and Georgia
- Cooperative and private milk processors
- More than 90% of the milk volumes processed in the EU





# Squadra lactosferica: EDA team in Brussels







"The role of the European Dairy Association in ensuring that the voice of your stakeholders is heard in Europe is key. I look forward to continuing our close cooperation to ensure that dairy has a promising future in Europe."

> Ursula von der Leyen President of the European Commission

# **Dairy Policy Conference**

European Dairy & Next Generation EU 2024 - 2029 IO April 2024

# MEP **Christophe Hansen** (EPP, LUX)

Nominated EU Commissioner for Agriculture & Food 2024 - 2029



# High Level Mission of EU Commissioner Janusz Wojciechowski to China 2018





Connect to the world of dalry\_

# Mozzarella at the EDA offices!

This week, EDA was surprised with a truly wonderful gift - none other than the exquisite fine cheese **Mozzarella di Bufala Campana** from the Consorzio in Caserta. The Mozzarella di Bufala Campana, is a Protected Designation of Origin (PDO), meaning that it has the strongest link to the place where it's made the Campania region of Italy - and stands as a testament to the rich heritage and unparalleled craftsmanship of the region – get inspired: https://www.mozzarelladop.it/territory



Mille grazie, dott Pier Maria Saccani, merci beaucoup, cher Pier Maria!



#### Welcome to our new trainees:

#### Marcella and Florian!



I love all varieties of cheese but my favourite one is for sure Stracciatella di bufala Florian Spiegelhalter Trade and Economics Trainee



That is like asking who your favourite child is but let's go with Mozzarella di bufala, PDO from Campania!!

Marcella Rosato

Food, Environment and Health Trainee

# **EDA Vision for Agriculture**

**15 November - Rome Italy** 

Strategic Autonomy in Food Production

### The Importance of the Dairy Sector

- Dairy as a Cultural and Economic Pillar
- Moving Beyond Commoditization

Single Market – The Fundamentals of Europe

Harmonized Legal Framework

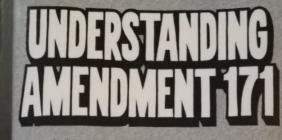
### Global Dairy Trade Leadership

- Reshoring and Diversification
- Prioritizing Trade Policy
- Fostering Competitiveness, Transition, and Resilience – CAP 2027

# Protection of Dairy Terms

# **Promotion Policy**

# **School Milk Scheme**



In October, the European Parliament voted YES on Amendment 171 which could make it illegal for plant-based foods to reference dairy in any way, shape, or form. "Dairy-free alternatives"? Banned. "Creamy texture"? Banned. The amendment could even make our current packaging illegal simply because it's the same type of packaging dairy products use. And even crazier, the amendment might prohibit climate footprint comparisons which awkwardly goes directly against the EU's own climate goals.

So who's behind such a silly law? Well, when AM 171 passed in the Parliament, the European Dairy Association said:

#### THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!

We're confident the European Council of Ministers will strike down this amendment. They're smart enough to see through the dairy lobby's tricks and they know that the people of Europe are not actually stupid. But just to be safe, join us in signing the petition:

# UNDERSTANDING AMENDMENT 171

In October, the European Parliament voted YES on Amendment 171 which could make it illegal for plant-based foods to reference dairy in any way, shape, or form. "Dairy-free alternatives" Banned. "Creamy texture"? Banned. The amendment could even make our current Jackaging illegal simply because it the same type of packaging dairy products use. And even crazier, the amendment might prohibit climate footprint comparisons which auko goes directly against the EU's out So who's behind such a silly l climate goals. when AM 171 passed in the Pa





So who's behind such a silly law? Well, when AM 171 passed in the Parliament, the European Dairy Association said:

# THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!



71 which could 11-based foods 14-based foods 14-based foods 15 way, shape, 17 anned. The 18 ke our current 19 because it's 19 dairy 19 razier, the 1 climate 11 hich awkwardly 19 EU's own

illy law? Well, e Parliament, ciation said:

#### THIS IS A GOOD DAY FOR THE EU LACTOSPHERE!

We're confident the European Council of Ministers will strike down this amendment. They're smart enough to see through the dairy lobby's tricks and they know that the people of Europe are not actually stupid. But just to be safe, join us in signing the petition:





# whey. for living. for life.





**Giuseppe Ambrosi** 



**Emmanuel Besnier** 



Rose O'Donovan



Paolo Zanetti



Jan Vreugdenhil



**James Neville** 



Gianpiero Calzolari



**Pat Murphy** 



14 - 15 November - Rome Italy



Associazione Italiana LATTIERO CASEARIA

www.eda2024.eu

# Origin Control and Quality Assessment in the Dairy Supply Chain through the Bludev® System

Biondi L., Gallo A., Garofalo F., Anzalone A., Montone AMI., Nappa M., De Carlo E., Sansone G., Cascone D., Ciardella G.

FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Powered by





AGRARIA





esearch i develop i innevati

To combat fraud and ensure the authenticity of the DOP (Protected Designation of Origin) mozzarella product within the dairy supply chains, while safeguarding the final consumer, a collaboration between the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM-Portici) and Farzati S.p.A. was established in 2023.

Farzati S.p.A., founded in 2014, is dedicated to the research and development of products aimed at improving individual quality of life. The company enables the implementation of non-invasive and non-destructive analytical techniques to ensure the quality and authenticity of food matrices

# INNOVATION

- The innovation of the BluDev® technology system lies in creating a BIOLOGICAL DIGITAL FOOTPRINT (BFP Bio-FingerPrint) for each batch of actual finished product, with the information obtained being stored in a virtual laboratory to create a digital twin, capable of tracing origin and quality throughout all stages of the production process.
- The acquisition of information regarding the analyzed matrices is based on spectroscopy.
- Artificial Intelligence, combined with predictive models, transforms the input data into unique digital objects, generating a DIGITAL BIO FINGER PRINT.
- All supported by safety systems to protect the data harvested



The aim of the following study is to develop an algorithm to discriminate between mozzarella produced with 100% buffalo milk and mozzarella produced with a mixed milk (buffalo and cow's milk).

- The analyses were carried out using two portable devices of the BluDev® system used in the laboratories of the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM) in Portici on known frozen mozzarella sam
- Specifically the analysis involved thawing the
- sample, taking a portion
- and scanning that portion.



**BluDev Pro**®

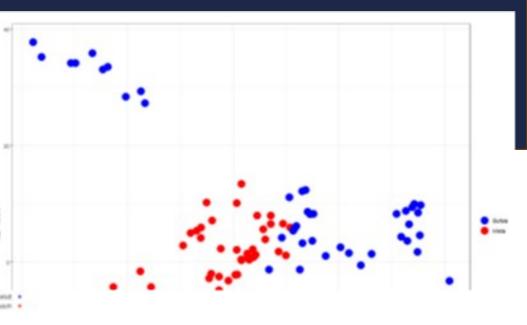
For the first BluDev Pro® device, a total of 12 aliquots from 12 samples (121 scans) were selected, with each sample being scanned at least 10 times. In the initial phase, a Principal Component Analysis (PCA) was performed for preliminary data exploration and to check for potential clustering

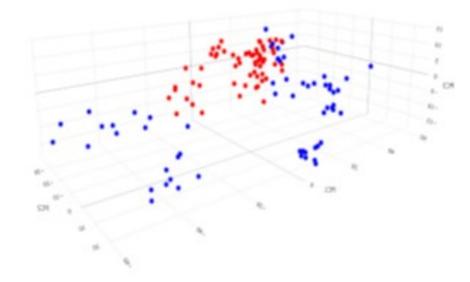
# PRELIMINARY RESULTS

6

The following images display the results of this exploratory in both a 2-dimensional and a 3-dimensional charts.

It is possible to observe a random distribution of buffalo mozzarella (indicated in blue) compared to mixed mozzarella (indicated in red). There does not appear to be a clear separation between the two clusters. Although this is a relatively common situation, it does not preclude further analysis and model development based on the available data. This is only a preliminary study





# **RESULTS BluDev Pro**®

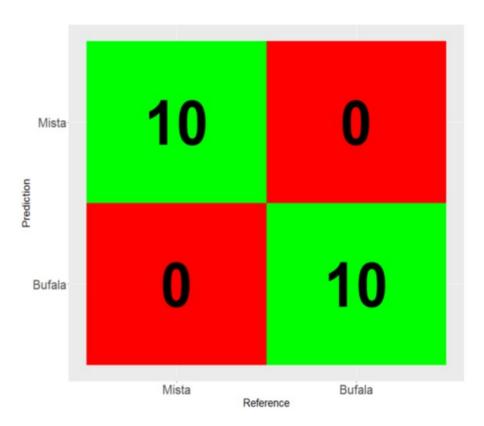
|             | Buffalo mozzarella scans<br>(n. of samples) | Mixed mozzarella scans<br>(n. of samples) | ans Total scans<br>(n. of samples) |  |
|-------------|---|---|------------------------------------|--|
| TRAIN (80%) | 51 (5)                                      | 50 (5)                                    | 101 (10)                           |  |
| TEST (20%)  | 10 (1)                                      | 10 (1)                                    | 20 (2)                             |  |
| TOTAL       | 61 (6)                                      | 60 (6)                                    | 121 (12)                           |  |

Before creating an actual model, all the scans were divided into two separate groups "Train" and "Test": the two groups represent 80% and 20% respectively. In order to have a dataset for training the model (Train) and another statistically significant dataset for its validation (Test). Thus, the larger group will contain samples not present in the smaller group, and vice versa. This approach ensures that the model is validated using data that were previously unknown and separate from the initial dataset

# **RESULTS BluDev Pro**®

The model correctly classified both scans of mozzarella produced with 100% buffalo milk (10 out of 10) and scans of mozzarella produced with mixed milk (10 out of 10).

In total, the model accurately classified 20 out of 20 scans (100%).



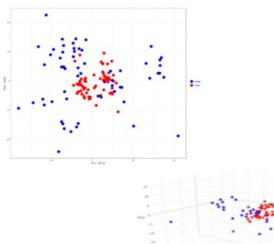
# **RESULTS BluDev Hand®**

The same type of analysis, including modeling, was conducted by a second portable device, BluDev Hand®. In this case, 12 aliquots from 12 samples (121 scans) were also used. The following figures display the results of the PCA analysis in both 2-dimensional and 3-dimensional formats, the detailed composition of the Train and Test datasets, and the confusion matrix related to the model validation.

|             | Buffalo<br>mozzarella<br>scans<br>(n. of samples) | Mixed mozzarella<br>scans<br>(n. of sample) | Total scans<br>(n. of<br>samples) |
|-------------|---|---|-----------------------------------|
| TRAIN (80%) | 50 (5)  | 51 (5)                                      | 101 (10)                          |
| TEST (20%)  | 10 (1)  | 10 (1)                                      | 20 (2)                            |
| TOTAL       | 60 (6)  | 61 (6)                                      | 121 (12)                          |



**BluDev Hand**®

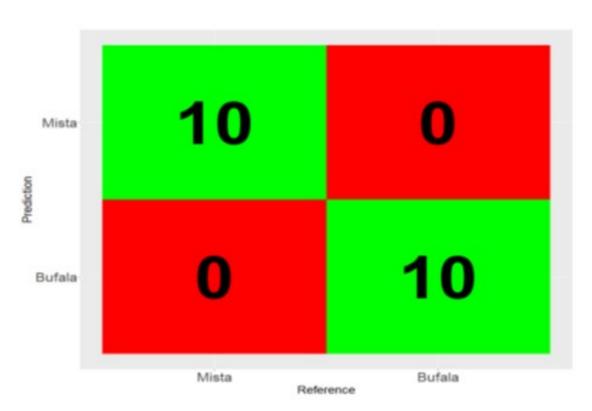


FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

-

# **RESULTS BluDev Hand®**

 The results obtained are similar to the previous ones. In this case as well, the model correctly classified both scans of mozzarella produced with 100% buffalo milk (10 out of 10) and scans of mozzarella produced with mixed milk (10 out of 10). In total, the model accurately classified 20 out of 20 scans (100%).



# CONCLUSION

We can conclude that this developed model suggests promising prospects for further improvement in near future: increasing the number of samples will improve the confidence of the method in order to discriminate between buffalo and mixed mozzarella.

We could imagine a *virtual* laboratory, performing a simple scan to screen mozzarella, identifying suspicious samples to be analysed to detect fraud.





# THANK YOU FOR YOUR ATTENTION

Loredana Biondi loredana.biondi@izsmportici.it



# **Development of a healthy dairy** custard based on buffalo milk and natural thickeners

### A. Fernández<sup>1</sup>, <u>A. Citro<sup>2</sup></u>, M. F. Mazzobre<sup>3</sup>, F. Vasile<sup>1</sup>

<sup>1</sup> INIPTA (CONICET-UNCAUS) Argentina <sup>2</sup> Veterinary Services, Local Health Unit of Salerno, Italy <sup>3</sup> ITAPROC (CONICET-UBA) Argentina

FIRST INTERNATIONAL CONFERENCE ON

# **Buffalo** Mozzarella **& Milk Products**

24/25 Sept. 2024

Powered by











# GRUPO DE INVESTIGACIÓN EN NANO Y MICRO **ENCAPSULACIÓN** DE COMPUESTOS BIOACTIVOS







UBA · CONICET





Dr. Franco Emanuel Vasile



Ing. Mirtha Marina Doval





Dr. Oscar Edgardo Pérez FCEyN - UBA



Ing. Andrea Beatriz Fernandez

CONICET



Dra. María Florencia Mazzobre FCEyN - UBA





 The main objective of this work was to advance in the development of a healthy buffalo milk-based food product as an innovative alternative to traditional buffalo cheeses.

We proposed the manufacture of a vanilla custard-type dessert intended for child nutrition.



# In a previous work...

#### Dairy Technology

### doi: 10.1111/1471-0307.129

RESEARCH ARTICLE

Exploring the sensory properties of buffalo (*Bubalus bubalis*) milk custards through a consumer-based study performed with children

- We tested three formulations varying the amount of cornstarch, sucrose, and food-grade flavor/colorant to get samples with distinctive textural, sweetness, and chromatic properties.
- A sensory evaluation performed with 100 children (8 to 12 age) allowed identify the most relevant attributes and get precise indications for reformulation and product optimization.
- Obtained product required attention because it was high in calories and non-stable for long period in cold-storage (1 week).







Buffalo Mozzarella & Milk Products

## In this context...

- The total replacement of sucrose with a non-caloric natural sweetener (Stevia) was proposed.
- The mixture of native starches from potato, cassava and corn was assessed as a healthier texturizer or stabilizer alternative to the modified starches commonly used in the industry.
- Natural additives as clear-label foods approach.

Calories reducing strategy.



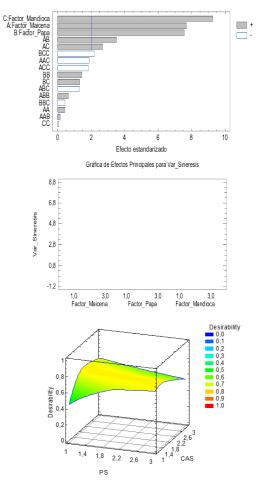
- The impact of formulation changes were assessed on textural and appearance properties by instrumental analysis.
- Cold-storage physical stability (water holding capacity and syneresis) was also monitored.



# **Outstanding results...**

- Potato, cassava and corn starches and their interactions exerted a significant positive effect on the firmness of custards as assessed through Texture profile analysis (TPA).
- Color parameters (L\*, a\* and b\*) and opacity did not show statistical differences.
- Cold-storage stability measured in terms of water holding capacity (accelerated water leakage) and syneresis (spontaneous water leakage) was mainly affected by cassava starch and starch mixtures.
- It is was possible to obtain an optimum that conserve the most preferred texture of custard, minimizing the time-depending changes over cold-storage.

#### Diagrama de Pareto Estandarizada para Var\_Firmeza



FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products



### CONCLUSIONS

Obtained results encourage the use of non-caloric sweetener and the mixture of native starches for making healthy and commercially attractive dairy desserts.

Manufacture of buffalo custards constitutes a promising alternative for using of buffalo milk in less explored dairy products.

# LET'S START WORKING TOGETHER

Caracas 13 th World Buffalo Congress 22-24 November 2023





FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products



# Gracias por su atención!





Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri* 

# A tailored-made model to predict the behaviour of *Listeria monocytogenes*

R. Condoleo, M.C. Campagna, M.L. De Marchis, T. Zottola, M.F. Iulietto

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri (IZSLT)

**Buffalo Mozzarella** & Milk Products

24-25 September 2024







# Roberto Condoleo – IZSLT **Epidemiology Unit**





# The villain

Name: *Listeria monocytogenes* Type: *Batterio Gram+* 

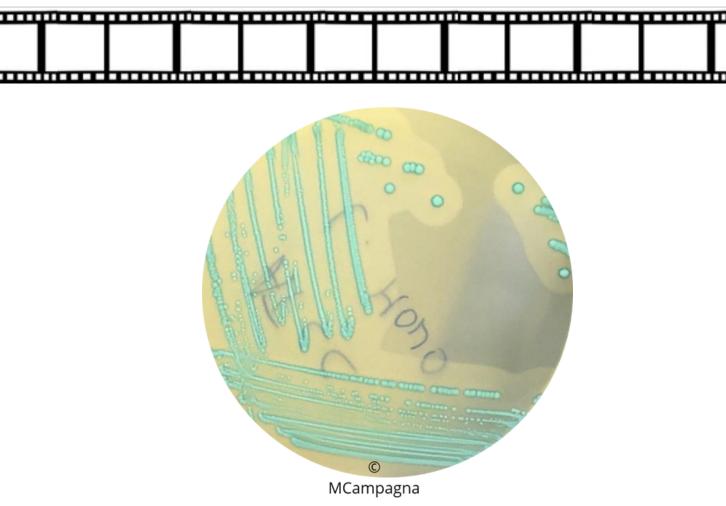
Seen last time: intestine, soil, farm, mastitic milk, dairy, any type of surface, kitchen... basically everywhere

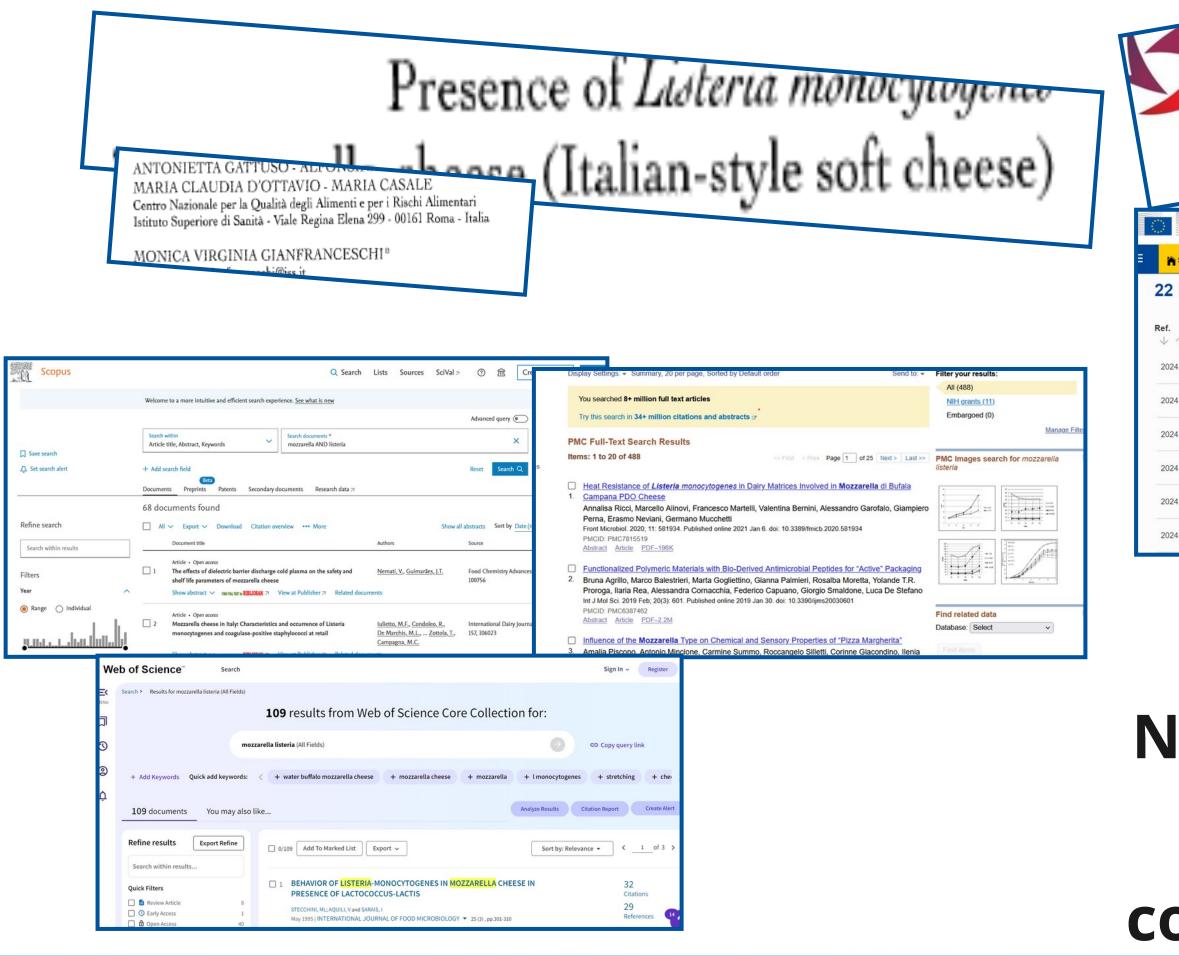
It loves 1) high pH foods 2) cold places 3) doing things slowly

It hates: 1) heat 2) good microorganisms

Particular signs: very evil with children, old people, pregnant women and sick people







**RASE INTOATION 2022.2972** 

# Listeria monocytogenes in mozzo

#### European Commission | RASFF Window

### 22 NOTIFICATIONS 🗟 🗎

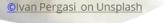
| Category<br>↓ ↑           | Type<br>↓ ↑   | Subject<br>↓ ↑  | Date<br>↓ ↑   | Origin   | Notifying<br>↓ ↑  | Class.<br>↓ ↑  | Decision<br>↓ ↑  |
|---------------------------|---|---|---|--|---|--|--|
| Milk and milk products    | food  | Presence of Listeria monocytogenes in<br>mozzarella from Italy  | 18 SEP 2024   | U.   | France  | alert<br>notification  | serious  |
| Milk and milk products    | food  | Listeria monocytogenes in Mozzarella Salami<br>from Luxembourg  | 16 AUG<br>2024  | =  |   | alert<br>notification  | serious  |
| Milk and milk products    | food  | Listeria in mozzarella from UK  | 23 JUL 2024   |  | France  | alert<br>notification  | serious  |
| Milk and milk products    | food  | Presence of Listeria monocytogenes in diced mozzarella from the UK  | 10 JUL 2024   | *  | France  | alert<br>notification  | serious  |
| Milk and milk products    | food  | Possible presence of pieces of hard plastic in<br>mozzarella, manufactured in Belgium   | 5 JUL 2024  |  | Belgium   | alert<br>notification  | serious  |
| Milk and milk<br>products | food  | Listeria monocytogenes in mozzarella cheese from Italy  | 10 JUN 2024   | U.   | France  | alert<br>notification  | serious  |
|                           | ↓ ↑     Milk and milk     products     Milk and milk     products | ↓ ↑     ↓ ↑       Milk and milk<br>products     food       Milk and milk<br>products     food | Image: Weight of the second | Image: transformation of transfo | Image: Weight and milk and milk products       food       Presence of Listeria monocytogenes in mozzarella from Italy       18 SEP 2024       Image: Weight and milk products         Milk and milk products       food       Listeria monocytogenes in Mozzarella Salami from Luxembourg       16 AUG 2024       Image: Weight and milk products       10 AUG 2024       Image: Weight and milk products       food       Listeria in mozzarella from UK       23 JUL 2024       Image: Weight and milk products       food       Presence of Listeria monocytogenes in diced mozzarella from UK       10 JUL 2024       Image: Weight and milk products       food       Presence of pieces of hard plastic in mozzarella, manufactured in Belgium       5 JUL 2024       Image: Weight and milk products         Milk and milk food       Listeria monocytogenes in mozzarella, cheese       10 JUN 2024       Image: Weight and milk products | Image: Constraint of the constra | Image: Constraint of the constra |

# No listeriosis cases associated to mozzarella consumption so far

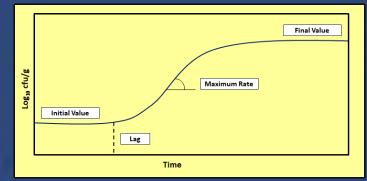


Istituto Zooprofilattico Spe del Lazio e della Toscana M





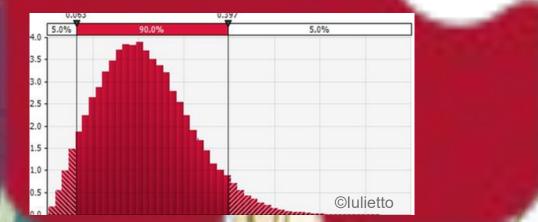
# Predictive microbiology model

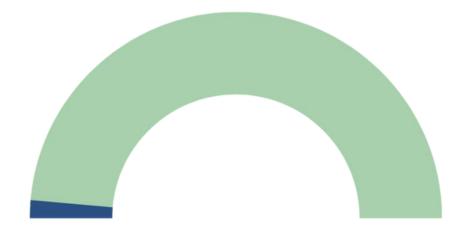


# Systematic review and meta-analysis

| Study                                     | Events       | Total   | Weight     | IV, Random, 95% CI      | IV, Random, 95% CI  |
|---|--------------|---------|------------|-------------------------|---------------------|
| Giordanelli et al., 2003                  | 0            | 94      | 12.7%      | 0.0000 [0.0000; 0.0385] |                     |
| Battisti et al., 2006                     | 0            | 64      | 10.3%      | 0.0000 [0.0000; 0.0560] |                     |
| Gattuso et al., 2008a                     | 0            | 120     | 14.3%      | 0.0000 [0.0000; 0.0303] |                     |
| Gattuso et al., 2008b                     | 11           | 320     | 19.8%      | 0.0344 [0.0173; 0.0607] |                     |
| Di Pinto et al., 2010                     | 0            | 186     | 17.0%      | 0.0000 [0.0000; 0.0196] |                     |
| Casalinuovo et al., 2014                  | 0            | 47      | 8.5%       | 0.0000 [0.0000; 0.0755] |                     |
| Condoleo & Palumbo, 2021                  | 1            | 200     | 17.4%      | 0.0050 [0.0001; 0.0275] | <b>—</b>            |
| Total (95% CI)                            |              | 1031    | 100.0%     | 0.0031 [0.0000; 0.0149] | -                   |
| Prediction interval                       |              |         |            | [0.0000; 0.0462]        |                     |
| Heterogeneity: Tau <sup>2</sup> = 0.0026; | $Chi^2 = 16$ | .48, df | = 6 (P = 0 | $1.01$ ; $l^2 = 64\%$   |                     |
|   |              |         |            | 0                       | 0.01 0.03 0.05 0.07 |

Risk assessment of listeriosis by mozzarella consumption

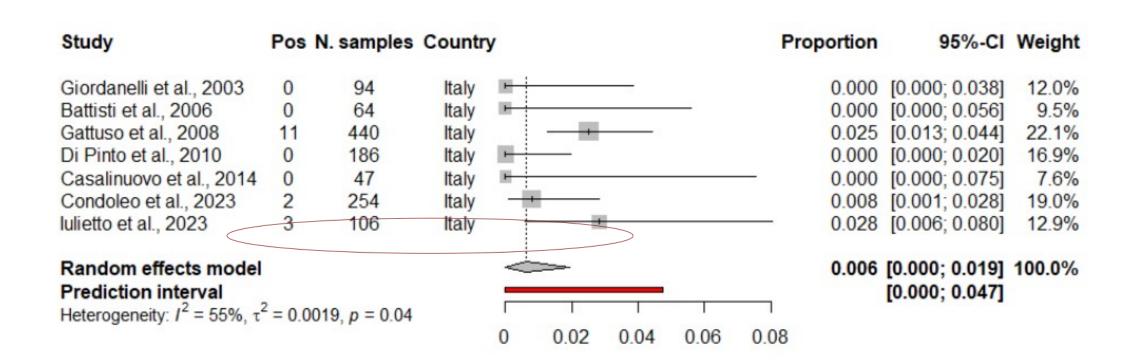




3 out of 106 batches were contaminated with L. *monocytogenes.* Occurence was 2.8% (IC 95% 0.6-8%)



Low concentration L. monocytogenes nei prodotti contaminati (< 10 CFU/g)

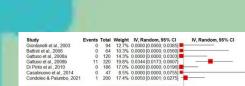


Mozzarella cheese in Italy: Characteristics and occurrence of Listeria monocytogenes and coagulase-positive staphylococci at retail

Maria Francesca Iulietto<sup>\*</sup>, Roberto Condoleo, Maria Laura De Marchis, Tatiana Bogdanova, Valeria Russini, Sonia Amiti, Roberta Zanarella, Tiziana Zottola, Maria Concetta Campagna tituto Zooprofilattico Sperimentale Lazio e Toscana M. Aleandri, Rome, Italy

International Dairy Journal 157 (2024) 106023

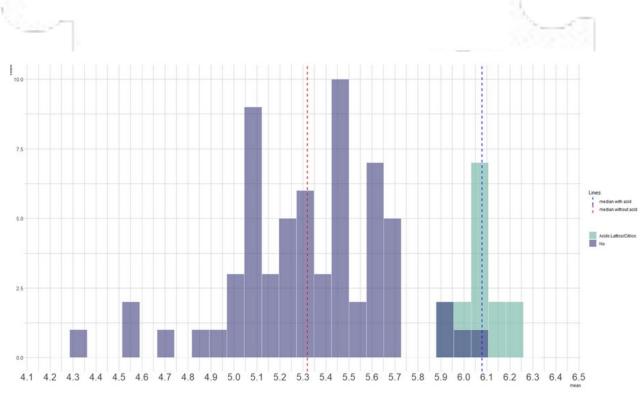
### Occurrence L. monocytogenes in mozzarella



Sytematic review

and meta-analysis

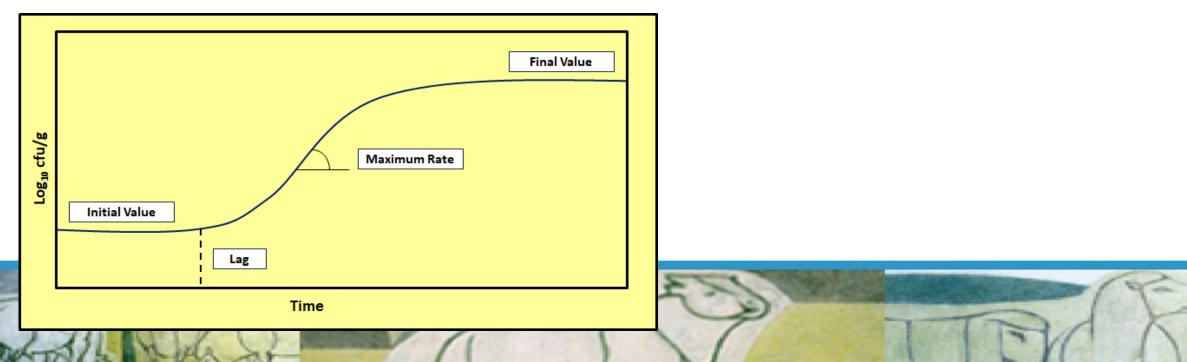


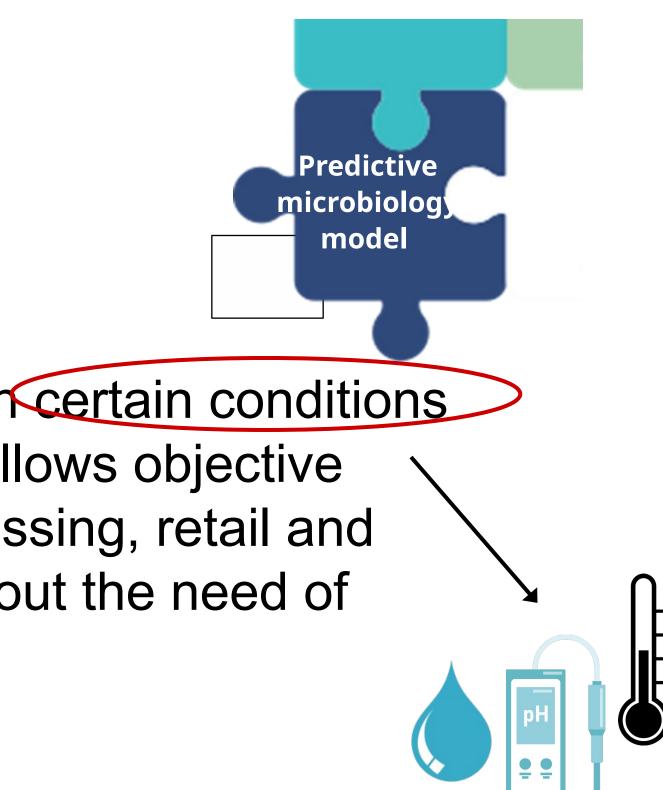


pН

# Predictive Microbiology in few words...

A detailed knowledge of microbial behaviour in certain conditions described in a mathematical modeDthat allows objective assessment of the food safety of the processing, retail and storage phases along the food chain without the need of microbiological tests







| Reference                 | Year | Temperature °C | Max rate (Log<br>CFU/day) | K  |
|---------------------------|------|----------------|---------------------------|----|
| Murru et al (2018)        | 2018 | 4              | 0.028                     |    |
| Murru et al (2018)        | 2018 | 20             | 0.271                     |    |
| Murru et al (2018)        | 2018 | 30             | 0.211                     |    |
| Murru et al (2018)        | 2018 | 4              | 0.066                     |    |
| Murru et al (2018)        | 2018 | 20             | 0.206                     | I  |
| Murru et al (2018)        | 2018 | 30             | 0.136                     |    |
| Kapetanakou et al. (2017) | 2017 | 7              | 0.213                     |    |
| Kapetanakou et al. (2017) | 2017 | 7              | 0.211                     |    |
| Kapetanakou et al. (2017) | 2017 | 7              | 0.114                     |    |
| Kapetanakou et al. (2017) | 2017 | 7              | 0.083                     | P  |
| Nava et al. (2016)        | 2016 | 12             | 0.020                     |    |
| Nava et al. (2016)        | 2016 | 12             | -0.003                    | ir |
| Nava et al. (2016)        | 2016 | 12             | 0.104                     |    |
| Nava et al. (2016)        | 2016 | 12             | 0.027                     | (6 |
| Han et al (2014)          | 2014 | 10             | 0.247                     | С  |
| Serraino et al (2012)     | 2012 | 5              | -0.014                    | Ŭ  |
| Serraino et al (2012)     | 2012 | 10             | -0.140                    |    |
| Serraino et al (2012)     | 2012 | 15             | -0.151                    |    |
| Serraino et al (2012)     | 2012 | 20             | -0.132                    |    |
| Finazzi et al. (2011)     | 2011 | 5              | -0.039                    |    |
| Finazzi et al. (2011)     | 2011 | 10             | 0.079                     |    |
| Finazzi et al. (2011)     | 2011 | 20             | 0.244                     |    |
| Finazzi et al. (2011)     | 2011 | 5              | 0.093                     |    |

ential growth of *Listeria hocytogenes* (*Log /g/day*) in mozzarella ese as affected by robiological and mical-physical fronment.

nary model fitting: takes account only the time perimental factors are not sidered)



| Reference                 | Year | Temperature °C | С  | ph 🦰 | Max rate at 8<br>°C |
|---------------------------|------|----------------|----|------|---------------------|
| Murru et al (2018)        | 2018 |                | 4  | 5.40 |                     |
| Murru et al (2018)        | 2018 | 2              | 0  | 5.39 | 0.05                |
| Murru et al (2018)        | 2018 | 3              | 0  | 5.37 | 0.02                |
| Murru et al (2018)        | 2018 |                | 4  | 5.40 | 0.19                |
| Murru et al (2018)        | 2018 | 2              | 0  | 5.39 | 0.04                |
| Murru et al (2018)        | 2018 | 3              | 0  | 5.37 | 0.01                |
| Kapetanakou et al. (2017) | 2017 |                | 7  | 6.23 | 0.26                |
| Kapetanakou et al. (2017) | 2017 |                | 7  | 6.19 | 0.26                |
| Kapetanakou et al. (2017) | 2017 |                | 7  | 6.19 | 0.14                |
| Kapetanakou et al. (2017) | 2017 |                | 7  | 6.14 | 0.10                |
| Nava et al. (2016)        | 2016 | 1              | 2  | 5.38 | 0.01                |
| Nava et al. (2016)        | 2016 | 1              | .2 | 5.38 | 0.00                |
| Nava et al. (2016)        | 2016 | 1              | 2  | 5.38 | 0.05                |
| Nava et al. (2016)        | 2016 | 1              | .2 | 5.38 | 0.01                |
| Han et al (2014)          | 2014 | 1              | 0  | 5.80 | 0.17                |
| Serraino et al (2012)     | 2012 |                | 5  | 4.96 | -0.03               |
| Serraino et al (2012)     | 2012 | 1              | 0  | 4.95 | -0.10               |
| Serraino et al (2012)     | 2012 | 1              | .5 | 4.89 | 0.05                |
| Serraino et al (2012)     | 2012 | 2              | 0  | 4.66 | -0.03               |
| Finazzi et al. (2011)     | 2011 |                | 5  | 4.79 | -0.08               |
| Finazzi et al. (2011)     | 2011 | 1              | 0  | 5.13 | 0.05                |
| Finazzi et al. (2011)     | 2011 | 2              | 0  | 4.66 | 0.05                |
| Finazzi et al. (2011)     | 2011 |                | 5  | 4.63 | 0.20                |
| Tirloni et al (2019)      | 2019 |                | 4  | 6.51 | 0.89                |
| Tirloni et al (2019)      | 2019 |                | 4  | 6.51 | 0.57                |
| Tirloni et al (2019)      | 2019 |                | 9  | 6.51 | 0.61                |
| Tirloni et al (2019)      | 2019 |                | 9  | 6.51 | 0.65                |
| Tirloni et al (2019)      | 2019 | 1              | .5 | 6.51 | 0.43                |
| Tirloni et al (2019)      | 2019 | 1              | .5 | 6.51 | 0.51                |
| Rivas et al (2022)        | 2022 | 1              | 0  | 5.36 | 0.01                |

### Square-root model modified from McMeekin (1992)

$$\mu_{ref} = \mu_{obs} * \frac{\left(T_{ref} - T_{min}\right)^2}{\left(T_{obs} - T_{min}\right)^2} * \frac{pH_{ref} - pH_{min}}{pH_{obs} - pH_{min}}$$

Modello gamma-concept modified from Zwietering et al., (1992)growth of *L*.

$$\mu_{max}(T, pH) = \mu_{opt}\tau(T)\rho(pH)$$

Modello cardinale from Rosso et al., (1995)

$$\mu_{opt} = \frac{\mu_{max}}{\gamma(T).\gamma(pH).\gamma(aw).\gamma(AH).\gamma(int)}$$

$$\gamma(T) = \begin{cases} 0 & , T \leq T_{\min} \\ \frac{(T - T_{\max})(T - T_{\min})^2}{(T_{opt} - T_{\min})[(T_{opt} - T_{\min})(T - T_{opt}) - (T_{opt} - T_{\max})(T_{opt} + T_{\min} - 2.T)]} & , T \leq T_{\max} \\ 0 & , T \geq T_{\max} \end{cases}$$

 $\rho(pH) = \frac{(pH)}{(pH - pH_{min})}$ 

Performance of three models have been assessed estimating the

$$\tau(T) = \left(\frac{T - T_{min}}{T_{opt} - T_{min}}\right)^2$$

)

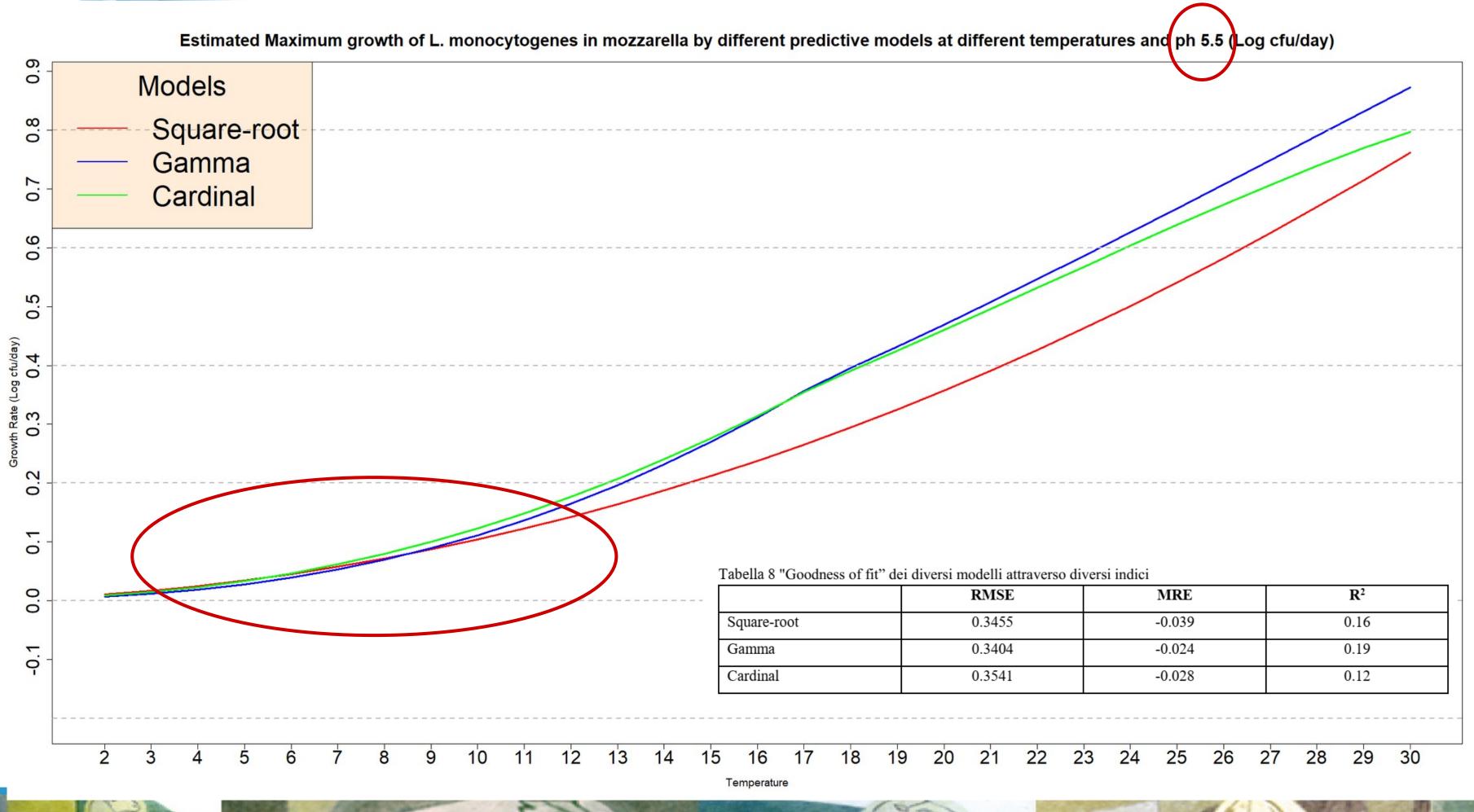
$$\rho(pH) = \left(\frac{pH - pH_{min}}{pH_{opt} - pH_{min}}\right)$$

(6)

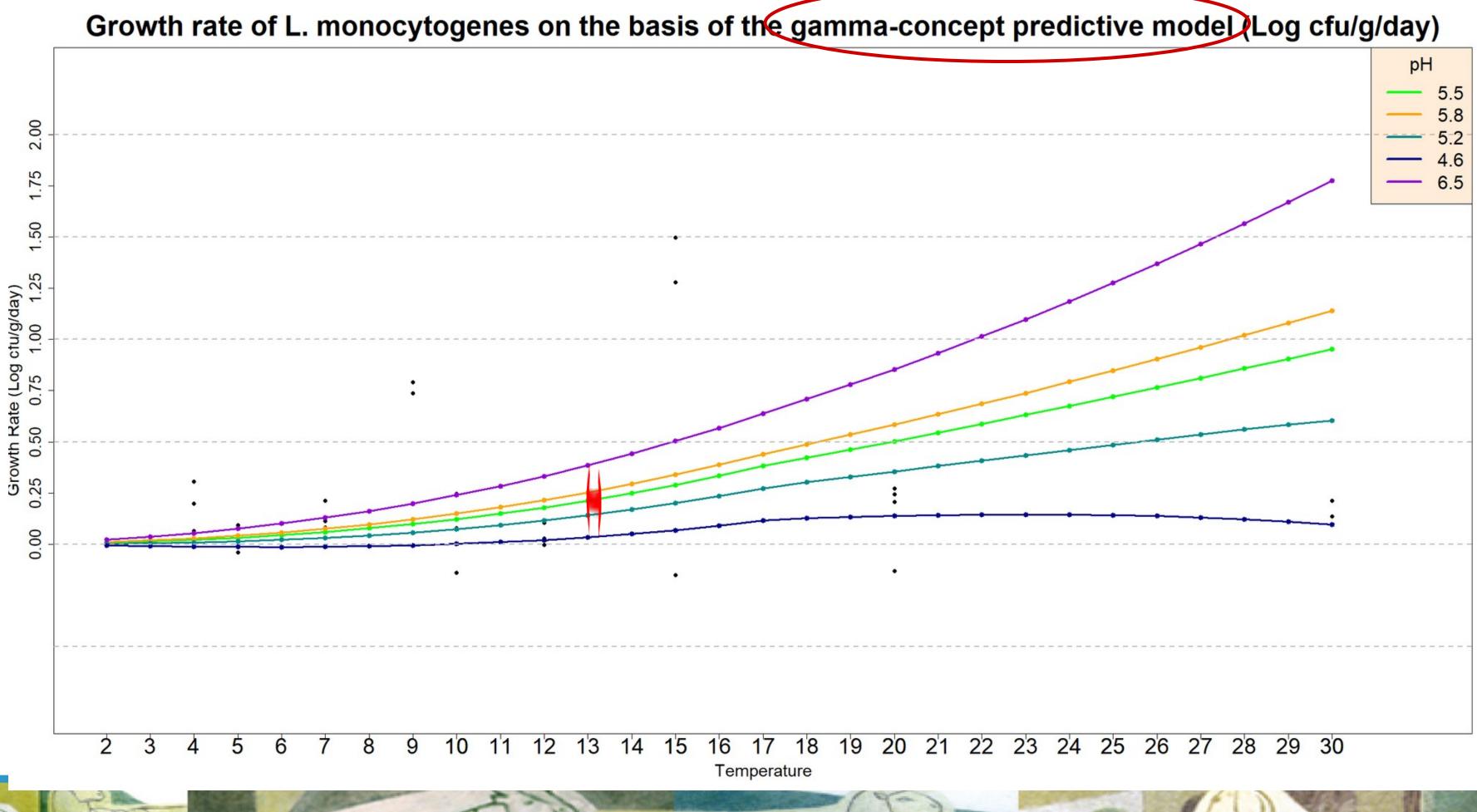
$$\frac{-pH_{min})(pH - pH_{max})}{(pH - pH_{max}) - (pH - pH_{opt})^2}$$

*monocytogenes* at the different experimental conditions of the selected curves and then evaluating the distribution of the residues with the following indexes:

RMSE (Root Mean Squared Error)
MRE (Mean Relative Error)
R2 (Coefficient of determination)

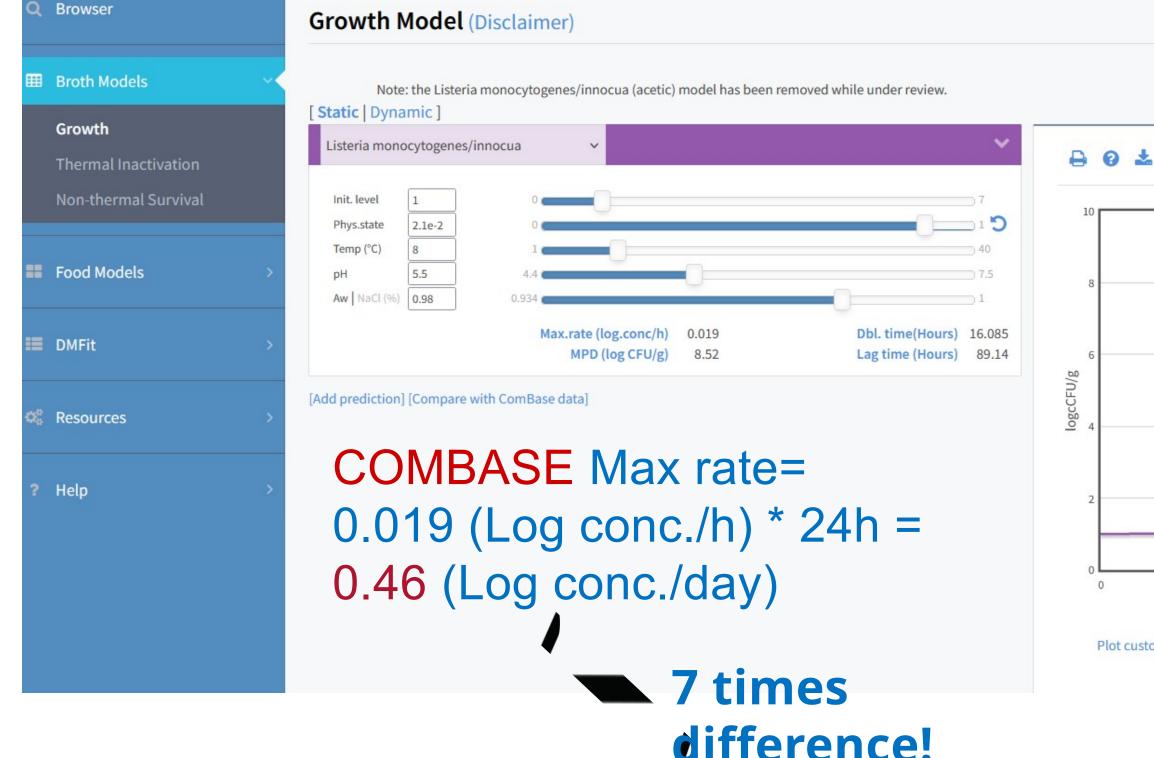






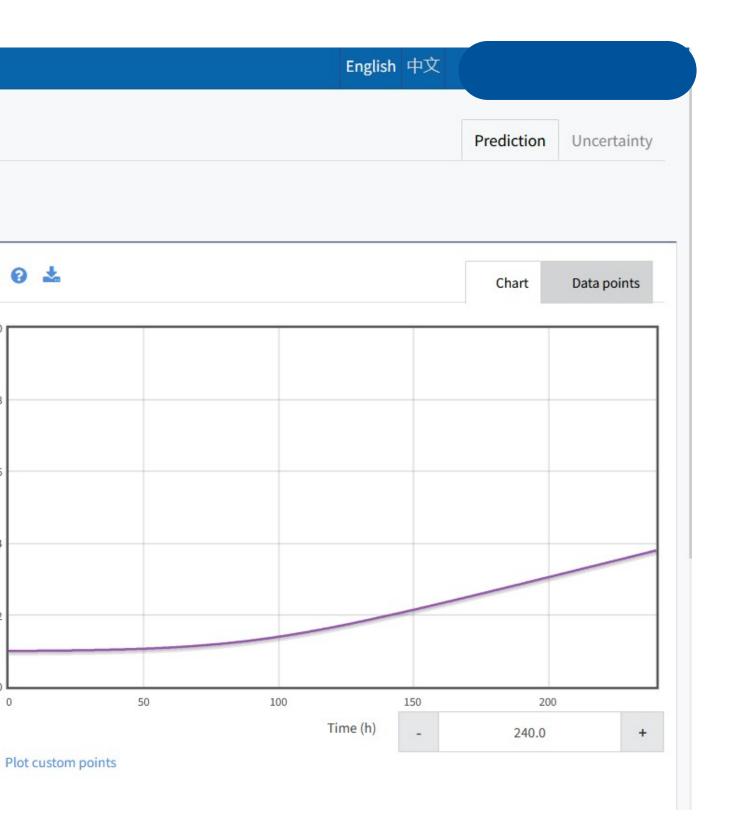


#### - ComBase



difference!Taylor-made model = 0.07 (Log conc./day)

# DARGE AND TOUR





# Take-home messages

The model is more robust than those developed using lab media and can support FBO to predict the concentration *L. monocytogenes* during the shelf life at certain conditions

pH of the product is an important factor to limit the growth of the microrganism

The model should be improved in term of accuracy with new experimental data







Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri* 

# THANK YOU FOR YOU ATTENTION...

My thanks to Dr. Maria Francesca Iulietto and the rest of the research group!

**Dr. ROBERTO CONDOLEO** 

ISTITUTO ZOOPROFILATTICO SPERIMENTALE LAZIO E TOSCANA «M. Aleandri» - EPIDEMIOLOGY UNIT -Via Appia Nuova 1411, Roma - Tel.06/7990360

Roberto.condoleo@izslt.it, www.izslt.it







# Effect of aging on probiotic bacteria in semi-hard buffalo cheese

**Valeria Vuoso** University of Naples Federico II Department of Veterinary Medicine and Animal Production, Italy FIRST INTERNATIONAL CONFERENCE ON

Buffalo Mozzarella & Milk Products

24/25 Sept. 2024

Powered by









# Introduction

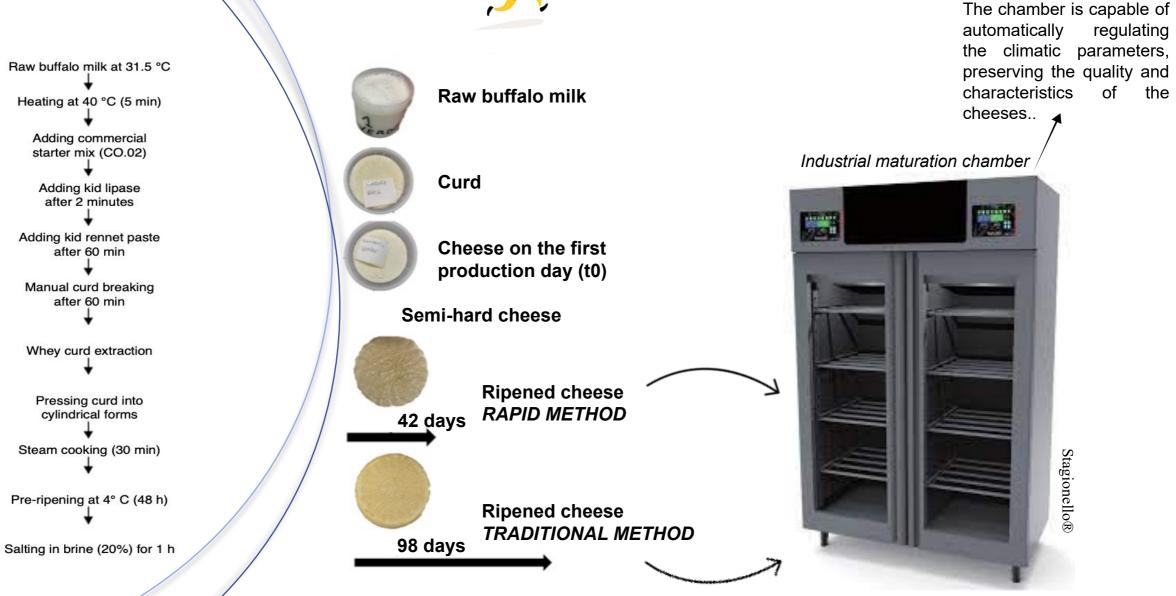


Cheese refinement relies on complex processes, with bacteria playing a key role in developing flavor and texture. These microorganisms can originate from milk, the environment, or starter cultures, but production methods often impact which bacteria dominate.

# Aim of the study

The aim of the study was to compare the effect of the traditional refinement method (**Trad; 98 days**) with the rapid method (**Fast; 42 days**) on probiotic bacteria in semi-hard raw milk buffalo cheese.

# Materials and methods

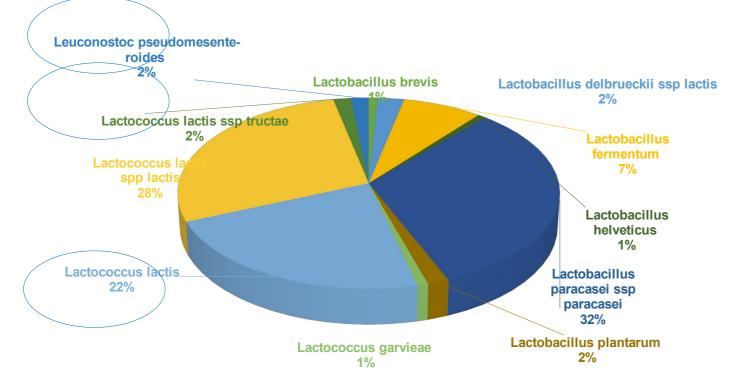


FIRST INTERNATIONAL CONFERENCE ON Buffalo Mozzarella & Milk Products

4

### **Results and discussion**

#### semi-hard cheese after 24h from production

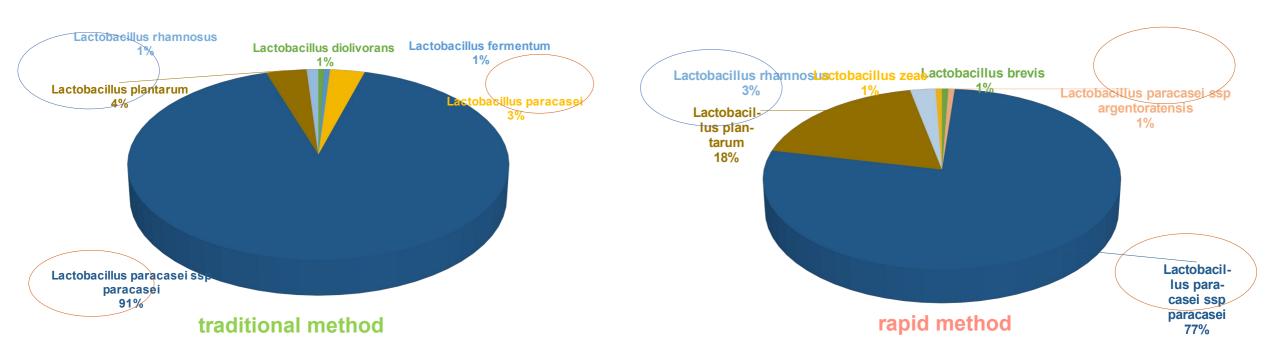




The **MALDI-TOF/MS** identification of the predominant bacterial populations isolated from pre-aged cheeses (after 24 hours from cheese production) indicates the presence of *heterofermentative bacilli.* 

*Lactococcus lactis* was the dominant species (52%).

## **Results and discussion**



After the ripening periods, indigenous lactic acid bacteria decreased in both experimental setups, allowing other microbial populations to prosper:

- the cocci were replaced by bacilli with Lactobacillus paracasei dominance (trad 94%; fast 78%);
- interesting was the isolation of *Lactobacillus plantarum*, which exhibited a higher prevalence in the
- <sup>6</sup> rapid method (**18%**) compared to the traditional one **(4%**).

## Conclusions



Certain strains of *Lactobacillus plantarum* are recognized for their capacity to produce natural antimicrobial compounds, such as bacteriocins, which can inhibit undesired bacteria sharing the same ecological niche.



The rapid refinement method not only shortened production times but also facilitated the selection of a diverse bacterial flora, promoting the prevalence of selected strains into the starter during the process.

# Grazie per l'attenzione!

Valeria Vuoso, PhD Student

