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DI PADOVA

Large-scale phenotyping through milk infrared spectroscopy for enhancing resilience in dairy cattle: the contribution of LEO project

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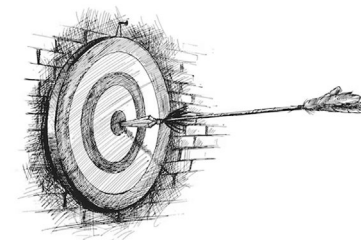
⁴ Italian Association of Breeders (AIA), Roma, Italy



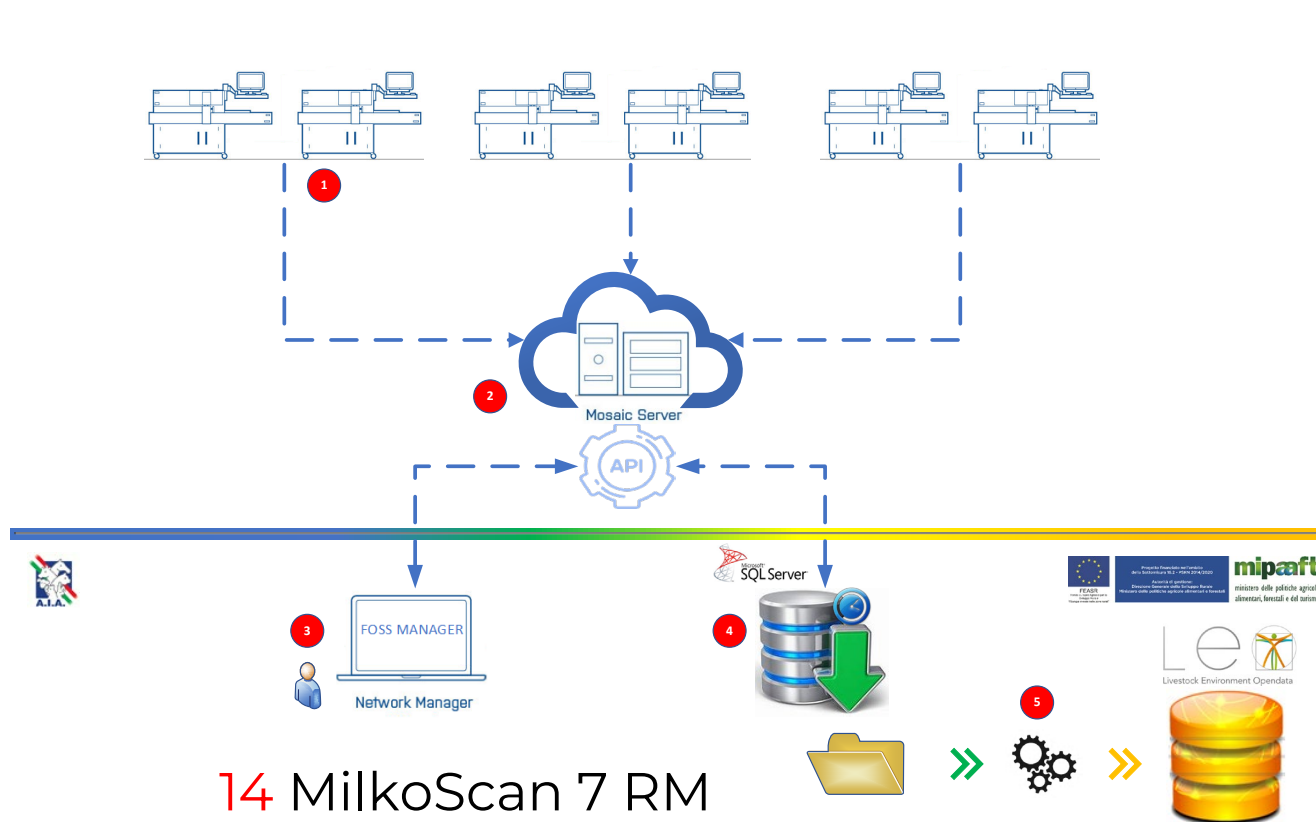
- ✓ Mid-infrared (MIR) spectroscopy as powerful predictive tool
- ✓ MIR to assess metabolic/inflammatory dysfunctions and stress response (Grelet et al., 2018; Luke et al., 2019; van den Berg et al., 2021; Giannuzzi et al., 2023)
- ✓ Good results in terms of R^2 , but what (practical) usefulness at the population level?
 - ✓ Study the behavior of predictions at the individual level according to the expected physiological response
 - ✓ Study the behavior of the predictions in terms of potential associations with health or fertility traits
 - ✓ Study the exploitable genetic variation (selective breeding?)

Show preliminary results related to the applications of prediction equations for serum biomarkers (pMARK) of stress response (resilience) on population databases extracted from the AIA database and the LEO project

- ✓ pMARK → physiological response over lactation
- ✓ pMARK → association with fertility traits
- ✓ pMARK → heritability (?)



Spectral data: MOSAIC



11 labs

- 14 MilkoScan 7 RM
- 12 Fossomatic 7 DC
- 1 Fossomatic 7
- 1 Fossomatic FC
- 4 NIRS DS3

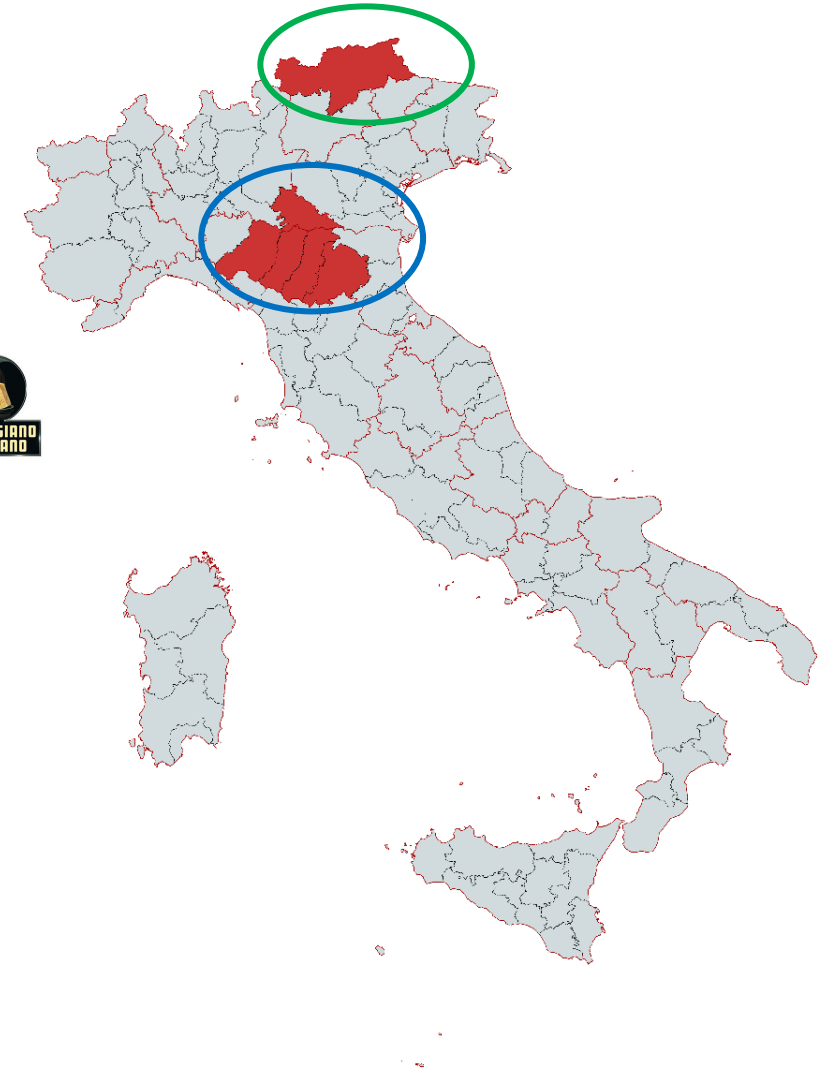
More than **45 million MIR spectra** stored since 2019

Areas of study – field data

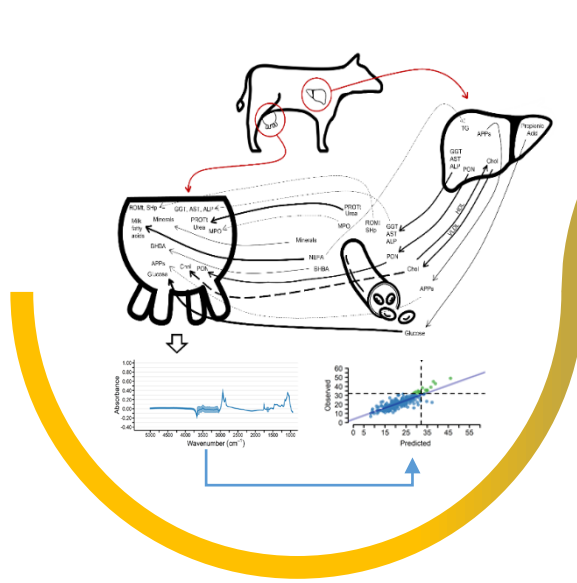
- ✓ Emilia Romagna and Lombardia (Italy)
- ✓ **1,869,687 MIR test-days** (2 years)
- ✓ 245,350 cows, 1,509 multi-breed farms (Holstein Friesian, Crossbreed, Brown Swiss, Simmental, Reggiana)
- ✓ Parmigiano Reggiano (PR) Consortium (farming practices, altitude zone, welfare assessment etc.)



- ✓ Bolzano Province (Italy)
- ✓ **1,856,200 MIR test-days** (7 years)
- ✓ 115,320 cows, 6,240 multi-breed farms (Holstein Friesian, Brown Swiss, Simmental, Alpine Gray)
- ✓ Breeders Federation of Alto Adige (fertility data)

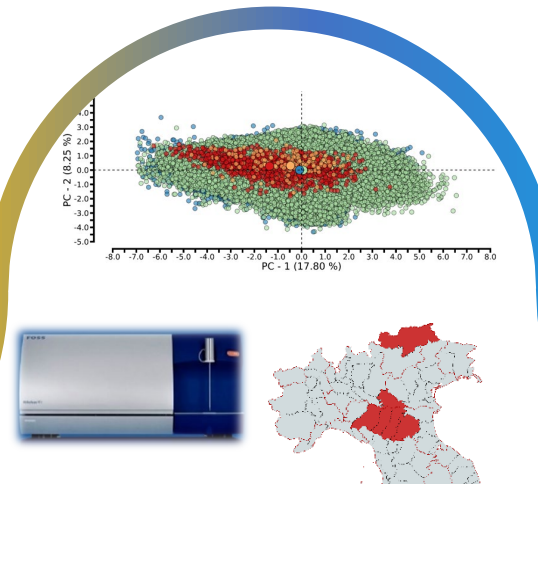


Study design flow



1) Development of MIR calibration equations (~1,367 cows, 5 herds)

Giannuzzi et al., 2023 J Dairy Sci
Mota et al., 2023 Genet Sel Evo



2) Equations applied to population databases:

- ✓ Emilia Romagna
- ✓ Bolzano Province



3) Merge with databases provided by:

PR Consortium (farming practices, welfare assessment etc. Breeders Federation of Alto Adige (fertility data)

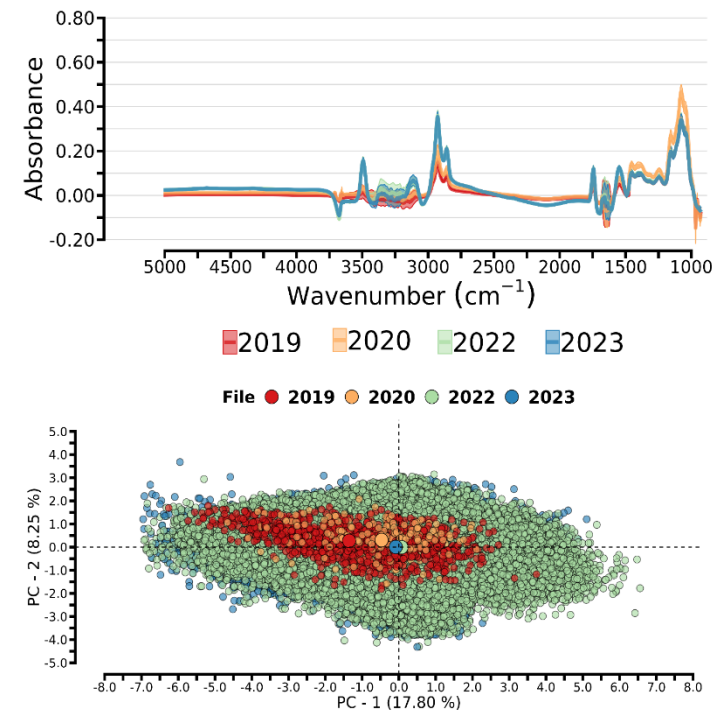


4) Statistical analyses on predicted traits:

ANOVA and Survival Analysis

Predicted biomarkers

Hepatic function and damage		R²
AST		0.62
ALP		0.55
GGT		0.68
Total bilirubin		0.68
Albumin		0.75
PON		0.58
Inflammation/ innate immunity		
Total proteins		0.81
Globulins		0.87
Haptoglobin		0.48
Ceruloplasmin		0.74
Myeloperoxidase		0.64
Oxidative stress		
ROMt		0.79
FRAP		0.56
AOPP		0.65
SHp		0.76

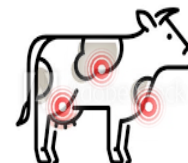
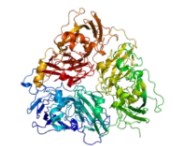
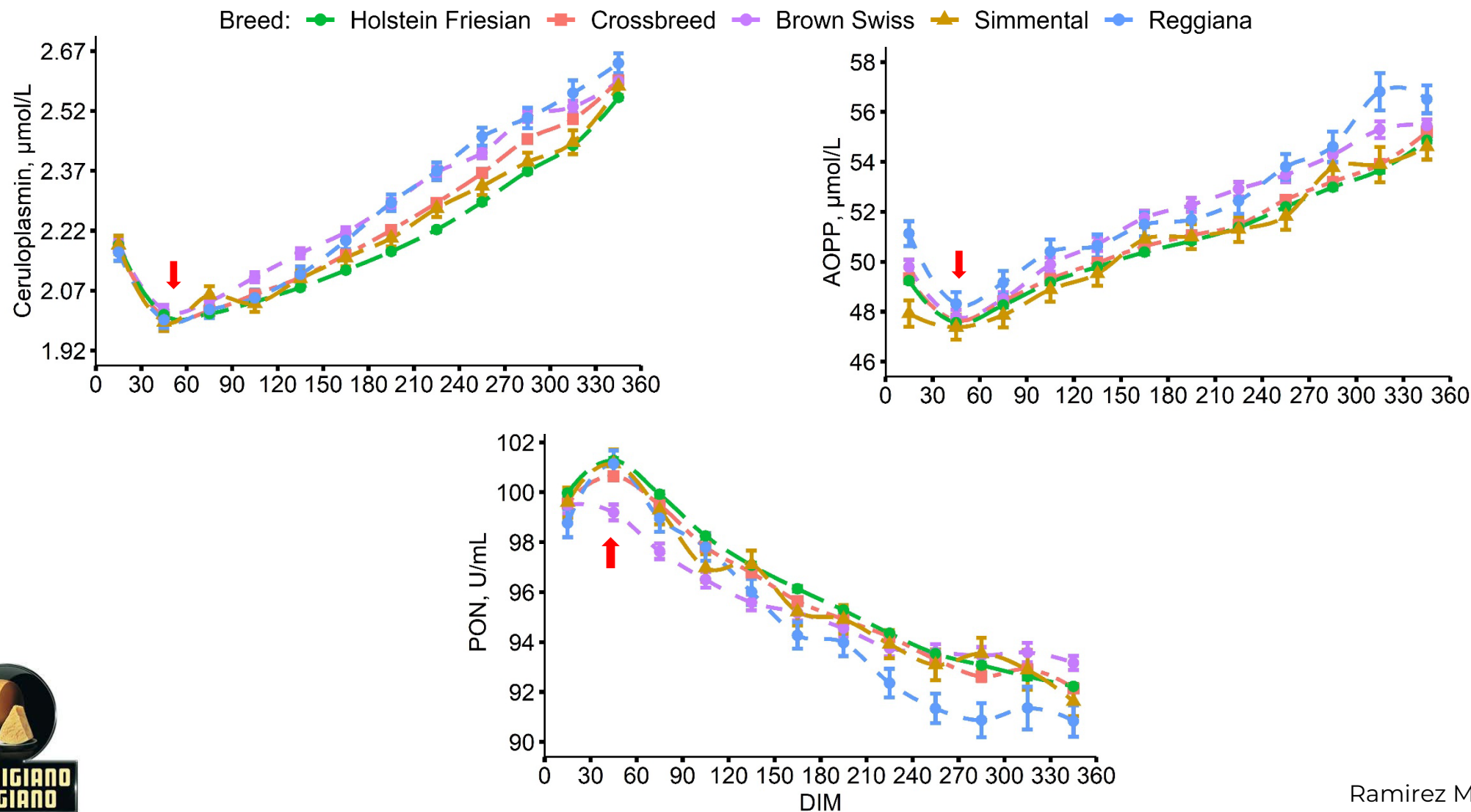


MIR quality control

- ✓ Mahalanobis distance
- ✓ Absorbances centered to a null mean and standardized to a unit sample variance within year periods (Toledo-Alvarado et al., 2018)

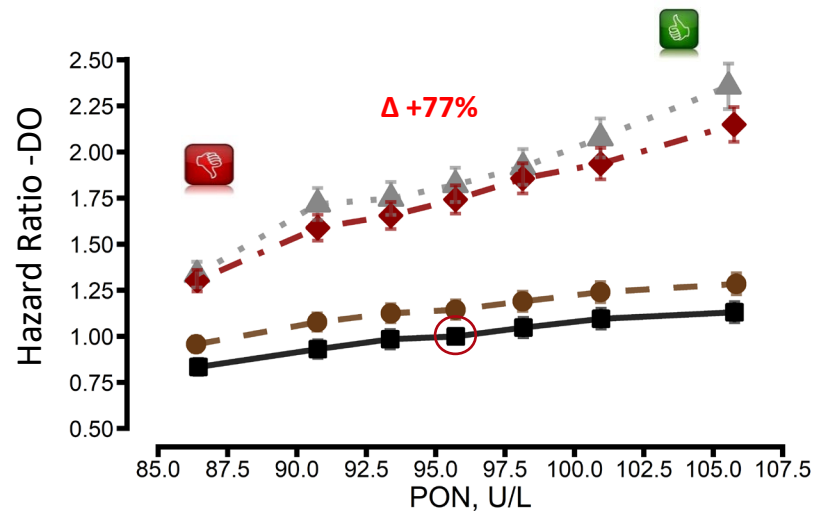
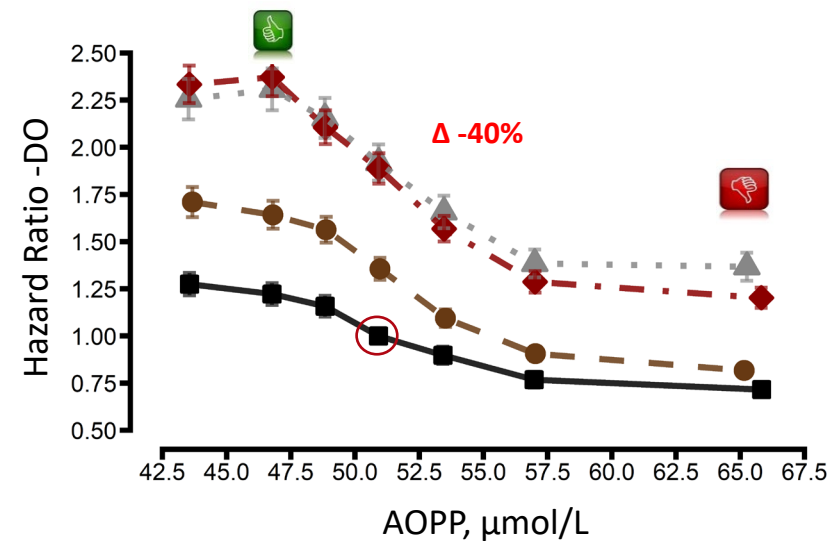
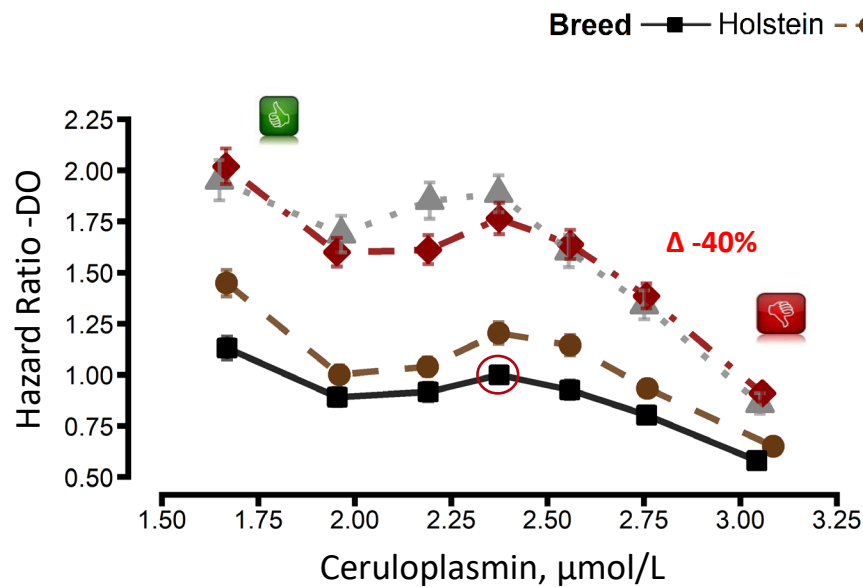
Giannuzzi et al., 2023 J Dairy Sci; Mota et al., 2023 Genet Sel Evo

LS means pMARK DIM × Breed – PR database ($P < 0.001$)



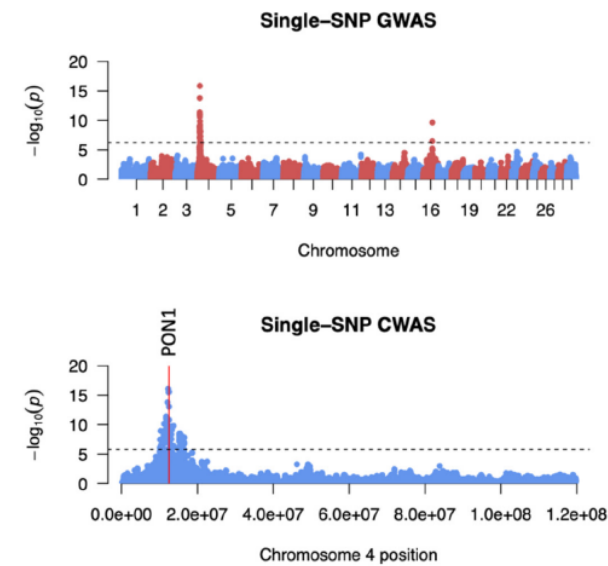
Ramirez Mauricio et al., 2024

Effect of pMARK on Days Open (DO) – Bolzano database



Cecchinato et al., 2024

Hepatic function and damage	h ² - measures	h ² - predictions
AST	0.28	
ALP	0.46	
GGT	0.28	
Total bilirubin	0.05	
Albumin	0.21	
PON	0.54	
Inflammation/ innate immunity		
Total proteins	0.06	?
Globulins	0.12	
Haptoglobin	0.19	
Ceruloplasmin	0.33	
Myeloperoxidase	0.29	
Oxidative stress		
ROMt	0.30	
FRAP	0.09	
AOPP	0.14	
SHp	0.09	



For more insights, please attend Matilde’s presentation 43.28, 540

Passamonti et al., 2024

Final remarks



Results of LS means of **pMARK over DIM** consistent with expected physiological patterns

▶ Results of effects of **pMARK on DO** consistent with the physiology of response to stressors

▶ Exploitable genetic variation exists among MARK



Future perspectives



- ▶ Estimate genetic parameters in populations using **pMARK** data across different breeds
- ▶ Estimate genetic correlations between **pMARK** and fertility traits
- ▶ Study the associations between **pMARK** and other indicators of resilience, such as functional longevity





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dairyomics.com



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FOSS



HR EXCELLENCE IN RESEARCH



Thank You!

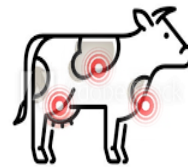
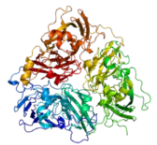
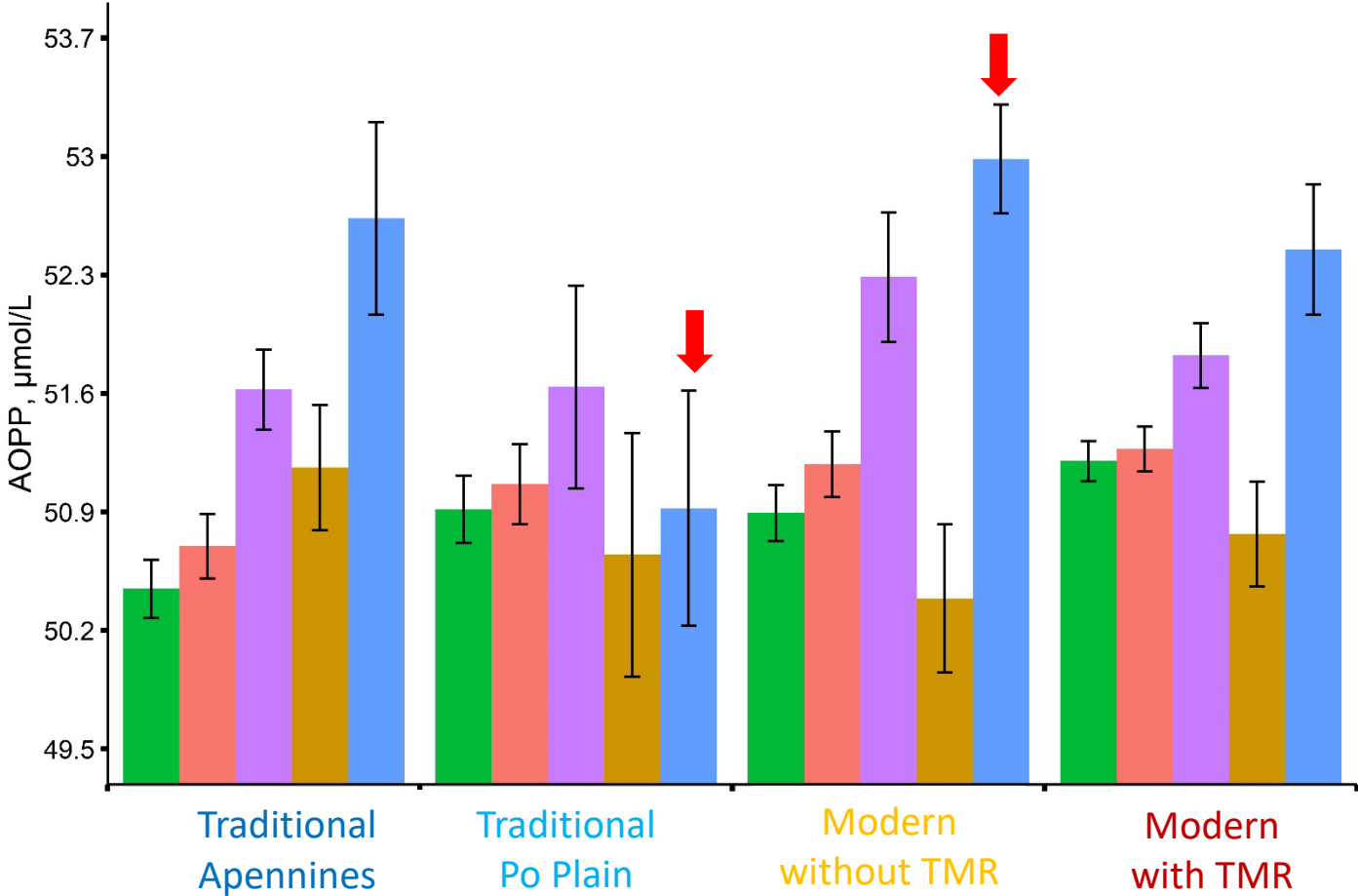
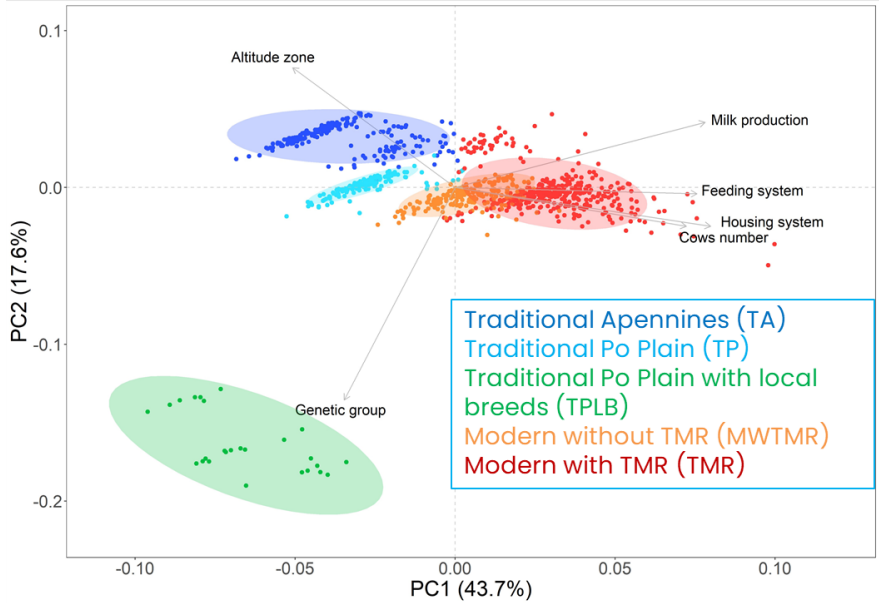


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DAFNAE
Department of Agronomy Food Natural
resources Animals and Environment

LS means pMARK Farming System × Breed – PR database ($P < 0.001$)

Breed: ■ Holstein Friesian ■ Crossbred ■ Brown Swiss ■ Simmental ■ Reggiana

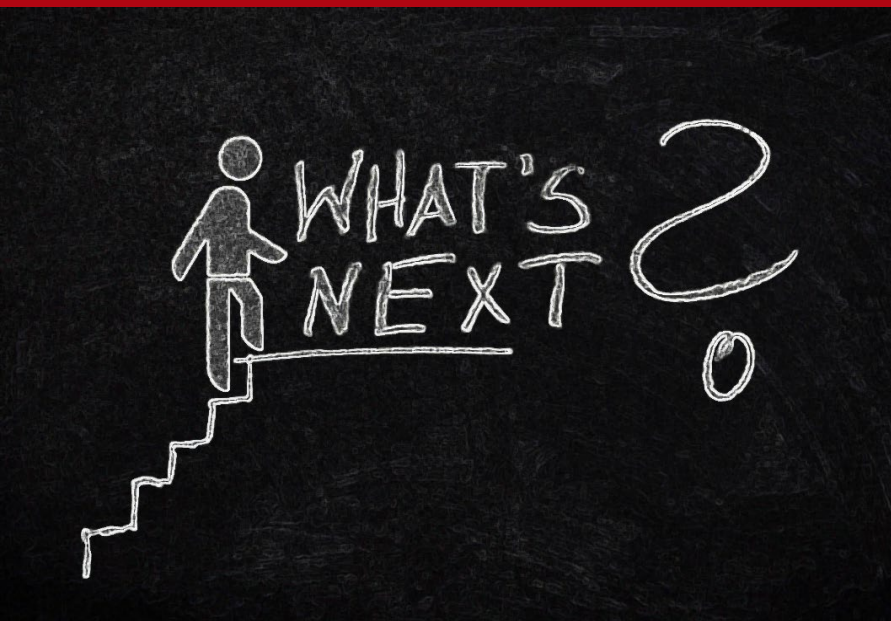
Five farming systems defined according to K-means clustering



Ramirez Mauricio et al., 2024

- ✓ MIR spectra outlier information was determined by the probability level for the chi-squared distribution of each sample's Mahalanobis distance, which was calculated using the incomplete gamma function with five degrees of freedom. Samples with a P -value of less than 0.01 were removed from the dataset (Shah and Gemperline, 1989)
- ✓ To explore spectral variation over time, the first 5 principal components were plotted over time and inspected. To overcome spectral variations, the absorbance values for every wave were centered to a null mean and standardized to a unit sample variance within year periods (Toledo-Alvarado et al., 2018)

The Next Steps



1

2

3