



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Phenomic data integration for the identification of biomarkers of subclinical mastitis in Holstein cattle

V. Bisutti¹, A. Lisuzzo¹, D. Giannuzzi¹, M.E. Gelain¹, E. Fiore¹, M. Giancesella¹, L. Laghi², A. Cecchinato¹, S. Pegolo¹

¹ University of Padova, viale dell'Università 16, Legnaro (PD), Italy

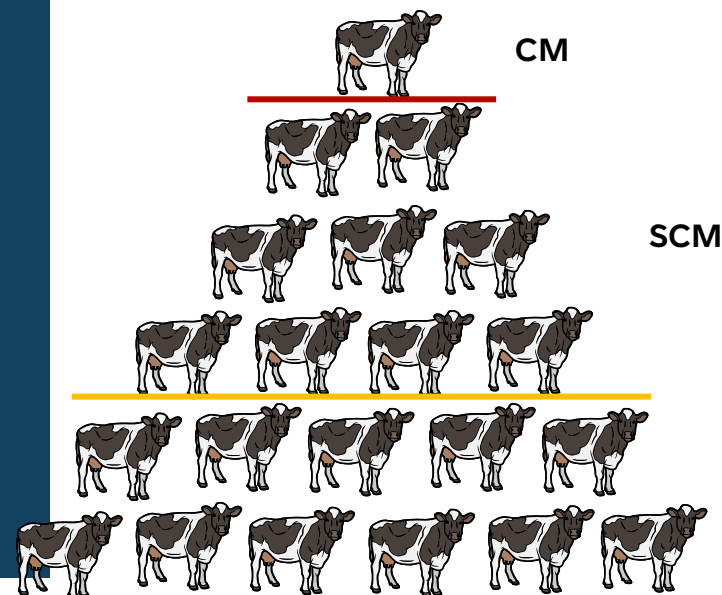
² University of Bologna, piazza Goidanich 60, 47023, Cesena (FC), Italy

Background

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- Introduction
- Aim
- Methods
- Results
- Conclusions

Subclinical Mastitis (SCM) is 15 to 40 times more frequent than clinical mastitis. It is a major source of **infection**, it causes a **decrease** in **milk production** and impairment in **composition**



CONSIDERATIONS

Fundamental to identify **reliable** and **reproducible** biomarkers for mastitis detection and resistance

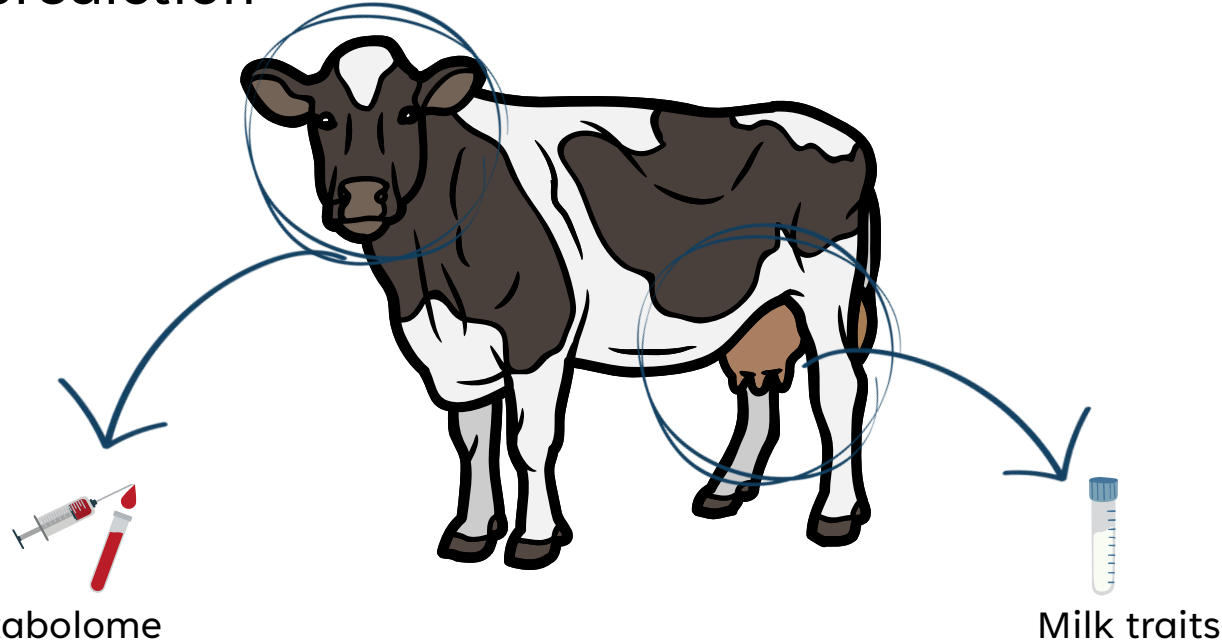
→ **System biology** approach provides a unique view for understanding the pathological processes behind this disease

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Integration of different sets of **phenotypic traits** for the identification of putative biomarkers for mastitis detection and prediction



Methods | Experimental design

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HERD SELECTION



Veneto region (Italy)

Presence of both *S.agalactiae* and *Prototheca spp.* (prevalence study by IZSve)

ANIMALS' SELECTION

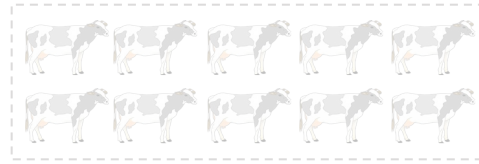


DIM $\geq$ 120 days
Parity $\geq$ 2

T0

Two weeks

T1



Aseptic manual milking on 188 Holstein cows

SAMPLING ACTIVITY



Bacteriological screening (IZSve) for the selection of the experimental groups

Negative animals: no history of mastitis

Positive animals having co-infections, treated with antibiotics, clinical signs of mastitis → **excluded from the trial**

Negative
n = 15



Prototheca spp.
n = 9



S.agalactiae
n = 10



Mastitis
n = 19

- ✓ Bacteriological confirmation
- ✓ Milk production, composition and udder health traits
- ✓ Flow cytometry analysis
- ✓ Omic analyses (metabarcoding, epigenomic, transcriptomic and peptidomic)
- ✓ Blood samples collection

Methods | Experimental design

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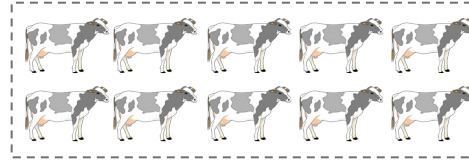
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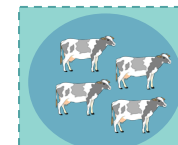
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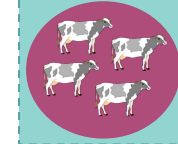
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Methods | The analyses

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MILK PHENOTYPES

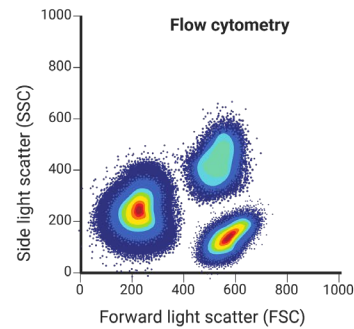
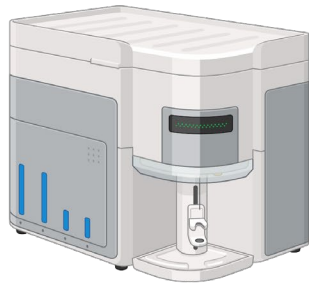
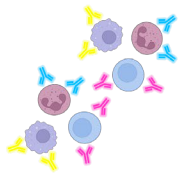
MILK COMPOSITION AND UDDER HEALTH TRAITS

Fat, protein, casein, lactose, urea, pH, conductivity
Milkoscan FT6000 (Foss) → ARAV

SCC AND DSCC

Fossomatic TM 7 DC analyser (Foss) → ARAV

MILK IMMUNE CELLS POPULATION



Leucocytes, T killer cells, T helper cells, macrophages, PMN, B cells

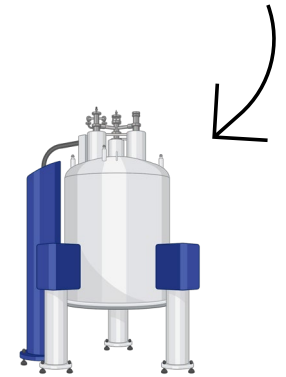
CyFlow Space flow cytometer → BCA department, UNIPD

BLOOD PHENOTYPES

METABOLOMIC ANALYSIS



Untargeted ^1H -NMR
metabolomic analysis
→ MAPS department,
UNIPD; UNIBO

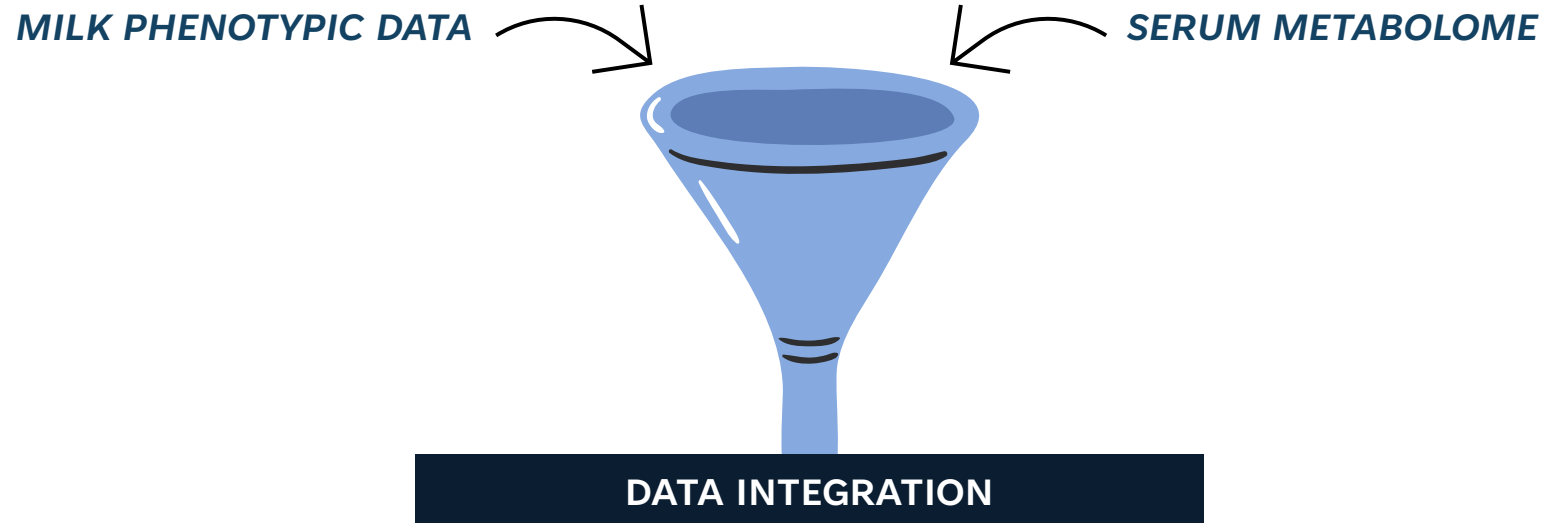


42 metabolites

Methods | Integration analysis

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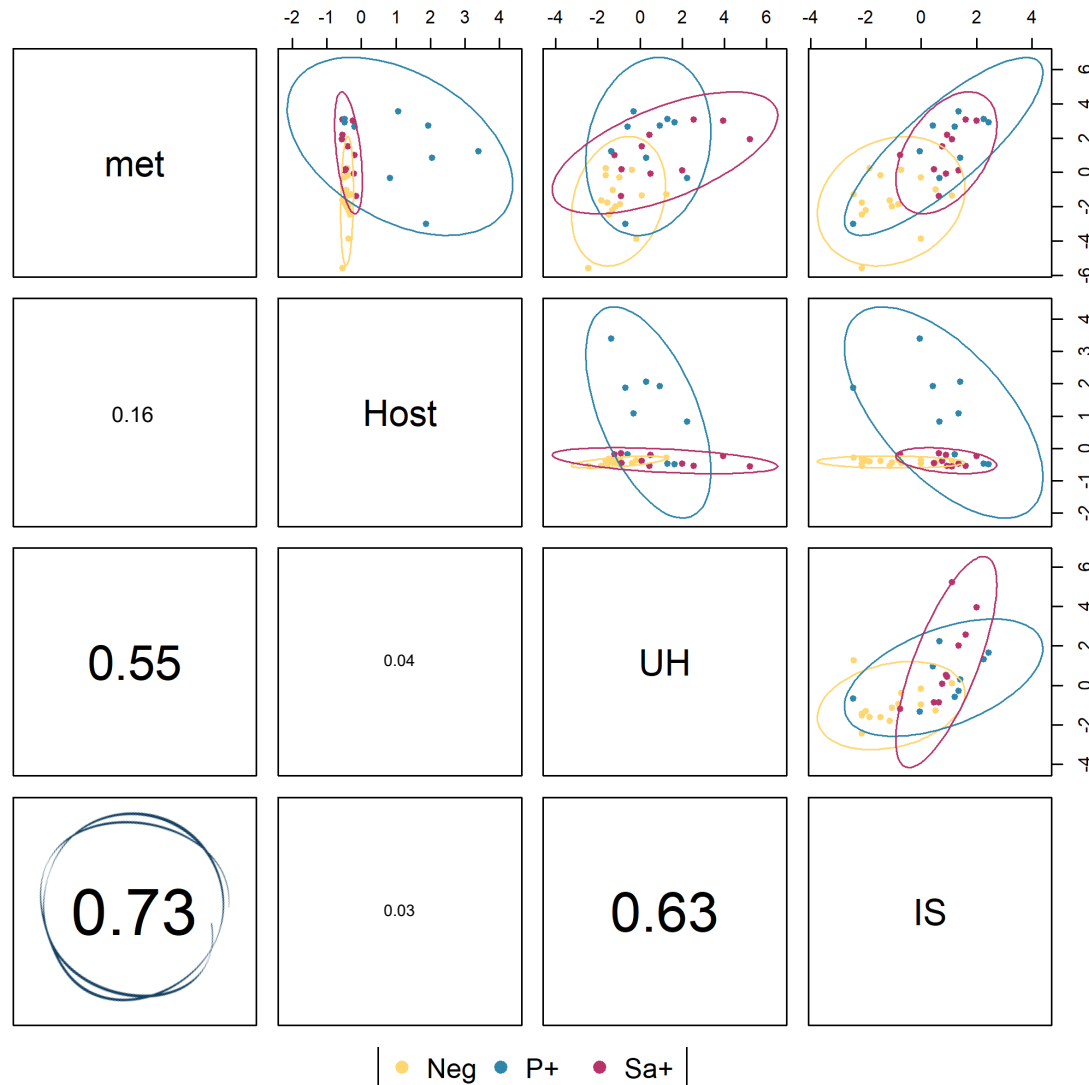
Data Integration Analysis for Biomarker discovery using Latent Components (DIABLO)
mixomics R package 

Receiver operating characteristics (ROC) analysis, pROC R package 
→ test the predictive performances of the identified putative hub variables

Results | Correlation matrix

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Met: 42 metabolomic variables

Host: DIM and Parity

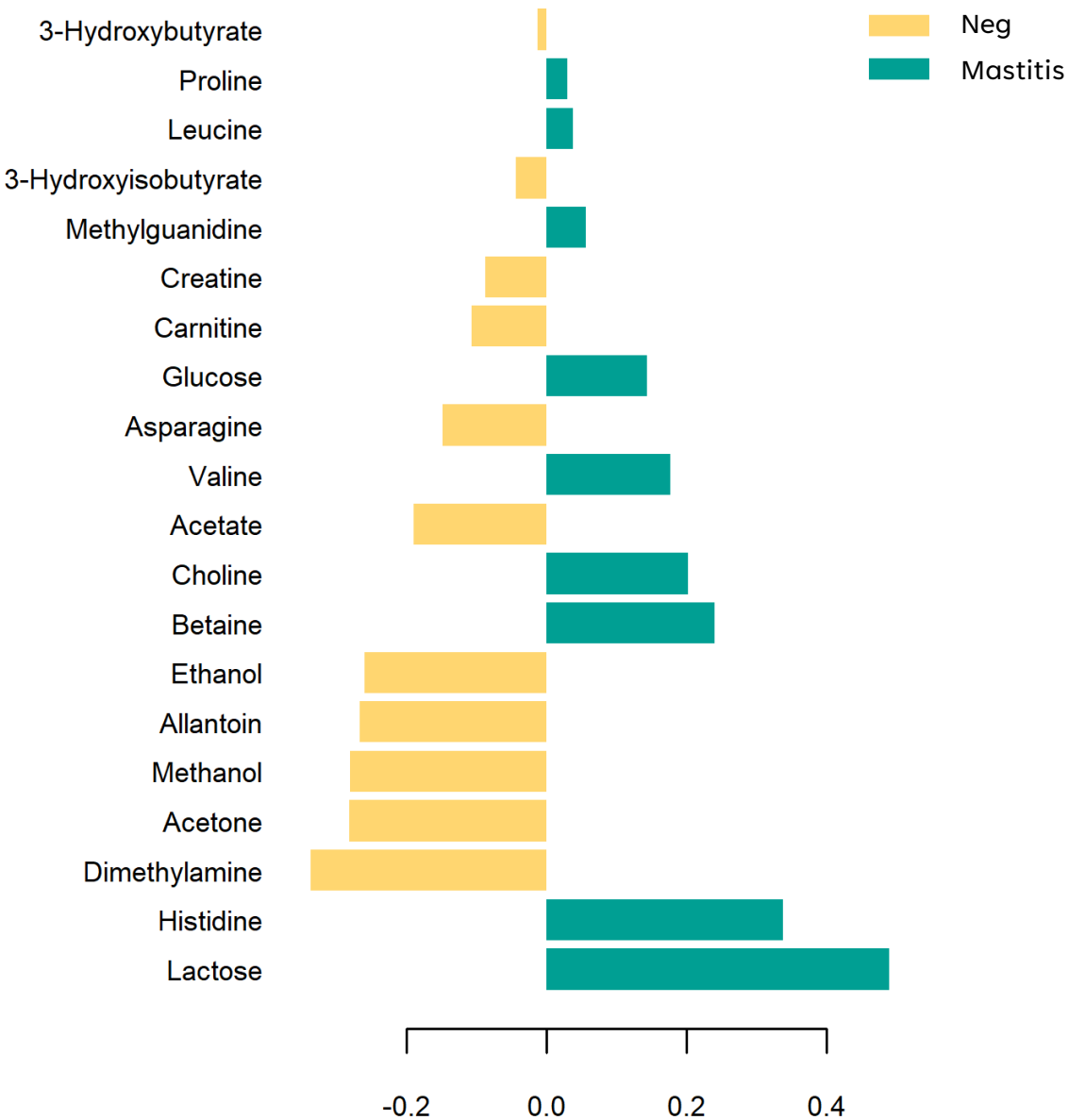
UH: Lactose, SCC, DSCC, CI, pH, conductivity

IS: Leucocytes, T killer cells, T helper cells,
macrophages, PMN, B cells

Results | Loadings plot

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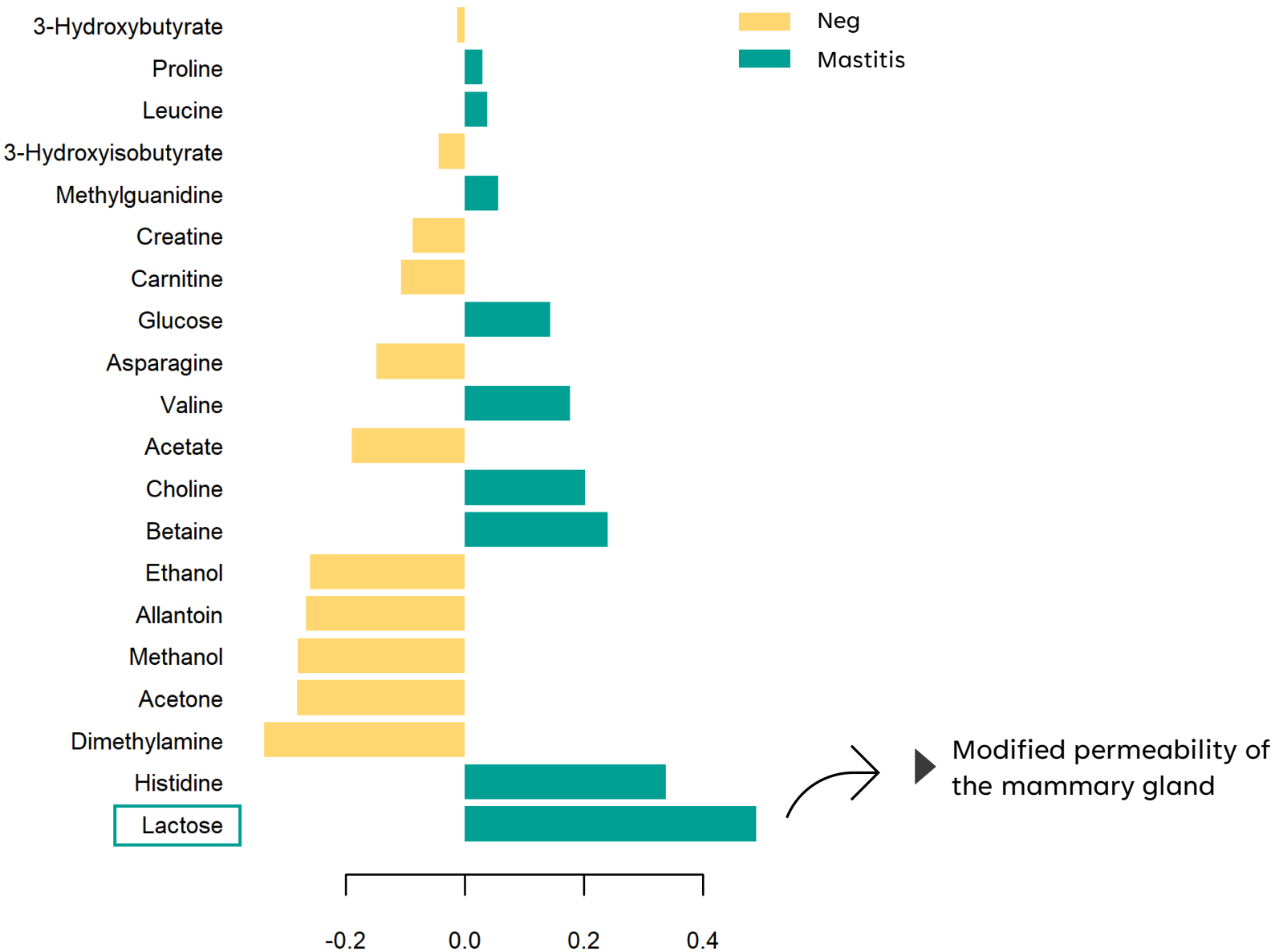
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Results | Loadings plot

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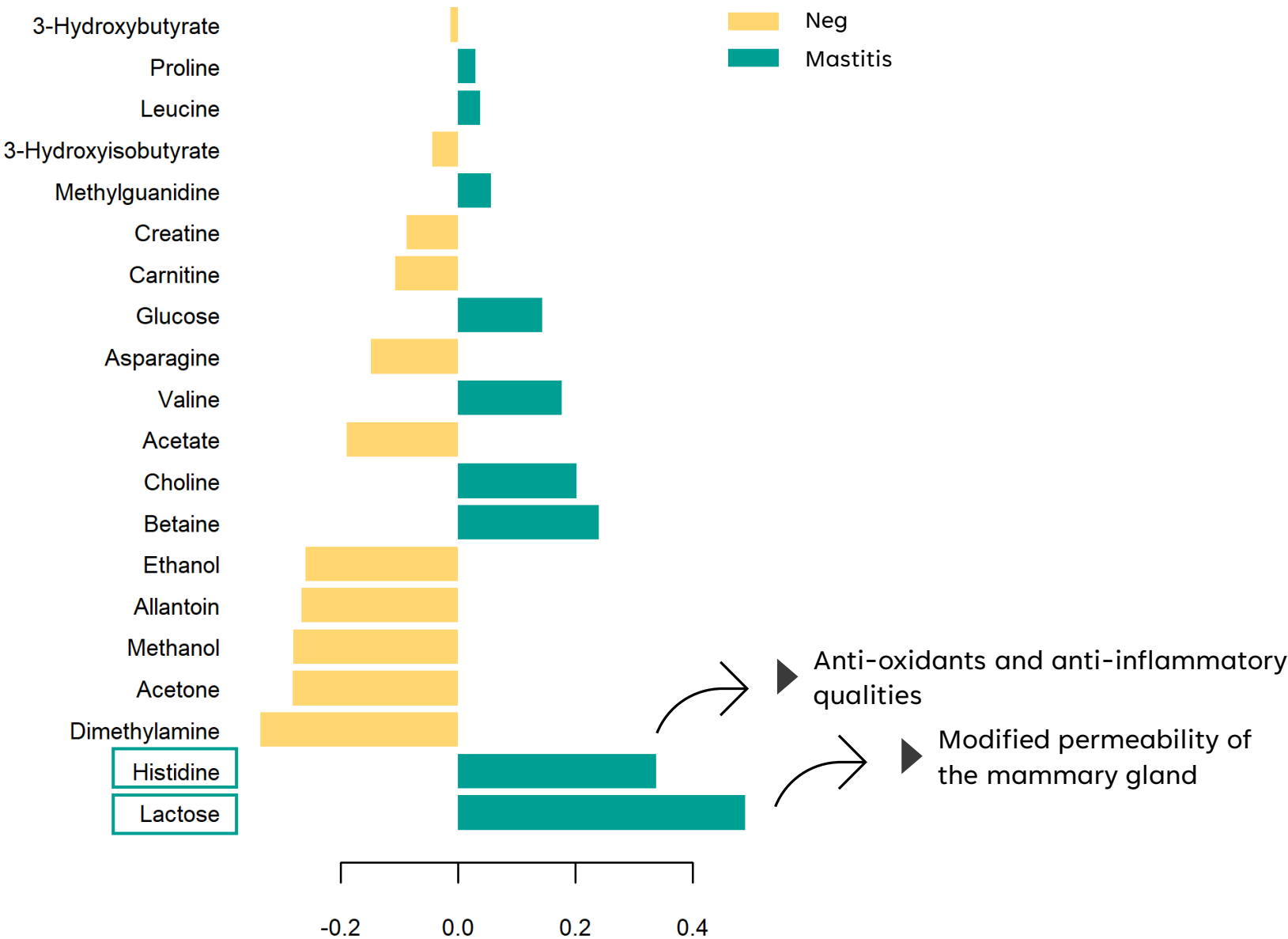
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Results | Loadings plot

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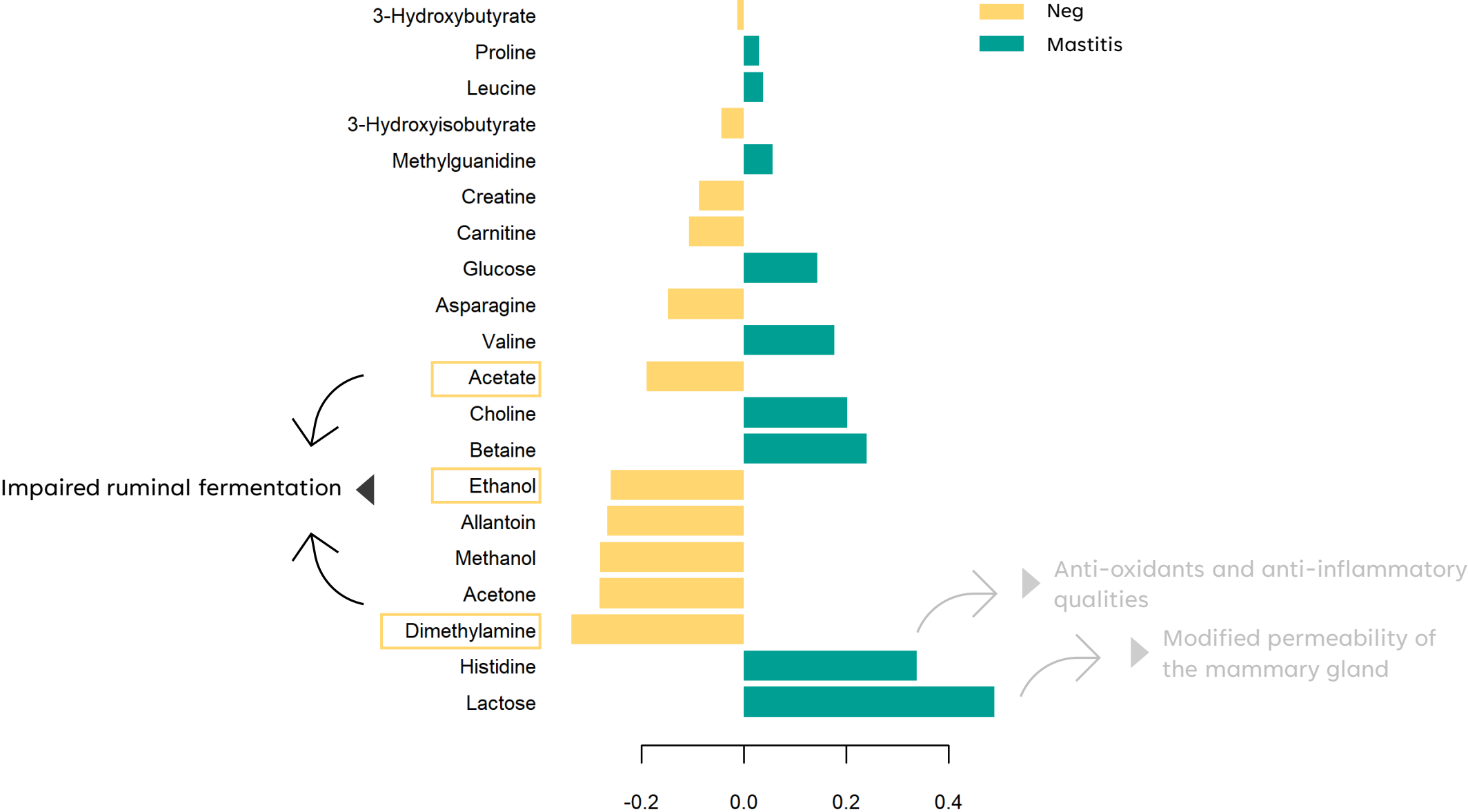
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Results | Loadings plot

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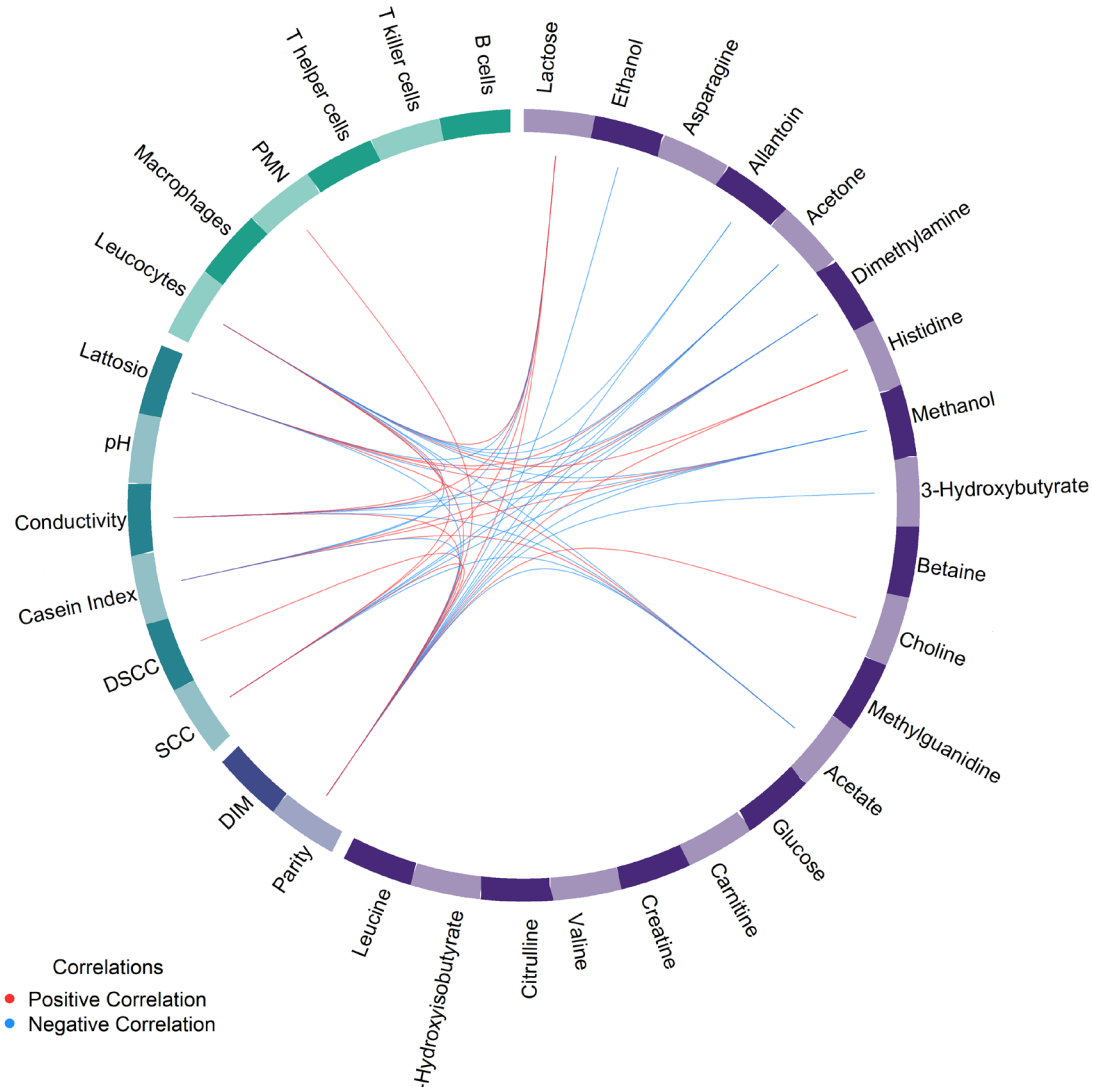
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Results | CIRCOS Plot

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Leucocytes – Allantoin (-0.61)
Leucocytes – Methanol (-0.67)
Leucocytes – Dimethylamine (-0.7)
Leucocytes – Acetone (-0.68)
Leucocytes – Acetate (-0.7)
Leucocytes – 3-Hydroxybutyrate (-0.57)

Leucocytes – Lactose (+ 0.60)

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PREDICTIVE PERFORMANCES OF THE HUB METABOLITES

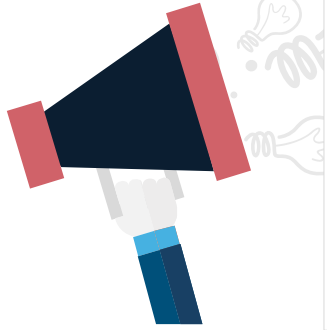


Metabolite	AUC	Sensitivity	Specificity	Accuracy	Precision
Acetate	0.67	0.93	0.53	0.71	0.61
Acetone	0.72	0.67	0.74	0.71	0.67
Allantoin	0.74	0.93	0.53	0.71	0.61
Betaine	0.78	0.60	0.89	0.76	0.82
Dimethylamine	0.87	0.67	0.95	0.82	0.91
Ethanol	0.67	0.67	0.68	0.68	0.63
Histidine	0.79	0.67	0.84	0.76	0.77
Lactose	0.91	1.00	0.74	0.85	0.75
Methanol	0.79	0.80	0.74	0.76	0.71

Conclusions

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TAKE-HOME MESSAGES

- ▶ Among the most informative MET variables we found lactose, histidine and metabolites linked with ruminal fermentation
- ▶ Total leucocytes negatively correlated with mostly all the metabolites, with the exception of lactose
- ▶ Blood lactose and methanol were found to be the most promising traits for prediction

THE NEXT STEPS

- ▶ **Milk** metabolome analysis
- ▶ **Multi-omics data integration** (transcriptomic, epigenomic, metagenomic, peptidomic, metabolomic) and validation on a larger dataset



European Partnership on
Animal Health and Welfare

THANK YOU!



vittoria.bisutti@unipd.it



www.dairyomics.it



@dairyomics_unipd



Results | Loadings plot

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