

Variations in amino acid and lipid profiles of honey bee hemolymph and gut microbiome composition in response to different dietary protein sources

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Nutritional deficiencies and biotic stressors compromise honey bee health and colony resilience. While pollen substitutes like Ultra Bee or Chlorella are used by beekeepers as dietary supplements, their metabolic effects remain insufficiently characterized. This study explores the influence of dietary protein sources on hemolymph amino acid and lipid composition, and gut microbiome structure to better understand their physiological impact.

Pollen-derived protein is crucial for honey bee immunity, metabolism, and overall health, but alternative protein sources may not offer the same benefits. Beyond protein, lipids in pollen play a key role in energy storage and immune function. To gain a comprehensive understanding of how pollen substitutes affect honey bees, we examined their influence from multiple perspectives by analyzing hemolymph amino acid and lipid profiles alongside gut microbiome composition.

Honey bees were fed diets containing natural pollen, Ultra Bee pollen substitute, or Chlorella as a protein source in *in vitro* conditions. Targeted metabolomic analysis of hemolymph amino acids was conducted using the MetAmino® kit (Chromservis) for LC-MS. Lipidomic profiling was performed using an untargeted LC-MS approach. Additionally, gut microbiome composition was determined through sequencing to investigate potential diet-related shifts in microbial communities.

Amino acid profiling revealed distinct differences between feeding groups. Notably, bees fed Chlorella exhibited elevated levels of valine and methionine, while those receiving Ultra Bee showed higher leucine and cysteine concentrations. Lipidomic analysis demonstrated that bees consuming natural pollen had higher lipid abundance and greater lipid diversity compared to those fed pollen substitutes. These findings suggest that natural pollen provides a more complex lipid profile that may support honey bee metabolic health. Preliminary gut microbiome analyses indicate dietary-dependent differences in microbial composition, which will be further explored.

Our findings emphasize the critical role of multi-floral pollen in supporting honey bee metabolic function, particularly due to its richer lipid composition. While pollen substitutes supply key amino acids, they may not fully replicate the metabolic benefits of natural pollen. Understanding how different protein sources influence honey bee physiology can inform better supplemental feeding strategies to enhance colony resilience and health.

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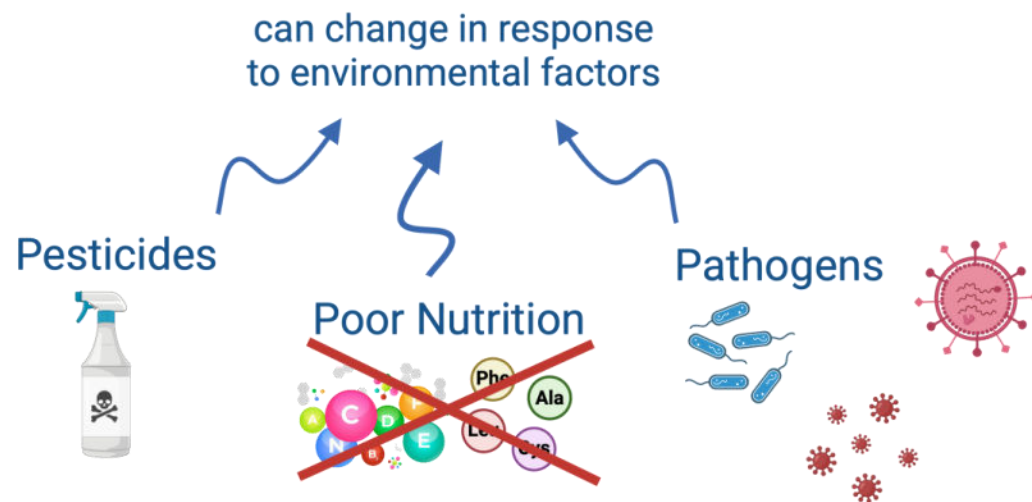
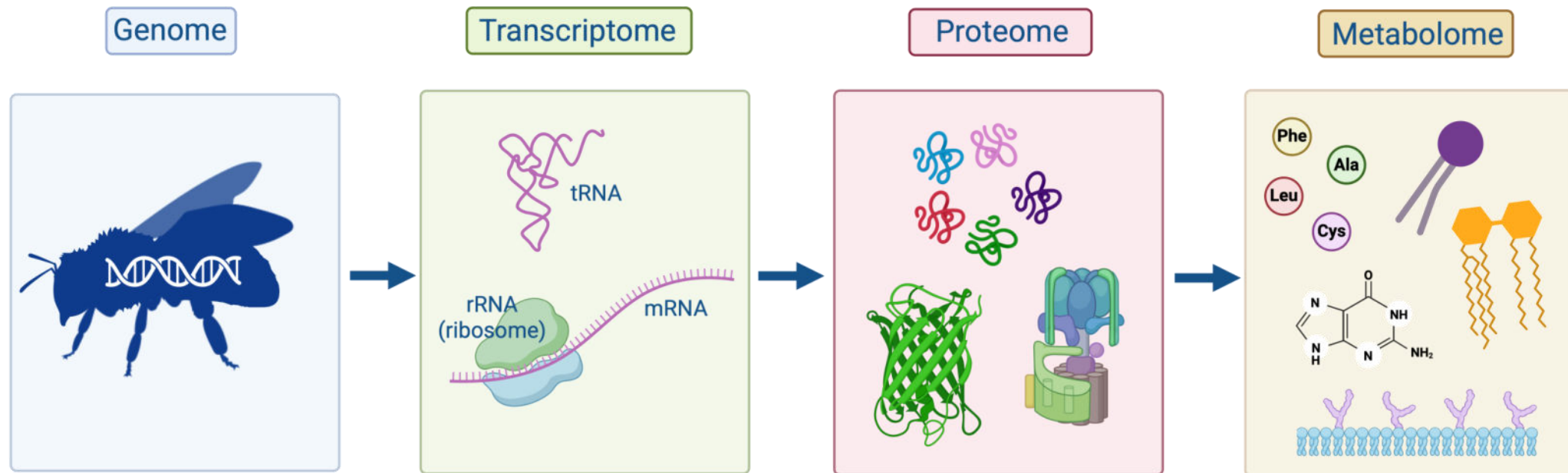
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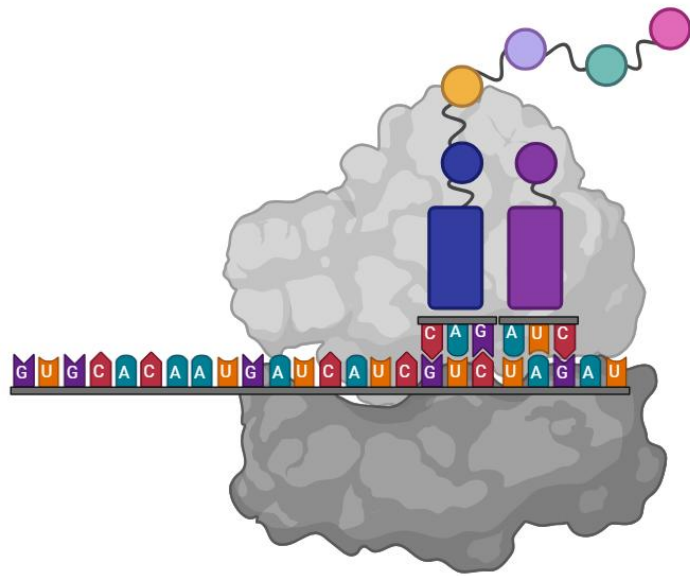
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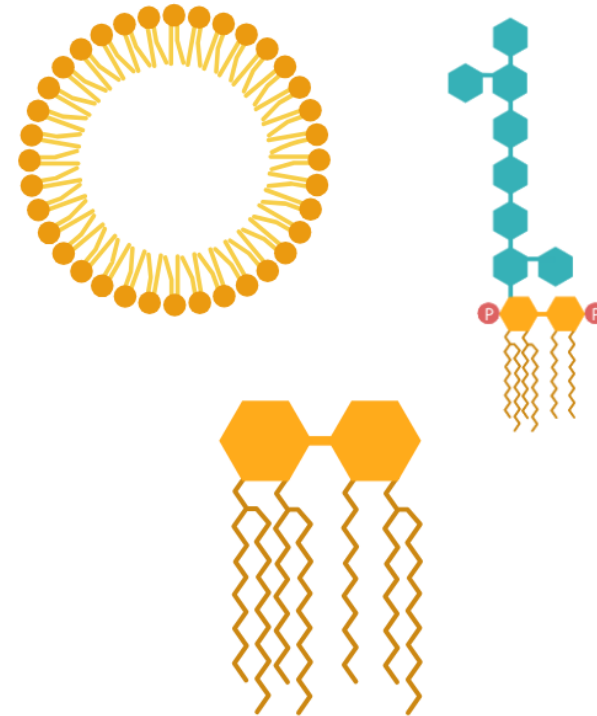


Pollen substitutes

Amino acids and proteins

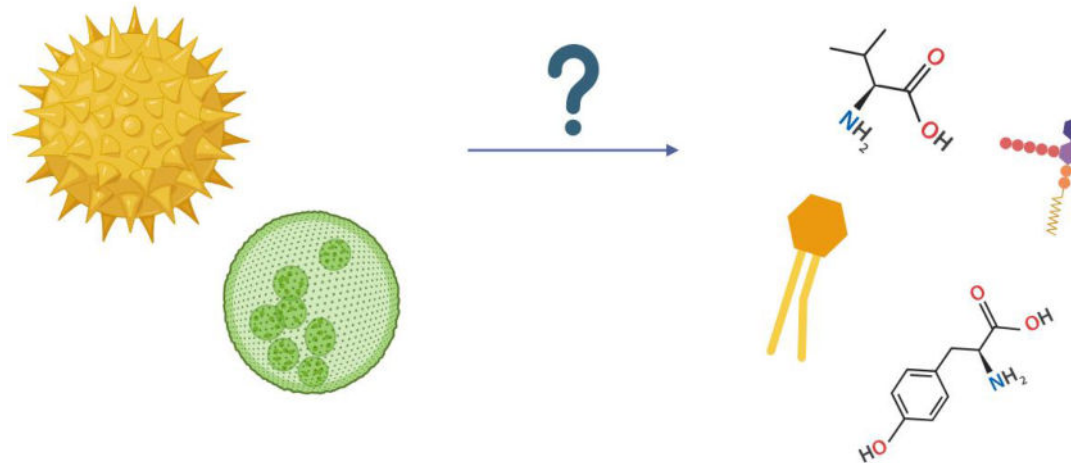


Lipids



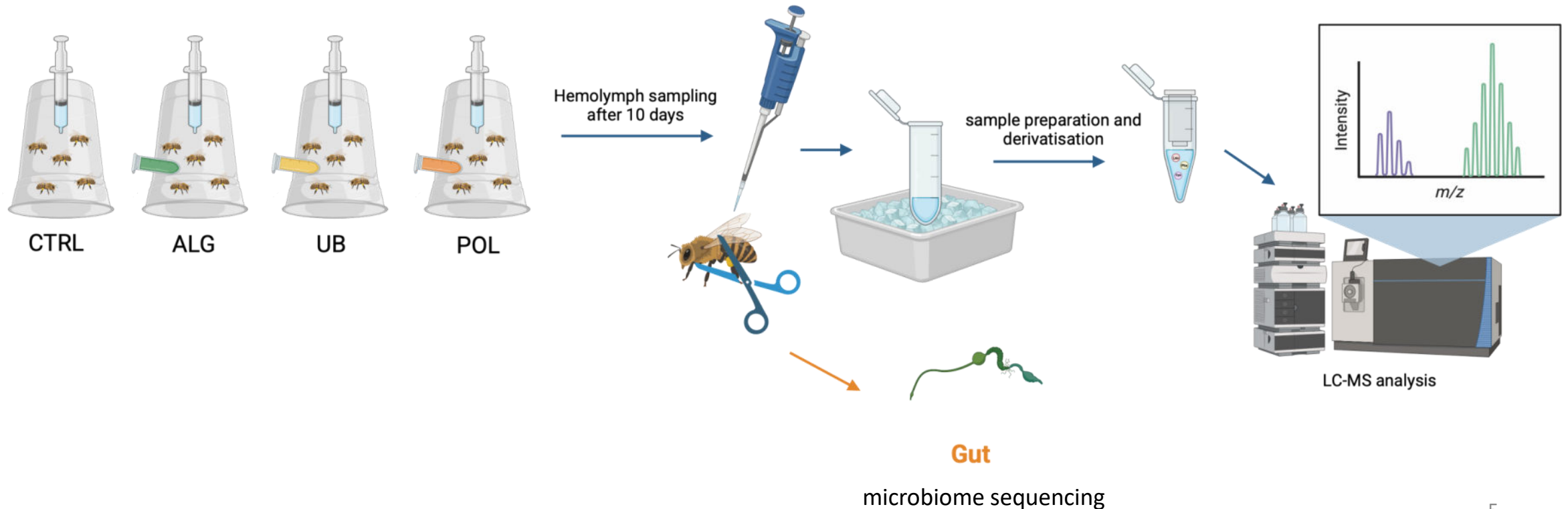
Goals of the study

- Compare metabolic responses of bees fed **pollen substitutes** to those fed **natural pollen**
- Are there differences in energy metabolism?
- Can we find **biomarkers** of poor nutrition?



Experiment Design and Methods

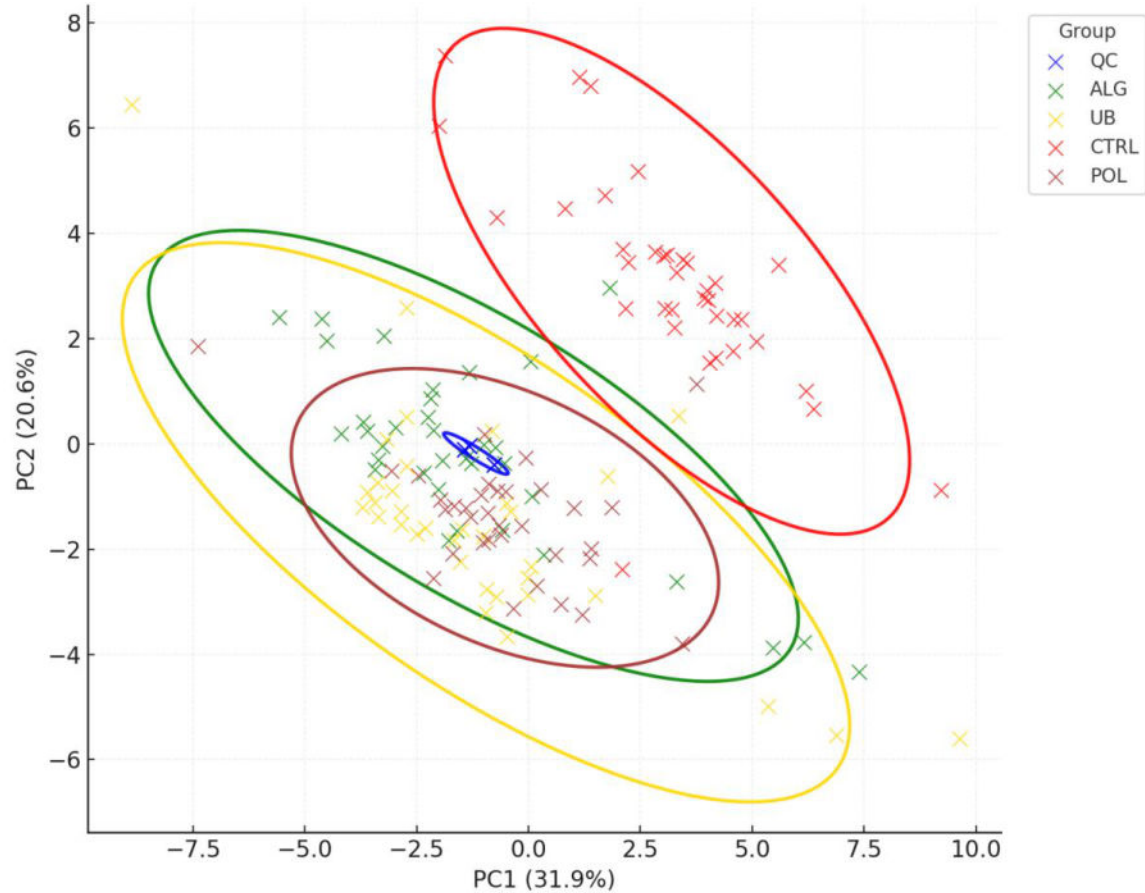
- CTRL = sucrose, ALG = chlorela, UB = ultrabee, POL = natural pollen
- In total 144 samples of hemolymph (pooled from 10 bees each)



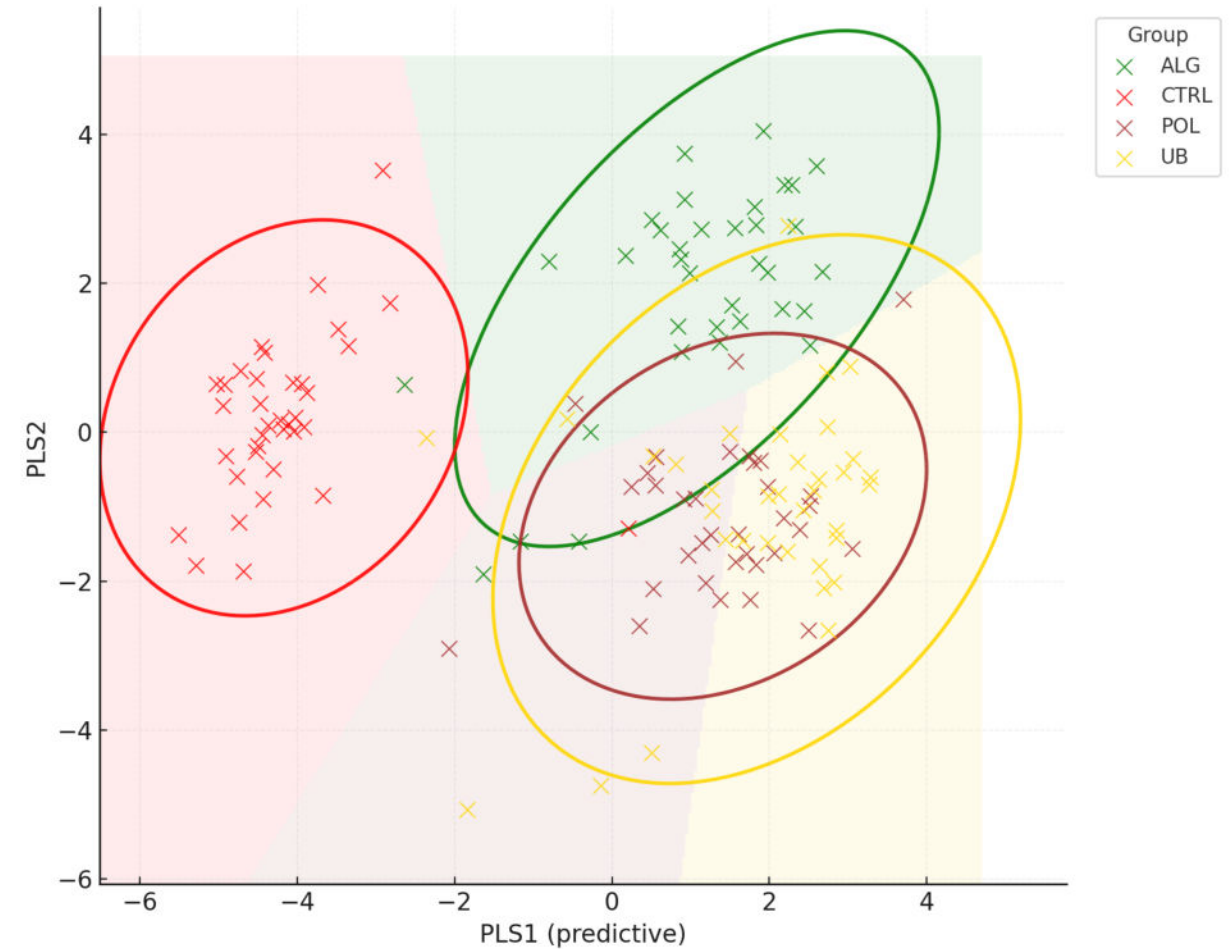
RESULTS

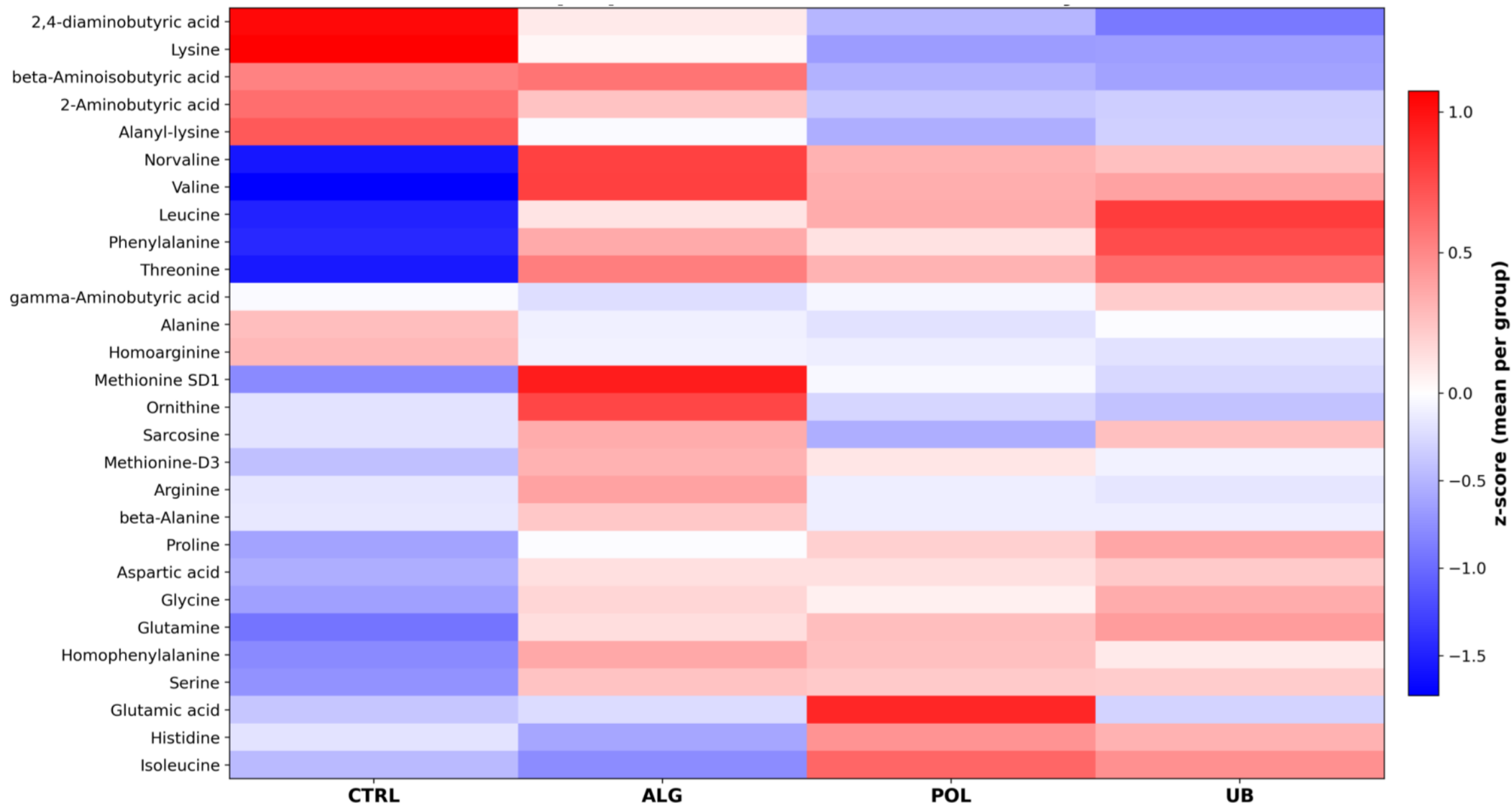
Amino acid profiles

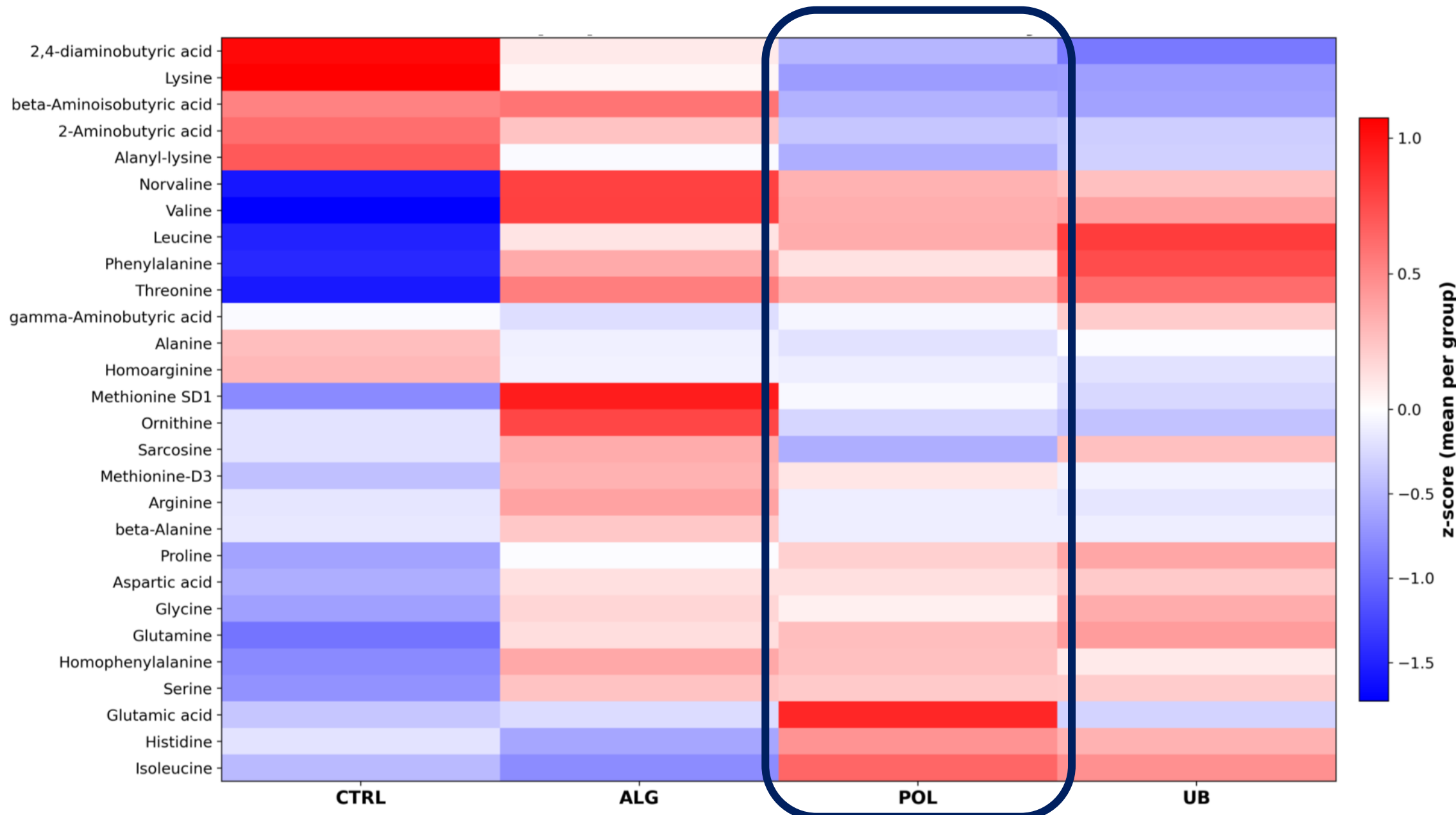
PCA – unsupervised analysis



PLS-DA – supervised analysis

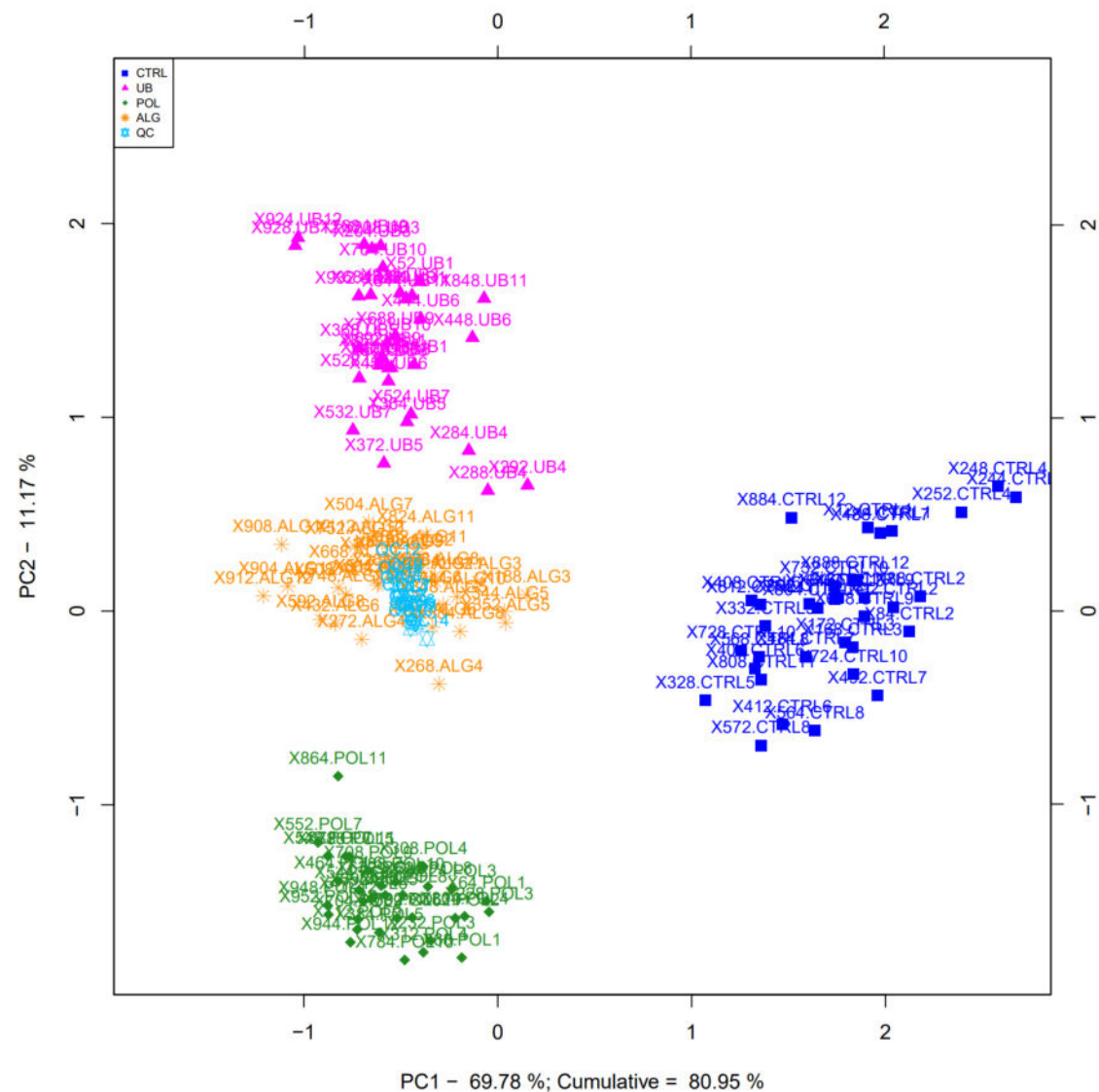




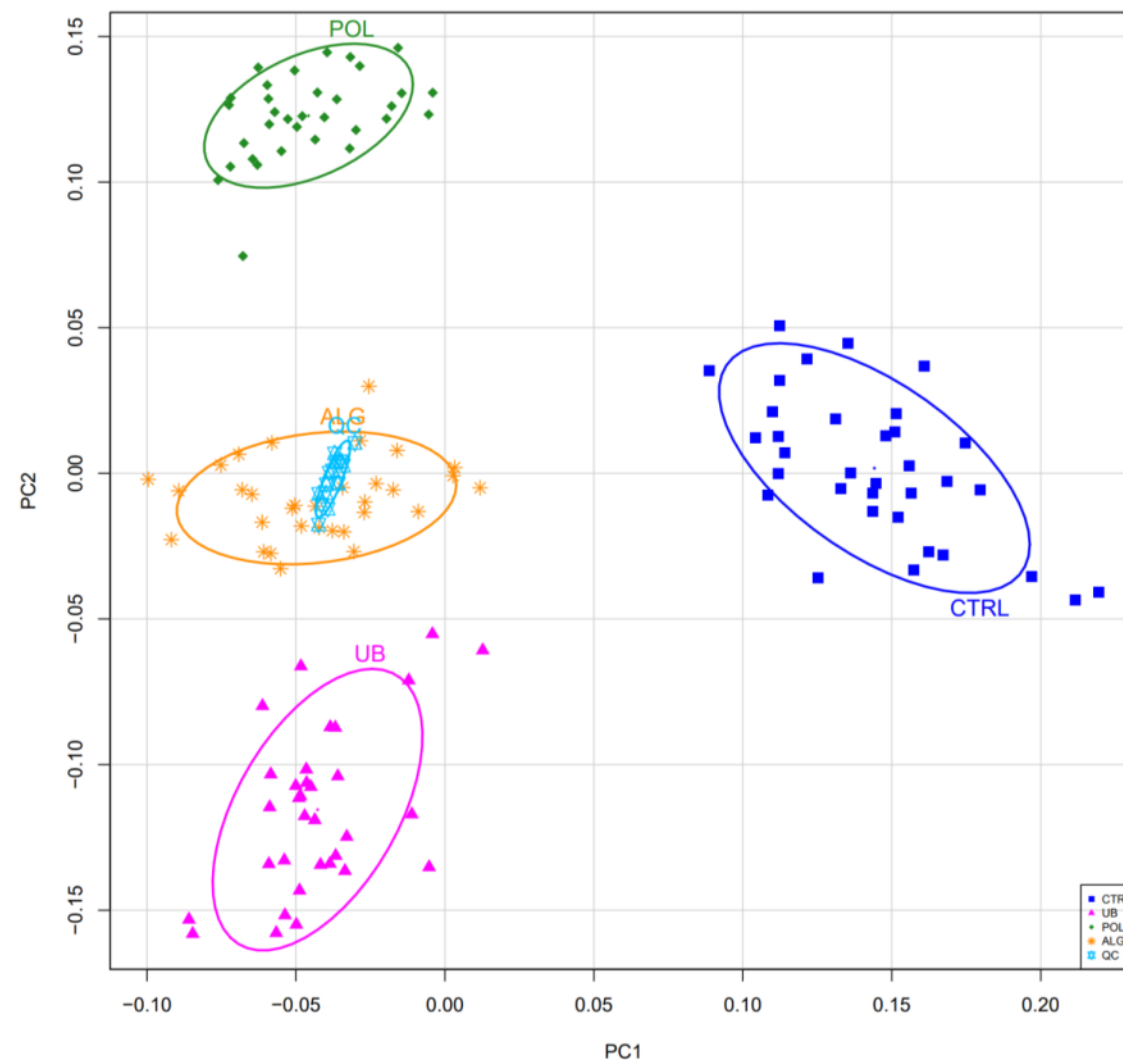


Lipid profiles

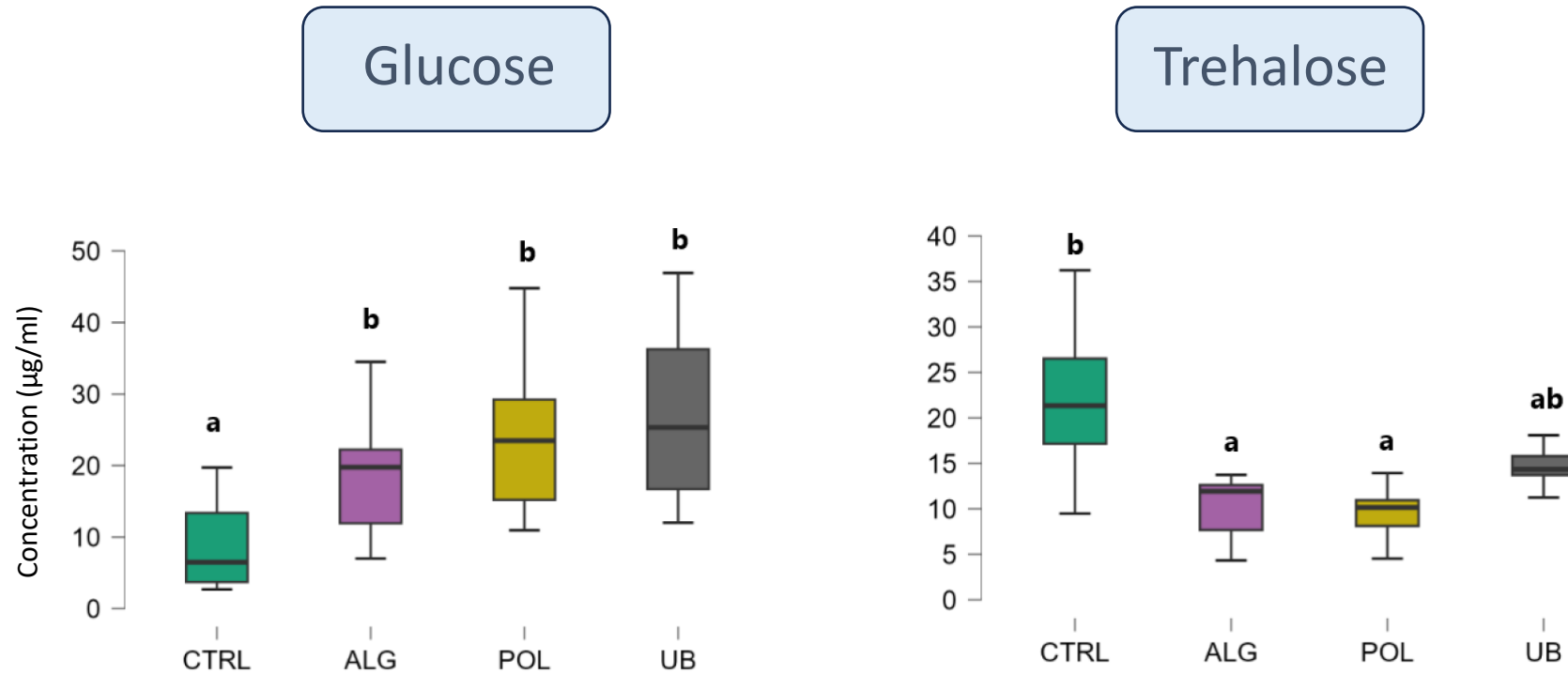
PCA



OPLS-DA



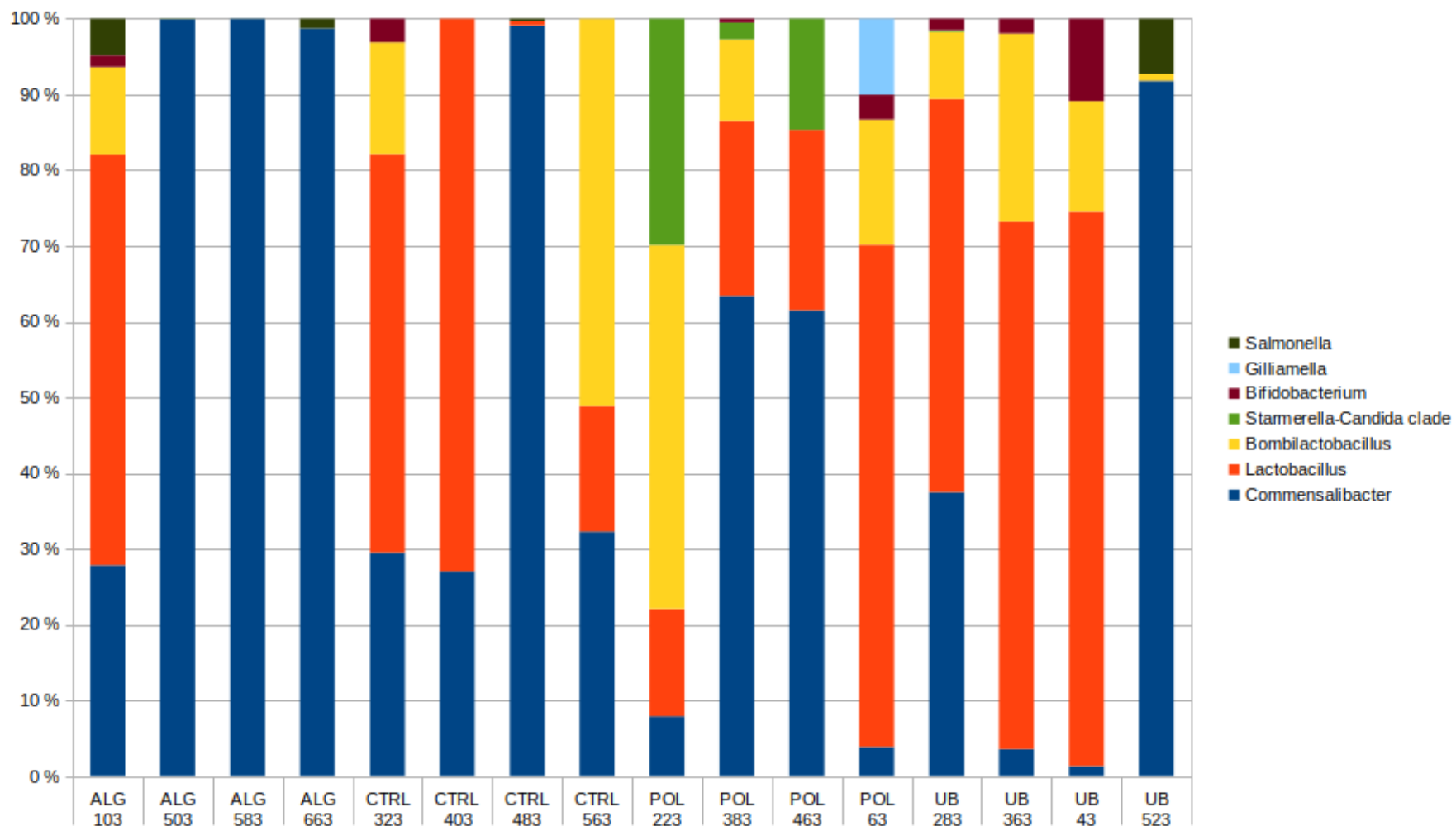
Carbohydrates



- Under high metabolic demands trehalose levels decrease – used for flight or foraging
- CTRL bees – reserve energy in form of trehalose in case of need
- Bees that have access to a protein source can better utilize energy – higher glucose

Microbiome

Genus



Summary and Key Points

- Metabolomic and lipidomic profiles differ between feeding groups
- Glucose availability higher with protein diets
- Microbiome not significantly affected
- **Comparing** substitutes with regional pollen via metabolomics

Thank you for your attention

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and Dentistry



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Laboratory for inherited metabolic disorders

University Hospital Olomouc | Palacký University | Institute of Molecular and Translational Medicine



Institute of Biophysics
of the Czech Academy of Sciences

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