

Innovative and integrated molecular approaches to assess honeybee health

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Wild and managed bees are facing increasing threats from environmental pollution, climate change, and pathogens, which together threaten their survival and the balance of ecosystems. In this scenario, veterinarians and researchers play a fundamental role in supporting beekeepers and addressing emerging challenges, while the need for reliable tools to monitor and protect bee health continues to grow. Conventional methods used to evaluate colony health offer general insights but tend to be subjective and lack precision. Conversely, hemolymph proteins have shown great potential as biomarkers of nutritional and health status (Isani et al., 2023). Now it's time to go a step further, following an integrated approach that links honeybee nutrition and health to the environment. It is recognized that honeybees are a paradigm of the One Health approach and are excellent biological indicators of environmental contamination. The aim of this research is to combine two omics techniques, namely proteomics and metabolomics, to evaluate the health of honeybees. This approach can make a real contribution to solving the problem of field assessment of the health status of honeybees by intercepting distressed colonies at an early stage in different environments and farm management.

In this study, hemolymph samples were collected from honeybees in different apiaries in the province of Bologna during different periods of the year, spanning from April to November. Hemolymph proteins were separated and quantified using 1D SDS-PAGE, while honeybees cytosolic extracts were fractionated using size exclusion chromatography (SEC) and metabolites were identified using mass spectrometry (Orbitrap Exploris 480, Thermo Fisher). Finally, copper was analyzed in SEC fractions by flame atomic absorption spectrometry.

The panel of hemolymph biomarkers used included apolipoprotein I, vitellogenin, apolipoprotein II, transferrin, hexamerin 70a, and total proteins. Their concentrations varied according to different physiological and environmental factors, such as age of honeybees (nurse bees had the highest vitellogenin levels compared to the other sub-castes), season (a peak in vitellogenin and transferrin concentrations was detected in November in winter bees), and parasitic infections (*Varroa*-infested honeybees showed a reduction in vitellogenin, apolipoprotein II, transferrin, and hexamerin 70a). One hundred and ninety-eight metabolic pathways and over 2,000 metabolites were identified, with the most prevalent belonging to the flavone pathway, followed by the lipoxygenase pathway. Many of these metabolites were of plant origin and may correlate with the environmental availability of nectar and pollen, both essential for honeybee nutrition, suggesting their possible role as biomarkers of nutritional status. In addition, these metabolites can be considered as indicators of the plant biodiversity around the hives. Finally, the copper content and speciation are related to the impact of agricultural practices around the hives.

In conclusion, this research presents an innovative integrated approach to assess colony health, and the relationship between honeybees and the environment, bridging gaps in current assessment methods and highlighting the importance of an integrated omics approach to address the challenges

in apiculture. These findings contribute to the advancement of honeybee monitoring and emphasize the intricate link between environmental, animal, and human health.

Isani, G.; Bellei, E.; Rudelli, C.; Cabbri, R.; Ferlizza, E.; Andreani, G. SDS-PAGE-Based Quantitative Assay of Hemolymph Proteins in Honeybees: Progress and Prospects for Field Application. *Int. J. Mol. Sci.* 2023, 24, 10216. <https://doi.org/10.3390/ijms241210216>

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