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The success of blueberry fruit set relies heavily on efficient bee pollination. In Canada, there is growing concern about the health of colonies involved in this process, with nutritional deficiencies suggested as a key contributing factor. This study aimed to determine the nutritional profile of bee bread and assess its impact on the larvae and bee metabolome using an untargeted metabolomics approach. Sixteen colonies from the Lac Saint-Jean region (QC), a polyfloral area, were distributed across two control sites (polyfloral; four hives per site) and two blueberry field sites (monofloral; four hives per site). Samples were collected at time 0 (before the hives were moved for pollination) and at time 1 (when pollination was nearing completion) and were analyzed by UHPLC coupled with mass spectrometry to perform untargeted metabolite profiling. After pollination, total polyphenol content in larvae and foraging bees decreased in the blueberry group. These results suggest that plant-derived secondary metabolites from blueberries, which help maintain the antioxidant status of bees, were reduced. In contrast, after pollination, bee bread from the blueberry group showed an increase in the intensity of flavonoids (isorhamnetin 3-galactoside, isorhamnetin-3,7-di-O-glucoside, isorhamnetin-3-glucoside-4'-glucoside, and syringetin-3-O-glucoside), organic compounds (hydroxypyruvate and citrate), and nucleotides (uridine and guanosine), indicating a distinct nutritional profile. The pollination service did not influence the larvae metabolome. However, after pollination, foraging bees in both groups showed decreased flavonoid content (cyanidin, rutin, nicotiflorin) and the phospholipid phosphatidylcholine. Homovanillic acid, an important biomarker of dopamine metabolism and pheromone regulation, specifically the queen mandibular pheromone, increased in the polyfloral group compared to the blueberry group. Overall, these results reveal that the untargeted metabolomic profile of bee bread presents distinct metabolites, differentiating the polyfloral from the blueberry group and suggesting higher nutritional diversity in the polyfloral group. Moreover, foraging bees exhibited temporal variations in their metabolic profiles before and after pollination, potentially influenced by growth, development, and nutrient exposure during the pollination service. Future studies will focus on the roles of bioactive metabolites in bee bread, larvae and the bee metabolome.