Effects of B-vitamins and probiotic supplementation on longevity and body weight of winter bees (*Apis mellifera*)

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Nutrition is a key factor for honeybee, *Apis mellifera*, health and all nutritional requirements are covered via nectar, which is stored as honey, and pollen. While beekeepers routinely replace stored honey with sugar solution and may supply pollen supplements, few data exist about micronutrients, e.g. B-vitamins, which can be produced by gut microbiota. To explore health benefits of supplementary feeding, we assessed the effects on individual longevity and body weight as a token of health by conducting a hoarding cage experiment with freshly emerged winter workers (N= 1865). All workers received antibiotics for 72 hours to inactivate the gut microbiota, received sucrose and were subjected to eight treatments (29 workers per cage, 8 cages per treatment): sucrose solution, sucrose & pollen, B-vitamins in low and high dosage (300 mg/L; 9000 mg/L), probiotics in low and high dosage (40’000 CFU/mL; 4’000’000 CFU/mL) and probiotics in low and high dosage combined with pollen for the entire duration of the study (i.e. 14 - 70 days depending on individual longevity). Treatment dosages for the B-vitamins represent lower and upper bounds of field-realistic ranges. For the probiotics, investigated dosages included one field-realistic as well as one considerably higher concentration to identify a potential dose-dependence of possible treatment effects. Two control groups could establish their gut microbiota on the brood frames for 72 hours and received then sucrose solution or sucrose and pollen (29 workers per cage, four cages per treatment). Body weight was significantly higher with pollen, almost certainly due to known positive effects on development, and B-vitamins had no impact. Despite antibiotics intoxication, the highest longevity was observed in the two control groups and in low probiotics dosage combined with pollen, highlighting the role of gut microbiota as well as suggesting low antibiotic toxicity at this dosage. No differential survival was observed in workers receiving sucrose, sucrose & pollen, probiotics & B-vitamins in the low dosage and probiotics in the high dosage. In contrast, workers fed with probiotic high dosage & pollen showed both reduced survival and no effect of the pollen treatment on body weight, which may be due to dysbiosis limiting other beneficial bacteria. The high dosages of B-vitamins showed significantly lower survival than all other experimental groups, probably due to B-vitamin toxicity. In conclusion, our data support the importance of gut microbiota, but the apparent dose-dependence should be considered for supplementation of both vitamins and bacteria.

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