Please cite this article as:

Staron M., Rajčáková L., Planý M., Abou-Shaara H. 2026. Assessing honeybee vulnerability to residue-level acetamiprid and thermal stress under different nutritional conditions. Journal of Plant Protection Research. DOI: 10.24425/jppr.2026.2998

Original article

Assessing honeybee vulnerability to residue-level acetamiprid and thermal stress under different nutritional conditions

Martin Staron[1], Ľubica Rajčáková[1], Matej Planý[1], Hossam Abou-Shaara[2*]

[1] Institute of Apiculture, Research Institute for Animal Production, Doktor J. Gašperíka, Liptovský Hrádok, Slovak Republic

[2] Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, Damanhour, Egypt

*Corresponding address: haboushaara@gmail.com

Received: December 10, 2025
Accepted: January 13, 2026
Online pree-Proof Publication: January 23, 2026
Responsible Editor: Rafał Kukawka
DOI: 10.24425/jppr.2026.2998

Abstract

Honeybees, which serve as vital pollinators, are often exposed to agrochemicals as non-target organisms. Among these substances, the widely used insecticide acetamiprid is of particular concern, as it is frequently detected in bee products. Honeybees are also routinely subjected to multiple stressors simultaneously, including pesticide exposure and heat stress—the latter becoming increasingly problematic under ongoing climate change. Nutrition adds an additional

layer of complexity to honeybee resilience, shaping how individuals respond to these concurrent stressors. In this study, the survival of nurse and forager honeybees was evaluated under two abiotic stressors—acetamiprid exposure at a residue level (52.78 µg kg^{-1}) and short-term heat stress (45°C for 2.5 h)—while providing bees with different dietary treatments: three types of honey and three commercial protein diets. Mortality of nurse bees was significantly higher in all acetamiprid-exposed groups than in the pesticide-free control, with honey-based diets providing better protection than protein-based diets. Regarding forager bees, mortality differed significantly between treatments, with buckwheat honey showing the lowest rates compared to protein diets and sugar candy. Age was a determining factor, as foragers exhibited higher mortality and lower consumption than nurses across treatments. Honey—particularly buckwheat —provided the most favorable outcomes, combining high consumption with reduced mortality under the study conditions. This study advances understanding of how nutrition can mitigate the impacts of multiple stressors on honey bees.

Keywords: honeybees, acetamiprid, nutrition, heat stress, residues

Introduction

Honeybees (*Apis mellifera* L.), well-known pollinators in global agriculture, continually face a growing suite of stressors—pesticides, environmental extremes, and nutritional deficits—that threaten their survival, particularly when these factors interact synergistically (e.g., Havard *et al.* 2019). In fact, the widespread use of pesticides causes various environmental contaminants and exposes non-target pollinators, such as honeybees, to harmful chemical residues (Stanley *et al.* 2016; Piechowicz *et al.* 2025; Staron *et al.* 2026). Climate change further intensifies these pressures by driving heat waves, altering flowering phenology, and increasing the frequency of erratic weather events (Neumann and Straub 2023). Farming practices—such as monocultures—often reduce floral diversity, diminish forage quality, and negatively affect the availability of

floral resources for honeybees (St. Clair *et al.* 2020). Together, these interacting stressors underscore the need to explicitly address the synergistic effects of multiple stressors on bee health. This perspective formed the foundation of the present study.

Regarding pesticides, numerous studies across different geographical regions have consistently detected pesticide residues in bee products (e.g., Sabo *et al.* 2024; Swiatly-Blaszkiewicz *et al.* 2025). Acetamiprid (a neonicotinoid insecticide; $C_{10}H_{11}ClN_4$) residues have been reported in unripe honey, bee bread, and beeswax (Ozols *et al.* 2024; Benčatová *et al.* 2025). Although acetamiprid is considered to be relatively fast-degrading, its extensive use in agricultural and domestic settings contributes to its persistence in bee-related matrices. It can be detected in areas beyond where it is directly sprayed (Capela *et al.* 2022). Acetamiprid is known for its systemic action against sap-feeding pests like aphids (Mokbel 2018). This chemical interferes with neuronal function in invertebrates, raising concerns about potential sublethal and lethal impacts on non-target pollinators, especially honeybees (Mackei *et al.* 2024; Shi *et al.* 2025). While research on its combined effects with other stressors - such as heat stress - is limited, the frequent detection of acetamiprid in bee-relevant environments underscores the pressing need for further studies on its ecological consequences for honey bee health.

Pesticide exposure may be further exacerbated by the intensifying heat stress experienced by honeybee colonies. Under normal conditions, honeybees can maintain brood-area temperatures from 32°C to 36°C—ideally close to 35°C—to ensure proper development (Abou-Shaara *et al.* 2017). Under elevated ambient temperatures, colonies deploy active cooling behaviors such as fanning, water collection for evaporative cooling, and bearding—where large groups of workers congregate outside the hive to dissipate heat (Rowe *et al.* 2025). Foraging activity can also be negatively affected under heat-stress conditions (Abou-Shaara *et al.* 2017). Honeybees are increasingly experiencing extreme heat under a changing climate, pushing their thermoregulatory capacities to their limits. These pressures underscore the urgent need to understand how honeybees may tolerate environmental and anthropogenic stressors.

Nutrition adds an additional layer of complexity to honeybee resilience under multiple stressors. Adequate and diverse nutrition underpins colony development, survival, and productivity (Tsuruda *et al.* 2021; Al-Esawy 2023). Adequate nutrients help honeybees endure environmental stressors. Honeybees require two primary feeding types: First, carbohydrates—sourced from nectar and later converted into honey—vary in their phytochemical composition depending on floral origin (Bernklau and Arathi 2023). Second, proteins—naturally derived from pollen—are sometimes supplemented by beekeepers during periods of seasonal pollen scarcity; however, these substitutes often differ in amino acid profiles and digestibility, leading to various impacts on colony health (Kim *et al.* 2024).

Given the limited understanding of how carbohydrate and protein diets—along with pesticide residue exposure and heat stress—interact to influence honeybee health, this study aimed to elucidate these dynamics. Feeding regimes incorporating both natural carbohydrate sources (various honey types) and commercially available protein supplements were examined, alongside assessments of exposure to acetamiprid residues. To simulate extreme temperature conditions, a short-term heat stress treatment at 45°C was applied. The experimental design included two age cohorts—nurse bees and foragers—and assessed their feeding preferences. This study provides key insights into the complex interactions between nutrition, pesticide residues, and thermal stress in honeybees.

Materials and Methods

Pesticides

In this study, acetamiprid was applied at a residue level previously detected in unripe honey in Slovakia (52.78 μg kg^{-1}; Sabo *et al.* 2024). The commercial formulation Mospilan (water-soluble powder; 20% acetamiprid as active ingredient; producer: NIPPON SODA, Tokyo, Japan; purchased from Floraservis, Bratislava, Slovakia) was used to prepare the target residue concentration in 50% sugar syrup (1 sucrose: 1 distilled

water, w/w). Sucrose was used in the preparation of syrup and candy in these experiments and is simply referred to as sugar.

Diet types

Three varieties of Slovakian bee honey were used in this study: buckwheat, rapeseed, and acacia honey. Additionally, three distinct commercial protein feeds were obtained from local retailers. The first feed, labeled as protein feeding 1, contained beet sugar, 8% pollen, and vitamins (A, D3, E, K3, B1, B2, B6, B12, and C), along with pantothenic acid and niacin. The second feed, referred to as protein feeding 2, contained sucrose, enzymatically inverted glucose-fructose syrup, 1% protein of vegetable origin, herbal extracts (thyme, stinging nettle, and sage), and essential oils (thyme, sage, and rosemary). The third variant, designated as protein feeding 3, contained sucrose, enzymatically inverted glucose-fructose syrup, and was enriched with amino acids (lysine, methionine, threonine, tryptophan, arginine, isoleucine, and valine), along with vitamins (E, B1, B2, and B6), pantothenic acid, folic acid, biotin, niacin, and minerals. For the control groups, bees were fed sugar candy (prepared from powdered sugar and 50% sugar syrup).

Honeybees

Honeybees, *Apis mellifera carnica* Pollmann, were sourced from three colonies situated at the apiary of the Apicultural Institute in Liptovský Hrádok, Slovakia (Latitude: 49.0417° N, Longitude: 19.7374° E) for the study. From each colony (replicate), bees from two age groups were collected: nurse bees taken from the middle comb, where an unsealed brood was present (i.e., larvae that are primarily attended by nurse bees), and forager bees gathered from outside the colony, i.e., those accumulated in front of the closed beehive entrance. This collection procedure across replicates of each treatment ensured an equal distribution of any residual age variation between the experimental groups. Additionally, the short-term exposure to heat stress minimized the potential influence of any occasional age-related differences on treatment comparisons within each age group.

The experimental setup

Two experiments were conducted—one with nurse bees and one with foragers—using the same experimental setup. Each experiment included two control groups and six dietary treatment groups. In the control groups, bees were fed sugar candy either without acetamiprid (hereafter referred to as the negative control) or with acetamiprid-based insecticide exposure (the acetamiprid-treated control). In all six dietary treatment groups—which consisted of three honey-based candy formulations (prepared at a 5:3 honey-to-powdered-sugar ratio by weight) and three protein-feeding formulations—bees were exposed to acetamiprid.

After the bees were collected from the colonies, they were kept at room temperature (approximately 24°C) for 1.5 h without access to food. Afterwards, groups of 30 individuals were confined in cages for experimental purposes. Each control and treatment group consisted of three cages, totaling 90 bees (30 bees per cage). Accordingly, three replicates were included for every control and treatment group, and the bees in each replicate originated from the same colony across all groups. A total of 24 cages were used, one for nurse bees and one for foragers.

Each cage was supplied with 3 ml of water held in a partially opened Eppendorf tube containing a small piece of cotton, 4 g of the assigned diet in a small container, and 0.5 ml of sugar syrup (50% sugar-to-water ratio by weight) containing acetamiprid at a residual concentration of 52.78 μg kg^{-1} in a separate container. This method of segregating pesticide residues from feeding ensured that all test groups were uniformly exposed to the specified quantity of pesticide residue. Thus, except for the three cages assigned to the negative control group, bees in all cages—including those in the positive control group—were exposed to acetamiprid.

Heat stress and survival

The cages were placed in an incubator at 35°C (RH >50%) for 72 h to allow enough time for food consumption as well as consuming the sugar syrup mixed with pesticide residue. Also, the survival of bee workers was checked prior to heat stress exposure to ensure that all bees were alive. This was followed by subjecting the bees to a brief period of heat stress at 45°C for 2.5 h. This temperature and duration were selected as the maximum heat stress that honeybees are likely to experience during extreme heat waves, and they fall within the tolerance capacity of honeybees (Abou-Shaara *et al.* 2017). Acute heat stress can also occur during hyperthermia treatments against Varroa mites, where colonies are exposed to approximately 42°C for 3 h (Xu *et al.* 2025), or to 43°C for 5 h under laboratory conditions when testing the interactive effects of other stressors (Gonzalez *et al.* 2022). In the present study, an acute heat stress limit comparable to these previous experiments was set by shortening the exposure time while increasing the temperature.

Following the heat stress treatment (45°C for 2.5 h), the number of dead bees was recorded. Feed consumption was measured using an electronic balance by subtracting the remaining diet from 4 g. In the present experiment, the primary objective was to evaluate how dietary treatments and pesticide residues influence bee performance under acute heat stress, rather than under benign temperature conditions. For this reason, all experimental groups—including the control groups, which served to isolate dietary effects—were intentionally subjected to the same acute heat-stress challenge to maintain full comparability across treatments. Prior to heat exposure, all bees were kept for 72 h at 35°C and exhibited normal survival without any observable impairment. Because this preliminary period confirmed that bees remained healthy at 35°C, there was no justified scientific rationale for creating an additional group exposed only to 35°C for 2.5 h, as such a group would not meaningfully contribute to interpreting treatment effects under the acute heat-stress conditions that were the focus of this study.

Statistical analysis

Statistical analysis was performed separately for each age group. Mortality (binary: 1 = dead, 0 = alive) and food consumption (g) were recorded for eight treatments: two controls, three honey types, and three protein diets. Mortality was analyzed using a binomial generalized linear model (GLM). Treatment effects were tested with likelihood ratio tests, and pairwise comparisons used Tukey-adjusted contrasts. Consumption data were checked for normality (Shapiro–Wilk) and homogeneity of variance (Levene's test). When assumptions were met, one-way ANOVA followed by Tukey's HSD was applied. The relationship between consumption and survival was evaluated using point-biserial correlation and logistic regression.

To assess age effects on mortality, a binomial GLM with a logit link was fitted, and Type II analysis of deviance tested the main effects of age, treatment, and their interaction. Estimated marginal means (EMMs) and pairwise comparisons were then calculated. For food consumption, an initial linear model was evaluated, but violations of normality and variance assumptions (Shapiro–Wilk, Levene's test) led to the use of a Gamma GLM with a log link. Type II ANOVA assessed main effects and interactions. To identify the most effective feeding treatment, mortality and consumption EMMs were combined into a composite ranking: within each age class, treatments were ranked by low mortality and high consumption, and an overall ranking was generated by integrating ranks across age groups.

Data visualization was performed using the ggplot2 package, with mortality and consumption summarized as bar plots (means ± standard errors) and pairwise groupings displayed as Compact Letter Display (CLD). Logistic regression predictions were visualized as probability curves with 95% confidence intervals. Statistical analyses were performed using R (version 4.5.1). All statistical tests were two-tailed, with significance set at $\alpha = 0.05$.

Results

Nurse honeybees

Mortality of nurse honeybees after 2.5 h of heat stress exposure differed significantly between treatments (χ^2 = 196.06; df = 7; $p < 0.0001$). Compared to the negative control, all other treatments showed a significantly higher mortality risk (all $p < 0.001$). Tukey-adjusted pairwise comparisons confirmed clear differences between several treatment groups (Fig. 1). Thus, mortality in the negative control was significantly lower than in all other treatments, with protein diets (protein feeding 1 and protein feeding 3) showing the highest mortality. Additionally, all honey diets exhibited lower mortality rates than protein diets as well as the acetamiprid-treated control. Mortality rates in the studied groups can be arranged in descending order as follows: protein feeding 3 (97.78 ± 1.56%), protein feeding 1 (94.44 ± 2.43%), sugar candy (82.22 ± 4.05%), protein feeding 2 (80.00 ± 4.24%), rapeseed honey (72.22 ± 4.75%), buckwheat honey (72.22 ± 4.75%), acacia honey (70.00 ± 4.86%), and the negative control (17.77 ± 4.05%).

Food consumption also varied significantly between treatments ($p < 0.05$). Tukey's test revealed distinct differences between diet types (Fig. 2). Based on food consumption amounts, treatments were categorized into five groups: I) protein feeding 3 (lowest consumption, mean = 1.17 ± 0.02 g), II) protein feeding 2 (1.36 ± 0.01 g), acacia honey and the acetamiprid-treated control (1.38 ± 0.02 g and 1.44 ± 0.02 g), III) protein feeding 1 (1.48 ± 0.03 g), IV) the negative control (1.80 ± 0.004 g), and V) buckwheat honey and rapeseed honey (highest consumption, 2.47 ± 0.02 g and 2.51 ± 0.03 g). Therefore, honey-based diets (buckwheat honey and rapeseed honey) exhibited the highest consumption, while the protein-based diet (protein feeding 3) showed the lowest.

There was a significant positive correlation between survival and food consumption (Pearson's r = 0.132; $p < 0.001$). Similarly, Spearman's rank correlation confirmed this positive association (ρ = 0.165; $p < 0.001$). Logistic regression further confirmed that food consumption was a significant predictor of mortality (β = -0.541 ± 0.154; $p < 0.001$). Increased consumption was associated with a reduced probability of death (Fig. 3).

Forager honeybees

Mortality of forager honeybees differed significantly between treatments (χ^2 = 38.36; df = 7; $p < 0.001$). The buckwheat honey group had significantly lower mortality than protein feeding 3 ($p < 0.05$), while other treatments did not differ significantly (Fig. 4). Mortality rates in the studied groups can be arranged in descending order as follows: the acetamiprid-treated control (100 ± 0.00%), protein feeding 3 (95.60 ± 2.18%), protein feeding 2 (91.10 ± 3.02%), protein feeding 1 (91.10 ± 3.00%), the negative control (88.90 ± 3.33%), acacia honey (89.99 ± 3.18%), rapeseed honey (85.60 ± 3.73%), and buckwheat honey (76.70 ± 4.48%).

Food consumption differed significantly between treatments ($p < 0.05$), demonstrating a clear treatment effect. Rapeseed honey showed the highest consumption, significantly exceeding all other treatments, while the controls consumed the least (Fig. 5). Consumption ranged from 0.583 g to 1.380 g, with the following arrangement from the highest to the lowest: rapeseed honey (1.38 ± 0.01 g), acacia honey (1.09 ± 0.01 g), protein feeding 1 (0.99 ± 0.01 g), protein feeding 2 (0.97 ± 0.01 g), buckwheat honey (0.90 ± 0.01 g), protein feeding 3 (0.85 ± 0.004 g), the negative control (0.66 ± 0.01 g), and the acetamiprid-treated control (0.58 ± 0.01 g).

Consumption was significantly associated with mortality. Pearson's correlation showed a weak but significant negative relationship between consumption and mortality (r = –0.115; p = 0.002). Similarly, Spearman's rank correlation confirmed this negative association (ρ = –0.111; p = 0.0028). Logistic regression further supported these results, indicating that consumption significantly predicted mortality (p = 0.0023), such that higher consumption was associated with a reduced likelihood of death (Fig. 6).

Effect of age

Mortality differed significantly by age (χ^2 = 76.11; df = 1; $p < 0.001$), treatment (χ^2 = 162.02; df = 7; $p < 0.001$), and their interaction (χ^2 = 72.40; df = 7; $p < 0.001$). Foragers showed higher mortality than nurses for the same treatment. Among foragers, honey diets

(especially buckwheat honey) reduced mortality relative to protein and control diets. Among nurses, mortality was lowest in the negative control and the highest in protein diets. The effect of treatment depended on age. The results demonstrated treatment-specific differences in survival, with generally higher mortality in foragers than in nurses across most feeding regimes (Fig. 7).

Food consumption differed significantly by age ($F = 6121.41$; $p < 0.001$), treatment ($F = 560.63$; $p < 0.001$), and interaction ($F = 289.39$; $p < 0.001$). Nurses consumed more than foragers across all diets. Honey diets generally promoted higher consumption than protein or control diets. Across both age groups, increased food consumption was associated with a lower probability of mortality, though the strength of this relationship differed between foragers and nurses (Fig. 8). By combining mortality and food consumption rankings, the overall best diets were identified. Honey diets, particularly buckwheat honey and rapeseed honey, achieved low mortality while maintaining high consumption, making them the most beneficial overall. Protein diets led to higher mortality and moderate consumption, indicating they were less optimal under these conditions.

Discussion

The present study showed that protein-based diets produced the highest mortality rates among nurse bees, whereas honey-based diets were consistently associated with lower mortality, even when bees were exposed to pesticides. A similar pattern was observed in foragers, which exhibited higher survival in honey diet groups than those receiving protein diets. These findings suggest that the nutritional composition of bee diets can significantly influence their resilience to environmental stressors. Previous research has demonstrated that natural honey provides essential phytochemicals, antioxidants, and micronutrients that enhance detoxification capacity and stress tolerance (e.g., Bernklau and Arathi 2023). Notably, honey types differ in their phenolic composition and antioxidant activity (Martinello and Mutinelli 2021; Hajian-Tilaki *et al.* 2024). The honey types used in this study are recognized for their antioxidant properties (Akgün *et al.* 2021; Derewiaka *et al.*

2024), with buckwheat honey being particularly well known in this regard (Deng *et al.* 2018; Dżugan *et al.* 2020). In contrast, artificial protein diets may lack balanced amino acid profiles or include components that are difficult for bees to digest, potentially imposing additional physiological stress (Ricigliano *et al.* 2022).

The two control groups showed contrasting effects depending on bee age. The control group exposed to acetamiprid exhibited the highest mortality rates in both nurse and forager bees. In contrast, the pesticide-free control group showed the highest mortality rates for foragers but the lowest for nurse bees. These findings affirm the toxic properties of acetamiprid as a neonicotinoid insecticide. Previous studies have documented its negative effects on honeybees, even at sublethal doses, including decreased survival (Manzer *et al.* 2024; Shi *et al.* 2025). Despite acetamiprid's rapid metabolism and degradation within the honeybee body (Zhang *et al.* 2021) and its relatively low acute toxicity (Barroso *et al.* 2025), exposure to heat stress may impair bees' ability to tolerate this chemical stressor. Thus, the present study highlighted additional negative effects when bees were simultaneously subjected to heat stress and acetamiprid.

When considering bee age as a factor, it was evident that across all treatments, foragers experienced substantially higher mortality than nurse bees under the same conditions. Supporting this pattern, forager *Apis cerana cerana* bees have been shown to exhibit lower survival than newly emerged bees when exposed to acetamiprid (Han *et al.* 2019). For both age groups, honey-based diets resulted in higher survival than all other dietary treatments, except for the pesticide-free control among nurse bees. Beyond the effect of bee age, the significant differences observed between diet types within pesticide treatments suggest that food quality interacted with pesticide toxicity, potentially by influencing detoxification pathways (Mao *et al.* 2013).

Nurse bees consumed more food than forager bees, which is to be expected given that younger bees have higher nutritional requirements than older foragers (e.g., Rodney and Purdy 2020). Across both age groups, higher food consumption correlated with

increased survival, underscoring the importance of diet palatability and nutritional adequacy. Honey-based diets generally promoted higher consumption than protein-based or control diets, suggesting a preference for natural honey over commercially tested protein diets under the experimental conditions. Likewise, colony-level studies have shown that natural honey diets yield superior outcomes, with colonies fed honey exhibiting better overall health than those receiving artificial diets such as sugar solution, inverted sugar, or wheat starch syrup (Papežíková *et al.* 2020). Together, these findings highlight the vital role of natural honey in supporting and preserving bee health. Unlike a previous study suggesting that sublethal doses of acetamiprid can increase heat tolerance under 43°C for 5 h of exposure (Gonzalez *et al.* 2022), this study revealed a contrasting effect at 45°C for 2.5 h. These differing outcomes highlight the need for further research under varied experimental conditions. Given that short-term heat stress (40–42°C for 1.5–3 h) has been proposed as a strategy for Varroa mite management (Xu *et al.* 2025), the present results suggest that the potential impacts of such hyperthermia treatments on pesticide-exposed colonies require careful evaluation.

Conclusion

This study showed that honeybee survival under acetamiprid-based insecticide exposure and short-term heat stress (45°C for 2.5 h) was strongly shaped by both diet type and bee age. Nurse bees consistently exhibited higher survival and food intake than foragers, and honey-based diets provided the greatest resilience across both groups. In contrast, protein-based diets combined with acetamiprid substantially increased mortality, indicating limited suitability under heat stress. These results highlighted the critical role of diet quality—particularly natural honey diets—in enhancing tolerance to combined chemical and environmental stressors. The findings emphasized the importance of honey availability for honeybee colonies in mitigating the negative effects of exposure to multiple stressors. Future field studies using experimental colonies, acetamiprid-contaminated diets, controlled hyperthermia, and varied dietary treatments are needed to clarify long-term impacts on colony development, survival, and productivity.

Acknowledgments We would like to thank Jaroslav Gasper and Štefan Tutka for their kind assistance during the experiments.

Funding This research was funded by the grant LIFE24-IPC-SK-NatAdaptSK – 101202890-2 and 1131/2024/MPRVSR–930 "Comparison of toxicological burden in eusocial and solitary bee species in agriculturally exposed and urban areas of Slovakia and Germany". This work was also supported by the MPRV SR under Ministry research and development project no. 42 based on contract 720/2023/MPRVSR-930 between MPRV SR and NPPC: Options for minimizing the risks of varroosis control for the honey bee and the quality of its products.

References:

Abou-Shaara H. F., Owayss A. A., Ibrahim Y. Y., Basuny N. K. 2017. A review of impacts of temperature and relative humidity on various activities of honey bees. Insectes Sociaux 64(4): 455-463. DOI: https://doi.org/10.1007/s00040-017-0573-8

Akgün N., Çelik Ö. F., Kelebekli L. 2021. Physicochemical properties, total phenolic content, and antioxidant activity of chestnut, rhododendron, acacia and multifloral honey. Journal of Food Measurement and Characterization 15(4): 3501-3508. DOI: https://doi.org/10.1007/s11694-021-00937-3

Al-Esawy M. T. 2023. Honey bee immunity and physiology are enhanced by consuming high-fat diets. Journal of Plant Protection Research 63(2):185-195. DOI: https://doi.org/10.24425/jppr.2023.145753

Barroso G., Pereira A. M., Bueno O. C., Nocelli R. C. F., Malaspina O. 2025. Behavioral impairments in Africanized Apis mellifera exposed to lethal and sublethal doses of acetamiprid, fipronil, and thiamethoxam. Ecotoxicology 1-13. DOI: https://doi.org/10.1007/s10646-025-02904-w

Bencatova S., Knazovická V., Staron M., Gasper J., Tutka S., Rajcakova L. 2025. Detection of the insecticide substance acetamiprid in honey bee population components under realistic field conditions. Journal of Environmental Science and Health Part B 1-12. DOI: https://doi.org/10.1080/03601234.2025.2511485

Bernklau E., Arathi H. S. 2023. Seasonal patterns of beneficial phytochemical availability in honey and stored pollen from honey bee colonies in large apiaries. Journal of Economic Entomology 116(4): 1069-1077. DOI: https://doi.org/10.1093/jee/toad096

Capela N., Xu M., Simões S., Azevedo-Pereira H. M., Peters J., Sousa J. P. 2022. Exposure and risk assessment of acetamiprid in honey bee colonies under a real exposure scenario in

Eucalyptus sp. landscapes. Science of the Total Environment 840: 156485. DOI: https://doi.org/10.1016/j.scitotenv.2022.156485

Deng J., Liu R., Lu Q., Hao P., Xu A., Zhang J., Tan J. 2018. Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. Food Chemistry 252: 243-249. DOI: https://doi.org/10.1016/j.foodchem.2018.01.115

Derewiaka D., Majewska E., Pruszkowska P. 2024. The effects of bee additives on the physico-chemical and antioxidant properties of rapeseed honey. Applied Sciences 14(3): 1292. DOI: https://doi.org/10.3390/app14031292

Dżugan M., Grabek-Lejko D., Swacha S., Tomczyk M., Bednarska S., Kapusta I. 2020. Physicochemical quality parameters, antibacterial properties and cellular antioxidant activity of Polish buckwheat honey. Food Bioscience 34: 100538. DOI: https://doi.org/10.1016/j.fbio.2020.100538

Gonzalez V. H., Hranitz J. M., McGonigle M. B., Manweiler R. E., Smith D. R., Barthell J. F. 2022. Acute exposure to sublethal doses of neonicotinoid insecticides increases heat tolerance in honey bees. PLoS One 17(2): e0240950. DOI: https://doi.org/10.1371/journal.pone.0240950

Hajian-Tilaki A., Kenari R. E., Razavi R., Farahmandfar R. 2024. Phenolic profile, antioxidant properties, and pollen spectra of Iranian-originated honeys. European Food Research and Technology 250(9): 2317-2329. DOI: https://doi.org/10.1007/s00217-024-04539-3

Han W., Yang Y., Gao J., Zhao D., Ren C., Wang S., Zhao S., Zhong Y. 2019. Chronic toxicity and biochemical response of *Apis cerana cerana* (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. Ecotoxicology 28(4): 399-411. DOI: https://doi.org/10.1007/s10646-019-02030-4

Havard T., Laurent M., Chauzat, M. P. 2019. Impact of stressors on honey bees (*Apis mellifera*; Hymenoptera: Apidae): Some guidance for research emerge from a meta-analysis. Diversity 12(1): 7. DOI: https://doi.org/10.3390/d12010007

Kim H., Frunze O., Lee J. H., Kwon H. W. 2024. Enhancing honey bee health: Evaluating pollen substitute diets in field and cage experiments. Insects 15(5): 361. DOI: https://doi.org/10.3390/insects15050361

Mackei M., Huber F., Sebők C., Vörösházi J., Tráj P., Márton R. A., Horváth E., Neogrády Z., Mátis G. 2024. Unraveling the acute sublethal effects of acetamiprid on honey bee neurological redox equilibrium. Scientific Reports 14(1): 27514. DOI: https://doi.org/10.1038/s41598-024-79274-6

Manzer S., Thamm M., Hilsmann L., Krischke B., Steffan-Dewenter I., Scheiner R. 2024. The neonicotinoid acetamiprid reduces larval and adult survival in honeybees (*Apis mellifera*) and interacts with a fungicide mixture. Environmental Pollution 360: 124643. DOI: https://doi.org/10.1016/j.envpol.2024.124643

Mao W., Schuler M. A., Berenbaum M. R. 2013. Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. Proceedings of the National

Academy of Sciences 110(22): 8842-8846. DOI: https://doi.org/10.1073/pnas.1303884110

Martinello M., Mutinelli F. 2021. Antioxidant activity in bee products: A review. Antioxidants 10(1): 71. DOI: https://doi.org/10.3390/antiox10010071

Mokbel E.S.M.S. 2018. Resistance risk assessment: realized heritability, cross resistance and resistance stability of acetamiprid in the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae). Journal of Plant Protection Research 58(4):328-334. DOI: https://doi.org/10.24425/jppr.2018.124641

Neumann P., Straub L. 2023. Beekeeping under climate change. Journal of Apicultural Research 62(5): 963-968. DOI: https://doi.org/10.1080/00218839.2023.2247115

Ozols N., Brusbārdis V., Filipovičs M., Gailis J., Radenkovs V., Rubene B., Zagorska V. 2024. Pesticide contamination of honey-bee-collected pollen in the context of the landscape composition in Latvia. Toxics 12(12): 862. DOI: https://doi.org/10.3390/toxics12120862

Papežíková I., Palíková M., Syrová E., Zachová A., Somerlíková K., Kováčová V., Pecková L. 2020. Effect of feeding honey bee (*Apis mellifera* Hymenoptera: Apidae) colonies with honey, sugar solution, inverted sugar, and wheat starch syrup on nosematosis prevalence and intensity. Journal of Economic Entomology 113(1): 26-33. DOI: https://doi.org/10.1093/jee/toz251

Piechowicz B., Łaś S., Łaś M., Łuc K., Zaręba L., Kobylarz D., Kuliga A., Grodzicki P., Zienkiewicz A., Romerowicz-Misielak M. 2025. The effects of deltamethrin (a synthetic pyrethroid insecticide), an anionic surfactant alone, and a co-formulated mixture of these substances on the honeybee (*Apis mellifera*) temperature preference, CO2 emission, and expression of detoxification-related genes. Journal of Plant Protection Research 65(2):187-199. DOI: https://doi.org/10.24425/jppr.2025.153285

Ricigliano V. A., Williams S. T., Oliver, R. 2022. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. BMC Veterinary Research 18(1): 52. DOI: https://doi.org/10.1186/s12917-022-03151-5

Rodney S., Purdy J. 2020. Dietary requirements of individual nectar foragers, and colony-level pollen and nectar consumption: a review to support pesticide exposure assessment for honey bees. Apidologie 51(2): 163-179. DOI: https://doi.org/10.1007/s13592-019-00694-9

Rowe E. B., Prathibha P., Marting P. R., Smith M. L. 2025. Honey bee beards: internal and external factors driving mass thermoregulatory evacuation. Insectes Sociaux 1-11. DOI: https://doi.org/10.1007/s00040-025-01046-w

Sabo R., Staroň M., Sabová L., Majchrák T., Bischoff G., Pistorius J., Janke M., Alkassab A. T. 2024. Honey bees for pesticide monitoring in the landscape: Which bee matrices should be used?. Chemosphere 364: 143130. DOI: https://doi.org/10.1016/j.chemosphere.2024.143130

Shi J., Wang X., Luo Y. 2025. Honey bees prefer moderate sublethal concentrations of acetamiprid and experience increased mortality. Pesticide Biochemistry and Physiology 208: 106320. DOI: https://doi.org/10.1016/j.pestbp.2025.106360

St. Clair A. L., Zhang G., Dolezal A. G., O'Neal M. E., & Toth A. L. 2020. Diversified farming in a monoculture landscape: effects on honey bee health and wild bee communities. Environmental Entomology 49(3): 753-764. DOI: https://doi.org/10.1093/ee/nvaa031

Stanley J., Chandrasekaran S., Preetha G., Kuttalam S., Jasmine R.S. 2016. Selective toxicity of diafenthiuron to non-target organisms: honey bees, coccinellids, chelonus, earthworms, silkworms and fish. Journal of Plant Protection Research 56(1):1-5. DOI: https://doi.org/10.1515/jppr-2016-0001

Staron M., Rajčáková Ľ., Planý M., Abou-Shaara H. 2026. Interactive effects of diet, tebuconazole, and elevated temperature on survival and body water loss in honey bee workers. Environmental Toxicology and Pharmacology, 121:104913. DOI: https://doi.org/10.1016/j.etap.2025.104913

Swiatly-Blaszkiewicz A., Klupczynska-Gabryszak A., Matuszewska-Mach E., Matysiak J., Attard E., Kowalczyk D., Adamkiewicz A.; Kupcewicz B., Matysiak J. 2025. Pesticides in honeybee products—determination of pesticides in bee pollen, propolis, and royal jelly from Polish apiary. Molecules 30(2): 275. DOI: https://doi.org/10.3390/molecules30020275

Tsuruda J. M., Chakrabarti P., Sagili R. R. 2021. Honey bee nutrition. Veterinary Clinics of North America: Food Animal Practice 37(3): 505-519. DOI: https://doi.org/10.1016/j.cvfa.2021.06.006

Xu X., Zhou S., Huang J., Geng F., Zhu X., Abou-Shaara H. F. 2025. Influence of hyperthermia treatment on varroa infestation, viral infections, and honey bee health in beehives. Insects 16(2): 168. DOI: https://doi.org/10.3390/insects16020168

Zhang Y., Chen D., Du M., Ma L., Li P., Qin R., Yang J., Yin Z., Wu X., Xu H. 2021. Insights into the degradation and toxicity difference mechanism of neonicotinoid pesticides in honeybees by mass spectrometry imaging. Science of the Total Environment 774: 145170. DOI: https://doi.org/10.1016/j.scitotenv.2021.145170

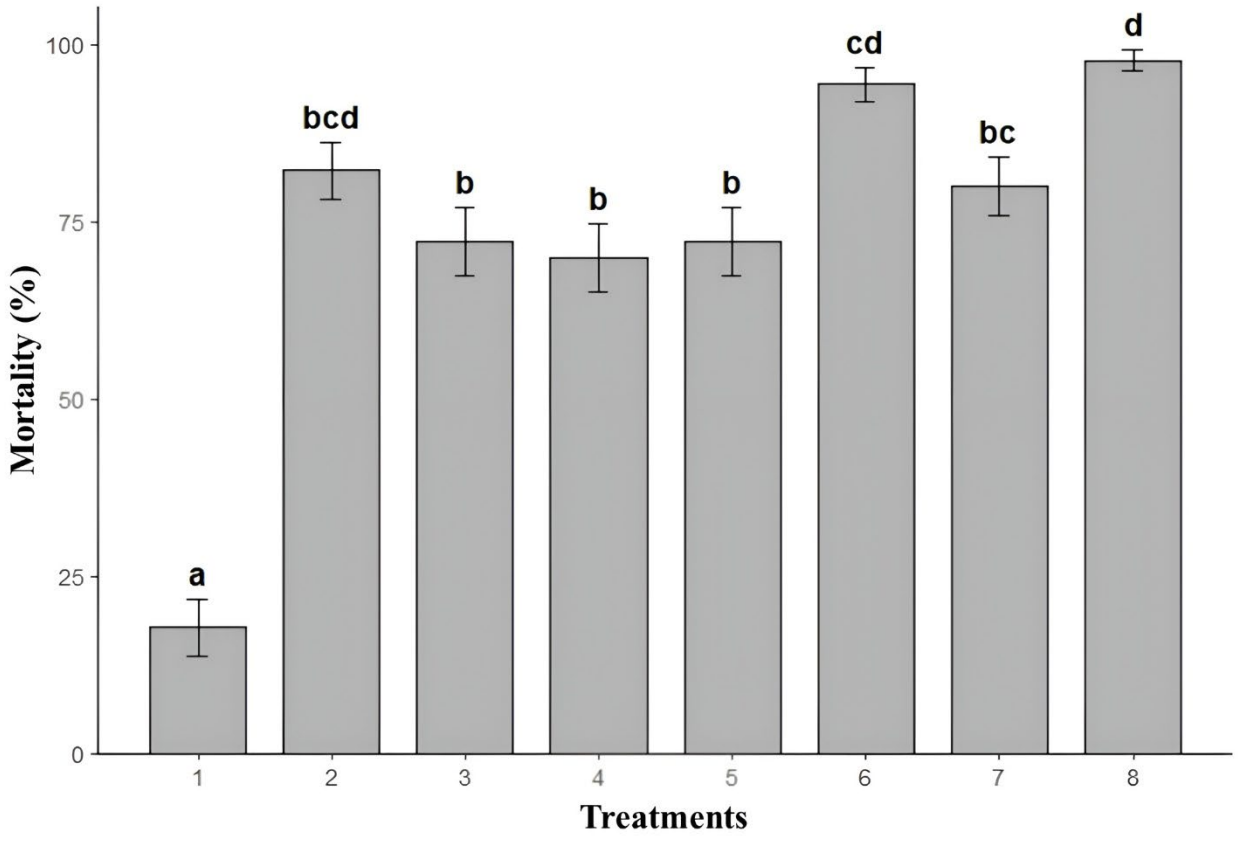

Fig. 1. The mortality rates (means ± SE) of nurse honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. Error bars represent the standard errors of the means, and those marked with different letters indicate significant differences between treatments according to Tukey's test.

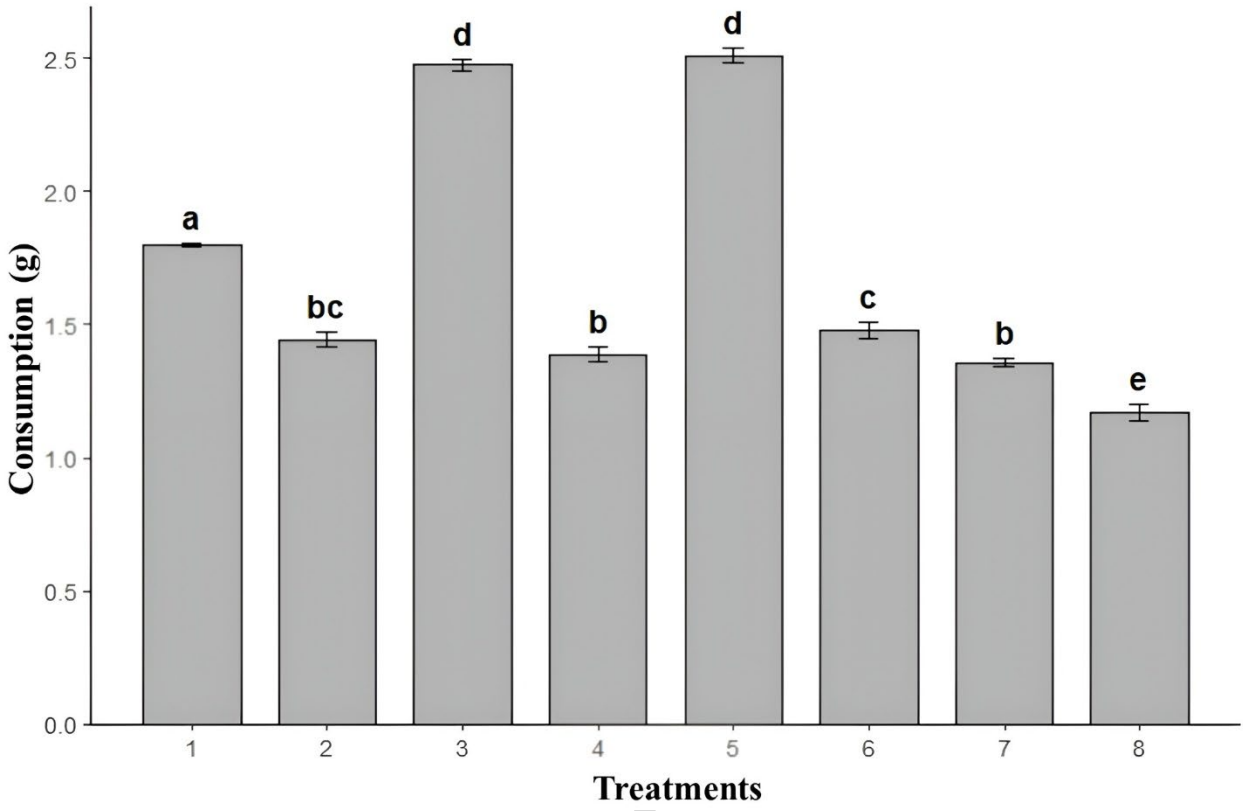

Fig. 2. Food consumption (mean ± SE) of nurse honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. Error bars represent the standard errors of the means, and those marked with different letters indicate significant differences between treatments according to Tukey's test.

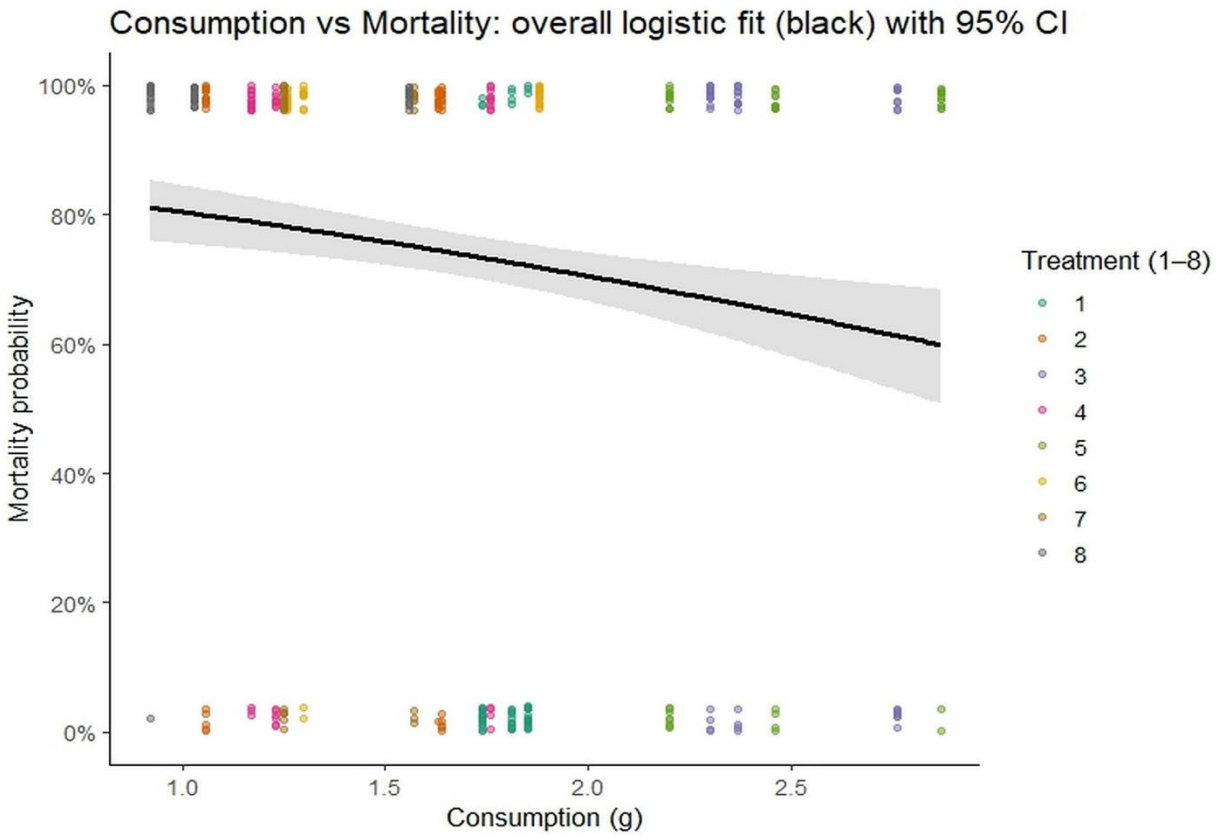

Fig. 3. The relationship between food consumption and mortality of nurse honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. The y-axis denotes mortality (1 = dead, 0 = alive). A logistic regression fit (GLM) is depicted by the black line encompassing all treatments, with the shaded area indicating the 95% confidence interval (CI). Generally, higher consumption correlated with increased survival, although patterns may vary across treatments.

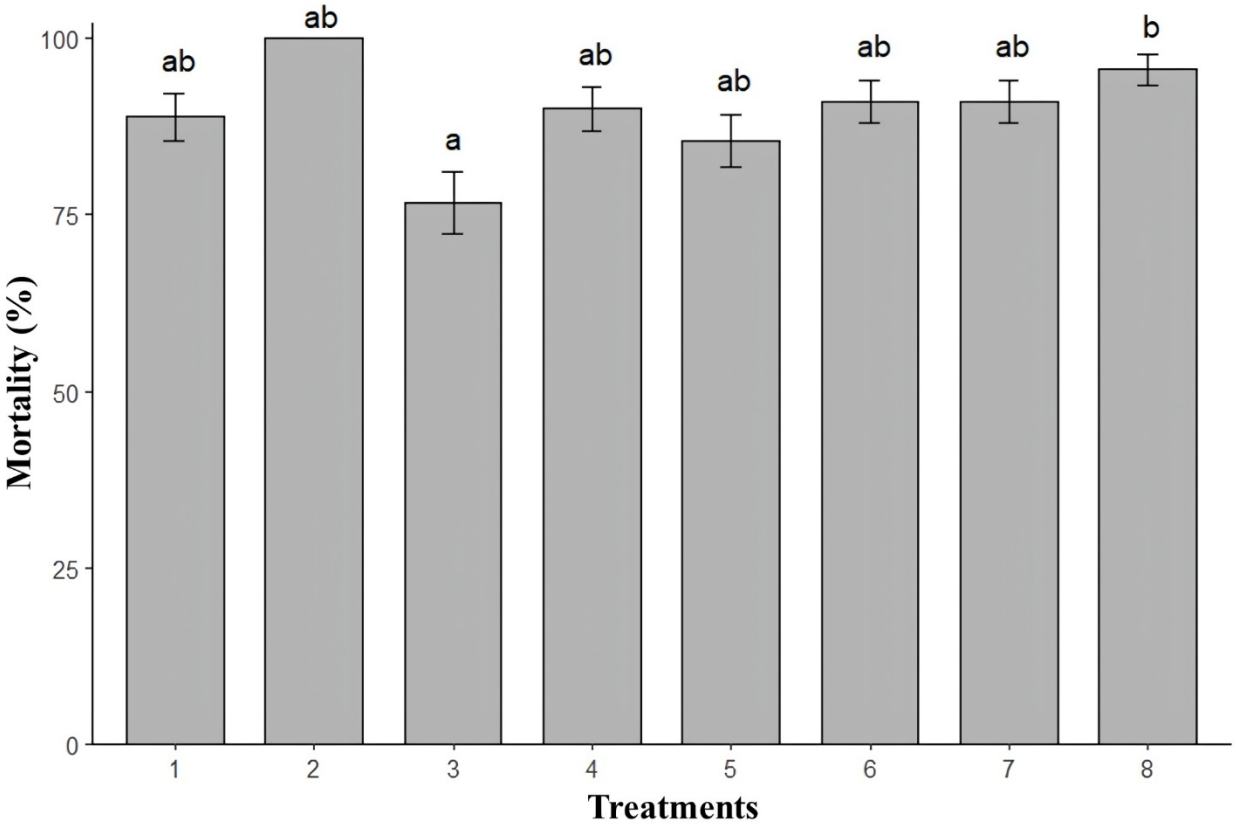

Fig. 4. The mortality rates (means ± SE) of forager honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. Error bars represent the standard errors of the means, and those marked with different letters indicate significant differences between treatments according to Tukey's test.

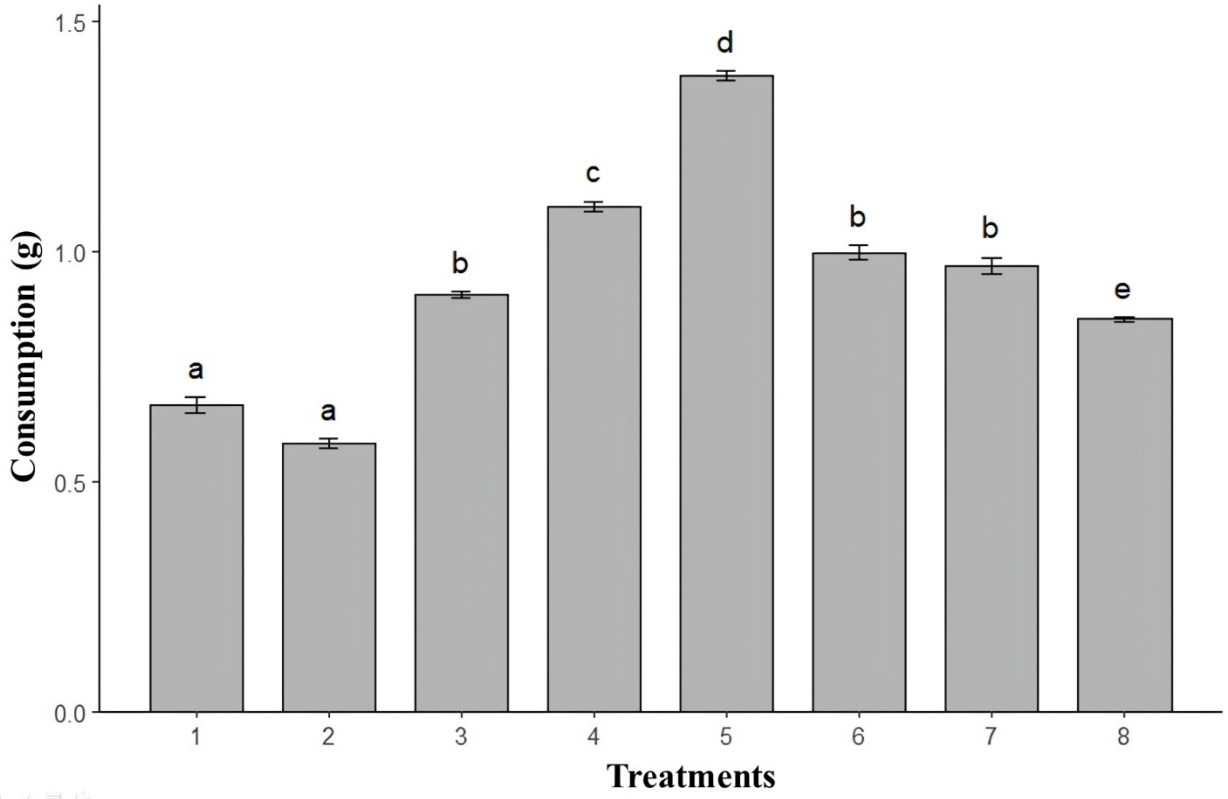

Fig. 5. Food consumption (mean ± SE) of forager honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. Error bars represent the standard errors of the means, and those marked with different letters indicate significant differences between treatments according to Tukey's test.

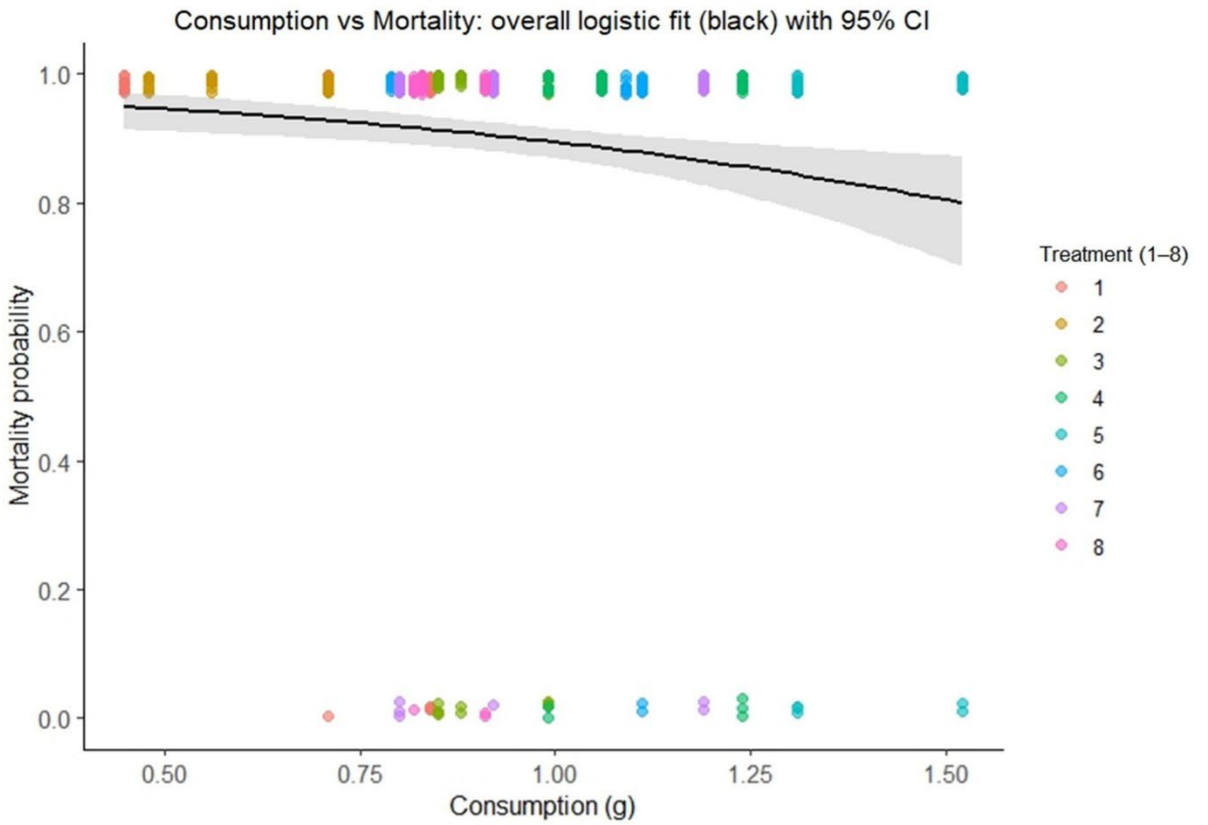

Fig. 6. The relationship between food consumption and mortality of forager honeybees across eight treatments: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. The y-axis denotes mortality (1 = dead, 0 = alive). A logistic regression fit (GLM) is depicted by the black line encompassing all treatments, with the shaded area indicating the 95% confidence interval.

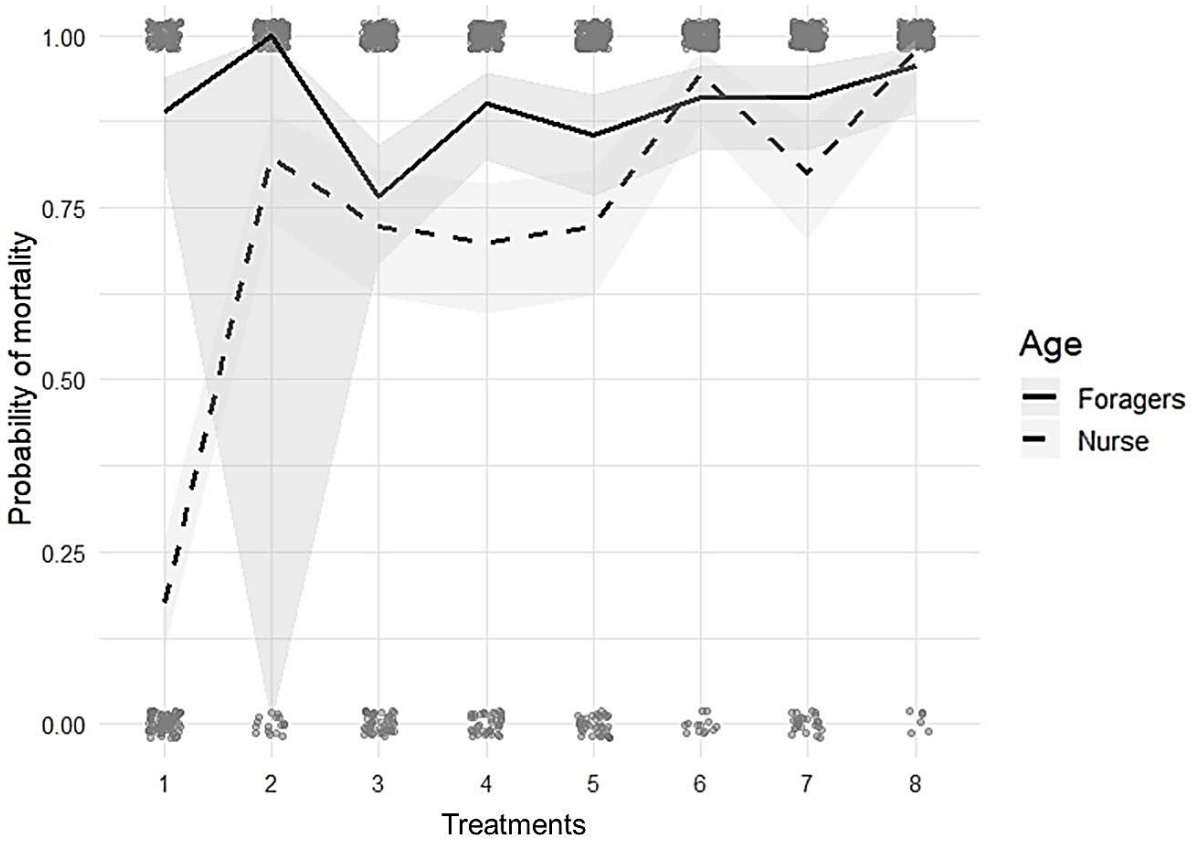

Fig. 7. Logistic regression analysis of the effect of age on mortality across eight feeding treatments in honeybees. Mortality was modeled as a function of age group (foragers vs. nurses) and feeding treatment: 1) negative control, 2) acetamiprid-treated control, and six dietary groups exposed to acetamiprid, 3) buckwheat honey, 4) acacia honey, 5) rapeseed honey, 6) protein feeding 1, 7) protein feeding 2, and 8) protein feeding 3. Gray points represent individual mortality outcomes within each treatment. Black lines show predicted mortality probabilities for foragers (solid) and nurses (dashed), with shaded gray regions indicating 95% confidence intervals.

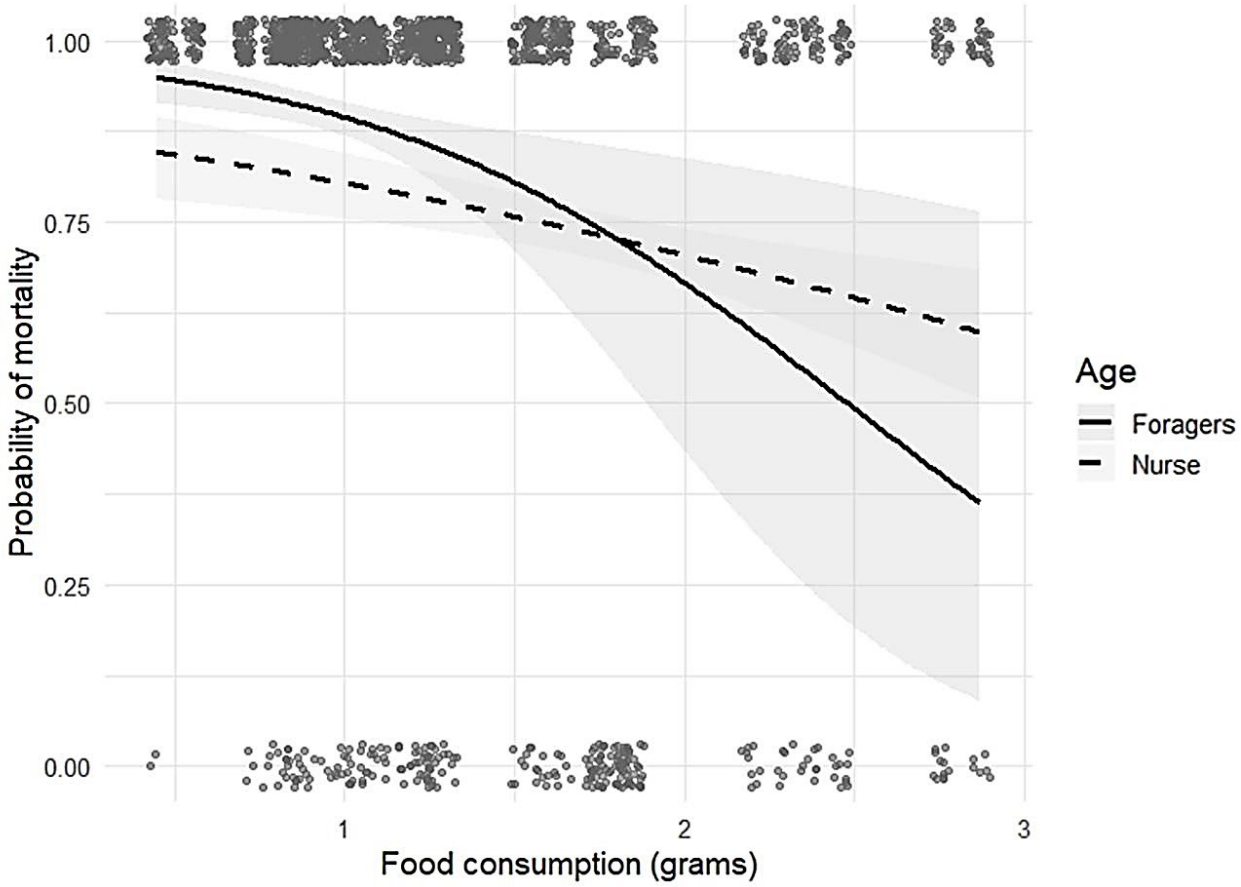

Fig. 8. Logistic regression analysis of the relationship between food consumption and mortality in honeybees. Mortality was modeled as a function of food consumption in two age groups: foragers (solid line) and nurses (dashed line). Points represent individual observations. Black lines indicate the fitted logistic regression curves, with shaded gray areas showing the 95% confidence intervals.