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Research Article

Effects of different concentrations of mineral supplements on certain biological, behavioral and biochemical traits of *Apis mellifera* L. (Hymenoptera: Apidae)

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Abstract. The nutritional status of honeybee (*Apis mellifera* L.) colonies plays a crucial role in their development, survival, and productivity. This study aimed to evaluate the effect of different concentrations of chelated mineral supplements including iron, zinc, copper, manganese, selenium, chromium, cobalt, magnesium, calcium, and phosphorus on the biological and biochemical characteristics of honeybee colonies. A total of 35 colonies were fed sugar syrup containing different levels (0, 0.5, 1, 1.5, and 2 g/L) of chelated minerals over 60 days, and then their population growth, pollen collection, overwintering success, honey production, and enzymatic activities were assessed. The results showed that colonies fed with 1.5 g/L of chelated minerals exhibited the highest population increase, brood area, and pollen collection. Overwintering success was also highest in colonies receiving 1.5 g/L of minerals, as indicated by the lowest hive weight loss and highest colony survival rates. Biochemical analyses revealed increased antioxidant enzyme activity and energy reserves in bees fed with 1.5–2 g/L of minerals. However, excessive supplementation (2 g/L) led to a decline in colony performance, where the mean number of combs, the population of pre-adult stages (cm^2), and collected pollens (cm^2) did not show significant differences from the control. These findings suggest that moderate mineral supplementation (1.5 g/L) enhances honeybee colony performance and productivity.

Keywords: Antioxidant enzymes, Honeybee, Overwintering, Pollen, Population

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Introduction

Honeybees (*Apis mellifera* L.) are among the most economically important social insects, playing a crucial role in ecosystems and human food security through their pollination activities (Lawal & Banjo, 2010). The honeybee's performance depends on the quality and quantity of nutrients, including carbohydrates, proteins, lipids, minerals, vitamins, and water, which are essential for their survival, activity, growth, and development. Honeybees primarily obtain their nutrition from collected nectar and pollen of flowers. Nectar provides the main source of carbohydrates, while pollen is rich in essential nutrients such as proteins, fats,

vitamins, sterols, and other micronutrients (Al-Kahtani *et al*., 2020). Micronutrients are particularly important for physiological processes, as they serve as precursors for molting hormones and structural components of cell membranes (Chakrabarti *et al*., 2020). Consequently, the energy required for tissue growth, body functions, and reproduction is derived from the minerals and nutrients found in plant-based sources and water (Filipiak *et al*., 2017; Ahmad *et al*., 2020). It should be considered that honeybees do not rely solely on pollen to obtain minerals; these nutrients are also provided through other sources, including water, nectar, and honeydew brought into the hive. Studies have shown that bees raised during periods of pollen scarcity maintained similar mineral levels to those raised under favorable flowering conditions. Indeed, they were able to regulate these nutrients from alternative sources. Although pollen has been known to contain 2.9%–8.3% mineral content, floral resources typically supply only small amounts of micronutrients. The essential minerals for honeybees include calcium, phosphorus, potassium, sodium, chlorine, sulfur, and magnesium (Somerville, 2005).

The nutritional requirements of honeybees vary with age, as larvae have higher nutritional needs than adults (Ghramh *et al*., 2020). To address micronutrient deficiencies, honeybees selectively forage on substances found in soil and water, thereby acquiring essential minerals and salts that are not typically available in floral resources (Hakami *et al*., 2020). Chakrabarti *et al*. (2020) demonstrated that when faced with a pollen shortage, honeybees utilized available minerals such as 24-methylenecholesterol to support their physiological functions and muscle activity.

Minerals are essential in small quantities to sustain the metabolic functions of honeybees. However, even a slight deficiency in these trace minerals can result in metabolic disturbance and negative effects on colony performance. It should be noted that minerals serve as critical structural components and enzymatic cofactors (Haraguchi, 2004). So, optimal mineral levels are necessary for tissue health and efficient excretory processes. However, elevated concentrations can cause significant complications and lead to toxicity. For example, a study conducted by Robinson (2003) found that excessive potassium and phosphorus levels, along with a calcium deficiency, were linked to paralysis in adult bees. Furthermore, high mineral concentrations caused diarrhea in adult bees. This highlights the importance of regulating micronutrients within an optimal range, as imbalances can lead to physiological disturbances.

Honeybees have co-evolved with flowering plants, developing specialized mechanisms to balance their intake of various macronutrients through a combination of pre- and post-ingestive processes. However, the regulation of micronutrient intake through these mechanisms remains relatively unexplored. The majority of research on honeybee nutrition has primarily focused on macronutrients (Olejnicek, 2004). Since all living organisms require a balanced diet that includes both macronutrients (proteins and carbohydrates) and micronutrients (minerals and salts) to support their daily activities, it is evident that regulating micronutrient levels within the body is crucial for optimal body function (Adgaba *et al*., 2020).

It is important to highlight that, in addition to the quantity of macronutrients and micronutrients, dietary diversity plays a crucial role in ensuring the optimal growth and development of honeybees. Based on Alaux *et al*. (2010), honeybees fed on a polyfloral diet displayed stronger immune responses compared to those on a monofloral diet. This suggests that a varied diet not only supports nutritional balance but also enhances the ability of the colony to tolerate environmental stressors and diseases.

The essential minerals, such as zinc, are particularly important due to their involvement in critical metabolic pathways. Zinc ions can penetrate the peritrophic membrane and midgut epithelial cells (Cousins *et al*., 2006). Zinc is a key component of over 200 metalloenzymes and other metabolic compounds, playing a vital role in biochemical processes, including maintaining cell membrane integrity, respiration, cell division, and other essential functions (Eisler, 1993). Studies indicate that in various insects, this trace element is transported from the gut to the hemolymph, where it participates in the transfer of vitellogenin to eggs (Eyer *et al*., 2016). Additionally, zinc improves the immune system (Zhang *et al*., 2015; Maret, 2017).

Calcium is another essential element for honeybee growth and development (Hebert & Shimanuki, 1997; Zhang & Xu, 2015). Under natural conditions, nurse bees feed larvae with royal jelly produced by the hypopharyngeal and mandibular glands, which provides essential elements, including calcium (Peters *et al.*, 2010). In the form of Ca^{2+} ions in hemolymph and tissues, calcium plays a critical role in cellular signaling and, by binding with calcium-binding proteins, regulates growth and stress responses (Yu *et al.*, 2012).

The role of manganese in honeybee bodies has been studied at various stages of ontogeny. Analysis of the chemical composition of muscle fibers in adults and different larval instars revealed that the concentration of manganese was notably high in these tissues. Research indicated that the manganese found in various tissues at different developmental stages of honeybees originated from their diet. (Kovalskyi *et al.*, 2020). Manganese is present in primary honeybee products such as royal jelly, honey, beeswax, and bee pollen. The manganese content varies significantly across different parts of the body, with the highest concentrations found in the cuticle. Additionally, chitinous structures that form the internal skeleton also contain manganese. On average, the manganese content is approximately 1.78 mg per 100 grams (Kuterbach *et al.*, 1986).

The ability to detect selenium seems to be crucial for pollinating insects. If honeybees cannot detect and avoid selenium compounds in pollen and nectar, it may lead to a reduction in colony performance. Inorganic forms of selenium are known to induce oxidative stress (Spallholz, 1997) and DNA damage (Combs Jr & Gray, 1998). Although selenium has been known as an essential micronutrient, excess amounts led to abnormality and toxicity (Jensen & Trumble, 2003).

Iron, as another essential element, has been known to influence honeybee performance. Trophocytes in adult worker honeybees contain substantial amounts of iron, which is distributed throughout the cell cytoplasm (Kuterbach *et al.*, 1986). Iron accumulates in the abdominal region and contributes to the orientation of honeybees (Wang *et al.*, 2013).

Numerous studies have reported the benefits of using mineral supplements including calcium for foraging capacity and antioxidant activities (Nation & Robinson, 1971; Zhang & Xu, 2015); zinc (at least 60 mg per kg of diet) for high-quality royal jelly production (Zhang *et al.*, 2015), and also iron for flight activity (Crabtree & Newsome, 1972). However, there is limited information regarding the combined effects and efficacy of these supplements. Since the quantity of pollen varies among different plant species and, also, they are limited or unavailable during certain seasons, incorporating minerals into artificial diets for honeybees seems to be necessary. This study, therefore, aimed to investigate the combined effects of various mineral concentrations on the biological, behavioral, and physiological characteristics of honeybee colonies.

Materials and methods

This study was conducted in the village of Darreh Jir (37.1698° N, 49.9939° E), located 5 kilometers southeast of Lahijan city, North of Iran, during the years 2022–2023. To minimize genetic differences among experimental treatments and standardize the experimental conditions, 35 honeybee colonies were used. All colonies had sister queens of the same age (June 2022), which were similar in population size and food reserves. The colonies were fed for 60 days (two periods from 2022-10-07 to 2022-12-06 (autumn) and 2023-03-06 to 2023-05-05 (spring), every other day, each period consisting of 30 days, for a total of 60 days) with syrup and chelated mineral supplements (Bonza Bee® (Iran)). It included iron (300 ppm), zinc (150 ppm), copper (150 ppm), manganese (200 ppm), selenium (0.3 ppm), chromium (0.1 ppm), cobalt (0.1 ppm), magnesium (3000 ppm), calcium (6000 ppm), and phosphorus (5000 ppm).

Five different concentrations of this mineral combination (five treatments) were prepared: (1) sugar syrup containing 0.5 g/L sugar with 0.5 g/L mineral levels, (2) sugar syrup containing 0.5 g/L sugar with 1 g/L minerals levels, (3) sugar syrup containing 0.5 g/L sugar with 1.5 g/L minerals levels, (4) sugar syrup

containing 0.5 g/L sugar with 2 g/L mineral levels, and (5) a control group (sugar syrup containing 0.5 g/L sugar without minerals) (Supplementary table). Each treatment was provided to seven colonies (replications). The amount of syrup administered was approximately half a liter per colony, provided every other day.

Sampling of young worker bees was randomly conducted at 15-day intervals after the first feeding and continued for 15 days after the last feeding (four sampling periods in total). Additionally, the population size, queen egg-laying activity, and occupied surface of immature stages were monitored. Given that the experimental conditions were identical for all colonies, the study was conducted using a completely randomized design with five treatments and seven replications.

Estimation of biological and behavioral indices

Estimation of queen egg-laying rate, occupied surface by immature bees, and pollen stores in the colony (cm^2)

To measure the queen's egg-laying rate and occupied surface by immature bees (egg, larva, and sealed brood), a grid frame divided into 5 × 5 cm squares was used. This grid was placed over the comb to quantify the area occupied by eggs, larvae, and pupae. For determining the amount of pollen collected by the bees, an empty frame of the same size as the other frames in the hive was used. This frame was divided into 5 × 5 cm square grids using wires, allowing for the measurement of the surface area covered by stored pollen (Sharma & Kumar, 2010).

Estimation of adult honeybee population

To estimate the adult population in the hive, the method of counting the number of combs fully covered with worker bees was employed. Sampling was conducted in the early morning (7-8:30 am). A comb was considered complete if both sides were entirely covered by bees. If the coverage was incomplete, a fraction of a full comb was recorded as an estimate of the population (Chabert *et al*., 2021).

Estimation of honey production in the colony

The honey production of the colonies was determined by weighing the honey-filled combs of each hive after extraction. First, the wax layer on honey-filled combs was scraped with a special scaler. Then, the combs were placed inside an extractor. Therefore, the honey was separated from the wax, and the pure honey was weighed for each hive.

Comparison of overwintered colonies

To examine the overwintering success of the colonies, two indicators -including hive weight and population- were estimated. As such, colonies were weighed at the end of experimental feeding in early winter (First of December: initial weight) and again at the beginning of spring activity on March 5 (final weight). The amount of food consumed by the colonies was calculated by subtracting the final weight from the initial weight (Delaplane *et al*., 2013).

To evaluate population changes during overwintering, the population was measured at the beginning of winter (first of December) and after overwintering on March 5, based on the number of combs covered with adult bees or fractions thereof (Burgett & Burikam, 1985). The population was then analyzed across the two periods (Döke *et al*., 2018).

Estimation of biochemical indicators

Sample preparation for biochemical assays

For biochemical analyses, five last-instar larvae were randomly selected from the hive in spring and then homogenized. A manual glass homogenizer was used for this process, with a 1:1 (weight/volume) ratio in phosphate buffer solution (pH 7) to ensure optimal enzyme stability. The homogenized mixture was then

centrifuged at 13,000 × g for 15 minutes at 4°C to separate insoluble components and obtain a clear supernatant. The resulting supernatant was used as the enzyme source for subsequent analyses.

Evaluation of antioxidant activities

To assess antioxidant activities, key antioxidant enzymes, including catalase, superoxide dismutase, peroxidase, and glucose-6-phosphate dehydrogenase, were analyzed. The activity of each enzyme was measured using standard biochemical methods and commercial assay kits specifically designed for each enzyme. Optical absorbance was recorded at different time points, and variations in absorbance were examined as an indicator of enzymatic activity. The obtained results were compared with standard values to determine the precise antioxidant activity levels in the samples.

Catalase (CAT)

Catalase activity was measured using the method described by Wang *et al.* (2001). In this assay, 500 µL of 1% hydrogen peroxide was added to 50 µL of the sample (both treated and control groups). The reaction mixture was incubated at 28°C for 10 minutes, after which absorbance was recorded at 240 nm.

Superoxide dismutase (SOD)

Superoxide dismutase activity was determined following the method of McCord & Fridovich (1969). A 500 µL solution of superoxide dismutase in phosphate buffer (pH 7) was prepared and then mixed with 100 µL of xanthine oxidase solution and 10 mg of fetal bovine serum in 2 mL of PBS. This mixture was added to 50 µL of the sample. The resulting reaction mixture was incubated in the dark at 28°C for 20 minutes, and absorbance was measured at 560 nm.

Peroxidase (POX)

Peroxidase activity was measured using the method of Addy & Goodman (1972). In this assay, 50 µL of the sample was added to 250 µL of buffered pyrogallol and 250 µL of 1% H_2O_2. Absorbance was recorded at 430 nm every 30 seconds for up to 2 minutes.

Glucose-6-phosphate dehydrogenase (GPDH)

Glucose-6-phosphate dehydrogenase activity was assessed following the method of Balinsky & Bernstein (1963). To initiate the reaction, 100 µL of Tris-HCl, 50 µL of NADP, and 30 µL of $MgCl_2$ were mixed. Subsequently, 100 µL of GPDH was added to the mixture, and absorbance was continuously measured at 340 nm using an ELISA reader.

Measurement of detoxification enzymes

General esterase (EST)

General esterase activity was measured following the method of Han *et al.* (1998). Initially, the midgut of larvae was homogenized in 1000 µL of 0.1 mM phosphate buffer containing 0.01% Triton in a microtube. The homogenate was centrifuged at 4°C for 10 minutes. Then, 10 µL of each of the two substrates, alpha-naphthyl acetate and beta-naphthyl acetate (10 mM), was separately mixed with 5 µL of RR-Salt blue, 40 µL of 20 mM phosphate buffer, and 5 µL of the enzyme sample. After incubation at 25°C for 5 minutes, absorbance was recorded at 450 nm.

Glutathione S-transferase (GST)

Glutathione S-transferase activity was determined based on the method of Habing *et al.* (1974) using two substrates: 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichlorotoluene (DCNB). A reaction mixture containing 50 µL of the sample, 135 µL of phosphate buffer (pH 7), 50 µL of reagent, and 100 µL of reduced glutathione was prepared. After incubation at 25°C for 5 minutes, absorbance was measured at 340 nm.

Measurement of energy reserves in young worker bees

Total protein

The total protein content was determined using a Biochem kit (Tehran, Iran) based on the Lowry *et al.* (1951) method. In this assay, 50 µL of reagent was mixed with 10 µL of the standard (bovine serum protein), and 50 µL of reagent was separately mixed with 10 µL of the sample. The mixtures were incubated for 15 minutes, and absorbance was measured at 545 nm. The obtained absorbance values were divided by the standard absorbance and multiplied by 50 (the standard concentration) according to the kit instructions. The final protein concentration was expressed as mg protein per mL.

Triglyceride

Triglyceride levels were measured using the method of Fossati & Prencipe (1982) with a Biochem kit (Tehran, Iran). For each sample, 50 µL of reagent and 30 µL of the supernatant from the centrifuged sample were added to an ELISA plate. A control sample consisting of 50 µL of reagent and 30 µL of distilled water was also prepared. The mixtures were incubated at 25°C for 15 minutes, and absorbance was recorded at 545 nm.

Glucose

Glucose content was determined following the method of Siegert (1987) using a Biochem kit (Tehran, Iran). This enzymatic method is based on the reaction of glucose oxidase with glucose, resulting in the formation of gluconic acid and hydrogen peroxide. The hydrogen peroxide then reacts with peroxidase in the presence of 4-aminophenazone and phenol, forming a colored complex. The absorbance of the resulting color was measured at 545 nm using an ELISA reader, and glucose concentration was calculated by comparing the sample absorbance to the standard solution.

Data analysis

The experiments were conducted using a completely randomized design. Statistical analyses were carried out using SAS software version 9 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was employed to determine the statistically significant differences among treatment means. Where significant differences were detected ($p<0.05$), mean comparisons were conducted using Tukey's Honest Significant Difference (HSD) test to identify specific group differences.

Results

Effect of autumn feeding with chelated minerals on the population (number of combs) of honeybee adults in the hive

Based on the results, there was a significant difference ($p < 0.05$) in the number of combs containing adult bees among the different concentrations of the tested mineral supplements. Throughout all four observations in the autumn season, the highest number of combs was recorded in hives that received a concentration of 1.5 g/L of minerals. Additionally, when comparing the mean effect of different mineral concentrations over the entire autumn season, significant differences were observed among treatments. The highest number of combs was found in hives fed with 1.5 g/L of minerals, averaging 5.53 ± 0.74 combs of bees ($F = 3.39$, $p = 0.0111$, $df = 4, 139$) (Table 1).

Effect of autumn feeding with chelated minerals on eggs, larvae, and pupae based on area (cm²) in the hive

The results indicated that the different concentrations of experimental diets had a significant effect ($p < 0.05$) on the area occupied by immature stages (eggs, larvae, and pupae) within the hive during all observations in the autumn. The lowest occupied area by immature individuals was recorded in the control (162.50 ± 4.60) (without mineral supplementation).

Although an increase in mineral concentration generally led to an increase in the area occupied by immature stages, a significant decrease was observed at the 2 g/L concentration. Furthermore, the overall comparison of the mean occupied area by immature stages showed a significant difference among treatments. The control treatment exhibited the lowest occupied area, with 5348.20 ± 110.17 cm² of eggs, larvae, and pupae ($F = 3.71$, $p = 0.0068$, $df = 4, 139$) (Table 2).

Effect of autumn feeding with chelated minerals on the amount of collected pollen (cm²) in the hive

Different concentrations of the experimental diet had a significant effect ($p < 0.05$) on the amount of pollen collected in the colonies. The highest pollen accumulation was observed in hives fed with 1.5 g/L of chelated minerals. As the concentration of minerals provided to the hives increased, the amount of collected pollen also increased. However, a significant decline was observed at the 2 g/L concentration, where the pollen levels were not significantly different from the control group.

A significant difference was also found in the mean pollen collection among treatments throughout the autumn season, with the highest collected pollen recorded at 264.29 ± 9.21 cm² in hives supplemented with 1.5 g/L of minerals ($F = 72.27$, $p < 0.0001$, $df = 4, 139$) (Table 3).

Effect of autumn feeding with chelated minerals on the population (number of combs) of honeybee adults in the hive after wintering

The analysis of the data revealed a significant difference ($p < 0.05$) in the number of adult bee combs among hives fed with different concentrations of chelated minerals during wintering. The highest population size (5.64 ± 0.21 combs) was recorded at 1.5 g/L of chelated minerals. Conversely, the lowest number of combs was observed in the control group and also in hives fed with 2 g/L of minerals, indicating a decline in population size at this concentration after wintering. A significant difference was also observed in the mean number of combs among treatments ($F = 4.14$, $p = 0.0087$, $df = 4, 34$) (Fig. 1).

Effect of autumn feeding with chelated minerals on honey consumption (hive weight loss) during overwintering

The results of the present study showed that different concentrations of mineral supplementation led to significant differences in hive weight before (initial weight) and after overwintering (final weight) ($p < 0.05$). Hive weight loss reflecting the amount of honey consumed during winter varied across different dietary concentrations and ranged from 1.45 ± 0.17 kg (at a concentration of 1.5 g/L chelated mineral) to 3.25 ± 0.22 kg (in the control group) ($F = 17.21$, $P < 0.0001$, $df = 4, 34$) (Fig. 2).

Effect of spring feeding with chelated minerals on the population (number of combs) of honeybee adults in the hive

The findings of this study indicate that different concentrations significantly influenced the population size of adult honeybees in the hive, measured as the number of occupied combs, across all observations during the spring season ($P < 0.05$). The highest number of combs was recorded in hives supplemented with minerals at a concentration of 1.5 g/L. Notably, continuous feeding throughout the entire spring season also resulted in significant differences among the treatments, with the lowest mean number of combs recorded in the control group (6.66 ± 0.18 combs) over four sampling periods. As the dietary concentration increased, the number of combs (population size) in the hive also increased, reaching its peak in the 1.5 g/L treatment. However, further increasing the mineral concentration from 1.5 to 2 g/L led to a significant decrease in the number of combs in the hive ($F = 10.03$, $P < 0.0001$, $df = 4, 139$) (Table 4).

Effect of spring feeding with chelated minerals on eggs, larvae, and pupae based on area (cm²) in the hive

Obtained data of different concentrations of chelated minerals revealed a significant difference among treatments in terms of the area occupied by immature stages (eggs, larvae, and pupae) ($P < 0.05$). Accordingly, the largest area occupied by immature stages during the first to fourth feeding periods ranged from 7307.1 to 10985.7 cm^2, observed at a concentration of 1.5 g/L. Moreover, a comparison of the overall

mean feeding response in spring revealed a significant variation among mineral concentrations, with the smallest area occupied by immature honeybee stages recorded in the control group (6817 ± 204.15 cm²) ($F = 18.06$, $P < 0.0001$, $df = 4, 139$) (Table 5).

Effect of spring feeding with chelated minerals on the amount of collected pollen (cm²) in the hive

The results of this study indicated a significant difference among dietary treatments in the amount of pollen collected in hives ($P < 0.05$). The lowest pollen collection during the first to fourth feeding periods ranged from 117.86 to 225.00 cm^2 and was observed in the control group. The highest collected pollen (215.18 cm^2) was recorded in hives fed with 1.5 g/L of chelated minerals ($F = 3.14$, $P = 0.0168$, $df = 4, 139$) (Table 6).

Effect of spring chelated mineral supplementation on honey production in the hive

The results indicated that different concentrations of chelated minerals did not have a significant difference in honey production ($P > 0.05$). Honey yield varied from 11.72 ± 1.07 kg in the control group to 15.29 ± 1.30 kg at a concentration of 1.5 g/L of minerals, but this variation was not statistically significant ($F = 2.44$, $P = 0.0684$, $df = 4, 34$) (Fig. 3).

Antioxidant enzyme activity in honeybee larvae under different treatments

The lowest peroxidase activity was observed at a concentration of 2 g/L of chelated minerals (0.002 ± 0.0002), which was significantly lower than the control group (0.005 ± 0.0002). Catalase activity at 2 g/L (0.156 ± 0.0057) was significantly higher than in other treatments, whereas the control group exhibited the lowest catalase activity (0.118 ± 0.0036). Superoxide dismutase activity did not show a significant difference among treatments; however, its levels varied from 0.132 ± 0.0109 in the control group to 0.160 ± 0.0091 at a concentration of 2 g/L. Furthermore, glucose-6-phosphate dehydrogenase activity in honeybee larvae showed a significant difference, with the highest activity recorded at 2 g/L (0.289 ± 0.0042) and the lowest in the control group (0.212 ± 0.0015) (Table 7).

Detoxifying enzyme activity in honeybee larvae under different treatments

The highest glutathione S-transferase (GST) activity with the DCNB substrate was observed at a concentration of 2 g/L of chelated minerals (0.260 ± 0.0042), which was significantly higher than in the control group (0.185 ± 0.0020). Similarly, GST activity with the CDNB substrate was also significantly elevated at 2 g/L treatment (0.142 ± 0.0045). This increase in GST activity suggests a negative impact of the 2 g/L mineral concentration by enhancing detoxification capacity in larvae.

A significant difference was also observed in α-naphthyl esterase activity among treatments, with the lowest activity recorded in the control group (0.109 ± 0.0036). In addition, β-naphthyl esterase activity was significantly higher at 2 g/L (0.614 ± 0.010) and 1.5 g/L (0.584 ± 0.007) of chelated minerals compared to other treatments (Table 8).

Energy reserves in honeybee larvae under different treatments

The results showed that protein levels of honeybee larvae were significantly influenced by different dietary concentrations. The highest protein content was observed at a concentration of 2 g/L of chelated minerals (1.70 ± 0.013), which was significantly higher than in the control group (1.50 ± 0.004). There was also a significant difference in glucose levels among treatments, with the lowest level recorded in the control group (0.065 ± 0.0016). Additionally, triglyceride levels in larvae were significantly higher at 1.5 g/L of chelated minerals (0.065 ± 0.0057) compared to the control group (0.033 ± 0.0026) (Table 9).

Energy reserves in honeybee adults under different treatments

The results indicated that the highest protein content in adult honeybees was observed at a concentration of 2 g/L of chelated minerals (1.65 ± 0.007), which was significantly higher than in the control group (1.45 ± 0.007). There was a significant difference in glucose levels among treatments, with the lowest level recorded in the control group (0.065 ± 0.0016) and the highest at 2 g/L of chelated minerals (0.083 ±

0.0017). Moreover, triglyceride levels were significantly higher at 2 g/L (0.061 ± 0.0042) and 1.5 g/L (0.057 ± 0.0008) of chelated minerals compared to other treatments (Table 9).

Discussion

To ensure the optimal utilization of nutritional resources in honeybee colonies, multiple internal factors should be considered to evaluate. Simply measuring the acceptance and consumption of a supplement by the bees seems not to be a reliable indicator of its quality. In addition to the quantity consumed, a comprehensive evaluation should include other indicators such as the supplement's effects on the protein and fat content of bee bodies, pollen storage levels, brood development (including eggs, larvae, and pupae), and overall colony population.

The findings of the current study showed that the highest adult population during the autumn season (measured by the number of occupied bee combs) was observed in colonies fed with a solution containing 1.5 g/L of minerals. Indeed, a clear positive correlation was evident between queen fecundity and colony population increase. Moreover, mineral supplementation significantly enhanced brood-rearing success, colony size, and honeybee longevity, all of which contributed to the increased number of occupied combs. Supporting this, De Grandi-Hoffman *et al.* (2008) demonstrated that providing essential supplements that promote the development of royal jelly-producing glands in worker bees improved royal jelly secretion. As a result, nurse bees were able to supply the queen with sufficient royal jelly, thereby enhancing her vitality, stimulating fecundity, and ultimately fostering colony expansion and improved hive productivity.

The present study also demonstrated that, during the autumn season, colony population -as measured by the number of occupied combs by adults- was influenced not only by the concentration of the dietary supplements but also by significant differences among the experimental diets in the proportion of comb area occupied by brood (eggs, larvae, and pupae). The greatest brood area was observed in colonies fed with 1.5 g/L of minerals. Overall, a strong positive relationship was found between queen egg-laying activity and colony population increase, which subsequently led to an increase in worker bee numbers and honey production. Notably, vitamin-mineral supplementation during autumn resulted in a substantial 181.7% increase in honey yield compared to the control group (Mladenović *et al.*, 2011).

Furthermore, the results indicated that increasing mineral concentrations enhanced pollen collection. However, at a concentration of 2 g/L, a significant decline in collected pollen was observed, where colonies fed with this concentration showed no significant difference from the control group. It seems that, other than quality, the quantity of minerals is also critical for honeybee performance. Indeed, minerals are generally required in small quantities to maintain the metabolic activity of honeybees. However, a deficiency in even these trace amounts can lead to metabolic disturbances and impair colony performance. Moreover, excessive levels of minerals can also result in toxicity (Haraguchi, 2004).

The quantity and quality of pollen collected by honeybees are influenced by several factors, including pollen availability, colony population, colony nutritional demand, and environmental conditions (Somerville, 2005). Furthermore, the number of eggs laid daily by the queen is closely linked to the amount of pollen brought into the hive (Mattila & Otis, 2006). In the present study, pollen availability, environmental conditions, and colony demand were held constant, while colony population varied across treatments. Therefore, the increased pollen collection observed in colonies receiving 1.5 g/L mineral supplementation can be attributed to the supplement's positive effects on brood rearing, colony population increase, and the resulting increase in nutritional demand. In essence, colonies that reared more brood and maintained larger populations also collected greater amounts of pollen.

It is noteworthy that the increase in pollen collection observed during the fourth sampling period, compared to other periods, can be attributed to the continuous feeding of colonies with experimental treatments. This sustained supplementation led to enhanced brood rearing and colony population growth. According to previous studies, zinc concentrations below 30 mg/kg in pollen are insufficient to maintain

colony health. Under such conditions, supplementation with zinc-containing additives is necessary to increase this concentration to 60 mg/kg (Zhang *et al*., 2015). It should be noted that in the current research, a complex of minerals rather than a single element was studied, and so the observed results might be related to additive, synergistic, or antagonistic interactions among the minerals. Therefore, additional research would be necessary to understand the effects of feeding periods and mineral interactions. Minerals play a vital role in maintaining tissue health and supporting the excretory system in insects. However, when present in excessive amounts, they can lead to numerous health issues. Based on research, an excess of potassium and phosphorus, combined with a calcium deficiency, led to paralysis in adult honeybees. Elevated mineral levels were also linked to diarrhea in adult bees. Therefore, it is expected that micronutrients in insect bodies are actively regulated within an optimal nutritional range; otherwise, physiological disorders may arise (Robinson, 2003).

The findings of the present study also revealed that, during the overwintering period, the highest colony population -measured by the number of occupied combs- was observed in colonies fed with a solution containing 1.5 g/L of minerals. In contrast, the lowest populations were recorded in colonies that received either the control or a diet supplemented with 2 g/L of minerals.

It is important to note that honeybees emerging in late summer (winter bees) have a longer lifespan and survive until early spring (Somerville & Nicol, 2002). Therefore, the provision of vitamins and minerals during this critical period likely stimulated queen fecundity, resulting in the emergence of a greater number of long-lived winter bees. Consequently, colonies were able to maintain stronger populations throughout the winter and into early spring (Somerville & Nicol, 2002). Moreover, previous research has shown that the timely provision of essential proteins and lipids stimulates the development and activity of the hypopharyngeal glands in worker bees, thereby ensuring adequate nutrition for larval growth and development. This, in turn, enhances the queen's egg-laying activity and contributes to the gradual expansion of colony populations. Notably, strong colonies are less vulnerable to pest infestations and diseases and are better equipped to endure severe and prolonged winter conditions (Toth & Robinson, 2005).

The results of the present study further indicated that increasing the concentration of minerals in colony diets led to an increase in pollen collection. However, at a concentration of 2 g/L, a significant decline in pollen collection was observed. In fact, the amount of pollen collected in colonies fed with this concentration showed no significant difference from the control group. The quantity and quality of pollen collected by honey bees depend on various factors, including pollen production timing, colony population size, colony nutritional demands, and environmental conditions (Somerville, 2005). Additionally, the daily egg-laying rate of the queen is directly correlated with the amount of pollen brought into the hive by foragers (Mattila & Otis, 2006). Given that pollen availability, colony nutritional requirements, and environmental conditions were consistent across all treatments in this study -while colony populations differed- it can be concluded that the increased pollen collection observed at the 1.5 g/L mineral concentration was due to its positive effects on brood rearing, colony population increase, and the resulting rise in nutritional demand. Furthermore, colonies that reared more brood and maintained larger populations consistently collected greater quantities of pollen.

Hive weight loss during winter reflects the amount of honey consumed when honeybees are unable to forage due to unfavorable weather conditions and limited availability of nectar as well as pollen (Nehzati, 2009). The results of the present study demonstrated that different concentrations of dietary minerals significantly influenced winter hive weight loss. The lowest weight loss was recorded in colonies supplemented with a 1.5 g/L concentration of chelated minerals. This finding suggests that bees fed with this mineral concentration utilized their energy reserves more efficiently, indicating improved ecological efficiency and better colony sustainability during winter.

Although the highest protein and fat levels in worker bees were observed in colonies supplemented with a 2 g/L mineral concentration, the lowest hive weight loss during the overwintering period was recorded in

colonies receiving the 1.5 g/L treatment. This discrepancy suggests that excessive mineral intake at the 2 g/L concentration—associated in this study with increased activity of detoxifying enzymes—may have impaired overall colony efficiency. Supporting this interpretation, previous studies have reported that elevated mineral concentrations in certain pollen sources led to negative effects on brood development and honey production (Herbert & Shimanuki, 1978).

Previous research has demonstrated that the protein content in honeybee bodies varies seasonally (Amdam *et al*., 2004). Otis (2004) further reported that the final generation of worker bees produced in late summer exhibits distinct physiological traits, including a significantly longer lifespan compared to those emerging in other seasons. During this period, these bees also possess higher levels of body protein and hemolymph. It is suggested that, in preparation for winter brood rearing, worker bees increase their pollen consumption in the autumn and accumulate essential proteins and lipids, such as vitellogenin and arylphorin, which play a critical role in sustaining colony function during periods of limited resource availability.

Based on the findings of this study, it can be concluded that honeybees from colonies supplemented with a 1.5 g/L mineral diet, under otherwise identical conditions, accumulated sufficient reserves of fats and proteins, which likely contributed to reducing honey consumption during winter. Furthermore, the highest number of combs occupied by bees during all autumn assessments -from the first to the fourth sampling- was consistently recorded in the 1.5 g/L mineral treatment group. A comparison of the average effects of the different mineral concentrations throughout the autumn season also revealed statistically significant differences among treatments, with the 1.5 g/L group achieving the highest colony population (5.53 combs). This increase is presumably attributed to enhanced brood rearing and colony population increase, given the well-established correlation between queen fecundity and colony expansion. These findings are in line with previous studies that have demonstrated the beneficial effects of vitamin and mineral supplementation on brood development and overall colony population increase in honeybees (Elbassiouny *et al*., 2005).

Given that all colonies were exposed to the same environmental conditions and received an identical protein source (pollen), the observed increase in colony population among the experimental groups receiving chelated minerals (ranging from 0.5 to 2 g/L) compared to the control group can be attributed to the beneficial effects of mineral supplementation. In particular, the notably higher population in treatment group 4 (1.5 g/L) is likely a result of the optimal mineral concentration provided, which may have offered a more balanced and bioavailable nutrient profile conducive to colony development.

The development of hypopharyngeal glands in worker bees is highly dependent on dietary intake (Maurizio, 1954), and the inclusion of vitamin and mineral supplements has been shown to enhance egg production. Brood rearing activity typically increases in response to experimental diets, rising ambient temperatures, and greater availability of pollen and nectar (De Grandi-Hoffman *et al*, 2008). In the present study, the 1.5 g/L mineral treatment yielded the highest number of immature bees among all mineral-supplemented groups, which is likely attributable to the more optimal mineral balance at this concentration. The slight decrease observed in the number of eggs, larvae, and pupae during the fourth sampling period may be explained by an increase in worker activity directed toward nectar storage, which potentially reduced the available space for queen oviposition on some combs.

The quantity and quality of pollen collected by honeybees are influenced by several factors, including the timing of pollen production, colony population size, nutritional demands of the colony, and prevailing weather conditions (Somerville, 2005). Honeybee foraging activity for pollen is typically observed within a temperature range of 18 to 30 °C, while extreme temperatures outside this range can hinder pollen collection. Furthermore, bee activity decreases markedly on cloudy or rainy days (Pearson & Braiden, 1990).

In the present study, colonies exhibiting greater population sizes and more intensive brood rearing also collected larger amounts of pollen, consistent with the findings of Somerville (2005). Colony strengthening

and the fulfillment of adult bees' nutritional requirements for brood development can be effectively supported through supplemental feeding with sugar syrup and the provision of natural pollen, particularly in early spring before the emergence of nectar-producing flora, as well as through autumn and winter-feeding strategies (De Grandi-Hoffman *et al*., 2008). Strong colony populations are essential for effective pollination and are positively correlated with increased productivity in honey and other hive products (Somerville, 2005).

Although the various chelated mineral treatments did not produce statistically significant differences in honey yield, an increase in honey production was observed—from 11.72 kg in the control group to 15.29 kg in the 1.5 g/L mineral treatment. This finding suggests that colonies with higher population levels in early spring are better able to exploit nectar sources, particularly those from fruit trees, thereby enhancing spring honey yields (Standifer, 1987).

Overall, the findings of this study indicate that dietary supplementation with chelated minerals -when combined with increased environmental pollen availability, rising ambient temperatures, and favorable weather conditions such as reduced rainfall compared to the previous year- contributed to improved colony growth, heightened foraging activity, and ultimately, enhanced honey production.

Reactive oxygen species (ROS) can initiate a range of deleterious reactions that contribute to cellular aging and apoptosis. To mitigate the detrimental effects of oxidative stress, living organisms -including insects- possess an array of antioxidant enzymes that safeguard cellular components by neutralizing reactive radicals and other oxidants, thereby preventing damage to metabolically active tissues. Among these enzymes, superoxide dismutase (SOD) plays a crucial role by catalyzing the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen. Catalase (CAT) further decomposes hydrogen peroxide (H_2O_2) into water and oxygen, thus preventing the accumulation of this potentially harmful oxidant. Peroxidase (POX), localized in the mitochondria, also contributes to the elimination of hydrogen peroxide. Additionally, glucose-6-phosphate dehydrogenase (GPDH) is vital due to its role in generating NADPH, a reducing agent essential for various biochemical pathways, including antioxidant defense mechanisms and lipid biosynthesis (Weirich *et al*., 2002; Erejuwa *et al*., 2012). The results of this study demonstrated that antioxidant enzyme activity in larvae fed with syrup supplemented with different concentrations of chelated minerals was elevated compared to the control group. Similarly, Zhang *et al*. (2015) reported a significant increase in antioxidant enzyme activity with higher dietary calcium levels. Given that metals are known to induce oxidative stress in insects by disrupting the balance between pro-oxidant species and antioxidant defenses (Puppel *et al*., 2014), the observed increase in antioxidant enzyme activity in larvae in this study is likely attributable to the metal content in their diet. Multiple studies have confirmed the induction of oxidative stress in bees exposed to various metal concentrations (Zhang *et al*., 2015; Gauthier *et al*., 2016; Behjatian-Esfahani *et al*., 2023). Furthermore, previous research has suggested an inverse relationship between insect longevity and antioxidant defenses (Nikolenko *et al*., 2011; Deepashree *et al*., 2022; Nemati *et al*., 2025). Consequently, the reduced hive efficiency observed at the 2 g/L mineral concentration in this study may be linked to the elevated activity of antioxidant enzymes (Hyrsl et al., 2007).

Although the primary diet of honeybees comprises honey, pollen, and water, detoxifying enzymes, particularly esterases secreted by midgut epithelial cells, play a vital role among digestive enzymes and are essential for intermediary metabolism (Gajger *et al*., 2013). General esterases, including both α- and β-esterases, constitute a large and diverse group of hydrolases capable of hydrolyzing a wide range of substrates, such as esters and certain non-ester compounds. These enzymes are involved in the regulation of juvenile hormone levels in the hemolymph, fat mobilization and metabolism, and contribute to insecticide resistance (Oakeshott *et al*., 2010). The present study revealed a significant increase in the activity of both α- and β-esterases in the treatment groups compared to the control. Notably, general esterase activity was significantly higher in colonies fed syrup supplemented with mineral zeolite than in the control group (Gajger *et al*., 2020).

Glutathione S-transferase (GST) is a detoxifying enzyme that catalyzes the conjugation of various toxic compounds to the thiol group of glutathione (GSH), thereby forming fewer toxic conjugates and facilitating their elimination from living organisms. In addition to its detoxification role, GST contributes to cellular protection against oxidative damage (Yan *et al*., 2013). In the present study, GST activity in the last instar larvae of *Apis mellifera* fed a diet containing 2 g/L of minerals was significantly elevated compared to the control group, whereas other treatment groups exhibited GST activity comparable to the control. GST likely plays a protective role against oxidative damage induced by elevated reactive oxygen species (ROS). The observed increase in GST activity in this treatment group may be associated with the concomitant elevation of oxidase and esterase activities, potentially regulated by shared genetic mechanisms (Rand *et al*., 2015; Afraze *et al*., 2020; Azizi and Jalali, 2024; Mojarab-Mahboubkar & Sendi, 2025). Similarly, Yu *et al*. (2012) reported that GST activity in third instar larvae of *Apis cerana* increased in a dose-dependent manner when exposed to $HgCl_2$. However, despite the induction of detoxifying enzymes such as GSTs, the ingestion of royal jelly, honey, or pollen contaminated with heavy metals by worker bees resulted in impaired growth, development, and even mortality under severe conditions. Conversely, Nikolić *et al*. (2019) found that feeding worker bees diets containing various minerals, including cadmium and lead, did not alter GST activity, while copper at a high concentration (1000 mg/L) caused a reduction in GST activity.

In insects, there is a close relationship between energy reserves and the activity of detoxifying enzymes. Energy stores such as glucose, glycogen, and lipids serve as essential fuel for various physiological processes, including detoxification. When these energy reserves are depleted or unavailable, the activity of detoxification enzymes declines due to insufficient energy to sustain their function. Conversely, when energy reserves are adequate, insects are better equipped to maintain and utilize detoxification pathways efficiently, resulting in enhanced tolerance to toxins and other harmful substances (Castañeda *et al*., 2009).

Energy reserves play a pivotal role in the growth, development, and reproduction of insects (Arrese and Soulages, 2010). Proteins are essential for supporting vital physiological processes, while triglycerides—stored forms of fats—serve as major energy sources necessary for cellular maintenance and metamorphosis. Carbohydrates also constitute a key energy resource in insect physiology, particularly contributing to processes such as molting (Wrigglesworth, 2015). The results of this study demonstrated that energy reserves, including protein, glucose, and triglycerides, were significantly higher in the larvae and adult bees fed diets containing 1.5 and 2 g/L of chelated minerals compared to the control and other treatment groups. Consistent with these findings, Sayed *et al.* (2022) reported that honeybee colonies fed a supplemental diet composed of 50 g of mixed brewer's yeast and chickpea cake enriched with 4.2% pollen, along with 250 mL of sugar syrup, showed the highest total protein content in adult bees during autumn. The researchers attributed this increase to reduced bee activity during autumn and the accumulation of excess food, resulting in protein storage that was utilized more gradually throughout the winter. Moreover, Behjatian-Esfahani *et al*. (2023) found that bees fed with organic zinc (40 and 60 mg) exhibited higher protein content in their tissues, longer lifespan, greater food intake, larger colony populations, and increased hive weight compared to those receiving mineral zinc. Similarly, Ghasemi *et al*. (2025) showed that energy reserves -including protein, glycogen, and lipids- increased significantly in bees fed diets supplemented with 0.2% and 0.4% chelated minerals compared to controls. Given that energy reserves directly influence the efficacy of insect detoxification systems, the enhanced activity of detoxifying enzymes observed in larvae fed the 2 g/L mineral diet in this study may be attributed to increased energy availability (Rand *et al*., 2015). Furthermore, the improved energy reserve levels in larvae fed the 1.5 g/L mineral diet appear to be positively correlated with increased queen oviposition and overall hive performance.

Conclusion

This study demonstrates that supplementary honeybee diets including chelated minerals significantly enhance colony population, brood development, pollen collection, overwintering survival, and enzymatic activity. The optimal mineral concentration (1.5 g/L) improved colony size, pollen foraging, and metabolic

efficiency without adverse effects. In contrast, higher concentrations (2 g/L) elevated detoxification and antioxidant enzyme activity but impaired colony performance, likely due to oxidative stress. These findings underscore the need for balanced mineral supplementation in beekeeping to maximize colony health and productivity. Future research should investigate long-term effects and mineral-specific interactions with bee physiology to refine supplementation strategies.

Author's Contributions

Sourena Chaiechi: Writing–original draft; Investigation; Data curation; **Azadeh Karimi-Malati:** Writing–review & editing; Supervision; Project administration; Methodology; Formal analysis; Conceptualization; **Jalal Jalali Sendi:** Writing–review & editing.

Data Availability Statement

The data availability statement is not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Effect of different concentrations of chelated minerals on the population size of honeybee adults (number of comb) in autumn.

Population	Treatment (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1st observation*	3.21 ± 0.10b	3.50 ± 0.15ab	3.71 ± 0.24ab	4.07 ± 0.25a	3.35 ± 0.14ab	0.0275	3.17	4, 34
2nd observation	4.14 ± 0.09b	4.42 ± 0.17ab	4.78 ± 0.21ab	5.14 ± 0.28a	4.21 ± 0.14b	0.0045	4.72	4, 34
3rd observation	5.07 ± 0.17b	5.57 ± 0.17ab	5.78 ± 0.28ab	6.07 ± 0.25a	5.35 ± 0.14ab	0.0237	3.30	4, 34
4th observation	5.71 ± 0.26b	6.07 ± 0.13ab	6.21 ± 0.18ab	6.85 ± 0.21a	5.85 ± 0.26b	0.0080	4.22	4, 34
Average	4.53 ± 0.24b	4.89 ± 0.44ab	5.12 ± 0.56ab	5.53 ± 0.74a	4.69 ± 0.39b	0.0111	3.39	4,139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 2. Effect of different concentrations of chelated minerals on eggs, larvae, and pupae (cm^2) of honeybees in autumn.

Egg, larva and pupa	Treatment (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1st observation*	3942.90 ± 103.75b	4278.60 ± 172.12b	4385.70 ± 172.07ab	4942.90 ± 201.01a	4075.00 ± 125.71b	0.0275	5.90	4, 34
2nd observation	4910.70 ± 110.17b	5378.60 ± 192.37ab	5435.70 ± 282.57ab	6007.10 ± 284.40a	5017.70 ± 560.88b	0.0067	4.36	4, 34
3rd observation	5942.90 ± 146.05b	6350.00 ± 188.35ab	6442.90 ± 330.12ab	7028.60 ± 164.33a	6058.70 ± 135.27b	0.0237	4.16	4, 34
4th observation	6596.40 ± 273.23b	7010.70 ± 130.91ab	7178.60 ± 113.31ab	7717.90 ± 168.38a	6689.30 ± 270.40b	0.0039	4.85	4, 34
Average	5348.20 ±110.17b	5754.50 ± 192.36ab	5860.70 ± 282.61ab	6424.10 ± 284.39a	5467.00± 560.87b	0.0068	3.71	4, 139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 3. Effect of different concentrations of chelated minerals on the collected pollens (cm^2) by honeybees in autumn.

Pollen	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1st observation*	121.43 ± 8.50c	142.86 ± 13.04bc	167.86 ± 7.14ab	185.71 ± 7.43a	125.00 ± 7.71c	<0.0001	9.42	4, 34
2nd observation	157.40 ± 4.61c	175.00 ± 7.71bc	200.00 ± 9.44b	264.29 ± 9.22a	160.71 ± 5.05c	<0.0001	34.61	4, 34
3rd observation	175.00 ± 7.71d	210.71 ± 5.05c	242.85 ± 7.14b	300.00 ± 5.45a	182.14 ± 7.14d	<0.0001	60.21	4, 34
4th observation	196.42 ± 6.52d	239.28 ± 5.05c	278.57 ± 6.52b	307.14 ± 7.14a	203.57 ± 6.52d	<0.0001	55.72	4, 34
Average	162.50 ± 4.60c	191.96 ± 7.71c	222.32 ± 9.61b	264.29 ± 9.21a	167.86 ± 5.04c	<0.0001	27.72	4,139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 4. Effect of different concentrations of chelated minerals on the population size of honeybee adults (number of comb) in spring.

Population	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1st observation*	5.07 ± 0.29b	5.28 ± 0.14b	5.71 ± 0.18ab	6.57 ± 0.20a	5.21 ± 0.18b	0.0001	8.43	4, 34
2nd observation	5.78 ± 0.18c	6.71 ± 0.18b	7.07 ± 0.20b	8.35 ± 0.17a	6.28 ± 0.28bc	<0.0001	21.26	4, 34
3rd observation	7.00 ± 0.18d	8.14 ± 0.23c	9.07 ± 0.25b	10.71 ± 0.18a	7.42 ± 0.25dc	<0.0001	42.88	4, 34
4th observation	8.78 ± 0.30d	10.42 ± 0.35c	11.92 ± 0.25b	11.64 ± 0.21a	9.00 ± 0.21d	<0.0001	56.07	4, 34
Average	6.66 ± 0.18c	7.64 ± 0.16bc	8.44 ± 0.25ab	9.82 ± 0.17a	6.98 ± 0.28bc	<0.0001	10.03	4,139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 5. Effect of different concentrations of chelated minerals on eggs, larvae, and pupae (cm^2) of honeybees in spring.

Egg, larva and pupa	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1st observation*	5632.10 ± 215.24b	6017.90 ± 138.09b	6157.10 ± 196.22b	7307.10 ± 172/92a	5896.40 ± 237.32b	<0.0001	11.01	4, 34
2nd observation	6394.40 ± 204.15c	7360.70 ± 151.76b	8482.10 ± 159.02b	9075.00 ± 288.98a	6942.90 ± 262.58bc	<0.0001	20.71	4, 34
3rd observation	7675.00 ± 134.29d	8839.30 ± 220.64c	9810.70 ± 89.11b	10985.70 ± 180.43a	8003.70 ± 249.90d	<0.0001	54.18	4, 34
4th observation	7564.30 ± 122.33d	8828.60 ± 239.25c	9685.70 ± 90.28b	10946.40 ± 212.30a	7953.60 ± 211.60d	<0.0001	54.67	4, 34
Average	6817.00 ± 204.15c	7761.60 ± 151.76bc	8283.90 ± 159.02b	9578.60 ± 288.91a	7199.10 ± 229.64c	<0.0001	18.06	4,139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 6. Effect of different concentrations of chelated minerals on the collected pollens (cm^2) by honeybees in spring.

Pollen	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
1stobservation*	117.86 ± 8.98a	125.00 ± 7.71a	132.14 ± 8.98a	153.57 ± 10.10a	121.43 ± 10.10a	0.0748	2.37	4, 34
2nd observation	146.43 ± 8.50a	171.43 ± 8.50a	175./0 ± 20.41a	196.43 ± 15.83a	153.57 ± 6.52a	0.0868	2.25	4, 34
3rd observation	189.29 ± 10.71b	203.57 ± 10.10ab	217.86 ± 13.04ab	239./29 ± 16.23a	196.43 ± 8.50ab	0.0483	2.72	4, 34
4th observation	225.00 ± 10.91a	250.00 ± 13.36a	253.57 ± 12.71a	271.43 ± 10.10a	232.14 ± 11.84a	0.0712	2.41	4, 34
Average	169.64 ± 8.53b	187.50 ± 8.51ab	194.64 ± 12.85ab	215.18 ± 15.87a	175.89 ± 8.59b	0.0168	3.14	4,139

Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.
* Fifteen-day interval

Table 7. Effect of different concentrations of chelated minerals on antioxidant enzymes of honeybee larvae in spring.

Antioxidant enzymes	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
Peroxidase	0.0058 ± 0.0002a	0.0043 ± 0.0001b	0.0042 ± 0.0003b	0.0030 ± 0.0003c	0.0020 ± 0.0002c	0.0001	31.06	4,14
Catalase	0.1187 ± 0.0036b	0.1293 ± 0.0025b	0.1361 ± 0.0087ab	0.1429 ± 0.0050ab	0.1562 ± 0.0057a	0.0076	6.5	4,14
Superoxide dismutase	0.1324 ± 0.0109a	0.1361 ± 0.0036a	0.1435 ± 0.0017a	0.1556 ± 0.0052a	0.1609 ± 0.0091a	0.0697	3.05	4,14
Glucose-6-phosphate dehydrogenase	0.2123 ± 0.0015d	0.2224 ± 0.0029c	0.2292 ± 0.0042c	0.2674 ± 0.0029b	0.2898 ± 0.0042a	<0.0001	97.39	4,14

Unit: (U/mg protein)
Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.

Table 8. Effect of different concentrations of chelated minerals on detoxifying enzymes of honeybee larvae in spring.

Detoxifying enzymes	Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
	Control	0.5	1	1.5	2			
Glutathione S-transferase (DCNB)	0.1857 ± 0.0020c	0.1992 ± 0.0100bc	0.2275 ± 0.0150ab	0.2277 ± 0.0010ab	0.2605 ± 0.0042a	0.0008	11.97	4,14
Glutathione S-transferase (CDNB)	0.0869 ± 0.0138b	0.0902 ± 0.0029b	0.1027 ± 0.0196ab	0.1190 ± 0.0032ab	0.1429 ± 0.0045a	0.0281	4.29	4,14
α-naphtyl esterase	0.1091 ± 0.0036b	0.1201 ± 0.0013ab	0.1239 ± 0.0039ab	0.1373 ± 0.0007ab	0.1462 ± 0.0130a	0.0148	5.31	4,14
β-naphtyl esterase	0.0410 ± 0.0045b	0.0447 ± 0.0124b	0.0597 ± 0.0051b	0.0951 ± 0.0071a	0.1181 ± 0.0051a	0.0001	20.06	4,14

Unit: (ΔOD/min/mg protein)
Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.

Table 9. Effect of different concentrations of chelated minerals on energy reserves of honeybee larva and adults in spring.

Energy reserves		Treatment Unit: (g/L mineral per sugar syrup)					P	F	*df*
		Control	0.5	1	1.5	2			
Adults	Protein	1.4545 ± 0.0074d	1.4743 ± 0.0074dc	1.5043 ± 0.0116c	1.5833 ± 0.0087b	1.6533 ± 0.0076a	<0.0001	89/76	4,14
	Glucose	0.0657 ± 0.0016d	0.0675 ± 0.0007dc	0.0734 ± 0.0011bc	0.0756 ± 0.0017b	0.0836 ± 0.0017a	<0.0001	23/74	4,14
	Triglyceride	0.0399 ± 0.0014b	0.0444 ± 0.0008b	0.0460 ± 0.0012b	0.0578 ± 0.0008a	0.0617 ± 0.0042a	0.0001	18/42	4,14
Larva	Protein	1.5021 ± 0.0043d	1.5256 ± 0.0074c	1.6184 ± 0.0075c	1.6578 ± 0.0131b	1.7066 ± 0.0076a	<0.0001	103.63	4,14
	Glucose	0.0682 ± 0.0017c	0.0878 ±0.0002b	0.1003 ± 0.0013a	0.0996 ± 0.0009a	0.1012 ± 0.0003a	<0.0001	160.47	4,14
	Triglyceride	0.0334 ± 0.0026c	0.0445 ± 0.0012bc	0.0506 ± 0.0003abc	0.0650 ± 0.0057a	0.0581 ± 0.0070ab	0.0036	8.07	4,14

Unit: (U/mg protein)
Means (±SE) within rows followed by the same letters are not significantly different ($p<0.05$) based on the Tukey test.

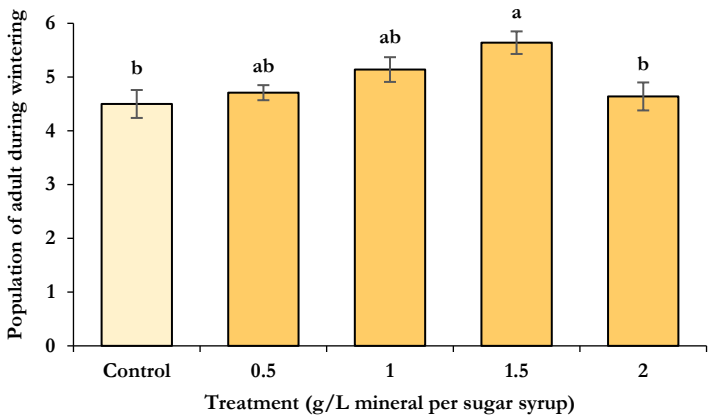

Fig 1. Effect of different concentrations of chelated minerals on the population size of honeybee adults (number of comb) during wintering.

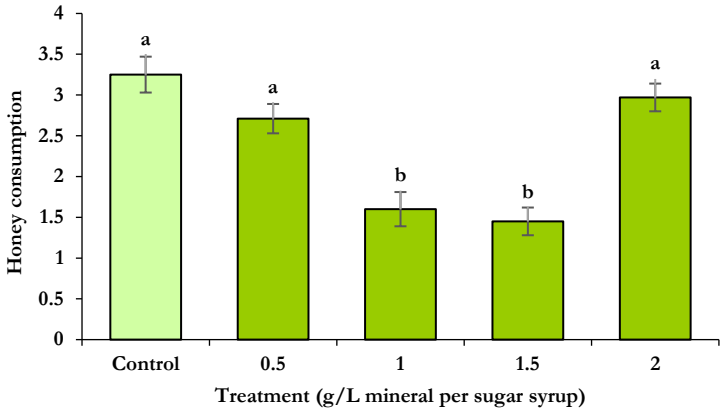

Fig 2. Effect of different concentrations of chelated minerals on the amount of honey consumed by the hive during the winter (kg).

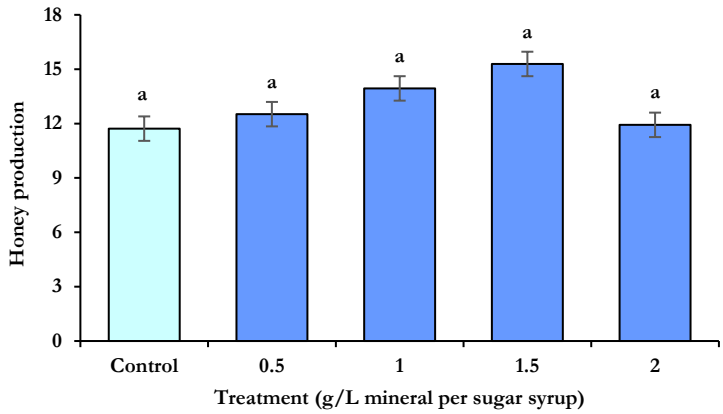

Fig 3. Effect of different concentrations of chelated minerals on honey production (kg).

تأثیر غلظت‌های مختلف مکمل‌های معدنی بر برخی ویژگی‌های زیست‌شناسی، رفتاری و بیوشیمیایی زنبورعسل *Apis mellifera* L. (Hymenoptera: Apidae)

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چکیده

شرایط تغذیه‌ای کلنی‌های زنبور عسل (*Apis mellifera* L.) نقش حیاتی بر رشد، زنده‌مانی و بهره‌وری آن‌ها دارد. این پژوهش با هدف ارزیابی اثر غلظت‌های مختلف مکمل‌های معدنی کلاته شامل آهن، روی، مس، منگنز، سلنیوم، کروم، کبالت، منیزیم، کلسیم و فسفر بر ویژگی‌های بیولوژیک و بیوشیمیایی کلنی‌های زنبور عسل انجام شد. در مجموع ۳۵ کلنی با شربت شکر حاوی غلظت‌های مختلف (صفر، ۰/۵، ۱، ۱/۵ و ۲ گرم در لیتر) مواد معدنی کلاته به مدت ۶۰ روز تغذیه شدند و رشد جمعیت، جمع‌آوری گرده، موفقیت در زمستان‌گذرانی، تولید عسل و فعالیت آنزیمی آن‌ها مورد ارزیابی قرار گرفت. نتایج نشان داد که کلنی‌های تغذیه‌شده با ۱/۵ گرم در لیتر مواد معدنی کلاته بیشترین رشد جمعیت، سطح اشغال شده توسط نوزادان و جمع‌آوری گرده را داشتند. موفقیت در زمستان‌گذرانی نیز در کلنی‌هایی که ۱/۵ گرم در لیتر مواد معدنی دریافت کرده بودند به بالاترین حد خود رسید، به طوری که کمترین کاهش وزن کندو و بالاترین نرخ زنده‌مانی را داشتند. تجزیه و تحلیل‌های بیوشیمیایی نشان داد که فعالیت آنزیم‌های آنتی‌اکسیدان و ذخایر انرژی در زنبورهای تغذیه‌شده با ۱/۵ و ۲ گرم در لیتر مواد معدنی افزایش یافت. در حالی که مکمل‌دهی بیش از حد (۲ گرم در لیتر) منجر به کاهش عملکرد کلنی شد. به‌طوری‌که میانگین تعداد قاب‌ها، جمعیت مراحل پیش از بلوغ (سانتی‌متر مربع اشغال شده) و گرده‌های جمع‌آوری شده (سانتی‌متر مربع) تفاوت معناداری با تیمار شاهد نداشتند. این یافته‌ها نشان می‌دهد که استفاده از مکمل‌های معدنی در غلظت متعادل (۱/۵ گرم در لیتر) عملکرد و بهره‌وری کلنی‌های زنبور عسل را بهبود می‌بخشد.

کلمات کلیدی: آنزیم‌های آنتی‌اکسیدان، زنبور عسل، زمستان‌گذرانی، گرده، جمعیت

دریافت:

پذیرش:

دبیر تخصصی:

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