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The Effect of Adding Different Concentrations of Chelated Mineral Supplement to Sugar Syrup on the Behavioral, Reproductive, and Functional Traits of Honey Bee (*Apis mellifera* L.) Colonies

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Abstract

The nutritional status of honey bee colonies plays a vital role in their development, survival, and productivity. This study aimed to evaluate the effect of different concentrations of chelated mineral supplement on the behavioral, reproductive, and functional traits of honey bee colonies. This study was conducted in a completely randomized design with five treatments and seven replications. The treatments included concentrations of 0.5, 0.75, 1, and 1.25 g/L of the chelated mineral supplement dissolved in sugar syrup, as well as a control treatment. The experimental colonies were fed for 60 days starting from the beginning of March 2025. Their honey production, population (adults and brood), palatability, aggressive behavior, calmness behavior, and pollen collection traits were then assessed. Variance analysis results showed that the treatment effect was significant for all studied traits ($p < 0.01$). Means comparison results showed that the highest mean of honey production (14.75 kg), adults (10.57 frames) and brood (7 frames) population, palatability (4), aggressive behavior (4), calmness behavior (4), and pollen collection (657.86 cells) traits of experimental honey bee colonies was observed in the treatment fed with sugar syrup containing a concentration of 0.75 g/L of the chelated mineral supplement, and the lowest mean of these traits was related to the treatment fed with sugar syrup containing a concentration of 1.25 g/L of this supplement. These findings indicate that adding the chelated mineral supplement at an optimal concentration (0.75 g/L) to sugar syrup during feeding improves behavioral, reproductive, and performance traits of honey bee colonies and increases apiary productivity.

Introduction

The honey bee, as one of the most effective natural pollinators, plays a crucial role in agricultural productivity, biodiversity conservation, and sustainable development (Lawal & Banjo, 2010; Rahimi et al., 2023). Honey bees require six major nutrient groups, including carbohydrates, proteins, fats, minerals, vitamins, and water, to survive and grow properly (Salehpor et al., 2023). Under conditions of abundant floral resources, honey bees acquire these nutrients through the collection of nectar and pollen from flowering plants. A shortage or reduction of nectar and pollen in the nature, often caused by unfavorable climatic conditions, can lead to decreased queen egg laying, reduced population growth, shortened lifespan of bees, lower resistance to pests and diseases, diminished colony food reserves, and ultimately the weakening and decline in the health of honey bee colonies (DeGrandi-Hoffman et al., 2008; Ahmadi, 2014; Tosi et al., 2017). Therefore, supplementary nutrition appears essential for overcoming periods of nectar and pollen scarcity, supporting colony health, enhancing productivity, and reducing the risk of disease outbreaks under stressful conditions (Brodschneider & Crailsheim, 2010; Avni et al., 2014). Studies have shown that pollen may not always meet all the nutritional requirements

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of a colony, even during periods of abundant floral resources in nature due to nutrient deficiencies (DeGrandi-Hoffman et al., 2016, 2018). Therefore, supplemental feeding appears essential to overcome periods of nectar and pollen scarcity and to support the growth and development of honey bee colonies under stressful environmental conditions.

The quality and availability of essential nutrients, including carbohydrates, proteins, lipids, minerals, vitamins, and water, strongly influences the performance and productivity of honey bee colonies. The nutritional requirements of honey bees are supplied by plants (nectar and pollen) and water sources. Nectar and pollen are the two primary sources of nutrition for honey bees. Nectar is the primary source of carbohydrates and provides the energy required for bee flight, whereas pollen is an important source of proteins, lipids, vitamins, and minerals (Abadi & Ahmadi, 2015). Most living organisms require a balanced diet composed of macronutrients (carbohydrates and proteins) and micronutrients (minerals and salts) to maintain normal physiological functions and support daily activities (Simpson & Raubenheimer, 2012; Hafeez et al., 2019; Adgaba et al., 2020). The composition and nutritional value of nectar and pollen produced by plants within a given geographical region vary considerably among plant species and across seasons. Consequently, maintaining a balance between the nutritional requirements of honey bees and the adequate supply of these nutrients is both challenging and critically important (Behmer, 2009).

Micronutrients play a critical role in physiological processes, serving as precursors of molting hormones and as structural components of cell membranes (Chakrabarti et al., 2020). Consequently, the energy needed for tissue development, physiological functions, and reproduction is derived from the minerals and nutrients obtained from plant-based sources and water (Filipiak et al., 2017; Ahmad et al., 2020). Honey bees do not rely solely on pollen to obtain minerals; they also acquire these nutrients from other sources including water, nectar, and honeydew (Lau & Nieh, 2016; Hakami et al., 2020). The role of minerals has been extensively investigated in the nutrition, physiology, and survival of many organisms, including the honey bee. Macro-minerals (phosphorus, calcium, magnesium, potassium, and sodium) and micro-minerals (zinc, copper, iron, chromium, manganese, and selenium) have been established in numerous studies as essential dietary components for the honey bee (De Groot, 1952; Somerville, 2005; Tosi et al., 2017; Hendriksma et al., 2019). Macro-mineral elements constitute the core components of biochemical reactions and pathways, while micro-mineral elements primarily contribute to enhancing the growth performance of honey bees. Studies have shown that plant-based resources (pollen) supply only a small portion of the minerals required by honey bees (Somerville, 2005). Consequently, honey bees selectively forage on soil and water-derived substrates to compensate for mineral deficiencies, thereby acquiring essential minerals and salts that are largely absent or insufficient in floral resources (Lau & Nieh, 2016; Hakami et al., 2020).

Studies have shown that minerals play an important role in many physiological activities in animals and in maximizing their health and productivity (López-Alonso, 2012). These elements play a vital role in enzymatic reactions, metabolic pathways, and the functioning of the honey bee's central nervous system (Haraguchi, 2004). Honey bee larvae and adults require minerals for optimal growth, development, and reproduction (Michener, 2007; Brodschneider & Crailsheim, 2010; Ahmad et al., 2020). Studies have shown that sodium, magnesium, and potassium are essential for the growth and development of honey bee larvae (Brodschneider & Crailsheim, 2010). Zinc ions can penetrate the peritrophic membrane and midgut epithelial cells of bees, playing a vital role in biochemical processes, including maintaining cell membrane integrity, respiration, cell division, and other essential functions (Eisler, 1993). Eyer et al. (2016) reported that zinc in various insects is transported from the gut to the hemolymph, where it participates in the transfer of vitellogenin to eggs. Additionally, zinc improves the immune system (Zhang et al., 2015; Maret, 2017). Calcium, manganese, and selenium are other essential elements for honey bee growth and development (Herbert & Shimanuki, 1997; Jensen & Trumble, 2003; Peters et al., 2010; Yu et al., 2012; Zhang & Xu, 2015; Kovalskyi et al., 2020). Also, honey bees consume minerals for muscle movement (Day et al., 1990; Wang et al., 2013; Chakrabarti et al., 2020). For example, iron accumulates in the abdominal region and plays a role in honey bee orientation (Wang et al., 2013). Honey bees consume sodium to regulate osmotic pressure (Nation, 2004). Additionally, various studies have shown that micronutrients play important roles in honey bee physiological processes, such as serving as precursors of molting hormones and as structural components of insect cell membranes (Cohen, 2015; Rupp, 2015; Chakrabarti et al., 2020; Al-Ghramh et al., 2020). Therefore, even a slight mineral deficiency can lead to metabolic disturbances and negative effects on colony performance. So, optimal mineral levels are essential for the health and vital physiological processes of honey bees. Studies have shown that concentrations either above or below the optimal level can cause significant complications and lead to toxicity in bees. For example, a study by Toth and Robinson (2005) found that excessive levels of potassium and phosphorus, together with a calcium deficiency, were associated with paralysis in adult bees. This highlights the importance of micronutrients and their regulation within an optimal range, as using them at levels above or below the optimal range can lead to physiological disturbances.

Most studies in the field of honey bee nutrition have mainly focused on the importance of sources providing macronutrients (including carbohydrates and proteins) (Olejnicek, 2004), while the honey bee's need for minerals and the effects of these minerals on their biology, growth, and improvement of behavioral and functional traits have received less attention. Therefore, despite adding minerals to bees' diet, maintaining optimal levels is essential for the proper functioning of their vital organs (Bonoan et al., 2018; Adgaba et al., 2020).

The chelated mineral supplement is synthesized and produced using advanced chelation technology. This supplement is absorbed by the honey bee's body more easily and more quickly compared with conventional forms of minerals. This supplement contains 10 micronutrients, including calcium, phosphorus, magnesium, iron, manganese, copper, zinc, selenium, chromium, and cobalt, which are formulated in chelated form. The present study aimed to investigate the effects of different concentrations of chelated mineral supplement dissolved in sugar syrup on the behavioral, reproductive, and performance traits of honey bee colonies in the climatic conditions of Kurdistan province, Iran.

Materials and Methods

Date and study site of the experiment

The present study was conducted at a private apiary located in Marivan city, Kurdistan province, Iran, from April 2024 to October 2025.

Setting up the honey bee colonies and experimental design

This study was conducted in a completely randomized design with five treatments and seven replications. The treatments included concentrations of 0.5, 0.75, 1, and 1.25 g/L of the chelated mineral supplement dissolved in sugar syrup (1:1) and a control treatment (sugar syrup (1:1) without supplement). Experimental colonies (35 colonies) were prepared in April 2024 in a private apiary located in Eisouli village, Marivan county, Kurdistan province, Iran (45°47′59.63″N, 39°05′53.05″E), and were subsequently standardized for queen age (all colonies had sister queens of the same age) and population (adults and brood) from May to July (2024) following Delaplane et al. (2013). The experimental colonies were fed 0.8 L of sugar syrup (1:1) containing 0.5, 0.75, 1, and 1.25 g/ L of the chelated mineral supplement every other day for 60 days from 1 April 2025. In the control treatment, sugar syrup (1:1) without the supplement was used. In the present study, honey production, population (brood and adults), palatability, aggressive behavior, calmness behavior, and pollen collection traits of the experimental colonies were measured as follows:

Evaluation of the studied traits

Honey production

To evaluate the honey production of the colonies during the regional harvesting season (August 2025), the weight of harvested honey as well as the remaining honey in each colony was measured. The difference in the weight of the honey frames of each hive before and after honey extraction was used to determine the amount of honey produced by the frames, and the total honey extracted from the frames of each colony was considered as the honey yield of its colony. To accurately estimate the remaining honey, the surface area of residual honey on the frames was assessed using special frame divided into 10 × 5 cm grids with galvanized wire (Fig 1). The remaining honey was estimated using the surface to weight conversion method based on previous studies by Iranian researchers (Yarahmadi et al., 2007), assuming that each square decimeter of honey on both sides of a frame is equivalent to 304 g of honey. The grids were placed on both sides of the remaining honey frames, and the total residual honey area per colony was calculated and recorded. Finally, total honey production per colony was calculated by summing the harvested and remaining honey, and the total was recorded for each colony.

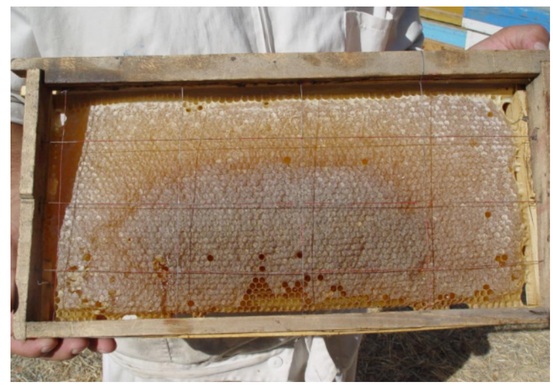

Fig 1. Evaluation of residual honey in the colony using a specially gridded frame

Adults population

The adults bee population was visually assessed in two stages: the first during May–June and the second during August–September 2025. In each stage, measurements were taken at 15-day intervals (twice per stage). During hive inspection, a frame fully covered with adult bees on both sides was considered one full bee frame. At the same time, partial coverage was recorded as a proportional fraction of a frame based on the observed bee population. The total number of frame populations was considered and recorded as the colony population.

Brood population

The brood population was evaluated in two phases, during May-June and June-September 2025, in all studied colonies. The brood population consisted of frames containing eggs, larvae, and pupae. During May-June, the brood population was quantified using the same method applied to the adult bee population assessment, by estimating the number of full brood frames and fractions thereof; the total was subsequently recorded as the colony brood population. In the late season (August–September), the brood population was estimated more accurately using an empty frame fitted with a galvanized wire grid that divided the frame area into 5 × 5 cm squares (Fig 2). By placing the grid frame over each brood frame in every colony, the number of grid squares containing brood was counted. The brood-covered area was subsequently calculated in square centimeters (Tahmasebi et al., 2017).

Fig 2. Evaluation of the brood area of colony using frame marked with 5 × 5 cm grids

Pollen collection

The stored pollen area in honey bee colonies in the experimental treatments was measured according to the method described by Page (2002). An empty frame fitted with a galvanized wire grid dividing the frame area into 2 × 2 cm squares was used to estimate the stored pollen area (Fig 3). The grid frame was placed over each comb of the colonies, and the number of grid squares containing pollen was counted to estimate the total stored pollen area. This trait was evaluated once per week for four consecutive weeks during May–June 2025 on the experimental colonies.

Fig 3. Measurement of stored pollen area in honey bee colony using a grid frame**.**

Aggressive behavior

The aggressive behavior of the honey bee colonies was evaluated using the visual assessment method in June 2025. In this method, the colony bee's behavior on the frames was visually evaluated during inspections and was assigned scores corresponding to their behavior as described below:

- ✓ Colonies that remained calm without the application of smoke were given a score of 4.
- ✓ Colonies that calmed with a small amount of smoke were assigned a score of 3.
- ✓ Colonies that remained agitated despite the application of smoke were assigned a score of 2.
- ✓ Colonies that exhibited persistent aggressive behavior despite the application of heavy smoke were assigned a score of 1.

Calmness behavior

For the assessment of calmness behavior on the comb surface in honey bee colonies, each colony was assigned a behavioral score based on the response of worker bees when the frame was removed from the hive according to the following criteria (Rahimi et al., 2023):

- ✓ During hive inspection, if most bees flew off the comb and abandoned it, and the worker bees moved rapidly across the comb surface, a score of 1 was assigned to that colony.
- ✓ During hive inspection, colonies were assigned a score of 2 when some worker bees flew off the comb. At the same time, the remainder stayed on the comb, and the bees present exhibited moderately rapid movement across the comb surface.
- ✓ During hive inspection, a score of 3 was assigned to a colony when the bees moved slowly on the comb, and most of them remained on the comb without flying.
- ✓ During hive inspection, a score of 4 was assigned to a colony when the bees exhibited very slow and calm movement on the comb and remained on the comb without flying.

The evaluation of calmness on the comb surface was conducted twice, in spring and summer of 2025, and the mean of these observations was recorded as the annual average for each colony.

Palatability

To assess palatability, the consumption of supplement-enriched sugar syrup by honey bee colonies in the experimental treatments was evaluated as follows (Salehpor et al., 2024):

- ✓ Colonies that consumed the sugar syrup within less than 12 hours were assigned a score of 4.
- ✓ Colonies that consumed the sugar syrup within 12 to 24 hours were assigned a score of 3.
- ✓ Colonies that consumed the sugar syrup within 24 to 36 hours were assigned a score of 2.
- ✓ Colonies that consumed the sugar syrup within 36 to 48 hours, or showed no inclination to consume the syrup within 36 hours after feeding, were assigned a score of 1.

Economic analysis

The costs and revenues were recorded throughout the study. The costs were categorized into two groups: feeding costs and other associated costs. Feeding costs associated with sugar syrup containing the chelated mineral supplement were recorded from the beginning to the end of the experiment. At the same time, the other expenses (labor, foundation sheets, and wax combs) were considered to constitute 35% of the total costs (Talebi et al., 2010). The income generated from the sale of honey and pollen at the end of the experiment was considered the total revenue. By subtracting the total costs from the combined revenue generated from the sale of honey and pollen, the gross profit per colony in each experimental treatment was calculated.

Data Analysis

After confirming the normality of the data distribution, analysis of variance (ANOVA) and means comparison (by Tukey's test at the 1% significance level) were performed using SAS software (V. 9.4).

The following statistical model was used to analyze the data obtained in the present study:

$$y_{ij} = \mu + h_{ij} + t_j + \varepsilon_{ij}$$

y_{ij} = Each observation from each experimental unit, μ = population mean, h_{ij} = the random effect of the hive in the experiment, t_j = the fixed effect of the treatment, and ε_{ij} = the effect of experimental error.

Results

Variance analysis and means comparison of the studied traits

Honey production

Variance analysis results of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the honey production trait of experimental honey bee colonies are presented in Table 1. The results showed that the treatment had a statistically significant effect on the honey production trait of the honey bee colonies in the present study ($p < 0.01$). The means comparison results of the honey production trait of the bee colonies are presented in Fig 4. The means comparison results showed that the highest mean honey production (14.57 kg) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement. In contrast, the lowest honey production value, with an average of 5.26 kg, was associated with the experimental treatment fed sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Adult population

Variance analysis results of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the adults population trait of experimental honey bee colonies are shown in Table 2. The results indicated that the treatment had a significant effect ($p<0.01$) on the adults population of the studied honey bee colonies. According to the means comparison results (Fig 5), the highest mean of adult's population (10.57 frames) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement. In contrast, the lowest mean for the adults population, with an average of 5.57 frames, was associated with the experimental treatment fed sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Table 1. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the honey production trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	94.113	87.66	0.0001
Error	30	1.964		

Table 2. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the adult's population trait of the studied honeybee colonies

Source of variation	df	Mean square	F	p-value
Treatment	4	62.042	81.94	0.0001
Error	30	0.757		

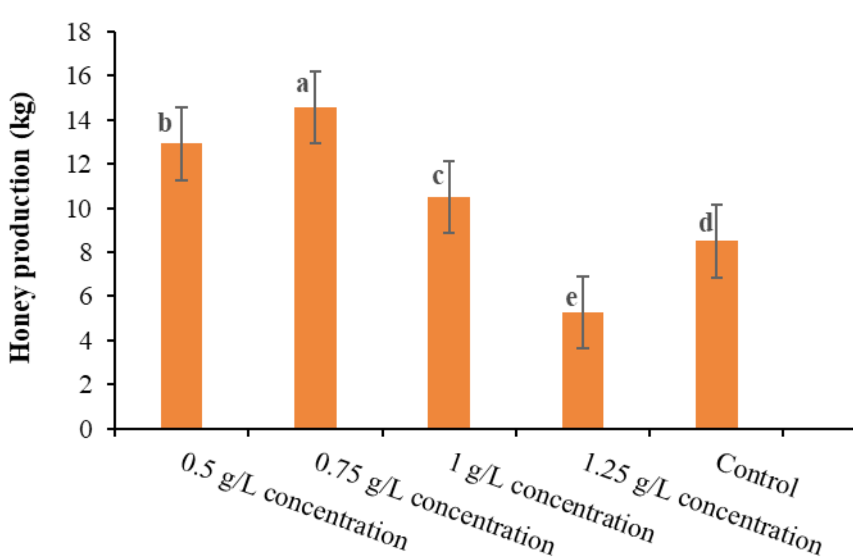

Fig 4. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the honey production trait of the studied honey bee colonies based on Tukey test ($p<0.01$).

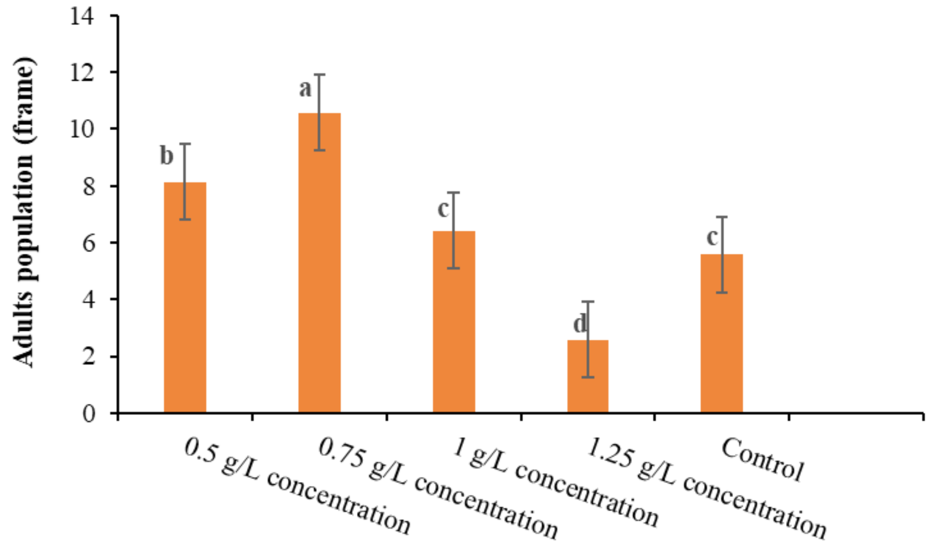

Fig 5. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the adult's population trait of the studied honey bee colonies based on Tukey test (p<0.01).

Brood population

The results of the analysis of variance of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the brood population trait of experimental honey bee colonies are presented in Table 3. The results indicated that the treatment effect on the brood population trait of the honey bee colonies in the present study was statistically significant (p<0.01). The means comparison results for the brood population trait of the bee colonies are shown in Fig 6. The results showed that the highest mean brood population (7 frames) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement. The lowest of the brood population mean (with an average of 2.5 frames) was related to the experimental treatment fed with sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Table 3. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the brood population trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	22.153	63.73	0.0001
Error	30	0.347		

Pollen collection

Variance analysis results of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the pollen collection trait of honey bee colonies in the experimental treatments are presented in Table 4. The results

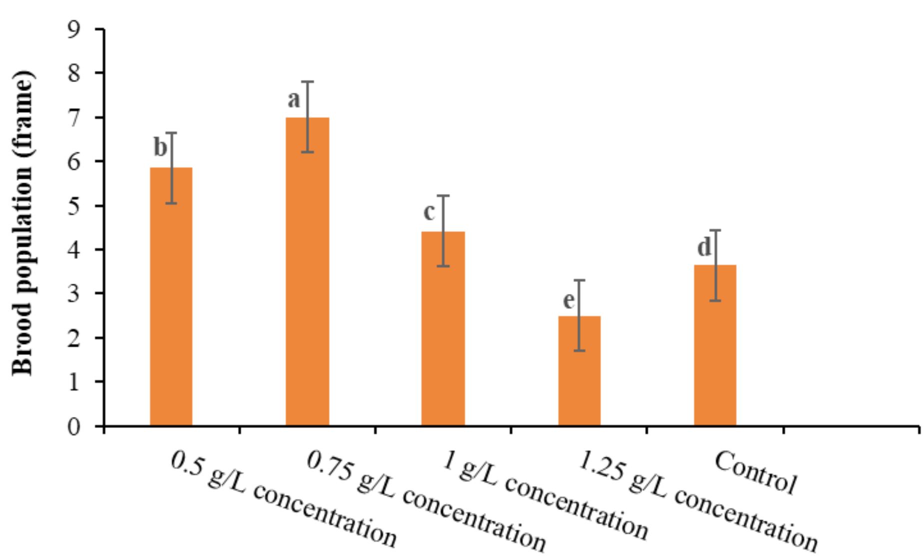

Fig 6. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the brood population trait of the studied honey bee colonies based on Tukey test (p<0.01).

showed that the treatment had a significant effect ($p<0.01$) on the pollen collection trait of the studied honey bee colonies. According to the means comparison results (Fig 7), the highest mean of pollen collection (657.86 cells) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement. The lowest mean pollen collection, with an average of 193.43 cells, was associated with the experimental treatment fed with sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Table 4. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the pollen collection trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	217790.829	1087.895	0.0001
Error	30	0.347		

Aggressive behavior

Variance analysis results of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the aggressive behavior trait of experimental honey bee colonies are presented in Table 5. The results indicated that the treatment effect on the aggressive behavior trait of the honey bee colonies was statistically significant ($p<0.01$). The means comparison results for the aggressive behavior trait of the bee colonies are shown in Fig 8. The results showed that the highest mean aggressive behavior (4)

Table 5. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the aggressive behavior trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	8.457	148	0.0001
Error	30	0.057		

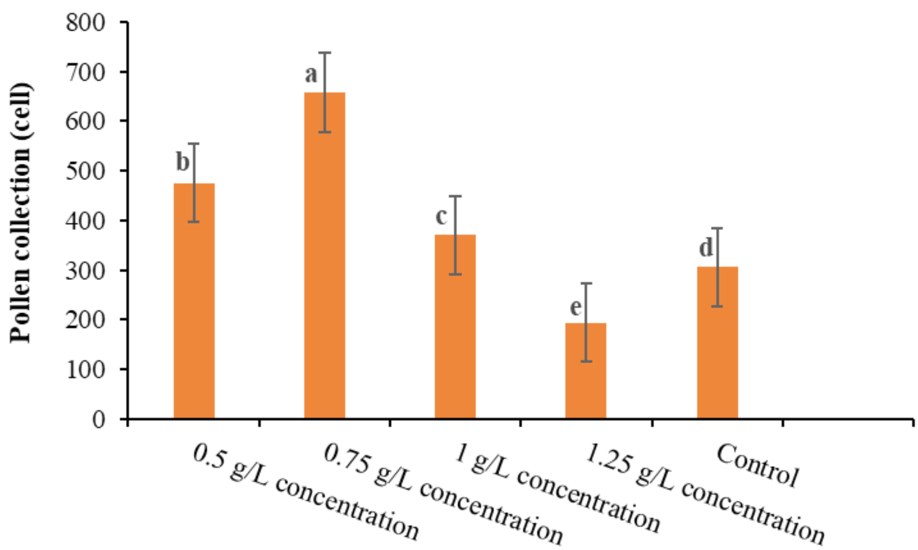

Fig 7. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the pollen collection trait of the studied honey bee colonies based on Tukey test ($p<0.01$)

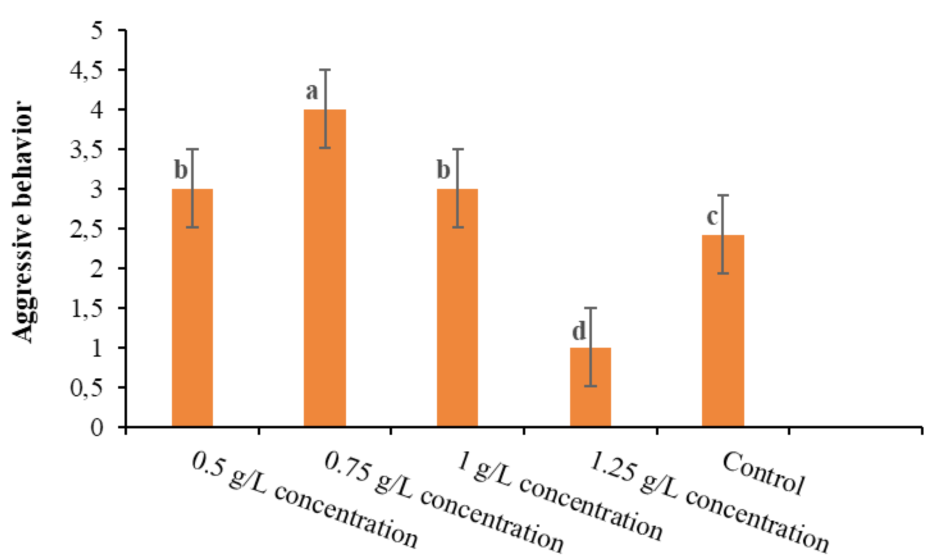

Fig 8. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the aggressive behavior trait of the studied honey bee colonies based on Tukey test ($p<0.01$).

was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement, and the lowest mean aggressive behavior (1) was related to the experimental treatment fed with sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Calmness behavior

Variance analysis results of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the calmness behavior trait of honey bee colonies in the experimental treatments are presented in Table 6. The results showed that the treatment effect had a significant effect ($p<0.01$) on the calmness behavior trait of the studied honey bee colonies. According to the means comparison results (Fig 9), the highest mean of calmness behavior (4) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement, and the lowest mean of pollen collection (1.14) was related to the experimental treatment fed with sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Palatability

The results of the analysis of variance of the effect of adding different concentrations of the chelated mineral supplement to sugar syrup on the palatability trait of honey bee colonies in the experimental treatments are presented in Table 7. The results indicated that the treatment effect on the palatability trait of the honey bee colonies in the present study was statistically significant ($p<0.01$). The means comparison results for the palatability trait of the bee colonies are shown in Fig 10. The results showed that the highest mean palatability (0.68) was observed in the experimental treatment fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement, and the lowest mean palatability (0.02) was related to the experimental treatment fed with sugar syrup containing 1.25 g/L of the chelated mineral supplement.

Table 6. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the calmness behavior trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	7.900	59.25	0.0001
Error	30	0.133		

Table 7. Variance analysis results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the palatability trait of the studied honey bee colonies.

Source of variation	df	Mean square	F	p-value
Treatment	4	10.457	54.90	0.0001
Error	30	0.190		

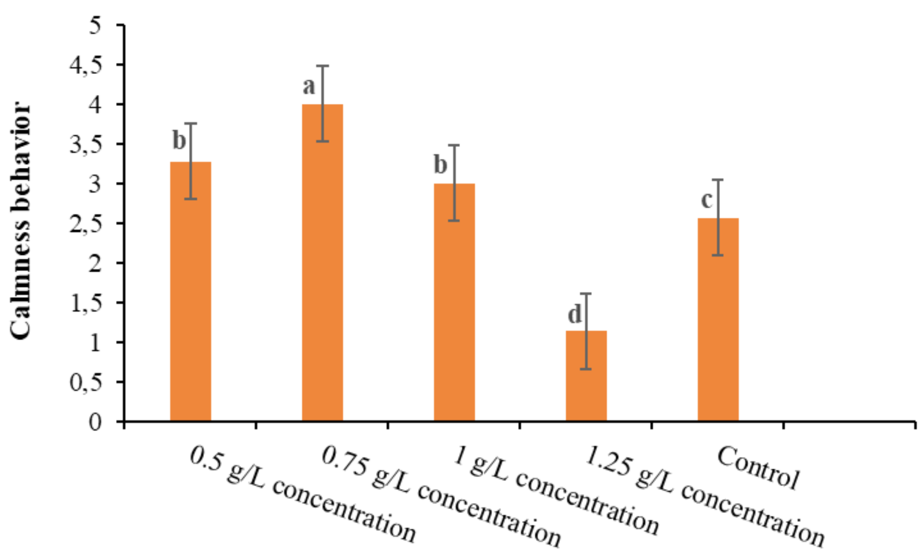

Fig 9. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the calmness behavior trait of the studied honey bee colonies based on Tukey test ($p<0.01$).

Economic analysis

In the present study, an economic analysis of the effect of adding the chelated mineral supplement to bees' nutrition on the performance of the experimental treatments was carried out based on two major honey bee products valued by beekeepers (honey production and pollen collection), and the results are presented in Table 8. The results showed that adding the chelated mineral supplement to bee nutrition increased the profit obtained from the sale of each colony's products (honey and pollen) compared with the control treatment. The results showed that the highest profit from adding the chelated mineral supplement to the experimental treatments was observed in the treatment fed sugar syrup containing 0.75 g/L. Therefore, the treatment with the highest profit per hive (58.21 $) was identified as the best treatment.

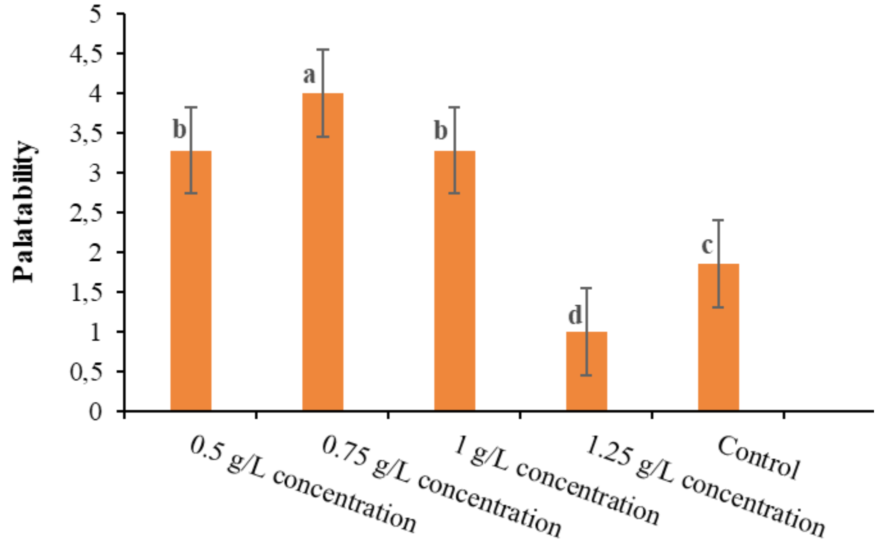

Fig 10. Means comparison results of the effect of adding different concentrations of chelated mineral supplement in sugar syrup on the palatability trait of the studied honey bee colonies based on Tukey test (p<0.01).

Table 8. Economic analysis of adding chelated mineral supplement (CMS) to the diet of honey bee colonies of the experimental treatments of the present study.

Variables	Experimental treatments				
	CMS 0.5%	**CMS 0.75%**	**CMS 1%**	**CMS 1.25%**	**Control**
Price per kilo of produced honey	4.61	4.61	4.61	4.61	4.61
Price per kilo of produced pollen	2.69	2.69	2.69	2.69	2.69
Cost of feeding each colony	10.84	10.90	10.97	11.04	10.70
Other costs (35% of total costs)	3.50	3.50	3.50	3.50	3.50
Total costs	14.35	14.40	14.48	14.55	14.21
Income from each colony	64.34	72.63	66.61	26.83	39.49
Profit	49.99	58.21	51.32	12.28	25.27

*Economic evaluation was calculated based on two traits: honey production and pollen collection traits in the present study.
**Prices are based on the September 2025 rate, and prices and costs are in US dollars.
***Calculations were performed per colony at the end of the experiment.

Discussion

Supplemental nutrition is valuable to overcome periods of resource scarcity and support colony development, bee growth, rearing, grazing behavior, and resistance to diseases and pests under stressful conditions. Providing a nutritious diet with optimal concentration is essential to stimulate colony strength and maximize honey production and plant pollination. Most studies in the field of honey bee nutrition have mainly focused on the importance of sources providing macronutrients (including carbohydrates and proteins) (Olejnicek, 2004), while the honey bee's need for minerals and the effects of these minerals on their biology, growth, and improvement of behavioral and functional traits have received less attention. The present study aimed to investigate the effects of different concentrations of chelated mineral supplement dissolved in sugar syrup on the behavioral, reproductive, and performance traits of honey bee colonies under the climatic conditions of Kurdistan province, Iran. Overall, the findings of the present study showed that the addition of chelated mineral supplement to the bees' diet had a favorable and significant effect ($p < 0.01$) on honey production, population (adults and brood), palatability, aggressive behavior, calmness behavior on the comb surface, and pollen collection traits in the experimental honey bee colonies. The results revealed that the highest performance was observed in the honey bee colonies fed with sugar syrup containing 0.75 g/L of the chelated mineral supplement. Therefore, feeding honey bee colonies with sugar syrup containing 0.75 g/L of the chelated mineral supplement is recommended to improve the behavioral, reproductive, and performance traits of honey bee colonies and to enhance the economic efficiency of apiaries, particularly during periods of nectar and pollen scarcity. This result indicates the existence of an optimal physiological range for mineral element consumption in honey bees. Minerals play essential roles, including enzyme activity, regulation of osmotic balance, strengthening of the immune system, reduction of oxidative stress, energy metabolism, and larval development. In insects, elements such as zinc, iron, copper, and manganese play key roles in the functioning of antioxidant enzymes such as superoxide dismutase and catalase. Therefore, feeding bees a chelated mineral supplement at a concentration of 0.75 g/L likely optimized enzymatic activity, thereby enhancing worker bee survival, improving brood rearing efficiency, and ultimately increasing the adult bee population and colony products, including honey and pollen. In contrast, the reduction in population observed in the treatment fed the 1.25 g/L concentration of this supplement may be attributed to metabolic stress. High levels of certain mineral elements may reduce nutrient absorption and bee longevity by increasing reactive oxygen species, damaging midgut epithelial cells, and disrupting gut microbiota homeostasis. Another factor that may have contributed to the observed results is the reduced palatability of the syrup containing the higher supplement concentration. Increasing the concentration of mineral components may have altered the syrup's taste and reduced its consumption by the bees, which could indirectly impair the colony's energy and nutrient supply. Consistent with the present findings, other researchers have also reported that adding the chelated mineral supplement to bee diets leads to increased colony population and improved apiary performance (Khalid et al., 2021; Besharati et al., 2022; Chaiechi et al., 2025). Additionally, Imdorf et al. (1998) examined the effects of mineral-containing diets on the population and performance of honey bee colonies. These researchers reported that a diet containing 1000 ppm potassium, 500 ppm calcium, 300 ppm magnesium, and 50 ppm sodium had favorable effects on the behavioral and performance traits of bees.

The results showed that bees did not show a strong preference for consuming diets containing high concentrations of the chelated mineral supplement. The bees of colonies fed diets containing high concentrations of this supplement were more aggressive than those from colonies fed diets with lower concentrations of the supplement. These findings suggest that bees preferentially consume mineral-supplemented diets only within a defined optimal range. The results of the present study also indicate that feeding bees diets containing high concentrations of mineral elements–particularly during periods of pollen scarcity in nature–may negatively affect bee behavior and survival. Honey bee colonies fed sugar syrup supplemented with 0.75 g/L of the chelated mineral supplement exhibited the most balanced level of aggressive behavior. This concentration likely met the bees' mineral requirements in a balanced manner, supporting neural and metabolic functions without excessive stimulation and thereby promoting calmer behavioral responses. In contrast, sugar syrup containing a higher concentration of the supplement (1.25 g/L) likely caused overstimulation of the bees' nervous system and disrupted mineral balance, leading to increased aggressive behavior. Using low concentrations of the supplement in the bees' diet resulted in behavioral instability and reduced functional performance due to insufficient levels of essential mineral elements. Therefore, the results of this study indicate that a precise balance of mineral elements in bee nutrition is essential for maintaining calm behavior and optimal colony performance. Consistent with the findings of the present study, Imdorf et al. (1998), Ghasemi (2022), and Ghasemi et al. (2025) also demonstrated that feeding bees diets containing high concentrations of mineral elements leads to physiological and behavioral disorders in bees and increased honey bee colony losses.

The results showed that the concentrations of the supplement used in this study also affected the palatability of the sugar syrup for bees. The treatments fed sugar syrup containing high concentrations of the supplement showed little tendency to consume of the syrup. Based on the results obtained, the highest palatability was observed in the treatment fed sugar syrup containing 0.75 g/L of the supplement. On the other hand, the lowest palatability occurred in the treatment receiving sugar syrup containing 1.25 g/L of the supplement. This finding indicates that the concentration of this supplement plays an important role in the palatability of the sugar syrup, as well as in the health of bees and apiary performance. Therefore, determining the optimal concentration of the supplement is essential, and based on the results of the present study, the highest palatability was observed in sugar syrup containing 0.75 g/L of the supplement.

In conclusion, the results showed that the addition of the chelated mineral supplement to the diet of honey bees had significant and favorable effects ($p<0.01$) on honey production, population (adults and brood), pollen collection, palatability, aggressive behavior, and calmness behavior in the experimental colonies. The best performance was observed in colonies fed sugar syrup (1:1) containing 0.75 g/L of the chelated mineral supplement. Therefore, administering 0.75 g/L of the chelated mineral supplement in sugar syrup is recommended as an effective nutritional strategy to enhance key behavioral and performance traits of honey bee colonies and to improve the economic efficiency of apiaries, particularly during periods of nectar and pollen scarcity in nature.

Authors' Contributions

A.R.: conceptualization, methodology, software, formal analysis, validation, investigation, data curation, writing-original draft, project administration, supervision.
L.Z.: methodology, validation, writing-original draft.

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