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Spirulina platensis improves the productive performance of honeybees (*Apis mellifera L.*) colonies

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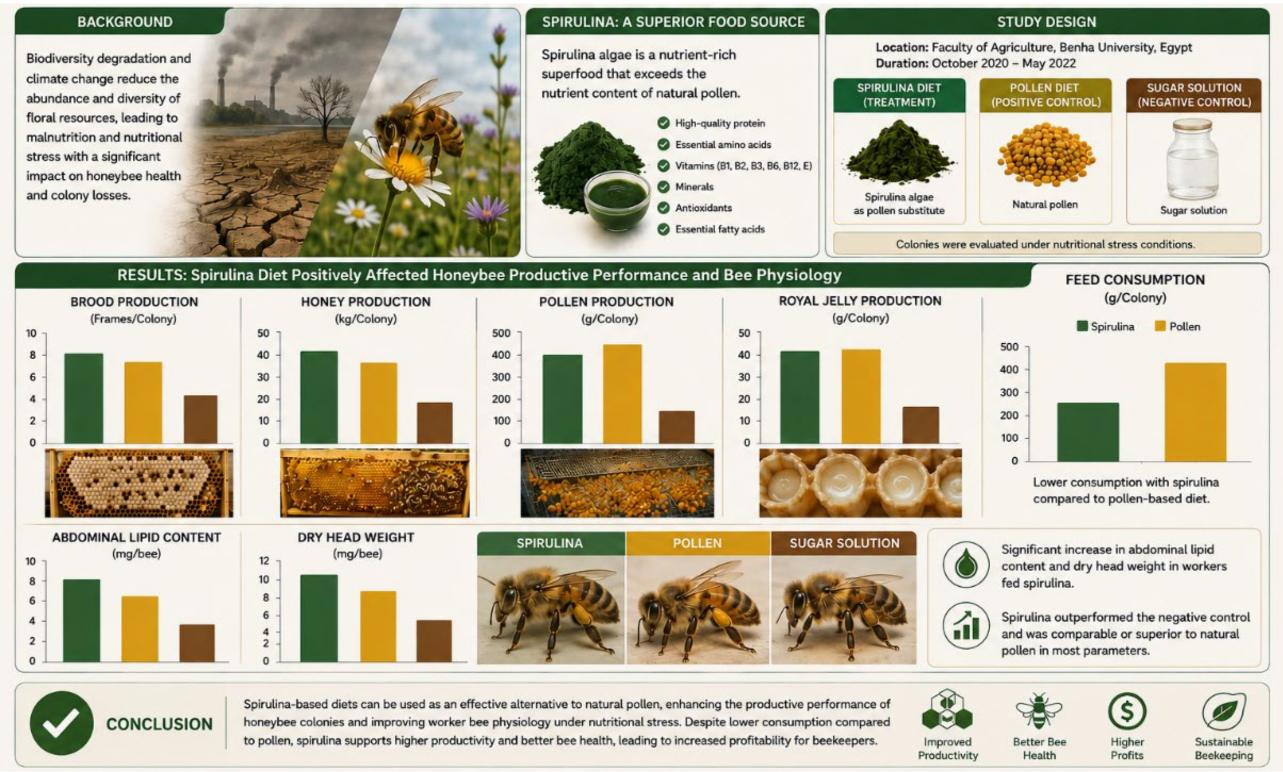
Abstract

Biodiversity degradation and climate change are factors that have a significant impact on the abundance and diversity of floral resources, leading to malnutrition, nutritional stress, honeybee health and colony losses. Spirulina algae is a good food source that can provide higher levels of certain nutrients than some natural pollen sources. This study evaluated spirulina as a pollen substitute for honey bee colonies under nutritional stress and compared its effects with those of natural pollen and a sugar solution control, from October 2020 to May 2022 at the Faculty of Agriculture, Benha University, Egypt. Results showed improved colony productivity, including brood production and yields of honey, pollen, and royal jelly, despite decreased consumption compared to a pollen-based diet (120.65±0.93 and 383.52±2.51g/colony, respectively). Furthermore, a significant increase in abdominal lipid content and dry head weight was observed in worker bees fed spirulina, compared to the positive and negative control groups. Hence, spirulina-based diets can serve as an alternative to pollen, enhancing honeybee productivity and increasing profits.

Keywords: Abdominal lipid content, *Apis mellifera*, Colony performance, Pollen substitute, *Spirulina platensis*

Graphical Abstract



INTRODUCTION

Honeybees (*Apis mellifera* L.) play a crucial role in conserving biodiversity and supporting global food production, as they are among the most important of the insect pollinators worldwide (Topitzhofer et al. 2019). Beyond this, they produce honey, beeswax, royal jelly, and propolis, which have numerous medicinal benefits (Edo et al. 2023). Therefore, honeybee conservation is important for maintaining food security and biodiversity (Patel et al. 2021, Van der Sluijs and Vaage 2016). Beekeepers suffer heavy colony losses due to various stressors, especially during periods of food scarcity (Ricigliano et al. 2022). Therefore, food scarcity is a major factor leading to impaired immunity and increased susceptibility to pests and diseases (López-Uribe et al. 2020), and it negatively affects brood production and colony survival (Brodschneider and Crailsheim 2010, Di Pasquale et al. 2010).

Basically, honeybees need flower nectar and pollen for complete nutrition (Noordyke and Ellis 2021). Nectar provides carbohydrates, and pollen is the main source of protein, minerals, and vitamins necessary to feed individual bees at different stages within the colony (Brodschneider and Crailsheim 2010). Proteins are necessary to maintain the life of honeybees because of their great role in strengthening their immune system (Sagona et al. 2022), also, helps in the development of hypopharyngeal glands (Watkins de Jong et al. 2019), which increases the production of royal jelly from these colonies (Chen et al. 2002). Furthermore, protein is necessary for brood rearing in honeybee colonies, which in turn supports worker density and, consequently, honey and pollen production (Ghazala and Nowar 2013).

A diversity of floral resources provides honey bees with a balanced diet of essential amino acids, lipids, and carbohydrates. On the other hand, poor nutrition resulting from the lack of availability or diversity of floral resources (monoculture) is one of the factors associated with the loss of honeybee colonies (Hristov et al. 2020, Stephen et al. 2024), it also weakens the immune systems of bees (Wheeler and Robinson 2014), making them more susceptible to diseases and parasites (Alaux et al. 2012, Di Pasquale et al. 2013). During dearth periods of natural pollen is scarce, beekeepers provide honeybee colonies with pollen substitutes comprised of proteins derived from animal (such as eggs or milk), microbes (such as brewer's yeast) and plants (such as soybean, corn, or wheat germ) sources (Elwakeil et al. 2025, Kim et al. 2024, Noordyke and Ellis 2021, Paray et al. 2021), these strategy is now common in commercial beekeeping, especially for maintaining brood rearing and colony strength during winter or periods of pollen scarcity.

Pollen substitutes should be inexpensive and easy to feed colonies through beekeeping practices, but they must be palatable to bees and beneficial to bees.

health (Noordyke and Ellis 2021). As a result, the microalgae *Chlorella sorokiniana* and *Spirulina platensis* (*Arthrospira platensis*) have recently emerged as promising food alternatives to pollen due to their high macronutrient content and bioavailability (Jehlík et al. 2019, Ricigliano 2020). Microalgae are used as a food source for humans (Caporgno and Mathys 2018) and insects (Qiu et al. 2019), especially honeybees, such as spirulina (Ricigliano and Simone-Finstrom 2020, Khan and Ghramh 2022) for their high content of protein 60-70% (Soni et al. 2017) and lipids including polyunsaturated fatty acids such as linoleic and alpha-linolenic acids (Ryckebosch et al. 2014, Xue et al. 2018), which are essential for honeybees (Arien et al. 2015). Studies have shown that they can serve as sustainable protein sources for bees due to their easy digestibility and amino acid richness (Khan and Ghramh 2022, Ricigliano 2020, Ricigliano and Simone-Finstrom 2020).

However, at the colony level, its effect as a feed supplement for enhancing the productive performance of honey, pollen, and royal jelly from these colonies has not been widely examined. Only one study has evaluated the effect of spirulina on brood production in commercial colonies (McMenamin et al. 2023). Due to the importance of protein diets for honeybee colonies nutrition, the main aim of this study to address this gap by investigating the influence of spirulina (*A. platensis*) as a promising feed alternative to pollen for honeybees by studying its effect on HPG development, abdominal lipid content of workers, and its effect on the productivity status (brood, royal jelly, honey, and pollen) of honeybee colonies.

MATERIAL AND METHODS

Honeybee colonies

Nine colonies of *A. mellifera carnica* L. headed by newly mated queens were selected from the apiary of the Faculty of Agriculture at Moshtohor, Benha University, Egypt (Elev. 52 ft., 30°21'22.4"N and 31°13'21.5"E), and were used from October 2020 to May 2022. These colonies were equal in strength (consisting of five combs covered with bees, three combs of sealed brood and two combs of honey and bee bread). All colonies were treated against Varroa mites with Mitac 20% (acaricide) and essential oils (peppermint, camphor, and garlic) at a 1:1 ratio and also maintained according to standard beekeeping techniques.

Diet preparation and chemical composition

Used pollen that was collected with pollen traps from a commercial apiary in Moshtohor, Toukh, Qalyoubia Governorate, Egypt, and dried in a drying oven at 50°C and then ground. Spirulina powder produced by Agriculture and Biology Research Institute, Fertilization Technology Department, National Research Center, Dokki, Cairo, Egypt. Spirulina and pollen diets were prepared by mixing between raw materials in the proportions shown in Table 1. Nutritional analyzes were performed on diets to estimate total protein, and lipids contents according to the methods (AOAC 2005).

Table 1. Raw ingredients and chemical composition of diets

Diets	Spirulina powder (g)	Pollen powder (g)	Soybean flour (g)	Powdered sugar (g)	Honey (ml)	Glycerin (ml)	Total protein (%)	Total lipid (%)
Spirulina patty	20	--	30	30	20	5	21.88	3.66
Pollen patty	--	20	30	30	20	5	17.5	4.29

Experimental design

In this study, nine honeybee colonies were selected and divided into three groups (three colonies for each treatment). The first group was fed a spirulina diet, the second group was a positive control fed a pollen patty, and the last group was not supplied with a protein diet as a negative control. All colonies were provided weekly with 1 liter of 50% sucrose solution, from October 2020 to May 2022. In all experimental colonies, biological measures were applied, including areas with sealed brood of workers, honey, and bee bread, before the start of the experiment, and these conditions were fairly equal across colonies.

- **Parameters**

1. Consumption of diets

Weekly, the colonies under study were provided with 150g of protein diets per colony above the brood nest on the combs' top and covered with a polyethylene sheet to prevent drying, from October 2020 to May 2022. Throughout the experiment, the amount of diet consumed by each colony was calculated as the difference in diet weight before and after feeding (g/colony/week).

2. Workers sealed the brood area

Throughout the experiment, at 13-day intervals, both sides of each brood comb were measured using a Langstroth frame, divided into square inches according to Jeffree (1958) and Büchler et al. (2013).

3. Abdominal lipid content (ALC) and dry head weight (DHW)

For this purpose, at the end of the experiment, 45 adult worker bees from each treatment (15 per colony) above unsealed brood cells were collected in glass vials and transported to the laboratory in a refrigerated container. The workers were dissected to separate the heads and abdomens. Measurements were performed in triplicate.

The abdomens were collected and the abdominal lipid content was tested by following the ether washing protocol (Chakrabarti et al. 2020). Each sample of 15 abdomens was dried at 45°C for 72 h in a drying oven to reach a stable dry mass and then weighed (WBE). After that, the lipid was dissolved by gently shaking each sample of 15 abdomens in 4.5 ml of ethyl ether for 24 h on a microplate shaker, dried for 3 days, and weighed again (WAE). The abdominal lipid content (mg/g) was calculated according to the formula described by (El-Ghabawy et al. 2022): ALC (mg/g) = $\frac{WBE}{WAE} \times 1000$.

While the heads were used to estimate the development of the hypopharyngeal gland (El Ghabawy et al. 2022). Samples were placed in a drying oven at 50°C for 48 h to determine the dry weight.

4. Honey yield

The honeycombs were pulled from the experimental colonies during the citrus and clover seasons of 2021 (April and June, respectively) and weighed before and after honey extraction. The extracted honey was estimated and recorded as kg per colony.

5. Pollen production

Wooden pollen front traps were fixed in all colonies under study for pollen gathering during the corn season 2021 (from 15 July to 30 August). They were left to collect the pollen for three days and removed for another three days. During this period, the trapped pollen grains were collected from the traps daily for three days, then weighed and recorded as grams per colony.

6. Royal jelly production

All colonies in each group were orphaned (queens were removed from their colonies for 5 days) only once in April 2022 to estimate royal jelly production using the dry grafting method as described by Doolittle (1909). One frame containing 54 cup cells was grafted with worker larva at the age of 24-36 h from each colony, then all the grafted frames were distributed and placed in the middle of the colony for three days, then at the end of the third day of grafting the number of accepted cups in each colony was recorded and the royal jelly was collected, weighed, and recorded as g/colony.

Statistical analysis

Using SPSS version 27 (IBM Corp., Released 2013), a two-way ANOVA was conducted to compare the feed consumption rates of spirulina and pollen and their effects on workers' sealed brood area. Also, used one-way ANOVA to compare its effects on dry head weight, abdominal lipid content, honey yield, pollen production and royal jelly production. The analysis was conducted under a completely randomized design, as detailed by Steel et al. (1997). For multiple comparisons, the Duncan test was used at a significance level of $p < 0.05$ to determine significant differences between group means. Results are presented as mean ± standard error of the mean (SEM). Visualizations were created using Microsoft Excel.

RESULTS

Complementing previous studies that used spirulina with honeybees, our study evaluated spirulina-based diets at the colony level and their effects on colony productivity.

Table 2: Effect of proteinaceous diets (spirulina and pollen) on colony performance and productivity of honeybee (mean±SE)

Patties	Diet consumption (g/colony)	Workers sealed brood area (inch^2/colony)	Honey yield (kg/colony)		Pollen production (g/colony)	Royal jelly production	
			Citrus season	Clover season		(mg/cell cup)	(g/colony)
Spirulina patty	120.65±0.93^{b}	678.34±25.59^{a}	5.48±0.57^{a}	6.59±0.47^{a}	566.37±63.12^{a}	304.00±15.37^{a}	10.55±0.71^{a}
Pollen patty	383.52±2.51^{a}	477.14±26.10^{b}	6.09±1.22^{a}	6.82±1.46^{a}	462.83±68.38^{a} ^{b}	297.67±25.98^{a}	8.90±0.73^{a}
Control	Not-diet	355.81±2.60^{c}	2.05±0.57^{b}	3.43±0.36^{b}	338.37±74.68^{b}	231.67±18.10^{a}	2.84±0.21^{b}
LSD at 0.05	7.44	83.05	3.32	3.57	55.36	79.78	2.35

The different small letters within each data represent statistically significant differences ($p<0.05$)

Feed consumption rate

Significant differences in consumption were observed between the two diets over 18 months ($P < 0.05$; Table 2 and Figure 1$_b$). The total mean of cumulative consumption was greater for pollen patty (383.52±2.51g/colony) than spirulina patty (120.65±0.93g/colony). Also, diet consumption was significantly different during the summer, winter, and spring feeding periods ($P < 0.05$). During these periods, the consumption was lower in summer than in late winter and spring (Figure 1$_a$).

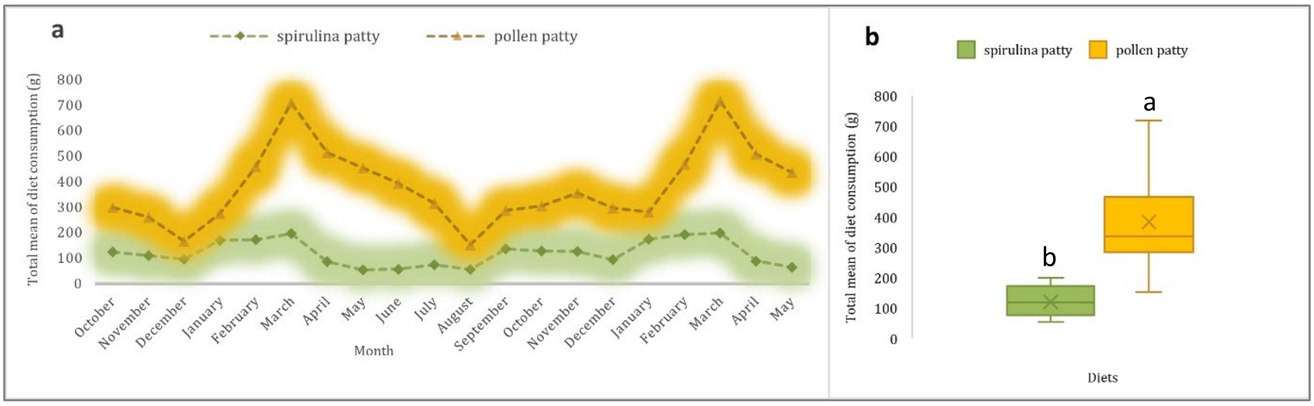


Figure 1. Mean weight (g) of protein diet consumed by treated honeybee colonies. (a), The mean weight of two diets (spirulina and pollen) consumed by a honeybee colony per month. (b), The cumulative weight (mean ± SE, n=3) of diet consumption by honeybee colonies over the time of observation. The different small letters within each data represent statistically significant differences ($p<0.05$).

Effect of protein diets on sealed brood area

Brood rearing is an indicator of individual population growth in honeybee colonies and production of newly emerging bees, followed by the highest individual population density in honeybee colonies. Data presented in Table 2 and Figure 3 show the sealed brood area of colonies fed with protein diets (spirulina and pollen) as compared to colonies fed

with sugar syrup only. These data revealed that spirulina increased brood production in honeybee colonies. Also, significant differences were shown among colonies fed protein diets and the control, as well as among colonies fed spirulina and pollen diet (spirulina diet>pollen diet>control, 678.34±25.59, 477.14±26.10 and 355.81±2.60 inch$_2$/colony, respectively, $p<0.05$; Figure 2$_b$).

On the other hand, results also demonstrated that the brood area pattern increased to a maximum during the experimental period in the spring and summer seasons, then decreased during the fall and was at its lowest in the winter (Figure 2$_a$).

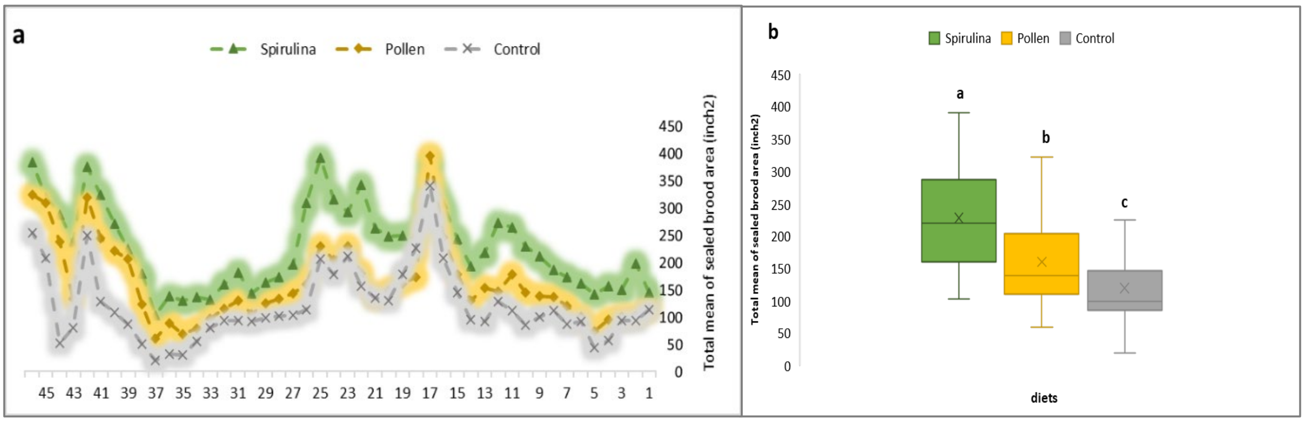


Figure 2. Total mean of sealed brood area in treated honeybee colonies. (a), Proportional change of sealed brood area in honeybee colonies (mean ± SE, n=3) fed with protein diets (spirulina and pollen) every 13 days. (b), The cumulative mean of sealed brood area in honeybee colonies (mean±SE) fed with protein diets over the time of observation. The different small letters within each data represent statistically significant differences (p<0.05).

Abdominal lipid content (ALC)

At the end of the experiment, bees supplemented with a spirulina diet had a significantly higher average of abdominal lipid content than those fed with pollen patty and control group bees (220.28±8.52, 134.48±26.48, and 115.08±18.22 mg/g, respectively, p>0.05; Figure 3).

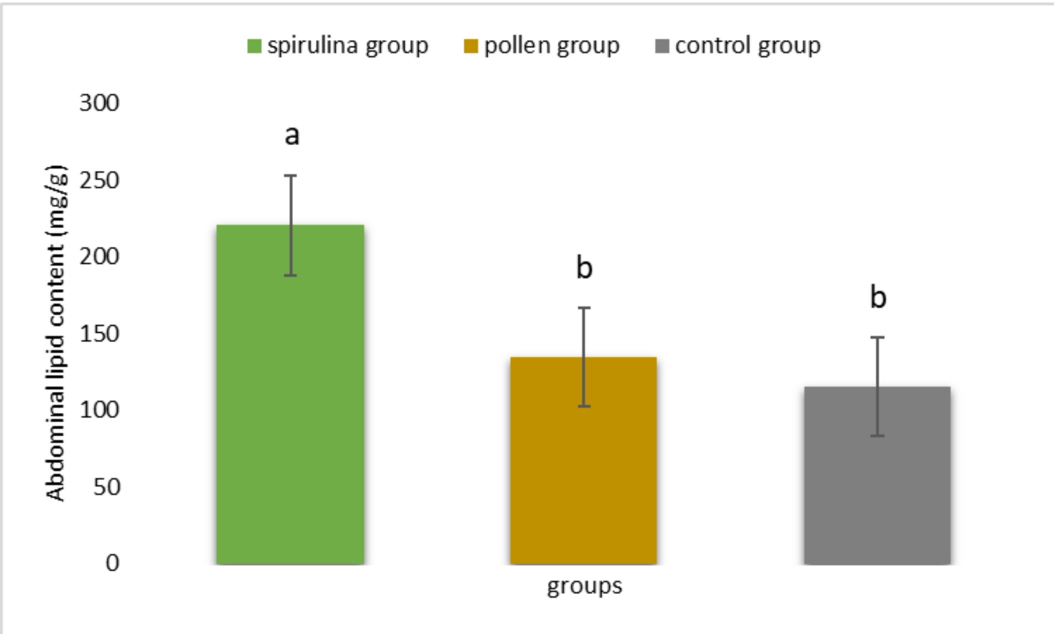


Figure 3. The average of abdominal lipid content in honeybee workers was supplied with protein diets (mean ± SE, n=3). The different small letters within each data represent statistically significant differences (p<0.05).

Dry head weight (HPG development)

Significant differences were found between the dry head weight of worker honey bees fed with protein diets over 18 months (P <0.05; Figure 4). The highest dry head weight was found in bees fed spirulina patty (8.7±0.887 mg/bee), followed by bees fed pollen patty (6.77±0.539 mg/bee). The lowest dry head weight was observed in the control (4.82±0.492 mg/bee).

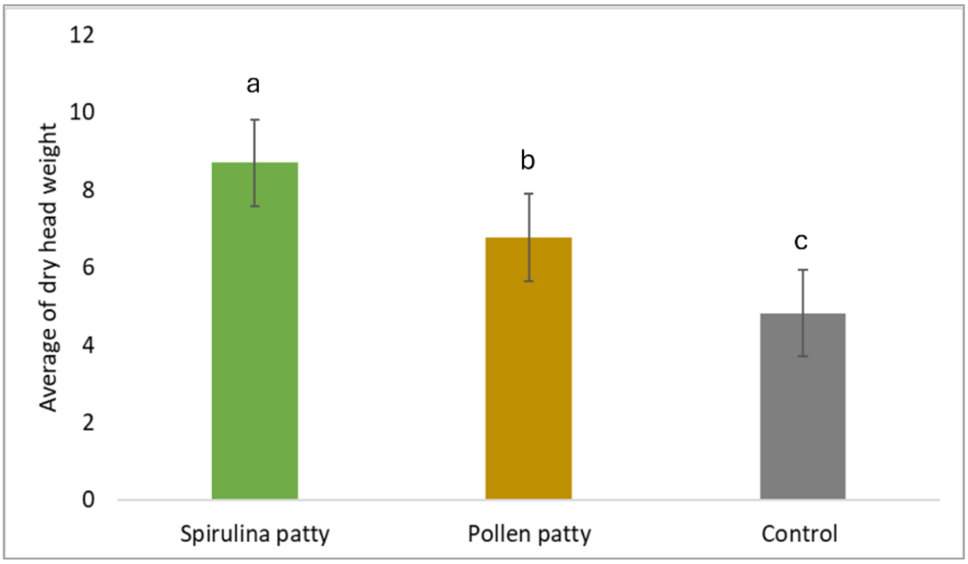


Figure 4. The average dry head weight in honeybee workers was supplied with protein diets (mean ± SE, n=3). The different small letters within each data represent statistically significant differences (p<0.05).

Productivity performance of honeybee colonies

The productivity status of honeybee colonies can be impacted by a variety of factors such as poor diet, which weakens colonies. Relative to honey yield, our findings showed significant differences in the honey wight among treated colonies with protein diets and control (p< 0.05) and no significant differences among treated colonies with spirulina and pollen patties (P<0.05), during citrus and clover seasons, were 5.48±0.57 and 6.59±0.47, 6.09±1.22 and 6.82±1.46, and 2.05±0.57 and 3.43±0.57 kg/colony, in spirulina, pollen and control groups, respectively, (Table 2 and Figure 5$_a$). In the same trend, the highest rate of pollen production was in colonies fed with spirulina patty, followed by those fed with pollen patty, and the lowest production was in the control group, which was 566.37±63.12, 462.83±68.38, and 334.37±74.68 g/colony, respectively (P<0.05, Table 2 and Figure 5$_b$).

Also, the highest mean weight of royal jelly for cell cup was in the colonies fed with spirulina patty, followed by pollen patty, then the control was 304.00±15.37, 297.67±25.98, and 231.67±18.10 mg/cell cup, respectively (P<0.05, Table 2 and Figure 6$_a$). Relative to production of royal jelly in gram per colony, there were significant differences among the colonies fortified with protein diets and control (p<0.05), but there were no significant differences between them (P>0.05), were 10.55±0.71, 8.90±0.73, and 2.84±0.21 g/colony in colonies fed with spirulina patty, pollen patty, and control, respectively, (Table 2 and Figure 6$_b$).

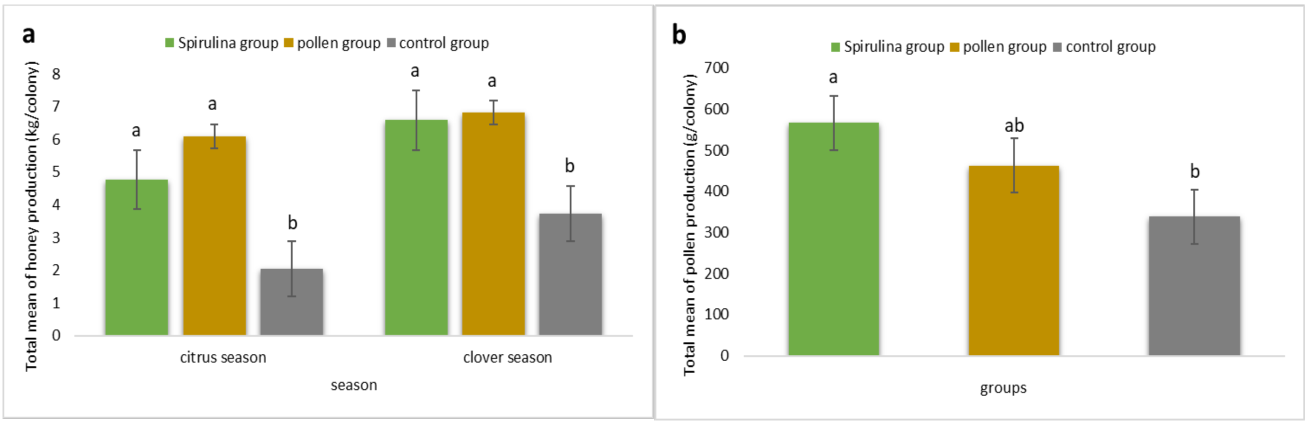


Figure 5. The total mean of honey yield and pollen collection weight (mean ± SE, n=3) by treated colonies with various diets. (a), The total mean of honey yield weight during the citrus and clover seasons. (b), The mean collected pollen weight during the maize season. The different small letters within each data represent statistically significant differences (p<0.05).

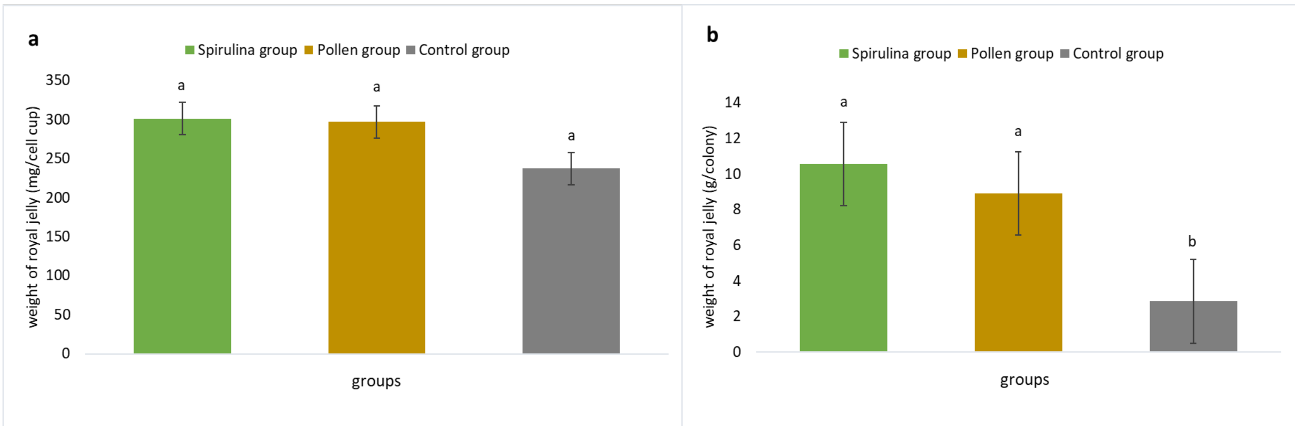


Figure 6. The total mean of royal jelly production weight (mean ± SE, n=3) by treated colonies with various diets. (a), The total mean of royal jelly production weight in mg per cell cup. (b), The total mean royal jelly production weight in grams per colony. The different small letters within each data represent statistically significant differences (p<0.05).

DISCUSSION

Consumption rate is an important metric in studies of feed comparison science, the amount of diet consumed determines the pool of available nutrients (Ricigliano et al. 2022b). In our study, a statistically significant difference in consumption was observed between the two diets over the observation period. The total mean of cumulative consumption was highest in pollen patty (383.52±2.51g/colony) (p>0.05) than spirulina patty (120.65±0.93g/colony), which is consistent with the observation of previous studies that bees consumed less spirulina in similar experiments (Ricigliano and Simone-Finstrom 2020, Ricigliano et al. 2022b) compared to natural and alternative pollen. This may be due to the higher protein content of the spirulina patty compared to the pollen patty, as well as the bees' greater palatability of pollen than spirulina. Moreover, this finding contradicts the finding reported in a previous study on chlorella (*C. sorokiniana*), a microalgal species (Jang et al. 2022), which found that consumption of chlorella-supplemented patty was significantly higher than that of rapeseed pollen patty. The presence of biostimulants or pollen as a stimulant may help bees consume more pollen-supplemented forage (Schmidt 1985). This explains the higher consumption of pollen patty than spirulina patty in our study.

On the other hand, seasonal variation and the availability of floral resources also influence the nutritional preferences of honeybees (Zasukha et al. 2022), leading them to adjust their food consumption accordingly. Our results indicate that the consumption rate of protein diets was lower in summer than in late winter and early spring (Figure 2_a), this is supported by findings (Khan et al. 2021, Norrström et al. 2021) that bee colonies consume a greater amount of food in the winter compared to the summer season; this is due to the local conditions of the study area, such as the scarcity of natural food sources in the winter periods compared to the summer.

Brood rearing is an indicator of individual population growth in honeybee colonies and production of newly emerging bees followed by the highest individual population density in honeybee colonies. The growth of colonies (sealed brood area) treated with protein diets (spirulina and pollen) was measured compared to colonies fed sugar syrup only. This protein supplementation is necessary for the worker bees to produce royal jelly, which is essential for feeding the young larvae. In our study, the results highlighted positive effects of a spirulina-based diet on brood rearing (Table 2 and Figure 3). This is consistent with the results of previous studies demonstrating that algae supplements increase brood production in a honey bee colony (Nichols and Ricigliano 2022), in an investigation by (Cebotari et al. 2017) beehives fed sugar paste containing the water algae *Oocystis borgei* Snow demonstrated a significant increase of 8%, whereas beehives fed sugar paste containing spirulina had an increase in a capped brood of 6.5% in comparison with controls.

Additionally, the sealed brood area showed a significant increase in colonies fed with paste supplemented with the alga *Scenedesmus quadricauda* (Cebotari et al. 2016), sugar syrup containing *C. vulgaris (Eremia et al. 2013),* and sugar syrup containing spirulina extracts (Toderaş et al. 2014) compared with control colonies. Also observed that colonies fed spirulina pancakes containing honey increased brood area by 54.2% compared to unfed controls (Kumar et al. 2013). On the other hand, the brood area increased to a maximum during the experimental period in the spring (Figure 3_a). This is consistent with previous results that showed that bee colonies that were fed with *C. sorokiniana* paste had a significant increase in spring brood rearing compared to control colonies (Jehlík et al. 2019). This may also be explained by the availability of a natural nectar supply from the abundant citrus orchards in the vicinity of the study area, which bloom in spring.

The adipose body is responsible for immunity, the production of antimicrobial molecules, and the storage of nutrients in bees (Arrese and Soulages 2010). Also, the amount of abdominal lipids reflects the effectiveness of the protein diets studied. The body's most lipid-rich organ is the abdomen, which suggests it may contribute to the body's defense against dehydration and starvation (Masuzzo and Royet 2018). Results showed that bees supplemented with a spirulina diet had a significantly higher average abdominal lipid content than bees fed a pollen patty or the control group. Corby-Harris et al. (2019) did not find a relationship between feed intake and fat body percentage, but they did find that a lower fat body percentage is associated with lower food quality. Our findings are consistent with those of El-Ghabawy et al. (2022), who found that bees fed protein diets had higher abdominal lipid content than the control group. Moreover, body fat mass in the abdomen was affected by diet, being higher in bees fed pollen and dry spirulina than in controls (Ricigliano and Simone-Finstrom 2020). Also, *C. sorokiniana*-fed adult bees had 1.7 times more body fat and 5.6 times more adipose body fat in their larvae (Jehlík et al. 2019); providing bees with chlorella increased fat body mass compared to the control (Jang et al. 2022). This suggests that spirulina is effective in supporting abdominal lipid storage in bees.

Changes in the dry weight of a bee's head reflect the development of glands located in its head (Hrassnigg and Crailsheim 1998). The hypopharyngeal gland is one of the most important glands in the heads of young worker bees, responsible for secreting royal jelly (Hu et al. 2019). Our results indicate that bees fed protein-rich diets with superior spirulina intake had the highest average dry head weight compared to the control group. In a similar study, El-Ghabawy et al. (2022) reported that bees fed azolla protein and pollen had higher head weight than the control group. Also, bees fed a 4:1 essential amino acid ratio had higher wet and dry head weights than the control (Hendriksma et al. 2019). Overall, the total weight of bees, or their different body parts, is affected by protein feeding and decreases when they are not fed pollen until the 7th day after adulthood (Brodschneider et al. 2022).

The production of honey and pollen in honeybee colonies can be impacted by a variety of factors such as inadequate diet, which weakens colonies. Comparison of colony productivity status under different nutritional regimens. Relative to honey yield, our findings showed significant differences in honey weight among treated colonies with protein diets and the control ($p < 0.05$), and no significant differences among treated colonies with spirulina and pollen patties ($P>0.05$), during citrus and clover seasons. These findings are consistent with several studies indicating that honey production in bee colonies supplemented with algae products increased by 38.7% in colonies fed on paste containing *Oocystis borgei* Snow (Cebotari et al. 2015), 27.6% in colonies fed with extracts of *Scenedesmus quadricauda* (Cebotari et al. 2016), 28.8% in colonies fed on pastes of *O. borgei* Snow, *S. apiculatus*, *S. quadricauda*, and *S. plantensis* (Toderash et al. 2017), 17.2% in colonies supplemented with *C. vulgaris* (Eremia et al. 2013), 12.1% in colonies fed on sugar syrup and chlorella suspension (Eremia et al. 2021), compared to control groups. In addition, the honey production rate increased in colonies fed with protein diets compared to the control that was fed with sugar syrup only (Ahmad et al. 2021).

Also, the honey storage area was greater in colonies fed with pollen substitutes compared to the control (Khan et al. 2022).

On the other hand, relative to pollen production, the results showed that the highest rate was in colonies fed a spirulina patty, followed by those fed a pollen patty, and the lowest in the control group. These results are in agreement with the findings by Ahmad et al. 2021) and Khan et al. 2022) that bee colonies supplemented with protein diets produced pollen in greater quantities than control colonies that were fed with sugar syrup only. This explains spirulina's role in supporting the production of high-density worker bees in treated colonies, which collect large quantities of honey and pollen.

Royal jelly production in honeybee colonies is influenced by various factors such as pollen trapping, cell acceptance rate, and the strength of the production unit. The acceptance rate of grafted queen cups is significantly impacted by pollen entrapment of honeybee colonies, and royal jelly output is decreased (Mohanny et al. 2022). Also, dietary protein levels significantly affect hypopharyngeal gland growth and royal jelly production (Wang et al. 2011). There were no significant differences in the average weight (mg per cell cup) of royal jelly among the experimental colonies; the highest average weight was in the colonies fed with spirulina patty, followed by pollen patty, then the control (Figure 7$_a$). Relative to the production of royal jelly in grams per colony, there were significant differences among the colonies fortified with protein diets and control ($p<0.05$), but there were no significant differences between them ($P>0.05$; Table 2 and Figure 7$_b$).

These findings are consistent with results showing that the different treatments significantly affected royal jelly production, with the control treatment having the lowest production rate (Nemati et al. 2022). Moreover, (Wang et al. 2011) found that a protein level of 30% has the best positive effects on hypopharyngeal gland development and royal jelly production compared to control and higher levels of dietary protein. Also, the diet supplemented with chlorella increased the amount of 10-HDA in royal jelly compared to the control (Jang et al. 2023). This indicates the effectiveness of spirulina in supporting the development of the hypopharyngeal gland and the final production of royal jelly from colonies. Further studies are needed to assess the quality of the royal jelly produced.

Finally, in practice, the bees' consumption of spirulina in commercial bee colonies was lower than their consumption of pollen, likely because pollen is more palatable and serves as their natural food source. Economically, in this study, spirulina costs as much as pollen, but the quantity used was significantly less (approximately 1/3 of the pollen quantity). Therefore, considering the results achieved, it is cost-effective. In the future, it is recommended to mix natural pollen with spirulina-based diets to increase bee consumption. Overall, after providing a spirulina diet from October 2020 to May 2022, a positive effect was observed on the productive performance of honeybee colonies in terms of the production of brood, honey, pollen, and royal jelly, in addition to hypopharyngeal glands and an increase in abdominal lipid content despite the lack of consumption. According to the results of this study, we concluded that spirulina enhances the productive performance of honeybee colonies.

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