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RESEARCH ARTICLE

Vital responses of caged honeybee (*Apis mellifera*) workers fed on new substitute diets in the laboratory

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Abstract

Malnutrition is a main threat to honey bees. Health and the inability to resist adverse conditions are the main losses resulting from nutritional deficiencies. To address this problem, substitute diets are provided to honey bee colonies at dearth times. The present study was conducted to examine the vital impacts of 2 new substitutes on caged honey bee workers. The main components of both diets (A and B) were sugar, powdered milk, and brewer's yeast fortified with chamomile extract. Diet A was distinguished with chickpeas and corn. Diet B was distinguished with soybeans and wheat germ. Palatability of both diets has been reported in this study with more tendencies towards soybeans and wheat germ components. In comparison with control workers fed on sugar syrup only (1:1), workers fed on diets A and B have shown higher significant survival rates, in addition to outstanding findings of thoraces weights and mandibular and hypopharyngeal glands development. Accordingly, palatability and nutritional diversity in diet proteinaceous sources should be considered in provided substitutes. Diets A and B are highly recommended to be applied in apiaries.

Keywords: *Apis mellifera*; Honey bees; Hypopharyngeal glands; Mandibular glands; Nutrition; Survival.

1. Introduction

Honeybees provide nature with priceless gifts. Plant pollination process comes first, as well as production of honey, royal jelly, and propolis. Despite their adaptive flexibility and ability to exist in a variety of habitats, climatic conditions, and flora, honey bees cannot keep up with growing demands (Aizen and Harder, 2009; Ruttner, 2013; Ahmad *et al.*, 2020; Hristov *et al.*, 2020; Jagdale *et al.*, 2021). Multiple natural and anthropogenic stressors cause notable declines in honey bee populations. Pathogens, pests, and pesticides are examples of these stressors (Ansari *et al.*, 2017; Belsky and Joshi, 2019; Iqbal *et al.*, 2019). Climatic changes also cast a shadow on the vegetation cover and negatively affected the availability of nutritive flowers. Resulted malnutrition has exacerbated the deterioration of honey bee viability (Le Conte and Navajas, 2008; Simone-Finstrom *et al.*, 2016; Phillips *et al.*, 2018).

Nutrition is the key for honey bees to maintain their health and to resist adverse circumstances (Brodschneider and Crailsheim, 2010). Accordingly, honey bee colonies are provided with fortified substitute diets in order to replenish food sources. Substitute diets have proven their effectiveness on honey bees viability including colony performance, survival rates, muscular strength, mandibular glands sizes and hypopharyngeal glands development (Hassan and Khater 2006; Pirk *et al.*, 2010; Ayoub *et al.*, 2015; Alhousini, 2020; Abdel-Rahman *et al.*, 2021 Khalid *et al.*, 2022).

The objective of this study is to apply new economic formulated diets, investigate their

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nutritional effects and discuss results in the context targeted optimal nutrition for honey bees.

2. Methodology

2.1. Date of work

Methodology was carried out between April 2022 to June 2022.

2.2. Diet preparation

Two diets (A, B) were prepared by commercial economic ingredients at determined ratios as shown in figures 1, 2. Diet A was distinguished with chickpeas (*Cicer arientinum*) and corn (*Zea mays*). Diet B was distinguished with soybeans (*Glycine max*) and wheat germ (*Triticum aestivum*). Percentages of macronutrients were reported for both diets accordimg to USDA 2022 (Table 1).

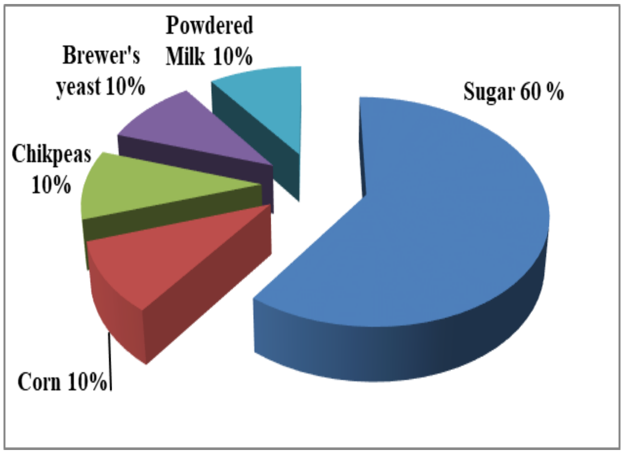

Figure 1. Proportions of diet A components.

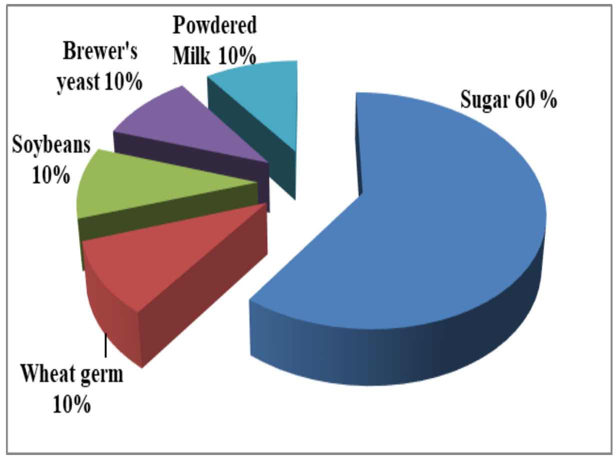

Figure 2. Proportions of diet B components.

Table 1. Percentages (%) of macronutrients of applied diets.

Macronutrients	Diet A	Diet B
Proteins	11.05	9.25
Carbohydrates	75.97	78.7
Fats	1.71	1.25

According to mentioned proportions, diets were blended and kneaded via drops of chamomile extract till they took dough texture. Subsequently, diets were left for 24 h to dry in room temperature.

2.3. Diet effect investigations

2.3.1. Diets preference test in field

At the apiary of South Valley University, three equal strength colonies of hybrid carniolan honey bees were elected to perform preference test. 20 grams of prepared diet pastes were provided to colonies. Consumption rates were calculated after 6 days.

2.3.2. Diets consumption rates in lab

Newly emerged workers were gathered in Insect laboratory, Zoology Department, Faculty of Science, South Valley University. Brood frames were kept overnight at 35 °C in incubator (Heraeus BK 600, Heraeus Instruments, Germany). and then 450 bees were taken and distributed into 3 groups of laboratory hoarding cages. Each cage was 760 ml volume occupied with 50 bees and supplied with holes, syringe filled with water and 2 eppendorf tubes with open ends, one filled with sugar solution (1:1) and the other filled with diet in diets groups or placed empty in control group (Figure 3). Two groups were determined for examined diets confronted with control group fed on sugar syrup only with 3 replicates for each one. Under fixed incubator conditions (35 °C and 55% humidity) and throughout 14 days, 1 gram of fresh diets A and B were replaced daily and consumption rates were determined by calculating the difference between weights before and after consumption.

2.3.3. Survival rates

The experiment has started with 150 individuals distributed into 3 replicates for each group. Survival rates were investigated by daily removing and counting of dead workers among all replicates of each group throughout experiment time (14 days).

2.3.4. Thoraces weights

Ten thoraces were separated from each replicate after experiment time and dried at 102°C in an electric oven (INS, LAB-LINE Instruments, India), After 24 hours, thoraces were weighed and the means for each group were calculated and compared.

2.3.5. Mandibular glands investigations

Mandibular glands were separated from 27 workers (9 from each group) after experiment time and then stained according to (Ayoub *et al.*, 2015) (Figure 4). Size of a mandibular gland (length x width) was recorded for both glands of each worker. The means of mandibular gland sizes for each group were calculated and compared.

Figure 3. Experimental hoarding cages.

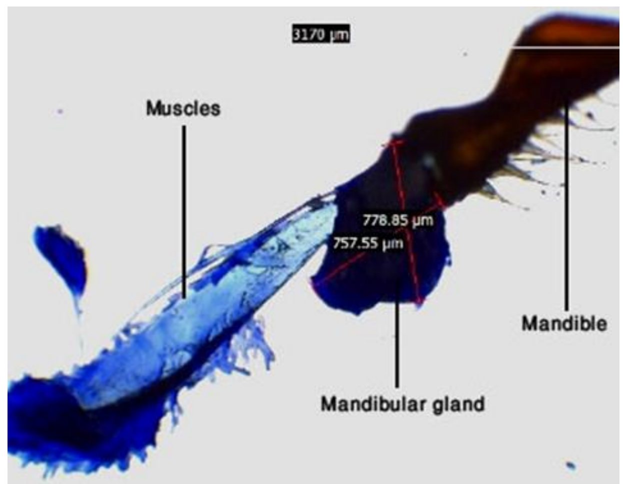

Figure 4. Mandibular gland of a honey bee worker (x 40).

2.3.6. Hypopharyngeal glands investigations

Hypopharyngeal glands were separated from 27 workers (9 from each group) after experiment time and then stained according to Ayoub et al., 2015 (Figure 5). Diameters of 30 acini from both glands of each worker were recorded and their mean was determined. The means of acini diameters for each group were calculated and compared.

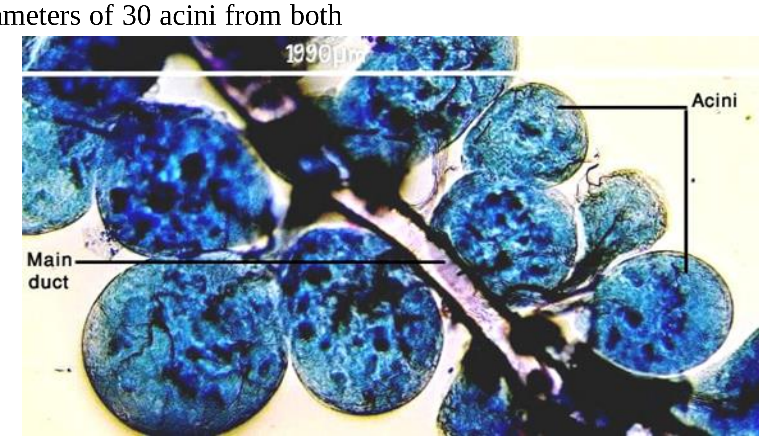

Figure 5. Hypopharyngeal gland of a honey bee worker (x 400).

2.4. Statistical analyses

Raw findings underwent Shapiro-Wilk test and D'Agostino and Pearson test to check normality. Normal data were then analyzed using t tests and one-way ANOVA followed by Tukey tests for multiple comparisons. Kaplan-Meier test was used for analyzing survival rates followed by Log-rank (Mantel-Cox) tests for comparisons. Significant difference was at level $P \leq 0.05$. Statistical analyses were performed via GraphPad Prism 8.0.2 software (2019).

3. Results

3.1. Diets preference test in field

Consumption rates of provided diets ranged from 50% for diet A to 90% for diet B throughout 6 days of test. Means of consumption rates showed more preference for diet B (70%) but with no significant variations when compared with diet A (55%), reflecting more tendency for soybeans and wheat germ components in comparison with chickpeas and corn (Table 2, Fig. 6).

3.2. Diets consumption rates in lab

Both diets A and B have been accepted from caged bees. Means of consumption rates of diets A (8.06%) and B (9.2%) are approximately equal. Statistically, no significant variations were recorded (Table 3, Fig. 7).

Table 2. Consumed amounts (%) of feeding diets after 6 days in apiary:

Colony	Diet A	Diet B
1	55	60
2	50	60
3	60	90
Means % (±SE)	55^{a} (± 2.89)	70^{a} (± 10)

Means followed by same letters are not significantly different (± SE)

Table 3. Consumption rates means (%) per day for (1 gram) provided diets throughout 14 days in laboratory:

Day	Diet A	Diet B
1	22.75 (± 2.02)	21 (± 2.08)
2	25 (± 2.65)	17.33 (± 1.45)
3	21.33 (± 2.03)	16.67 (± 2.73)
4	8.83 (± 1.92)	20 (± 2.51)
5	6.67 (± 2.03)	15.5 (± 1.89)
6	4 (± 1.26)	6.67 (± 1.2)
7	4.83 (± 1.64)	3.67 (± 0.88)
8	3.1 (± 0.95)	2.67 (± 0.88)
9	1.67 (± 0.33)	1.33 (± 0.33)
10	2.33 (± 0.88)	3.33 (± 0.33)
11	5.17 (± 1.3)	1.67 (± 0.67)
12	4.1 (± 0.1)	12 (± 1.15)
13	1.67 (± 0.88)	1.67 (± 0.67)
14	1.33 (± 0.88)	5.33 (± 0.88)
Means	8.06^{a} (± 2.24)	9.2^{a} (± 2)

Means followed by same letters are not significantly different (± SE)

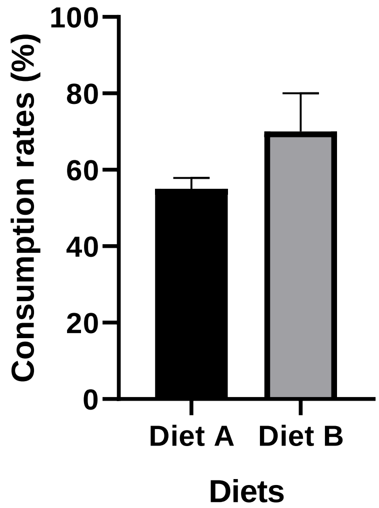

Figure 6. Consumed amounts (%) of feeding diets after 6 days in apiary.

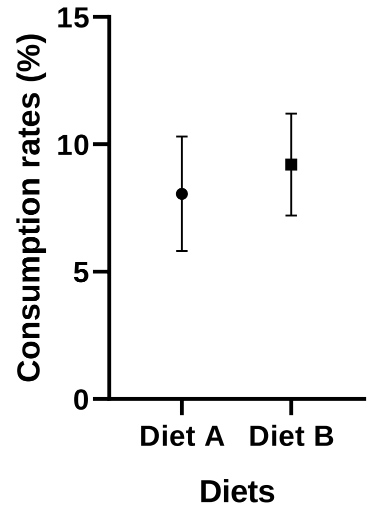

Figure 7. Consumption rates means (%) per day for (1 gram) provided diets throughout 14 days in laboratory.

3.3. Survival rates

Higher survival rates were reported for groups fed on provided diets with significant variations when compared with control group. At day 14, the percent survival values were 92.67% and 89.33% for diets A and B respectively confronted with the lowest value 74% for control group (Fig. 8).

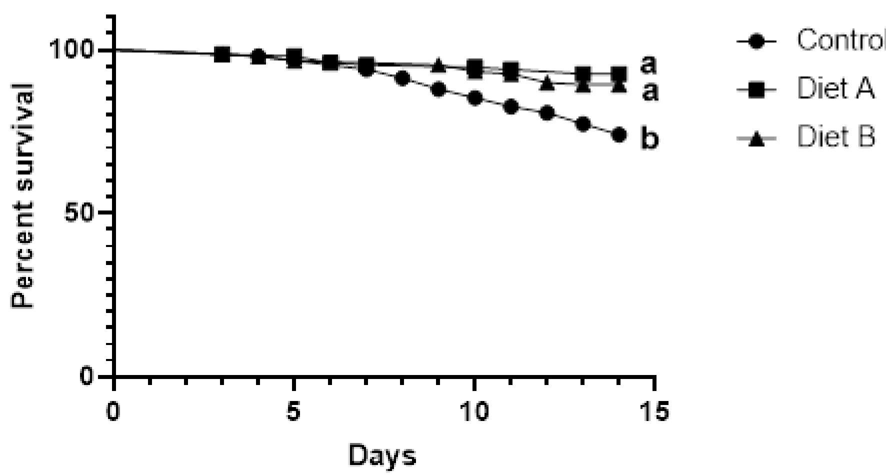

Figure 8. Percent survival for control and fed groups throughout 14 days (same letters are not significantly different).

3.4. Thoraces weights

Higher values of thoraces weights were recorded by means 18.67 mg/bee and 21 mg/bee for workers fed on diets A and B respectively, and 17.67 mg/bee for control group. No significant variations were reported (Table 4, Fig. 9).

3.5. Mandibular gland sizes

The highest mean for mandibular gland sizes was observed for workers fed on Diet A by 0.28 mm^2 followed by workers fed on Diet B by 0.2 mm^2, on the other hand, the lowest mean was observed for control workers by 0.15 mm^2. No significant variations were recorded (Table 5, Fig. 10).

Table 4. Thoraces weights means (mg/bee) of control and fed workers after 14 days of feeding:

Replicates	Control	Diet A	Diet B
1	17	18	23
2	18	19	18
3	18	19	22
Means % (±SE)	17.67^a (± 0.33)	18.67^a (± 0.33)	21^a (± 1.53)

Means followed by same letters are not significantly different (± SE)

Table 5. Means of mandibular gland sizes (mm^2) of control and fed workers after 14 days of feeding:

Replicates	Control	Diet A	Diet B
1	0.17	0.22	0.22
2	0.12	0.3	0.1
3	0.17	0.31	0.28
Means % (±SE)	0.15^a (± 0.02)	0.28^a (± 0.03)	0.2^a (± 0.05)

Means followed by same letters are not significantly different (± SE)

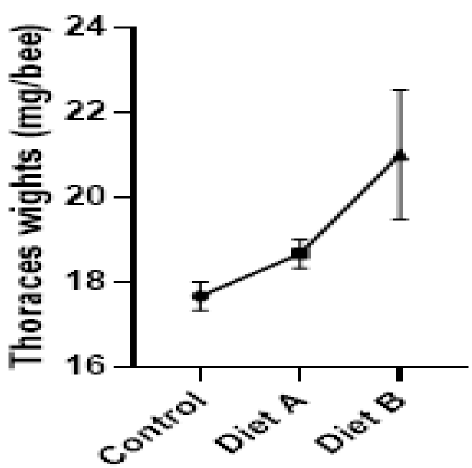

Figure 9. Thoraces weights means (mg/bee) of control and fed workers after 14 days of feeding.

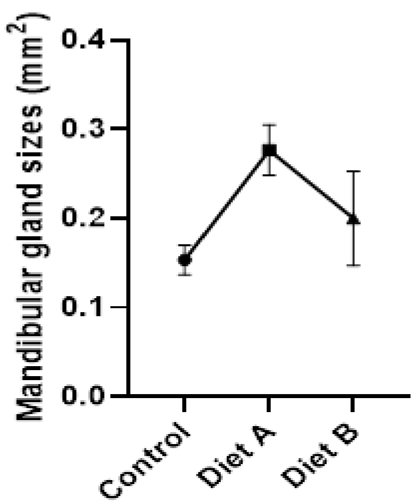

Figure 10. Means of mandibular gland sizes (mm^2) of control and fed workers after 14 days of feeding.

3.6. Hypopharyngeal acini diameters

Although there were no significant variations, higher means of hypopharyngeal acini diameters were noted by 142.5 µm and 108.2 µm for workers fed on diets A and B respectively. The lowest mean was reported for control group by 99.55 µm (Table 6, Fig. 11).

Table 6. Means of hypopharyngeal acini diameters (µm) of control and fed workers after 14 days of feeding:

Replicates	Control	Diet A	Diet B
1	92.13	116.52	92.35
2	92.87	178.99	103.88
3	113.64	132.06	128.28
Means % (±SE)	99.55^a (± 7.05)	142.5^a (± 18.78)	108.2^a (± 10.59)

Means followed by same letters are not significantly different (± SE)

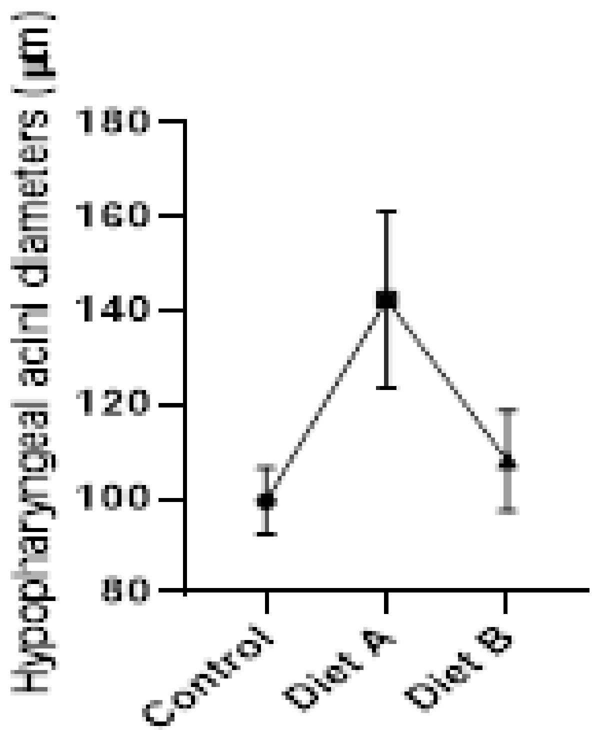

Figure 11. Means of hypopharyngeal acini diameters (µm) of control and fed workers after 14 days of feeding.

4. Discussion

Although, provided diets A and B have been accepted, diet B was more preferred than diet A. This can be interpreted by the variety and richness of nutrients content of soybeans and wheat germ confronted with chickpeas and corn. Aly *et al.* (2019) have shown promising consumption rates of soybeans and wheat germ components when provided to honey bee colonies either separated or combined.

Consumption rates findings confirm positive conclusions of earlier studies on palatability and impacts of soybeans, wheat germ, chickpeas and corn components in different forms (Sammataro and Milagra, 2013; Aly *et al.*, 2019; Younis, 2019). The initial rise in consumption rates compared to the last recorded ones for both diets can be attributed to the progressive mortality in caged individuals. Eshbah *et al.* (2021) have observed that diet consumption was highest in day 6 for old bees across all diets. Diets consumption gradually decreased from day 9 for old bees till the end of the experiment

No doubt that, supplementary nutrition has a vital role in preserving survival rates. Higher significant survival rates were noticed for groups fed on provided diets confronted with control group. Pirk *et al.* (2010) have approved that regardless of protein component type, carbohydrate component is very important for honey bees survival.

The presence of protein content in examined diets was reversed in the means of thoraces muscle mass of workers fed on diets A and B. In the same context, the notable effect of artificial feeding on improving muscle structure was previously discussed (Esmael *et al.*, 2016; Alhousini, 2020)

The higher rates of mandibular gland sizes in workers fed on diets A and B can be attributed to the nutritional value presented by these diets. In addition to nutritional effect, mandibular gland sizes may be negatively affected by other factors as *Varroa* infestation (Ayoub *et al.*, 2015).

Findings have shown affirmative effects of examined diets A and B on the development degree of hypopharyngeal glands. In the same context, (Abdel-Rahman *et al.*, 2021) evaluated how the hypopharyngeal gland changed as workers ages and diets changed. This explained the idea that feeding has a great influence on how hypopharyngeal gland develops according to diet nutritional values.

5. Conclusion

Diets A and B are highly recommended in honey beekeeping due to their reported palatability rates, nutritional diversity and promising impacts on honey bees health and viability at dearth times.

Authors' Contributions
All authors are contributed in this research.

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Institutional Review Board Statement
All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement
Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate
Not applicable

Consent for Publication
Not applicable.

Conflicts of Interest
The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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