

Influence of different proteinaceous diets on consumption, brood rearing, and honey bee quality parameters under isolation conditions

Abdulraouf AMRO*, Mohamed OMAR, Ahmed Al-GHAMDI

Chair of Engineer Abdullh Ahmad Bagshan for Bee Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

Received: 07.07.2015 • Accepted/Published Online: 02.02.2016 • Final Version: 27.06.2016

Abstract: This study was carried out to evaluate the efficacy of 5 proteinaceous diets on honey bees under isolation conditions. The diets were soybean meal, mesquite pod powder, date paste, Feedbee®, and corn gluten, along with control colonies (bees fed naturally on pollens). These 5 diets were supplied to honey bee colonies under isolation conditions, except for the control group. Food consumption amount, brood rearing activity, and some physiological parameters of the honey bees were measured. The best consumption amount was for date paste, followed by Feedbee, mesquite, and corn gluten, respectively. Concerning brood rearing activity, the highest mean of sealed brood cells was 174.7 cells/colony in colonies fed on Feedbee, while no brood was reared in colonies fed on soybean meal. The fresh weight, dry matter, and protein content of fully grown larvae and newly emerged workers were higher in colonies fed on bee bread than the colonies fed on the artificial diets. Total soluble solids %, total hemolymph count, and hemolymph protein % were varied among tested diets. Feedbee was able to enhance brood rearing, but Feedbee is expensive and not available in the local markets of Saudi Arabia. Hence, beekeepers are advised to use mesquite pod flour as a pollen substitute in dearth periods.

Key words: Pollen substitutes, Feedbee®, hemolymph, mesquite pod powder

1. Introduction

Honey bees, *Apis mellifera*, depend mainly on nectar and pollen as sources of nutrients. Nectar provides the bees with carbohydrates while pollen supplies them with the remaining dietary requirements such as proteins, lipids, vitamins, and minerals (1). Lack of pollens in the field is a critical problem for beekeepers in Saudi Arabia, especially in the central region due to the elevated temperature and drought conditions during the summer. Supplying bee colonies with an alternative artificial source of protein (pollen substitutes) is essential to boost colony survival and development (2). Various studies have been performed to develop an ideal protein diet for honey bees with positive impacts on honey bee physiology, and considering the hemolymph of honey bee workers is an accurate method to evaluate the efficacy of different types of diets (3). Some criteria such as food consumption rate, brood rearing activity, and quality of honey bees were used as indicators for the quality of the artificial diets presented to honey bee colonies during periods of pollen shortage. The adequacy of pollen or pollen substitutes is normally evaluated by determining the quantity of diet consumed or by measuring brood production (4). Herbert et al. (5)

compared pollen substitutes with varying quantities of protein using caged colonies. They demonstrated that 20%–23% protein is ideal for normal growth of honey bee workers.

Shortage of pollen can cause abnormal development of the brood, a decrease in the length of workers' life, and low honey production (6). Omar (7) concluded that feeding honey bee colonies with different pollen substitutes or supplements was not sufficient for the requirements of larvae older than 3 days. On the other hand, the body protein can be influenced by a combination of factors including quantity of pollen, protein content, and level of essential amino acids in pollen protein (8). Mostafa (9) found that when honey bee colonies were fed on pollen substitutes under isolation conditions, the fresh weight and dry matter of newly emerged bees were less than those of control colonies (reared under free flight conditions with natural feeding). Food source can impact the percentages of glycogen, lipids, and protein of honey bees (10), as well as hemolymph composition. Glinski and Klimont (11) reported that the total hemocyte count, differential hemocyte count, and total solid content are indicators for the hemolymph functional efficiency. However, the highest

* Correspondence: abamro@ksu.edu.sa

number of hemocytes occurred in the hemolymph of bees that received improved pollen substitute lacking L-amino acids (12). Cremones et al. (13) found that the protein values reflected the quantity and usability of protein in the diets, and not the consumption, which was similar for all protein diets used. Successful pollen substitutes should contain valid and cheap materials as well as a high protein percentage, and should have high palatability and attractiveness to honey bees.

Therefore, the aim of the present study was to develop pollen substitutes with high efficacy to boost physiological and productive characteristics of honey bees.

2. Materials and methods

This investigation was carried out at the apiary of the Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh Region, Saudi Arabia.

2.1. Experimental colonies

Hybrids of Carniolan honey bees were used in the experiments. The study was performed inside an isolator (3.5 × 6.5 × 4 m) covered with muslin net (16 × 16 mesh). Fifteen colonies were introduced into the isolator and painted with different colors to facilitate the orientation of honey bee workers. Each colony contained 5 empty combs. Each colony was then provided with 1000 g of young worker bees, and sister queens (aged less than 1 year). The colonies were divided into 5 groups (3 colonies per group), and besides these groups 3 colonies were kept outside the isolator under free flight conditions to serve as a control group and were provided with 2 bee bread combs. The experiment was carried out over 6 consecutive weeks.

2.2. Proteinaceous diets

Five proteinaceous diets were compared to select the best one to serve as an alternative to pollens or bee bread (the natural protein source for honey bees). These 5 diets were made of 7 raw materials and the total protein percentages of these materials are shown in Table 1. These raw materials were selected because they were rich in protein content according to the Kjeldahl method (14) of determination of protein and were available in the study region. The 5 diets were then made by mixing different amounts of the raw materials as shown in Table 2. The main component in each diet was soybean meal in diet 1 (traditional pollen substitute), mesquite pod powder in diet 2 (described by Odoul et al. (15) as a rich source of carbohydrates and proteins for humans and animals), date paste in diet 3 (a very common and cheap source of carbohydrates in Saudi Arabia), Feedbee® (16) in diet 4, and corn gluten in diet 5 (a very rich protein source).

Each group of colonies was provided with a specific diet throughout the experiment. Each colony was provided with 100 g of the diet in a perforated polyethylene bag

Table 1. Total protein % in 7 materials used for making the proteinaceous diets (n = 3).

Materials	Total protein %
Soybean meal (<i>Glycine max</i>)	39.88 ± 0.13 ^c
Brewer's dried yeast	40.57 ± 0.19 ^b
Skimmed milk powder	29.87 ± 0.13 ^d
Corn gluten	55.94 ± 0.07 ^a
Date paste (<i>Phoenix dactylifera</i>)	24.55 ± 0.26 ^f
Flour of mesquite pods (<i>Prosopis juliflora</i>)	16.58 ± 0.27 ^g
Feedbee®	28.82 ± 0.19 ^e

Means followed by the same letter do not differ significantly at the 5% level of probability.

above the middle comb every 6 days. Supplementary feeding with sugar syrup (250 mL, 1:1, w/v) was presented to each colony every 3 days to stimulate honey bee activity. The colonies were also provided with a water source.

2.3. Measurements

2.3.1. Food consumption amount

The food consumption amount was calculated as the difference between the fresh weight of the diet and the weight 6 days after providing it to the colony (g/colony). The calculations were repeated every 6 days for each diet type. The total consumption for each diet during the experimental period (42 days) was also calculated.

2.3.2. Brood rearing activity

The number of sealed brood cells was counted after 2 generations (42 days) for each colony.

2.3.3. Honey bee quality

Three samples of fully grown larvae and newly emerged bee workers were taken after 2 generations from each colony. Fresh weight (g), dry weight (g), water content (%), and total protein (%) were determined according to Hanna and Azab (17). The fresh samples were dried in an oven at 65 °C for 36 h until they reached a constant weight. The water content in worker larvae and adult bees' bodies was calculated by subtracting the dry weight from the fresh weight. Three dry samples of larvae and honey bee workers were ground into a fine powder. One hundred milligrams of powdered material was then used for total nitrogen determination using the Kjeldahl method and the total nitrogen was multiplied by a constant factor of 5.6 to obtain the total protein content (18).

2.3.4. Hematological studies

To test the suitability of pollen substitutes for honey bee feeding, 3 hemolymph samples were collected from 3 fully

Table 2. Composition of the diets used in the experiments.

Materials	Composition of the diets, g/kg				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Soybean meal (<i>Glycine max</i>)	252				
Mesquite pods powder (<i>Prosopis juliflora</i>)		258			
Date paste (<i>Phoenix dactylifera</i>)			274		
Feedbee**				379	
Corn gluten					424
Dried skim milk	84	86	91		
Brewer's yeast	84	86	91		
Sugar powder	378	386	411		381
Fresh mixed pollen pellets	42	43	46		42
Multivitamins and minerals (Centrum)**		4	5		
Coriander oil		9	9		
Sucrose solution (2:1)				606	
Honey	17	17	18	15	17
Water	143	112	55		136
Total	1000	1000	1000	1000	1000

*Commercial substitute tested by Saffari (16).

**Produced by Pfizer (formerly Wyeth).

grown larvae (6 days old) from each colony by puncturing the larval cuticle with a fine hypodermic needle. The total soluble solids percentage (TSS%) in the hemolymph sample was then determined using a hand refractometer (Euromex Brix, USA). Three readings of TSS% per treatment were taken. The total hemocyte count (THC) per mm³ of 3 samples from the hemolymph was determined using a Neubauer hemocytometer. The total number of hemocytes per mm³ of hemolymph was calculated using the following formula (19): $THC = \frac{a \times 4000 \times b}{C}$,

where:

THC: the number of hemocytes per mm³ of hemolymph,

a: the number of hemocytes in 100 large squares,

b: the hemolymph dilution,

C: the number of small squares in 100 large squares.

Hemolymph pools of 20–100 individuals were sampled from different experimental colonies and were continuously held in an ice bath. A few crystals of phenylthiourea were added to each pool to prevent melanization. The hemolymph pools were stored at –20 °C. The Lowry method (20) was used to determine the protein

concentration of hemolymph spectrophotometrically at 500 nm using standard curve determination.

2.4. Statistical analysis

The experimental design used was a random complete block design. Three replicates (colonies) were used per treatment (5 treatments or diets) and control group. Means ± standard deviation (SD) were calculated for studied parameters. Analysis of variance (ANOVA) was then performed and means were compared using Duncan's multiple range tests at a 5% level of probability (21).

3. Results

3.1. Food composition

The tested diets were consumed in different amounts (Table 3). The greatest amount of diet consumption was recorded during the second feeding period (days 7–12), but the amount of food consumption of all diets decreased gradually through other feeding periods. The highest consumption was with diet 3 (date paste) with 213.2 g/colony in 42 days, followed by Feedbee, mesquite, corn gluten, and soybean meal with differences of 39.6, 89.1, 117.5, and 125.8 g/colony in 42 days, respectively, from

Table 3. Food consumption by honey bee colonies fed with 5 diets (g/colony in 6 days) under isolation conditions (n = 3).

Diets	Mean of food consumption (g/colony in 6 days)							Total g/colony in 42 days	Order
	1-6	7-12	13-18	19-24	25-30	31-36	37-42		
Diet 1 (soybean meal)	29.36 ± 1.18 ^f	27.40 ± 1.82 ^{fg}	14.77 ± 1.46 ^{kl}	7.13 ± 1.12 ^{no}	4.73 ± 0.67 ^{op}	2.70 ± 0.62 ^p	1.33 ± 1.00 ^p	87.4 ± 9.61 ^D	5
Diet 2 (mesquite)	33.67 ± 1.68 ^{de}	31.07 ± 2.38 ^{ef}	16.73 ± 0.96 ^{jk}	14.13 ± 0.83 ^{kl}	11.73 ± 0.56 ^{lm}	9.53 ± 0.27 ^{mn}	7.20 ± 0.30 ^{no}	124.1 ± 7.81 ^C	3
Diet 3 (date paste)	37.86 ± 1.62 ^{bc}	58.83 ± 3.06 ^a	33.87 ± 1.26 ^{de}	29.43 ± 0.98 ^f	22.33 ± 0.63 ^{hi}	16.80 ± 0.68 ^{ik}	14.10 ± 0.80 ^{kl}	213.2 ± 2.40 ^A	1
Diet 4 (Feedbee®)	27.47 ± 1.79 ^{fg}	40.70 ± 1.95 ^b	29.03 ± 1.36 ^f	24.37 ± 0.55 ^{gh}	19.90 ± 1.45 ^{ij}	17.47 ± 0.75 ^{jk}	14.67 ± 1.67 ^{kl}	173.6 ± 13.62 ^B	2
Diet 5 (corn gluten)	14.13 ± 1.66 ^{kl}	36.53 ± 1.72 ^{cd}	17.17 ± 0.43 ^{jk}	12.10 ± 1.15 ^{lm}	9.43 ± 1.09 ^{mn}	4.47 ± 0.14 ^{op}	1.83 ± 0.17 ^p	95.7 ± 3.73 ^D	4

Means followed by the same letter do not differ significantly at the 5% level of probability.

diet 3. Significant differences were detected among the diets, but diet 3 had the highest consumption mean and differed significantly from the other diets.

3.2. Brood rearing activities

The results showed that the highest total number of sealed brood cells (1066.7 cells/colony) was recorded in the control group under free flying conditions. The mean number of sealed brood cells in colonies fed with Feedbee was 1.57-, 1.86-, and 4.48-fold of those fed with diet 2, diet 3, and diet 5, respectively (Table 4). The colonies fed with diet 1 (soybean meal) under isolation conditions were not able to rear the brood until the emergence of the adults (Figure). The queens of this treatment continued to lay eggs during the 7 weeks of investigation, but there were no sealed brood cells during the period of the experiment.

3.3. Physical and chemical analysis of honey bees

Fresh and dry weight, water content (%), and total protein percentage of fully grown larvae and newly emerged honey bee workers fed with different proteinaceous diets under isolation conditions are shown in Table 5. The results show that the fresh weight and dry matter content of fully grown larvae (6 days old) were lower in the tested individuals fed with all examined diets than the control larvae reared under natural conditions. The fresh weight and dry weight of fully grown larvae reared under natural conditions were 132.7 and 31.9 mg/larva. However, the fresh weight and dry weight of honey bee larvae fed with diet 4 (Feedbee) was recorded as 123.6 and 29.5 mg/larva. The results showed that diet 2 (mesquite), diet 3 (date paste), and diet 5 (corn gluten) had lower values than the

Table 4. Effect of tested proteinaceous diets on number of sealed brood cells (cells/colony) under isolation condition after feeding period of 42 days (n = 3).

Treatments	Number of sealed brood cells (n/cell)	Order
Diet 1 (soybean meal)	0.00 ± 0.00*	6
Diet 2 (mesquite)	111 ± 3.0 ^{cd}	3
Diet 3 (date paste)	94 ± 5.0 ^d	4
Diet 4 (Feedbee®)	174.7 ± 8.0 ^b	2
Diet 5 (corn gluten)	39.0 ± 3.0 ^e	5
Control	1066.7 ± 62.9 ^a	1

Means followed by the same letter do not differ significantly at the 5% level of probability
*No sealed brood was recorded.

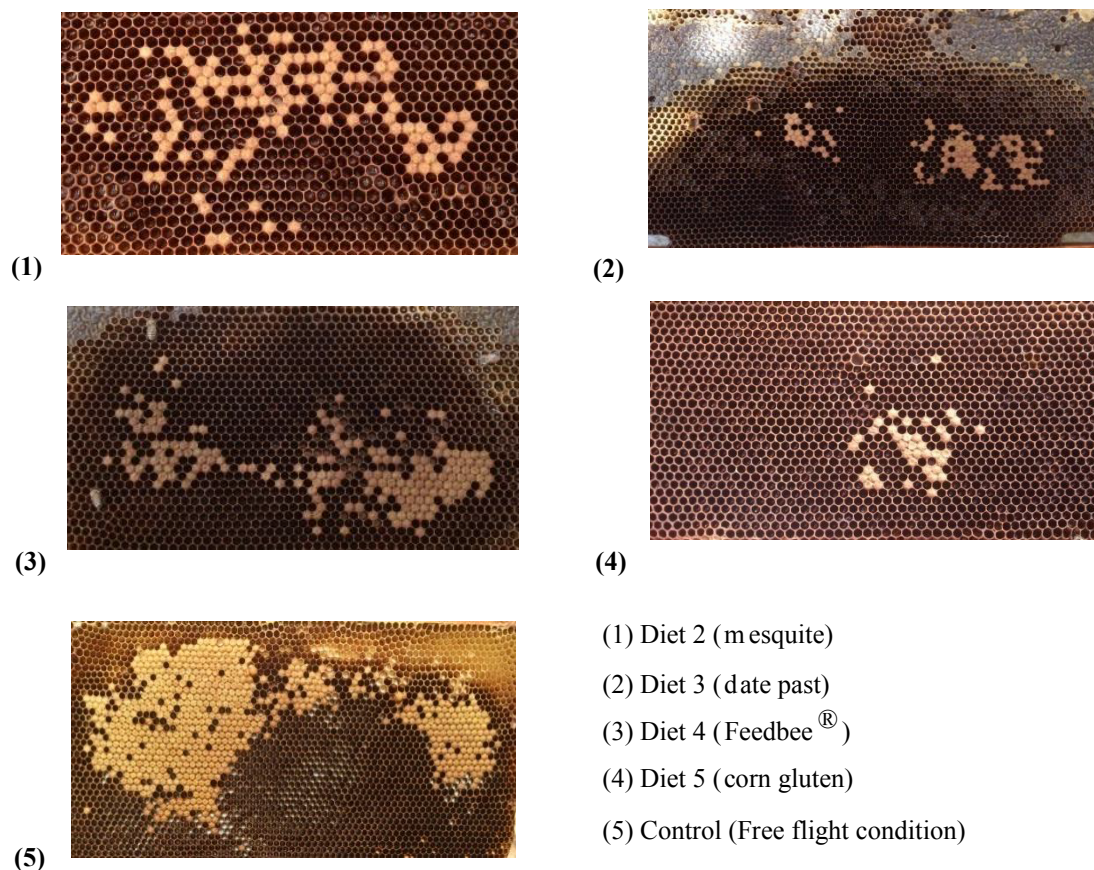


Figure. Brood rearing areas after administration of proteinaceous diets to honey bee colonies under isolated condition.

Table 5. Fresh, dry weight, water content (%), and total protein percentage of fully grown larvae and newly emerged honey bee workers fed with different proteinaceous diets under isolation conditions (n = 3).

Treatment	Six-day-old larvae				Newly emerged adults			
	Fresh weight (mg)	Dry weight (mg)	Water content (%)	Total protein (%)	Fresh weight (mg)	Dry weight (mg)	Water content (%)	Total protein (%)
Diet 2 (mesquite)	124.7 ± 2.0 ^{bc}	28.0 ± 1.3 ^d	77.55	31.62 ± 0.31 ^c	95.9 ± 2.8 ^c	14.7 ± 0.8 ^{cde}	84.63	51.22 ± 0.39 ^c
Diet 3 (date paste)	113.2 ± 2.8 ^{de}	28.1 ± 1.1 ^{cd}	75.18	28.35 ± 0.28 ^d	89.8 ± 2.0 ^d	14.0 ± 0.7 ^e	84.43	48.55 ± 0.46 ^d
Diet 4 (Feedbee®)	123.6 ± 1.9 ^c	29.5 ± 1.0 ^{bc}	76.13	34.67 ± 0.35 ^b	96.5 ± 1.5 ^{bc}	15.0 ± 0.8 ^{bcd}	84.41	53.66 ± 0.29 ^b
Diet 5 (corn gluten)	112.4 ± 2.4 ^e	26.4 ± 1.3 ^e	76.51	23.89 ± 0.50 ^e	82.8 ± 1.9 ^e	14.2 ± 0.5 ^{de}	82.87	44.08 ± 0.43 ^e
Control	132.7 ± 2.9 ^a	31.9 ± 1.2 ^a	75.93	38.05 ± 0.38 ^a	111.8 ± 2.0 ^a	16.9 ± 1.7 ^a	84.88	55.92 ± 0.27 ^a

Means followed by the same letter do not differ significantly at the 5% level of probability.

abovementioned two diets with significant differences. On the other hand, the fresh weight and dry matter content of the adults were lower in the individuals reared on the tested diets compared with the control. The fresh weight of the newly emerged bees reared under natural conditions

was recorded as 111.8 mg/adult. The fresh weight of honey bee workers fed with diets 4, 2, 3, and 5 were consequently lower than the adult weight of the control by 15.3, 15.9, 22.0, and 29.0 mg/adult, respectively. Similar results were obtained when the dry matter of all tested diets was

compared with the control. The differences were 1.9, 2.2, 2.7, and 2.9 for diets 4, 2, 5, and 3, respectively, compared with the control.

The protein contents of fully grown larvae and newly emerged adult bees were directly influenced by the types of diet provided to the bee colonies. The protein percentage of larval dry matter (6 days old) was recorded as 38.05% for the control. This percentage was reduced significantly in all treated colonies that depended on artificial diets for 6 weeks, which was recorded as 34.67%, 31.62%, 28.35%, and 23.89% in respect to diets 4, 2, 3, and 5, respectively. The protein percentage determined in the newly emerged honey bee workers of the control was recorded as 55.92%. The protein percentage was reduced significantly by feeding honey bee colonies artificial diets under isolation conditions. The highest percentage (53.66%) was obtained when honey bee colonies were fed with diet 4 (Feedbee), followed by diet 2 (mesquite) with a mean of 51.22%. Diet 3 (date paste) and diet 5 (corn gluten) showed the lowest effects on the total protein percentage of the newly emerged adults.

3.4. Hematological studies

The effects of some proteinaceous diets on TSS%, THC, and total protein concentration (mg/100 mL) in 6-day-old larval hemolymph are shown in Table 6. The results revealed that the highest total protein concentration (2080 mg/100 mL) was estimated in larval hemolymph collected from honey bee colonies outside the isolator (control group). Larvae fed on diet 4 (Feedbee) contained 2034 mg/100 mL, followed by diet 2 (mesquite) with 1974 mg/100 mL, while the remaining treatments exhibited less effect on the protein concentrations.

4. Discussion

4.1. Food consumption

The results showed that diet 1 (soybean meal) had a lower rank of consumption than the other diets, while diet 3 (date

paste) had the highest consumption amount. This finding reflects the fact that honey bee workers prefer diets with high sucrose content, which serves as a phagostimulant and thus increases honey bees' response towards this patty (9). The addition of 5% pollen grains and coriander oil in small amounts (0.3%) likely increased the palatability of the diets and positively increased the consumption rate for diets 2 and 3. The results of Feedbee consumption amount were in line with a previous study (16), in which honey bees consumed a greater amount of Feedbee than natural pollen.

4.2. Brood rearing activity

The activity of honey bee colonies to rear a brood is highly dependent on the contribution of a suitable protein in food, as well as on its quality, to activate their hypopharyngeal glands (9). The highest brood rearing activity was recorded in honey bee colonies fed on bee bread (the natural protein source for honey bees). On the other hand, the lowest results were recorded in honey bee colonies fed on pollen substitutes. These results were in line with a previous study (9), which reported that bee bread is the best source of protein for honey bee workers. It has also been reported that brood rearing activity of honey bee colonies with few pollen foragers was reduced by placing them under a mesh tent, and finally the colonies stopped brood rearing (22). As a consequence, natural pollens are very important for brood rearing activity. There were highly significant differences between tested proteinaceous diets and brood rearing activity. Herbert et al. (5) found that brood rearing activity was only possible when using proteinaceous diets containing suitable proteins. That could explain why diet 5 (corn gluten) did not give the highest brood cell number. On the other hand, diet 2 (mesquite pod powder), which contains only 16.58% protein, encouraged brood rearing in honey bees more than the other diets. These results are in agreement with a previous study (15) that described how the protein content of mesquite pods was useful for honey bees.

Table 6. Effect of feeding colonies with some proteinaceous diets on TSS%, THC, and total protein concentration (mg/100 mL) in hemolymph of 6-day-old larvae (n = 3).

Diets	THC	TSS%	Protein concentration (mg/100 mL)	Order
Diet 2 (mesquite)	3580 ± 55.69 ^c	14.83 ± 0.11 ^c	1974.51 ± 10.55 ^c	3
Diet 3 (date paste)	3156 ± 42.35 ^d	14.07 ± 0.03 ^d	1862.35 ± 21.56 ^d	4
Diet 4 (Feedbee)	3792 ± 48.00 ^b	15.19 ± 0.04 ^b	2034.12 ± 14.31 ^b	2
Diet 5 (corn gluten)	2936 ± 27.45 ^e	13.21 ± 0.05 ^e	1783.14 ± 3.92 ^e	5
Control	4184 ± 46.65 ^a	15.81 ± 0.12 ^a	2080.00 ± 5.30 ^a	1

Means followed by the same letter within the column do not differ significantly at the 5% level of probability.

4.3. Physical and chemical analysis of honey bees

In the present study, the highest protein level of the hemolymph was recorded in bees fed on pollens. This result is in agreement with previous studies (23,24). Colonies fed on Feedbee had the highest hemolymph protein content, followed by mesquite pod powder. This result was in line with a previous study (7) that clarified that protein content in the hemolymph increased when the dietary protein content was increased. This reflects how bees gained benefits from diets 2 and 4.

According to another study (23), the protein content of newly emerged bees can be influenced by the quality of the proteinaceous diet. It was clear in the present study that the highest body protein contents were recorded in the control colonies. Adult bees from broods reared under protein shortage conditions (fed on pollen substitutes) had less protein content. The highest workers' body protein content was recorded in colonies fed with diet 4, while the lowest was recorded in colonies fed with diet 5. This result is in agreement with those obtained by previous studies (25,26). It was concluded that high protein content in the surrogate feeding was not enough to compensate honey bees for natural pollen absence. It should also be absorbed and have satisfactory physiological results for honey bees.

The highest protein percentage and dry weight was recorded in larvae taken from control colonies (fed on bee bread), while the lowest values were recorded in colonies fed on the other pollen substitutes. These results were in accordance with Wille and Imdorf (27), who reported that low dry weight and nitrogen contents may result from having no or little access to pollen after honey bee workers' emergence. Neither corn gluten nor date paste achieved the benefits required for larval quality. However, both Feedbee and mesquites pod powder achieved the main objective for larval quality. Colonies fed on Feedbee had the best results among the other substitutes in regard to dry matter and protein percentage of emerged adult bees. This reflects the high utilization of the protein content of diets 2 and 4 for honey bees.

4.4. Hematological studies

Natural protein food is an important factor in the functioning of the hemolymph cellular system of bees. According to the results obtained in the present study, the hemolymph of bees fed with pollen substitutes had a low THC in all the treatments when compared with the control group (bees fed on bee bread). Similar results were obtained by Szymas and Jedruszuk (28), who found that pollen substitutes caused a decrease in THC. The pollen substitute in their study, which had an ingredient makeup very similar to the surrogate pollen investigated in this study, and especially diet 3 (mesquite pod powder), turned out to be a very valuable food comparable to natural pollen. The lack of suitable protein in the 5 diets caused

major disturbances in the structure and functioning of the hemolymph cellular system. However, the number of hemocytes in bee hemolymph increased with the increased nutritional value of substitute protein in diets 4, 3, and 2.

According to a study (28), the determination of TSS% and protein concentration in the hemolymph of fully grown larvae of honey bees can be used as an accurate method to evaluate the efficiency of protein diets. In line with another study (29), the results of the total soluble solids obtained by colonies fed on Feedbee were the nearest to that of bee bread. There were significant differences between diets 2 and 3. These reflect the impact of mesquite pod powder as a good source of protein for bee hemolymph as described by Oduol et al. (15).

Considerable variations in total protein percentage in the hemolymph of bees fed on different diets were found. The protein percentages of larval hemolymph were high when bees were fed on Feedbee. This result was in agreement with Gregory (30), who reported that bees fed on Feedbee supplement had higher hemolymph protein levels than bees fed on a Bee-Pro diet. Results of De Jong et al. (29) were in line with the present study. They indicated the important role of mesquite pod flour protein content for honey bees and recorded that it gave nearly the same protein titers in hemolymph as did the bee-collected pollen.

Providing honey bee colonies with protein is very important, especially when no natural protein sources (pollens) are available for them. Using diet 4 (containing mainly the commercial product Feedbee) was found to enhance brood rearing activity and some physiological characteristics of honey bees. Although diet 4 was not consumed rapidly by the bees, it had the highest brood rearing activity compared to the other tested diets. A soybean diet was not good in regard to the investigated parameters, and this diet is not recommended. However, due to the high expense and nonavailability of Feedbee in the markets of Saudi Arabia, beekeepers are advised to use diets 2 and 3 (mesquite pod flour and date paste) when no or few natural pollen sources are available for their bee colonies.

Acknowledgments

Thanks are given to the Deanship of Scientific Research at the College of Food and Agriculture Science Research Center as well as the Bee Research Unit for providing the necessary materials for the research. We would also like to thank Dr Hossam F Abou-Shaara for his assistance during the research period. Special thanks to Dr Boris C Kondratieff (Professor of Entomology and Director of the C. P. Gillette Museum of Arthropod Diversity, Colorado State University) for linguistic and grammar editing.

References

1. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. *Apidologie* 2010; 41: 278-294.
2. Herbert EW Jr. Honey bee nutrition. In: Graham JM, editor. *The Hive and the Honey Bee*. 1st ed. Hamilton, IL, USA: Dadant and Sons; 1992. pp. 197-233.
3. Bounias M, Morgan MRJ. Effect of sucrose feeding on the induction of honey bee haemolymph alpha-glucosidase. *J Apic Res* 1990; 29: 181-186.
4. Herbert EW, Bickley WG, Shimanuki H. The brood rearing capacity of caged honey bees fed dandelion and mixed pollen diets. *J Econ Entomol* 1970; 63: 215-218.
5. Herbert EW, Shimanuki H, Caron D. Optimum protein levels required by honey bees (*Hymenoptera, Apidae*) to initiate and maintain brood rearing. *Apidologie* 1977; 8: 141-146.
6. Winston ML, Chalmers WT, Lee PC. Effects of two pollen substitutes on brood mortality and length of adult life in the honey bee. *J Apic Res* 1983; 22: 49-52.
7. Omar MOM. Influence of supplementary honeybee feeding with pollen and yeast paraffin to development haemolymph composition and enzymatic activity of *Apis mellifera* L. PhD. Institute of Agronomy, Bucharest, Romania, 1984.
8. Kleinschmidt GJ, Kondos AC. The influence of crude protein levels on colony performance. *Aust Bee J* 1977; 79: 357-361.
9. Mostafa AM. Influence of some supplementary feeding on physiological characters and productivity of honey bees. PhD, Assiut University, Assiut, Egypt, 2000.
10. Hrassnigg N, Crailsheim K. Differences in drone and worker physiology in honey bees (*Apis mellifera* L.). *Apidologie* 2005; 36: 255-277.
11. Glinski Z, Klimont S. Effect of *Varroa jacobsoni* invasion on the cells in the haemolymph of worker bees *Apis mellifera* L. *Med Weter* 1987; 43: 546-549.
12. Rogala R, Szymas B. Nutritional value for bees of pollen substitute enriched with synthetic amino acids. Part II. Biological methods. *J Apic Sci* 2004; 48: 29-36.
13. Cremonese T, DeJong D, Bittondi M. Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees (*Hymenoptera: Apidae*). *J Econ Entomol* 1998; 91: 1284-1289.
14. Kirk PL. Kjeldahl method for total nitrogen. *Anal Chem* 1950; 22: 354-358.
15. Oduol PA, Felker P, McKinley CR, Meier CE. Variation among selected *Prosopis* families for pod sugar and pod protein contents. *Forest Ecol Manag* 1986; 16: 423-431.
16. Safari AM, Kevan PG, Atkinson JK. Feeding colonies with a nutritious pollen supplement is beneficial. *Bee Culture* 2006; 134: 30-31.
17. Hanna HM, Azab SG. Effects of larval population density on weights and components of *Spodoptera exigua*. *Bull Soc Ent* 1973; 57: 55-65.
18. Rabie AL, Wells JD, Dent LK. The nitrogen content of pollen protein. *J Apic Res* 1983; 22: 119-123.
19. Predetshensky VE, Parovska VM, Margolina LT. Microtechnique methods. Goso Uzd Medgez Moskva 1950; 125: 115-121 (in Russian with an English abstract).
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
21. Duncan DB. Multiple range and multiple tests. *Biometrics* 1955; 11: 1-42.
22. Imdorf A, Kilchenmann V, Maquelin C. What is the effect of spring pollen feeding on the development of colonies? *Liebfeld Apiculture* 1988; 85: 67-76 (in German with an English abstract).
23. Konopacka Z. Quality of honeybee families, strong and weak. *Pszczeln Zesz Nauk* 1974; 18: 161-173 (in Russian with an English abstract).
24. Konopacka Z, Muszynska J. Changing in the honeybee worker condition during growing season. *Pszczeln Zesz Nauk* 1981; 25: 3-14 (in Russian with an English abstract).
25. Dustmann JH, von der Ohe W. Influence of cold snap in the spring development of honeybee colonies (*Apis mellifera* L.). *Apidologie* 1988; 19: 245-254.
26. Kunert K, Crailsheim K. Seasonal changes in carbohydrate, lipid and protein content in emerging worker honey bees and their mortality. *J Apic Res* 1988; 27: 13-21.
27. Wille H, Imdorf A. The nitrogen supply of honeybee colonies. *Allg Dtsch Imkerztg* 1983; 17: 37-50 (in German with an English abstract).
28. Szymas B, Jedruszuk A. The influence of different diets on haemocytes of adult worker honey bees, *Apis mellifera* L. *Apidologie* 2003; 34: 97-102.
29. De Jong D, Da Silva EJ, Kevan PG, Atkinson JL. Pollen substitutes increase honey bee haemolymph protein levels as much as or more than does pollen. *J Apic Res* 2009; 48: 34-40.
30. Gregory P. Protein diets and their effects on worker weight, longevity, consumption and haemolymph protein levels of *Apis mellifera* L. In: *Proceedings of the 19th American Bee Research Conference*. Baton Rouge, LA, USA: ABRC; 2006. pp. 9-10.