



Studying the effect of feeding honey bees with natural materials and pollen supplements on the chemical composition and amino acid ratio in the worker body

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Received: December 24th 2022; Accepted: January 13th 2023

Abstract

Nutritional and chemical experiments were conducted on honey bee workers (*Apis mellifera* L.) in the apiary of the College of Agriculture / Tikrit University for the period from January to April 2022. The percentage of moisture content and chitin in the water treatment amounted to 68.27% and 46.81% respectively, and the treatment of pollen, honey and palm pollen was superior to the protein content in the body of the works, which amounted to 48.68%, while the fats gave the treatment of pollen, honey and palm pollen the highest percentage, which amounted to 10.28. It showed the effect of feeding with natural nutrients on the chemical content, as the water treatment recorded the highest percentage in moisture content and the percentage of chitin, which amounted to 7.15%, 58.70%, and the treatment (sugar solution + spirulina) and the treatment (sugar solution + *Ganoderma lacidum* + spirulina) recorded the highest percentage in fats. It reached 7.23%, which gave the highest percentage of protein, reaching 56.11%. As for the effect of the feeding materials with pollen supplements on the percentage of amino acids in the bodies of workers. The (pollen + honey) treatment gave the highest percentage of cysteine acid, which amounted to 5.32%, and the treatment (pollen + protein + vitamins) gave the highest percentage of lysine acid, which amounted to 5.78%, and the treatment (pollen + honey + protein) was superior in the percentage of Tryptophan acid, which amounted to 6.22%, and the treatment of (pollen + honey + palm pollen) gave the highest percentage of histidine acid, which amounted to 5.87%, while the control treatment excelled in giving the highest percentage of valine acid, which amounted to 4.23%. As for the effect of feeding with natural foodstuffs, treatment (sugar solution) gave the highest percentage in fultine acid, which amounted to 5.07%, and treatment (sugar solution + *Ganoderma lacidum*) gave the highest percentage of lysine acid, which amounted to 5.05%, and treatment (*Ganoderma lacidum* + spirulina) gave the highest percentage of acid Lysine was 5%, and the treatment of sugar solution and reishi mushroom gave the highest percentage of cysteine, which amounted to 5.01%, and the comparison treatment (water only) gave the highest percentage of lysine and tryptophan, which amounted to 5%.

Keywords: Honey bees, Amino acids, Fats, Moisture, Protein.

Introduction

Honey bees (*Apis mellifera* L.) belong to the hymenoptera insects that live in social groups. Social living bees have been domesticated to produce bee products in sufficient quantities. Breeding has gone through a long development. Although modernization retains some details that are from the instincts of bees such as the acquisition of bee products that depend on the free movement of worker bees over the landscape. As well as the ability to communicate and direct. The main communication methods are chemical

communication via pheromones. Therefore, one of the conditions for his upbringing is health care.

And making sure that it is free from diseases and feeding at the appropriate times (Rajchard, 2011) honey bees contribute significantly to the pollination of agricultural crops and local plants, and thus sustainable agriculture that depends on biodiversity. This, along with honey, makes these insects both ecological and economically important. Can several factors affect the vitality of honey bee colonies, including disease (Murilhas 2002; Higes et al. 2010), availability of adequate food resources, weather

conditions, adult size, number of bees and brood, quantity and quality (Tlak et al., 2015).

The natural food of bees is the nectar of flowers, which they convert into honey, which is the main source of carbohydrates. As for the pollen that bees collect from the male organs of the flowers (the anthers), it is a source of proteins, fats, minerals and vitamins. It is the food for the development of bee larvae, as it contributes to building muscles, glands and tissues and helps in food production. The royal brooding workers fed the royal larvae for five days and the males during the first three days of the larval stage (Abdullah, 2018).

Materials and Methods

The research was carried out in the apiary of the Faculty of Agriculture - Tikrit University during the period from January 2021 to April 2021, which included fifteen colonies of hybrid Carniolan bees. *Apis Mellifera L* The research included a study of pollen supplements and honey nutritional supplements

The aim of the study is to evaluate the natural, artificial and local nutrients on the percentage of amino acids in the body of whole insects and to study the effect of feeding on the activity of honey bee colonies.

Selecting cells and installing them in the study site

Choosing honey bee colonies: Fifteen medium-strong colonies of 4-5 bee-covered frames were selected from the local hybrid bee breed *Apis Mellifera L*. They were distributed at equal distances (75) cm, taking into account that they were free from pathogens and infestation with *Varroa* mites, lice and waxworms.

The denominations were distributed to five random groups, each group containing three replicates that were randomly distributed within the apiary.

Preparing the mixtures

Preparing the pollen mixture: I weighed 20 g of pollen and an antibiotic was added to it to prevent the growth of mold spores. I added 15 g of honey and a sugar solution until it became a soft and cohesive paste. The weight was then 26 g and was placed in a petri dish over the tires for the period from 1/1/2120 to 1/ 4/2120 Another quantity was added whenever the first quantity was exhausted.

Preparing a mixture of pollen, protein and vitamins: Weigh 20 g of pollen and 50 mg of Vitamin Beeagra, to which an antibiotic was added to prevent the growth of mold spores, and 15 g of honey and sugar solution were added to it until it was a soft and cohesive paste. Then it weighed 26 g and was placed in a petri dish over the tires for the period from 1/1/

2020 to 1/4/2020, and another quantity was added whenever the first quantity was used up.

Preparing a mixture of pollen and palm pollen: I weighed 20 g of pollen and 10 g of palm pollen, to which an antibiotic was added to prevent the growth of mold spores, and 17 g of honey and a sugar solution were added to it until it was a soft and cohesive paste. The weight was then 26 g and was placed in a petri dish over the tires for the period from 1/1/ 2021 to 1/4/2021, and there will be another quantity whenever the first quantity runs out.

Preparing a mixture of pollen, palm pollen and protein: I weighed 20 g of pollen, 10 g of palm pollen and 50 mg of protein, and an antibiotic was added to it to prevent the growth of mold spores, and 17 g of honey and sugar solution were added to it until a soft and cohesive dough was formed. The weight was then 26 g and was placed in a petri dish over the tires for a period of 1 1/2020 to 1/4/2021, and another quantity was placed whenever the first quantity was exhausted.

Preparation of the sugar solution: Prepare the sugar solution in a ratio of 1:1: water: sugar. The sugar was mixed with water and an acidic substance (limondoze) was added so that the sugar would not crystallize. Stir the mixture until the sugar is completely dissolved, heat the mixture until it boils, leave to cool, and divide it into three 500ml plastic-made feeders, and put the dry grass in order to Do not drown the bee and give it twice a week per colony for the period from 1/1/2021 to 1/4/2021, at a rate of 0.5 liters per colony each time

Preparation of the sugar solution and reishi mushrooms: The sugar solution was prepared in a ratio of 1:1 (water: sugar) 1 and 5 gm of reishi mushrooms was added to it and divided into three feedings of 500 ml plastic-made capacity. Dry weeds were placed in order not to drown the bee. It was served three times a week per hive starting from 1/1/2021 until 1 /4/2021.

Preparation of the sugar solution and spirulina: The sugar solution was prepared in a ratio of 1:1: water: 1 sugar, 2 gm of spirulina was added to it and divided into three feedings of 500 ml plastic-made capacity. Dry weeds were placed in order not to drown the bee. It was served three times a week for one colony for the period from 1/1/2021 to 1/4/2021. .

Preparation of the sugar solution, reishi mushrooms and spirulina: The sugar solution was prepared in a ratio of 1:1 (water: sugar) and 5 gm of reishi mushrooms and 2 gm of spirulina were added

to it and divided into three feedings of 500 ml plastic-made capacity. Dry weeds were placed so that the bee would not drown. It was served twice a week for each colony from 1/1/2021 to 1 /4/2021. (Al Basha, 2019)

Study of the effect of nutrition on the chemical composition of the bee body Determination of the moisture content of bees: Five samples of domestic workers of different ages were taken for each sample and were weighed, then the samples were dried in an electric oven at a temperature of 100 ° C for 90 minutes. The weight was taken after drying with the same scale and the percentage of moisture was calculated, which is called coefficient.

The questioner according to the following equation:

$$\text{Moisture percentage (\% in the body)} = \frac{\text{live weight of bees} - \text{weight of dry bees}}{\text{live bee weight}} \times 100$$

Determination of the percentage of fat in bees:

After extracting the moisture content of the workers, a mixture of ether and chloroform (1:1) was added to it at the laboratory temperature for 24 hours to get rid of the fat. The percentage of fat from the following equation: -

$$\text{Fat \%} = \frac{\text{total weight of the cuticle-dry weight without fat}}{\text{Total weight of the kite}} \times 100$$

(Borderau and Anderson, 1978)

Determination of chitin and protein content in bees: After extracting the fats, the treatments were weighed and then treated with sodium hydroxide (NaOH) 50% and dried in an electric oven of the same type at a temperature of 100°C for two hours to dissociate the protein. The insects were weighed a second time, washed with 100% ethyl alcohol, and then dried again in the electric oven at a temperature of 60 °C for two hours. They were re-weighed until we obtained a third weight. The same scale was used and the percentage of chitin and protein was calculated according to the following equations:

$$\text{Chitin (\%)} = \frac{\text{last weight after protein removal (mg)}}{\text{Weight (chitin + protein) (mg)}} \times 100$$

(Borderau and Anderson, 1978)

$$\text{Protein (\%)} = \frac{\text{weight before protein removal-weight after removal}}{\text{Weight after protein removal (mg)}} \times 100$$

(Borderau and Anderson, 1978)

Study of the effect of feeding on the proportion of amino acids in the insect's body: The experiment was carried out with three replicates, one replicator included seven domestic workers, three replicates

for each sample. All samples were dried in an electric oven at a temperature of 60-70 ° C for 80 minutes and grinded by a ceramic mortar, then acid digestion of the sample - HCL was carried out at a temperature of 110 m for 24 hours (AOAC, 1990).

Standard amino acids 0.1 mg were dissolved in 100 ml NaoH sodium hydroxide (1000 ppm) and were read in a spectrometer at a wavelength of 264 n.m and the equation was applied:

%amino acids = reading of the insect solution at a wavelength of 264 * ppm concentration / reading of standard acids at a wavelength of 264 nm.

Results and Discussion

Study of the effect of mixtures with different protein materials on the chemical compounds of honey bee workers in the third season: The results of the statistical analysis, as shown in Table (1), showed the effect of mixtures and seasons on the chemical composition of the worker's body, where the comparison treatment excelled in moisture content. The results of the statistical analysis showed that there were significant differences in the effect of the mixtures on the percentage of protein in the insect's body if the treatment was given water only and the treatment (pollen + honey) with the highest percentage reaching 54.16 and 54.19%, respectively. And the percentage of protein decreased in the comparison treatment, reaching 43.60%. For fats, the treatment (pollen + honey + palm pollen + protein) outperformed by 10.04% and decreased in the control treatment if it reached 7.15% and the percentage of chitin in the water treatment only (the comparison) was superior to 56.40%, the results also showed that the artificial feeding worked to reduce moisture in the bodies of bees, due to the increase of solid substances in relation to the liquid in the food medium, the moisture content in the bodies of bees is called the Sap Coefficient, as it helps the bees to withstand low temperatures, the more the liquid coefficient is a little, the more the insect tolerates the cold. As for chitin, the process of its manufacture and deposition is carried out by many regulating hormones, such as the hormone of youth and molting, which are secreted from inside the body of the insect (Mansour, 2015). That the bees accepting mixtures containing pollen and palm pollen led to an increase in the percentage of fat. As the worker bees in the winter keep protein inside the fatty bodies and carbohydrates, as the sugar trehalose is a reserve for energy production and its concentration is constant in the blood and in balance with the glycogen stored in the fatty bodies. Brood food, and amino acids

participate in most of the vital activities of the body, as they enter the synthesis of hormones and enzymes, and honey bee workers consume pollen to

obtain protein and amino acids to complete their growth, development and longevity linked to the amount of proteins in fatty bodies (Huang, 2010).

Table (1) Effect of mixtures with different protein materials on the chemical compounds of honey bee workers

Transactions	moisture content %	protein %	fat %	chitin %
Pollen + Honey	68.27 ^b	57.16 ^{ed}	10.08 ^a	48.84 ^c
Pollen + honey + palm pollen	60.67 ^{cb}	56.19 ^{ed}	10.28 ^b	46.81 ^{cb}
Pollen + Honey + Protein	60.10 ^a	55.28 ^d	9.11 ^{cd}	48.72 ^{cb}
Pollen + honey + palm pollen + protein	54.30 ^b	58.13 ^{ed}	10.09 ^e	49.87 ^{cb}
Water only	70.10 ^d	48.0 ^{cd}	7.28 ^{cd}	55.40 ^{de}
Average	37.97			
LSD 0.05		2.43		
LSD 0.05 for overlaps		5.44		

The effect of mixtures with natural nutritional supplements on the chemical components of honey bee workers:

The results of the statistical analysis, as shown in Table (2), showed the superiority of the comparison treatment in moisture content and chitin, which amounted to 96.15 and 56.70%, respectively. And the moisture percentage in the treatment (sugar solution + spirulina) was 69.30%. And the treatment (diabetic solution + spirulina) achieved the highest protein percentage and outperformed the control treatment with a value of 43.30%. As for fats, the treatment (sugar solution + *Ganoderma lacidum* + spirulina) outperformed it, which amounted to 10.14%, and it decreased in the treatment of sugar solution, which amounted to 5.20%. The results showed that the lowest value in

the percentage of chitin gave the treatment (sugar solution + spirulina) and it was 55.81.

The reason for the decrease in humidity may be due to the change in the proportions of the components of the cuticle according to the nutrition and environmental conditions, which leads to an increase in the permeability of the cuticle and the loss of water. The reason for the increase in protein and fat in the treatments that reishi mushrooms and spirulina enter into the food mixture is because they contain many active substances, which are more than 200 active substances, including organic germanium, Ergosterols, adenosate compounds and polysaccharides, as well as containing ganoderic acids, vitamins and other important substances (Al-Atabi and Abdel-Wahid, 2009).

Table (2) The effect of mixtures with natural nutritional supplements on the chemical components of honey bee workers

Transactions	moisture content %	protein %	fat %	chitin %
sugar solution	73.27 ^{cb}	50.12 ^a	6.20 ^a	55.88 ^{ed}
Sugar solution + <i>Ganoderma lacidum</i>	80.61 ^{ed}	54.30 ^{ab}	9.15 ^b	49.70 ^{cb}
Sugar solution + spirulina	70.30 ^{ad}	56.11 ^{ac}	6.23 ^c	46.89 ^a
Sugar solution + <i>Ganoderma lacidum</i> + spirulina	80.27 ^{ab}	52.18 ^d	10.45 ^e	50.82 ^{cd}
Water only	97.15 ^b	45.30 ^e	7.23 ^{cd}	58.70 ^{cb}
Average		40.65		
LSD 0.05		2.33		
LSD 0.05 for overlaps		5.22		

The effect of feeding with pollen supplements on the proportion of amino acids in the insect's body:

The results of the statistical analysis in Table (3) showed that there is a clear difference in the

percentage of the main amino acids in the domestic workers according to the type of food provided to them. The treatment (pollen + honey + palm pollen) recorded the highest percentages of amino acids

(valine, lysine, vitolinine, talrin, Histidine (which amounted to (4.15%, 5.84,4.78,4.78%) respectively, while the comparison treatment water only gave the lowest value for amino acids (valine, lysine, tryptophan, vitolin, tellnin, cysteine, tyrosine, isoleucine histidine), which amounted to (0.01) %,5%,2%, 4.23%, 3.02%, 3.98%, 0.32%, 4.08%,3.99%) respectively, and the treatment of (honey + pollen) recorded the highest percentage of lysine acid as it reached 5.11% and the lowest value for terrine It reached 2.96%, while the treatment of (pollen, honey, vitamins and protein) had the highest percentage of lysine acid, which amounted to 5.78%, and the lowest percentage of tryptophan acid was 2.07%, and the results of the statistical analysis recorded that the treatment

(pollen + honey + protein) had the highest percentage of tryptophan acid, as the percentage reached 6.22% and the lowest percentage of valine, which reached 3.56%. The reason is due to the bees benefiting from protein materials, vitamins and amino acids that are included in the composition of pollen, honey and palm pollen.

The amino acids and proteins are important for all living organisms and constitute half of its dry weight. They are found in the skin and hair, muscles to provide protection for movement, and in the bones to give them protection Structure and in the blood and nerves. It has an important role in stimulating reactions that provide the body with energy and growth materials, these proteins are called enzymes that catalyze these reactions.

Table (3) The effect of feeding with natural nutrients on the percentage of amino acids in the insect's body

Iterators	Valine	lysine	tryptophan	vitolin	tyrenin	cysteine	tyrosine	isoleucine	histidine
Pollen + honey	3.72 ^a	5.11 ^d	5 ^a	4.43 ^d	2.96 ^e	5.32 ^c	4.08 ^d	4.32 ^e	4.32 ^e
Pollen + honey + protein + vitamin	3.84 ^b	5.78 ^a	2.03 ^e	5.35 ^d	4.35 ^b	4.98 ^c	5.67 ^a	4.26 ^b	4.32 ^b
Pollen + honey + palm pollen	4.15 ^a	5.84 ^a	6.17 ^a	5.49 ^c	4.78 ^b	5.26 ^a	5.78 ^a	4.78 ^b	5.87 ^a
Pollen + honey + protein	3.56 ^d	5.32 ^c	6.22 ^a	4.82 ^b	4.99 ^b	5.36 ^c	5.85 ^a	4.56 ^b	4.32 ^b
water only	0.01 ^e	5 ^a	2 ^b	4.23 ^b	3.02 ^d	3.98 ^e	0.32 ^e	4.08 ^d	3.99 ^e

The effect of feeding with natural nutrients on the percentage of amino acids in the insect's body: The results of the statistical analysis in Table (4) showed the effect of sugary nutrition, *Ganoderma lacidum* and spirulina on the percentage of amino acids, as the treatment (sugar solution) gave the highest percentage of phytolenin acid, which amounted to 5.07%, and gave the lowest value of valine acid, which amounted to 2.58%, while the treatment (diabetic solution) recorded + Reishi solution) gave the highest percentage of acid 5.05% and the lowest value of valine acid, which amounted to 2.85%, and the results of the statistical analysis recorded the superiority of lysine acid in the treatment of (sugar solution + spirulina) which amounted to 5% and it

decreased in tryptophan acid, which amounted to 0.09%, and the treatment of (The sugar solution + *Ganoderma lacidum* + spirulina) had the highest percentage in cysteine acid, which amounted to 5.01%, and the lowest value in tyrosine acid, which amounted to 0.01%, and the comparison treatment (water only) recorded the highest percentage of lysine and tryptophan, which amounted to 5%, and gave the lowest value in valine acid. It amounted to 0.02%.

Amino acids are of great importance to the body of insects, as they are found in the cuticle layer, bristles and muscles to ensure movement and also have a role in interactions where they provide the body with energy.

Table (4) The effect of natural mixtures on the proportion of amino acids in the body of honey bee workers

Iterators	Valine	lysine	tryptophan	vitolin	tyrenin	cysteine	tyrosine	isoleucine	histidine
sugar solution	2.58 ^a	4.17 ^a	5 ^d	5.07 ^d	3.04 ^c	3.75 ^b	4.88 ^b	4.76 ^b	4.85 ^b
Sugar solution + <i>Ganoderma lacidum</i>	2.85 ^b	5.05 ^d	4.52 ^b	4.52 ^b	3.51 ^b	2.96 ^b	3.32 ^b	4.85 ^b	0.23 ^c

Sugar solution + spirulina	2.48 ^a	5 ^d	0.09 ^c	3.42 ^b	3.70 ^b	3.43 ^c	4.86 ^b	4.85 ^b	4.85 ^b
Sugar solution + <i>Ganoderma lacidum</i> + spirulina	2.86 ^b	3.84 ^b	5 ^d	3.84 ^c	3.07 ^b	5.01 ^a	0.01 ^c	4.86 ^b	4.85 ^b
Water	2.46 ^b	4.23 ^b	5 ^d	3.69 ^b	3.01 ^b	2.69 ^a	0.01 ^c	4.23 ^b	0.89 ^c

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