Arab Universities Journal of Agricultural Sciences
(2022) 30 (1) 147-155
Website: http://ajs.journals.ekb.eg

Impacts of Natural and Supplementary Foods on Some Biochemical Activities in the Bodies of Honeybee Workers

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https://doi.org/10.21608/AJS.2022.108947.1440

Received 29 December 2021; Accepted 14 February 2022

Keywords:

Honeybee,
Protein,
Invertase,
ATPase,
Alkaline phosphatase

Abstract: The experiments were conducted during seasons (summer, autumn, winter, and spring) 2020–2021 to study the activities of invertase, adenosine triphosphatase (ATPase) and alkaline phosphatase (AlkP) as well as the determination of total proteins. These activities were determined in the bodies of adult honeybee workers, *Apis mellifera*, (house and forager bees) after feeding on three different diet groups (A) pollen cake, (B) supplementary diet and (C) control group during the four seasons. Group (A) received 50 g pollen cake + 250 ml honey syrup (2 honey: 1 water)/colony/three-day intervals, group (B) received 50 g Brewer's yeast chickpea cake fortified with 4.2% pollen + 250 ml sugar syrup (2 sugar: 1 water) while group (C) received 250 ml sugar syrup (1 sugar: 1 water) only. Results indicated that the highest increase in total proteins was found in group B during the autumn season with house bees. Invertase activity was high in group A during the summer season with forager bees. The highest ATPase activity was observed in group B during the spring season with house bees, whereas the highest AlkP activities were found in group A during the autumn season with house bees.

1 Introduction

Honeybees, similar to all living organisms, need energy sources, which they obtain from nectar (the main source of carbohydrates) and pollen (the main source of proteins, minerals, and vitamins) **(**Brodschneider and Crailsheim 2010). Carbohydrates are important to honeybees as an energy source, especially during winter for colony and brood thermoregulations (Kleinhenz et al 2003, Sammataro and Weiss 2013). Nutrition is understood to play an important role in bee development and antiviral immunity (DeGrandi- Hoffman and Chen 2015), where low levels of macronutrients have been associated with reduced worker lifespan (Knox et al 1971) and capacity for energy-intensive tasks, such as flight, thermoregulation, comb building, and colony vigor (Brodschneider and Crailsheim 2010, Zheng et al 2014). Forager bees (field bees) have less fat (responsible for energetic metabolism) than nurse bees (house bees). The decrease in fat in forager bees is not attributable to age. The strong association between low-fat stores and the transition to foraging suggests that the nutritional status may be involved in regulat-

ing the division of labor in honeybee colonies (Toth and Robinson 2005, Arrese and Soulages 2010). Twenty proteins have been discovered in the brain of a honeybee. These proteins may be highly experienced in proteins involved in energy production, iron binding, metabolic signaling, and neurotransmitter metabolism. These differential expressions of proteins may be related to genetic and behavioral changes in *Apis mellifera* (Garcia et al 2009). The variability in invertase enzyme activities found in different honey types is probably due to a series of factors such as nectar collection period abundance of nectar flow and its sugar content, age, and pollen consumption of bees (Persano et al 1999). In Carniolan bees, the invertase secretion of cephalic and thoracic salivary glands increases gradually with age. In Egyptian bees, invertase increases with age only in the cephalic gland. In 10–15-day-old bees, the highest secretion activity is detected in the thoracic gland (Al-Sherif et al 2017). The invertase enzyme activity in the hypopharyngeal gland, which is determined in Egyptian and Carniolan honeybees on three ages of worker bees (newly emerged, 10–15-day-old, and forager bees), establishes a different view of the secretion trend of the enzyme in the two honeybee races, which may be considered race-specific characteristics (Al-Sherif et al 2012). The progressive increase of adenosine triphosphatase ATPase (mitochondrial in origin) is related to developmental changes during the pharate adult and early adult stages of honeybee workers in a colony at 32°C. The enzyme activity in the young adult stage is greater than that in the pharate adult stage (Cheng et al 1980). Alkaline phosphatase (AlkP) activities are localized on the elongate microvilli of the striated border and within large electron-lucent microbodies. The association of AlkP activities with the peroxisomal microbodies and their relationship with phospholipid metabolism have been discussed in existing studies (Jimenez and Gilliam 1990). During the shortage of pollen and nectar, beekeepers tend to feed honeybee colonies with pollen substitutes or supplementary diets (Saffari et al 2010a, b). Moreover, sugar syrup or honey candy showed the highest ability to improve the tolerance of honeybees in cold climates (Abou-Shaara 2017).

The aim of the present study is to evaluate the effects of three types of diets (pollen cake, Brewer's yeast chickpea cake fortified with pollen and sugar syrup) during the four seasons (summer, autumn, winter, and spring) for encouraging honeybee colonies to produce the energy they need to do all their work, whether making hives for house bees or collecting nectar and pollen for forger bees and heating in winter.

2 Material and Methods

2.1 Experimental treatments

This work was performed in the apiary of the Plant Protection Research Institute at El-Quanater, Qaluobia Governorate and at the Agricultural Microbiology Department of the National Research Center. The experiments were conducted during the four seasons (summer, autumn, winter, and spring). Fifteen honeybee colonies headed with open-mated local Carnica queens, which had relatively the same strengths, were used. The bee colonies were divided into three groups, and each group comprised five colonies (replicates). The colonies received one of the following diets during the summer, autumn, winter, and spring seasons.

Group (A) Pollen Cake: 50g pollen cake as a protein supplement (3 parts pollen + 3 parts sugar + 1 part water) + 250 ml concentrated honey solution (2 honey: 1 water)/colony/three-day intervals.

Group (B) Supplementary Diet: 50g Brewer's yeast chickpea cake fortified with 4.2% pollen as a protein supplement (2 parts dried Brewer's yeast + 3 parts chickpea meal + 3 parts honey + 1 part pollen + 15 parts sugar) + 250 ml concentrated sugar solution (2 sugar:1 water)/colony/three-day intervals.

Group (C) Control: 250 ml sugar solution (1 sugar: 1 water)/colony/three-day intervals.

2.2 Bee worker collection

Adult honeybee workers (house and forager) were collected from colonies at the end of each season. They were identified by their specific behaviors (e.g., nursing behavior toward larvae). The bees were taken from the combs of the brood nest. To ensure the selection of fully mature ones, forager bees were collected from the hive door. Bee samples were placed in a freezer until chemical analysis was performed. The samples were sent to the Agricultural Microbiology Department of the National Research Center for analysis.

2.3 Preparation of homogenate samples for biochemical analysis

The samples were homogenized in the volume of sodium phosphate buffer (0.2M, pH 6.6) in the ratio of 10 mg/1ml. The homogenates were centrifuged at 10,000 xg for 15 minutes/4°C, and the supernatant was used as the enzyme source. All samples were kept frozen for enzyme activity determination (Taha and Al Hadek 2017).

The invertase, ATPase, and AlkP activities were determined in homogenates made from the entire bodies of honeybee workers and expressed as units (µg) of enzyme activity per mg of tissue or mmol per mg of tissue.

2.3.1 Determination of total proteins

Protein determination was based on the observation that Coomassie brilliant Blue G-250 exists in two different forms, red and blue. The red form is converted to the blue form upon binding of dye to protein. The protein-dye complex was read at 595 nm. Total proteins were determined by using the method of (Bradford 1976).

2.3.2 Determination of carbohydrate-hydrolyzing enzyme activities

The activities of invertase (which hydrolyzes sucrose) were selected to reflect the effects of the studied bulk and nano-formulations on the physiological functions of the digestive system of the affected instar larvae. The determination procedure of the three enzymes was performed according to the method described by (Ishaaya and Swiriski 1976).

2.3.3 Determination of ATPase activity

The specific activity of sodium and potassium dependent on ATPase in the insect's homogenate was determined according to the method described by (Shiosaka et al 1971).

2.3.4 Determination of AlkP activities

AlkP activities were determined according to the method described by (Powell and Smith 1954).

2.4 Statistical analysis

The one-way and three-way Anova test was applied for analysis of variance and Duncan's Multiple Range Test with a level of 0.05 was used to determine the significance of differences among the means, using SAS (SAS Institute 2006).

3 Results and Discussion

3.1 Biochemical activities in the bodies of honeybee workers during the four seasons (summer, autumn, winter, and spring)

The data in **Tables (1, 2, 3 and 4)** show the determination of total proteins and the activities of invertase, ATPase, and AlkP in the bodies of adult bee workers (house and forager) after feeding on three different diets (pollen cake, supplementary diet, and control) during the four seasons.

3.1.1 Total proteins

The data in **Table 1** indicate that the highest total protein in the bodies of honeybee workers (µg protein/mg tissue) was recorded in the bees that received the supplementary diet (2292.49 µg), followed by pollen cake (1948.79 µg). The lowest total protein was recorded in the control group (1873.65 µg). The proteins in the bodies of honeybee workers were influenced by the seasons, where 3167.80, 2263.80, 2112.50 and 609.10 µg were recorded in autumn, spring, winter, and summer, respectively, with significant differences among them. House bee workers exhibited higher protein content (2201.55 µg) than forager bee workers (1875.04 µg), with significant differences between them.

3.1.2 Invertase activities

The data in **Table 2** reveal that the highest invertase activities in the bodies of honeybee workers were found in bees that received pollen cake (7.25µg glucose/minute/mg tissue), followed by supplementary diet and control (6.73 and 5.54µg glucose/minute/mg tissue), respectively. These activities were affected by the four seasons; specifically, 13.79, 8.29, 3.10 and 0.85µg glucose/minute/mg tissue were recorded in summer, autumn, winter, and spring, respectively, with significant differences among them. The influence of the type of worker on the invertase activities in the bodies of honeybee workers was higher in forager bees than in house bees (i.e., 7.74 and 5.27µg glucose/minute/mg tissue), respectively with significant differences between them.

Table 1. Rates of total proteins (µg protein/mg tissue) in the bodies of honeybee workers (house and forager) after being fed on three different diets during the four seasons

Treatment / Season	Pollen Cake		Supplementary Diet		Control		F value	L.S.D	Mean (Season)	F value	L.S.D
	House bees	Forager bees	House bees	Forager bees	House bees	Forager bees					
Summer	636.95^{ab} ±37.07	566.85^{ab} ±32.96	630.85^{ab} ±51.91	676.57^{a} ±65.93	530.28^{b} ±32.96	613.03^{ab} ±27.73	1.46	134.07	609.08^{C} ±18.92	187.14	220.46
Autumn	3497.59^{ab} ±351.62	2761.59^{bc} ±500.47	2354.28^{c} ±164.76	3359.53^{abc} ±321.73	3061.94^{abc} ±419.67	3972.11^{a} ±223.29	2.68	1075.3	3167.8^{A} ±174.10		
Winter	2100.34^{bc} ±29.54	1903.99^{c} ±192.13	2063.03^{cb} ±143.11	2494.62^{a} ±55.84	1853.71^{c} ±75.00	2259.19^{ab} 24	5.02	325.81	2112.5^{B} ±63.76		
Spring	2393.59^{b} ±120.68	1729.36^{c} ±58.29	5642.51^{a} ±140.27	1118.16^{d} ±87.17	1653.48^{c} ±69.64	1045.48^{d} ±7959	316.63	298.8	2263.8^{B} ±383.48		
Mean (Tr)	1948.79^{B}±206.23		2292.45^{A}±326.26		1873.65^{B}±239.81					11.06	190.93
Mean (House)	2201.55^{A}±235.41									17.73	155.89
Mean (Forager)	1875.04^{B}±188.20										

Where: **Pollen Cake:** 50g pollen cake as a protein supplement (3 parts pollen + 3 parts sugar + 1 part water) + 250 ml concentrated honey solution (2 honey: 1 water)/colony/three-day intervals.
Supplementary Diet: 50g Brewer's yeast − chickpea cake fortified with 4.2% pollen as a protein supplement (2 parts dried Brewer's yeast + 3 parts chickpea meal + 3 parts honey + 1 part pollen + 15 parts sugar) + 250 ml concentrated sugar solution (2 sugar:1 water)/colony/three-day intervals.
Control: 250 ml sugar solution (1 sugar: 1 water)/colony/three-day intervals.
Small letters: (a, b,…) significant among treatments within seasons
Capital letters: (A, B,) significant among treatments, seasons, and types of workers

Table 2. Rates of invertase activities (µg glucose/minute/mg tissue) in the bodies of honeybee workers (house and forager) after being fed on three diet groups during the four seasons

Treatment / Season	Pollen Cake		Supplementary Diet		Control		F value	L.S. D	Mean (Season)	F value	L.S. D
	House bees	Forager bees	House bees	Forager bees	House bees	Forager bees					
Summer	5.90^{c}±1.07	16.97^{b} ±3.71	16.72^{b} ±0.20	17.45^{ab} ±2.56	2.35^{c} ±0.28	23.35^{a} ±1.82	15.19	6.29	13.79^{A} ±1.89	158.26	1.30
Autumn	12.76^{a} ±1.15	12.49^{a} ±1.79	9.94^{ab} ±0.25	2.00^{c} ±0.44	4.51^{c} ±0.23	8.06^{b} ±0.42	22.28	2.83	8.29^{B} ±1.01		
Winter	4.00^{b} ±0.21	4.54^{a} ±0.25	3.59^{b} ±0.02	2.7^{c} ±0.06	1.42^{d} ±0.13	2.38^{c} ±0.10	54.13	0.48	3.10^{C} ±0.26		
Spring	0.710^{b} ±0.04	0.676^{b} ±0.04	0.75^{b} ±0.10	0.693^{b} ±0.02	0.673^{b} ±0.01	1.6^{a} ±0.19	15.33	0.28	0.850^{D} ±0.08		
Mean (Tr.)	7.25^{A}±1.27		6.73^{A}±1.39		5.54^{B}±1.48					4.89	1.12
Mean (House)	5.27^{B}±0.85										
Mean (Forager)	7.74^{A}±1.32									28.90	0.92

Where: **Pollen Cake:** 50g pollen cake as a protein supplement (3 parts pollen + 3 parts sugar + 1 part water) + 250 ml concentrated honey solution (2 honey: 1 water)/colony/three-day intervals.
Supplementary Diet: 50g Brewer's yeast − chickpea cake fortified with 4.2% pollen as a protein supplement (2 parts dried Brewer's yeast + 3 parts chickpea meal + 3 parts honey + 1 part pollen + 15 parts sugar) + 250 ml concentrated sugar solution (2 sugar:1 water)/colony/three-day intervals.
Control: 250 ml sugar solution (1 sugar: 1 water)/colony/three-day intervals.
Small letters: (a, b, …) significant among treatments within seasons
Capital letters: (A, B, …) significant among treatments, seasons, and types of workers

3.1.3 ATPase activity

Table 3 presents that the highest ATPase activity in the bodies of honeybee workers was observed in bees that received a supplementary diet (51.73 mmol/mg tissue) followed by pollen cake and control (49.91 and 44.70 mmol/mg tissue), respectively, with significant differences among them. The seasons influenced the ATPase activity in the bodies of honeybee workers specifically, 90.40, 83.48, 16.28, and 4.96 mmol/mg tissue were respectively recorded in spring, winter, autumn, and summer, with significant differences among them. The influence of the type of worker on this variation is that ATPase activity was higher in-house bees than in forager bees (48.98 and 48.58 mmol/mg tissue), respectively with no significant differences between them.

3.1.4 AlkP activities

The results in **Table 4** indicate that the diets influenced the AlkP activities in the bodies of honeybee workers; pollen cake, supplementary diet, and control (2.89, 2.24, and 1.67 µg Phenol/minute/mg tissue), respectively, with significant differences among them. The seasons influenced the AlkP activities in the bodies of honeybee workers; 4.76, 3.27, 0.61, and 0.43 µg Phenol/minute/mg tissue were respectively recorded in autumn, summer, winter, and spring, with significant differences among them. The type of worker influenced the AlkP activities of house and forager bees (2.57 and 1.95 µg Phenol/minute/mg tissue), respectively, with significant differences between them.

This experiment, where honeybee colonies were fed on a supplementary diet recorded the highest total protein in the bodies of honeybee workers. The reason for this highest record may be because it contains 2 parts dried Brewer's yeast, 3 parts chickpea, 3 parts honey, 1 part pollen, and 15 parts sugar + sugar syrup (2:1). Due to the lack of activity during the autumn season, with a surplus store of diets, protein appeared in a high percentage and was extended at a lower rate in winter; then, it began to gradually increase in spring and decrease significantly in summer due to the activity of regulating temperatures in critical processes. The total protein in the bodies of house bee workers was higher than that in the bodies of forager bee workers. These results agree with (Reeves et al 2018, Crailsheim 1990, Hernandez et al 2012) who reported that nurse bee brains exhibit increased expression of proteins implicated in translation, ribosomal structure, and biogenesis (14.5%) compared with forager bee brains (1.8%). In the colonies fed with supplemental diet in different periods, a gradual increase was observed in the protein content of the new workers over the study period to reach the maximum level on March 6. By contrast, the protein content of the new workers in the control group gradually decreased (Tawfik et al 2020).

Feeding honeybee colonies on pollen cake recorded the highest invertase activity in the bodies of honeybee workers, followed by supplementary diet, without significant differences between them. The reason may be that both diet groups, in addition to having the same amount of carbohydrate supplements, are composed of 250 ml of honey and 250 ml of concentrated sugar syrup, respectively. Due to the scarcity of plants and crops that bees depend on for collecting nectar in the experimental area during spring, the results indicated that the percentage of invertase enzymes during the spring season was minimal. On the contrary, the summer season, which is characterized by an abundance of flowering plants (alfalfa), recorded the highest degree of invertase enzyme. Considering the presence of some medicinal and aromatic plants and flowers that bloom in early autumn, it was ranked second in the degree of invertase enzyme. The presence of invertase enzyme during the winter period ranked third because bees were fed with sugar solutions. The invertase activities in the bodies of honeybee workers were higher in forager bees than in house bees. This result agrees with (Persano et al 1999) who argued that when a honeybee becomes a forager, its glands produce more digestive enzymes. Invertase is not detected in newly emerged bees of the Carniolan strain and in general, the excretion of newly emerged bees is significantly less pronounced than the age of nurse and forager bees (Al-Sherif et al 2012, Al-Sherif et al 2017, Ament et al 2008).

The supplementary diet recorded the highest ATPase activity in the bodies of honeybee workers; the reason may be because it contains a large amount of carbohydrates, which are a major source of energy production. This result agrees with (Abd El-Naby and Zidane 2014) who indicated an increase in the activities of some enzymes in the bodies of honeybee workers, as a result of feeding on different diets. Given that forager bees began their activities, the energy enzymes also began to be active during the spring season, which recorded the highest number of enzymes, following the winter season. The percentage of enzymes was high because the bees needed energy in winter to

Table 3. Rates of ATPase activity (mmol/mg tissue) in the bodies of honeybee workers (house and forager) after being fed on three diet groups during the four seasons

Treatment / Season	Pollen Cake		Supplementary Diet		Control		F value	L.S. D	Mean (Season)	F value	L.S.D
	House bees	Forager bees	House bees	Forager bees	House bees	Forager bees					
Summer	11.9^{a} ±0.28	3.45^{cd} ±0.62	2.46^{d} ±0.13	5.13^{b} ±0.46	3.91^{bc} ±0.52	2.91^{cd} ±0.169	73.71	1.26	4.96^{D} ±0.79	6151.26	1.60
Autumn	19.79^{b} ±1.01	13.91^{c} ±0.34	23.33^{a} ±2.01	22.35^{ab} ±0.28	8.83^{d} ±0.44	9.5^{d} ±0.28	44.20	2.97	16.28^{C} ±1.45		
Winter	84.37^{ab} ±0.55	86.16^{ab} ±1.50	86.8^{ab} ±1.89	89.00^{a} ±1.14	71.93^{c}± 1.89	82.69^{b} ±2.48	12.785.02	5.21	83.49^{B} ±1.46		
Spring	90.41^{ab} ±1.51	89.33^{ab} ±2.28	93.12^{a} ±1.31	91.7^{ab} ±0.85	90.92^{ab} ±1.14	86.91^{b} ±3.32	1.22	5.94	90.39^{A} ±0.81		
Mean (Tr)	49.91^{B}±7.91		51.73^{A}±8.14		44.70^{C}±8.10					55.45	1.39
Mean (House)	48.98^{A}±6.42										
Mean (Forager)	48.58^{A}±6.66									0.49	1.13

Where: **Pollen Cake:** 50g pollen cake as a protein supplement (3 parts pollen + 3 parts sugar + 1 part water) + 250 ml concentrated honey solution (2 honey: 1 water)/colony/three-day intervals.
Supplementary Diet: 50g Brewer's yeast − chickpea cake fortified with 4.2% pollen as a protein supplement (2 parts dried Brewer's yeast + 3 parts chickpea meal + 3 parts honey + 1 part pollen + 15 parts sugar) + 250 ml concentrated sugar solution (2 sugar:1 water)/colony/three-day intervals.
Control: 250 ml sugar solution (1 sugar: 1 water)/colony/three-day intervals.
Small letters: (a, b, …) significant among treatments within seasons
Capital letters: (A, B, …) significant among treatments, seasons, and types of workers

Table 4. Rates of AlkP activities (µg Phenol/minute/mg tissue) in the bodies of honeybee workers (house and forager) in after being fed on three diet groups during the four seasons

Treatment / Season	Pollen Cake		Supplementary Diet		Control		F value	L.S. D	Mean (Season)	F value	L.S. D
	House bees	Forager bees	House bees	Forager bees	House bees	Forager bees					
Summer	6.32^{a} ± 0.54	2.80^{bc} ± 0.16	3.75^{b} ±0.24	1.98^{cd} ± 0.45	1.55^{d} ± 0.05	3.21^{b} ± 0.46	21.34	1.13	3.26^{B} ± 0.39	421.79	0.29
Autumn	8.43^{a} ± 0.78	3.37^{cd} ± 0.10	3.56^{cd} ± 0.06	6.49^{b} ± 0.07	4.12^{c} ±0.05	2.60^{d} ± 0.22	42.90	1.05	4.76^{A} ± 0.50		
Winter	0.52^{b} ± 0.03	0.83^{a} ± 0.02	0.81^{a} ± 0.02	0.60^{b} ± 0.02	0.52^{b} ± 0.04	0.36^{c} ± 0.01	42.03	0.08	0.60^{C} ± 0.04		
Spring	0.37^{bc} ±0.04	0.47^{ab} ±0.06	0.43^{ab} ±0.01	0.29^{c} ±0.01	0.52^{a} ±0.02	0.48^{ab} ± 0.04	4.57	0.12	0.43^{C} ±.0.02		
Mean (Tr)	2.89^{A}± 0.60		2.24^{B}± 0.43		1.67^{C}± 0.29					47.03	0.25
Mean (House)	2.57^{A}±0.43									36.22	0.20
Mean (Forager)	1.95^{B}± 0.30										

Where: **Pollen Cake:** 50 g pollen cake as a protein supplement (3 parts pollen + 3 parts sugar + 1 part water) + 250 ml concentrated honey solution (2 honey: 1 water)/colony/three-day intervals.
Supplementary Diet: 50 g Brewer's yeast − chickpea cake fortified with 4.2% pollen as a protein supplement (2 parts dried Brewer's yeast + 3 parts chickpea meal + 3 parts honey + 1 part pollen + 15 parts sugar) + 250 ml concentrated sugar solution (2 sugar:1 water)/colony/three-day intervals.
Control: 250 ml sugar solution (1 sugar: 1 water)/colony/three-day intervals.
Small letters: (a, b, …) significant among treatments within seasons
Capital letters: (A, B, …) significant among treatments, seasons, and types of workers

regulate the thermoregulation within honeybee colonies. It ranged between 30° and 36°. In summer, the degree of ATPase was recorded as the lowest one. It might be related to the few activities of honeybee colonies, as a result of the availability of food within them. No significant differences were found in the ATPase activity in the bodies of honeybee workers between house and forager bees because the functional role of house beeswax is no less important than that of forager bees. These results are in agreement with those of previous studies (Baumann and Takeyasu 1993, Nam et al 2014, Emery et al 1998, Nicodemo et al 2014, Cheng et al 1980).

Pollen cake came first in the order of influencing AlkP activities. The reason might be that it contains many minerals that stimulate enzyme activities. The AlkP activities in the bodies of honeybee workers were higher in summer and continued to increase in autumn then decreased in winter and continued to decrease in spring. This result was because honeybee colonies had fewer activities during the summer and autumn seasons than during the winter and spring seasons. House bees recorded higher AlkP activities than forager bees. These results agree with (Suarez et al 1996, Gregorc et al 1998, Zheng et al 2017, Jimenez and Gilliam 1990) who reported that AlkP activities are localized on the elongated microvilli of epithelia Midgut from 5-day-old to 30-day-old honeybees. They also discussed the relationship of these honeybees with phospholipid metabolism.

A positive relationship was observed between invertase and AlkP. By contrast, a negative relationship was found among ATPase and both invertase and AlkP. AlkP somewhat stimulated an invertase secretion.

4 Conclusion

In this research, honeybee colonies were fed on either diet (A), which is 50 g of pollen cake + 250 ml of honey solution/colony/three-day intervals or diet (B), which is 50 g of Brewer's yeast chickpea cake fortified with 4.2% pollen+250ml of concentrated sugar solution in periods when natural food is scarce during the four seasons of the year (i.e., summer, autumn, winter, and spring). Both diets helped honeybee colonies produce the energy they needed to do all their work, whether making hives for house bees or collecting nectar and pollen for Forger's forager bees and heating in winter.

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