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Impact of Winter Feeding with Some Protein Pollen Supplement Diets on the Biological Activities of Honeybees

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ABSTRACT

Different protein sources, defatted soybean flour, casein or whey protein concentrate, were used for the preparation of three protein pollen supplement diets to feed colonies of *Apis mellifera carnica*, during the winter season. The effect of studied protein pollen supplement diets on food consumption (%), sealed brood area (In^2), pollen stores (In^2) and honeybee strength (in terms of mean number of combs covered with bees) of the colonies were recorded. The results indicated that, the defatted soybean flour or whey protein concentrate pollen supplement diet was more consumed by the honeybee colonies than casein pollen supplement diet. Also, the highest areas of worker sealed brood, stored pollen and colony population density were observed with defatted soybean flour or whey protein concentrate pollen supplement diet. Casein pollen supplement diet was found to be the lowest consumed and showed the lowest biological activities among three tested pollen supplement diets. There was a positive relationship between the amount of diet consumed and the changes in worker sealed brood, stored pollen and colony population density. Defatted soybean flour or whey protein concentrate in pollen supplement diets of honeybees during winter season proved to be very effective and could be valuable to improve colony parameters.

Keywords: defatted soybean flour, casein or whey protein concentrate, pollen supplement diets, food consumption, sealed brood area, pollen stores, honeybee strength

INTRODUCTION

Honeybees feed upon pollen and nectar of flowers. Pollen is the major source of protein, vitamins and minerals and nectar fulfils their carbohydrate requirement. During certain periods of the year, weather conditions are not suitable for bees and availability of food (nectar and pollen) resources is very low (Kumar and Agrawal, 2014). The problems of hurtful influence of adverse weather conditions and non-availability of bee flora all round the year have been realized by earlier workers [Saffari *et al*. (2004); Sihag & Gupta (2011) and Kumar *et al*. (2013)].

During the shortage or complete absence of pollen, or in the presence of only poor quality pollen, beekeepers often feed colonies of honey bees with either pollen substitute (with no pollen) or supplement (with pollen) diets (Saffari *et al*., 2006). Providing colonies with pollen supplement or substitute helps to survive and maintain throughout dearth period and help the colony to start early brood production (Prakash, *et al*., 2007).

Different types of pollen substitutes are used to feed the honeybee colonies during floral dearth. Pollen substitutes include powdered skimmed milk, soybean products, brewer's yeast, fish meal and meat scraps (Sihag and Gupta, 2011).Soybean products are still the most popular ingredients of the honeybee diet. Pollen supplement diets containing more than 20% of soybean flour are highly palatable to bees and have the nutritive requirements for their growth and reproduction (Mattila and Otis, 2006). There is paucity of information on casein and whey protein concentrate to form pollen supplement diets as protein sources. Whey is a term that generally refers the translucent liquid part of the milk which remains in the process of cheese manufacturing. Proteins are the nitrogen-containing substances which are formed by the amino acids. Whey proteins are separated and also purified by using various techniques to obtain different concentrations of whey proteins. Whey protein provides high level of branched and essential chain amino acids. In addition to these, whey protein is rich in minerals and vitamins (Shankar and Bansal, 2013). Casein is the main protein of milk, making up 80% of proteins in cow milk. It is a source of essential amino acids.

So, this study was carried out to evaluate three pollen supplement diets, protein rich, i.e. soybean flour, casein and whey protein concentrate by measuring consumption and colony parameters (brood, pollen stores and honeybee strength) and comparing it with the sugar syrup to recommend one pollen supplement diet as "Best" out of three tested pollen supplement diets for feeding honeybees during winter season.

MATERIALS AND METHODS

Study site

The study was conducted at apiary of Honey Bee Research Center, Environmental Agricultural Sciences Faculty, Al-Arish, Suez Canal University, Egypt from December 2015 to February 2016. Twelve colonies (each 4 combs) in the same strength of honeybee (*Apis mellifera carnica*) were used in this study. The tested colonies were classified into four groups; three colonies were used for each treatments and control.

Treatments and feeding

Three tested protein pollen supplement diets were prepared as shown in Table (1) and used to feed the bee under investigation. Sugar syrup was used as a control and prepared by dissolving one- kilo gram of crystal sugar in one liter of fresh water (1:1). The chemical composition of various components used in supplement diets was presented in Table (2).

Every 12 days, from 17 December 2015 until 15 February 2016, 50g of supplement diets (patties) were placed on a plastic grid above the brood area in each hive. The grid had openings of 6.5 mm, which allowed bees' access to the patty from below. Also, the colonies of control and treatments received one liter of 50

percent sugar syrup every 12 days. The unused portion of the diet was collected and weighed.

Table (1): The composition of tested protein pollen supplement diets (%).

Pollen supplement diet	Defatted soybean flour	Casein	Whey protein concentrate	Pollen	Honey
A	50			10	40
B		50		10	40
C			50	10	40

Table (2): Chemical composition of different components used in protein pollen supplement diets.

Component	Defatted soybean flour	Casein	Whey protein concentrate	Honey
Moisture	6.6	11.4	3.93	17.20
Protein	47.0	75	45.0	0.26
Fat	1.2	0.8	2.93	0.0
Ash	6.3	7.8	5.1	0.17
Sugar	33.2	0.1	41.3	79.59

Measurements:

1-Food consumption percentage

The food consumption percent (%) was calculated as the difference between the fresh weight of the diet and the weight after 12 days from providing it to the colony, and then divided by fresh weight. The calculations were repeated every 12 days for each diet type.

2-Brood area and stored pollen estimates

The areas (square inches) of stored pollen and the worker's sealed brood were measured at an interval of 12 days using a grid with 5 cm x 5 cm squares that covered the entire side of a comb. The grid was placed over each side of a comb and the number of squares with brood or pollen stores was counted. Measurements of all frames with brood or pollen stores were summed for each colony (Taha, 2007).

3- Colony population density (Honeybee strength)

Number of combs covered with bees/colony was recorded every 12 days to determine the colony population density (DeGrandi-Hoffman *et al*., 2008).

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) through SPSS computer program. Means were compared using Duncan's Multiple Range tests.

RESULTS AND DISCUSSION

Consumption of tested protein pollen supplement diets:

Data presented in Table (3) showed that, the consumption of tested protein pollen supplement diets. It could be observed that the percent consumption of all diets was found to be progressive. Maximal consumption rate was noticed either in the defatted soybean flour or whey protein concentrate diets. While, the lowest consumption rate was recorded with casein diet. It believed that the honeybees not preferred casein diet due to their poorness in carbohydrate. Results obtained for the consumption rates of tested diets are in accordance with that of (Sihag and Gupta, 2011) who reported that the pollen supplements have been consumed significantly and the soybean was the most preferred.

Table (3): Food consumption rate (%) for bees fed on tested protein pollen supplement diets

Protein supplement diets	Measurement interval				
	29/12/2015	11/1/2015	23/1/2016	03/2/2016	15/2/2016
Defatted soybean flour	45 ± 1.29 ^{ih}	53± 1.54 ^{fed}	67± 1.67 ^{c}	74± 1.78 ^{b}	80± 1.86 ^{a}
Casein	28± 0.76 ^{m}	35± 0.94 ^{l}	43± 1.29 ^{ji}	50± 1.50 ^{gf}	58± 1.60 ^{ed}
Whey protein concentrate	40± 1.24 ^{kj}	48± 1.33 ^{hg}	59± 1.60 ^{d}	67± 1.67 ^{c}	78± 1.82 ^{a}

***Means followed by the same letters are not significantly different at the 5% level of probability**

Sealed worker brood area:

The obtained results in Table (4) showed that, significant effect of tested protein pollen supplement diets on sealed worker brood area compared with the control. It could be seen that, the all tested protein pollen supplement diets improved sealed worker brood area compared with control. The sealed worker brood area increased at the last two periods in this study for control and treatments. Defatted soybean flour diet had the highest sealed worker brood area compared with other pollen supplement diets and control through all experiment period. Also, the sealed worker brood area of honeybee colonies fed whey protein concentrate diet was significantly higher than those of fed casein diet or sugar syrup (control) during experiment period. The highest sealed worker brood area was recorded for defatted soybean flour and protein concentrate diets at the end of experiment period. The dietary protein sources and formulations are attractive to bees and will support growth and development of emerging bees and brood rearing (Standifer *et al*.,1977).Similar results were obtained by Chhuneja *et al*. (1993); Saffari *et al*. (2010); Sihag and Gupta (2011) who reported an increase in brood area in the colonies provided various pollen supplements. Also, Abusabbah *et al* (2012) who indicates that feeding with soybean flour had high potential for improving build brood production during a shortage of natural maintenance.

Table (4): Effect of tested protein pollen supplement diets on sealed worker brood area (In^2)

Measurement interval	Tested protein supplement diets			Control
	Defatted soybean flour	Casein	Whey protein concentrate	
29/12/2015	204.00 ± 4.16 ^{fg}	128.00 ± 10.06 ^{j}	156.00 ± 2.00 $^{g-j}$	140.00 ± 1.15 ^{hij}
11/1/2015	226.66 ± 16.22 ^{def}	132.66 ± 7.33 ^{j}	192.66 ± 11.56 ^{fgh}	136.66 ± 2.90 ^{ij}
23/1/2016	272.66 ± 32.46 ^{cd}	176.66 ± 9.61 $^{f-j}$	210.00 ± 20.81 ^{efg}	171.33 ± 10.41 $^{f-j}$
03/2/2016	336.00 ± 29.14 ^{b}	223.33 ± 19.05 ^{def}	270.00 ± 18.47 ^{cd}	196.00 ± 12.16 ^{fg}
15/2/2016	384.66 ± 32.95 ^{a}	320.00 ± 15.09 ^{bc}	382.00 ± 5.29 ^{a}	188.00 ± 20.42 $^{f-I}$

***Means followed by the same letters are not significantly different at the 5% level of probability**

Stored pollen:

The area of stored pollen for colonies that received different protein pollen supplement diets was shown in Table (5).No significantly different stored pollen area was observed for every tested supplement diet and control during the first three periods in the experiment and then rose. Also, the results indicated that the maximum stored pollen area 172.00 ± 36.16 and 168.66 ± 11.68 In^2 were recorded in colonies fed defatted soybean flour and protein concentrate diets, respectively which gathered pollen better than casein diet and control. The colonies fed casein diet or sugar syrup had statistically equal stored pollen area. The amount of pollen stored in the colonies depends on the amount of collected pollen as well as the rate of pollen consumption by brood and bees (Taha and Al-kahtani,2013). The amount of pollen in the colony increased in parallel to the amount of brood (Georgijev *et al*.,2003).

Table (5): Effect of tested protein pollen supplement diets on stored pollen (In^2)

Measurement interval	Tested protein supplement diets			Control
	Defatted soybean flour	Casein	Whey protein concentrate	
29/12/2015	59.33 ± 2.40 $^{d-g}$	28.66 ± 1.76 ^{g}	54.00 ± 1.15 ^{efg}	46.66 ± 16.59 ^{fg}
11/1/2015	71.33 ± 9.68 $^{c-f}$	41.33 ± 4.05 ^{fg}	69.33 ± 10.34 $^{c-f}$	37.33 ± 3.33 ^{fg}
23/1/2016	86.66 ± 5.45 ^{cde}	52.66 ± 2.40 ^{efg}	77.33 ± 3.33 $^{c-f}$	52.66 ± 7.33 ^{efg}
03/2/2016	130.00 ± 16.16 ^{b}	74.00 ± 4.61 $^{c-f}$	130.66 ± 13.53 ^{b}	87.33 ± 13.13 ^{cde}
15/2/2016	172.00 ± 36.16 ^{a}	97.33 ± 9.82 ^{bcd}	168.66 ± 11.68 ^{a}	107.33 ± 6.35 ^{bc}

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

Colony population density (Honeybee strength)

Data presented in Table (6) cleared the effect of tested protein pollen supplement diets number of combs covered with bees. It could be noticed from the results that the bee populations significantly different ($p< 0.05$) with an overall average of 6.00 ± 0.00 frames of bees fed on defatted soybean flour or whey protein concentrate diet compared to 5.00 ± 0.00 frames of bees fed on sugar syrup or casein diet at the end of experiment period. This result is in close agreement with Kumer and Agrawal (2013) who reported that number of bee covered on frames are positively affected by feeding protein diet to bees. Also, Kumova (2000) reported that the different artificial diets had a positive effect on total number of frames covered by bees.

Positive correlation was found between worker broad area and each of food consumption, stored pollen area and colony population. These results agreed with the findings of (Taha and Al-kahtani, 2013) who stated that in winter, there was a significant positive correlation among brood area, stored pollen area and colony population. Gemeda (2014) found that there was a positive relationship that was highly significant ($p< 0.001$) between the amounts of food consumed and increase brood area and change in adult population. Also, Dodologlu *et al*. (2004) reported that there was a direct relation between brood production and the number of frames of bees and an increase in brood activity caused an increase in the number of mature bees.

Table (6): Effect of tested protein pollen supplement diets on the number of combs covered with bees

Measurement interval	Tested protein supplement diets			Control
	Defatted soybean flour	Casein	Whey protein concentrate	
29/12/2015	5.00 ± 0.00 ^{b}	4.00 ± 0.00 ^{c}	4.00 ± 0.00 ^{c}	4.00 ± 0.00 ^{c}
11/1/2015	6.00 ± 0.00 ^{a}	4.00 ± 0.00 ^{c}	5.00 ± 0.00 ^{b}	4.00 ± 0.00 ^{c}
23/1/2016	6.00 ± 0.00 ^{a}	4.00 ± 0.00 ^{c}	5.00 ± 0.00 ^{b}	4.00 ± 0.00 ^{c}
03/2/2016	6.00 ± 0.00 ^{a}	5.00 ± 0.00 ^{b}	6.00 ± 0.00 ^{a}	5.00 ± 0.00 ^{b}
15/2/2016	6.00 ± 0.00 ^{a}	5.00 ± 0.00 ^{b}	6.00 ± 0.00 ^{a}	5.00 ± 0.00 ^{b}

***Means followed by the same letters are not significantly different at the 5% level of probability**

CONCLUSION

There was a different effect of tested supplemental protein diets on food consumption and biological activities of honeybee colonies. It can be concluded from the results that, the soybean flour or whey protein concentrate supplemental diet is the best diet for feeding bee colonies during winter season. It provided best results with regard to net consumption and positive influence on colony parameters

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تأثير التغذية الشتوية ببعض مكملات حبوب اللقاح البروتينية علي الانشطة البيولوجية لنحل العسل

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تم دراسة تاثير تغذية نحل العسل *Apis mellifera carnica* بثلاث مكملات حبوب لقاح بروتينية (دقيق الفول الصويا منزوع الدسم ،كازين ،مركز بروتينات الشرش) اثناء موسم الشتاء علي معدل استهلاك الغذاء و مساحة الحضنة المغلقة بالبوصة المربعة ومساحة حبوب اللقاح بالبوصة المربعة والكثافة النحلية (متوسط عدد البراويز المغطاه بالنحل) .وقد اوضحت النتائج ان مكملات حبوب اللقاح المحتوية علي دقيق فول الصويا منزوع الدسم او مركز بروتينات الشرش كان اكثر استهلاكا بواسطة النحل من مكملات حبوب اللقاح المحتوية علي الكازين .ايضا لوحظ ان اكبر مساحة للحضنة المغلقة ومساحة حبوب اللقاح المخزنة وكثافة النحل كانت مع مكملات حبوب اللقاح التي تحتوي دقيق الفول الصويا منزوع الدسم او مركز بروتينات الشرش بينما كانت مكملات حبوب اللقاح المحتوية علي الكازين اقل استهلاكا كما اظهرت اقل نشاط بيولوجي مقارنة بباقي مكملات حبوب اللقاح المختبرة .وقد وجد علاقة طردية ما بين كمية الغذاء المستهلك وما بين التغيرات في مساحة الحضنة المغلقة وحبوب اللقاح وكثافة النحل.ونستنتج من الدراسة ان تغذية النحل على مكملات حبوب اللقاح التى تحتوى على دقيق فول الصويا منزوع الدسم او مركز بروتينات الشرش اثناء موسم الشتاء تكون اكثر فاعلية كما تحسن من الانشطة البيولوجية لخلايا النحل.