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Nutritional Effects of High Protein Feeds on Growth, Development, Performance and Overwintering of Honey Bee (*Apis mellifera L.*)

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ABSTRACT

In order to evaluate eight different protein sources in honey bee nutrition, some experiments were conducted as a completely randomized designs with four replicates and three steps incubator, field and overwintering in Isfahan region. Protein sources were lentil flour, soybean flour, soybean meal, bread yeast, wheat gluten, skim milk powder, fish meal and Pollen. Results showed that the longevity of caged honey bees in incubator was significantly affected by dietary treatments ($P<0.05$). Wheat gluten supplement and lentil substitute resulted in the highest (61 days) and lowest (9.2 days) longevity of bees in 50% mortality, respectively; while in 100% morality pollen and lentil substitutes caused the highest (143.5 days) and lowest (20.7 days) longevity of bees, respectively. Field experiments showed a significant difference between feed intake in experimental colonies. Honey bees consumed the most amounts of pollen cakes, while bread yeast and soybean cakes were consumed more than soybean meal and wheat gluten; however, fish meal cakes were not accepted by bees. Brood area of experimental colonies was significantly different and varied from 13050 to 22680 cm^2. Honey production in test colonies was also significantly ($P<0.05$) different and varied from 5.98 to 9.56 kg. After 90 days of overwintering the experimental colonies fed with pollen supplement and substitute cakes showed a significant ($P<0.05$) difference in honey consumption, total bee mass reduction and laying area of queens. Dietary inclusion of pollen and wheat gluten supplements caused the lowest bee mass reduction, and respective colonies had the greatest laying area at the end of winter. Overall, the present results showed that soybean flour and bread yeast can be used as pollen supplements and substitutes cakes.

Key words: Honeybee, Pollen, Bee Longevity, Overwintering, Egg laying of queen

INTRODUCTION

Honey bees (*Apis mellifera L.*), like other live organisms, need carbohydrates, proteins, fats, vitamins, minerals and water for brood rearing. The only protein source in the diet of honey bees is pollen that contains all of the lipids, vitamins and minerals necessary for normal growth and development of a bee colony (Herbert and Shimanuki, 1978). Honeybees obtain most of the necessary carbohydrates from nectar, whereas the proteins, fats, minerals and

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vitamins usually come from pollen. Pollen is a food of complex chemical makeup, the protein being the ingredient of the greatest importance for bees. Breaks of prolonged duration in the supply of that food to bee colonies may negatively affect the development and the functioning of a bee colony (DeGroot, 1953; Manning, 2001). In such cases pollen collected with bee traps during high pollen flow or pollen substitutes should be fed to colonies (Doull, 1980a, 1980b; Peng et al., 1984; Zahra and Talal, 2008). A colony of honey bee needs to collect about 17 and 34 kg of pollen yearly (Crailsheim et al., 1992; Keller et al., 2005), to meet their requirements for these nutrients. The growth of colony can be restricted by either a lack of pollen or by the available pollen not containing the necessary nutrients (Crailsheim, 1990; Herbert, 2000). Thus, it is advantageous under some circumstances to feed pollen supplements or substitutes (Doull, 1980a; Skubida et al., 2008). The most effective pollen substitutes and supplements are those that are most similar in chemical composition and physical consistency to stored pollen (Herbert and Shimanuki, 1979; Schmidt et al., 1987; Wilson et al., 2005 Saffari et al., 2010). In the research for formulation of potential use as pollen substitute's individual feed ingredients or formulations made up of different high-protein feeds have been tested (Herbert and Shimanuki, 1978; Zahra and Talal, 2008; Saffari et al., 2010). Laboratory tests that exclude natural food sources provide the basis for the assessment of the nutritional value of pollen substitutes for honeybees (Kullincevic et al., 1982). Pollen and honey are the natural diets of adult honey bees. Sometimes, however, when nectar is not available, bees collect sweet-tasting juices from overripe fruit and plant exudates. Also, bees may collect and store honeydew as honey, which secreted by certain insects. Bees may collect powdery animal feed or spores from plants and store this material as they would pollen during periods when no pollen is available. This material is considered as a poor substitute for pollen and it may have some food value but does not sustain brood rearing. In attempts to find substitutes to replace pollen in honey bees' natural diet, many kinds of animal and plant products have been evaluated (Haydak, 1967; Herbert and Shimanuki, 1979; Saffari et al., 2010). None has been found that is a complete replacement for natural pollen. Certain protein foodstuffs, however, will improve nutrition and ensure continued colony development in places and times of shortage of natural pollen. Soybean flour, and several brewer's yeast-products fed singly or in combination are palatable to bees and contain the quality and quantity of proteins and amino acids, lipids, vitamins, and minerals required for growth and development of individuals and reproduction of the colony (Standifer et al., 1977). The objective of present study was to determine, by means of biological methods, the nutritional effects of high protein feeds on growth, development, performance and overwintering of honey bee (*Apis mellifera L.*).

MATERIALS AND METHODS

A. Incubator Experiments

The trials were performed at Agricultural Research Center of Isfahan University of Technology (Isfahan, Iran). To produce enough newly emerged adult bees, 10 queens were selected and each was placed in a specially-designed cage along with a black comb between the frames in the center of the hive and the date was registered on each cage. The queens were set free after forty-eight hours and the frames were removed from the cage and were placed in the center of the hive frames. One day before hatching, all the frames were removed from the hives and placed in an incubator to be hatched. Incubator was a $2.5\times1.8\times1.5$ m^3 small room insulated by aluminum sheets. The temperature at the incubator

kept at 34±1°C with a relative humidity of 60%. The experimental small cages consisting wooden boxes of 15×11×15 cm^3 covered with superfine wire screen on two sides. There was a 2.8 cm in diameter circular hole on top of each small cage, provided for water container. In each small cage, there was a small black comb for the bees to rest and a L-shaped feeder for feeding. The method of Gary was used to count and transfer the honeybee larvae into the small cages (Gary and Lorenzen, 1987; Haydak, 1945). The feed was mixed in a rate of 10% with sugar syrup (50% sugar concentration) that provided daily for the bees (in different treatments) and the 50 cc water bottle was placed on the top of the small cages upside down. The dead honeybees were counted daily and were removed from the small cages. This experiment continued until all honeybees died. The experimental diets in the incubator test were:

1. Pollen substitutes provided by the following two formulas: a. 10% skim milk powder, 30% honey and 60% from one of the protein ingredients including lentil flour, soybean flour, soybean meal, wheat gluten, and fish meal were used. In this paper, these treatments were introduced as substitutes for lentil, soybean flour, soybean meal, wheat gluten, and fish meal; b. Honey, skim milk powder, and soybean powder were used by 30%, 10%, and 30%, respectively, in addition to one of the protein ingredients (lentil flour, wheat gluten, and fish meal) by 30% to prepare the diets. The treatments of this group comprised soybean-lentil substitute, soybean-wheat gluten substitute, and soybean-fish meal substitute.

2. Pollen supplements consisting of honey and pollen by 30 and 20%, respectively, as well as one of the protein ingredients such as lentil flour, soybean flour, soybean meal, wheat gluten flour, and fish meal by 50% were used. The treatments were lentil flour supplement, soybean supplement, soybean meal, wheat gluten supplement, and fish meal supplement.

3. The control group was also prepared by three different formulas: a. honey (30%) and pollen (70%); b. honey (30%) and skim milk powder (70%); c. honey (100%). Furthermore, the percentage of crude protein, ether extract, crude fiber, and ash of the experimental feed ingredients were measured by approximate analysis method as described by AOAC International (2002) and were then used in the feed of the caged honeybees on the basis of the above formulas.

B. Field Experiments

In field experiments, 12 treatments with four replicates were performed during 105 days experimental period from the months of July until mid November. First, 48 honeybee colonies with one year-old sister queens were selected and coordinated on the basis of their population and honey storage rate. Different protein ingredients prepared according to the above formulas in part “a” in the form of 200-gram patties, was wrapped in waxed paper and placed on the top of frames near the brood rearing area. The hives were inspected once every three days and in case of feed need a cake was placed in the proper place. Protein nutrients were thoroughly ground into powder, screened by a 50-mesh sieve and completely mixed according to the above-mentioned formulas to prepare a cake. Honey and sufficient amount of water were mixed according to the specified formula in another container and heated over a gas oven to about 80°C. The powder mix was gradually added to it and whipped to make a homogenous cake. The baked cakes were transferred to plastic containers with lid on them and packaged after being cooled. One liter of sugar syrup with a concentration of 1:1 or 2:1 (two parts of sugar and one part of water) was fed to all the colonies whenever the nectar supply was not sufficient. The amount of the feed

consumption by the bees was calculated from the total weight of the cakes fed to the hives during the experiment. The rate of the eggs laid by the queen was measured once every 15 days by measuring the surface of the comb containing broods (eggs, larvae, and pupae) by graduated 20×42.5 cm^2 talc. By weighing the honey frames and subtracting the average weight of the empty combs and the initial honey, the amount of the accumulated honey at the end of the summer was calculated. Furthermore, samples of different cakes sent to livestock feed Lab (Laboratory of Animal Nutrition, Isfahan University of Technology) to measure the amount of crude protein, ether extract, crude fiber, and their ash by standard analysis method (AOAC, 2002).

C. Overwintering Experiments:

At the end of the field experiment and before the beginning of winter, all preventive measures required to control Nosema disease, American and European foulbrood and Varroa parasite were equally taken on the hives and with the onset of the experiment, the net weight of the bee mass, the amount of honey and the rate of egg laying of the queens in the hives were measured and the colonies were ready for overwintering. After the expiration of winter (90 days), all the weighing operations performed before overwintering were carried out again. The weight of honey frames were measured before and after winter, and the net weight of the bee mass was calculated by subtracting the weight of the hive with adult bee from the empty hive. In this stage, consumption of the honey, increase or reduction of bee mass weight, the egg-laying rate of the queen at the end of winter, disease or any noticeable conditions in the experimental colonies were recorded. The rate of winter feed consumption and variations in bee mass weight were measured by subtracting the related weights before winter from those after winter. At the end, the collected data were analyzed through analysis of variance by SAS software (SAS Institute, 2001) and the means were compared by Duncan's Multiple Range Test at $P<0.05$ level (Duncan, 1955).

RESULTS AND DISCUSSION

A. Incubator Experiments

The mortality data for the honeybees in the incubator after feeding with pollen substitutes and supplements is presented in Table 1. A significant difference ($P<0.01$) was observed between the average time of 50% and 100% mortality after feeding with pollen substitutes and supplements. The average longevity of bees treated with soybean-lentil substitute, raw soybean substitute, lentil supplement, soybean substitute and lentil substitute was the least ($P<0.05$) and 50% of the bees died after 9 to 11 days. In 100% mortality, the average longevity of bees treated with raw pollen (143.5 days) was longer, but its difference with baked pollen (139.5 days), wheat gluten supplement (135.5 days) and raw soybean meal (127.5 days) was not significant ($P>0.05$). The average longevity of the bees treated with raw soybean supplement, soybean lentil substitute, lentil supplement and lentil substitute was the least and 100% of the bees died after 20 to 26 days (Table 1). The comparison of mean mortality according to the type of protein feed ingredient showed that pollen was the best protein source for honeybee feeding. Next to pollen, wheat gluten compared to soybean meal had more effect on the longevity of honeybees in the incubator, but the difference was not significant. Fish meal, bread yeast, and baked soybean had no significant differences, while raw soybean and lentil had adverse effect on the longevity of honeybees in the incubator. Skim milk powder mixed in small quantity with other protein

nutrients was suitable for the feeding honeybees. Although honey was the best sugar source for honeybees, but by itself it was not enough for the feeding of the newly emerged honeybees.

B. Field Experiments

The mean consumption rate of the cakes in different treatments was significant ($P<0.01$) different (Table 2). Pollen was more appetitive to honeybees than other protein feed ingredients (1631 g). The consumption rates for bread yeast supplement and its substitute with a significant difference between them ($P<0.01$) were 1032 and 869.1 g, respectively, and were easily accepted by the bees. Although soybean supplement was consumed more than its substitute by honeybees (449.5 versus 410.6 g), but this difference was not significant ($P>0.05$). A significant ($P<0.01$) difference was observed between the mean of the egg-laying rate in the experimental colonies, so that the means of egg-laying rate for the queen in the colonies consumed pollen cakes was the most (22636 cm^2) and it was the least for fish meal supplement (13052 cm^2). The differences between wheat gluten supplement with soybean meal substitute and fish meal substitute, as well as fish meal supplement and the control group (not received any protein cake) were significant ($P<0.05$). The mean difference of honey production in the experimental colonies was significant ($P<0.01$). The colonies which consumed pollen produced the most amount of honey (9562 g), although their honey production mean was not significant compared with hives fed with soybean supplement, soybean substitute (Table 2). Similarly, bread yeast supplement and wheat gluten treatments were not significant; however, its difference with soybean meal supplement and fish meal substitute, soybean meal substitute, bread yeast substitute, fish meal supplement, and control group (with no protein cake) were significant ($P<0.05$). Colonies fed with fish meal had the least amount of honey production (5894 g).

C. Overwintering Experiments:

The means of honey consumption and reduction rate of honeybee mass weight in winter, as well as the queen egg-laying rate at the end of winter were significantly ($P<0.01$) different among the experimental treatments (Table 3). Honey consumption in wheat gluten supplement treatment (with the most amount, 4266 g) had no significant difference with pollen (3773 g), soybean substitute (3503 g), bread yeast substitute (3081 g), and bread yeast supplement (3009 g); its difference with other treatments, however, was significant ($P<0.05$). Apart from wheat gluten supplement treatments and pollen, the means of honey consumption in other dietary treatments was not significant (Table 3). The weight reduction rate of bees on different dietary treatments was significantly ($P<0.05$) different. The soybean supplement showed the least weight reduction rate (208.1 g), and had significant difference ($P<0.05$) with bread yeast substitute (366 g), soybean meal substitute (374.4 g), wheat gluten substitute (378.9 g) and fish meal substitute (406.9 g), but not compared with others. The means difference between egg-laying rate of test colonies was significant ($P<0.05$) during winter (Table 3). The colonies fed with pollen and soybean meal supplement had the most egg-laying rates (1069 and 970 cm^3, respectively), while the control colonies as well as colonies fed with soybean meal substitute had the least rate of egg-laying (264.5 and 221.5 cm^2, respectively). In general, the most egg-laying rate during winter period was assigned to colonies fed on pollen cakes in seasons before winter began. When compared to control groups without protein cake and wheat gluten substitute, egg-laying rate of queens fed fish meal substitute, soybean meal substitute, as well as fish meal supplement was significant ($P<0.05$). During overwintering, none of the experimental

colonies experienced queen mortality and no disease or complications was noticed either (Table 3).

A. Incubator Experiments

The results of incubator experiment showed that the effect of feed protein materials on the longevity of honeybees was variable because it depended on the supply of the required nutrients and lack of poisonous materials. The pollen which is a natural food for honeybees provides most of the protein, vitamins and minerals for honeybees resulting longer longevity of bees, but raw soybean flour led to the reduction of longevity probably due to its poisonous materials such as trypsin inhibitor (Johansson and Johansson, 1977; Wilson et al., 2005; Saffari et al., 2010). It is, therefore, recommended that the cakes containing trypsin inhibitors be heated for 15 min at 100°C to inactivate their inhibitors (Haydak, 1961). Pollen containing up to 40% crude protein, is collected by honeybees and consumed. The average level of crude protein in pollen collected by honeybees has reported to be 23% (Iannuzi, 1993a, 1993b). Shaw (1990) reported that honeybees which consumed diet with more than 23% protein could rear honeybee larvae. The problem was solved when soybean meal with more crude protein (51.09%) in comparison with raw soybean flour (40.88%) was heated. The present findings indicated that soybean meal could compete with pollen as a protein source. Feed protein ingredients such as wheat gluten with 84.26% and pollen with 21.14% crude protein (CP) resulted in the most longevity in periods of 50% and 100% mortality. The feeding of newly-emerged honeybees with honey without feed protein ingredients resulted in an early mortality, indicates that although honey is the best source of sugar for honeybee, but can not satisfy all of the nutritional requirements of young honeybees (Johansson and Johansson, 1977). Complete mortality of honeybees consuming skim milk powder lasted more than 100 days which show that skim milk powder could be used in the honeybees' nutrition. This feed supplements was prepared according to Herbert et al. (1977) in which lactose level did not exceed 5% of the feed. Fish meal could also improve the longevity of honeybees in contrast to honey, lentil, and raw soybean in incubator experiments. This might be, in part, due to the lack of poisonous elements in fish meal; however, when fish meal was used in combination with raw soybean, the time for the complete mortality of the honeybees decreased from 112 days to about 65 days which could happen as the result of adverse effect of raw soybean (Haydak, 1961). Wheat gluten was easily accepted by the honeybees and it took a long time for their mortality, probably due to lacked poisonous and harmful elements in given feed supplement. Although most of reports showed that a mixture of different feed ingredients was better than a specific feed ingredient alone (Herbert and Shimanuki, 1976); in this experiment, however, the addition of raw soybean flour to substitute cakes of lentil, wheat gluten and fish meal resulted in faster mortality which was probably due to the enzyme inhibitors contained in mentioned ingredient.

B. Field Experiments

With a glance at Table 2, it is evident that the pollen supplement cakes were consumed more than the pollen substitute cakes by honeybees because of the presence of pollen in their mixture (Doull, 1974). In consistent with previous reports (Doull, 1980a; Herbert and Shimanuki, 1976; Iannuzi, 1993a, 1993b) honeybees consumed more pollen cakes than other feed ingredients, indicates that honeybees prefer pollen to other feed ingredients. The probable reasons for this observation can be traced to the attractive chemicals in the pollen

(Iannuzi, 1993a, 1993b), soft and pleasant nature of the cakes containing pollen and their moisture maintaining potential (Schmidt et al., 1987), and their being delicious (Doull, 1980a). Similar to previous reports (Haydak, 1945, 1961), the colonies consuming pollen reared more broods and collected more honey, probably due to an increase in the population within the colonies. Ruttner (1973) reported that the population of the hives was one of the important factors in the production of honey with a positive relation with egg-laying of the queen. Herbert et al. (1977) reported that the brood rearing in the colonies fed pollen substitutes or pollen supplements were less than those fed by fresh pollen. Although cakes containing bread yeast were consumed more than others due to their suitable consistency and their being appetitive (Schmidt et al., 1987), but the rate of egg-laying and honey production was not much different from the cakes containing soybean flour, soybean meal, and wheat gluten. This finding is in conformity with reports by Kullincevic et al. (1982). The present results indicated that in spite of the temporary presence of pollen in nature, honeybees prefer pollen to some cakes (Herbert and Shimanuki, 1976; Schmidt and Hanna, 2006; Saffari et al., 2010b). Cakes containing soybean meal were consumed less than other cakes, probably due to their becoming dry and hard soon. Cakes containing fish meal, in spite of their suitable texture and relative consistency, were not appetitive, indicates that other limiting factors contained in fish meal might have been functioning. It appears that the repulsive smell of fish meal and high ash content of these cakes was the reasons for unacceptability of them. The ash content of fish meal substitute and fish meal supplement cakes were 13.89 and 11.78%, respectively, which was approximately more than twice than that of the ash contained in other cakes. It is reported that the smelling sense is the most important sense organ in honeybees and high amounts of minerals are also harmful to them (Herbert and Shimanuki, 1976). Cakes containing wheat gluten soon got hard due to the high viscosity properties of gluten and because wheat gluten was poor in fat content (1.9%). The ether extract of cakes containing wheat gluten were 1.2 and 2.78%, in comparison with pollen (6.35%). On the other hand, it seems that the presence of high amounts of protein in wheat gluten (84.26%) could be the reason for less consumption rate of these cakes (Haydak, 1945). Pollen supplement cakes had more effects on egg-laying of the queens which is in agreement with Standifer et al. (1977), who suggested that feeds containing pollen supplement were very delicious for honeybees and satisfied the requirements of honeybees for development and reproduction. Different researchers have confirmed that the presence of a little amounts of pollen or its extract in the feed would make the supplement delicious (Doull, 1980a), and it seems that the more the chemical composition of the protein feed is nearer to the chemical composition mean of pollen, the more the honeybee is inclined to consume it. The rate of honey production was different among the treatments and it was evident that in a beekeeping field disregarding the queen breed, honey production in each hive was also greatly dependent on the population of the hive (Ruttner, 1973). The more the population of the colony, in case there was an access to nectar plants, the more the amount of collected honey was. Therefore, the colonies that consumed pollen or its supplement and had more egg-laying could also collect more honey. Of course, Herbert and Shimanuki (1976) reported that if feeding by pollen supplement started in the beginning of April, the usual active period for egg-laying, the production of honey could be increased up to 40%; however, since the incubator experiments lasted longer in the present study, the protein cake feeding to the field colonies started at the last week of July.

C. Overwintering Experiments:

The results for overwintering showed that the most honey was consumed in winter by those colonies which had the least mortality or reared more brood during winter time,

resulting in an increase in population and concomitantly increase in feed consumption. On the other hand, the means reduction rate of honeybee mass weight for different overwintered colonies was significantly affected by protein cakes consumed before winter. The dietary treatments that had satisfactory results concerning feed consumption and queen egg-laying rate in field experiments could keep their superiority during winter as well. Soybean supplement, wheat gluten, and pollen treatments, for example, kept the most honeybee population during winter time. Macicka (1987) reported that the honeybees which consumed sufficient and suitable feed protein ingredients before winter could rear more brood during winter due to more fat they stored in their bodies and as a result they had more winter feed consumption and less weight reduction as well, which was evident in our study. In overall, the present results indicate that the protein feed materials such as soybean flour and bread yeast could be used in protein cakes for honeybees. However, if these materials were used along with pollen (between 5 to 25%), honeybees consumed them with more appetite. It seems that the various protein materials have some deficiencies or their individual use is limited. In order to compensate for that, therefore, it is recommended that a mixture of several feed protein materials be used in combination. Eventually, whenever the conditions are favorable for queen egg-laying and brood rearing, but pollen is not sufficiently accessible, the use of pollen substitute and supplement is recommended; however, to provide the requirements of honeybees for protein and other nourishments present in pollens, these cakes are recommended so as to have their effects during the active egg-laying months and during the rearing of brood as well as during overwintering.

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Table 1: The mean days of 50% and 100% honey bee mortalities after feeding with pollen supplements and substitutes in incubator.

Dietary treatments	50% mortality (days)	100% mortality (days)
Pollen supplements		
Wheat gluten	61.0 ^{a}* ± 1.41	135.5 ^{ab}* ± 4.20
Soybean meal, caked	38.4 ^{c} ± 2.73	117.5 ^{cd} ± 22.78
Soybean, raw	38.2 ^{c} ± 3.82	127.2 ^{abc} ± 21.41
Bread yeast, cooked	36.8 ^{c} ± 5.97	111.0 ^{cdef} ± 11.86
Fish meal	34.6 ^{c} ± 3.78	112.7 ^{cdef} ± 4.11
Soybean flour, cooked	23.7 ^{de} ± 2.44	102.1 ^{def} ± 8.56
Soybean flour, raw	10.1 ^{g} ± 1.08	26.5 ^{i} ± 2.08
Lentil flour	10.0 ^{g} ± 1.18	22.2 ^{i} ± 2.63
Pollen substitutes		
Wheat gluten	47.0 ^{b} ± 9.17	119.5 ^{bcd} ± 11.44
Soybean meal	36.8 ^{c} ± 3.71	114.7 ^{cde} ± 17.13
Fish meal	34.3 ^{c} ± 5.60	97.5 ^{ef} ± 4.80
Soybean-wheat gluten	33.7 ^{c} ± 3.59	95.5 ^{f} ± 12.40
Soybean-fish meal	19.2 ^{ef} ± 2.61	64.7 ^{g} ± 19.45
Soybean-lentil	11.1 ^{g} ± 1.84	25.8 ^{i} ± 2.06
Soybean flour	9.7 ^{g} ± 1.44	25.7 ^{i} ± 2.06
Lentil flour	9.2 ^{g} ± 1.63	20.7 ^{i} ± 2.50
Controls		
Pollen, raw	46.1 ^{b} ± 1.78	143.5 ^{a} ± 15.93
Pollen, cooked	37.9 ^{c} ± 7.12	139.5 ^{a} ± 6.45
Honey	26.6 ^{d} ± 1.61	44.2 ^{h} ± 0.54
Skim milk	15.4 ^{fg} ± 3.04	101.3 ^{def} ± 12.97

*Means ± SE with no common superscripts differ significantly (P<0.05) as measured by Duncan's multiple range test.

Table 2: Mean of feed consumption, egg laying and honey production in supplemented colonies with pollen supplements and substitutes during summer.

Dietary treatments	Feed consumption (g DM)	Egg laying (cm^2)	Honey production (g)
Pollen supplements			
Wheat gluten	151.0^{ef} ±11.37	18235^{b}±1545	8311^{abc}±770
Soybean meal	196.5^{e}±16.54	15578^{bcd}±1320	7500^{bcd}±630
Bread yeast	1032.0^{b}±9.83	17058^{bcd}±1470	8793^{ab}±745
Fish meal	52.0^{ef}±4.36	13052^{d}±1140	5984^{d}±510
Soybean flour	449.5^{d}±3.74	17546^{bc}±1565	8973^{ab}±715
Pollen substitutes			
Wheat gluten	95.6^{ef}±7.35	14834^{bcd}±1210	6021^{d}±545
Soybean meal	100.2^{ef}±6.92	13715^{cd}±1465	6750^{cd}±495
Fish meal	26.5^{f}±1.85	13439^{cd}±1070	7088^{cd}±530
Bread yeast	869.1^{c}±7.78	16026^{bcd}±1280	6643^{cd}±610
Soybean flour	410.6^{d}±3.63	15586^{bcd}±1115	8823^{ab}±785
Controls			
Pollen	1630^{a}±95.75	22635^{a}±1890	9562^{a}±810
Without any cake	0.00^{f}	13349^{cd}±1165	6509^{d}±625

$^{a-f}$ Means with no common superscripts differ significantly ($P<0.05$) as measured by Duncan's multiple range test.

Table 3: Mean of honey consumption, laying area of queens and total bee mass reduction in supplemented colonies with pollen supplements and substitutes during overwintering

Dietary treatments	Honey consumption (g DM)	Total bee mass reduction (g)	Laying area of Queens (cm^2)
Pollen supplements			
Wheat gluten	$4266^{a}\pm280$	$211^{cd}\pm12.54$	$880^{ab}\pm33.97$
Soybean meal	$2488^{bc}\pm135$	$217^{bcd}\pm15.76$	$970^{ab}\pm65.43$
Bread yeast	$3009^{abc}\pm185$	$256^{bcd}\pm18.71$	$570^{abc}\pm43.27$
Fish meal	$2613^{bc}\pm115$	$282^{abcd}\pm16.37$	$280^{c}\pm38.66$
Soybean flour	$2835^{bc}\pm165$	$208^{d}\pm13.85$	$615^{abc}\pm43.27$
Pollen substitutes			
Wheat gluten	$2297^{c}\pm170$	$379^{ab}\pm25.23$	$338^{c}\pm25.42$
Soybean meal	$2252^{c}\pm155$	$374^{abc}\pm17.98$	$222^{c}\pm18.45$
Fish meal	$2375^{c}\pm180$	$407^{a}\pm33.74$	$308^{c}\pm16.58$
Bread yeast	$3081^{abc}\pm245$	$366^{abc}\pm28.50$	$860^{ab}\pm53.18$
Soybean flour	$3503^{ab}\pm270$	$323^{abcd}\pm17.54$	$865^{ab}\pm44.63$
Controls			
Pollen	$3773^{ab}\pm245$	$211^{cd}\pm11.51$	$1069^{a}\pm78.37$
Without any cake	$2357^{c}\pm155$	$282^{abcd}\pm16.68$	$265^{c}\pm15.82$

^{abcd}Means with no common superscripts differ significantly ($P<0.05$) as measured by Duncan's multiple range test.