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THE EFFECTS OF DIFFERENT LEVELS OF PROTEIN AND SILYMARIN ON THE POPULATION GROWTH AND HYPOPHARYNGEAL GLAND SURFACE OF HONEY BEE WORKERS (*Apis mellifera meda*)

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Abstract: A pollen substitute is a valuable resource to maintain bee colonies strong and healthy, in the absence of pollen in sufficient quantities in nature. Hence, the current study was performed to investigate the effects of different levels of dietary proteins and silymarin (SM) as a natural antioxidant, on honey bee worker colonies. The study was carried out as a completely randomized design in an experiment conducted using 36 honey bee colonies in a completely randomized design with nine experimental treatments (four levels of crude protein 0, 20, 30 and 40%, two levels of silymarin 0 and 0.2 mM and pure pollen (control)), and four replications at Agricultural Sciences and Natural Resources University of Khuzestan in autumn 2015. In current study parameters such as workers in sealed broods, newborn workers bee weight, body protein and, the amount of development of Hypopharyngeal gland surface were studied. Soybean meal, maize and wheat gluten were included as pollen substitutes. Based on the results of the pre-experiment, SM supplement at a dose of two-tenths mM improved the survival of bees. Capped broods number using the divided box into squares with 2 × 2 cm, newborn workers bee weight using the balance, body protein based on the percentage of body weight and development of the Hypopharyngeal gland surface using microscopes and micrometers were measured. The results showed that there was a significant difference between treatments in terms of laying eggs ($P < 0.05$). The highest and lowest rate of workers in sealed broods were related to treatment containing 30% protein and SM (12467 cells) and sucrose treatment (2042 cells), respectively. Also, the highest and lowest newborn workers bee weight were related to pollen treatment and the sucrose treatment,

respectively (P <0.05). Body protein of worker bees in studied treatments had significant differences (P <0.05), so that the widest and narrowest percent body protein were observed in a diet containing 30% protein and SM and sucrose treatment, respectively. The Hypopharyngeal gland surface in the colonies fed with different diets was significantly different (P<0.05) and the widest and narrowest of its surface were observed in the diet containing 30% protein and SM and sucrose treatment, respectively. According to the current results, to maximize the reproduction of bees, a diet containing 30% crude protein is proposed.

Keywords: antioxidants, diet protein, pollen substitute, longevity, sealed broods.

Introduction

According to the study of *van Engelsdorp et al. (2009)* honey bees population as major pollinators of many edible plants have declined. A poor diet, due to land use changes reducing the availability and diversity of floral resources, might help drive these declines *(Vanbergen and Initiative, 2013)*. In keeping with this idea, nutrition is a key determinant of honeybee survival *(Altaye et al., 2010; Archer et al., 2014b)*. Understanding the association between diet and honeybee survival is essential to protect declining and threatened honeybee populations *(Pirk et al., 2014)*.

Oxidative stress suppresses animal health, performance, and production, subsequently impacting economic feasibility; hence, maintaining and improving oxidative status especially through natural nutrition strategy is essential for normal physiological process in animals *(Li et al., 2012)*. Antioxidants, which neutralize Reactive Oxygen Species (ROS), help to maintain this balance and a poor diet could, in theory, disrupt it. Diets, high or low in proteins could push cells into oxidative stress by increasing ROS production from the mitochondria, impairing antioxidant defenses against ROS or reducing the repair of oxidized molecules *(López-Torres and Barja, 2008)*. Silymarin (SM) as a natural antioxidant can be considered as one of the most promising materials used in animal diets in various forms *(Surai, 2015)*. SM is the bioactive extract from *Silybum marianum* L. seeds (*Asteraceae*) and contains 65-85% flavonolignans like silychristin, isosilychristin, silydianin, silybin A and B, isosilybin A and B, and also 20- 35% fatty acids, flavonoids, and other polyphenolics. The major source of SM is fruits and seeds from this plant, but traces of these compounds can occur in all plant parts *(Ramasamy and Agarwal, 2008)*.

Honey bee workers start to consume pollen just a few hours after emerging *(Hagedorn and Moeller, 1967; Dietz, 1969)* and enough supply of proteins particularly during the first two weeks after emergence is required to sustain their

normal growth and development, and for them to be able to rear larvae *(Haydak, 1963)*. Pollen as a natural and protein-rich food source for honey bees *(Schäfer et al., 2006)* is essential for the production of royal jelly; a high-protein food used to feed bee larvae and adult queens *(Crailsheim, 1992)*. Royal jelly is secreted by two hypopharyngeal glands (HPGs), situated in the head, reaching maximum development in nurse bees, around 6–12 days after emergence, and afterward degenerate in forager bees *(Deseyn and Billen, 2005)*. A decline in accessibility and proteins content of bee-collected pollen might result in the lower development of HPGs *(DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013)*, less brood reared *(Herbert et al., 1977; DeGrandi-Hoffman et al., 2008)*, shorter longevity *(Schmidt et al., 1987; Di Pasquale et al., 2013)* and recruitment of bees at a young age *(Sagili and Pankiw, 2007)*, eventually entailing a decreased lifespan *(Khoury et al., 2011)*.

During times of pollen scarcity, the pollen reserves in the combs and protein reserves in bees are quickly expended. Consequently, supplementary pollen or pollen substitutes are needed to preserve the colony's strength for pollination services or honey production *(Herbert et al., 1977)*. The availability of pollen depends on the plants' growing seasons during the year. Brood production could decrease or even stop completely in the colonies if there is no pollen or not available at desirable pollen substitute.

The pollen might contain spores of pathogens causing diseases such as chalk brood *(Flores et al., 2005)* or American foulbrood in bees and larvae. Provision of artificial diets is a safe way to feed bees protein. Beekeepers often provide pollen substitute diets to colonies, although these are often formulated without considering the costs of the diet components versus the benefits of providing such diets *(Herbert et al., 1977; Li et al., 2012; Morais et al., 2013)*.

This study aims to investigate the effects of dietary proteins levels and antioxidants (silymarin) on honey bee colonies, especially the population growth, hypopharyngeal gland, emergent weight and proteins content of honey bee workers during fall.

Material and methods

Experiment 1: Evaluation of silymarin dose on the survival of honey bee workers fed with sucrose solution

To identify an appropriate dose of SM supplementation, a pre-trial was conducted based on completely randomized design with six treatments and three replications in the cage rearing and an incubator. The cages volume was 2000 cm3 (12.5 × 10.7 × 15 cm), enclosed by wire mesh on one side. They contained two larger holes for inserting 40.0 ml Eppendorf tubes, each with fifteen small holes

through which bees could feed. One of these tubes was used to provide water and the other was used to provide one of the 6 liquid diets. We established three replicate cages, for each of the six treatments and three colonies. In this experiment, survival was monitored for six weeks. Brood frames were collected from three different colonies at the Department of the honey bee, Animal Sciences Research Institute of Iran and incubated at 34°C in constant darkness. On the day of their emergence from the brood comb, freshly emerged (<24 h) honey bee workers were caged in groups of 100 individuals. Each group received a diet consisting of 0.68 M sucrose solution and one of six SM doses (0, 0.2, 0.4, 0.6, 0.8 and 2.4 mM). Diets were made every week to ensure that the silymarins did not deteriorate and were frozen in aliquots at -20 °C and defrosted on the day of use. Each colony received all six diets; therefore, a total of 18 groups were fed in standard laboratory hoarding cages *(Köhler et al., 2013)* following standard procedures *(Köhler et al., 2012).* The liquid diet and water were provided fresh daily when survival was also measured and dead bees removed from cages.

Experiment 2: Evaluation of different levels of protein and silymarin on physiological processes

The current study was performed at Agricultural Sciences and Natural Resources University of Khuzestan, Ahwaz, Iran from November to February 2015. A set of equalized honey bee *(Apis mellifera Meda)* colonies was selected during the fall. The colonies were randomly assigned to different protein-level treatments. Before the experiment, each colony consisted of a sorority 6-month-old queen that had been reared and mated naturally and the same quantity of bees. Three empty comb frames were applied for each brood chamber, and two comb frames were full of honey. According to *Burgett (1985),* the population bees were evaluated. To prevent pollen from entering the ventilation, all of the hives were installed with a pollen trap.

Experimental diets

The effects of four diets containing different levels of crude protein (CP) were tested (Table 1). The dietary formulas and the approximate compositions of four isocaloric test diets (diet 1-diet 4); two levels of SM (0 or 0.2 Mm). The CP of the diets was measured using the Kjeldahl nitrogen procedure. The experiment was consisted of testing 32 colonies (8 diets by four colonies). The defatted soybean meal, corn gluten, wheat gluten and mixed pollen components of the diet were sieved through a 185 μm mesh. The diets were supplemented by providing the colonies with prepared feed patties weighing 400 g each consisting of the dry

feeds. The patties were wrapped in ordinary waxed paper and placed on top of the frames over the brood clusters. Also, the honey and patties were checked every 3-4 d, and new honey and patties were supplied when the levels were inadequate.

Table 1. Ingredients and chemical compositions of the experimental diets (on dry matter basis)

Ingredients	Dietary proteins levels				
Ingredients (%)	0% (diet 1)	20% (diet 2)	30% (diet 3)	40% (diet 4)	Control diet (control)
Mixed pollen	0	5.00	5.00	5.00	100
sucrose	100	65.0	49.5	33.5	0
Wheat gluten	0	10.0	15.1	20.5	0
Corn gluten	0	10.0	15.2	20.5	0
Soybean meal	0	10.0	15.2	20.5	0
Total Proximate analysis	0	100	100	100	100
Dry matter (%)	99.5	96.4	95.2	93.9	86.7
Crude protein (%)	0	20.1	30.0	39.8	22.5
Gross energy (MJ/kg)[a]	16.9	17.7	18.0	18.4	19.5

[a] Gross energy (kJ/g diet) = (% Crude protein × 23.6) + (% Crude lipids × 39.5) + (% Carbohydrates × 17.3).

Coding and sampling the bees

Emerging brood combs were put in single-combs isolator from the colonies, to obtain bees of defined ages. Fifty newly emerged adult honey bee workers were collected per colony within 6 h, and their thoraces were marked with shellac paint *(von Frisch, 1965)*. To minimally disturb the colonies, the sampling was carried out without the use of smoke, inducing honey bee workers to feed on honey *(Free, 1968; Hrassnigg and Crailsheim, 1998b)* and affected the trophallactic behavior of the honey bee workers *(Farina and Núñez, 1991).*

Monitoring brood rearing activity in the colonies

Capped broods number was evaluated to determine the honey bee workers number reared in each colony. Assessments were made at 12-d intervals, once before and four times after treatment. The pupating honey bee workers remained in the sealed cells for 12 d *(Winston 1991)*, and the sealed broods mortality was so little (generally, the honey bee workers mortality in sealed broods was lower than 3%; according to *Fukuda and Sakagami (1968)*. Thus, a new set of pupating honey bee workers was counted in each assessment. The capped brood number was

monitored via a modified square grid system *(Mattila and Otis, 2007)*. The grids with a characteristic of 189 squares and each with an area of 4 cm^2were placed over the brood frames, and the comb area occupied by the capped brood was measured. The total number of bees reared in each colony was evaluated using a factor of 4.29 worker cells per square centimeter *(Seeley and Visscher, 1985).*

Newly emerged honey bee workers

The wet weight of 20 bees per colony was obtained within 2 h after emergence. Nutrition quality was measured via CP level and this index is correlated with the physiological conditions of honey bee workers *(Standifer et al., 1960; Pernal and Currie, 2000)*.

Hypopharyngeal gland measurements

Five honey bee workers from each colony were selected at 9 d of age to assess HPG development. The HPGs were removed and placed in a Petri dish with wax depressions each containing a droplet of ice-cold sodium chloride solution (0.85%, isotonic to the hemolymph). Micrographs of the HPGs were taken using a microscope equipped with a camera. For calibrating, an image of a 1µm scale bar was obtained at the same magnification. The analysis of HPG acini was carried out by measuring the areas of five acini cells selected randomly for each bee using the Photoshop (Adobe) pixel counting routine. Hereupon, 25 acini cells were surveyed for each colony for a total of 100 acini cells per treatment.

Statistical analysis

The data were analyzed by completely randomized design by one-way ANOVA using Proc MIXED. All statistical analyses were performed with SAS. Protein level and SM was the main effect and colony in each group was the random factor. The significance difference among treatments ($P < 0.05$) was tested using Duncan's new multiple range test of SAS.

$$\mu ij = \mu + \alpha i + \beta j$$

Results

Effects of silymarin dose on survival of honey bee workers fed with a single, pure carbohydrate diet

In current study, the effect of silymarin was evaluated on the lifespan (Fig. 1), survival (Fig. 2) and overall survival of honey bees. The dose of SM that bees were fed affected their survival (Fig. 1). SM with dosage of 0.2 mM was improved survival, compared to other SM doses and had significant effect on lifespan (Fig. 2).

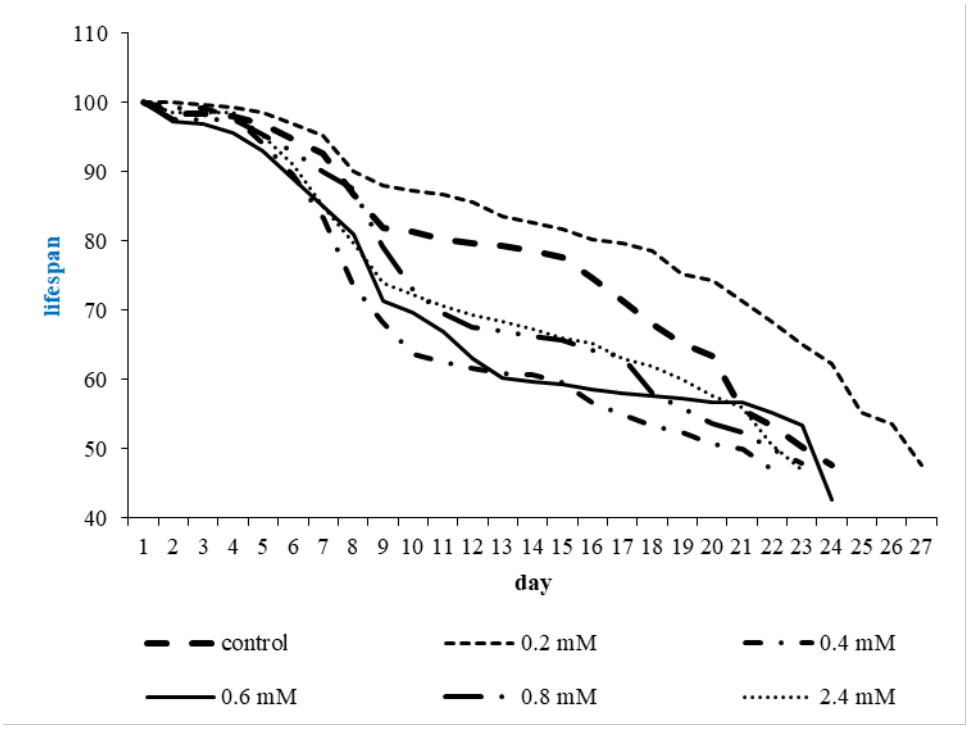

Figure 1. Honey bee workers survival fed with 0.68 M sucrose without SM (control) and with five SM concentrations until bee population decline by half. Diet 1: sucrose, diet 2: sucrose with 0.2 Mm SM, diet 3: sucrose with 0. 4 Mm SM, diet 4: sucrose with 0. 6 Mm SM, diet 5: sucrose with 0.8 Mm SM and diet 6: sucrose with 2.4 Mm SM.

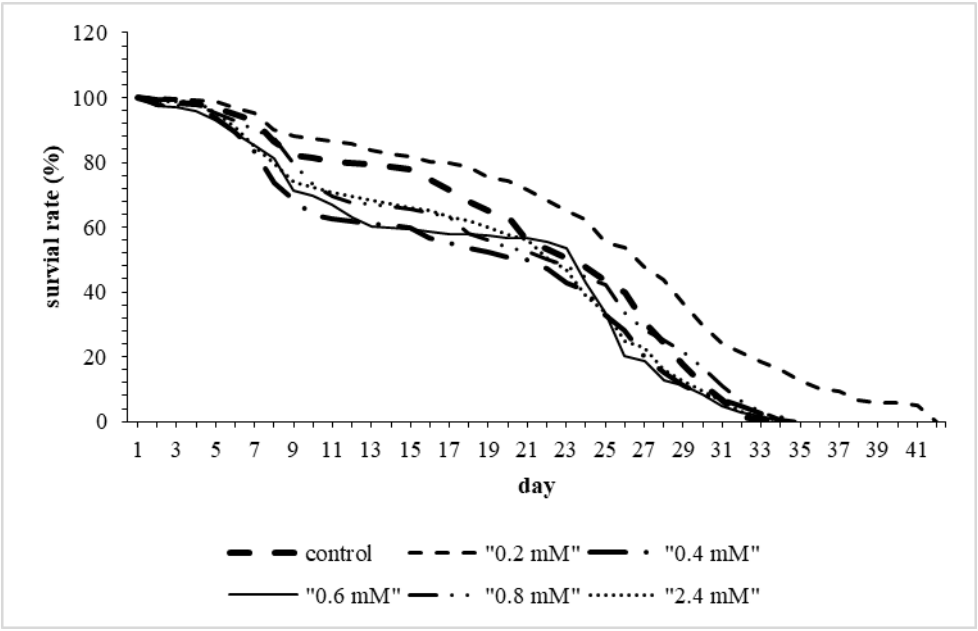

Figure 2. Honey bee workers survival with fed 0.68 M sucrose without SM (control) and with five SM concentrations. Diet 1: sucrose, diet 2: sucrose with 0.2 Mm SM, diet 3: sucrose with 0. 4 Mm SM, diet 4: sucrose with 0. 6 Mm SM, diet 5: sucrose with 0.8 Mm SM and diet 6: sucrose with 2.4 Mm SM.

Number in sealed broods

The brood rearing number in sealed broods during the experiment is shown in Fig. 3. Furnishing diets with different CP levels to the experimental colonies had a significant effect on the timing of the increase in brood rearing activity as the season progressed ($P < 0.05$). The average number of honey bee workers brood cell by the colonies quickly increased. At the beginning of the experiment, all of the brood colonies were similar. Treatments supplements with 30% CP with SM and 30% CP colonies tended to have the greatest number of honey bee workers in sealed broods at each colony census, and brood rearing activity was not significantly different from that observed with other treatments ($P > 0.05$). Treatment of 20% CP and 40% CP colonies represent more honey bee workers than sucrose treatment, but were statistically insignificant ($P > 0.05$).

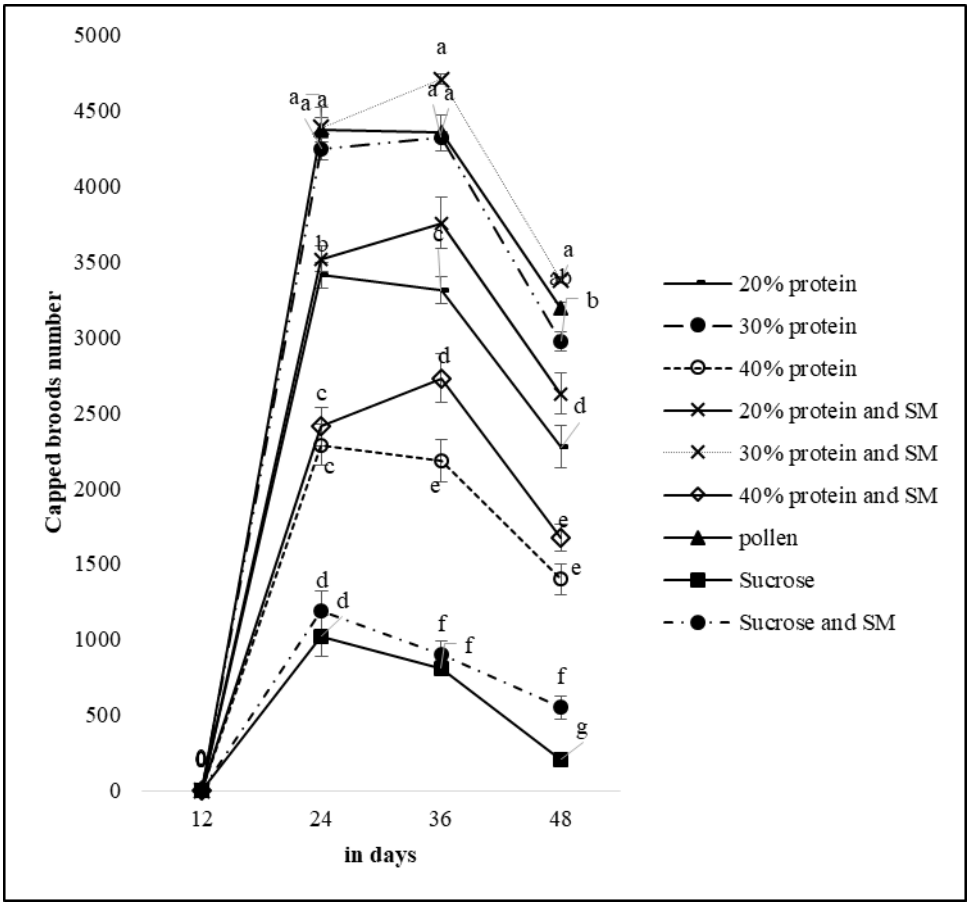

Figure 3. Mean number of Sealed pupa by colonies fed mix pollen and diets with different protein levels (0, 20, 30 and 40 %) during fall (N = 4 colonies per treatment). Different letters signify significant differences at P < 0.05.

Newly Emerged honey bee workers-Emergent Worker Weight

The average emergent worker weight was significantly affected by dietary proteins levels ($P < 0.05$; Fig. 4). The greatest emergent worker weights were obtained with honey bees fed with 30% CP diet (0.115 g), but there was no significant difference between the weights of honey bees fed with 30% CP and 30% CP with SM diet, and the lowest emergent worker weights (0.098 g) was observed in the treatment of sucrose, in compared to other treatments ($P > 0.05$).

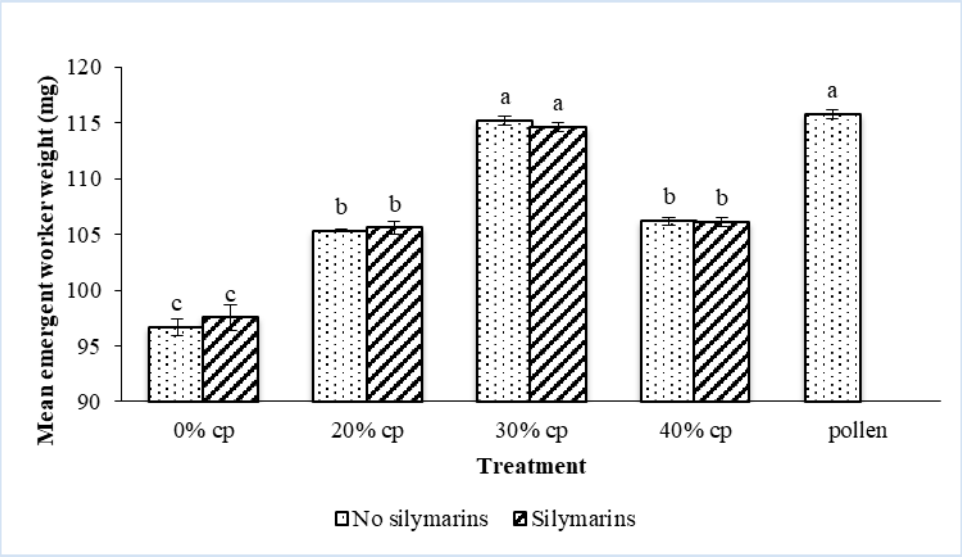

Figure 4. Mean emergent worker weight (±SE) bees fed pollen and diets containing different protein levels (0, 20, 30, and 40), two levels of silymarin (0 or 0.2 Mm) N=4 colonies per treatment). Different letters signify significant differences at P<0.05.

Protein Concentration

The highest protein concentrations in body were obtained using the 30% CP with SM diet (Fig. 5), but there was no significant difference between protein in body of the bees fed with 30% CP diet and 20% CP diet. There was a significant difference between the protein in the body, the 40% CP diet and 0% diet against other treatments.

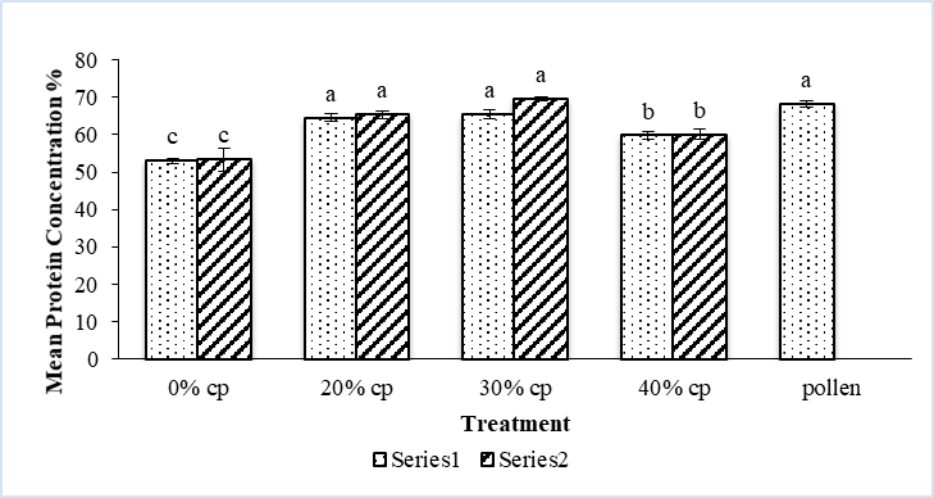

Figure 5. The mean Protein Concentration (±SE) of workers fed pollen and the various dietary proteins levels (0, 20., 30, and 40%), two levels of silymarin (0 or 0.2 Mm) N=4 colonies per treatment). Different letters signify significant differences at P<0.05.

Development of hypopharyngeal glands

The largest HPG acini in the nurse bees were obtained at 30.0% CP ($P < 0.05$). There were significant differences in HPG development in bees fed with 30.0% CP with other treatments ($P > 0.05$; Fig. 6).

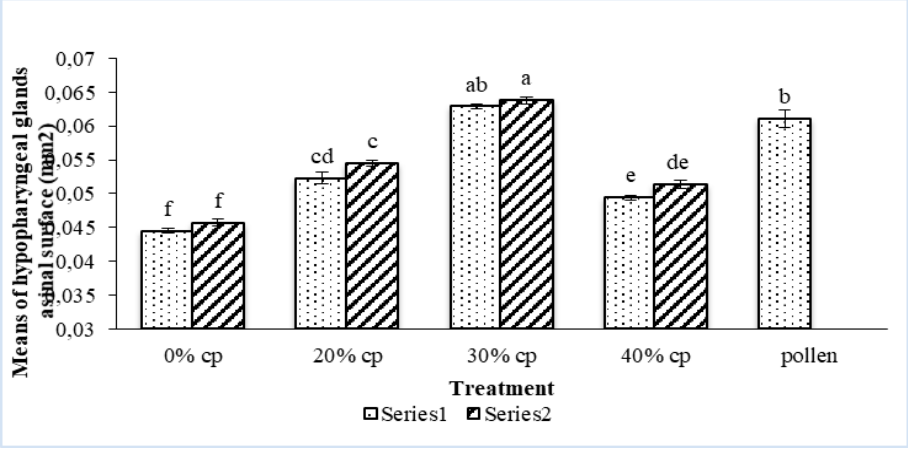

Figure 6. Development of hypopharyngeal glands during the nursing period of honey bee workers (at 9 d; N=4 colonies per treatment). The control diet is mixed pollen, and the dietary proteins (0, 20., 30, and 40%), silymarin (0 or 0.2 Mm). Different letters signify significant differences at P<0.05.

Discussion

Caged honey bees survived favorably when fed with sucrose and 0.2 mM SM solutions. Antioxidant supplementation at low doses improved honey bee survival (Fig. 1 and Fig. 2). Similar to our results using the major antioxidant in green tea epigallocatechin-3-gallate supplementation improved honeybee survival but only at an intermediate dose (0.3–0.5 mM) *(Archer et al., 2014a)*. It seems likely that antioxidants are among the major regulators of many physiological processes and, therefore, a redox balance between antioxidants and prooxidants in the diet, gastro-intestinal tract, plasma and tissues is an important determinant of the state of our health. Plants consumed by humans and animals contain thousands of phenolic compounds. Among them, the effects of dietary polyphenols including SM are of great current interest. Indeed, various phytochemicals, including flavonoids, are an essential part of our diet, which is responsible for turning on and maintaining the optimal status of our antioxidant defenses. Since flavonoids are not well absorbed in the gut, their active concentration in the plasma and target tissues are comparatively low, but probably sufficient for Nrf2 activation and NF-κB suppression as well as vitagene activation.

In the current study, we provided experimental evidence for a link between dietary proteins and important parameters in honey bees. Based on the current findings, there is a strong association between dietary proteins content and worker proteins content.

Current results showed that an increase in dietary proteins from 20% to 30% consequently caused an increase in the number of honey bee workers in sealed broods at each colony census. Also, brood rearing activity was noticeable. However, increase in dietary proteins from 30% to 40% was associated with decrease number of honey bee workers in sealed broods at each colony census, and brood rearing activity was observed (Fig. 3). Providing pollen substitutes had good impact on colony growth parameters like honey production, pollen storage, sealed brood and adult population *(Mitta and M R, 2016)*. Autumn is a critical period for honey bee colonies and the weak colonies probably die during winter. The colonies need good pollen sources during this period to foster enough brood and increase the survive-ability of colony during winter *(Abou-Shaara, 2015)*. Similar findings were observed by *Zheng et al. (2014)* and *Morais et al. (2013)*, reporting the colonies fed with artificial diets had a significant improvement in population development, colony weight, honey production and increases the efficiency of the colonies. *Somerville* (2000) showed that what is probably more important for the growth rate and development of bees is the total protein intake of a colony but not simply food consumption. The high-protein diets also increased colony growth parameters during periods of scarcity of pollen resources, in the field experiments, as also reported by *Mattila and Otis (2007)*. Bee bread and the artificial protein diets were well accepted by the bees. *Cremonz et al. (1998)* also found the highest

protein values in bees fed with bee bread. *Garcia et al. (1986)* supplied various protein foods with 20, 30 and 40% crude protein; they reported that CP negatively affects food collection. *Herbert et al. (1977)* reported that pollen substitutes containing 50% protein depressed brood rearing. Although the 40% CP diet is protein rich, the excess protein might inhibit the absorption of other nutrients or otherwise result in fitness costs. The current study demonstrated that 30% protein level is optimal for meeting the nutritional requirements for brood rearing during fall.

In the current study, average emergent bee honey worker weight fed with 30% protein and protein with silymarin ($P > 0.05$) was heavier than other treatments (Fig. 4). The heaviest honey bee workers were reared when pollen is readily available *(Kunert and Crailsheim, 1988)*. The bee development enhancement with increase in protein intake is probably due to honey bees requiring protein for producing cuticle *(Campbell, 1929)*, muscle, and other tissues *(Somerville 2000)*.

Proteins are responsible for 66-74% of the dry matter of adult honey bee workers *(Hrassnigg and Crailsheim, 1998a)*. This proteins content increases during the first days due to protein anabolism and decreases as the honey bee workers age *(Crailsheim, 1992)*. Measurement proteins content in honey bees is an effective method to evaluate the dietary proteins quality *(De Jong et al., 2009)*. To evaluate the diet efficiency precisely, total proteins content in newly emerged honey bee workers was tested. Increase in dietary proteins from 20% to 30% of colonies in the present study had a significantly positive effect in the protein concentrations of body, but increase in dietary proteins from 30% to 40% substantially decreased the protein concentrations of body. A significant difference was not observed in the protein concentrations of body between protein supplement of 30% treatment (Fig. 5). Similar to current results bees fed with 15% protein supplement diet had lower body protein concentration than bees fed with 30.5% and 35% protein supplement diet, indicating that protein level in protein supplement 15% diet was not supportive for the optimum growth and development of bees *(Li et al., 2012)*

The results indicated that an increase in dietary proteins from 20% to 30% of colonies had a significant effect on HPG acini, whereas an increase in dietary proteins from 30% to 40% significantly decreased the HPG acinal surface. *Di Pasquale et al. (2013)* found that certain aspects of nurse bee physiology, such as HPG development, were affected by pollen quality. Similar results were obtained when comparing honey bees fed pollen patties versus Mega Bee patties *(DeGrandi-Hoffman et al., 2010)*. Contrary to current results, *Zheng et al. (2014)* reported that nurse bees fed with pollen supplements had significantly larger HPG acini than the control group, which was fed pure pollen and confirmed that the superficial area of HPG acini increased with the bee's age regardless of whether the colonies were fed pollen or pollen supplements. It seems that the pollen supplements activated HPG development similarly to that activated by bee bread.

Our observations indicated that bees fed with 30% CP diet had the largest HPG acini and brood rearing activities. Current results suggest that an adequate provision of protein is required to sustain normal development of bees. The results of the current study demonstrate that dietary proteins levels strongly affect the population growth, performance, and physiological status of worker bees.

Conclusion

In present study, protein supplements can closely resemble pollen in nutritional value and because of their effects on protein concentrations and HPG development can play a significant part in colony. Diet containing 30% protein was recognized as an excellent one to promote honey bee colonies development. The optimum proteins content in field applications might differ according to dietary ingredient compositions and feeding methodology. These findings are particularly important for the successful bee keeping (colonies management) using pollen supplements when natural pollen is unavailable. In conclusion, population growth, body proteins content and surface HPG and worker quality of emerging honey bee workers were significantly affected by dietary composition and could be manipulated as metabolic tools to assess the optimal concentration of dietary proteins in the honey bees feeding.

Efekti različitih nivoa proteina i silimarina na rast populacije i površinu hipofaringealne žlezde pčela radilica (*Apis mellifera meda*)

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Rezime

Zamena za polen je dragoceni resurs za održavanje pčelinjih društava jakim i zdravim, u odsustvu polena u dovoljnim količinama u prirodi. Stoga je ovo istraživanje sprovedeno kako bi se istražili efekti različitih nivoa dijetetskih proteina i silimarina (SM) kao prirodnog antioksidansa na kolonije medonosnih pčela radilica. Istraživanje je izvedeno u ogledu sa 36 društava medonosnih pčela u potpuno randomiziranom dizajnu sa devet eksperimentalnih tretmana (četiri nivoa sirovih proteina 0, 20, 30 i 40%, dva nivoa silimarina 0 i 0,2 mM i čisti polen

(kontrola), i četiri ponavljanja na Univerzitetu za poljoprivredne nauke i prirodne resurse u Khuzestanu u jesen 2015. U navedenim parametrima studije, kao što su radilice u zapečaćenom leglu, masa novorođenih pčela radilica, telesni proteini i razvoj površine hipofaringealne žlezde su proučavani. Sojino brašno, kukuruz i pšenični gluten bili su uključeni kao zamene polena. Na osnovu rezultata prethodnog eksperimenta, dodatak sojine sačme u dozi od dve desetine mM poboljšao je preživljavanje pčela. Brojnost omeđenog legla pomoću podeljenog okvira na kvadrate veličine 2 × 2 cm, težina novorođenih radilica pomoću vage, telesni proteini na osnovu procenta telesne mase i razvoj površine hipofaringealne žlezde su mereni pomoću mikroskopa i mikrometara. Rezultati su pokazali da postoji značajna razlika između tretmana u pogledu polaganja jaja ($P<0,05$). Najveća i najniža stopa radilica u zatvorenom leglu odnosila se na tretman koji sadrži 30% proteina i SM (12467 ćelija), odnosno na tretman saharozom (2042 ćelije). Takođe, najveća i najmanja masa novorođenih pčela radilica bile su povezane sa tretmanom sa polenom, odnosno sa saharozom ($P<0,05$). Proteini u telu pčela radilica u ispitivanim tretmanima imali su značajne razlike ($P <0,05$), tako da su najveći i najniži procenat telesnih proteina primećeni u ishrani koja sadrži 30% proteina, odnosno SM i saharozi. Površina hipofaringealne žlezde u kolonijama hranjenim različitim obrocima bila je značajno različita ($P <0,05$), a najšira i najuža njena površina zabeležene su u ishrani koja sadrži 30% proteina, odnosno SM i saharozu. Prema trenutnim rezultatima, kako bi se reprodukcija pčela maksimizirala, predlaže se ishrana koja sadrži 30% sirovih proteina.

Ključne reči: antioksidanti, protein u obroku, zamena za polen, dugovečnost, zatvorene kolonije

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