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EFFECT OF DIETARY THIAMINE ON GROWTH OF THE IRANIAN HONEY BEE COLONIES (*Apis mellifera meda*) IN DIFFERENT SEASONS

SUMMARY

Three trials were conducted using completely randomized designs to evaluate the influence of concentration of thiamine supplementation on honey bee colony growth (brood and adult population) in different seasons. Twenty Iranian honey bee colonies (*Apis mellifera meda*), divided into 5 experimental groups, were used in the study. Control groups were fed sugar syrup. The experimental colonies were fed in the same manner but with syrup supplemented with different levels of thiamine (100, 200, 300 and 400 ppm). Each diet was tested on a set of four colonies selected for each treatment. Changes in brood areas had significantly difference between colonies fed thiamine (200 ppm) and sugar syrup (control) in summer season ($P<0.05$). In summer season the lowest brood production occurred in colonies fed sugar syrup. In winter season, adult population sizes and brood areas in colonies fed syrup supplemented with thiamine (200 ppm) were significantly larger than colonies fed sugar syrup ($P<0.05$). No significant differences were found between treatments on brood area and adult population size in spring season when some pollen was being collected by colonies.

Keywords: Brood, Iranian honey bee, population, season, thiamine.

INTRODUCTION

Honey bees need a range of elements to satisfy their nutritional requirements for normal growth and development (Haydak, 1970). These elements include water, proteins, carbohydrates, minerals, fats and vitamins. Lack of one or more of these substances will potentially lead to a serious reduction in the population of the colony, reduced longevity of honey bees, increased disease susceptibility and ultimately, death of the colony (DeGrandi-Hoffman *et al*., 2010). Not a great deal is known about the vitamin requirements of honey bees, although they are essential for all animals. It appears vitamins are not linked to longevity of the adult bee (Pain, 1956) but are intrinsically linked to brood development (Haydak and Dietz, 1965). The key indicators for the proper health and development of bees are vitamin A, B1, B2, B6 and folic acid and

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minerals such as iron, manganese and zinc in the pollen (Vivino and Palmer, 1994). The B complex vitamins have been demonstrated to be essential for most insects. B-vitamin complex are necessary for normal brood rearing (Back, 1956; Haydak and Dietz, 1965; Anderson and Dietz, 1976). Many vitamins are not very stable and will deteriorate in stored pollen (Hagedorn and Moeller, 1968b). This may be one of the factors contributing to the reduced food value of pollen stored for lengthy periods (Hagedorn and Moeller, 1968a). To compensate of this loss, vitamin supplementation is necessary.

Thiamine or vitamin B_1 is a water-soluble vitamin of the B complex. Its phosphate derivatives are involved in many cellular processes. The best-characterized form is thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. All living organisms use thiamine, but it is synthesized only in bacteria, fungi, and plants. Animals must obtain it from their diet, and thus, for them, it is an essential nutrient (Du *et al*., 2011). ThDP is a coenzyme for several enzymes. The enzymes include transketolase, pyruvate dehydrogenase (PDH), and α-ketoglutarate dehydrogenase all of which participate in carbohydrate catabolism and all of which, to one extent or another show reduced activity during thiamine deficiency (Singleton and Martin, 2001; Song and Singleton, 2002; Makarchikov *et al*., 2003). Thiamine in the diet is often not included, and precise functions and requirements of thiamine for honey bees have not been demonstrated. The levels of thiamine in bee-collected pollen have been reported by several investigators (Campos *et al*., 2008). The levels of thiamine ranged from 6-13 ($mgkg^{-1}$). In bees fed the complete vitamin diets, hypopharyngeal gland development, body nitrogen, and longevity were greater than in newly emerged bees fed diets deficient in either thiamine or riboflavin (Herbert and Shimanuki, 1978). Topolska (1983) reported that Administration of anti-vitamin B1 resulted in bees with lower body weights and underdeveloped wings. Hartwig and Krol (1989) concluded that adding vitamin B_1 to syrup fed to queen rearing colonies, starting 6 days before rearing starts, is beneficial, but further investigations are needed. Krol (1993) tested the effect of dietary vitamin B_1 on the condition and development of honey bees and observed that experimental colonies produced more brood (by 40%) compared with control ones.

The exact role played by thiamine in the growth and development of honey bees is not known also limited data are available. The purpose of the present paper is to study the effect of thiamine supplements on brood area and adult population size in Iranian honey bee colonies.

MATERIAL AND METHODS

Colonies and trials

Three trials were carried out at Honey Bee Department of Animal Science Research Institute of Iran. The first trial was conducted in summer season (Jul. 5, 2012 through Aug. 6, 2012), the second was in winter season (Feb. 19, 2013 through Mar. 20, 2013) and finally, third trial was conducted in spring season

(Apr. 27, 2013 through May. 28, 2013). The duration of the experiments was 30 days. Twenty Iranian honey bee (*Apis mellifera meda*) colonies were used. In three seasons we prepared five groups of four colonies fed with different levels of thiamine. Feeding treatments were assigned randomly to the colonies in trials. Honey bee colonies of the same strength and queen of similar age were selected to feeding treatments. Measurements of brood and bee population size were made at the start of the study. Recommended colony management practices were regularly followed in all these colonies.

Thiamine (Sigma® thiamine hydrochloride T-4625) was used for feeding in the honey bee colonies. Control colonies received sugar syrup (1 part sugar to 1 part water by weight) without thiamine (1 Lit/day/colony). The experimental colonies were fed in the same manner but syrup supplemented with different levels of thiamine (100,200,300 and 400 ppm).

Brood area and adult bee population estimates

Brood measurements were made just prior of feeding and three weeks after the last feeding. The total area under brood comprising eggs, larvae and sealed brood was measured in all the experimental colonies with using a grid with 5 cm × 5 cm squares that covered the entire side of a comb. The grid was placed over each side of a brood comb and the number of squares with brood was counted. Measurements of all frames with brood were summed for each colony. Adult bee populations were made by estimating the number and area of comb covered with bees. We totaled the area covered with bees for all frames to estimate the number of frames in the colony covered with bees (DeGrandi-Hoffman *et al*., 2008). Bee strength of all the experimental colonies was recorded beginning and 30 days later of feed.

Statistical analysis

The data were considered by a one-way analysis of variance for the response variables changes in brood area and adult population. Means of response variables resulting in a significant F test were further analyzed using Duncan's multiple range test (Duncan, 1955; SAS Institute 1991).The analyzing was conducted for each season separately. Change in brood area (B) or adult worker population (A) was estimated in colonies using the equation: [B (t+1) / B (t)] and [A (t+1) / A (t)] where A (t) or B (t) is the population or brood estimate at the start of the study and A (t+1) or B (t+1) is the estimate at the end (DeGrandi-Hoffman *et al*., 2008).

RESULTS AND DISCUSSION

The average brood area and adult population size at the beginning of the study did not differ among groups of colonies chosen for each level of thiamine in summer season (adult population: F=0.500, df=4, P=0.737; brood area: F=0.77, df=4, P=0.564), winter season (adult population: F=1.44, df=4, P=0.269; brood area: F=0.430, d.f.=4, P=0.785) and spring season (adult population: F =

0.81, df=4, P=0.535; brood area: F=0.20, df=4, P=0.931). In summer season, colonies had an average of 5 frames of adult bees and 2600 cm^2 of brood. In winter season, colonies had an average of 5 frames of adult bees and 104 cm^2 of brood and in spring season, 5 frames of adult bees and 3200 cm^2 of brood.

The effect of dietary thiamine on brood and adult populations of the Iranian honey bee colonies (*Apis mellifera meda*) in different seasons is presented in Figure 1. Changes in brood areas were significantly difference between colonies fed with the 200 ppm thiamine and sugar syrup in summer season (F=2.701, df=4, P=0.042). In this season the lowest brood production occurred in colonies fed sugar syrup. However, there were no significant differences between the other treatments on brood areas. In summer season, changes in adult population sizes did not differ between colonies fed sugar syrup and syrup supplemented with thiamine (F=0.386, df=4, P=0.814).

In winter season, adult population size and brood area in colonies fed syrup supplemented with the 200 ppm thiamine were significantly greater than colonies fed sugar syrup alone (adult population: F=2.92, df=4, P=0.05; brood area: F =2.177, df=4, p=0.046). There were no significant differences between the other treatments on adult population size and brood area.

In spring season, no significant differences were found between treatments for brood area and adult population size (adult population: F=0.275, df=4, P=0.890; brood area: F=0.893, df=4, P=0.492). Although in this season the colonies fed syrup supplemented with thiamine had a numerically superior brood areas and adult population sizes.

In the present study, the average initial adult population size was similar in all treatments, so the effects should have been the same regardless of the diet being fed. In a supplementary feeding program, sugar is a most effective bee feed, because it stimulates the bees into breeding, foraging for pollen, and metabolizing stored honey and protein. However, feeding sugar only is a stimulus to the bees and not a balanced diet. In all three experiments, our study indicates that add different levels of thiamine to sugar syrup could be stimulated the brood rearing and growth of the Iranian honey bee colonies (*Apis mellifera meda*). Results of the present study are in agreement with reports by Back (1956), Haydak and Dietz (1965), Anderson and Dietz (1976) and Krol (1993). They suggested that B vitamins are essential for brood rearing.

In the Iran there is likely to be a broodless period during January and February. In the second trial, declining population and low-laying queen had occurred. In this trial, when brood rearing rates are at their lowest, the only food source available to the bees was the diets we fed. In late winter, the absence of pollen or nectar caused by rapid consumption of diets (Shimickl and Crailsheim, 2001; Vander Steen, 2007) and thus brood rearing and growth of colonies were stimulated.

In the Iran the main nectar flow is often over a short four-week period in May and June. In spring season, when some flowering plants were in bloom and bees could collect pollen and nectar from the field, brood rearing and adult

population growth did not differ among the diets. It is known that the number of eggs reared in a colony is related to the number of bees in the hive. Big colonies raise more brood (Khoury *et al*., 2011). Apparently if some pollen is available, diets can work equally well provided they are consumed at sufficient rates.

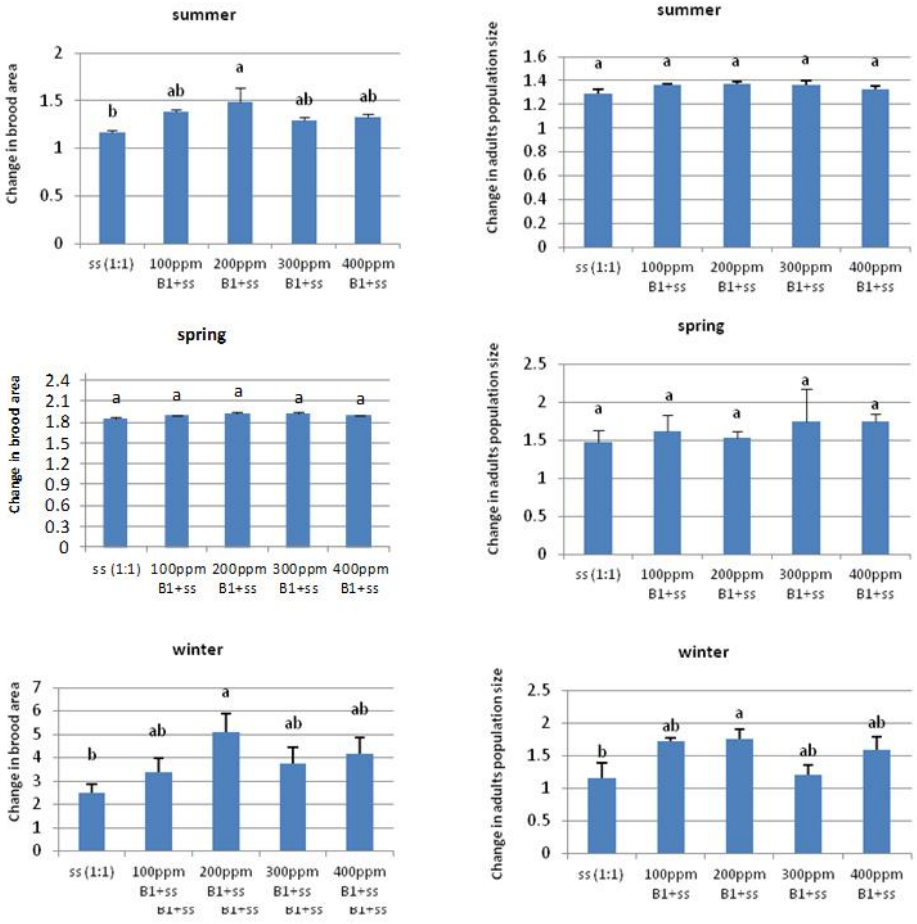

Figure 1. Average proportional change in brood area and adult population size in Iranian honey bee colonies fed sugar syrup supplemented with different levels of thiamine in different seasons. Proportional change in brood area (B) or adult worker population (A) was estimated incolonies using the equation: [B(t+1)/ B(t)] and [A(t+ 1)/ A(t)] where B(t) or A(t) are the population estimate at the start of thestudy and (t+1) is the estimate at the end. Columns labelled with the same letters are not significantly different at the 0.05 level as determined by a Duncan's multiple range test. ss:sugar syrup.

There are several possible explanations for this result. In addition to nutritional factors, brood rearing and population growth in colonies are affected by the quality of the queen and the size of the adult worker population (Winston,

1987; DeGrandi-Hoffman *et al.*, 1989). The availability of natural pollen differed during the three Trials and this might have affected the response of the colonies in terms of brood rearing and adult population growth. These factors are interrelated and because of the difficulty of controlling each independently of the others in free flying colonies, many predications about colony development are, in fact, only estimates (Herbert and Shimanuki, 1982; DeGrandi-Hoffman *et al*., 2008).

CONCLUSIONS

Our study indicates that colonies fed sugar syrup supplemented with different levels of thiamine had better growth than control colonies which fed sugar syrup alone in all seasons. This result shown that thiamine could be affective in the metabolism of food sources collected by honey bees. It was found that low levels of thiamine are effective on colony growth. Effect of dietary thiamine on colony growth due to a deficiency or absence of pollen in summer and winter, respectively, were significantly different. Because of the abundance of pollen in the spring, there was no significant difference in colony growth. These observations suggest that thiamine supplementation is effective on colony growth in summer and winter.

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UTICAJ ISHRANE OBOGAĆENE TIJAMINOM NA RAST DRUŠTVA IRANSKE MEDONOSNE PČELE (*Apis mellifera meda*) TOKOM RAZLIČITIH GODIŠNJIH DOBA

APSTRAKT

Izvršena su tri ogleda uz upotrebu potpuno nasumičnih obrazaca za procjenu uticaja koncentracije tijamina kao suplementa ishrani na rast društva iranske medonosne pčele (leglo i odraslu populaciju) tokom različitih godišnjih doba. Studijom je obuhvaćeno dvadeset društava iranske medonosne pčele (*Apis mellifera meda*), podijeljenih u 5 eksperimentalnih grupa. Kontrolne grupe su hranjene šećernim sirupom. Eksperimentalna društva su hranjena na isti način, ali je sirup obogaćen različitim količinama tijamina (100, 200, 300 i 400 ppm). Svaki od ovih načina ishrane testiran je na grupi od četiri društva odabrana za svaki tretman. Promjene u leglu su pokazale značajnu razliku između društava hranjenim tijaminom (200 ppm) i šećernim sirupom (kontrola) tokom ljeta ($p<0,05$). Tokom ljeta najniža proizvodnja legla utvrđena je u društvu koje je hranjeno šećernim sirupom. Tokom zime, veličina populacije odraslih i legla u društvima čiji je sirup obogaćen tijaminom (200 ppm) bila je značajno veća nego kod društava hranjenih šećernim sirupom ($p<0,05$). Nijesu uočene značajne razlike između ovih tretmana na leglo i veličinu odrasle populacije tokom proljeća, kada su društva prikupljala izvjesnu količinu polena.

Ključne riječi: leglo, iranska pčela, populacija, godišnje doba, tiamin