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## The effect to various oleic acid levels on reproductive parameters in queen bee

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### ABSTRACT

Queen bee larva needs nutrients such as proteins, carbohydrates, minerals, vitamins and water to provide its energy, growth, and development. These materials are provided by royal jelly. The jelly is produced by worker bees and poured in queen cell. This study was conducted to produce queens with desired physiological specifics and high reproduction ability. 0.02, 0.04, and 0.06 mg of oleic acid were mixed with royal jelly and added to queen cell in five days after grafting. Twelve days after grafting queens were born and birthweight was measured. Five days after queens spawning, ovary weight and the number of oocytes were measured. The spawning amount was measured in two stages: 20 days after spawning and 60 days after spawning. According to results, oleic acid levels affected birth weight, ovary weight, the number of oocytes in each ovary and spawning amount which were increased 41.1, 59, 29.3, and 57.4%, respectively ( $p < 0.05$ ) in proportion to control group. Significant differences were observed also between the groups in second spawning. Considering the results, adding oleic acid affected reproduction parameters and will increase reproduction ability of queen.

**Keywords:** Oleic acid, Queen bee, Queen egg

### INTRODUCTION

Bees need nutrients such as proteins (amino acids), carbohydrates (sugars), fats (fatty acids), minerals, vitamins and water to natural growth and development. Bees collect three types of compounds including water, nectar and pollen of flowers to meet their food needs. Nectar is the most important source of energy in carbohydrate form and pollen also contains a variety of chemical compounds that are essential for survival and producing good colonies. It also provides necessary proteins [1].

In recent years, amounts of fats, vitamins, and minerals in pollen have been considered and importance of pollen has been proved. Lipids which are found in bees' body contains a variety of free and bonding fatty acids, short-chain and long-chain alcohols, monoacylglycerol, diacylglycerol and triacylglycerol, steroids and other esters, phospholipids and several other combinations [2]. Lipids are synthesized in bees' body and stored in triglyceride form. These fats are distributed throughout the body cavities. Fatty acids are from essential components of phospholipids which play important role in strengthening the structure and function of cell membranes. Dominant fatty acids in the fat stores of bees in order of importance are oleic acid, linoleic acid, palmitic acid, linolenic acid, stearic acid and palmitoleic acid [3].

Researches showed that well fed bees have sufficient lipid reserves in their body which allows longer flights, as well as fatty acids such as capric, lauric and linoleic acids have antibacterial properties. It has been proven that there is a difference between quality of queen's and workers' royal jellies which makes physiological differences [4]. Fatty acids such as capric, lauric and linoleic acids have antibacterial properties and prevent secondary infections in American Luc disease by using linoleic acid therapy and sucrose [3]. Dominant fatty acids in the fat stores of bees in order of importance are oleic acid, linoleic acid, palmitic acid, linolenic acid, stearic acid and palmitoleic acid. Maning (2006) announced that well fed bees have sufficient reserves in their body which allows longer flights [5].

Robinson (1970) reported the range of changes of body's fatty acids for six days old larva and matures bees (male, workers and queen) [6].

Manning (2006) studied the effects of oleic and linoleic acids on bee's lifetime and reported that 2% group of oleic acid and 2% and 6% groups of linoleic acid had better results than higher levels. Researches have shown that using linoleic acid therapy and sucrose prevented secondary infections in American Luc disease [1]. Comparing the growth rate of ovary in queen and worker bees showed that the highest weight increment of queen larva was obtained from fourth day so that in fifth day it was 2.5 times bigger than worker [7]. Due to the limited research in this area it necessary to pay more attention to this important issue and do more researches in beekeeping centers. Extant necessary compounds in bee nutrition are directly related to reproduction physiology which can affect oogenesis and reproduction in the hive in this way.

This study was carried out to investigate the effect of different levels of oleic acid on ovarian characteristics, birth weight and spawning amount of queen.

## MATERIALS AND METHODS

Sixty female queen bees were studied in four treatments and fifteen replications. Oleic acid in 0.02, 0.04, and 0.06 mg amounts was added to queen cells. After birth small nursing beehives were considered with seven frames in each beehive. The situation was similar for all treatments.

### - Treatments groups:

- Control group: including 15 queens to measure base amounts of birth weight, ovarian weight, number of eggs per spawn, and the rate off spawning
- First experimental group: 0.02 mg of oleic acid was added (using sampler) to queen cell for five days
- Second experimental group: 0.04 mg of oleic acid was added to queen cell for five days
- Third experimental group: 0.06 mg of oleic acid was added to queen cell for five days

Obtained data were analyzed using SPSS (18) program.

## RESULTS AND DISCUSSION

### - Birth weight

Mean comparison of queen's ovary weight is presented in figure 1. Experimental groups had more ovary weights than control group ( $p < 0.05$ ). The least and the highest weights were belonging to 0.04mg levels.

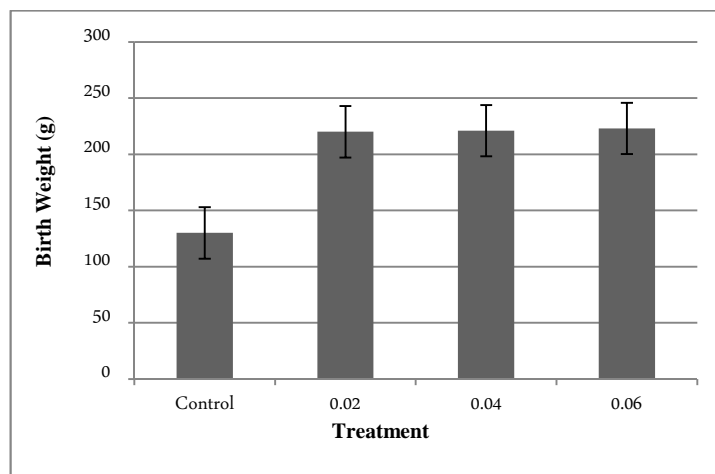


Figure 1. Birth weight in all groups

### - Ovary weight

Results of ovary weight's mean comparison is presented in figure 2. Ovary weight was significantly higher in experimental groups ( $p < 0.05$ ) than control group but treatments were not statistically different.

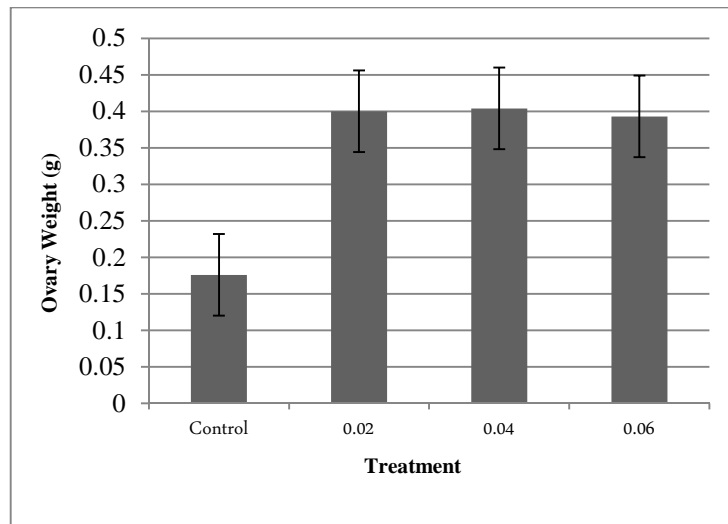


Figure 2. Ovary weight in all groups

- **The number of oocytes per ovary**

Mean comparison results of the number of oocytes are presented in figure 3. Oocytes of experimental groups were significantly more than control group ( $p < 0.05$ ) but there was no significant difference between treatments.

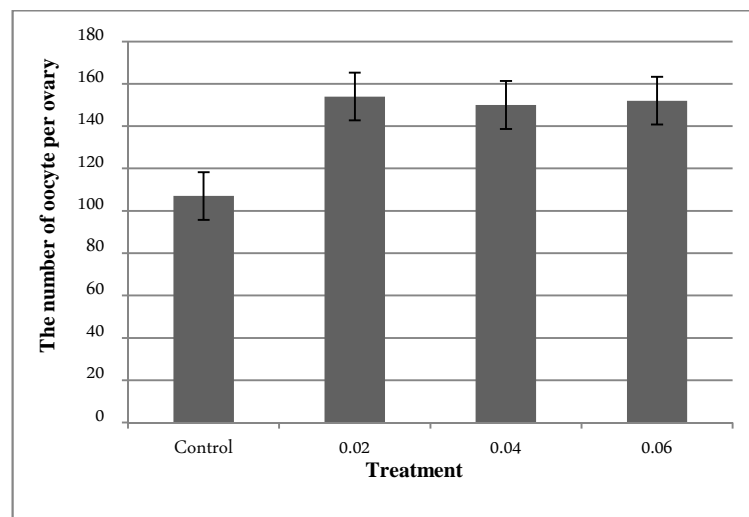


Figure 3. The number of oocytes per ovary in all groups

- **Spawning amount of queen- first stage**

Results of queen's spawning are presented in figure 4. The amounts of first spawning in experimental groups were significantly more than control group ( $p < 0.05$ ), but treatments were not different statistically.

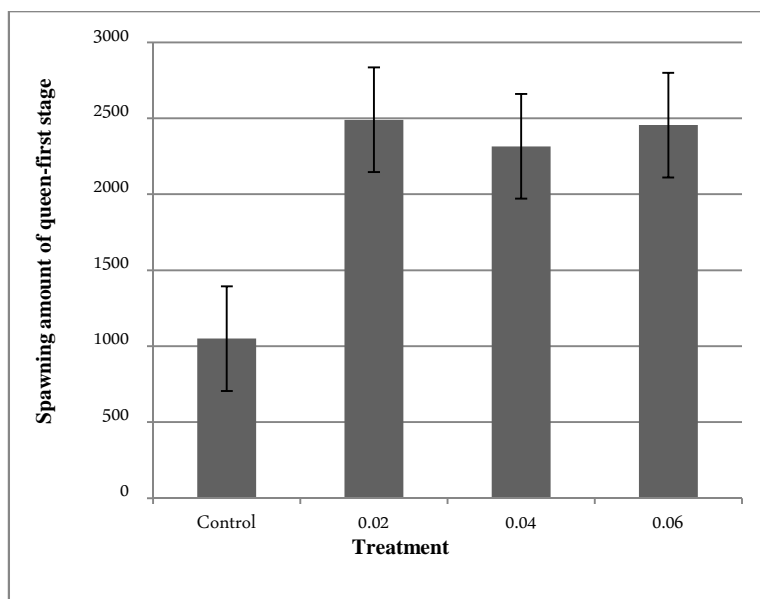


Figure 4. Spawning amount of queen- first stage in all groups

- **Spawning amount of queen-second stage**

Mean comparison of second spawning amount is shown in figure 5. Among experimental groups, the highest amount was belonging to 0.02% level of oleic acid ( $p < 0.05$ ).

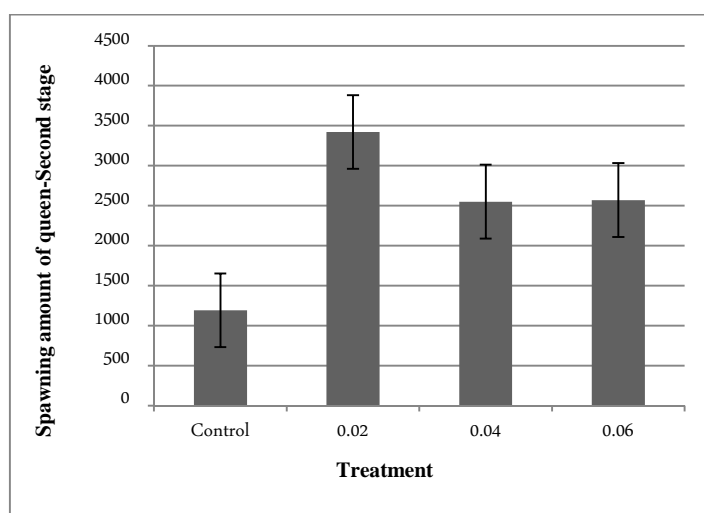


Figure 5. Spawning amount of queen- second stage in all groups

According to results, oleic acid had positive effects on birth date, ovary weight, oocytes number of each ovary, and spawning amount of queen.

- **Birth weight**

Birth weights of experimental groups were significantly more than control group, and the highest amount was belonging to 0.02 and 0.06mg group. There was no obvious relationship between birth weight and ovary weight, the number of oocytes and the first spawning amount, but 0.06 mg group which had the highest weight among treatments (225.82 mg) had the highest spawning amount (3310  $\text{cm}^2$ ). Therefore there is positive correlation between birth weight and second spawning [5].

Fatty acids are activated by reaction with the coenzyme A and ATP and make acyl coenzyme fatty acid which this molecule is broken under oxidative reactions of Krebs cycle and produces acid, water and energy which is used for physiological processes [7].

Body fats plus hypopharyngeal gland cause development of head and other inner organs of bees' larva. Extant fatty acids in jelly are only 8 to 10 carbon fatty acids. Insects cannot synthesize estrole which is an essential fat. This particular fat is needed to molting. Adding unsaturated fatty acid in the diet some insects improved growth. It seems in the present study also fatty acid could affect this biological factor [8].

#### - Ovary weight

Ovary weight was significantly higher in experimental groups ( $p < 0.05$ ) than control group but treatments were not statistically different. Therefore, adding this fatty acid increased the ovary weight of queen. Experimental groups had 59% increases in proportion to control group.

Different nutrition of worker bees and queen causes the difference. Researches showed that growth is similar at first but after third day queens grow faster and at the end of larval period the ovary of queen is different from workers [7]. Therefore, adding fatty acids (oleic and linoleic acid) which are from royal jelly compounds can increase ovary's weight.

Considering the previous researches, royal jelly of queen is more nutritional than workers jelly; therefore slower growth can be expected in case of shortage of these materials. Studies have shown that there are differences between the quality of queen and workers' larvae jellies that is the reason of physiological differences [4]. Supplementary nutrition is very important in case of pollen shortage. All pollens contain lipids which are really important for bee because it is used as a source of energy primarily and after that, fat ingredients such as fatty acids and sterols are important in development, nutrition and reproduction of bees. In this study, adding fatty acids (oleic and linoleic acid) could be a useful source of energy which increased ovary weight and the number of sexual cells [8].

#### - The number of oocytes in each ovary

Oocytes of experimental groups were significantly more than control group ( $p < 0.05$ ) but there was no significant difference between treatments. The highest (154.5) and the least (107.8) amounts were obtained in 0.02 mg and control group, respectively. Adding fatty acids to cow's diet improved sexual performance [7]. Adding fats increase synthesis of hormones such as progesterone and changes blood insulin concentration and stimulating the growth of ovarian follicles. In some animals acids play important role in sexual processes including ovulation, fertilization and childbirth. Fatty acids of diet may affect follicular growth, corpus luteum activity and production of progesterone [7]. It has reported that omega-3 enriched diet caused delay of calving and increased incidence of retained placenta in sheep and cow, respectively. Increased unsaturated omega-3 fatty acid in diet after birth increased the amount of pregnancy of cows. In this study also, adding mentioned fatty acid stimulated oogenesis via stimulating reproduction system and therefore oocytes amount was increased which was the reason of increase in ovary weight.

#### - Spawning amount of queen

Spawning amount of first stage was higher in experimental groups than control ( $p < 0.05$ ) but treatments were not different. The highest and the least spawning were belonging to 0.02 mg (2490 cm<sup>2</sup>) and control group (107.8 cm<sup>2</sup>). In second spawning which was from day 40 to day 60, groups show differences whereas in first stage there was no significant difference between treatments [9].

The importance of fats in bees' diet has been reported by Herbert et al. (1970) [10]. In colonies with 2, 4, 8 and 8% extra fat more larva reached to pupa stage in proportion to no fat die [9].

In view of increase in oocyte in experimental groups, increase in spawning is normal which is considered as secondary effect of adding fatty acid to queen cell.

### CONCLUSION

On the whole, using this fatty acid had positive effect on queen growth and increased birth weight, the weight of ovaries, the number of oocytes and the spawning amount. Among experimental groups 0.04 mg group showed the highest effects.

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