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Effect of Varying Diets on Growth, Development and Survival of Queen Bee (*Apis mellifera* L.) in Captivity

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***Abstract*—**Keeping in view the increasing demand, queen of *Apis mellifera* L. (Hymenoptera: Apidae) was reared artificially in this experiment at varying diets including royal jelly. Larval duration, pupal duration, weight, and size of pupae were evaluated at different diets including royal jelly. Queen larvae were raised by Doo Little grafting method. Four different diets were mixed with royal jelly and applied to larvae. Fructose, sugar, yeast, and honey were provided to rearing queen larvae along with same amount of royal jelly. Larval and pupal duration were longest (6.15 and 7.5 days, respectively) at yeast and shortest on honey (5.05 and 7.02 days, respectively). Heavier and bigger pupae were recorded on yeast (168.14 mg and 1.76 cm, respectively) followed by diets having sugar and honey. Due to production of heavier and bigger pupae, yeast was considered as best artificial diet for the growing queen larvae. So, in the second part of experiment, different amounts of yeast were provided to growing larvae along with fixed amount (0.5 g) of royal jelly. Survival rates of the larvae and queen bee were 70% and 40% in the 4-g food, 86.7% and 53.3% in the 6-g food, and 76.7% and 50% in the 8-g food. Weight of adult queen bee (1.459±0.191 g) and the number of ovarioles (41.7±21.3) were highest at 8 g of food. Results of this study are helpful for bee-keepers in producing fitter queen bees.

Keywords*—Apis melifera* L., dietary effect, survival and development, honey bee queen.

I. Introduction

QUEEN bee, *Apis mellifera* L. (Hymenoptera: Apidae), is mated female developed from fertilized egg living in hive or colony and refer as the mother of colony [1]. Queen bees are developed from selected larvae by worker bees and are fed on special feed for their sexual maturity. Honey bee larvae are fed with the secretions produced by hypopharyngeal and mandibular glands of nurse bees [2]. These secretions contain all necessary nutrients required for development of workers, drones, and queens. All larvae of honey bees are initially fed on royal jelly for few days, but later, only queen larvae are fed on royal jelly. This difference in diet in form of royal jelly enables queen to develop into a sexually mature female [2].

Queen larvae are fed on fresh royal jelly and grow at least 1400-1600 times more than egg weight, i.e. from 0.12-0.20 mg up to 250-346 mg. All honey bees larvae receive the same feed up to age of 2.5 days and they float on the food [3]. Food of the worker larvae and queen larvae is changed afterwards. Worker larvae is fed 143 times during its larval stage by nurse bees [4]. Nutrition, cell size, season, and genotype affect the adult worker weight and size. It is estimated that larvae of worker grow about 800-1200 times the egg weight or newly hatched larva [5].

Rhein [6] reared adult bees in an incubator. He fed the larvae with fresh larval food which he collected from different cells of same aged larvae from colony. He was not successful in rearing worker bees by feeding fresh worker larval food. In his attempt of rearing adult bees, he was able to rear large workers with many overioles. After that, many scientists had made attempts to rear honey bees in vitro for 80 years [7]-[13]. Many scientists tried to rear *A. mellifera* in the laboratory, but this requires intensive labor and results in small number of viable individuals, and the resultant individuals were not of specific castes. Rembold and Lackner [10] tried to rear queens in vitro. They prepared a diet by mixing 20 g of royal jelly, 2.5 g glucose, 2 g of fructose, and 20 ml of distilled water. Their results were 70% survival of adults and most of them were workers. Adding of 0.5 difcobacto yeast extract to this prepared diet results in increased survival rate to 80% and 30% of these were queens. Hanser and Ruttner [12] reared honey bee queens in vitro using artificial diet prepared by mixing 30 g of honey, 12 g of yeast, and 0.5 g of nipagin in 100 ml of water. Younger larvae were fed on royal jelly diluted at 2:1 and older at 1:1. One to two-day old larvae were grafted on 0.30 ml of royal jelly solution. Vandenberg and Shimanuki [14] reared honey bee larvae in artificial queen cell cups made of plastic and beeswax. Larvae were fed on diet prepared by 50% royal jelly, 1% yeast, 6% fructose, 6% glucose, and 37% distilled water in an incubator kept at 35 °C and 98% RH during the larval stage and 72% RH during pupal stages. They obtained 90% larval survival in queen cell cups made of beeswax and 55% in cells cup of plastic. Peng et al. [7] modified the Vandenberg method of raising queens and determined the effects of chlortetracycline on development of worker larvae. Diet consisted of 4.3 g royal jelly powder, 0.8 g glucose, 0.7 g

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fructose, 0.2 g yeast and 14 g double distilled water. They obtained results of 6.3% and 18.1% larval mortality and post-defecation mortality, respectively.

A new practice for rearing honeybee in vitro was developed by feeding larvae only once instead of feeding every day [15]. This method was very beneficial; it reduced the labor and in vitro mass production of honey bee increased. However, the larvae were given a common basic larval diet, and the food competition resulted in a big variation on weight of larvae, adult weight and number of overioles [15]. Crailsheim et al. [2] showed in-vitro techniques for rearing larvae for standard toxicological studies. Hladun et al. [16] summarized the mass feeding technique and studied the effects of selenium on larval development in vitro. In-short, diets have effects on queen development and growth. Different studies have been conducted on different diets and produced varying results. In this study, we tried to rear better honeybee queen on different artificial diets, i.e. yeast, sugar, fructose, and honey. Larval and pupal duration of queen bees is evaluated at these different diets. Furthermore, queen size and reproductive potential (number of ovarioles) were also measured at varying level of applied artificial diets. We hypothesize that yeast may play a positive role in diet and our diet may help in producing fitter queen with better reproductive potential.

II. Materials and Methods

Preparation of Breeding Colonies

Healthy and open mated European honey bee colonies were used as larval source for queen rearing. Breeding colony was prepared with five frames; two frames of pollen, one frame of honey, and two frames with regular brood and it was shifted three kilometers away from the main apiary and placed in brassica field (one foot above ground level in a tree shadow) for provision of nectar and pollen. Queen cells were attached on a wooden frame with two vertical bars. Artificial queen cells cup was prepared by hot wax and attached on the bars.

Preparation of Variable Diets

Four different artificial diets were prepared by mixing 0.5 g royal jelly with 0.2 g of yeast /sugar/fructose, or natural honey. Royal jelly was collected from different queen cells in colonies. Prepared diets were kept in centrifuge tubes at -18 °C in freezer until they were used. Before using, the diets were defrosted and brought to 34 °C in a water bath.

III. Experiment No. 1

A total of 20 larvae were grafted; there were four treatment groups, four replications, and four larvae in each replication. The Doolittle method of grafting was used to transfer the larvae to artificial queen cups, as this method was easy and quick. Already prepared artificial diets were transferred to queen cell cups. Larvae were transferred to cells 24 hours after diet transfer. Brood frame with 24-hour larvae was brought to lab to graft cells with the help of grafting tool. The larvae were scooped up from cell with nib of tool and placed in artificial queen cell cup. As age of grafted larvae plays vital role in grafting, so cells were marked up at time of egg laying to ensure that the 24-hour old larvae are grafted. After grafting all cells, the frame was put back in the colony. Extra feed was made with sugar and water with ratio 1:1 was placed in colony for nurse bees. The grafted cells were checked (20 hours after grafting) for acceptance by nursing bees. Accepted cells have pool of royal jelly in cells. Six cells were not accepted by bees, these cells were re-grafted, and 2 g of artificial feed was given to each larva. Cells were checked after four days of grafting. Earlier grafted cells were sealed up after four days and were converted into pupae. Re-grafted cells were sealed one day later.

IV. Experiment No. 2

In this experiment, most effective diet composition (yeast) was used in different amount to evaluate the growth, survival, and fecundity of adult queen bee. In this study, queen was put in a single fully drawn frame by excluder in order to get 1-day old larvae [7]. Distinctive queen cell cups were placed in 60 × 15 mm disposable petri dishes. Larval diet (0.5 g of RJ and Yeast) was placed in queen cell cups in amount of 2 g, 4 g, 6 g, 8 g, and 10 g. There were five replicates of each group. Larvae were fed once as specified by Kaftanoglu et al. [15]. Bees were raised in incubator at 34 °C and 90% RH. The brood comb was removed from hive from which larvae were obtained for grafting. Emerged adult bees were weighed and dissected to count their number of ovarioles.

A. Statistical Analysis

Effects of artificial diets on larval duration, pupal duration, pupa weight and size were evaluated using two-way ANOVA and means were separated using LSD test (as the number of replications were less). Statistical software “R” (2.15.3) was used to perform the statistical analysis among different treatments.

V. Results

Effects of Artificial Diets on Larval and Pupae Duration

Different artificial diets affected the larval duration significantly. Larvae fed with RJ+Yeast had maximum larvae duration (6.15±0.12 days) (ANOVA F84.42, *P<0.001)* followed by RJ+Fructose (5.50±0.14 days). Minimum larvae duration was observed in diet RJ+Honey (5.05 ± 0.14 days). Different artificial diets also affected the pupal duration as well. Maximum pupal duration was observed in RJ+Yeast (7.50± 0.12 days) (ANOVA F72.75, *P<0.001)* followed by RJ+Fructose (7.22±0.12 days). Minimum larvae duration was observed in diet RJ+Honey (7.02±0.14 days).

Effects of Artificial Diets on Pupae Weight and Size

Different artificial diets also affected the weight and size of pupae significantly. Larvae that fed with diet RJ+Yeast had the highest pupae weights (168.14±1.36 mg) and they were heavier than other groups (ANOVA F101.83, P<0.001) followed by RJ+Fructose and RJ+ Sugar respectively

(157.50±4.50, 162.26 ±3.78). Minimum results were found in diet RJ+Honey (152.23 ±4.28).

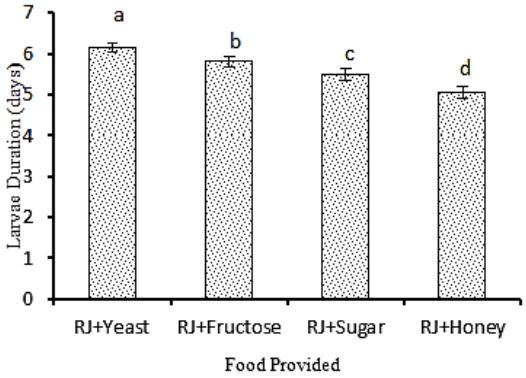

Fig. 1 Effect of different artificial diets on larval duration of queen of *A. mellifera*

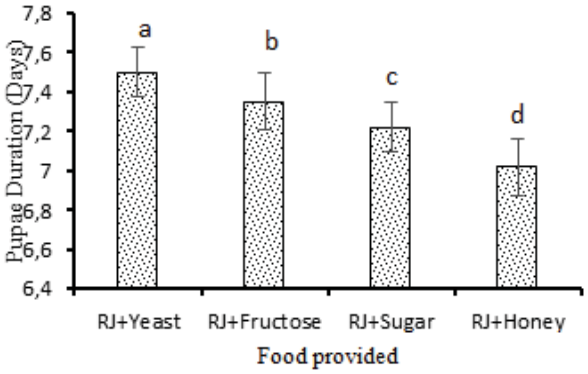

Fig. 2 Effect of different artificial diets on pupal duration of queen of *A. mellifera*

Pupae size was also affected by the different diets Larvae that fed with diet RJ+Yeast had the highest pupae weights (1.76 ± 0.02 mg) and they were heavier than the other groups (ANOVA F101.83, P<0.001) followed by RJ+Fructose and RJ+ Sugar, respectively (1.37±0.02, 1.56±0.02 mg). Minimum results were found in diet RJ+Honey (1.19±0.02 mg).

TABLE I
EFFECT OF FOOD QUALITY ON LONGEVITY AND GROWTH OF LARVAL AND PUPAL STAGE

	Larvae			Queen	
Groups	**Grafted**	**Survived**	**% survival**	**N**	**%**
2 g	30	12	40	6	20
4 g	30	21	70	12	40
6 g	30	26	86.7	16	53.3
8 g	30	23	76.7	15	50
10 g	30	17	56.7	10	66.7
Total	150	99		59	

According to Table I, survival percentage of larvae and queen emergence was observed minimum at 2 g diet. While both were increased with increasing food rate as 30%, 20% of 4 g diet and 46.7%, 33.3% of 6 g diet, respectively. A sudden decline was observed at 8 g and 10 g of diet after 6 g of diet but not in the case of queen. As compared with the 6-g diet, both survival of larvae and queen emergence were decreased with increasing food quantity as 3%, 3.3% of 8 g diet and 9% and 16.7% of 10 g of diet, respectively.

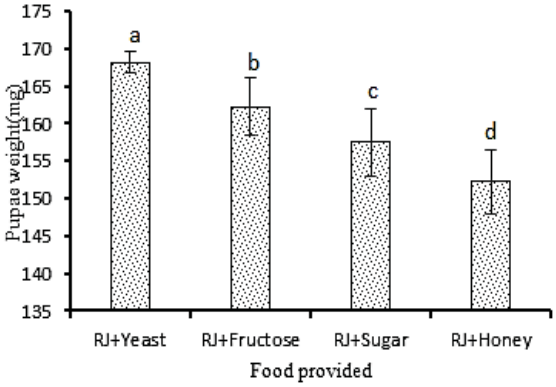

Fig. 3 Effect of different artificial diets on pupal weight of queen of *A. mellifera*

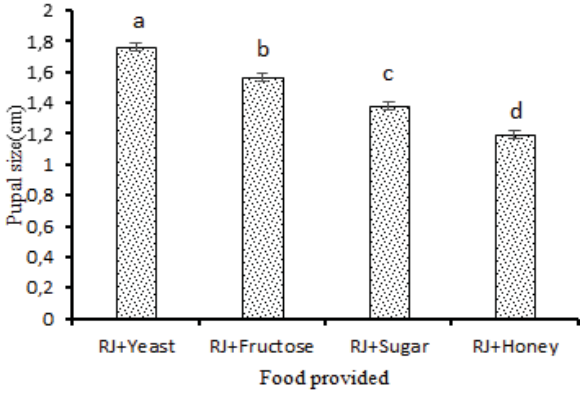

Fig. 4 Effect of different artificial diets on pupal size of queen of *A. mellifera*

In captivity, quantity of best suited food was evaluated. it was observed that food quantity affected the adult weighs significantly. As the amount of larval diets increased, weights of the bees also increased. The larvae fed on 2-g larval diet were lighter (0.863±0.081 g) than the larvae fed on 10 mg larval diet (1.398±0.163 g). The average weights of the hive reared control bees (1.09±0.113 g) were between the 2-g and 4-g larval diet groups. Similarly, quantity of food also affected number of ovarioles, bees that were fed by 2 g of larval food had the lowest (23.6±7.33) and 10 g of larval food had the highest (42.9±26.8) number of ovarioles.

TABLE II
EFFECTS OF FOOD QUANTITY ON ADULT WEIGHTS AND OVARIOLE NUMBERS

Food Quantity	Total No.	Newly hatched queen bee weight (g)			Total No.	No. of Ovariole/Queen		
		X±SE	Min	Max		X±SE	Min	Max
2 g	06	0.863±0.081a	0.71	0.93	06	23.6±7.33a	12	41
4 g	12	1.163±0.211b	0.69	1.37	11	29.3±9.46b	14	76
6 g	16	1.397±0.311c	0.89	1.84	16	35.9±16.4bc	23	109
8 g	15	1.459±0.191c	0.83	1.96	14	41.7±21.3c	21	156
10 g	10	1.398±0.163c	0.95	1.89	09	42.9±26.8c	18	183
control	11	1.09±0.113ab	0.86	1.42	11	15.8±6.4a	11	37

VI. Discussion

Queen of *A. mellifera* can be raised on artificial diets composed of RJ with fructose, yeast, sugar, and honey. Diet prepared with RJ+Honey did not affect well on queen larvae. However, the diet prepared by mixture of yeast and RJ showed maximum results. Thompson et al. [17] reared queen bees to check queen weight and size in queenright and queen less colonies. Their graph acceptance of 6666 larvae was 81%. They observed that weight of queen pupae and length of queen pupae were not significantly different from means of larvae raised in queen right and queen less colony. Their data showed that the mean length (30.82 mm) of queen cells raised in queenless colony was significantly greater than the mean length (26.70 mm) of cells raised in queen right colony.

Scientists tried to rear *A. mellifera* L. queen larvae in vitro on seven different diets prepared with different concentration of glucose and fructose. Larval and adult weight rate showed that sugar concentration affected the weight of larvae. The average weight was 204+19.27 mg [15].

For endurance of in vitro nurture bees, those were fed once in bunch, 4-g, 6-g, 8-g, and 10-g food was adequate for their development. Growth and developmental rates of larvae and adult were almost alike or somehow high as compared to [7], [13], [14]. However, the survival of larvae and queen emergence percentage was minimum at 2-g diet, while both were increased with increasing food rate as 30%, 20% of 4-g diet and 46.7%, 33.3% of 6-g diet, respectively. A sudden decline was observed at 8 g and 10 g of diet after 6 g of diet. As compared with the 6-g diet, both survival of larvae and queen emergence were decreased with increasing food quantity as 3%, 3.3% of 8-g diet and 9% and 16.7% of 10-g of diet, respectively.

Adult weight was significantly affected by the mass feeding. Larval weight was continuously increased by increasing food quantity because the body size of the larvae bees was smaller on 2-g food quantity and larger on 4-g food. In control environment, average weight of bees (1.09±0.113 g) was recorded between 2-g and 4-g diet groups. Ovariole number was also overstated by the food rate fluctuation as the bees that were treated with 2 g had low number of ovariole, while highest number of ovarioles was observed on 10-g of diet. It is suggested by Aupinel et al. [13] and Crailsheim et al. [2] that, for standard growth and development of bees, 160 mg food is sufficient. In order to determine the growth of bees in control conditions, high quality of royal jelly is the most imperative. Even 6 g diet may fulfill their development and growth process; however, if there is any calamity in royal jelly like having high moisture contents, the development and longevity will definitely be decreased. So, it is subjected to increase the food rate capable of 10 g or use of fresh royal jelly lot for survival of the bees.

Rembold and Lackner [10] developed larval diet for rearing of queen larvae in vitro. Diet consists of 20 g royal jelly, 2.5 g glucose, 2.5 g of fructose, and 20 ml of distilled water. They observed that addition of 0.5 g of yeast to larval food increased the survival rate of 80-90%, and the individual were grown to queens.

Rearing of larval honeybees just once in separate cell cups with identified food quantity is very novel and easily practicable for expansion, development, growth, evolution, and honeybee genome projects. One can easily rear to determine their varying sizes and also for forecasted ovariole number and their behavior. Practice is simple and does not require man power/labor for grafting and food preparation.

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