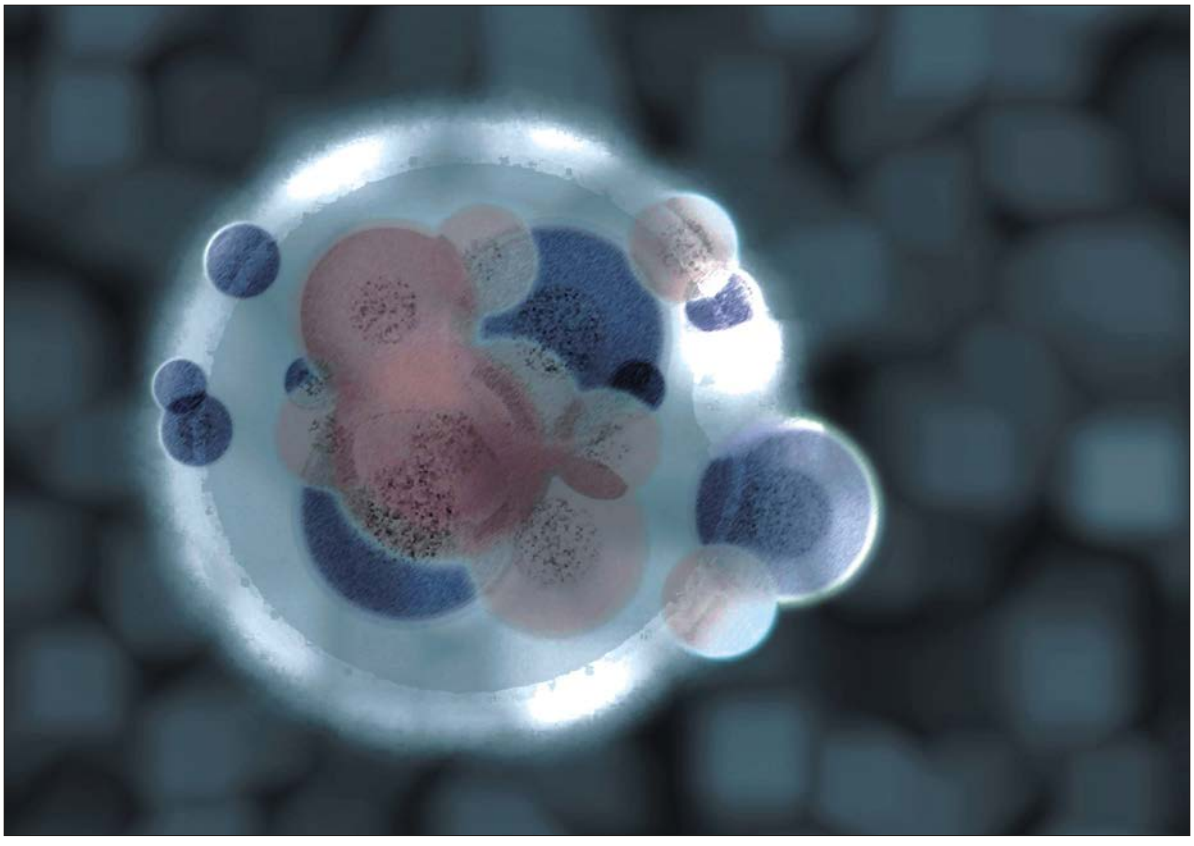

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Studies on the Artificial Feeding Conditions of Queen Bee Larvae

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Abstract: In order to realize the artificial incubation of honeybee larvae, queen bee larvae of *Apis mellifer* were fed artificially from early larval stage in an incubator under controlled temperature and relative humidity. Different proportions of royal jelly, honey and water were added into the diets. Results showed that when queen bee larvae were incubated in a temperature of 35.5±0.1°C and relative humidity of 90±2% and fed diet, which contained 70% water and 10% honey their nutritional needs could be met. Before queen bee larvae entered into the pupal stage, their body weight had no differences with that of larvae developed in natural condition. Extra honey and water besides royal jelly must be added into the diets to promote the growth of larvae.

Key words: Queen bee larvae, royal jelly, honey, artificial feeding conditions

INTRODUCTION

Honeybees (*Apis mellifer ligustica*) experience four distinct life stages: the egg, larva, pupa and adult. Larval stage of queen bee lasts for about five days. It is also the critic stage that determines the caste differentiation of fertilized eggs (Zhijiang *et al*., 2004). Up till now, the technique of artificial incubation has already been successfully applied in such organisms as chicken and silkworm. However, relative research in honeybee has been conducted very little (Bin *et al*., 2003; Fehler *et al*., 2007). We only know that in the whole developmental period queen bee larvae take royal jelly as their principal food. However, the optimum environmental and nutritional conditions for larval growth are still not clear. Honeybee lives a social life. In natural condition, honeybee colony has the ability to modulate relative humidity and temperature in the beehive to keep appropriate environment for the development of honeybees at different stages. In order to realize the artificial incubation of larvae, we must create an artificially ideal environment and provide nutritional factors to meet the basic survival needs of larvae. We took this study to find a suitable artificial incubating condition for larvae, which can help us to study the biological characteristics of honeybee deeply.

MATERIALS AND METHODS

Preparation of larvae: Queen bee larvae of *Apis mellifer ligustica* were obtained from the apiary in Yangzhou University. An empty honeycomb was put into a healthy colony. Four to six hours after the queen laying eggs we took out the comb and put it into the upper honey drawer. Eggs were hatched to larvae in three days. Larvae of 36 h old were picked up with a plastic earspoon and gently laid in aseptic culture dishes with diet spread evenly at the bottom. Larvae in tested groups were developed in an incubator with controlled temperature of 35.5±0.1°C and relative humidity of 90±2%. Larvae were also transferred into queen cups filled with royal jelly and developed in natural condition, which were set as a control group.

Obtain the weight of larvae: Larvae in tested groups were weighed every day. Each larva was rinsed quickly by sterile distilled water pre-warmed to 35°C to remove remaining diet on its body. Aseptic filter paper was used to absorb the remaining water on the body. After being weighed, larvae were transferred to a new aseptic culture dishes with fresh diet spread on it. Twenty queen bee larvae developed in natural condition were also weighed everyday and discarded until the end of the experiment. All the data were analyzed by SPSS software (SPSS, 10.0). Significance of the difference was analyzed by one-way classification ANOVA.

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Composition of diets: Basic diets were a mixture of royal jelly, honey and water. Fresh royal jelly and mature honey were obtained from the same colony where larvae were taken from. Water content was 66 and 22%, respectively, in fresh royal jelly and honey. Lyophilized royal jelly, which contained 6% water, was also used to reduce the water content in the diet. Compositions of diets used in the five treatments were showed in Table 1. Different ingredients were mixed thoroughly with a superfine homogenizer (F6/10, FLUKO, Germany). Diets in culture dishes were pre-warmed in the incubator for at least 30 min before use.

RESULTS

Since body weight is an important indicator reflecting the developmental conditions of larvae, control group was set to evaluate whether the artificial condition adopted could match the developmental needs of queen bee larvae. Each day 20 queen bee larvae were picked out to be weighted. We could see that larvae had a much higher growth rate at the later larval stage compared with earlier larval stage (Table 2).

Each day 20 queen bee larvae were selected from different tested groups and weighted (Table 3).

Statistical results showed that at larval stage of 36 h old, larvae in different groups had no difference in weight, which indicated that larvae we selected to be artificially fed in the incubator were of the same size at the beginning of our experiment. Larvae in group A always had significantly higher weight than larvae in group D from larval stage of 60 to 106 h ($p \leq 0.01$)and from 130 to 150 h ($p \leq 0.05$). We inferred from this phenomenon that larvae needed extra water in their diets besides royal jelly and honey for their growth. In natural condition queen bee larvae might be fed water frequently of worker bees. When artificially incubated, larvae can only take in nutritional factors contained in the diets. All of their needs for water come from diets. We concluded that when water content in the diets fell down below 60%, the growth of larvae might be impeded. Larvae in group C were fed by fresh royal jelly only in the whole larval stage. We found out that although at the early larval stage of 60 h, larvae in group C had a significantly higher weight than that in groups B and D ($p \leq 0.01$), this advantage disappeared from the larval stage of 82 h compared with group B. At the end of larval stage, larval weight in group C was significantly lower than that in group B ($p \leq 0.01$). This was coincident with the known fact that royal jelly was not the only food for queen bee larvae during larval stage. Honey and water are indispensable for their growth. Larval weight in group E was always significantly lower than that of the other groups ($p \leq 0.01$). We concluded that water was another nutritional factor which had important effects on the development of larvae. When the content of water in the diet dropped below that in fresh royal jelly, it would impede the growth of larvae severely. Compared with the control group, larvae in group B had the closest weight to that in natural condition at different larval stages, which leads us to conclude that incubational condition in this group is the most optimum one for the growth of queen bee larvae in our experiment.

We could see clearly from Fig. 1 that larvae in group B had almost the same growth curve as that in control group. We also noted that larvae in group B had a lighter weight than larvae in the control group, although this difference did not reach a significant level. This indicated that the most optimum condition for the development of queen bee larvae still needs to be adjusted finely. Elabrate

Table 1: Composition of daily diet for tested groups

Group	Fresh royal jelly (g)	Honey (g)	Distilled water (g)	Lyophilized royal jelly (g)	Water content (%)
A	9.00	1.85	7.65	-	75.66
B	9.00	1.41	3.69	-	70.50
C	Only	-	-	-	66.00
D	8.80	1.00	-	0.20	60.40
E	8.00	1.00	-	1.00	55.60

Table 2: Average weights of queen bee larvae in the control group

Larval stage	36 h	60 h	82 h	106 h	130 h	150 h
Weight (mg)	2.11±0.55	7.83±2.71	28.75±3.60	83.78±12.61	172.70±19.18	234.30±26.64

Table 3: Average weight of queen bee larvae artificially incubated

Larval stage (h)	Group A (mg)	Group B (mg)	Group C (mg)	Group D (mg)	Group E (mg)
36	2.16±0.62	2.08±0.57	2.16±0.62	2.20±0.58	2.16±0.69
60	11.08±2.48	7.52±1.70	10.71±2.16	7.52±3.06	4.88±1.78
82	29.05±9.94	26.81±5.59	26.05±3.68	22.55±7.37	12.37±4.22
106	68.07±37.97	69.26±16.82	68.55±13.43	46.06±20.33	17.06±6.56
130	119.00±66.59	164.74±36.88	141.60±22.69	94.87±47.82	28.87±15.75
150	155.92±75.24	227.05±37.95	181.63±26.97	123.00±39.81	55.50±31.95

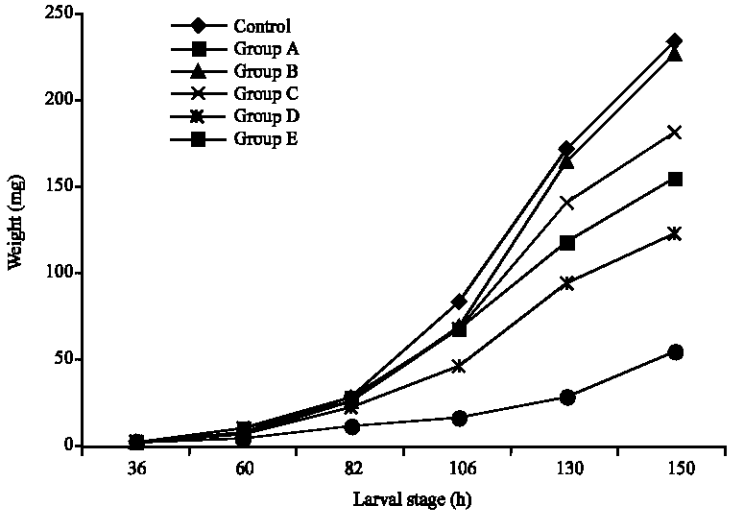

Fig. 1: Larval weight at different larval stages

manipulation of environmental condition and concentration of different nutritional factors in the artificial diets should be studied in the near future.

DISCUSSION

In this experiment, we fed queen bee larvae by a mixture of royally jelly, honey and water. Present results showed these nutritional factors were indispensable for the development of larvae. Firstly, royal jelly is the main food for queen bee larvae during the whole larval period. It was always a principal component in the diets we used to feed larvae artificially. It is also a pivotal component that determine the castification of fertilized eggs. Secondly, honey was another nutritional factor we added in the diets. As we know larvae tend to take in more if there is more honey in their food. In natural condition, worker bees may give different amount of honey to worker bee and queen bee larvae, which results in the significant difference in weight between worker bee and queen bee larvae. (Xiaowu and Yumin, 2005; Barchuk *et al*., 2004). In this study 10% mature honey was added in the diets to stimulate larvae to take in more food and promote their growth. Thirdly, we found out that extra water was needed for the growth of larvae, which was coincident with the fact that larvae body had a relatively high content of water (Bin *et al*., 2003). When larvae were developed in an incubator, larvae were spread on the top of diets and took food *ad libitum*. We could not feed them water frequently. Water in the diet must meet their daily needs in the whole larval stage. We concluded that when incubated in a relative humidity of 90±2% and in a temperature of 35.5±0.1°C, diets that contained 70% water and 10% mature honey (with 22% water) could match the growth needs of larvae.

Queen grafting is commonly used for mass production of queens. This practice normally happens before the honey flow begins, when the bees are building up. In this study we used this technique to study the artificial conditions for the growth of queen bee larvae. Since almost every bee larva has the potential to become a queen if properly nourished. The success in the artificial incubation of queen bee larvae could help apiarists obtain more queen and bee products. On the other hand, many research papers are focused on the molecular mechanisms of cast differentiation and important economic traits of honeybee (Evans and Wheeler, 1999, 2000; Hepperle and Hartfelder, 2001; Chen *et al*., 2005). We are currently conducting studies on the screen of specific expressed genes in the castification progress. This can not only facilitate relative studies on the mechanism of larval development but also provide a useful tool to elucidate honeybee social behaviors at a molecular level. Research results will help to clarify the exact function of certain nutritional factors and promote the progress of honeybee breeding.

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