Effect of Supplementary Folic Acid on Queen Quality in Western Honey Bees (Apis mellifera) During Queen Rearing: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to determine the effects of supplemental folic acid at different concentrations on the quality of Western honey bee (Apis mellifera) queens. Queen oviposition was controlled for 8 h, and after hatching, artificial larval grafting was performed for queen rearing. From the second day post-grafting, 2 L of sugar solution containing 0 (Group I), 0.05 (Group II), 0.25 (Group III), and 1.00 mg/kg (Group IV) folic acid was fed daily to the young larvae in queen cells using a microsyringe for 3 consecutive days. At queen emergence, newborn weight and thorax width were measured, and real-time quantitative PCR was employed to detect the relative expression levels of storage protein 110 (hex110), storage protein 70b (hex70b), and vitellogenin (Vg) genes in queen ovaries. The results showed that the newborn weight of queens in Group II was significantly higher than that in Groups I, III, and IV ($P<0.05$), while no significant differences were observed among Groups I, III, and IV ($P>0.05$). No significant differences in queen thorax width were detected among the four groups ($P>0.05$). The relative expression level of Vg gene in queen ovaries was significantly higher in Groups I and III compared to Group IV ($P<0.05$); however, no significant differences were found among Groups I, II, and III ($P>0.05$), nor between Groups II and IV ($P>0.05$). With increasing folic acid concentration, the relative expression level of hex110 gene in queen ovaries exhibited an initial increase followed by a decrease, with significant differences among the four groups ($P<0.05$), peaking in Group II. The relative expression level of hex70b gene in queen ovaries was significantly higher in Groups I, II, and III compared to Group IV ($P<0.05$), but no significant differences were observed among Groups I, II, and III ($P>0.05$). In conclusion, supplemental folic acid during queen rearing affects the quality of Western honey bee queens. Supplemental low-concentration folic acid can increase queen newborn weight and the expression of hex110 gene in ovaries, whereas supplemental high-concentration folic acid inhibits the expression of Vg, hex110, and

hex70b genes in queen ovaries. Therefore, appropriate supplementation of low-concentration folic acid can be applied to improve queen quality during queen rearing.

Full Text

Effects of Supplemental Folic Acid on Queen Quality in Western Honey Bees (Apis mellifera) during Rearing

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Abstract

This study investigated the effects of supplemental folic acid at different concentrations on the quality of Western honey bee (Apis mellifera) queens during rearing. Queens were confined to lay eggs for 8 hours, and the resulting larvae were transferred to queen cells for artificial rearing. Beginning on the second day after grafting, larvae were fed 2 L of sugar syrup containing 0 (Group I), 0.05 (Group II), 0.25 (Group III), or 1.00 mg/kg (Group IV) folic acid daily for three consecutive days using a micro-syringe. At emergence, queen birth weight and thorax width were measured, and the relative expression levels of storage protein 110 (hex110), storage protein 70b (hex70b), and vitellogenin (Vg) genes in queen ovaries were quantified by real-time fluorescence quantitative PCR.

The results demonstrated that Group II queens had significantly higher birth weight compared to Groups I, III, and IV ($P<0.05$), while no significant differences were observed among Groups I, III, and IV ($P>0.05$). Thorax width did not differ significantly among the four groups ($P>0.05$). The relative expression of Vg gene in queen ovaries was significantly higher in Groups I and III compared to Group IV ($P<0.05$), but no significant differences were detected among Groups I, II, and III, nor between Groups II and IV ($P>0.05$). With increasing folic acid concentration, the relative expression of hex110 gene in queen ovaries first increased then decreased, with significant differences among all four groups ($P<0.05$) and the highest expression in Group II. The relative expression of hex70b gene in queen ovaries was significantly higher in Groups I, II, and III compared to Group IV ($P<0.05$), though no significant differences were found among Groups I, II, and III ($P>0.05$).

In conclusion, supplemental folic acid during queen rearing significantly affects queen quality. Low-concentration folic acid supplementation increased queen birth weight and hex110 gene expression in ovaries, whereas high-concentration folic acid inhibited expression of Vg, hex110, and hex70b genes in queen ovaries. Therefore, appropriate supplementation with low-concentration folic acid may improve queen quality during rearing.

Keywords: Western honey bee (Apis mellifera); queen rearing; folic acid; queen ovary; gene expression

Introduction

Honey bees are social insects intimately connected with humans and the natural world. They not only provide nutritious bee products but also enhance crop yields through pollination, maintain ecological balance, and play a crucial role in biodiversity conservation [1]. The queen is the sole reproductive female in a honey bee colony, and her quality is decisive for colony performance. High-quality queens exhibit superior egg-laying capacity and disease resistance, making the cultivation of premium queens essential for apiculture [2].

During both larval and egg-laying stages, queens consume royal jelly, which primarily contains proteins, various free amino acids, fatty acids, sugars, and vitamins [3]. Vitamins are micronutrients essential for insect growth and metabolism and constitute major components of coenzymes. Vitamin deficiency affects cellular metabolism, impedes insect growth and development, and can cause tissue and cellular pathologies. Vitamins participate in oxidation-reduction reactions and metabolism of the three major nutrients in honey bees, closely relating to bee health, growth, and reproduction. Insects generally cannot synthesize vitamins and must obtain them from food [4].

Folic acid is an essential organic compound for normal life activities. After absorption, it participates in one-carbon unit transfer as at least five active coenzyme forms, playing particularly important roles in biosynthesis of purines, pyrimidines, nucleic acids, and proteins, as well as in cell division and growth [5]. Royal jelly contains 0.16–0.50 g/g folic acid. Although insects require minimal folic acid during development, deficiency can affect development of older larvae and pupae [6]; however, the impact of folic acid on queen quality remains poorly documented.

Queen quality encompasses birth weight, thorax length, thorax width, egg-laying capacity, and related gene expression. Vitellogenin (Vg) is a pleiotropic gene correlated with ovarian activity, lifespan, and immunity in honey bees [7]. Storage protein 110 (hexamerin 110, hex110) and storage protein 70b (hexamerin 70b, hex70b) are important storage proteins in honey bees involved in metamorphosis, caste differentiation, sex determination, oviposition, lifespan, and immunity [8-14]. Therefore, to elucidate the effects of folic acid on queen quality, this study used Western honey bees (Apis mellifera) as experimental subjects to investigate the impacts of supplemental folic acid on queen birth weight, thorax width, and expression of Vg, hex110, and hex70b genes in queen ovaries, providing reference for rearing higher-quality queens.

1.1 Experimental Animals

Experimental animals were Western honey bees (Apis mellifera) maintained at the Honeybee Research Institute of Jiangxi Agricultural University. The experiment was conducted from May to [date incomplete in original].

1.2 Main Reagents and Equipment

Folic acid (purity $ $98%) and diethyl pyrocarbonate (DEPC) water were purchased from Beijing Solarbio Science & Technology Co., Ltd. Sodium chloride (analytical grade) was obtained from Beijing Xilong Chemical Co., Ltd. Trizol total RNA extraction kit and RNase inhibitor were from Beijing TransGen Biotech Co., Ltd. Reverse transcriptase M-MLV (200 U/ L), dNTP Mixture (2.5 mmol/L), and fluorescent dye were from TaKaRa Corporation. Oligo(dT) was synthesized by Invitrogen Life Technologies. Equipment included a fluorescence quantitative PCR instrument (iQTM2, Bio-Rad, USA), a nucleic acid-protein analyzer (Implen, Germany), and a biochemical incubator (GZ-250-GSI, Guangzhi Science and Technology Equipment Development Co., Shaoguan).

1.3.1 Queen Rearing and Folic Acid Treatment

A strong colony was selected as the donor colony, and the queen was confined to lay eggs for 8 hours. Three days later, Western honey bee queens were reared using standard artificial queen-rearing methods in four colonies with similar strength. Each colony received one frame containing two rows of 20 queen cells each. Beginning on the second day after larval grafting, larvae in queen cells were fed 2 L of sugar syrup containing 0, 0.05, 0.25, or 1.00 mg/kg folic acid according to randomized zones on each frame, designated as Groups I, II, III, and IV, respectively. This feeding continued for three consecutive days.

1.3.2 Measurement of Queen External Characteristics

Upon emergence, queen birth weight was measured using an electronic balance. The thorax was then excised, wings and legs removed, and thorax width measured using a morphological observation system.

1.3.3 Quantitative PCR Analysis of Vg, hex110, and hex70b Gene Expression in Queen Ovaries

1.3.3.1 Sample Collection

Immediately after measuring birth weight and thorax width, newly emerged queens were dissected to collect ovaries, which were placed in 1.5 mL RNase-free EP tubes and rapidly frozen in liquid nitrogen for subsequent analysis.

1.3.3.2 Total RNA Extraction and cDNA Synthesis

Total RNA was extracted following the method of Qiuqiu Hong [15]. RNA purity was assessed using a nucleic acid-protein analyzer (OD260/280 ratio 1.9-2.1, meeting quality standards), and RNA integrity was evaluated by agarose gel

electrophoresis of 28S, 18S, and 5S bands. Total RNA was reverse transcribed using a reverse transcription kit, and the resulting cDNA products were stored at -80°C.

1.3.3.3 Primer Design and Quantitative PCR

Primer sequences were designed using Primer 5.0 software based on GenBank sequences and synthesized by Shanghai Biotechnology Co., Ltd. (Table 1). β-actin served as the internal reference gene. The quantitative PCR reaction system (10 L) contained 1 L cDNA, 5 L SYBR® Premix ExTaqTM II, 0.2 L Rox, 0.4 L each of forward and reverse primers, and 3 L ultrapure sterile water. Reactions were performed in a fluorescence quantitative PCR instrument under the following conditions: 95°C for 30 s; 40 cycles of 95°C for 10 s and 60°C for 1 min; followed by heating from 50°C to 90°C (increasing 1°C every 6 s). Melting curves were generated, and Ct values for target and reference genes were collected [16]. Relative expression levels of each target gene were calculated using the method of Qiang et al. [17].

1.4 Data Processing

Experimental data were analyzed for significant differences using the ANOVA procedure in SPSS 17.0 software.

Results

2.1 Effects of Folic Acid on Queen Birth Weight and Thorax Width

As shown in Table 2 , Group II queens exhibited significantly higher birth weight compared to Groups I, III, and IV ($P<0.05$), while no significant differences were observed among Groups I, III, and IV ($P>0.05$). Thorax width measurements were similar across all four groups, with no significant differences ($P>0.05$).

2.2 Effects of Folic Acid on Relative Expression of Vg Gene in Queen Ovaries

Figure 1 [Figure 1: see original paper] shows that the relative expression of Vg gene in queen ovaries was significantly higher in Groups I and III compared to Group IV ($P<0.05$). However, no significant differences were detected among Groups I, II, and III, nor between Groups II and IV ($P>0.05$).

2.3 Effects of Folic Acid on Relative Expression of hex110 Gene in Queen Ovaries

As illustrated in Figure 2 [Figure 2: see original paper], the relative expression of hex110 gene in queen ovaries increased initially then decreased with increasing folic acid concentration. Group II showed significantly higher expression than Groups I, III, and IV ($P<0.05$), Group III was significantly higher than Groups I and IV ($P<0.05$), and Group I was significantly higher than Group IV ($P<0.05$).

2.4 Effects of Folic Acid on Relative Expression of hex70b Gene in Queen Ovaries

Figure 3 [Figure 3: see original paper] reveals that the relative expression of hex70b gene in queen ovaries was significantly higher in Groups I, II, and III compared to Group IV ($P<0.05$), though no significant differences existed among Groups I, II, and III ($P>0.05$).

Discussion

Nutrition is a critical factor affecting honey bee health. Adequate, balanced nutrient supply and appropriate environmental conditions are essential for healthy bee development. Honey bee nutritional requirements primarily include carbohydrates, proteins, minerals, lipids, and vitamins [18]. Although vitamin requirements are relatively small, vitamins are crucial for bee growth and development. Queen quality determines colony strength, and queen birth weight positively correlates with queen quality. Heavier queens possess well-developed ovaries with more ovarioles and exhibit stronger egg-laying capacity [19]. This study found that low-concentration folic acid supplementation increased queen birth weight, while high-concentration supplementation showed no significant effect. This may occur because folic acid, as an essential organic compound for normal life activities, is converted into coenzymes after absorption to participate in one-carbon unit transfer, playing vital roles in biosynthesis of pyrimidines, purines, nucleic acids, and proteins, as well as cell division and growth [5], thereby promoting gene expression and cellular proliferation. Excess folic acid may not be absorbed by queen larvae and could interfere with other metabolic processes, affecting other functions such as ovarian gene expression.

Vitellogenin (Vg) is a high-molecular-weight glycolipoprotein widely present in hemolymph, fat body, and eggs of oviparous vertebrates and invertebrates. It is a key substance for vitellogenesis in honey bees, with multiple functions including ovarian activation, reproductive competition, behavioral regulation, lifespan extension, and food conversion. Vg serves as a precursor to yolk proteins that provide proteins and essential amino acids for embryonic development. Queens with well-developed ovaries exhibit higher Vg gene expression, making Vg expression an important indicator of queen quality [20-28]. This study found that high-concentration folic acid (Group IV) significantly reduced Vg gene expression in queen ovaries compared to the control (Group I), while low- and medium-concentration groups showed no significant differences from the control. This indicates that excessive folic acid inhibits Vg gene expression in queen ovaries, thereby affecting queen development.

Storage proteins are nutrients stored for later use in insects. They typically consist of six subunits forming hexameric proteins synthesized by larval fat body cells and secreted into hemolymph. Honey bees possess four storage protein genes named according to molecular weight: hex110, hexamerin 70a (hex70a), hex70b, and hexamerin 70c (hex70c) [13]. Research has shown that storage

proteins are associated with oviposition in many insects, and their expression correlates with oviposition capacity [29-32]. This study found that hex110 gene expression in queen ovaries increased then decreased with rising folic acid concentration, peaking in Group II. This suggests that low-concentration folic acid supplementation during queen rearing promotes hex110 gene expression in ovaries, while high concentrations inhibit it. hex110 is an important gene in queen ovaries [33] that is highly expressed in egg-laying queens. High hex110 expression facilitates ovarian development and improves reproductive performance [13], whereas relatively low expression is detrimental to ovarian development.

This study also revealed that high-concentration folic acid significantly reduced hex70b gene expression in queen ovaries compared to the control, low-, and medium-concentration groups, though the latter three groups did not differ significantly. This indicates that excessive folic acid also inhibits hex70b gene expression. hex70b is a methionine- and leucine-rich storage protein associated with caste differentiation and gonadal development in both queens and drones [13]; decreased hex70b expression negatively impacts ovarian development quality. Notably, high-concentration folic acid (1.00 mg/kg) significantly reduced expression of Vg, hex110, and hex70b genes in queen ovaries compared to the control. Pickell et al. [34] reported that excessive folic acid causes DNA hypermethylation, altering epigenetic characteristics, and that high folic acid intake disrupts embryonic development and affects gene expression.

Conclusion

Supplemental folic acid during queen rearing significantly affects Western honey bee queen quality. Supplementing with 1.00 mg/kg folic acid significantly decreased relative expression of Vg, hex110, and hex70b genes in queen ovaries. Supplementing with 0.25 mg/kg folic acid significantly increased hex110 gene expression in queen ovaries, while 0.05 mg/kg folic acid significantly increased both queen birth weight and hex110 gene expression in ovaries. Therefore, appropriate supplementation with low-concentration folic acid can improve queen quality during rearing.

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Note: Figure translations are in progress. See original paper for figures.