Optimal Dietary Lysine Level for Italian Honey Bee Worker Larvae Postprint

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Abstract

This experiment aimed to investigate the optimal lysine level in the diet of Italian honeybee (Apis mellifera ligustica) worker larvae, providing a theoretical basis for elucidating the lysine nutritional requirements during the larval development stage of Italian honeybee workers. A total of 1,200 one-day-old Italian honeybee worker larvae were selected and randomly divided into 5 groups, with 5 replicates per group and 48 larvae per replicate. The five groups of worker larvae were fed diets with measured lysine levels of 6.08 (control), 11.08, 16.08, 21.08, and 26.08 mg/g, respectively, and were reared until adult bee eclosion. Growth indices including pupation rate (at 6 days old), total body protein content (at 6 days old), and eclosion rate (at 21 days old), as well as hemolymph biochemical indices and immune-related indices (at 6 days old) were measured at specific ages. The results showed that: 1) The pupation rate and eclosion rate of worker larvae in the 11.08 mg/g lysine group were extremely significantly higher than those in all other groups ($P<0.01$). 2) Compared with the control group, the 11.08-26.08 mg/g lysine groups showed significantly increased total body protein content and free lysine content in hemolymph of 6-day-old worker larvae ($P<0.05$), with the highest values observed when dietary lysine level was 26.08 mg/g. 3) The triglyceride content in hemolymph of 6-day-old worker larvae exhibited a trend of first decreasing then increasing with increasing dietary lysine levels, reaching its lowest value at a dietary lysine level of 16.08 mg/g, which was extremely significantly lower than all other groups ($P<0.01$). 4) The lysozyme activity in hemolymph of 6-day-old worker larvae in the 11.08-21.08 mg/g lysine groups was significantly higher than that in the control group ($P<0.05$); however, as dietary lysine level increased from 21.08 mg/g to 26.08 mg/g, lysozyme activity decreased sharply, with the 26.08 mg/g lysine group being significantly lower than all other groups ($P<0.05$). 5) When dietary lysine level was 11.08 mg/g, the relative expression level of the lysozyme gene in 6-day-old worker larvae was the highest, significantly higher than at all other lysine levels ($P<0.05$). Compared with the control group, the 11.08-16.08 mg/g lysine

groups showed significantly increased relative expression level of the defensin 1 gene in 6-day-old worker larvae (P<0.05). In conclusion, lysine can promote the growth of Italian honeybee worker larvae, enhance protein and lysine deposition, increase eclosion rate, and exert regulatory effects on lipid metabolism and larval immune capacity to a certain extent. Based on comprehensive consideration of the above indices, the recommended optimal lysine level in the diet of Italian honeybee worker larvae is 11.08-16.08 mg/g.

Full Text

Appropriate Dietary Lysine Level for *Apis mellifera ligustica* Worker Bee Larvae

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Abstract

This experiment was conducted to investigate the appropriate lysine level in the diet for *Apis mellifera ligustica* worker bee larvae and to provide a theoretical basis for understanding lysine nutritional requirements during the larval development stage. A total of 1,200 one-day-old *Apis mellifera ligustica* worker bee larvae were randomly divided into 5 groups with 5 replicates per group and 48 larvae per replicate. The five groups were fed diets with measured lysine levels of 6.08 (control), 11.08, 16.08, 21.08, and 26.08 mg/g, respectively, until adult emergence. Growth indices including pupation rate (at 6 days old), total body protein content (at 6 days old), and eclosion rate (at 21 days old), as well as hemolymph biochemical parameters and immune-related indicators (at 6 days old), were measured at specific ages.

The results showed: 1) The pupation rate and eclosion rate of worker bee larvae in the 11.08 mg/g lysine group were extremely significantly higher than those in all other groups (P<0.01). 2) Compared with the control group, the total body protein content and free lysine content in hemolymph of 6-day-old worker bee larvae in the 11.08–26.08 mg/g lysine groups were significantly increased (P<0.05), with the highest values observed in the 26.08 mg/g lysine group. 3) The hemolymph triglyceride content of 6-day-old worker bee larvae showed a trend of first decreasing then increasing with dietary lysine level, reaching its lowest value at a dietary lysine level of 16.08 mg/g, which was extremely significantly lower than all other groups (P<0.01). 4) The hemolymph lysozyme activity of 6-day-old worker bee larvae in the 11.08–21.08 mg/g lysine groups was significantly higher than that of the control group (P<0.05); however, as dietary lysine level increased from 21.08 to 26.08 mg/g, hemolymph lysozyme activity decreased sharply, with the 26.08 mg/g lysine group being significantly lower than all other groups (P<0.05). 5) When dietary lysine level was 11.08

mg/g, the relative expression level of the lysozyme gene in 6-day-old worker bee larvae was highest, significantly higher than at other lysine levels ($P<0.05$). Compared with the control group, the relative expression level of the defensin 1 gene in 6-day-old worker bee larvae in the 11.08–16.08 mg/g lysine groups was significantly increased ($P<0.05$). In conclusion, lysine can promote the growth of *Apis mellifera ligustica* worker bee larvae, enhance protein and lysine deposition, increase eclosion rate, and regulate lipid metabolism and larval immunity to a certain extent. Based on these comprehensive indicators, the recommended appropriate dietary lysine level for *Apis mellifera ligustica* worker bee larvae is 11.08–16.08 mg/g.

Key words: *Apis mellifera ligustica*; worker bee; larvae; lysine

Introduction

Lysine is an essential amino acid in honey bees that plays a vital role in their growth and development, contributing significantly to promoting animal growth, fat metabolism, and immunity enhancement. Research on the appropriate dietary lysine level for *Apis mellifera ligustica* worker bee larvae is of great significance for scientific and healthy beekeeping. Lysine is one of the essential amino acids required for animal growth and development. It cannot be completely synthesized in the animal body and must be obtained from the diet. Lysine exists as two isomers: L-type and D-type. D-type lysine is almost non-absorbable and non-utilizable; the biologically active form is primarily L-lysine. Numerous studies have demonstrated that dietary supplementation with appropriate levels of lysine can improve or enhance body weight, feed intake, weight gain, feed conversion ratio, and carcass quality in poultry. Research has found that appropriate dietary lysine levels can significantly reduce serum uric acid, triglyceride, and very low-density lipoprotein contents in broiler chickens. Kornegay et al. investigated the relationship between dietary lysine level and immune function in weaned piglets, finding that antibody levels after ovalbumin immunization increased with increasing lysine levels. Konashi et al. found that dietary lysine deficiency inhibited protein synthesis and limited lymphocyte proliferation, thereby reducing immunity in broiler chickens and increasing morbidity and mortality. Chen et al. discovered that lysine could increase Newcastle disease virus antibody levels in broiler chickens, but lysine deficiency reduced antibody response and cellular immunity. Appropriate dietary protein types can significantly affect small intestine morphology, increasing villus length and crypt depth. De Groot estimated the lysine requirement of adult honey bees to be approximately 0.6%. Currently, there is no recommended standard for lysine requirement in *Apis mellifera ligustica*, and research on lysine nutritional function and requirements constitutes an important component of honey bee protein nutrition studies and forms the basis for scientifically formulating larval diets. Therefore, this study investigated the effects of dietary lysine levels on growth indices, hemolymph biochemical parameters, and immune-related indicators of

Apis mellifera ligustica worker bee larvae to determine the appropriate dietary lysine level and provide a basis for scientific beekeeping.

1.1 Experimental Materials

The experimental animals were *Apis mellifera ligustica* worker bee larvae obtained from healthy colonies with similar colony strength. The lysine used in the experiment was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., product number L103479-25g, with a purity of 98%.

1.2 Diet Formulation for *Apis mellifera ligustica* Worker Bee Larvae

Five artificial larval diets with different lysine levels were used to feed *Apis mellifera ligustica* worker bee larvae, labeled as A, B, C, D, and E. Diet A was the basal diet without additional lysine (control) with a measured lysine level of 6.08 mg/g. Diets B, C, D, and E were experimental diets with 0.5%, 1.0%, 1.5%, and 2.0% lysine added to the basal diet, respectively, with measured lysine levels of 11.08, 16.08, 21.08, and 26.08 mg/g. The composition and nutrient levels of the five diets for *Apis mellifera ligustica* worker bee larvae are shown in Table 1 .

Table 1 Composition and nutrient levels of five diets for Italian worker bee larvae (as-fed basis)

Item	Diet
Ingredients	
Royal jelly	
Glucose	
Fructose	
Yeast extract	
Lysine	
Sterile water	
Total	
Nutrient levels	
GE/(MJ/kg)	
Lysine/(mg/g)	

Note: GE and lysine were measured values.

1.3 Experimental Groups and Management

The experiment was conducted in 2016 at the College of Animal Science and Technology, Shandong Agricultural University, using the experimental apiary at the South Campus of Shandong Agricultural University. A total of 1,200 one-day-old *Apis mellifera ligustica* worker bee larvae were randomly divided into 5 groups with 5 replicates per group and 48 larvae per replicate. The five groups

were fed diets with measured lysine levels of 6.08, 11.08, 16.08, 21.08, and 26.08 mg/g, respectively. One-day-old worker bee larvae were placed in 48-well plates with 150 µL of diet added to each well. The plates were placed in a desiccator (relative humidity 95%, 15% glycerol solution), which was then placed in an incubator (35°C, relative humidity 90%) for rearing, with diet changed once daily. At 6 days old, the mature larvae were transferred to 24-well plates lined with sterile paper for pupation (feeding was stopped). At 9 days old, the pupae were transferred to new 24-well plates lined with sterile paper and reared until adult emergence. Growth indices including pupation rate, body protein content, and eclosion rate, as well as hemolymph biochemical parameters and immune-related indicators, were measured at specific ages.

1.4 Sample Processing and Measurement Methods

1.4.1 Sample Processing Methods Three 6-day-old *Apis mellifera ligustica* worker bee larvae were randomly selected from each group as molecular biology samples to study the relative expression of genes related to pupation. Three 6-day-old worker bee larvae were randomly selected from each group for tissue homogenization and prepared into 1% homogenate with physiological saline. One portion was centrifuged at 3,000 r/min for 10 min at 4°C to collect the supernatant, while another portion was used to determine body protein content in worker bee larvae. One hundred 6-day-old larvae were randomly selected from each group, and hemolymph was collected using a 20 µL capillary tube to determine free lysine, triglyceride content, and lysozyme activity in hemolymph.

1.4.2 Calculation of Pupation Rate and Eclosion Rate Using the replicate as the unit, the number of pupated larvae was recorded at 6 days old, and the total number of emerged adults was recorded at 21 days old to calculate pupation rate and eclosion rate.

Pupation rate (%) = 100 × number of pupated larvae / total number of larvae
Eclosion rate (%) = 100 × total number of emerged adults / number of pupated larvae

1.4.3 Determination of Total Protein Content in Worker Bee Larvae Total protein content in 1% tissue homogenate of 6-day-old larvae was determined using a total protein quantification kit (product number A045-2) from Nanjing Jiancheng Bioengineering Institute, with 3 replicates per group.

1.4.4 Determination of Free Lysine Content in Hemolymph Free lysine content in hemolymph of 6-day-old worker bee larvae was determined using an insect lysine kit (product number SU-B97004) from Quanzhou Kenodi Biotechnology Co., Ltd., with 3 replicates per group.

1.4.5 Determination of Triglyceride Content in Hemolymph Triglyceride content in hemolymph of 6-day-old worker bee larvae was determined using

an insect triglyceride kit (product number SU-B97002) from Quanzhou Kenodi Biotechnology Co., Ltd., with 3 replicates per group.

1.4.6 Determination of Lysozyme Activity in Hemolymph Lysozyme activity in hemolymph of 6-day-old worker bee larvae was determined using an insect lysozyme kit (product number SU-B97001) from Quanzhou Kenodi Biotechnology Co., Ltd., with 3 replicates per group.

1.4.7 Determination of Relative Expression Levels of Lysozyme and Defensin 1 Genes Total RNA was extracted from samples using the Trizol method, and the extracted total RNA was immediately reverse-transcribed into cDNA using a reverse transcription kit (TaKaRa). After adjusting the cDNA concentration to the same level, samples were stored at -20°C. One microliter of cDNA (5-fold dilution) was added to a 20 μL fluorescence quantitative PCR system. According to the fluorescence quantitative PCR kit (TaKaRa) operation guidelines, a PCR instrument was used to detect the relative expression of target genes. Primer sequences for target genes were designed based on sequences from the NCBI database, with β-actin as the internal reference gene. Primers were designed using Primer 5.0 and synthesized by Shanghai Sangon Biotech Co., Ltd. The primer sequences are shown in Table 2 .

Table 2 Primer sequences of genes

Target genes	Primer sequence (5 -3)	GenBank accession number
Lysozyme	F: TGGTAATGGCAACA-GAAATGGR: TATACGAAATGGTCCG-CAAAC	NC_{007075}
Defensin 1	F: CGTGCCGACAGA-CATAGAAGR: TCCTTTCTCGCAAT-GACCTC	NC_{007082}

1.5 Data Analysis

Data were analyzed using SAS 9.2 software for one-way ANOVA and Duncan' s multiple comparison tests. $P<0.01$ indicated extremely significant difference, and $P<0.05$ indicated significant difference. Results were presented as bar charts or line graphs.

Results

2.1 Effect of Dietary Lysine Level on Pupation Rate of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 1 [Figure 1: see original paper], the pupation rate of 6-day-old worker bee larvae showed a trend of first increasing then decreasing with increasing dietary lysine level. When dietary lysine level was 11.08 mg/g, the pupation rate of 6-day-old worker bee larvae reached its peak, and the 11.08 mg/g lysine group was extremely significantly higher than all other groups ($P<0.01$). As dietary lysine level gradually increased from 11.08 to 26.08 mg/g, the pupation rate of 6-day-old worker bee larvae showed a gradient decreasing trend, with extremely significant differences among the 16.08, 21.08, and 26.08 mg/g lysine groups ($P<0.01$). The 21.08 and 26.08 mg/g lysine groups were extremely significantly lower than the control and 11.08 mg/g lysine groups ($P<0.01$).

2.2 Effect of Dietary Lysine Level on Eclosion Rate of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 2 [Figure 2: see original paper], the eclosion rate of 21-day-old worker bee larvae showed a trend of first increasing then decreasing with increasing dietary lysine level. When dietary lysine level was 11.08 mg/g, the eclosion rate of 21-day-old worker bee larvae reached its peak, and the 11.08 mg/g lysine group was extremely significantly higher than all other groups ($P<0.01$). As dietary lysine level gradually increased from 11.08 to 26.08 mg/g, the eclosion rate of 21-day-old worker bee larvae showed a gradient decreasing trend, with extremely significant differences among the 16.08, 21.08, and 26.08 mg/g lysine groups ($P<0.01$). The 21.08 and 26.08 mg/g lysine groups were extremely significantly lower than the control and 11.08 mg/g lysine groups ($P<0.01$).

Note: Data columns or data points with different capital letters indicate extremely significant difference ($P<0.01$), and different small letters indicate significant difference ($P<0.05$). The same as below.

2.3 Effect of Dietary Lysine Level on Total Protein Content in Whole Body of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 3 [Figure 3: see original paper], total protein content in whole body of 6-day-old worker bee larvae showed an overall increasing trend with increasing dietary lysine level. Compared with the dietary lysine level of 6.08 mg/g, total protein content in whole body of 6-day-old worker bee larvae was significantly increased at dietary lysine levels of 11.08–26.08 mg/g ($P<0.05$), with the highest value observed at the lysine level of 26.08 mg/g, which was significantly higher than all other groups ($P<0.05$).

2.4 Effect of Dietary Lysine Level on Free Lysine Content in Hemolymph of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 4 [Figure 4: see original paper], free lysine content in hemolymph of 6-day-old worker bee larvae showed an overall increasing trend with increasing dietary lysine level. When dietary lysine level increased from 6.08 to 11.08 mg/g, free lysine content in hemolymph of 6-day-old worker bee larvae significantly increased ($P<0.05$). At dietary lysine levels of 11.08–26.08 mg/g, free lysine content in hemolymph of 6-day-old worker bee larvae increased with dietary lysine level, and although differences among groups were not significant ($P>0.05$), all were significantly higher than the control group ($P<0.05$).

2.5 Effect of Dietary Lysine Level on Triglyceride Content in Hemolymph of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 5 [Figure 5: see original paper], triglyceride content in hemolymph of 6-day-old worker bee larvae showed an overall trend of first decreasing then increasing with increasing dietary lysine level. When dietary lysine level was 16.08 mg/g, triglyceride content in hemolymph of 6-day-old worker bee larvae reached its lowest value, which was extremely significantly lower than all other groups ($P<0.01$).

2.6 Effect of Dietary Lysine Level on Lysozyme Activity in Hemolymph of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 6 [Figure 6: see original paper], lysozyme activity in hemolymph of 6-day-old worker bee larvae showed a parabolic trend with increasing dietary lysine level. When dietary lysine level increased from 6.08 to 11.08 mg/g, lysozyme activity in hemolymph of 6-day-old worker bee larvae increased significantly ($P<0.05$). At dietary lysine levels of 11.08–21.08 mg/g, lysozyme activity showed no significant change with increasing dietary lysine level ($P>0.05$) but remained significantly higher than the control group ($P<0.05$). When dietary lysine level was 26.08 mg/g, lysozyme activity in hemolymph decreased sharply to its lowest value, significantly lower than all other groups ($P<0.05$).

2.7 Effect of Dietary Lysine Level on Relative Expression Level of Lysozyme Gene in Whole Body of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 7 [Figure 7: see original paper], the relative expression level of the lysozyme gene in whole body of 6-day-old worker bee larvae showed a trend of first increasing then decreasing with increasing dietary lysine level. When dietary lysine level was 11.08 mg/g, the relative expression level of the lysozyme gene reached its peak, significantly higher than all other groups ($P<0.05$). As dietary lysine level gradually increased from 11.08 to 26.08 mg/g, the relative expression level of the lysozyme gene showed a gradient decreasing

trend, with the 16.08 mg/g lysine group significantly higher than the control group ($P<0.05$), while the 21.08 and 26.08 mg/g lysine groups showed no significant difference from the control group ($P>0.05$).

2.8 Effect of Dietary Lysine Level on Relative Expression Level of Defensin 1 Gene in Whole Body of *Apis mellifera ligustica* Worker Bee Larvae

As shown in Figure 8 [Figure 8: see original paper], the relative expression level of the defensin 1 gene in whole body of 6-day-old worker bee larvae showed a trend of first increasing then decreasing with increasing dietary lysine level. The relative expression level of the defensin 1 gene reached relatively high levels at dietary lysine levels of 11.08–16.08 mg/g, significantly higher than the control group ($P<0.05$). When dietary lysine level continued to increase to 21.08 and 26.08 mg/g, the relative expression level of the defensin 1 gene showed a decreasing trend, significantly different from the 11.08 and 16.08 mg/g lysine groups ($P<0.05$) but not significantly different from the control group ($P>0.05$).

Discussion

3.1 Effect of Dietary Lysine Level on Pupation Rate and Eclosion Rate of *Apis mellifera ligustica* Worker Bee Larvae

The pupation rate and eclosion rate of *Apis mellifera ligustica* are important indicators that directly reflect larval growth and development status. Studies on the effects of essential amino acid deficiency or protein deficiency on insect growth and reproduction have found that nutritional deficiency in *Apis mellifera ligustica* shortens lifespan, reduces fecundity, inhibits gland development, decreases body weight, and reduces disease resistance. Nutritional factors are important factors affecting caste differentiation in *Apis mellifera ligustica* and can influence larval development. For example, under certain spatial conditions, nutritional levels can determine whether larvae develop into queens or workers. It can be inferred that many factors may affect bee pupation and eclosion, among which nutritional factors cannot be ignored. Malnourished *Apis mellifera ligustica* larvae may not be able to pupate and eclose normally, while well-nourished larvae may be more likely to eclose and develop normally. The results of this study showed that dietary lysine level had an extremely significant effect on both pupation rate and eclosion rate of *Apis mellifera ligustica* worker bee larvae. Both too low and too high dietary lysine levels may prevent normal pupation and eclosion, with a dietary lysine level of 11.08 mg/g being most conducive to normal pupation and eclosion.

3.2 Effect of Dietary Lysine Level on Total Protein Content in Whole Body of *Apis mellifera ligustica* Worker Bee Larvae

Lysine is primarily used for protein deposition and participates in the synthesis of enzyme proteins and certain peptide hormones, thus being closely related to

growth. As an essential amino acid, lysine is involved in the synthesis of various proteins including skeletal muscle, enzymes, serum proteins, and peptide hormones. Early animal studies have confirmed that long-term consumption of low-lysine diets inhibits growth in rats, reduces serum albumin and β-globulin contents, and suppresses liver protein synthesis. Research has shown that lysine can inhibit degradation of some muscle fiber proteins through the autophagosome-lysosome system, maintaining protein stability. Haunerland et al. found that insect storage proteins play important roles during insect metamorphosis, primarily serving as amino acid storage pools for architectural organization during development. Therefore, the amount of insect storage protein is an important indicator for measuring whether insects can develop completely. It can also be inferred that the richer the total protein in insect body, the more complete the insect development may be. This experiment found that dietary lysine level had a significant effect on total protein content in whole body of 6-day-old *Apis mellifera ligustica* worker bee larvae, with total protein content showing an overall increasing trend with dietary lysine level. When dietary lysine level was 6.08–11.08 mg/g, total protein content was in the increasing range. At dietary lysine levels of 11.08–21.08 mg/g, total protein content showed no significant change with increasing dietary lysine level but remained significantly higher than the control group. At a dietary lysine level of 26.08 mg/g, total protein content reached its peak, significantly higher than all other groups. In summary, total protein content in whole body of *Apis mellifera ligustica* worker bee larvae was highest at a dietary lysine level of 26.08 mg/g.

3.3 Effect of Dietary Lysine Level on Free Lysine Content in Hemolymph of *Apis mellifera ligustica* Worker Bee Larvae

As an essential amino acid, lysine participates in the synthesis of various proteins including skeletal muscle, enzymes, serum proteins, and peptide hormones. Lysine is a precursor for carnitine production, which is responsible for converting some unsaturated fatty acids into energy, participating in fat metabolism and helping to reduce cholesterol levels. Honey bee immune mechanisms require lysine participation, and lysine is crucial for the honey bee immune system. Ma et al. showed that dietary lysine level had a significant effect on serum lysine content in goats. It can be inferred that lysine nutrients within a certain range may gradually increase in body fluid content with increasing nutritional levels, but absorption efficiency and utilization may decrease after exceeding the optimal level required by the body. This study found significant differences in the effect of dietary lysine level on free lysine content in hemolymph of 6-day-old *Apis mellifera ligustica* worker bee larvae. When dietary lysine level was 6.08–11.08 mg/g, free lysine content in hemolymph increased with dietary lysine level. However, when dietary lysine level increased to 11.08–26.08 mg/g, free lysine content in hemolymph remained consistently high without significant further increase, but all groups remained significantly higher than the control group. It is concluded that free lysine content in hemolymph of *Apis mellifera ligustica* worker bee larvae can be maintained at relatively high levels at dietary lysine

levels of 11.08–26.08 mg/g.

3.4 Effect of Dietary Lysine Level on Triglyceride Content in Hemolymph and Immune Function of *Apis mellifera ligustica* Worker Bee Larvae

Si Rileng studied the effect of dietary lysine level on growth performance of 0–3-week-old broiler chickens and found that appropriate lysine levels significantly affected serum triglyceride content, which first decreased then increased with increasing dietary lysine level. Chen Zhimin et al. found that appropriate dietary lysine levels could reduce serum uric acid, triglyceride, and low-density lipoprotein contents in 0–3-week-old male broiler chickens. This study found that dietary lysine level had an extremely significant effect on triglyceride content in hemolymph of *Apis mellifera ligustica* worker bee larvae, showing an overall trend of first decreasing then increasing with dietary lysine level. Triglyceride content reached its lowest value at a dietary lysine level of 16.08 mg/g, which was extremely significantly lower than the control group. As dietary lysine level continued to increase to 16.08–26.08 mg/g, triglyceride content showed a gradient increasing trend, with extremely significant differences among these three groups. The 11.08, 16.08, and 26.08 mg/g lysine groups were all extremely significantly different from the control group. This may be because appropriate dietary lysine levels can enhance fat metabolism in *Apis mellifera ligustica* worker bee larvae, improving fat utilization and thereby reducing triglyceride content in hemolymph. It is concluded that triglyceride content in hemolymph of *Apis mellifera ligustica* worker bee larvae was lowest at a dietary lysine level of 16.08 mg/g.

Regarding immune function, Kornegay et al. studied the relationship between dietary lysine level and immune function in weaned piglets, finding that antibody levels after ovalbumin immunization increased with lysine level. Konashi et al. found that dietary lysine deficiency inhibited protein synthesis and limited lymphocyte proliferation, reducing immunity in broiler chickens and increasing morbidity and mortality. Chen et al. also found that lysine could increase Newcastle disease virus antibody levels in broiler chickens, but lysine deficiency reduced antibody response and cellular immunity. Honey bee immune mechanisms include cellular immunity and humoral immunity. Cellular immunity primarily relies on enhanced phagocytosis, nodule formation, and encapsulation by hemocytes, while humoral immunity mainly functions by increasing the content of certain substances in hemolymph. Humoral immunity includes innate immune factors and acquired immune factors. Innate immune factors include lysozyme, lectins, hemocyte aggregation inhibitors, and phenoloxidase, while acquired immune factors include defensins, proline-rich antimicrobial peptides, and antifungal peptides. All insect defensin sequences contain strongly cationic amino acids (arginine and lysine) between the α-helix and the first β-sheet strand. Most defensins also have cationic amino acids in the turn region and C-terminus, suggesting that these charged regions in the sequence are meaningful

for defensin function. Therefore, honey bee immune mechanisms require lysine participation, and lysine is crucial for the honey bee immune system. This experiment found that when dietary lysine level was 6.08–11.08 mg/g, hemolymph lysozyme activity in *Apis mellifera ligustica* worker bee larvae increased with dietary lysine level. At dietary lysine levels of 11.08–21.08 mg/g, hemolymph lysozyme activity showed no significant change with increasing dietary lysine level but remained significantly higher than the control group. When dietary lysine level was 21.08–26.08 mg/g, hemolymph lysozyme activity decreased with increasing dietary lysine level. Additionally, this experiment found that the relative expression level of the lysozyme gene in whole body showed a trend of first increasing then decreasing with dietary lysine level, reaching its peak at a lysine level of 11.08 mg/g. The relative expression level of the defensin 1 gene also showed a trend of first increasing then decreasing, reaching relatively high levels at dietary lysine levels of 11.08–16.08 mg/g. It is concluded that dietary lysine levels of 11.08–16.08 mg/g can maximize the enhancement of immunity in *Apis mellifera ligustica* worker bee larvae.

Conclusions

Based on the comprehensive results of this study, the following conclusions can be drawn:

1. A dietary lysine level of 11.08 mg/g can significantly improve pupation rate, eclosion rate, and expression of lysozyme and defensin 1 genes in *Apis mellifera ligustica* worker bee larvae.
2. At dietary lysine levels of 16.08–21.08 mg/g, hemolymph lysozyme activity and defensin 1 gene expression in *Apis mellifera ligustica* worker bee larvae were improved to varying degrees. However, when dietary lysine level exceeded 16.08 mg/g, pupation rate, eclosion rate, and expression of immune-related genes were inhibited.
3. Total protein content in whole body and free lysine content in hemolymph of *Apis mellifera ligustica* worker bee larvae showed a gradual increasing trend with dietary lysine level, reaching maximum values at a dietary lysine level of 26.08 mg/g.
4. Considering all the above indicators comprehensively, the recommended appropriate dietary lysine level for *Apis mellifera ligustica* worker bee larvae is 11.08–16.08 mg/g.

References

[1] DE GROOT A P. Protein and amino acid requirement of the honeybee (*Apis mellifera* L.)[J]. Physiologia Comparata et Oecologia, 1953, 3: 197–285.

[2] SI R. Effect of Different Dietary Lysine Levels on Growth Performance and Carcass Quality of 0-3 Week-Old Broiler Chickens[D]. Master' s Thesis. Hohhot:

Inner Mongolia Agricultural University, 2004.

[3] CHEN Z, CAI H, YU H, et al. Effect of Different Lysine Supplementation Levels on Blood Physiological and Biochemical Indices of Broiler Chickens[J]. Heilongjiang Animal Science and Veterinary Medicine, 2006(11): 68-69.

[4] KORNEGAY E T, LINDEMAN M D, RAVINDRAN V. Effects of dietary lysine levels on performance and immune response of weanling pigs housed at two floor space allowances[J]. Journal of Animal Science, 1993, 71(3): 552-556.

[5] KONASHI S, TAKAHASHI K, AKIBA Y. Effect of dietary essential amino acid deficiency on immunological variables in broiler chickens[J]. British Journal of Nutrition, 2000, 83(4): 449-456.

[6] CHEN C, SANDER J, DALE N M. The effect of dietary lysine deficiency on the immune response to Newcastle disease vaccination in chickens[J]. Avian Diseases, 2003, 47(4): 13

[7] LIU Y. Study on Digestible Methionine and Lysine Requirements in Meat Duck Diets[D]. Master' s Thesis. Wuhan: Huazhong Agricultural University, 2009.

[8] BONS A, TIMMLER R, JEROCH H. Lysine requirement of growing male Pekin ducks[J]. British Poultry Science, 2002, 43(5): 677-686.

[9] CHEN D, YANG F, CHEN K, et al. Effect of different dietary protein types on small intestine morphology in piglets, increasing villus length and crypt depth[J]. Acta Zoonutrimenta Sinica, 1996, 8(2): 18-24.

[10] AVNI D, HENDRIKSMA H P, DAG A, et al. Nutritional aspects of honey bee-collected pollen and constraints on colony development in the eastern Mediterranean[J]. Journal of Insect Physiology, 2014, 69: 65-73.

[11] ALAUX C, DUCLOZ F, CRAUSER D, et al. Diet effects on honeybee immune competence[J]. Biology Letters, 2010, 6(4): 562-565.

[12] SHI Y. Effect of Nutrition and Space Factors on Female Honey Bee Development[D]. Master' s Thesis. Nanchang: Jiangxi Agricultural University, 2011: 1-20.

[13] CANFIELD L, CHYTIL F. Effect of low lysine diet on rat protein metabolism[J]. Journal of Nutrition, 1978, 108(8): 1343-1347.

[14] SATO T, ITO Y, NAGASAWA T. Regulation of skeletal muscle protein degradation and synthesis by oral administration of lysine in rats[J]. Journal of Nutrition Science and Vitaminology, 2013, 59(5): 412-419.

[15] HAUNERLAND N H. Insect storage proteins: gene families and receptors[J]. Insect Biochemistry and Molecular Biology, 1996, 26(8/9): 755-765.

[16] WANG Y. Insect Physiology[M]. Beijing: China Agriculture Press, 2004.

[17] MA H, ZHANG W, SONG W H, et al. Effects of tryptophan supplementation on cashmere fiber characteristics, serum tryptophan, and related hormone concentrations in cashmere goats[J]. Domestic Animal Endocrinology, 2012, 43(3): 239–250.

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