Effects of Dietary Cholesterol and Lecithin Levels and Surfactin Supplementation on the Intermolt Period of Litopenaeus vannamei Postprint

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Abstract

Two experiments were conducted to investigate the effects of dietary cholesterol and lecithin levels on the molting interval of Pacific white shrimp (Litopenaeus vannamei) and the effects of dietary surfactin supplementation on molting interval and hepatopancreas antioxidant capacity of L. vannamei. Experiment 1: One hundred shrimp with an average weight of (0.61$±0.02)gwererandomlydividedinto10groups(10replicatespergroup, 1shrimpperreplicate), includingacon
g were randomly divided into six groups (15 replicates per group, 1 shrimp per replicate). The control group was fed the test diet containing 0.4% cholesterol and 2.0% lecithin from Experiment 1, while five surfactin-supplemented groups were fed test diets supplemented with 10, 20, 40, 80, and 160 mg/kg surfactin based on the control diet. The 28-day trial investigated the effects of dietary surfactin on molting interval and hepatopancreas antioxidant capacity. The results showed that dietary cholesterol level significantly affected molting interval of L. vannamei (P<0.05), while lecithin level had no significant effect (P>0.05). There was a significant interaction between dietary cholesterol and lecithin levels on molting interval (P<0.05). The groups with 0.4% cholesterol + 1.0% lecithin, 0.4% cholesterol + 2.0% lecithin, and 0.6% cholesterol + 2.0% lecithin had significantly shorter molting intervals than other groups (P<0.05), with the 0.4% cholesterol + 2.0% lecithin group showing the shortest molting interval. Compared with the control group, only the 10 mg/kg surfactin group significantly shortened molting interval (P<0.05) and significantly increased hepatopancreas total antioxidant capacity (P<0.05). All surfactin-supplemented groups showed significantly higher hepatopancreas superoxide dismutase activity than the control group (P<0.05). The 10 and 20 mg/kg surfactin groups exhibited increased hepatopancreas catalase activity (P<0.05), while other surfactin groups showed significantly decreased catalase activity (P<0.05). Except for the 10 mg/kg surfactin group, all surfactin-supplemented groups had significantly higher hepatopancreas glutathione peroxidase activity

than the control group (P<0.05). Only the 10 and 20 mg/kg surfactin groups showed significantly lower hepatopancreas malondialdehyde levels than the control group (P<0.05). In conclusion, under the experimental conditions, dietary cholesterol level and its interaction with lecithin level significantly affected molting interval of L. vannamei, with 0.4% cholesterol and 2.0% lecithin resulting in the shortest molting interval. Supplementation of 10 mg/kg surfactin in the diet containing 0.4% cholesterol and 2.0% lecithin could shorten molting interval and improve hepatopancreas antioxidant capacity of L. vannamei.

Full Text

Effects of Dietary Cholesterol and Lecithin Levels and Surfactin Supplementation on Intermolt Period of Pacific White Shrimp (Litopenaeus vannamei)

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Abstract

Two experiments were conducted to investigate the effects of dietary cholesterol and lecithin levels on the intermolt period, and surfactin supplementation on the intermolt period and hepatopancreatic antioxidant capacity of Pacific white shrimp (*Litopenaeus vannamei*). In trial 1, one hundred shrimp with an average body weight of $(0.61\$\pm0.02)gwererandomlyallocatedinto10groups(10replicatespergroup,1shrimpperreplicate$
g were randomly divided into 6 groups (15 replicates per group, 1 shrimp per replicate). The control group received the diet containing 0.4% cholesterol and 2.0% lecithin from trial 1, while five surfactin groups were fed this basal diet supplemented with 10, 20, 40, 80, and 160 mg/kg surfactin. This 28-day trial evaluated surfactin supplementation effects on intermolt period and hepatopancreatic antioxidant capacity.

The results showed that dietary cholesterol level significantly affected the intermolt period (P<0.05), whereas lecithin level did not (P>0.05). However, the interaction between cholesterol and lecithin levels significantly influenced the intermolt period (P<0.05). Groups receiving 0.4% cholesterol with 1.0% lecithin, 0.4% cholesterol with 2.0% lecithin, and 0.6% cholesterol with 2.0% lecithin exhibited significantly shorter intermolt periods than other groups (P<0.05), with the 0.4% cholesterol and 2.0% lecithin group showing the shortest intermolt period.

Compared with the control, only the 10 mg/kg surfactin group showed a significantly shortened intermolt period (P<0.05) and significantly increased total antioxidant capacity in hepatopancreas (P<0.05). All surfactin-supplemented groups demonstrated significantly elevated superoxide dismutase activity in hepatopancreas relative to the control ($P<0.05$). The 10 and 20 mg/kg groups showed increased catalase activity ($P<0.05$), while other surfactin groups exhibited significantly decreased catalase activity ($P<0.05$). Except for the 10 mg/kg group, all surfactin groups displayed significantly higher glutathione peroxidase activity than the control ($P<0.05$). Only the 10 and 20 mg/kg groups showed significantly reduced malondialdehyde levels compared with the control ($P<0.05$).

In conclusion, under the present experimental conditions, dietary cholesterol level and its interaction with lecithin level significantly affected the intermolt period of Pacific white shrimp, with the shortest intermolt period observed at 0.4% cholesterol and 2.0% lecithin. Supplementation of 10 mg/kg surfactin in diets containing 0.4% cholesterol and 2.0% lecithin can shorten the intermolt period and enhance hepatopancreatic antioxidant capacity.

Keywords: cholesterol; lecithin; surfactin; Pacific white shrimp; intermolt period

Introduction

Cholesterol is an essential nutrient for many crustaceans, serving as a precursor for sex hormones, adrenal cortical hormones, and molting hormones. Lecithin plays a vital role in maintaining cellular structure and function, as well as in animal growth and differentiation. Crustaceans cannot synthesize cholesterol *de novo* and can only partially synthesize phospholipids, a capacity insufficient to meet the metabolic needs of larvae, thus requiring dietary supplementation. Previous studies have shown that dietary cholesterol significantly improves weight gain and survival in kuruma shrimp (*Penaeus japonicus*), while lecithin supplementation promotes growth in banana shrimp (*Penaeus merguiensis*), Pacific white shrimp (*Litopenaeus vannamei*), and black tiger shrimp (*Penaeus monodon*). Crustacean development is invariably accompanied by molting, and increased molting frequency can enhance growth. However, most previous research has focused on the individual effects of cholesterol or lecithin on crustacean molting, with few reports examining their combined dietary addition.

Dietary emulsifiers can promote further dispersion of lipids into emulsified microparticles, increasing the contact area between lipids and lipase or intestinal mucosal cells, thereby improving lipid digestion and absorption. Lecithin, besides its nutritional role, is added to feed as an emulsifier, though its emulsifying capacity is limited. Whether adding substances with stronger emulsifying capacity could improve lipid digestion and absorption in crustaceans and consequently promote molting warrants investigation. In recent years, surfactin, a lipopeptide produced through secondary metabolism by certain *Bacillus subtilis* strains, has attracted considerable attention due to its strong emulsifying capacity. Its molecular structure consists of a fatty acid chain with 13-15 carbon atoms and a peptide chain with 7 amino acid residues. The fatty acid chain and peptide residues L-Leu2, D-Leu3, L-Val4, D-Leu6, and L-Leu7 constitute the lipophilic groups, while the cyclic skeleton and two acidic amino acid residues (L-Glu1 and L-Asp5) form the hydrophilic groups. This structure allows surfactin to form micelles at low concentrations, exhibiting excellent emulsifying properties and ranking among the most potent biosurfactants known. Our previous research demonstrated that dietary surfactin supplementation promotes lipid digestion and absorption in fish and enhances liver antioxidant capacity. The hepatopancreatic antioxidant capacity of Pacific white shrimp decreases during the intermolt period, potentially causing physiological stress, making improvement of hepatopancreatic antioxidant capacity important for molting and growth. Whether dietary surfactin supplementation affects the intermolt period and hepatopancreatic antioxidant capacity in Pacific white shrimp remains unclear. Therefore, this study investigated the effects of different cholesterol and lecithin levels on the intermolt period, and changes in intermolt period and hepatopancreatic antioxidant capacity following surfactin supplementation at appropriate cholesterol and lecithin levels, aiming to provide a basis for nutritional regulation of molting in Pacific white shrimp.

1.1 Experimental Design

Trial 1 was conducted in the Ecology Laboratory of the Fisheries College at Jimei University. After a 2-week acclimation, 100 healthy, uniform-sized Pacific white shrimp with an average weight of (0.61$±$0.02) g were randomly divided into 10 groups with 10 replicates each (1 shrimp per replicate). Nine experimental groups (D1-D9) were fed diets supplemented with 0.2%, 0.4%, and 0.6% cholesterol combined with 1.0%, 2.0%, and 3.0% lecithin, while the control group (D10) received a basal diet without cholesterol or lecithin supplementation. The trial lasted 35 days.

Trial 2 was conducted at the Jimei University Aquaculture Experimental Station. Following a 2-week acclimation, 90 healthy, uniform-sized Pacific white shrimp with an average weight of (0.48$±$0.03) g were randomly divided into 6 groups with 15 replicates each (1 shrimp per replicate). The control group was fed the diet containing 0.4% cholesterol and 2.0% lecithin from trial 1, while five surfactin-supplemented groups received this basal diet with added surfactin at 10, 20, 40, 80, and 160 mg/kg. The trial lasted 28 days.

1.2 Feed Composition and Management

Experimental diets were formulated using fish meal, soybean meal, and wheat gluten meal as protein sources, and fish oil and soybean oil as lipid sources. Dietary composition and nutrient levels are presented in Table 1 . Feed ingredients were ground through a 60-mesh sieve, mixed using the stepwise expansion method, then combined with fish oil, soybean oil, and water before being extruded into 0.8 mm diameter pellets using a twin-screw extruder (CD4$×$1TS, South China University of Technology Science and Technology Industry General

Factory). The pellets were air-dried and stored in sealed bags at -20°C. Cholesterol and lecithin were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (analytical grade). Surfactin was provided by Fujian Zhengyuan Co., Ltd. with an effective content of 80%.

Table 1 Composition and nutrient levels of experimental diets (air-dry basis) %

The premix provided the following per kg of diet: $Ca(H_2PO_4)_2 \cdot H_2O$ *20,000 mg,* $NaH_2PO_4 \cdot 2H_2O$ *5,000 mg,* KH_2PO_4 *8,000 mg, calcium lactate 5,000 mg, KCl 1,000 mg, NaCl 1,000 mg,* $MgSO_4 \cdot 7H_2O$ *6,000 mg, ferric citrate 800 mg,* $CuSO_4 \cdot 5H_2O$ *24 mg,* $ZnSO_4 \cdot 7H_2O$ *190 mg,* $MnSO_4 \cdot 4H_2O$ *100 mg,* $CoSO_4 \cdot 7H_2O$ *50 mg, KI 8 mg,* Na_2SeO_3 *2 mg,* $Al(SO_4)_3 \cdot 18H_2O$ *25 mg, cellulose 30,030.6 mg, VA 10 mg, VD 10 mg, VC 2,000 mg, VK 40 mg, VE 500 mg,* VB_1 *60 mg,* VB_2 *70 mg,* VB_6 *80 mg,* VB_{12} *0.4 mg, nicotinic acid 200 mg, calcium pantothenate 200 mg, biotin 2 mg, inositol 500 mg, folic acid 8 mg, para-aminobenzoic acid sodium salt 90 mg, Ser 47.48 mg, Gly 359.80 mg, Val 288.49 mg, Met 81.86 mg, Leu 54.93 mg, Tyr 16.01 mg, Lys 173.71 mg, His 16.19 mg, choline chloride 50,000 mg, nucleic acid 10,000 mg, sodium alginate 10,000 mg, mold inhibitor 1,500 mg, antioxidant 500 mg.*

1.3 Feeding Management

During the acclimation period, shrimp were reared in 350 L circular tanks (diameter 100 cm, height 85 cm) and fed the basal diet three times daily, with regular siphoning of waste and water exchange. During the experimental period, shrimp were cultured in 2 L transparent plastic beakers with continuous aeration. They were fed to satiation at 08:30, 12:30, and 18:30 daily. After 0.5 h, residual feed and feces were siphoned out and approximately one-third of the water was exchanged. Experimental water was fresh seawater from the station with salinity of 24‰-28‰, pH 8.0-8.2, nitrite below 0.02 mg/L, and ammonia nitrogen below 0.2 mg/L, with continuous aeration. Lighting was provided by natural and fluorescent sources on a 12L:12D photoperiod. Molting was monitored every 2 h from 00:00-08:00 and every 4 h from 08:00-24:00 daily, with exuviae promptly removed and recorded.

1.4 Sample Collection and Tissue Homogenate Preparation

At the end of trial 2, shrimp were fasted for 24 h before sampling. Hepatopancreas was dissected, placed in cryovials, and stored at -80°C. For each sample, 0.1 g of hepatopancreatic tissue was homogenized with 0.86% cold physiological saline at a ratio of 1:9 (g/mL) using a homogenizer for 3-5 cycles. The homogenate was centrifuged at 3,000 r/min for 10 min at 4°C, and the supernatant was aliquoted for analysis.

1.5 Measurement Indicators and Methods

The intermolt period was defined as the time interval between two consecutive molts (in hours), calculated as the average interval of the first three molts occurring after the initial week of the experiment, following methods described by Long et al. and Guan et al. Hepatopancreatic total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GSH-Px) activity, and malondialdehyde (MDA) level were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.6 Statistical Analysis

Data were processed using Excel 2003 and expressed as mean±SD. Trial 1 data were analyzed by two-way ANOVA, while trial 2 data were analyzed by one-way ANOVA using SPSS 17.0 statistical software, with significance set at $P<0.05$.

2.1 Effects of Dietary Cholesterol and Lecithin Levels on Intermolt Period

As shown in Table 2 , dietary cholesterol level significantly affected the intermolt period of Pacific white shrimp ($P<0.05$), whereas lecithin level did not ($P>0.05$). The interaction between cholesterol and lecithin levels significantly influenced the intermolt period ($P<0.05$). The intermolt periods of groups D2, D5, and D6 were significantly shorter than those of other groups except D1 and D7 ($P<0.05$), with group D5 showing the shortest intermolt period.

Table 2 Effects of dietary cholesterol and lecithin levels on intermolt period of Pacific white shrimp (h)

In the same column, values with the same letter superscripts indicate no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05).

2.2 Effects of Surfactin Supplementation on Intermolt Period

Table 3 shows that compared with the control, only the 10 mg/kg surfactin group significantly shortened the intermolt period ($P<0.05$), while the 20, 40, 80, and 160 mg/kg groups showed no significant differences ($P>0.05$).

Table 3 Effects of surfactin supplementation on intermolt period of Pacific white shrimp (h)

In the same row, values with the same letter superscripts indicate no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05). The same applies below.

2.3 Effects of Surfactin Supplementation on Hepatopancreatic Antioxidant Capacity

Table 4 reveals that only the 10 mg/kg surfactin group exhibited significantly higher hepatopancreatic T-AOC than the control ($P<0.05$), while the 20, 40, 80, and 160 mg/kg groups showed no significant differences ($P>0.05$). All surfactin-supplemented groups displayed significantly increased SOD activity compared with the control ($P<0.05$), with no significant differences among surfactin groups. The 10 and 20 mg/kg groups showed significantly higher CAT activity ($P<0.05$), while other surfactin groups had significantly lower CAT activity than the control ($P<0.05$). Except for the 10 mg/kg group, all surfactin groups demonstrated significantly elevated GSH-Px activity compared with the control ($P<0.05$). Only the 10 and 20 mg/kg groups showed significantly reduced MDA levels ($P<0.05$), while the 40, 80, and 160 mg/kg groups did not differ significantly from the control ($P>0.05$).

Table 4 Effects of surfactin supplementation on antioxidant ability in hepatopancreas of Pacific white shrimp

3.1 Effects of Dietary Cholesterol and Lecithin Levels on Intermolt Period

This study demonstrated that dietary cholesterol significantly affected the intermolt period, with the shortest period observed at 0.4% cholesterol. These findings align with Tao et al. and Sheen, who reported that dietary cholesterol at 0.32% and 0.51% significantly improved molting frequency in Chinese mitten crab and mud crab, respectively. They also correspond with studies showing cholesterol requirements of 0.5% for banana shrimp and black tiger shrimp. Cholesterol cannot be synthesized from sterol precursors in crustaceans and must be obtained from the diet. After absorption, cholesterol is converted by the Y-organ first to 5β-diketol and then to ecdysteroids, which antagonize the molt-inhibiting hormone secreted by the X-organ-sinus gland complex in the eyestalk, thereby regulating molting cycle duration. The shortened intermolt period in this study may result from appropriate cholesterol levels promoting ecdysteroid synthesis while inhibiting molt-inhibiting hormone production, though the specific pathways require further investigation.

Appropriate dietary lecithin levels showed a trend toward shortening the intermolt period, with 2.0% being optimal, consistent with findings in banana shrimp by Thongrod et al. Wang et al. observed that 0.5% lecithin did not significantly affect molting frequency in swimming crab, while 1% and 2% levels showed increasing trends, similar to our results. This study also found a significant interaction between dietary cholesterol and lecithin levels, which has been observed in American lobster research showing cholesterol requirements of 0.5% without lecithin but only 0.25% with lecithin supplementation. This interaction may relate to lecithin's emulsifying effect enhancing intestinal cholesterol absorption. However, Briggs et al. found no significant interaction between cholesterol (0, 0.5%, 1.0%) and lecithin (0, 5%) on molting frequency in freshwater prawn, differing from our results possibly due to variations in shrimp species, experimental conditions, and nutrient levels. The mechanism underlying this interaction in Pacific white shrimp requires further investigation.

3.2 Effects of Surfactin Supplementation on Intermolt Period and Hepatopancreatic Antioxidant Capacity

In this study, 10 mg/kg surfactin supplementation shortened the intermolt period, likely by enhancing lipid digestion and absorption through emulsification, particularly promoting storage of molting-related cholesterol and lecithin. Differences between these results and trial 1 may relate to variations in initial molting timing, shrimp quality, and body weight between the two batches, requiring confirmation in future studies.

Under normal conditions, free radical production and elimination maintain equilibrium. SOD, CAT, and GSH-Px are crucial antioxidant enzymes that scavenge reactive oxygen species. T-AOC reflects overall antioxidant capacity, while higher MDA levels indicate greater lipid oxidative damage. This study showed that appropriate surfactin supplementation significantly increased SOD, CAT, GSH-Px activities and T-AOC while decreasing MDA levels, demonstrating enhanced hepatopancreatic antioxidant capacity. These results align with Li and Zhai et al., who reported improved antioxidant capacity in GIFT tilapia with 50 mg/kg surfactin, and Sun' s similar findings in orange-spotted grouper. The antioxidant effect may relate to surfactin' s aspartic and glutamic acid residues chelating Fe^{2+} and Cu^{2+}, reducing metal-catalyzed reactions and free radical generation, thereby improving hepatopancreatic health.

As surfactin supplementation increased, the intermolt period tended to lengthen while antioxidant capacity declined, possibly due to surfactin's emulsifying properties. Below the critical micelle concentration, emulsifying capacity increases with concentration, but stabilizes beyond this threshold, limiting further enhancement.

Conclusions

1. Under the present experimental conditions, dietary cholesterol level and its interaction with lecithin level significantly affected the intermolt period of Pacific white shrimp, with the shortest intermolt period achieved at 0.4% cholesterol and 2.0% lecithin.
2. Supplementation of 10 mg/kg surfactin in diets containing 0.4% cholesterol and 2.0% lecithin can shorten the intermolt period and improve hepatopancreatic antioxidant capacity in Pacific white shrimp.

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