# Effects of Dietary Chromium Source and Supplementation Level on Growth Performance, Serum Biochemical Indices, and Non-Specific Immune Enzyme Activity in Juvenile Litopenaeus vannamei (Postprint)

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## Abstract

This experiment aimed to investigate the effects of dietary chromium sources and supplementation levels on growth performance, serum biochemical indices, and non-specific immune enzyme activities in juvenile Pacific white shrimp (Litopenaeus vannamei), and to determine the optimal supplementation levels of three chromium sources in feed. A two-factor experimental design was adopted, with chromium sources being chromium chloride (CrCl3), chromium picolinate (Cr-Pic), and chromium methionine (Cr-Met), and chromium supplementation levels being 0, 0.3, 0.6, 0.9, 1.2, and 2.0 mg/kg, formulated into 16 experimental diets, which were fed to juvenile Pacific white shrimp for 8 weeks. A total of 1,920 juvenile Pacific white shrimp with an initial body weight of (0.895±0.001) g were selected and randomly divided into 16 groups, with 3 replicates per group and 40 shrimp per replicate. The results showed that chromium source, chromium supplementation level, and their interaction had significant effects on final body weight, weight gain rate, feed conversion ratio, and protein efficiency ratio of Pacific white shrimp ($P<0.05$). The final body weight and weight gain rate of shrimp in groups with chromium supplementation levels of 0.3–2.0 mg/kg were significantly higher than those in the group without chromium supplementation ($P<0.05$), and these indices reached maximum values when chromium was supplemented at 0.9 mg/kg in the form of Cr-Met. The CrCl3 and Cr-Met groups had the lowest feed conversion ratio at a chromium supplementation level of 0.9 mg/kg, but this did not differ significantly from that at 1.2 mg/kg ($P>0.05$); the Cr-Pic group had the lowest feed conversion ratio at a chromium supplementation level of 1.2 mg/kg. Under the three chromium sources, protein efficiency ratio reached maximum values at a chromium supplementation level of

0.9 mg/kg, but this did not differ significantly from that at 1.2 mg/kg (P>0.05). Chromium source, chromium supplementation level, and their interaction had significant effects on whole-body crude lipid and crude ash contents (P<0.05), but had no significant effects on whole-body crude protein and moisture contents (P>0.05). Chromium source, chromium supplementation level, and their interaction had significant effects on serum total protein, glucose, cholesterol, and triglyceride contents (P<0.05), with the highest serum total protein content and lowest serum glucose content observed when chromium was supplemented at 0.9 mg/kg in the form of Cr-Met. Chromium source, chromium supplementation level, and their interaction had significant effects on serum alkaline phosphatase, acid phosphatase, phenoloxidase, and total superoxide dismutase activities (P<0.05), with the highest serum phenoloxidase and total superoxide dismutase activities observed when chromium was supplemented at 0.9 mg/kg in the form of Cr-Met. Using weight gain rate as the evaluation index, the appropriate dietary chromium supplementation levels for CrCl3, Cr-Pic, and Cr-Met were determined to be 1.33, 1.27, and 1.04 mg/kg, respectively, through the broken-line model. Through comparison, Cr-Met had the highest relative bioavailability, followed by Cr-Pic, and CrCl3 had the lowest.

## Full Text

# Effects of Dietary Chromium Source and Supplemental Level on Growth Performance, Serum Biochemical Indices and Non-Specific Immune Enzyme Activities of Juvenile Litopenaeus vannamei

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**Abstract:** This experiment was conducted to investigate the effects of dietary chromium source and supplemental level on growth performance, serum biochemical indices, and non-specific immune enzyme activities of juvenile *Litopenaeus vannamei*, and to determine the optimal supplemental levels of three chromium sources in feed. A two-factor experimental design was employed with three chromium sources [chromium trichloride (CrCl ), chromium picolinate (Cr-Pic), and chromium methionine (Cr-Met)] and six supplemental levels (0, 0.3, 0.6, 0.9, 1.2, and 2.0 mg/kg), resulting in 16 experimental diets that were fed to juvenile *L. vannamei* for 8 weeks.

A total of 1,920 juvenile *L. vannamei* with an initial body weight of (0.895±0.001) g were randomly allocated into 16 groups with 3 replicates per group and 40 shrimps per replicate. The results showed that chromium source, supplemental level, and their interaction significantly affected final body weight (FBW), weight gain rate (WGR), feed conversion ratio (FCR), and protein effi-

ciency ratio (PER) of *L. vannamei* (P<0.05). The FBW and WGR of shrimps in groups supplemented with 0.3–2.0 mg/kg chromium were significantly higher than those in the group without chromium supplementation (P<0.05), with the Cr-Met group at 0.9 mg/kg chromium showing the maximum values for these parameters. The lowest FCR was observed in CrCl and Cr-Met groups at 0.9 mg/kg chromium, which did not differ significantly from the 1.2 mg/kg groups (P>0.05), while the Cr-Pic group showed the lowest FCR at 1.2 mg/kg chromium. The highest PER for all three chromium sources was achieved at 0.9 mg/kg chromium, though this was not significantly different from the 1.2 mg/kg groups (P>0.05). Chromium source, supplemental level, and their interaction significantly affected crude lipid and crude ash contents in whole body (P<0.05), but had no significant effects on moisture or crude protein contents (P>0.05). Serum total protein, glucose, cholesterol, and triglyceride contents were significantly affected by chromium source, supplemental level, and their interaction (P<0.05). The highest serum total protein content and lowest serum glucose content were observed in the Cr-Met group supplemented with 0.9 mg/kg chromium. The activities of alkaline phosphatase (AKP), acid phosphatase (ACP), phenoloxidase (PO), and total superoxide dismutase (T-SOD) in serum were significantly affected by chromium source, supplemental level, and their interaction (P<0.05), with the highest serum PO and T-SOD activities found in the Cr-Met group at 0.9 mg/kg chromium. Using WGR as the evaluation index and applying broken-line model analysis, the optimal dietary chromium supplemental levels were determined to be 1.33, 1.27, and 1.04 mg/kg for CrCl , Cr-Pic, and Cr-Met, respectively. Comparative analysis revealed that Cr-Met had the highest relative bioavailability, followed by Cr-Pic, with CrCl showing the lowest.

**Keywords:** *Litopenaeus vannamei*; chromium; growth performance; serum biochemical indices; non-specific immune enzyme activities

## Introduction

Chromium (Cr) is an essential trace element for animals and a vital active component of glucose tolerance factor (GTF). Through GTF, chromium synergizes with insulin to influence carbohydrate, lipid, protein, and nucleic acid metabolism, thereby affecting animal growth, immunity, reproduction, carcass quality, stress reduction, immune function improvement, and overall production performance and reproductive capacity [1].

Chromium nutrition was initially studied primarily in livestock and poultry, but recent research in aquaculture has yielded promising results, particularly in fish species. Studies have demonstrated that dietary chromium supplementation can promote growth and feed utilization in various aquatic animals, including common carp (*Cyprinus carpio* Linnaeus) [2], grass carp (*Ctenopharyngodon idellus*) [3-4], Chinese mitten crab (*Eriocheir sinensis*) [5], Nile tilapia × blue tilapia hy-

brid (*Oreochromis niloticus* × *O. aureus*) [6], GIFT tilapia (*Oreochromis niloticus*) [7], and blunt snout bream (*Megalobrama amblycephala*) [8]. However, research findings vary considerably among different species. Studies on chromium requirements in Pacific white shrimp (*Litopenaeus vannamei*) have primarily focused on chromium picolinate (Cr-Pic) as the chromium source. Yang et al. [9] reported that the dietary chromium requirement for *L. vannamei* ranges from 1.2 to 1.6 mg/kg when supplemented as Cr-Pic.

Chromium absorption is highly dependent on its valence state and chemical form. Mertz et al. [10] suggested that organic chromium is more readily absorbed than inorganic chromium, with absorption rates of 25–30%. Chromium trichloride (CrCl ), an inorganic chromium source, is chemically stable and resistant to hydrolysis, serving as an important raw material for synthesizing other chromium salts and constituting a major component of feed additives. Both Cr-Pic and chromium methionine (Cr-Met) are chelates of trivalent chromium ($Cr^3$ ) with picolinic acid and methionine, respectively, which can alleviate antagonistic competition among mineral elements and facilitate chromium absorption. This study aims to compare the effects of three different chromium forms—inorganic chromium, organic acid chromium, and amino acid chelated chromium—at various supplemental levels on growth performance, serum biochemical indices, and immunity of *L. vannamei*, the most economically important crustacean species in Chinese aquaculture, thereby providing a theoretical basis for chromium application in shrimp formulated feeds.

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## Materials and Methods

### 1.1 Experimental Diets

A basal diet was formulated using casein and fish meal as protein sources, and fish oil, corn oil, and lecithin as lipid sources (Table 1 ). Based on this basal diet, a two-factor experimental design was employed with three chromium sources [CrCl (provided by Foshan Haina Chemical Co., Ltd.), Cr-Pic (provided by Mianyang Xinyimei Chemical Co., Ltd., containing 0.1% $Cr^3$ ), and Cr-Met (provided by Guangzhou Tianke Technology Co., Ltd., containing 0.1% $Cr^3$ )] at six supplemental levels (0, 0.3, 0.6, 0.9, 1.2, and 2.0 mg/kg), resulting in 16 experimental diets. All feed ingredients were ground to pass through an 80-mesh sieve. The three chromium sources were first diluted with zeolite powder to achieve a $Cr^3$ concentration of 0.1%, then weighed according to the required supplemental levels for each treatment group and mixed thoroughly with other micro-components in the feed formulation using a stepwise expansion method. The complete feed ingredients were blended in a V-type mixer, after which approximately 30% water was added and mixed again using a blender. The mixture was then pelleted into two particle sizes (1.0 and 1.5 mm diameter), conditioned at 60 °C for 30 minutes, air-dried, sealed in plastic bags, and stored at -20 °C until use.

### 1.2 Experimental Animals and Culture Management

Juvenile shrimp were purchased from Zhanjiang Zhonglian Aquaculture Co., Ltd. and acclimated for a period during which they were fed the basal diet three times daily to satiation. Prior to the experiment, 1,920 healthy juveniles with uniform size and an initial body weight of (0.897±0.001) g were selected and randomly distributed into 16 groups, with 3 replicates per group and 40 shrimps per replicate. Each replicate was cultured in a 0.3 $m^3$ fiberglass tank. Shrimp were fed at 8–10% of body weight daily, divided into four feedings at 07:00, 11:00, 17:00, and 21:00 hours, with each meal provided to near satiation (consumed within 30 minutes). Seawater used in the experiment was settled and filtered. During weeks 1–4, water was exchanged every two days, while during weeks 5–8, daily water exchange was implemented at 30–50% of the total volume. Daily observations were made regarding feeding, molting, and growth, with feed consumption recorded. The experiment was conducted at the indoor aquaculture system of Donghai Island Marine Biology Research Base, Guangdong Ocean University, following the methodology of Yang et al. [9]. Throughout the experimental period, continuous aeration was maintained with water temperature at 28–31 °C, dissolved oxygen concentration >7.0 mg/L, salinity at 26–28, pH at 7.8–8.2, and ammonia nitrogen concentration <0.03 mg/L. The experiment lasted for 8 weeks, with feeding ceased 24 hours before final weighing and counting.

### 1.3 Sample Collection

At the conclusion of the experiment, all shrimps in each tank were weighed and counted for calculation of survival rate, weight gain rate, and other growth parameters. Ten shrimps were randomly selected from each tank and hemolymph was collected from the pericardial cavity using a 1 mL sterile syringe. The hemolymph was placed in 1.5 mL centrifuge tubes, allowed to stand overnight at 4 °C, then centrifuged at 8,000 r/min for 10 minutes at 4 °C. The supernatant was collected, aliquoted, and stored at -80 °C for serum biochemical analysis. An additional five shrimps per tank were randomly selected and stored at -20 °C for whole-body composition analysis.

### 1.4 Analytical Methods

**1.4.1 Whole-Body Composition Analysis** Whole-body composition of feed and shrimp samples, including moisture, crude protein, crude lipid, and crude ash, was determined according to AOAC (1995) methods [11]. Moisture content was measured by drying to constant weight in an oven at 105 °C. Crude protein content was determined using the Kjeldahl method (KjeltecTM 8400, Sweden). Crude lipid content was measured by Soxhlet extraction using ether as the solvent. Crude ash content was determined by incineration in a muffle furnace at 550 °C.

**1.4.2 Serum Biochemical Indices Determination** Serum total protein, glucose, triglyceride, and cholesterol concentrations were measured using a Hitachi 7020 automatic biochemical analyzer with reagents purchased from Wittman (Nanjing) Biotechnology Co., Ltd.

**1.4.3 Serum Non-Specific Immune Enzyme Activity Assay** The activities of total superoxide dismutase (T-SOD), acid phosphatase (ACP), and alkaline phosphatase (AKP) in serum were determined using assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s instructions. Serum phenoloxidase (PO) activity was measured according to the methods of Wang et al. [12] and Huang et al. [13].

### 1.5 Growth Performance Calculations

The following formulas were used to calculate growth performance indices: Survival rate (%) = 100 × (final shrimp number / initial shrimp number); Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight; Feed conversion ratio (FCR) = feed intake / (final body weight - initial body weight); Protein efficiency ratio (PER) = (final body weight - initial body weight) / protein intake.

### 1.6 Statistical Analysis

Results are presented as mean ± standard deviation. Data were analyzed using two-way ANOVA with SPSS 17.0 statistical software. When significant differences were detected, Duncan' s multiple range test was applied to examine differences among groups, with $P<0.05$ considered statistically significant. The optimal dietary chromium supplemental level was determined through broken-line model regression analysis, and the relative bioavailability of CrCl , Cr-Pic, and Cr-Met for *L. vannamei* was compared using the slope ratio below the breakpoint.

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## Results

### 2.1 Effects of Dietary Chromium Source and Supplemental Level on Growth Performance of Juvenile *L. vannamei*

As shown in Table 3 , chromium source and supplemental level significantly affected final body weight (FBW), weight gain rate (WGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of *L. vannamei* ($P<0.05$), with significant interactions between these two factors ($P<0.05$). Chromium source significantly affected survival rate ($P<0.05$), whereas supplemental level did not ($P>0.05$), and no interaction was observed between source and level for this parameter ($P>0.05$).

Final body weight: For CrCl  and Cr-Met sources, the 0.9 and 1.2 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Pic, the 1.2 mg/kg group was significantly higher than all other groups (P<0.05). At supplemental levels of 0.3, 0.6, and 0.9 mg/kg, the Cr-Met group was significantly higher than CrCl  and Cr-Pic groups (P<0.05), while at 1.2 and 2.0 mg/kg, the CrCl  group was significantly lower than Cr-Pic and Cr-Met groups (P<0.05).

Weight gain rate: For CrCl  and Cr-Met, the 0.9 and 1.2 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Pic, no significant difference was observed between 1.2 and 2.0 mg/kg groups (P>0.05), but both were significantly higher than remaining groups (P<0.05). At 0.6 and 0.9 mg/kg, the Cr-Met group was significantly higher than CrCl  and Cr-Pic groups (P<0.05), while at 1.2 and 2.0 mg/kg, the CrCl  group was significantly lower than Cr-Pic and Cr-Met groups (P<0.05).

Survival rate: The Cr-Pic group exhibited significantly higher survival than CrCl  and Cr-Met groups (P<0.05).

Feed conversion ratio: For CrCl , the 0.9 mg/kg group showed the lowest FCR, with 0 and 0.3 mg/kg groups being significantly higher than 0.9 and 1.2 mg/kg groups, while other groups showed no significant differences (P>0.05). For Cr-Pic, the 1.2 mg/kg group had the lowest FCR and the 0 mg/kg group the highest, with significant differences among all groups except between 0.3 and 0.6 mg/kg (P>0.05). For Cr-Met, the 0.9 mg/kg group showed the lowest FCR and the 0 mg/kg group the highest, with the 0.9 mg/kg group being significantly lower than all groups except 0.6 and 1.2 mg/kg (P<0.05). At 0.6 and 0.9 mg/kg, the Cr-Met group was significantly lower than CrCl  and Cr-Pic groups (P<0.05), while at 1.2 and 2.0 mg/kg, the CrCl  group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05).

Protein efficiency ratio: For CrCl , the 1.2 mg/kg group showed the highest PER and the 0 mg/kg group the lowest, with the 2.0 mg/kg group being significantly higher than all other groups (P<0.05). For Cr-Pic and Cr-Met, the 0.9 mg/kg group showed the highest PER, followed by the 1.2 mg/kg group, with the 0 mg/kg group being the lowest, and both 0.9 and 1.2 mg/kg groups being significantly higher than 0 and 0.3 mg/kg groups (P<0.05). Across all supplemental levels, the CrCl  group was significantly lower than Cr-Pic and Cr-Met groups (P<0.05).

**Table 3** Effects of dietary chromium source and supplemental level on growth performance of juvenile *Litopenaeus vannamei* (n=3)

### 2.2 Effects of Dietary Chromium Source and Supplemental Level on Whole-Body Composition of Juvenile *L. vannamei*

As shown in Table 4 , chromium source and supplemental level significantly affected crude lipid and crude ash contents in whole body (P<0.05), with significant interactions between these factors (P<0.05). However, no significant

effects were observed on moisture or crude protein contents (P>0.05), and no interactions were detected for these parameters (P>0.05).

Crude lipid content: For CrCl , the 1.2 mg/kg group was significantly lower than all other groups (P<0.05). For Cr-Pic and Cr-Met, the 0.9 and 1.2 mg/kg groups were significantly lower than remaining groups (P<0.05). At 0.6 mg/kg, the Cr-Met group was significantly lower than CrCl and Cr-Pic groups (P<0.05), while at 0.9 mg/kg, the CrCl group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05).

Crude ash content: For CrCl , the 0 mg/kg group was significantly lower than all other groups (P<0.05). For Cr-Pic, the 0 and 0.3 mg/kg groups were significantly lower than remaining groups (P<0.05). For Cr-Met, the 0.9 mg/kg group showed the highest value, significantly exceeding all other groups (P<0.05). At supplemental levels of 0.6, 0.9, 1.2, and 2.0 mg/kg, the Cr-Pic group was significantly higher than CrCl and Cr-Met groups (P<0.05).

**Table 4** Effects of dietary chromium source and supplemental level on body composition of juvenile *Litopenaeus vannamei* (DM basis, n=3)

### 2.3 Effects of Dietary Chromium Source and Supplemental Level on Serum Biochemical Indices of Juvenile *L. vannamei*

As shown in Table 5 , chromium source and supplemental level significantly affected serum total protein, glucose, cholesterol, and triglyceride contents (P<0.05), with significant interactions between these factors (P<0.05).

Total protein content: For CrCl and Cr-Pic, the 0.9, 1.2, and 2.0 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Met, the 0.9 and 1.2 mg/kg groups were significantly higher than remaining groups (P<0.05). At 0.6, 0.9, and 1.2 mg/kg, the Cr-Met group was significantly higher than CrCl and Cr-Pic groups (P<0.05), while at 2.0 mg/kg, the CrCl group was significantly lower than Cr-Pic and Cr-Met groups (P<0.05).

Glucose content: For CrCl and Cr-Pic, the 0 and 0.3 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Met, the 0 mg/kg group was significantly higher than all other groups (P<0.05). Across all supplemental levels, the Cr-Met group was significantly lower than CrCl and Cr-Pic groups (P<0.05).

Cholesterol content: For CrCl , the 0 mg/kg group was significantly higher than all other groups (P<0.05). For Cr-Pic, the 0.3 mg/kg group was significantly higher than remaining groups (P<0.05). For Cr-Met, the 0 and 0.3 mg/kg groups were significantly higher than other groups (P<0.05). At 0.3 mg/kg, the Cr-Met group was significantly higher than CrCl and Cr-Pic groups (P<0.05), while at 0.9 and 2.0 mg/kg, the CrCl group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05).

Triglyceride content: For CrCl , the 0 and 0.3 mg/kg groups were significantly

lower than other groups (P<0.05). For Cr-Pic, the 2.0 mg/kg group was significantly higher than all other groups (P<0.05). For Cr-Met, the 1.2 and 2.0 mg/kg groups were significantly higher than remaining groups (P<0.05). At 0.6 mg/kg, the CrCl group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05), while at 1.2 and 2.0 mg/kg, the Cr-Met group was significantly higher than CrCl and Cr-Pic groups (P<0.05).

**Table 5** Effects of dietary chromium source and supplemental level on serum biochemical indices of juvenile *Litopenaeus vannamei* (n=3)

### 2.4 Effects of Dietary Chromium Source and Supplemental Level on Serum Non-Specific Immune Enzyme Activities of Juvenile *L. vannamei*

As shown in Table 6 , chromium source and supplemental level significantly affected serum phenoloxidase (PO), total superoxide dismutase (T-SOD), alkaline phosphatase (AKP), and acid phosphatase (ACP) activities (P<0.05), with significant interactions between these factors (P<0.05).

Phenoloxidase activity: For CrCl , the 0.9, 1.2, and 2.0 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Pic, the 0.9 and 1.2 mg/kg groups were significantly higher than remaining groups (P<0.05). For Cr-Met, the 0.6 and 0.9 mg/kg groups were significantly higher than other groups (P<0.05). Across all supplemental levels, the Cr-Met group was significantly higher than CrCl and Cr-Pic groups (P<0.05).

Total superoxide dismutase activity: For CrCl , the 1.2 mg/kg group was significantly higher than all other groups (P<0.05). For Cr-Pic and Cr-Met, the 0.9 mg/kg group was significantly higher than remaining groups (P<0.05). At 0.3 and 0.9 mg/kg, the Cr-Met group was significantly higher than CrCl and Cr-Pic groups (P<0.05), while at 1.2 mg/kg, the CrCl group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05).

Alkaline phosphatase activity: For CrCl , the 0.9, 1.2, and 2.0 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Pic, the 1.2 mg/kg group was significantly higher than all other groups (P<0.05). For Cr-Met, the 1.2 and 2.0 mg/kg groups were significantly higher than all groups except the 0.9 mg/kg group (P<0.05). At 0.3 and 1.2 mg/kg, the CrCl group was significantly lower than Cr-Pic and Cr-Met groups (P<0.05), while at 0.9 mg/kg, the Cr-Pic group was significantly lower than CrCl and Cr-Met groups (P<0.05).

Acid phosphatase activity: For CrCl and Cr-Pic, the 0.9 and 1.2 mg/kg groups were significantly higher than other groups (P<0.05). For Cr-Met, the 1.2 mg/kg group was significantly higher than all other groups (P<0.05). At 0.3 mg/kg, the Cr-Pic group was significantly higher than CrCl and Cr-Met groups (P<0.05). At 0.6 and 2.0 mg/kg, the Cr-Pic group was significantly lower than CrCl and Cr-Met groups (P<0.05), while at 1.2 mg/kg, the CrCl group was significantly higher than Cr-Pic and Cr-Met groups (P<0.05).

**Table 6** Effects of dietary chromium source and supplemental level on serum non-specific immune enzyme activities of juvenile *Litopenaeus vannamei* (n=3)

### 2.5 Comparison of Relative Bioavailability of Three Chromium Sources

As shown in Table 7 and Figure 1 [Figure 1: see original paper], using weight gain rate as the evaluation index, broken-line model analysis determined the optimal dietary chromium supplemental levels to be 1.33, 1.27, and 1.04 mg/kg for CrCl , Cr-Pic, and Cr-Met, respectively. When CrCl was used as the standard, the relative bioavailability values of Cr-Pic and Cr-Met were 124.20% and 184.32%, respectively.

**Table 7** Relative bioavailability of Cr-Pic and Cr-Met for WGR as an evaluation index

**Figure 1** Relationship of dietary chromium supplemental level and WGR of juvenile *Litopenaeus vannamei*

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## Discussion

### 3.1 Effects of Chromium Source and Supplemental Level on Growth Performance of Juvenile *L. vannamei*

Chromium is an essential trace element for animals, and dietary chromium supplementation has been shown to enhance growth performance in aquatic species. As the active component of glucose tolerance factor (GTF), $Cr^3$ synergizes with insulin [14] to participate in the metabolism of the three major nutrients [15], thereby promoting growth and weight gain [16].

Limited research exists on the effects of dietary chromium on shrimp growth performance, though numerous studies have been conducted in fish species. Previous investigations have reported that dietary supplementation with 1.2–1.6 mg/kg Cr-Pic improved growth and feed utilization in *L. vannamei* [9], while 2.0% Cr-Pic enhanced growth and feed utilization in Nile tilapia × blue tilapia hybrids [6]. Additionally, supplementation with 1.7 mg/kg chromium from chromium nicotinate, chromium glycinate, or Cr-Met promoted growth and reduced feed conversion ratio in the same tilapia species [17]. Chromium picolinate at 0.8 mg/kg significantly improved growth and feed utilization in GIFT tilapia [7], enhanced glucose tolerance and promoted growth in grass carp [3], and improved growth performance in blunt snout bream at 600 g/kg [8].

The present study demonstrated that supplementation with 1.2 mg/kg chromium as CrCl or Cr-Met, and 0.9 mg/kg chromium as Cr-Met, significantly improved weight gain rate, survival rate, and protein efficiency ratio while reducing feed conversion ratio in *L. vannamei*. These findings align

with previous research, confirming that appropriate dietary chromium supplementation enhances animal growth and feed utilization, with efficacy varying among chromium forms. The complexity of factors influencing trace element absorption and utilization in animals extends beyond digestive physiology to include molecular structure, molecular weight, and solubility of different mineral forms. Current understanding of trace element absorption mechanisms in crustaceans remains limited, necessitating further research to address these knowledge gaps and optimize practical application technologies.

### 3.2 Effects of Chromium Source and Supplemental Level on Whole-Body Composition of Juvenile *L. vannamei*

In this study, no significant differences were observed in whole-body moisture or crude protein contents among treatments, consistent with findings in tilapia [18] and grass carp [3]. However, research on chromium effects on crude lipid and ash contents has yielded variable results. Xi et al. [19] reported reduced fat deposition in growing-finishing pigs fed chromium-supplemented diets due to decreased lipogenic enzyme activity. Liu et al. [3] found no significant changes in whole-body crude lipid content but significantly higher ash content in grass carp fed chromium-supplemented diets. Yang et al. [9] observed decreasing trends in whole-body crude lipid and increasing trends in ash content with increasing Cr-Pic levels in *L. vannamei*. Cui [20] reported reduced body fat content in juvenile Jian carp supplemented with Cr-Pic.

The present study demonstrated a decreasing trend in whole-body crude lipid content with increasing chromium levels across all three sources, corroborating the findings of Xi et al. [19], Yang et al. [9], and Cui [20]. This effect may be attributed to chromium' s regulatory role in insulin function and lipid metabolism. Whole-body ash content increased with chromium supplementation across all sources, consistent with Liu et al. [3] and Yang et al. [9]. Variations in research outcomes regarding chromium effects on body composition may be attributed to differences in species, size, feed composition, and culture environment, warranting further investigation.

### 3.3 Effects of Chromium Source and Supplemental Level on Serum Biochemical Indices of Juvenile *L. vannamei*

Serum glucose content decreased with increasing chromium supplementation across all three sources in this study, consistent with reports by Yang et al. [7], Liu et al. [3], and Cai et al. [21]. Cai et al. [21] demonstrated that dietary chromium significantly improved glucose tolerance in gibel carp fed glucose-based diets and showed some beneficial effects in starch-based groups. Liu et al. [3] reported significantly enhanced glucose tolerance in grass carp fed 0.8 mg/kg Cr-Pic. Yang et al. [7] found that 0.8 mg/kg Cr-Pic significantly reduced serum glucose in GIFT tilapia. As the primary component of GTF, chromium increases insulin receptor numbers on cell surfaces, promotes insulin-receptor binding, and stimulates cellular glucose uptake, thereby reducing blood glucose

levels [22]. While chromium' s glucose-regulating effects have been extensively studied in humans and terrestrial animals, further research is needed in aquatic species.

Serum total protein content reflects dietary protein nutritional value and animal protein digestion and absorption capacity, correlating with protein synthesis and nitrogen deposition [23]. Amoikon et al. [24] suggested that chromium-enhanced insulin function promotes amino acid uptake into cells, thereby stimulating protein synthesis. In this study, serum total protein increased with chromium supplementation, with the highest values observed in the 0.9 and 1.2 mg/kg Cr-Met groups. This may be attributed to increased essential amino acid content, optimized essential amino acid ratios, and enhanced $Cr^3$ absorption associated with amino acid chelate supplementation. These results are generally consistent with Wu et al. [25] and Yang et al. [9], though Lin et al. [26] reported lower serum total protein in chromium-supplemented common carp, possibly due to enhanced conversion of amino acids to tissue proteins, reducing serum levels. The underlying mechanisms require further investigation.

Chromium maintains normal blood cholesterol levels [27], influences hepatic lipid and cholesterol synthesis and clearance, and promotes lipid redistribution. Livestock studies have shown that organic chromium supplementation reduces serum cholesterol [28] while increasing triglyceride levels [29]. Liu [30] reported that Cr-Pic and chromium nicotinate significantly reduced serum cholesterol and increased triglycerides in grass carp. Yang et al. [9] found that Cr-Pic reduced serum cholesterol without significantly affecting triglycerides in *L. vannamei*, while Lin et al. [26] confirmed cholesterol-lowering effects of chromium. The present study demonstrated cholesterol reduction and triglyceride elevation in *L. vannamei*, consistent with Liu [30]. Reduced serum cholesterol may result from both decreased accumulation and enhanced mobilization of deposited cholesterol from the aorta [31]. The mechanisms underlying chromium effects on serum triglycerides remain unclear, with some suggesting enhanced -oxidation reduces triglyceride synthesis [25], while Mertz et al. [10] proposed that GTF-enhanced insulin promotes glucose conversion to glycogen and triglycerides rather than fat deposition, potentially explaining the observed triglyceride increase. Further investigation is needed to clarify chromium' s effects on lipid metabolism.

### 3.4 Effects of Chromium Source and Supplemental Level on Serum Non-Specific Immune Enzyme Activities of Juvenile *L. vannamei*

Chromium not only promotes animal growth but also enhances immune function. This study demonstrated that appropriate dietary chromium supplementation significantly increased serum AKP, ACP, PO, and T-SOD activities.

Alkaline phosphatase is a non-specific membrane-bound enzyme widely distributed in animal tissues, participating in phosphate transport and metabolism and skeletal mineralization in aquatic animals [32]. Acid phosphatase, a lysosomal marker enzyme in macrophages, catalyzes phosphate monoester hydrolysis

and participates in phosphate transfer and metabolism [33]. This study showed that all three chromium sources significantly increased AKP and ACP activities in *L. vannamei* serum, with activities plateauing as chromium levels increased, consistent with Zhou et al. [34] and Yang et al. [9].

Phenoloxidase is a crucial defense enzyme involved in foreign substance recognition and sensitive reflection of immune status [35-36]. Superoxide dismutase scavenges free radicals and catalyzes dismutation of superoxide radicals to peroxides and oxygen [37]. This study demonstrated that all chromium sources significantly enhanced serum PO and T-SOD activities. The highest PO activity was observed at 0.9 mg/kg for CrCl and Cr-Met, and at 1.2 mg/kg for Cr-Pic. Maximum T-SOD activity occurred at 1.2 mg/kg for CrCl and at 0.9 mg/kg for Cr-Pic and Cr-Met. Yang et al. [9] reported peak PO and T-SOD activities at 1.6 mg/kg Cr-Pic in *L. vannamei*, while Yang et al. [5] found no significant effects of chromium on PO activity in Chinese mitten crab. These discrepancies may be attributed to variations in species, size, chromium source, form, and supplemental level, requiring further investigation.

Using weight gain rate as the evaluation index, broken-line model analysis determined optimal dietary chromium supplemental levels of 1.33, 1.27, and 1.04 mg/kg for CrCl , Cr-Pic, and Cr-Met, respectively. Comparative analysis revealed that Cr-Met exhibited the highest relative bioavailability, followed by Cr-Pic, with CrCl showing the lowest.

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