

Effects of Dietary Zinc Content on Growth Performance, Body Composition, Zinc Content in Coelomic Fluid, and Alkaline Phosphatase Activity in Juvenile *Sipunculus nudus* (Postprint)

Authors: Xu Mingzhu, Zhang Qin, Tong Tong, Dong Lanfang, Yang Jialin, Jiang Yan, Huang Guoqiang

Date: 2017-10-10T00:00:00+00:00

Abstract

Using zinc methionine as the zinc source, six experimental diets with actual zinc contents of 9.3, 31.7, 49.9, 90.1, 168.6, and 326.5 mg/kg were formulated by supplementing different levels of zinc to an isonitrogenous and isoenergetic basal diet. These diets were fed to juvenile *Sipunculus nudus* with an average body weight of (14.54±0.10) mg for 8 weeks to investigate the effects of dietary zinc content on growth, body composition, coelomic fluid zinc content, and alkaline phosphatase activity. Each experimental diet had three replicates, with 400 juvenile *Sipunculus nudus* per replicate. The results showed that dietary zinc content significantly affected the survival rate, weight gain rate, and specific growth rate of *Sipunculus nudus* ($P<0.05$). Both weight gain rate and specific growth rate reached their maximum values at a dietary zinc content of 49.9 mg/kg, which were significantly higher than those of the groups with the lowest (9.3 mg/kg) and highest (326.5 mg/kg) dietary zinc levels ($P<0.05$). Dietary zinc content significantly affected the crude lipid and crude ash contents of the worms ($P<0.05$). The crude lipid content exhibited a trend of initially increasing and then decreasing with increasing dietary zinc content, while the crude ash content showed the opposite trend. Dietary zinc content had no significant effect on the moisture and crude protein contents of the worms ($P>0.05$). Dietary zinc content significantly affected both the zinc content and alkaline phosphatase activity in the coelomic fluid of the worms ($P<0.05$). The zinc content in the coelomic fluid continuously increased with increasing dietary zinc content, reaching its maximum value at a dietary zinc content of 326.5 mg/kg. In contrast, the alkaline phosphatase activity in the coelomic fluid initially increased and then stabilized with increasing dietary zinc content, reaching its maximum value at a dietary zinc content of 49.9 mg/kg. Using weight gain

rate as the evaluation criterion, regression analysis determined that the optimal dietary zinc content for juvenile *Sipunculus nudus* was 41.93 mg/kg.

Full Text

Effects of Dietary Zinc Content on Growth Performance, Body Composition, Coelomic Fluid Zinc Content and Alkaline Phosphatase Activity of Juvenile Peanut Worm, *Sipunculus nudus* Linnaeus

XU Mingzhu, ZHANG Qin*, TONG Tong, DONG Lanfang, YANG Jialin, JIANG Yan, HUANG Guoqiang

(Guangxi Institute of Oceanology, Key Laboratory of Marine Biotechnology of Guangxi, Beihai 536000, China)

Abstract: This study investigated the effects of dietary zinc content on growth performance, body composition, coelomic fluid zinc content, and alkaline phosphatase (AKP) activity in juvenile peanut worm (*Sipunculus nudus*). Six isonitrogenous and isoenergetic experimental diets were formulated using zinc methionine as the zinc source, with measured zinc concentrations of 9.3, 31.7, 49.9, 90.1, 168.6, and 326.5 mg/kg. Juvenile peanut worms with an average initial weight of (14.54±\$0.10) mg were fed these diets for 8 weeks. Each dietary treatment had three replicates, with 400 juvenile worms per replicate. The results demonstrated that dietary zinc content significantly affected survival rate (SR), weight gain rate (WGR), and specific growth rate (SGR) ($P < 0.05$). Both WGR and SGR reached their maximum values at a dietary zinc level of 49.9 mg/kg, which were significantly higher than those in the lowest (9.3 mg/kg) and highest (326.5 mg/kg) zinc groups ($P < 0.05$). Dietary zinc content also significantly influenced crude lipid and ash contents ($P < 0.05$). Body crude lipid content initially increased then decreased with rising dietary zinc levels, while ash content showed the opposite trend. No significant differences were observed in moisture or crude protein contents among all groups ($P > 0.05$). Coelomic fluid zinc content and AKP activity were significantly affected by dietary zinc content ($P < 0.05$). Coelomic fluid zinc content continuously increased with dietary zinc supplementation, reaching its highest value at 326.5 mg/kg. In contrast, coelomic fluid AKP activity initially increased then plateaued, with the maximum activity observed at 49.9 mg/kg dietary zinc. Using WGR as the evaluation criterion, regression analysis determined that the optimal dietary zinc content for juvenile peanut worm was 41.93 mg/kg.

Keywords: juvenile peanut worm; zinc; growth performance; body composition; coelomic fluid zinc content; AKP activity

Introduction

Trace elements can be classified as essential or non-essential, both serving as indispensable nutrients that play irreplaceable roles in animal physiology and biochemistry. Although present in minute quantities, trace elements directly or indirectly participate in nearly all physiological and biochemical processes within the organism. Zinc is an essential trace element for most animals, often referred to as the “element of life” [1-2], with extensive physiological and biochemical functions. First, zinc participates in protein metabolism as a component or activator of numerous enzymes, including carbonic anhydrase, carboxypeptidase, alkaline phosphatase (AKP), and lactate dehydrogenase. By the 1990s, over 250 zinc-containing enzymes had been identified, which participate in energy metabolism, protein synthesis, and redox reactions through various enzymes such as carbonic anhydrase, lactate dehydrogenase, glutamate dehydrogenase, and DNA polymerase. Second, zinc is involved in immune regulation, playing a crucial role in modulating antioxidant capacity and immune function. Zinc deficiency can impair immune function and disease resistance [3-4]. Therefore, determining the optimal zinc content in artificial feed formulations is of great significance.

The peanut worm (*Sipunculus nudus*), commonly known as “sandworm,” is a characteristic economic seafood product in Guangxi, prized by consumers for its high protein, low fat, and rich nutritional profile [5-6]. Previous research on feed and nutrition for juvenile peanut worms has investigated optimal requirements for protein, lipid, carbohydrate, and trace elements including iron, manganese, and selenium [7-12]. To complement and complete the understanding of trace element nutrition, studies on the optimal dietary zinc requirement are essential. This research examines the effects of zinc on growth, body composition, coelomic fluid zinc content, and AKP activity in juvenile peanut worms, providing a theoretical foundation for improving artificial feed formulations for large-scale aquaculture.

1.1 Experimental Diets

Prior to diet preparation, the crude protein, crude lipid, and gross energy content of each ingredient were measured to guide diet formulation. The composition and nutrient levels of the experimental diets are presented in . Zinc methionine (containing 19% zinc) served as the zinc source, with supplementation levels of 105.25, 210.55, 421.05, 842.10, and 1,684.20 mg/kg (dry matter basis), corresponding to zinc additions of 0, 20, 40, 80, 160, and 320 mg/kg (dry matter basis). The measured zinc concentrations were 9.3, 31.7, 49.9, 90.1, 168.6, and 326.5 mg/kg (dry matter basis), respectively. Diet preparation followed the method described by Blair et al. [13]. Primary ingredients were ground to pass through a 200-mesh sieve, weighed in ascending order of supplementation level, and thoroughly mixed. The mixture was blended with fish oil, then with a small amount of water, dried, and ground into microparticle feed passing through a 150-mesh sieve before being bagged and labeled for storage.

1.2 Feeding Management

The feeding trial was conducted from August to October 2013 for a duration of 8 weeks. Eighteen identical tanks (65 cm × 55 cm × 45 cm) were used, each containing a thin layer (3–4 cm thick) of fine sand on the bottom. The sand had been thoroughly washed with freshwater, disinfected, and soaked in seawater for one day to serve as habitat for the juvenile worms. Water depth was maintained at approximately 20 cm. A total of 7,200 healthy juvenile peanut worms from the same batch, with an average initial weight of (14.54 ± 0.10) mg and natural body length of 1.17–1.65 cm, were randomly distributed into 6 groups. Each group comprised three replicates of 400 worms each, fed one of the six experimental diets with varying zinc content. The feeding method involved excessive feeding to ensure a small amount of uneaten feed remained on the sand surface. The experiment was conducted under natural photoperiod with continuous micro-aeration for 24 hours. Water temperature was maintained at 26–30 °C, salinity at 18–22, and dissolved oxygen concentration above 5.0 mg/L.

1.3 Index Determination

At the end of the feeding period, juvenile worms were transferred to sand-free tanks with approximately 10 cm of seawater and starved for 2–3 days to empty their guts. Worms from each tank were then collected and weighed, and survival numbers were recorded to calculate survival rate for each group.

The following formulas were used: - Survival rate (SR, %) = $100 \times N_t / N_0$ - Weight gain rate (WGR, %) = $100 \times (W_t - W_0) / W_0$ - Specific growth rate (SGR, %/day) = $100 \times (\ln W_t - \ln W_0) / t$

Where N_0 is the initial number of worms, N_t is the final number, W_0 is the initial body weight, W_t is the final body weight, and t is the experimental duration in days.

Body composition and feed proximate composition were determined using AOAC (1995) methods. Moisture content was measured by drying to constant weight in a 105 °C oven. Crude protein content was determined by the Kjeldahl method (Kjeltec 8400, Sweden). Crude lipid content was measured by Soxhlet extraction (Soxtec 2050, Switzerland). Ash content was determined by incineration in a muffle furnace at 550 °C for 12 hours. Coelomic fluid AKP activity was measured using a kit from Nanjing Jiancheng Bioengineering Institute, with enzyme activity expressed as specific activity (U/mg prot). Zinc content in coelomic fluid was determined according to GB 13885-2003.

1.4 Statistical Analysis

Data were analyzed using SPSS 19.0. One-way ANOVA was performed, and if significant differences were detected, Tukey' s multiple comparison test was applied with a significance level of $P < 0.05$.

2.1 Effects of Dietary Zinc Content on Growth Performance of Juvenile Peanut Worm

As shown in , SR of juvenile worms ranged from 63.83% to 85.50%. The 9.3 and 326.5 mg/kg zinc groups exhibited lower SR, significantly different from the other groups ($P < 0.05$), indicating that both deficient and excessive zinc levels adversely affected survival. WGR and SGR initially increased then decreased with rising dietary zinc content. The 9.3 and 326.5 mg/kg zinc groups showed no significant difference between them ($P > 0.05$) but were significantly lower than the other groups ($P < 0.05$). The four groups receiving 31.7, 49.9, 90.1, and 168.6 mg/kg zinc showed no significant differences in SR, WGR, or SGR ($P > 0.05$).

Linear regression analysis ([Figure 1: see original paper]) revealed that WGR (y_1) formed a linear relationship with dietary zinc content (x_1 : 9.3, 31.7, and 49.9 mg/kg), described by the equation $y_1 = 3.13x_1 + 127.04$ ($R^2 = 0.892$). Additionally, WGR (y_2) formed a linear relationship with the latter five dietary zinc levels (x_2 : 31.7, 49.9, 90.1, 168.6, and 326.5 mg/kg), described by $y_2 = -0.4519x_2 + 277.24$ ($R^2 = 0.9233$). The intersection of these two lines determined the optimal dietary zinc content for maximum WGR (258.29%) to be 41.93 mg/kg.

2.2 Effects of Dietary Zinc Content on Body Composition of Juvenile Peanut Worm

shows that dietary zinc content significantly affected crude lipid and ash contents ($P < 0.05$). Body crude lipid content initially increased then decreased with rising dietary zinc, reaching its maximum value of 2.85% at 90.1 mg/kg dietary zinc, which was significantly higher than the 326.5 mg/kg zinc group ($P < 0.05$). Body ash content showed the opposite trend, initially decreasing then increasing. The highest ash content occurred in the 9.3 mg/kg zinc group, followed by the 326.5 mg/kg zinc group, with both being significantly higher than the other groups ($P < 0.05$). No significant differences were observed among the remaining groups ($P > 0.05$). Body moisture content ranged from 73.74% to 74.53%, and crude protein content ranged from 60.46% to 63.27%, with no significant differences among groups ($P > 0.05$).

2.3 Effects of Dietary Zinc Content on Coelomic Fluid Zinc Content and AKP Activity of Juvenile Peanut Worm

demonstrates that coelomic fluid zinc content increased with dietary zinc levels. The lowest value (11.32 mg/L) occurred in the 9.3 mg/kg zinc group, significantly lower than the 90.1, 168.6, and 326.5 mg/kg zinc groups ($P < 0.05$). The highest coelomic fluid zinc content was observed in the 326.5 mg/kg zinc group, significantly higher than all other groups ($P < 0.05$). In contrast, coelomic fluid AKP activity initially increased then plateaued with rising dietary zinc. The lowest AKP activity occurred in the 9.3 mg/kg zinc group (5.61 U/mg prot),

followed by the 31.7 mg/kg zinc group, with both being significantly lower than the other groups ($P < 0.05$).

3.1 Effects of Dietary Zinc Content on Growth of Juvenile Peanut Worm

Zinc is the most abundant intracellular trace element and serves as a component or activator of numerous enzymes, making it an indispensable essential trace element [1]. Dietary zinc is primarily supplemented in two forms: inorganic zinc (e.g., $ZnSO_4$, $Zn(NO_3)_2$, $ZnCl_2$) and organic zinc, typically amino acid chelates such as zinc methionine. Studies have shown that rainbow trout (*Oncorhynchus mykiss*) utilization efficiency decreases progressively for these three inorganic sources [14]. Organic zinc, chelated with amino acids, can be absorbed through amino acid transport pathways in the intestine, avoiding competition and antagonism with other trace elements in mineral absorption channels [12]. Research on channel catfish (*Ictalurus punctatus*) demonstrated that organic zinc utilization is substantially higher than inorganic zinc, with zinc methionine showing 3–5 times higher utilization than zinc sulfate in purified diets [15]. Therefore, this study utilized zinc methionine as the zinc source for feeding juvenile peanut worms.

Previous studies have reported that aquatic animals require an appropriate range of dietary zinc, with both deficiency and excess affecting growth and development [16–17]. Qiao [18] found that WGR in cobia (*Rachycentron canadum*) was affected by dietary zinc content, with WGR initially increasing then decreasing as dietary zinc methionine levels rose, establishing an optimal dietary zinc content of 41.7 mg/kg based on WGR. Similarly, Wu et al. [16] observed that WGR in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) increased then decreased with rising dietary zinc, with faster growth and enhanced antioxidant capacity at 5–10 mg/kg dietary zinc. Our results align with these findings. When dietary zinc was deficient (9.3 mg/kg), WGR and SGR were low. Supplementation with zinc methionine significantly improved growth, confirming that juvenile peanut worms can absorb and utilize zinc methionine. However, further increasing dietary zinc to 326.5 mg/kg caused a significant decline in WGR and SGR. The 9.3 and 326.5 mg/kg zinc groups both showed significantly lower WGR and SGR than other groups, indicating that while zinc is essential for growth and development, an optimal dosage range exists. Within this range, zinc promotes growth; below it, insufficient zinc absorption may limit development, while excessive levels may cause mineral antagonism, inhibiting iron and copper absorption [12,19–20], or lead to adverse effects from zinc overload that impair nutrient absorption [18]. The specific mechanisms require further investigation.

In contrast, studies on other aquatic species including large yellow croaker (*Pseudosciaena crocea*) [21], Japanese seabass (*Lateolabrax japonicus*) [22], babylon snail (*Babylonia areolate*) [4], and oriental river prawn (*Macrobrachium nipponense*) [23] found that WGR increased with dietary zinc then plateaued rather

than declining beyond the optimal level, unlike the results observed in juvenile peanut worms.

Additionally, SR in this study was relatively low (63.83%–85.50%), possibly attributable not only to dietary zinc content but also to the use of purified diets, which may have affected palatability for juvenile peanut worms.

3.2 Effects of Dietary Zinc Content on Body Composition of Juvenile Peanut Worm

In this study, increasing dietary zinc content did not significantly affect body moisture or crude protein content, consistent with findings in cobia [18], large yellow croaker [21], Japanese seabass [22], and babylon snail [4]. However, dietary zinc significantly influenced crude lipid and ash contents. The high ash and low crude lipid contents observed at 326.5 mg/kg dietary zinc suggest that excessive zinc may inhibit growth and disrupt nutrient metabolism. Similarly, the diet without zinc methionine supplementation also showed high ash and low crude lipid contents, likely due to insufficient zinc failing to meet developmental requirements and thereby affecting nutrient metabolism [18]. The specific mechanisms underlying these effects on body composition require further investigation.

3.3 Effects of Dietary Zinc Content on Coelomic Fluid Zinc Content and AKP Activity of Juvenile Peanut Worm

In mineral nutrition research, tissue mineral content serves as an important indicator for evaluating mineral requirements [17,24-25]. Cui et al. [17] found that whole-body and vertebral zinc content in starry flounder increased continuously with dietary zinc, with both deficient and excessive levels significantly affecting tissue zinc accumulation. Qiao [18] reported significant effects of dietary zinc on serum zinc in cobia, with the high-zinc group (328.2 mg/kg) showing significantly higher serum zinc than low-zinc groups (13.2, 36.6, and 52.5 mg/kg). Guo et al. [23] observed that zinc content in carapace and whole body of oriental river prawn increased gradually with dietary zinc, plateauing at 36.89 mg/kg, while hepatopancreas zinc peaked at 33.00 mg/kg then stabilized, and muscle zinc remained relatively low and stable. Zhang [21] measured zinc content in vertebrae, whole body, serum, and liver of large yellow croaker, finding that all tissues except liver increased with dietary zinc and gradually stabilized.

Our results align with these studies. Juvenile worms fed the basal diet showed the lowest coelomic fluid zinc content, which increased continuously with dietary zinc supplementation, remaining relatively stable at 31.7–168.4 mg/kg but increasing significantly at 326.5 mg/kg. This suggests that zinc methionine can be absorbed and accumulated in coelomic fluid, but excessive dietary zinc may disrupt nutrient metabolism, leading to excessive coelomic fluid zinc levels that impair growth.

AKP is a homodimeric protein that dephosphorylates substrates by hydrolyzing

phosphate monoesters to generate phosphate ions and free hydroxyl groups. As a zinc-containing metabolic regulatory enzyme with broad substrate specificity including nucleic acids, proteins, and alkaloids, AKP activity reliably reflects zinc nutritional status in aquatic animals [24]. In this study, coelomic fluid zinc content increased with dietary zinc, but AKP activity did not follow the same pattern. AKP activity initially increased then plateaued, with the lowest activity (5.61 U/mg prot) in the 9.3 mg/kg zinc group, followed by the 31.7 mg/kg zinc group, both significantly lower than other groups ($P < 0.05$). Maximum AKP activity occurred at 49.9 mg/kg dietary zinc, with no further increase upon additional zinc supplementation, and no significant differences among groups receiving 49.9–326.5 mg/kg zinc. Similar plateauing of AKP activity has been observed in channel catfish [26], rainbow trout [27], babylon snail [4], and cobia [18]. Low AKP activity at low dietary zinc levels may result from insufficient zinc for AKP synthesis, while the plateau at 49.9 mg/kg suggests that AKP activity is regulated by physiological mechanisms and does not increase indefinitely with zinc content, though the specific regulatory mechanisms require further investigation.

Appropriate dietary zinc content is highly beneficial for WGR and SGR in juvenile peanut worms, while both deficiency and excess inhibit growth. Based on WGR as the evaluation criterion, the optimal dietary zinc content for juvenile peanut worm is 41.93 mg/kg.

References

- [1] Cao ZJ, Wang LM, Dong W, et al. Discussion on trace element requirements and nutritional physiology of fish and shrimp [J]. Heilongjiang Fisheries, 2006(4): 40–42.
- [2] Chen Q, Cheng GP. Trace mineral requirements of fish and shrimp [J]. China Feed, 2001(20): 20–22.
- [3] Wang ZM. Biological selection law of elements [J]. Chinese Journal of Nature, 1997, 19(1): 47–49.
- [4] Yang YZ, Wu YY, Dong XH, et al. Dietary zinc requirement of babylon snail, *Babylonia areolate* [J]. Chinese Journal of Animal Nutrition, 2013, 25(3): 643–650.
- [5] Li FL, Kong QL, Shi GT, et al. Study on the genus *Sipunculus* (Sipuncula) from Chinese coasts [J]. Journal of Ocean University of Qingdao, 1990, 20(1): 93–99.
- [6] Zhang GH, Li L, Zhao MM, et al. Analysis of nutritional components and anti-fatigue effect of *Sipunculus nudus* [J]. Acta Nutrimenta Sinica, 2008, 30(3): 318–320.
- [7] Zhang Q, Tong WP, Dong LF, et al. Effects of dietary lipid level on growth performance, body composition and digestive enzyme activities of juvenile

- peanut worm, *Sipunculus nudus* [J]. Progress in Fishery Sciences, 2011, 32(6): 99-106.
- [8] Zhang Q, Tong WP, Dong LF, et al. Effects of dietary protein level on growth and body composition of juvenile peanut worm, *Sipunculus nudus* [J]. Progress in Fishery Sciences, 2012, 33(1): 86-92.
- [9] Xu MZ, Zhang Q, Tong WP, et al. Effects of dietary carbohydrate level on growth, body composition and digestive enzyme activities of juvenile peanut worm, *Sipunculus nudus* [J]. Chinese Journal of Animal Nutrition, 2013, 25(3): 534-542.
- [10] Xu MZ, Zhang Q, Tong T, et al. Effects of dietary selenium content on growth, body composition, tissue selenium content and related enzyme activities of juvenile peanut worm, *Sipunculus nudus* [J]. Chinese Journal of Animal Nutrition, 2015, 27(6): 1733-1739.
- [11] Xu MZ, Zhang Q, Tong T, et al. Effects of dietary iron level on growth performance, body composition, enzyme activities and tissue iron content of juvenile peanut worm, *Sipunculus nudus* [J]. Chinese Journal of Animal Nutrition, 2014, 26(11): 3325-3331.
- [12] Xu MZ, Zhang Q, Tong T, et al. Effects of dietary manganese content on growth performance, body composition, coelomic fluid Mn-SOD activity and tissue manganese content of juvenile peanut worm, *Sipunculus nudus* [J]. Chinese Journal of Animal Nutrition, 2015, 27(10): 3077-3083.
- [13] Blair T, Castell J, Neil S, et al. Evaluation of microdiets versus live feeds on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*) [J]. Aquaculture, 2003, 225(1/2/3/4): 451-461.
- [14] Sato S. Utilization of zinc in white fish meal and several zinc compounds by rainbow trout [J]. Nippon Suisan Gakkaishi, 1987, 53(4): 63-65.
- [15] Paripatananont T, Lovell RT. Chelated zinc reduces the dietary zinc requirement of channel catfish, *Ictalurus punctatus* [J]. Aquaculture, 1995, 133(1): 73-82.
- [16] Wu HY, Chen XX, Yang HJ, et al. Effects of dietary zinc methionine supplementation on growth and antioxidant function of juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) [J]. Freshwater Fisheries, 2007, 37(6): 28-32.
- [17] Cui LJ, Zhang LM, Wang JY, et al. Effects of dietary zinc level on growth, tissue accumulation and antioxidant function of juvenile starry flounder (*Platichthys stellatus*) [J]. Journal of Fisheries of China, 2010, 34(9): 1420-1428.
- [18] Qiao YG. Studies on nutritional physiology of trace elements zinc, iron and copper in cobia (*Rachycentron canadum*) [D]. Master's thesis. Qingdao: Ocean University of China, 2007.

- [19] Nan XY. Effects of Cu^{2+} on Zn^{2+} and hemoglobin content in crucian carp [J]. Chinese Journal of Public Health, 2006, 22(1): 62-63.
- [20] Hilton JW. The interaction of vitamins, minerals and diet composition in the diet of fish [J]. Aquaculture, 1989, 79(1/2/3/4): 223-244.
- [21] Zhang JM, Ai QH, Mai KS, et al. Dietary zinc requirement of juvenile large yellow croaker (*Pseudosciaena crocea*) [J]. Journal of Fisheries of China, 2008, 32(3): 417-424.
- [22] Zhang JM. Studies on nutritional physiology of trace elements zinc and iron in Japanese seabass (*Lateolabrax japonicus*) and large yellow croaker (*Pseudosciaena crocea*) [D]. Master' s thesis. Qingdao: Ocean University of China, 2007.
- [23] Guo JL, Chen JM, Sun LH, et al. Dietary zinc requirement of juvenile oriental river prawn (*Macrobrachium nipponense*) [J]. Chinese Journal of Animal Nutrition, 2013, 25(3): 661-668.
- [24] Xu ZX, Dong XH, Liu CW. Optimal dietary zinc supplementation level for juvenile cobia (*Rachycentron canadum*) [J]. Fisheries Science, 2007, 26(3): 138-141.
- [25] Wei WQ, Li AJ, Li DS. Effects of dietary zinc supplementation on growth and biochemical indices of Japanese flounder (*Paralichthys olivaceus*) [J]. Journal of Ocean University of Qingdao, 1999, 29(1): 60-66.
- [26] Gatlin III DM, Wilson RP. Dietary zinc requirement of fingerling channel catfish [J]. The Journal of Nutrition, 1983, 113(3): 630-635.
- [27] Apines MJ, Satoh S, Kiron V, et al. Bioavailability of amino acids chelated and glass embedded in rainbow trout, *Oncorhynchus mykiss*, fingerlings [J]. Aquaculture Nutrition, 2001, 7(4): 221-228.

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

Source: ChinaXiv –Machine translation. Verify with original.