Effects of Feed Cobalt Source and Cobalt Content on Growth Performance, Hematological Indices, and Tissue Cobalt Deposition in Juvenile Cobia (Postprint)

Authors: Huang Qincheng, Jiaquan Nie, Dong Xiaohui, Tan Beiping, Shuyan Chi, Yang Qihui, Liu Hongyu, Zhang Shuang, Yang Yuanzhi, Zhang Haitao

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Abstract

This study aimed to investigate the effects of dietary cobalt (Co) content on growth performance, hematological indices, and tissue Co deposition in juvenile cobia fed two Co sources, cobalt chloride (Co-Cl) and cobalt methionine (Co-Met), and to compare the biological efficacy of the two Co sources. A basal diet was formulated using vitamin-free casein and fish meal as the main protein sources. Co-Cl or Co-Met was added to the basal diet at levels of 0 (control), 2, 4, 8, 16, and 32 mg/kg (as Co) to prepare 11 experimental diets (with a shared control). Nine hundred ninety healthy juvenile cobia with an initial body weight of (22.18±0.35) g were selected and randomly divided into 11 groups, with 3 net cages (replicates) per group, each cage stocked with 30 fish and fed the same experimental diet for 10 weeks. The results showed: 1) Under both Co sources, specific growth rate (SGR) and weight gain rate (WGR) initially increased and then decreased with increasing dietary Co content. Dietary Co content had extremely significant effects on SGR, WGR, and feed conversion ratio (FCR) of juvenile cobia ($P<0.01$), and the interaction between dietary Co source and Co content significantly affected SGR, WGR, and survival rate (SR) ($P<0.05$). 2) Dietary Co source had an extremely significant effect on red blood cell count (RBC) ($P<0.01$), dietary Co content had extremely significant effects on RBC, hemoglobin concentration (HGB), and hematocrit (HCT) ($P<0.01$), and the interaction between dietary Co source and Co content had an extremely significant effect on HCT ($P<0.01$). 3) Dietary Co source had an extremely significant effect on vertebral Co content ($P<0.01$), dietary Co content had extremely significant effects on vertebral and whole-body Co content ($P<0.01$), and the interaction between dietary Co source and Co content significantly affected vertebral and whole-body Co content ($P<0.05$). It was

concluded that appropriate dietary Co content could improve growth performance and hematological indices, and increase tissue Co deposition in juvenile cobia. When using Co-Cl and Co-Met as Co sources, juvenile cobia achieved maximum SGR at dietary Co contents of 17.75 and 19.40 mg/kg, respectively. Based on SGR, RBC, and vertebral Co content as criteria, the biological efficacy of Co-Met was 1.47, 1.49, and 1.12 times that of Co-Cl, respectively.

Full Text

Effects of Cobalt Source and Cobalt Content on Growth Performance, Hematological Indexes and Cobalt Accumulation in Tissues of Juvenile Cobia (Rachycentron canadum)

HUANG Qincheng[12], **NIE Jiaquan**[1], **DONG Xiaohui**[12*], **TAN Beiping**[12], **CHI Shuyan**[12], **YANG Qihui**[12], **LIU Hongyu**[12], **ZHANG Shuang**[12], **YANG Yuanzhi**[1], **ZHANG Haitao**[2]

[1]Laboratory of Aquatic Animal Nutrition and Feed, Guangdong Ocean University, Zhanjiang 524088, China
[2]Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang 524000, China

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Abstract

This experiment was conducted to investigate the effects of dietary cobalt (Co) content on growth performance, hematological indexes, and tissue Co deposition in juvenile cobia (*Rachycentron canadum*) fed diets supplemented with two Co sources—cobalt chloride (Co-Cl) and cobalt methionine (Co-Met)—and to compare their relative bioavailability. A basal diet was formulated using vitamin-free casein and fish meal as the primary protein sources. Eleven experimental diets were prepared by supplementing the basal diet with 0 (control), 2, 4, 8, 16, or 32 mg/kg Co (as Co) from either Co-Cl or Co-Met, with the control diet shared between both source groups. A total of 990 healthy juvenile cobia with an initial body weight of (22.18 ± 0.35) g were randomly allocated to 11 groups, each with three replicate cages. Fish in each cage (30 fish per cage) were fed one of the experimental diets to satiation twice daily for 10 weeks.

The results showed: (1) For both Co sources, specific growth rate (SGR) and weight gain ratio (WGR) increased initially and then decreased as dietary Co content increased. Dietary Co content exerted highly significant effects on SGR, WGR, and feed conversion ratio (FCR) ($P < 0.01$), while the interaction between Co source and Co content significantly affected SGR, WGR, and survival rate (SR) ($P < 0.05$). (2) Dietary Co source had a highly significant effect on red blood cell count (RBC) ($P < 0.01$), and dietary Co content highly significantly

affected RBC, hemoglobin concentration (HGB), and hematocrit (HCT) ($P < 0.01$). The interaction between Co source and Co content highly significantly affected HCT ($P < 0.01$). (3) Dietary Co source highly significantly affected vertebral Co content ($P < 0.01$), while dietary Co content highly significantly affected Co content in vertebrae and whole body ($P < 0.01$). The interaction between Co source and Co content significantly affected Co content in vertebrae and whole body ($P < 0.05$).

In conclusion, appropriate dietary Co supplementation improved growth performance, hematological indexes, and tissue Co accumulation in juvenile cobia. Based on SGR, the optimal dietary Co levels were 17.75 mg/kg and 19.40 mg/kg for Co-Met and Co-Cl, respectively. Using SGR, RBC, and vertebral Co content as criteria, the relative bioavailability of Co-Met was 1.47, 1.49, and 1.12 times that of Co-Cl, respectively.

Keywords: juvenile cobia (*Rachycentron canadum*); cobalt; growth performance; hematological indexes; cobalt accumulation; bioavailability

Introduction

Cobalt (Co) is a silver-white ferromagnetic metal belonging to Group VIII of the periodic table and the iron family, with physicochemical properties similar to iron and nickel. It is an essential trace mineral element for animals. Vitamin B12, also known as cobalamin due to its Co content, plays crucial biological roles in promoting erythrocyte maturation and development, fatty acid metabolism, homocysteine methylation, and normal folate cycling. Vitamin B12 deficiency often leads to folate deficiency syndrome [1]. Previous studies have demonstrated that Co can promote vitamin B12 synthesis by gastrointestinal microbiota in animals, thereby meeting the normal growth requirements of fish and compensating for insufficient dietary vitamin B12 [2]. Additionally, Co plays important roles in enhancing heme catabolism, antioxidant activity, and anti-inflammatory responses in animals [3].

Unlike terrestrial animals, fish can absorb some mineral elements from water; however, the concentrations in aquatic environments are often inadequate. Therefore, appropriate mineral supplementation in feed is necessary to meet the growth requirements of fish [1]. Cobalt deficiency in animals can lead to reduced appetite, growth retardation, and impaired synthesis of hemoglobin or erythrocyte maturation factors [4-5]. Current research on Co requirements, growth-promoting effects, and relationships with vitamin B12 has been reported in several species, including malabar grouper (*Epinephelus malabaricus*) [2], Asian seabass (*Lates calcarifer*) and walking catfish (*Clarias batrachus*) [4], red tilapia (*Tilapia zillii*) [6], Nile tilapia (*Oreochromus niloticus*) [7], rohu (*Labeo rohita*) [8], and grass carp (*Ctenopharyngodon idella*) [9].

Biological value is commonly used to evaluate the efficiency of nutrient absorp-

tion, retention, and utilization [10-11], with different mineral sources exhibiting varying bioavailability. Studies have shown that manganese hydroxymethionine is more effective than manganese sulfate and manganese glycinate in improving growth, antioxidant capacity, and vertebral mineral deposition in cobia [12]. In abalone (*Haliotis discus hannai* Ino), the relative bioavailability value (RBV) of zinc methionine is approximately three times that of zinc sulfate for enhancing weight gain ratio or alkaline phosphatase activity [13]. In juvenile cobia, the RBV of selenium methionine is 1.2 and 2.9 times that of sodium selenite based on specific growth rate and whole-body selenium content, respectively [14]. However, other studies have reported no significant differences between lysine copper and copper sulfate in affecting growth, serum ceruloplasmin activity, and immunity in cattle [15]. Most current research on Co has focused on forms such as cobalt chloride (Co-Cl), cobalt carbonate, cobalt oxide, and cobalt acetate or propionate salts [9,16]. To date, no studies have investigated the Co requirement of juvenile cobia or compared the bioavailability of Co-Met and Co-Cl.

Cobia (*Rachycentron canadum*), belonging to the order Perciformes, family Rachycentridae, and genus *Rachycentron*, is also known as black kingfish, sergeant fish, or ling. It is a warm-water demersal fish species primarily distributed in Hainan, Guangdong, and Taiwan provinces of China. As a large carnivorous fish with rapid growth and delicate, delicious flesh, cobia is a popular seafood product and has become an important species for marine cage culture in southern coastal China. Extensive research has been conducted on the nutritional and feed requirements of cobia, including studies on protein [17], lipid [17], carbohydrate [18], vitamin [19], and mineral requirements [20], as well as fish meal replacement [21]. This study aims to investigate the effects of different dietary Co sources and concentrations on the nutritional and physiological functions of juvenile cobia and to compare the bioavailability of the two Co sources, thereby providing fundamental data for determining Co requirements and developing efficient formulated feeds for cobia.

Materials and Methods

1.1 Experimental Materials Cobalt chloride (Co-Cl) used in the experiment was analytical grade. Cobalt methionine (Co-Met) contained 0.2% Co and 1.7% methionine (Met). Both were purchased from Changsha Xingjia Bioengineering Co., Ltd.

1.2 Experimental Diets and Design A basal diet was formulated using vitamin-free casein and fish meal as protein sources, corn starch as carbohydrate source, and corn oil, fish oil, and soybean phospholipid oil as lipid sources. The basal diet contained no vitamin B12. Eleven experimental diets were prepared by supplementing the basal diet with 0 (control), 2, 4, 8, 16, or 32 mg/kg Co

(as Co) from either Co-Cl or Co-Met, with the control diet shared between both source groups.

Feed ingredients were ground to pass through a 60-mesh sieve. After weighing according to the formulation, micro-ingredients were mixed using the progressive enlargement method. Fish oil, corn oil, and soybean phospholipid oil were then added and mixed evenly, followed by blending in a V-type vertical mixer for 5 minutes. Water (30–40% by weight) was added before processing the mixture into 3.0 mm diameter pellets using an F-26 twin-screw extruder (South China University of Technology, Guangzhou). The pellets were air-dried in a ventilated, light-shaded area to a moisture content of approximately 10%, sealed in bags, and stored at -20°C. Dietary Co content was determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies, USA). The experimental design is presented in Table 1 , and the composition and nutrient levels of the basal diet are shown in Table 2 .

1.3 Experimental Animals and Husbandry The feeding trial was conducted at a floating marine cage farm in Nansan Island, Zhanjiang, Guangdong Province. Juvenile cobia were purchased from a fish farmer in Yingli Town, Zhanjiang. The fish were acclimated for two weeks and fed commercial diets (crude protein 55%, crude lipid 8%) during this period. After 24 hours of fasting, healthy juvenile cobia with uniform size and an initial body weight of (22.18 ± 0.35) g were randomly selected and distributed into 11 groups with three replicate cages per group. Each cage (2.5 m × 1.2 m × 1.4 m) was stocked with 30 fish, totaling 33 cages. To minimize experimental error, all cages were randomly arranged. Fish were hand-fed to satiation twice daily (07:00 and 18:00) at 6–9% of body weight. During the 10-week experimental period, water temperature ranged from 28 to 33°C, pH was 7.6–7.8, salinity was 29–31, and dissolved oxygen concentration was >6.0 mg/L. The Co concentration in seawater was 0.12 μg/L.

1.4 Sample Collection and Analysis At the end of the experiment, fish were fasted for 24 hours before sampling. Fish in each cage were counted and weighed collectively. Five fish per cage were randomly selected (anesthetized with eugenol), and blood was collected from the heart using 2.5 mL syringes and placed in 1.5 mL Eppendorf tubes containing anticoagulant for hematological analysis. Another five fish per cage were dissected to collect liver samples, which were immediately frozen in liquid nitrogen and stored at -80°C. Eight fish per cage were randomly selected, eviscerated, boiled for 3 minutes, and then muscle tissue was removed to isolate vertebrae, which were rinsed with ultrapure water to remove attached muscle. The processed vertebrae were dried at 105°C, ground to pass through an 80-mesh sieve, defatted with ether for 12 hours, and redried at 105°C.

Proximate analysis of diets [22]: Moisture content was determined by oven drying at 105°C; crude protein content was measured by the Kjeldahl method

(Kjeltec™ 8400, Sweden); crude lipid content was determined by Soxhlet extraction using ether; and ash content was measured by combustion in a muffle furnace at 550°C.

Mineral analysis: Diets, whole fish, liver, and vertebrae samples were dried at 105°C, ground to pass through an 80-mesh sieve, and digested with nitric acid and hydrogen peroxide. Cobalt content in diets, whole fish, vertebrae, and liver was determined by inductively coupled plasma-mass spectrometry [22].

Hematological indexes: Red blood cell count (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) were measured using a PENTRA 80 automatic hematology analyzer (France).

1.5 Growth Performance Calculations

- Final body weight (FBW, g) = total final weight (g) / number of fish
- Weight gain ratio (WGR, %) = 100 × [FBW (g) - initial body weight (g)] / initial body weight (g)
- Specific growth rate (SGR, %/d) = 100 × [ln FBW (g) - ln initial body weight (g)] / feeding days (d)
- Feed conversion ratio (FCR) = dry feed intake (g) / [FBW (g) - initial body weight (g)]
- Survival rate (SR, %) = 100 × final number of fish / initial number of fish

1.6 Statistical Analysis Data are presented as means ± standard error (SE). Two-way ANOVA was performed using the General Linear Model procedure in SPSS 17.0, with Co source, Co content, and their interaction as main effects. When significant differences were detected, Duncan' s multiple range test was used for post-hoc comparisons. Significance was set at $P < 0.05$, and highly significant differences were defined as $P < 0.01$. The relative bioavailability of Co-Met compared to Co-Cl was calculated using the slope-ratio method [23-24].

Results

2.1 Effects of Dietary Co Source and Co Content on Growth Performance of Juvenile Cobia As shown in Table 3 , SGR, FBW, and WGR under both Co sources increased initially and then decreased with increasing dietary Co content. The SGR and WGR of the C-0 group were significantly lower than those of Co-Cl-4, Co-Cl-8, Co-Cl-16, and Co-Cl-32 groups (Co-Cl source) and Co-Met-4, Co-Met-8, Co-Met-16, and Co-Met-32 groups (Co-Met source) ($P < 0.05$). The SGR and WGR of Co-Cl-32 and Co-Met-32 groups were significantly lower than those of Co-Cl-16 and Co-Met-16 groups ($P < 0.05$). In the Co-Cl groups, WGR and FBW were highest in the Co-Cl-16 group, while in the Co-Met groups, WGR and FBW peaked in the Co-Met-8 group. In the Co-Cl groups, SR was lowest in the Co-Cl-32 group, which was significantly lower than all other groups except Co-Cl-4 ($P < 0.05$), and FCR was significantly higher

than in Co-Cl-2, Co-Cl-8, and Co-Cl-16 groups ($P < 0.05$). In the Co-Met groups, SR in the Co-Met-8 group was significantly lower than in the C-0 group ($P < 0.05$), but other Co-Met groups showed no significant differences from C-0 ($P > 0.05$). FCR in the Co-Met-2 group was significantly higher than in the Co-Met-16 group ($P < 0.05$).

Two-way ANOVA revealed that dietary Co content had highly significant effects on FBW, SGR, WGR, and FCR ($P < 0.01$) but no significant effect on SR ($P > 0.05$). Dietary Co source had no significant effects on FBW, SGR, WGR, FCR, or SR ($P > 0.05$). The interaction between Co source and Co content significantly affected SGR, WGR, and SR ($P < 0.05$) but had no significant effects on FBW or FCR ($P > 0.05$). Based on SGR, quadratic regression analysis indicated that maximum SGR could be achieved at dietary Co concentrations of 19.40 mg/kg and 17.75 mg/kg for Co-Cl and Co-Met, respectively (Figure 1 [Figure 1: see original paper]).

As shown in Figure 2 [Figure 2: see original paper], using SGR as the criterion, the relative bioavailability of Co-Met (relative to Co-Cl) in juvenile cobia was 1.47 times that of Co-Cl (linear regression: Co-Cl source, $y = 0.0188x + 2.5276$, $R^2 = 0.9968$; Co-Met source, $y = 0.0277x + 2.5108$, $R^2 = 0.9752$; relative bioavailability = 0.0277/0.0188 = 1.47).

2.2 Effects of Dietary Co Source and Co Content on Hematological Indexes of Juvenile Cobia As shown in Table 4 , in the Co-Cl groups, RBC in Co-Cl-8, Co-Cl-16, and Co-Cl-32 groups was significantly higher than in the C-0 group ($P < 0.05$). HGB in the C-0 group was the lowest and significantly lower than all other groups ($P < 0.05$). HCT in C-0 and Co-Cl-2 groups was significantly lower than in other groups ($P < 0.05$). In the Co-Met groups, RBC, HGB, and HCT in the C-0 group were significantly lower than in all Co-supplemented groups ($P < 0.05$). HGB in the Co-Met-32 group was significantly lower than in the Co-Met-16 group ($P < 0.05$). RBC, HGB, and HCT under both Co sources increased initially and then decreased with increasing dietary Co content.

Two-way ANOVA showed that dietary Co content had highly significant effects on RBC, HGB, and HCT ($P < 0.01$). Dietary Co source had a highly significant effect on RBC ($P < 0.01$) but no significant effects on HGB or HCT ($P > 0.05$). The interaction between Co source and Co content had a highly significant effect on HCT ($P < 0.01$) but no significant effects on RBC or HGB ($P > 0.05$). Using RBC as the criterion, the relative bioavailability of Co-Met (relative to Co-Cl) was 1.49 times that of Co-Cl (linear regression: Co-Cl source, $y = 0.0543x - 0.4328$, $R^2 = 0.9473$; Co-Met source, $y = 0.0807x - 2.9138$, $R^2 = 0.7780$; relative bioavailability = 0.0807/0.0543 = 1.49).

2.3 Effects of Dietary Co Source and Co Content on Tissue Co Content of Juvenile Cobia As shown in Table 5 , vertebral and whole-body Co content increased with increasing dietary Co content under both Co sources.

Two-way ANOVA indicated that dietary Co content had highly significant effects on vertebral and whole-body Co content ($P < 0.01$) but no significant effect on hepatic Co content ($P > 0.05$). Dietary Co source had a highly significant effect on vertebral Co content ($P < 0.01$) but no significant effects on whole-body or hepatic Co content ($P > 0.05$). The interaction between Co source and Co content had significant effects on vertebral and whole-body Co content ($P < 0.05$) but no significant effect on hepatic Co content ($P > 0.05$).

Using vertebral Co deposition as the criterion, the relative bioavailability of Co-Met (relative to Co-Cl) was 1.12 times that of Co-Cl (linear regression: Co-Cl source, $y = 0.0511x + 0.0721$, $R^2 = 0.9659$; Co-Met source, $y = 0.0571x + 0.0990$, $R^2 = 0.9937$; relative bioavailability = 0.0571/0.0511 = 1.12).

Discussion

3.1 Effects of Dietary Co Source and Co Content on Growth Performance of Juvenile Cobia Under the experimental conditions (vitamin B12-free diets), SGR and WGR of juvenile cobia increased initially and then decreased with increasing dietary Co content. Based on SGR, the Co requirements of juvenile cobia were 19.40 mg/kg and 17.75 mg/kg for Co-Cl and Co-Met, respectively. The Co requirement in fish may be influenced by species (marine vs. freshwater), basal dietary vitamin B12 content, ambient water Co concentration, Co source, fish size, basal diet composition, and evaluation criteria. Previous studies reported that the Co requirement for malabar grouper was 10 mg/kg based on WGR [2]. For grass carp juveniles, the optimal dietary Co level was approximately 0.88 mg/kg when vitamin B12 was absent from the diet [25], but only 0.20 mg/kg when vitamin B12 was present. For Pacific white shrimp (*Penaeus vannamei*), the fastest growth was observed at 15 mg/kg Co supplementation [26]. Nile tilapia juveniles required 0.3–3.0 mg/kg dietary Co [27], while Japanese flounder (*Paralichthys olivaceus*) required 0.8 mg/kg Co supplementation when the basal diet contained 1.43 mg/kg Co.

The growth-promoting effects of Co may be attributed to several mechanisms: (1) Co stimulates vitamin B12 synthesis by intestinal microbiota, and vitamin B12 is a hematopoietic vitamin essential for animal growth [2,28]; (2) Co participates in hematopoiesis and nutrient metabolism (protein, carbohydrate, lipid) as a coenzyme, promoting nitrogen absorption and growth [5]; (3) Optimal Co levels improve intestinal histological structure and protect organ tissues [7]; and (4) Co enhances antioxidant capacity and anti-inflammatory responses by affecting gene expression [3,29].

However, growth performance declined and SR decreased significantly in the high-dose (32 mg/kg) groups, indicating Co intolerance at this level in juvenile cobia. This is consistent with results from grass carp, where growth slowed during the later stages in groups receiving 0.95 and 1.63 mg/kg Co [25]. Excessive dietary Co may cause toxicity, leading to intestinal hemorrhage or damage and

abnormal leukocyte changes [30]. Additionally, Co shares transport pathways with iron and manganese, and excessive Co can inhibit their absorption and utilization [31-32]. In the Co-Met groups, SR decreased significantly only in the Co-Met-8 group, while WGR and SGR improved markedly, suggesting that the reduced SR was not Co-induced. In practical aquaculture, SR is influenced by multiple factors including feed quality, environmental conditions, disease outbreaks, and other unknown stochastic factors, warranting further investigation.

3.2 Effects of Dietary Co Source and Co Content on Hematological Indexes of Juvenile Cobia Cobalt enhances hematopoietic function through several pathways: (1) inhibiting key enzymes (e.g., cytochrome oxidase) or increasing erythropoietin (Epo) to stimulate hematopoiesis; (2) promoting iron absorption and utilization in bone marrow; and (3) participating in RNA and hematopoietic substance metabolism via vitamin B12 [33]. Studies have shown that Co significantly increased HCT, HGB, and RBC in Nile tilapia [27] and improved HGB, platelet count (PLT), HCT, and RBC in grass carp juveniles [25]. Cobalt can stabilize hypoxia-inducible factor-1 (HIF-1), activate the erythropoietin gene, and enhance hemoglobin and erythrocyte synthesis [34]. In this study, RBC, HGB, and HCT increased to varying degrees after Co supplementation under both sources, consistent with previous findings. The C-0 group exhibited lower hematological indexes than Co-supplemented groups, indicating that ambient seawater and basal dietary Co were insufficient for normal physiological needs. However, RBC, HGB, and HCT decreased in the 32 mg/kg Co groups compared to the 16 mg/kg groups, suggesting that excessive Co levels adversely affect hematopoietic capacity.

3.3 Effects of Dietary Co Source and Co Content on Tissue Co Deposition in Juvenile Cobia Co content in fish tissues such as liver, muscle, kidney, and vertebrae is commonly used to evaluate nutritional status [9,14,29,35-36]. In pearl gentian grouper (*Epinephelus lanceolatu* × *E. fuscoguttatus*), Co deposition in liver, intestine, vertebrae, muscle, and whole body increased significantly with dietary Co level, with the highest deposition rate in liver reaching 2.27 mg/kg in the highest supplementation group [29]. In Nile tilapia, hepatic Co deposition increased significantly with dietary Co and stabilized at 1.73 mg/kg when dietary Co reached 2.67 mg/kg or higher [27]. In Pacific white shrimp, dietary Co from Co-Met did not significantly affect muscle or hepatopancreas Co content, whereas Co from Co-Cl significantly affected hepatopancreas Co content, with the highest level (15 mg/kg) reaching 6.05 mg/kg, significantly higher than the control [26]. These results indicate that hepatic Co content is influenced by dietary Co level.

In this study, vertebral and whole-body Co content in juvenile cobia increased with dietary Co level, consistent with previous reports. The liver is typically the primary site for nutrient metabolism or serves as the main storage organ for Co [35]. However, hepatic Co content was not significantly affected by dietary Co source or level in this study. Comparing with previous studies suggests that

different fish species have varying hepatic Co storage capacities. Hepatic Co content in juvenile cobia may reach saturation easily and remain stable. Similar results were observed in grass carp juveniles, where hepatic Co content did not differ significantly across dietary Co levels ranging from 0.17 to 1.57 mg/kg [25]. This may indicate that fish liver possesses a homeostatic regulatory mechanism for Co, preventing further accumulation once saturation is reached.

3.4 Comparison of Biological Value of Different Co Sources Antagonistic interactions exist among mineral elements in vivo, such as between iron and copper, zinc and iron/manganese/copper, and cobalt and manganese [30,36]. Additionally, feed ingredients contain phytic acid, cellulose, and phosphate that interfere with trace element absorption. For example, high levels of hydroxyapatite in white fish meal can reduce zinc utilization in fish [37]. Biological value is commonly used to compare the efficiency of nutrient absorption and utilization [10]. Organic chelates generally exhibit higher bioavailability than inorganic compounds [12-14]. Studies have reported that the bioavailability of manganese hydroxymethionine is 1.09–2.47 times that of manganese sulfate [12], zinc methionine is approximately 3 times that of zinc sulfate [13], and selenium methionine is 1.20–2.90 times that of sodium selenite [14].

In this study, using SGR, RBC, and vertebral Co content as criteria, the bioavailability of Co-Met was 1.47, 1.49, and 1.12 times that of Co-Cl, respectively. Co-Met is a novel amino acid chelate with a 2:1 Met to Co ratio, forming a non-ionic complex that can readily pass through anion-rich cell membranes. Amino acid chelates of trace elements may be absorbed as amino acids, avoiding antagonism among trace elements and improving not only Co utilization but also absorption of other minerals [29]. Additionally, Co-Met provides Met, which is the first limiting amino acid in fish. Therefore, Co-Met offers dual nutritional functions and is efficiently utilized by juvenile cobia.

Conclusion

1. Appropriate dietary supplementation of Co as either Co-Cl or Co-Met improved growth performance and hematopoietic capacity in juvenile cobia, with Co-Met demonstrating higher efficiency in promoting growth, hematopoiesis, and tissue Co deposition.
2. Based on SGR, the optimal dietary Co levels for juvenile cobia were 19.40 mg/kg and 17.75 mg/kg when supplemented as Co-Cl and Co-Met, respectively.
3. The relative bioavailability of Co-Met for juvenile cobia was 1.12–1.49 times that of Co-Cl.

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