Effects of Different Dietary Vitamin D Levels on Growth Performance and Calcium-Phosphorus Metabolism in Juvenile Perch (Postprint)

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

Abstract

This study aimed to investigate the effects of different dietary vitamin D levels on growth performance and calcium-phosphorus metabolism in juvenile largemouth bass. Juvenile largemouth bass with an initial body weight of (2.26$±$0.03) g were randomly divided into 6 groups (3 replicates per group, 15 fish per replicate) and fed isonitrogenous and isoenergetic diets with measured vitamin D levels of 34.2, 219.4, 393.8, 775.9, 1,534.1, and 3,091.2 IU/kg for a 9-week feeding trial. The results showed: 1) When dietary vitamin D levels ranged from 34.2 to 393.8 IU/kg, the weight gain rate of bass increased significantly with increasing dietary vitamin D content ($P<0.05$); however, when dietary vitamin D levels exceeded 393.8 IU/kg, the weight gain rate showed no significant change and reached a plateau ($P>0.05$). Similarly, specific growth rate, feed efficiency, and protein efficiency ratio exhibited trends similar to those of weight gain rate. 2) Dietary vitamin D levels significantly affected whole-body crude ash, calcium, and phosphorus contents, crude ash contents in vertebrae, operculum, and scales, as well as calcium and phosphorus contents in vertebrae ($P<0.05$), but had no significant effects on whole-body crude protein, crude lipid, and moisture contents ($P>0.05$). 3) Dietary vitamin D levels significantly affected serum alkaline phosphatase activity and contents of hydroxyproline, calcium ions, and inorganic phosphorus in bass ($P<0.05$). 4) Dietary vitamin D levels significantly affected hepatosomatic index and liver lipid and vitamin D contents in bass ($P<0.05$). With increasing dietary vitamin D levels, liver vitamin D content showed an increasing trend and stabilized when dietary vitamin D level reached 1,534.1 IU/kg. Using weight gain rate as the evaluation index, broken-line model analysis indicated that the dietary vitamin D requirement for maximum growth in juvenile largemouth bass was 431.0 IU/kg. Using liver vitamin D content as the evaluation index, quadratic model analysis indicated that the dietary

vitamin D requirement for maximum liver vitamin D deposition in juvenile largemouth bass was 2,444.4 IU/kg.

Full Text

Effects of Different Dietary Vitamin D Contents on Growth Performance and Calcium-Phosphorus Metabolism in Juvenile Japanese Seabass

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Abstract: This study investigated the effects of varying dietary vitamin D levels on growth performance and calcium-phosphorus metabolism in juvenile Japanese seabass (*Lateolabrax japonicus*). Juvenile seabass with an initial body weight of (2.26$±$0.03) g were randomly allocated into six groups (three replicates per group, 15 fish per replicate) and fed six isonitrogenous and isoenergetic diets containing measured vitamin D levels of 34.2, 219.4, 393.8, 775.9, 1,534.1, and 3,091.2 IU/kg for nine weeks. The results showed: (1) Weight gain rate (WGR) increased significantly with dietary vitamin D supplementation from 34.2 to 393.8 IU/kg ($P<0.05$), but plateaued without further significant changes when vitamin D exceeded 393.8 IU/kg ($P>0.05$). Specific growth rate (SGR), feed efficiency ratio (FER), and protein efficiency ratio (PER) exhibited similar trends. (2) Dietary vitamin D significantly affected whole-body ash, calcium, and phosphorus contents, as well as ash content in vertebrae, opercula, and scales, and calcium and phosphorus levels in vertebrae ($P<0.05$), but had no significant impact on crude protein, crude lipid, or moisture contents ($P>0.05$). (3) Serum alkaline phosphatase (AKP) activity and hydroxyproline, calcium ion, and inorganic phosphorus concentrations were all significantly influenced by dietary vitamin D ($P<0.05$). (4) Hepatosomatic index (HSI) and hepatic lipid and vitamin D contents were significantly affected by dietary vitamin D ($P<0.05$). Hepatic vitamin D content increased with dietary vitamin D and stabilized at 1,534.1 IU/kg. Broken-line model analysis based on WGR indicated that the optimal dietary vitamin D requirement for maximum growth was 431.0 IU/kg. Quadratic curve model analysis based on hepatic vitamin D content suggested a requirement of 2,444.4 IU/kg for maximal vitamin D deposition.

Keywords: Japanese seabass; vitamin D; growth; requirement; calcium-phosphorus metabolism

Introduction

The primary forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Ergocalciferol is derived from plant ergosterol, while cholecalciferol originates from animal 7-dehydrocholesterol, both converted via ultraviolet irradiation. Vitamin D_3 functions not only as a nutrient but also as a prohormone that becomes biologically active only after hepatic conversion to 25-hydroxyvitamin D_3 [25(OH)D_3] and subsequent renal metabolism to 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2D_3$] or 24R,25-dihydroxyvitamin D_3 [24R,25(OH)$_2D_3$]. Vitamin D regulates calcium-phosphorus homeostasis and modulates parathyroid hormone (PTH) effects on bone [1]. Recent research indicates additional physiological roles in metabolism, cell proliferation and differentiation, autoimmunity, cardiovascular function, neuromuscular activity, and cancer prevention [2].

Limited information exists regarding vitamin D requirements in fish. Previous studies suggest that rainbow trout require 1,600–2,400 IU/kg [3], channel catfish require 1,000 IU/kg [4] or 250 IU/kg [5], while Pacific salmon and yellowtail show no dietary vitamin D requirement [6-7]. Vitamin D deficiency causes rickets, calcium imbalance, white muscle spasms, and altered muscle fiber structure in low-calcium environments [8], whereas excess vitamin D reduces growth and causes lethargy and darkening in brook trout [1].

Japanese seabass (*Lateolabrax japonicus*) is a carnivorous fish valued for its delicate flesh and rapid growth. It tolerates wide salinity and temperature ranges, requires no indoor overwintering, and represents a major aquaculture species in China. Previous research has established optimal dietary protein and lipid levels [9-12], evaluated effects of lipid sources and lipid-lowering factors [13], and investigated protein-to-energy ratios [14], mineral requirements [15-16], and exogenous enzyme supplementation [17]. Vitamin C effects on growth and immunity have been studied [18], but vitamin D requirements remain unexamined. This study quantified the vitamin D requirement in purified diets and investigated its effects on whole-body composition, hepatic vitamin D content, serum physiological and biochemical parameters, and skeletal mineralization to provide theoretical support for formulated feed development.

Materials and Methods

1.1 Diet Preparation A purified basal diet was formulated using casein and gelatin as protein sources, soybean and menhaden oils as lipid sources, dextrin as carbohydrate source, sodium alginate as binder, and supplemented with an amino acid mixture simulating seabass muscle amino acid profile . Six experimental diets were prepared by adding 0, 200, 400, 800, 1,600, and 3,200 IU vitamin D_3 per kg basal diet, yielding measured vitamin D levels of 34.2, 219.4, 393.8, 775.9, 1,534.1, and 3,091.2 IU/kg.

All ingredients were ground through an 80-mesh sieve, mixed thoroughly, blended with water, soybean oil, and menhaden oil, then extruded using

an F-26 twin-screw extruder. The extruded diets were dried at 45°C to 9-10% moisture content, crumbled, and sieved to produce two pellet sizes (1.5 mm$\times$3.0$mm and 2.5mm\times$4.0 mm). The diets were sealed in plastic bags and stored at -20°C until use.

1.2 Feeding Management Wild-caught juvenile seabass were acclimated in concrete tanks (3.0 m$\times$2.0$m \times 1.5m$) $and fed the basal diet to adapt to experimental conditions. After acclimation, L fiberglass tank (250 L water volume) in a flow-through system. Fish were hand-fed to apparent satiation twice daily (07:00, 17:00). Pellet size was 1.5$mm \times$3.0$mm during weeks 1–4 and 2.5$mm \times$ mm during weeks 5–9. Uneaten feed was collected 1 h after feeding, dried, and weighed; feces were removed 1 h before feeding. Daily feed intake was recorded, and mortalities were documented. Seawater was settled and sand-filtered, with continuous aeration at 1 L/min flow rate. Water temperature was 24.0–27.0°C, salinity 28.0–29.5‰, pH 8.0–8.1, and dissolved oxygen approximately 7 mg/L.

After nine weeks, fish were fasted for 24 h, anesthetized with eugenol (1:10,000), counted, and weighed. Three fish per tank were randomly selected for blood collection from the caudal vein using sterile syringes; serum was separated and stored at -80°C. Remaining fish were weighed, sampled, and stored at -20°C.

1.3 Sample Analysis Proximate composition of ingredients, diets, and fish was analyzed using AOAC (1995) methods [19]: moisture by oven drying at 105°C to constant weight, crude protein by semi-micro Kjeldahl method (N$\times$6.25), $crude lipid by Soxhlet extraction with ether, and ash by combustion at 600°C for 12 h. Hepatic lipid conten$ and Pi concentrations were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute). Dietary and hepatic vitamin D contents were analyzed by HPLC [21]. Whole-body amino acid composition was determined using Zhang et al.' s method [22]. Calcium and phosphorus in whole body and vertebrae were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian VISTA-MPX). All samples were analyzed in triplicate.

1.4 Calculation Formulas Weight gain rate (WGR) = 100 × (Wt -W0) / W0
Specific growth rate (SGR) = 100 × (ln Wt -ln W0) / t
Feed efficiency ratio (FER) = 100 × (Wt × Nt -W0 × N0) / Id [23]
Protein efficiency ratio (PER) = 100 × (Wt × Nt -W0 × N0) / Ip
Survival rate (SR) = 100 × Nt / N0
Hepatosomatic index (HSI) (%) = 100 × WL / WB

Where: W0 = initial body weight; Wt = final body weight; t = experimental days; Nt = final fish number; N0 = initial fish number; Id = feed intake (dry weight basis); Ip = crude protein intake (dry weight basis); WL = liver weight; WB = body weight.

1.5 Statistical Analysis Data were analyzed using SPSS 16.0. One-way ANOVA was performed, and if significant differences were detected ($P<0.05$), Tukey' s test was applied for multiple comparisons. Results are expressed as means ± standard error.

Results

2.1 Effects of Dietary Vitamin D on Growth Performance Dietary vitamin D did not significantly affect survival ($P>0.05$) but significantly influenced WGR, SGR, FER, and PER ($P<0.05$) . The unsupplemented group (34.2 IU/kg) showed the lowest survival (73.3%) but did not differ significantly from other groups (82.2–93.3%) ($P>0.05$). WGR increased significantly as dietary vitamin D rose from 34.2 to 393.8 IU/kg ($P<0.05$), then plateaued without further significant changes at higher levels ($P>0.05$). SGR, FER, and PER showed similar trends. The relationship between dietary vitamin D (X) and WGR (Y) was described by the broken-line model: $Y = 652.5 - 1.24(431.0 - X)$, indicating a vitamin D requirement of 431.0 IU/kg for optimal growth [Figure 1: see original paper].

2.2 Effects of Dietary Vitamin D on Whole-Body Composition Dietary vitamin D significantly affected whole-body ash, calcium, and phosphorus contents ($P<0.05$) but not crude protein, crude lipid, or moisture ($P>0.05$) . Whole-body ash increased with dietary vitamin D, with the highest level (3,091.2 IU/kg) being significantly greater than the unsupplemented group ($P<0.05$). Calcium and phosphorus showed similar trends, though calcium in the highest group was slightly lower than the second-highest group (1,534.1 IU/kg) without significant difference ($P>0.05$).

2.3 Effects of Dietary Vitamin D on Serum Parameters Serum AKP activity and Ca^{2+} concentration increased initially then declined, while Pi concentration increased continuously with dietary vitamin D . Serum HPro peaked in the unsupplemented group, significantly higher than the 219.4 and 775.9 IU/kg groups ($P<0.05$) but not different from others ($P>0.05$).

2.4 Effects of Dietary Vitamin D on Skeletal Mineralization Vertebral, opercular, and scale ash contents, and vertebral calcium increased significantly as dietary vitamin D rose from 34.2 to 1,534.1 IU/kg ($P<0.05$), then decreased slightly but not significantly at 3,091.2 IU/kg ($P>0.05$) . Vertebral phosphorus correlated positively with dietary vitamin D, with the two highest groups significantly exceeding lower groups ($P<0.05$).

2.5 Effects of Dietary Vitamin D on Hepatic Indices Dietary vitamin D significantly affected HSI and hepatic lipid and vitamin D contents ($P<0.05$) . HSI and hepatic lipid decreased with increasing dietary vitamin D, while hepatic vitamin D increased and stabilized at 1,534.1 IU/kg. The quadratic

relationship between dietary vitamin D (X) and hepatic vitamin D (Y) was: Y = (-9E-07)X^2 + 0.0044X + 0.8816, yielding a requirement of 2,444.4 IU/kg for maximal hepatic vitamin D deposition [Figure 2: see original paper].

Discussion

The broken-line model based on WGR indicated a vitamin D requirement of 431.0 IU/kg for juvenile Japanese seabass, similar to Lovell and Li' s [24] findings for channel catfish (500 IU/kg) but lower than Barnett et al.' s [3] report for rainbow trout (1,600–2,400 IU/kg) and Andrews et al.' s [4] report for channel catfish (1,000 IU/kg). Hepatic vitamin D accumulation followed a similar trend to growth, increasing then plateauing. The quadratic model better fitted the relationship between hepatic and dietary vitamin D (higher R^2), yielding a requirement of 2,444.4 IU/kg, which aligns with Barnett et al.' s [3] rainbow trout requirement. Requirements based on tissue saturation exceed those based on growth [25], consistent with our findings. Model selection significantly affects requirement estimates, with quadratic models typically yielding higher values than broken-line models [26]. Standardizing model selection criteria in aquaculture nutrition research would facilitate comparison across studies. Variations in reported requirements may reflect differences in species, life stage, physiological status, vitamin D form, environment, and culture system.

After nine weeks, the unsupplemented group showed lower survival without significant differences and lacked overt deficiency symptoms, though opercula were fragile and brittle. This contrasts with Halver' s [27] report that excess vitamin D causes opercular fragility, as our highest vitamin D group showed no such symptoms. Opercular ash content was lowest in the unsupplemented group and significantly lower than the highest group. The lack of fragility in the high vitamin D group may reflect insufficient dosage or experimental duration.

Serum AKP activity and Ca^{2+} and Pi concentrations increased with dietary vitamin D, but AKP activity and Ca^{2+} declined slightly at 3,091.2 IU/kg. AKP, present in bone, liver, and intestine, indicates active bone metabolism and serves as a bone turnover marker in human medicine. The active vitamin D metabolite $1\alpha,25(OH)_2D_3$ stimulates osteoclast activity and formation, promoting bone resorption, while synergizing with PTH to enhance osteoclast proliferation and function. $1\alpha,25(OH)_2D_3$ also stimulates osteoblast collagen secretion and bone formation, and increases intestinal calcium and phosphorus absorption to promote mineralization. Under adequate calcium and phosphorus, $1\alpha,25(OH)_2D_3$ primarily promotes bone formation; when calcium is limiting, it enhances bone resorption to maintain blood calcium. In this study, increasing dietary vitamin D elevated $1\alpha,25(OH)_2D_3$ production, stimulating osteoblast metabolism and releasing AKP into serum. However, the decline in AKP at the highest vitamin D level reflects dose-dependent effects—physiological doses stimulate while toxic doses inhibit bone cells. Zhou et al. [28] reported similar findings in abalone, where optimal vitamin D increased soft tissue AKP activity but excessive or deficient levels were inhibitory. Shiau and Hwang [29] observed comparable

results in shrimp. Human studies confirm that $1\alpha,25(OH)_2D_3$ increases blood calcium and phosphorus by enhancing intestinal absorption and renal reabsorption, consistent with our serum results. Elevated serum calcium and phosphorus enhanced skeletal mineralization, as evidenced by increased ash, calcium, and phosphorus in vertebrae, opercula, and scales, and higher whole-body ash content, consistent with Lovell and Li' s [24] channel catfish findings. Bone consists of mineral salts (calcium and phosphorus) and organic matrix (Type I collagen). Vitamin D deficiency causes osteomalacia or osteoporosis with reduced mineral and matrix content, confirmed by elevated serum HPro in our unsupplemented group, indicating severe organic matrix loss.

Interestingly, maximal values for vertebral, opercular, and scale ash, and vertebral calcium occurred at the second-highest vitamin D level (1,534.1 IU/kg) rather than the highest (3,091.2 IU/kg), with slight non-significant reductions at the highest level. Human research shows that excessive vitamin D causes widespread bone demineralization and increases fracture risk. Although we observed no fractures, the highest group showed slight bone mineral reduction, while the unsupplemented group exhibited the most severe organic matrix loss, explaining why opercular fragility occurred in the deficient rather than the excess group.

HSI and hepatic lipid followed identical trends, peaking in the unsupplemented group (HSI 2.69%, lipid 15.67%) and declining with increasing dietary vitamin D. George et al. [30] reported that vitamin D-deficient rainbow trout showed reduced growth and increased hepatic lipid, consistent with our results. However, whole-body lipid increased with dietary vitamin D, being lowest in the group with highest hepatic lipid (5.3%). This suggests that elevated hepatic lipid does not result from increased dietary lipid intake or synthesis capacity, but likely from impaired phospholipid or apolipoprotein synthesis due to vitamin D accumulation, preventing triglyceride export and causing hepatic steatosis, which corresponds with reduced whole-body lipid content.

In conclusion, the vitamin D requirement for juvenile Japanese seabass is 431.0 IU/kg based on growth performance, but 2,444.4 IU/kg based on hepatic vitamin D deposition.

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