Postprint: Dietary Vitamin E Requirement for Juvenile Lenok (Brachymystax lenok)

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Abstract

This study aimed to investigate the effects of different dietary vitamin E levels on growth performance, serum biochemical indices, and vitamin E accumulation in juvenile lenok (Brachymystax lenok), and to determine the dietary vitamin E requirement of juvenile lenok. A total of 270 juvenile lenok with an initial mean weight of (40.2$±$3.6) g were randomly divided into 6 groups, with 3 replicates per group and 15 fish per replicate. Each group was fed one of six isonitrogenous and isoenergetic experimental diets containing different vitamin E levels (actual measured values of 16.6, 64.9, 165.5, 316.2, 615.5, and 1214.7 mg/kg). The experimental period lasted 120 days. The results showed: 1) The final mean weight (FW), weight gain rate (WGR), and specific growth rate (SGR) of lenok all exhibited a trend of initially increasing and then decreasing with increasing dietary vitamin E level. The FW of lenok in the 165.5 mg/kg group was significantly higher than that in all other groups ($P<0.05$), and the WGR and SGR of lenok in the 165.5 mg/kg group were significantly higher than those in the 16.6 and 1214.7 mg/kg groups ($P<0.05$). There were no significant differences in feed intake, feed conversion ratio, or protein efficiency ratio among all groups ($P>0.05$). 2) With increasing dietary vitamin E level, serum triglyceride (TG) and total cholesterol (TC) contents both showed a gradual decreasing trend, and the 615.5 and 1214.5 mg/kg groups were significantly lower than all other groups ($P<0.05$). With increasing dietary vitamin E level, serum high-density lipoprotein cholesterol (HDL-C) content showed a trend of initially increasing and then decreasing, and the 165.5 mg/kg group was significantly higher than all other groups ($P<0.05$), while the 64.9 and 316.2 mg/kg groups were significantly higher than the 16.6, 615.5, and 1214.5 mg/kg groups ($P<0.05$). In contrast, serum low-density lipoprotein cholesterol (LDL-C) content showed an opposite trend to HDL-C content, and the 165.5 and 316.2 mg/kg groups were significantly lower than all other groups ($P<0.05$). There were no significant differences in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities among all groups ($P>0.05$). 3) With increasing dietary vitamin E level, hepatic vitamin E accumulation in lenok showed a significant increasing trend (P<0.05), except that there was no significant difference between the 615.5 and 1214.5 mg/kg groups (P>0.05). Muscle vitamin E accumulation also showed an increasing trend, plateauing at a constant level when dietary vitamin E level exceeded 165.5 mg/kg, and the muscle vitamin E deposition in the 165.5, 316.2, 615.5, and 1214.5 mg/kg groups was significantly higher than that in the 16.6 and 64.9 mg/kg groups (P<0.05). These results indicate that supplementation of appropriate dietary vitamin E can improve growth performance and serum biochemical indices, and increase vitamin E accumulation in liver and muscle of lenok. Using weight gain rate and muscle vitamin E accumulation as evaluation indices, broken-line model analysis indicated that the optimal dietary vitamin E requirements for lenok were 145.87 and 180.98 mg/kg, respectively.

Full Text

Requirement of Dietary Vitamin E for Juvenile Manchurian Trout (Brachymystax lenok)

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Abstract: This study investigated the effects of dietary vitamin E levels on growth performance, serum biochemical parameters, and vitamin E accumulation in juvenile Manchurian trout (*Brachymystax lenok*), and determined the optimal dietary vitamin E requirement. A total of 270 juvenile trout with an initial mean weight of (40.2$±$3.6) g were randomly allocated into six groups with three replicates each (15 fish per replicate). Six isonitrogenous and isoenergetic experimental diets were formulated with graded levels of vitamin E (measured values: 16.6, 64.9, 165.5, 316.2, 615.5, and 1214.7 mg/kg). The feeding trial lasted 120 days. The results showed: (1) Final weight (FW), weight gain rate (WGR), and specific growth rate (SGR) increased initially and then decreased with rising dietary vitamin E levels. The 165.5 mg/kg group exhibited significantly higher FW than all other groups (P<0.05), while WGR and SGR in this group were significantly higher than those in the 16.6 and 1214.7 mg/kg groups (P<0.05). No significant differences were observed in feed intake rate, feed conversion ratio, or protein efficiency ratio among groups (P>0.05). (2) Serum triglyceride (TG) and total cholesterol (TC) concentrations decreased progressively with increasing dietary vitamin E, with the 615.5 and 1214.5 mg/kg groups showing significantly lower values than other groups (P<0.05). Serum high-density lipoprotein cholesterol (HDL-C) increased initially then decreased, peaking at 165.5 mg/kg, which was significantly higher than all other groups (P<0.05); the 64.9 and 316.2 mg/kg groups also showed significantly higher HDL-C than the 16.6, 615.5, and 1214.5 mg/kg groups (P<0.05). Conversely, low-density lipoprotein cholesterol (LDL-C) displayed the opposite trend, with the 165.5 and 316.2 mg/kg groups showing significantly lower values than other groups ($P<0.05$). No significant differences were detected in serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) activities among groups ($P>0.05$). (3) Liver vitamin E accumulation increased significantly with dietary vitamin E level ($P<0.05$), except between the 615.5 and 1214.5 mg/kg groups ($P>0.05$). Muscle vitamin E accumulation also increased, plateauing when dietary vitamin E exceeded 165.5 mg/kg; the 165.5, 316.2, 615.5, and 1214.5 mg/kg groups showed significantly higher muscle vitamin E deposition than the 16.6 and 64.9 mg/kg groups ($P<0.05$). These findings demonstrate that appropriate dietary vitamin E supplementation improves growth performance and serum biochemical parameters while enhancing vitamin E deposition in liver and muscle. Based on broken-line regression analysis using WGR and muscle vitamin E accumulation as criteria, the optimal dietary vitamin E requirements for juvenile Manchurian trout were estimated to be 145.87 mg/kg and 180.98 mg/kg, respectively.

Keywords: *Brachymystax lenok*; vitamin E; growth performance; serum biochemical indicators; requirement

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Vitamin E is a crucial fat-soluble vitamin that protects cell membranes from oxidative damage, prevents formation of reactive membrane phospholipid peroxides, and exhibits antioxidant properties while enhancing humoral and cellular immunity and modulating immune system function. Elucidating vitamin E' s role in lipid metabolism and fat deposition regulation holds significant biological importance. Li et al. reported that dietary vitamin E supplementation at appropriate levels increased intramuscular fat content in broiler breast and thigh meat while reducing abdominal fat. Zhang et al. demonstrated that vitamin E improved lipid metabolism in broilers by decreasing serum triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) concentrations while increasing high-density lipoprotein cholesterol (HDL-C). Our previous research showed that supplementing high-fat diets with 124–243 mg/kg vitamin E improved growth performance, feed utilization, hepatosomatic and viscerosomatic indices, regulated lipid metabolism, modulated lipid metabolic enzyme activities, and enhanced tissue vitamin E accumulation and immune function in turbot (*Scophthalmus maximus*).

Manchurian trout (*Brachymystax lenok* Pallas), belonging to the family Salmonidae and genus *Brachymystax*, is a valuable cold-water fish species with high economic and nutritional value, distributed primarily in streams, rivers, and lakes of Heilongjiang, Jilin, Liaoning, Hebei, Inner Mongolia, Xinjiang, and the Qinling region in China. The species is listed as a second-class nationally protected aquatic animal. In recent years, artificial breeding and seedling cultivation have been successfully implemented in various regions including Northeast China, Hanzhong in the Qinling region, Hebei, and Shandong, followed by large-scale stocking and farming. However, limited research on nutritional requirements has hindered development of precise formulated feeds, with farmers currently using rainbow trout commercial feeds or small fish instead. Investigating the nutritional physiology of Manchurian trout is essential for developing comprehensive, high-quality formulated feeds for healthy precision aquaculture. Therefore, this study examined the effects of graded dietary vitamin E levels on growth performance, serum biochemical parameters, and tissue vitamin E accumulation to provide theoretical reference for determining optimal vitamin E requirements.

1.1 Experimental Diets

The basal diet composition and nutrient levels are presented in Table 1 . Microcrystalline cellulose was used to adjust vitamin E levels based on Atlantic salmon requirements, with DL-α-tocopheryl acetate (50% vitamin E content) added at 0, 100, 300, 1200, and 2400 mg/kg to produce six isonitrogenous and isoenergetic experimental diets with measured vitamin E levels of 16.6, 64.9, 165.5, 316.2, 615.5, and 1214.7 mg/kg, respectively. All ingredients were ground through an 80-mesh sieve and mixed; micro-ingredients were added using the progressive enlargement method. The mixture was extruded into pellets (2.5-4.0 mm diameter, 3.0 mm length) using a DS32-II twin-screw extruder (Jinan Saixin Extrusion Machinery Co., Ltd.), air-dried at 65°C, and stored at -20°C until use.

1.2 Experimental Design and Husbandry

Juvenile Manchurian trout were obtained from the Heilongjiang River Fisheries Research Institute experimental base (Fengcheng, Liaoning) and acclimated in an indoor cold-water recirculating aquaculture system (equipped with a chiller) at Inner Mongolia University for Nationalities for 12 days. A total of 270 uniform-sized juveniles with initial mean weight of (40.2$±3.6)$gwererandomlydistributedintosixgroupswiththreereplicateseach(15fishperreplicate),witheachr$ 7.5$mg/L, ammonia < 0.25mg/L, andpH$7.6±$0.2. One-third of the water volume was exchanged or replenished every three days. The 120-day feeding trial involved three daily feedings (08:00, 13:30, 18:30) to apparent satiation.

1.3 Sampling and Analysis

At the end of the trial, fish were fasted for 24 hours before counting, length measurement, and weighing. Five fish per tank were anesthetized with MS-222, and blood was collected from the caudal vein using a 2.5 mL syringe. Fish were then dissected, and muscle samples were collected in labeled bags and stored at -20°C for subsequent nutrient analysis.

1.3.1 Growth Performance Growth parameters were calculated as follows:
Weight gain rate (WGR, %) = [(final mean weight - initial mean weight) / initial mean weight] × 100
Specific growth rate (SGR, %/d) = [(ln final mean weight - ln initial mean

weight) × 100] / feeding days
Feed intake rate (FIR, %/d) = 100 × [feed intake / feeding days × (initial mean weight + final mean weight) / 2]
Feed conversion ratio (FCR) = feed intake / (final mean weight - initial mean weight)
Protein efficiency ratio (PER) = [(final mean weight - initial mean weight) / protein intake] × 100

1.3.2 Serum Sample Preparation and Biochemical Analysis Blood samples were allowed to clot at room temperature for 2 hours, then centrifuged at 4,000 r/min for 10 minutes at 4°C to obtain serum. Serum biochemical parameters including TC, TG, HDL-C, LDL-C, ALT, and AST were measured using a Pronto-E automatic biochemical analyzer (BPC, Italy).

1.3.3 Determination of Feed Nutrients and Tissue Vitamin E Content Feed moisture, crude protein, crude lipid, and ash contents were analyzed according to AOAC (2000) methods. Vitamin E concentrations in feed, muscle, and liver were determined by high-performance liquid chromatography following Xue et al. and Salo-Väänänen et al., respectively.

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0 software with one-way ANOVA and t-tests for significance testing, followed by Duncan' s multiple comparison test ($P<0.05$). Broken-line regression analysis was used to determine the optimal dietary vitamin E requirement for juvenile Manchurian trout.

2.1 Effects of Dietary Vitamin E Level on Growth Performance

As shown in Table 2 , final weight (FW), weight gain rate (WGR), and specific growth rate (SGR) increased initially then decreased with rising dietary vitamin E levels. The 165.5 mg/kg group exhibited significantly higher FW than all other groups ($P<0.05$), while WGR and SGR in this group were significantly higher than those in the 16.6 and 1214.7 mg/kg groups ($P<0.05$) but did not differ significantly from other groups ($P>0.05$). Dietary vitamin E level had no significant effect on feed intake rate, feed conversion ratio, or protein efficiency ratio ($P>0.05$). Broken-line regression analysis of the relationship between dietary vitamin E level and WGR indicated an optimal dietary vitamin E requirement of 145.87 mg/kg (Figure 1 [Figure 1: see original paper]).

2.2 Effects of Dietary Vitamin E Level on Serum Biochemical Parameters

As presented in Table 3 , serum TG and TC concentrations decreased progressively with increasing dietary vitamin E, with the 615.5 and 1214.5 mg/kg groups showing significantly lower values than other groups ($P<0.05$). Serum HDL-C concentration increased initially then decreased, peaking at 165.5 mg/kg, which was significantly higher than all other groups ($P<0.05$); the 64.9 and 316.2 mg/kg groups also showed significantly higher HDL-C than the 16.6, 615.5, and 1214.5 mg/kg groups ($P<0.05$). Conversely, LDL-C displayed the opposite trend, with the 165.5 and 316.2 mg/kg groups showing significantly lower values than other groups ($P<0.05$). Serum ALT and AST activities showed slight decreasing trends with increasing dietary vitamin E but did not differ significantly among groups ($P>0.05$).

2.3 Effects of Dietary Vitamin E Level on Tissue Vitamin E Accumulation

As shown in Figure 2 [Figure 2: see original paper], liver vitamin E accumulation increased significantly with dietary vitamin E level ($P<0.05$), except between the 615.5 and 1214.5 mg/kg groups ($P>0.05$). Muscle vitamin E accumulation increased initially then plateaued when dietary vitamin E exceeded 165.5 mg/kg (approximately 43 µg/g wet muscle weight), with the 165.5, 316.2, 615.5, and 1214.5 mg/kg groups showing significantly higher values than the 16.6 and 64.9 mg/kg groups ($P<0.05$).

Broken-line regression analysis of the relationship between dietary vitamin E level and muscle vitamin E accumulation indicated an optimal dietary vitamin E requirement of 180.98 mg/kg (Figure 3 [Figure 3: see original paper]).

3.1 Vitamin E Requirement of Manchurian Trout

Numerous studies on vitamin E requirements in aquatic animals have demonstrated that appropriate dietary levels improve growth performance, while deficiency or excess reduces immunity and stress resistance, inhibits growth, and excessive supplementation may decrease HDL synthesis, impair lipid metabolism, and cause hepatic vitamin E deposition and toxicity. Pan et al. reported that dietary vitamin E supplementation at 0–225 mg/kg in grass carp (initial weight 266 g) for 10 weeks yielded an optimal requirement of 116.2 mg/kg for best growth performance, with deficiency inhibiting growth, reducing survival, and increasing skin lesions. Lin et al. found that 100 mg/kg dietary vitamin E significantly improved growth of grouper (*Epinephelus malabaricus*) compared to 25 and 50 mg/kg. Zhang et al. reported that appropriate dietary vitamin E enhanced growth performance in juvenile Chinese sucker (*Myxocyprinus asiaticus*), while excessive levels inhibited growth. Our results showed that appropriate dietary vitamin E (145.87–180.98 mg/kg) improved growth performance in juvenile Manchurian trout, while excessive levels inhibited growth, consistent with previous findings. No obvious deficiency symptoms or toxicity were observed during the trial, though one fish in the 16.6 mg/kg group developed deformities and tail curvature, and another exhibited belly-up swimming behavior in the later stage; no toxicity was observed in the highest 1214.7 mg/kg group. However, vitamin E effects on growth vary among species and levels, with different aquatic animals and different life stages of the same species showing varying requirements. Additionally, vitamin E requirements are influenced by dietary nutrients (PUFA, vitamins A and C, trace elements) and environmental factors.

Tissue vitamin E content is affected by dietary vitamin E level, showing positive correlation within a certain range, though accumulation in tissues such as muscle and liver approaches saturation at high intake levels, with differential accumulation among tissues. In aquatic animal nutrition research, liver and muscle vitamin E accumulation serves as an important indicator of vitamin E requirement. Our study determined an optimal dietary vitamin E requirement of 180.98 mg/kg based on muscle vitamin E accumulation using broken-line regression. Liver vitamin E accumulation was higher than muscle, with both increasing with dietary vitamin E level, but muscle accumulation plateaued at a threshold, suggesting that vitamin E absorption and metabolism primarily occur in the liver, which may function as a "vitamin E reservoir." When dietary vitamin E level matches metabolic demand, tissue accumulation correlates positively with dietary level; when dietary level exceeds metabolic demand, excess vitamin E may deposit in the liver and be transported via circulation to various tissues.

3.2 Effects of Dietary Vitamin E Level on Serum Biochemical Parameters

Serum biochemical parameters reflect changes in growth, health status, and immune function, serving as early indicators of tissue and organ damage and diagnostic markers for disease. Multiple factors influence serum biochemical parameters in fish, including environmental conditions, dietary composition, and stress levels.

Serum TG and TC concentrations are important physiological indicators of lipid metabolism and fat deposition. Excess dietary energy elevates blood TC and TG, promoting hepatic fat accumulation. Our results showed that appropriate vitamin E levels reduced serum TG and TC concentrations and improved lipid metabolism in Manchurian trout, consistent with findings in turbot fed high-fat diets, black porgy, and broilers. This may be due to enhanced activity of TG and TC catabolic enzymes, facilitating transport and excretion. Appropriate dietary vitamin E increased serum HDL-C and decreased LDL-C in Manchurian trout, indicating effects on blood and liver function, consistent with Anwar et al.' s report that vitamin E is transported via chylomicrons to HDL, increasing serum HDL-C. Elevated HDL-C is considered beneficial, while decreased HDL-C may indicate hepatic dysfunction or metabolic issues warranting further investigation. Appropriate vitamin E supplementation slightly reduced serum ALT and AST activities compared to the unsupplemented group, consistent with El-Demerdash et al. and Li et al. Dietary vitamin E levels above 165.5 mg/kg reduced serum TC, TG, LDL-C, ALT, and AST while increasing HDL-C, possibly by elevating serum vitamin E content and reducing lipid peroxidation, thereby minimizing cellular structural damage. Limited reports exist on vitamin E effects on fish serum biochemistry, though vitamin E reduced TC and TG in grass carp and black porgy fed oxidized fish oil; the underlying mechanisms require further exploration.

Appropriate dietary vitamin E supplementation improved growth performance and serum biochemical parameters while increasing vitamin E accumulation in liver and muscle of Manchurian trout. Based on broken-line regression analysis using weight gain rate and muscle vitamin E deposition as criteria, the optimal dietary vitamin E requirements were 145.87 mg/kg and 180.98 mg/kg, respectively.

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