# Postprint: Tolerance of Largemouth Bass to Dietary Selenium Yeast

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**Date:** 2017-10-23T00:00:00+00:00

## Abstract

This experiment aimed to evaluate the tolerance of largemouth bass to dietary yeast selenium by investigating its effects on growth performance, plasma biochemical indices, tissue antioxidant indices, and liver histological structure. Yeast selenium was supplemented to the basal diet at 0 (Y0), 0.5 (Y0.5), 2.5 (Y2.5), and 5.0 mg/kg (Y5.0) (expressed as selenium), where 0.5 mg/kg is the maximum recommended dose, and 2.5 and 5.0 mg/kg represent 5 and 10 times the maximum recommended dose (0.5 mg/kg), respectively. The background selenium content of the basal diet was 0.76 mg/kg. Largemouth bass with an initial body weight of (12.99$±$0.01) g were randomly divided into 4 groups, with 6 replicates per group and 20 fish per replicate, and the experimental period lasted for 10 weeks. The results showed that the Y0 group had the lowest weight gain rate and feed intake rate, and its feed conversion ratio was also the lowest, all significantly lower than those of the other groups ($P<0.05$). The activity of alkaline phosphatase in plasma of the Y0 group was significantly higher than that of the other groups ($P<0.05$). The high-density lipoprotein cholesterol content in plasma of the Y0.5 group was significantly higher than that of the other groups ($P<0.05$). The urea nitrogen content in plasma of the Y2.5 group was significantly higher than that of the other groups ($P<0.05$). The immunoglobulin M content in plasma of the Y2.5 and Y5.0 groups was significantly higher than that of the Y0 and Y0.5 groups ($P<0.05$). Compared with the Y0 group, the addition of yeast selenium significantly reduced the malondialdehyde content in plasma ($P<0.05$) and significantly increased the activity of glutathione peroxidase in plasma ($P<0.05$). The hepatic selenium content in the Y5.0 group was significantly higher than that in the Y0 and Y0.5 groups ($P<0.05$), with no significant difference from the Y2.5 group ($P>0.05$). Daily selenium intake and hepatic selenium content showed a significant linear correlation ($P<0.05$), with hepatic selenium content increasing linearly with increasing daily selenium intake. All groups of largemouth bass exhibited varying

degrees of liver damage, but the addition of 0.5 mg/kg yeast selenium had an alleviating effect on liver injury. Based on these results, dietary supplementation with 0.5 mg/kg yeast selenium (total selenium content of 1.29 mg/kg) promoted lipid metabolism to a certain extent, provided antioxidant protection, and was safe for largemouth bass. Under the conditions of this experiment, integrating growth performance, plasma biochemical indices, tissue antioxidant indices, and liver histological structure, when the background selenium content of the diet was 0.76 mg/kg, the tolerable dose of dietary yeast selenium for largemouth bass was 0.5 mg/kg (expressed as selenium), which is the maximum recommended dose for selenium, with a safety factor of 1. Animal protein sources such as fish meal and krill meal contain relatively high levels of selenium; therefore, supplementation of selenium in high-fish-meal aquatic animal feeds should be approached with caution.

## Full Text

# Tolerance of Selenium-Yeast in Diets of Largemouth Bass (Micropterus salmoides)

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### Abstract

This study investigated the tolerance of selenium-yeast in largemouth bass (*Micropterus salmoides*) by examining its effects on growth performance, plasma biochemical indices, tissue antioxidant capacity, and liver histology. Four experimental diets were formulated with selenium-yeast supplementation at 0 (Y0), 0.5 (Y0.5), 2.5 (Y2.5), and 5.0 mg/kg (Y5.0) (as Se). The 0.5 mg/kg level represented the maximum recommended dose, while 2.5 and 5.0 mg/kg were 5- and 10-fold multiples of this recommendation, respectively. The basal diet contained 0.76 mg/kg background selenium. Juvenile largemouth bass with initial body weight of (12.99$±$0.01) g were randomly assigned to four groups (n=6 replicates, 20 fish per replicate) and fed for 10 weeks. The results showed that fish in the Y0 group exhibited the lowest weight gain rate, feeding rate, and feed conversion ratio, all significantly lower than other groups ($P<0.05$). Plasma alkaline phosphatase activity in Y0 was significantly higher than in all other groups ($P<0.05$). The Y0.5 group showed significantly higher plasma high-density lipoprotein cholesterol content compared to other groups ($P<0.05$). Plasma urea nitrogen content in Y2.5 was significantly higher than in other groups ($P<0.05$). Plasma immunoglobulin M content in Y2.5 and Y5.0 groups was significantly higher than in Y0 and Y0.5 groups ($P<0.05$). Compared with

Y0, selenium-yeast supplementation significantly decreased plasma malondialdehyde content ($P<0.05$) and increased plasma glutathione peroxidase activity ($P<0.05$). Liver selenium content in Y5.0 was significantly higher than in Y0 and Y0.5 groups ($P<0.05$), but did not differ significantly from Y2.5 ($P>0.05$). Daily selenium intake showed a significant linear correlation with liver selenium content ($P<0.05$), with liver selenium increasing linearly as daily intake increased. All groups exhibited varying degrees of liver damage, though supplementation with 0.5 mg/kg selenium-yeast alleviated hepatic injury. These findings indicate that dietary supplementation with 0.5 mg/kg selenium-yeast (total Se content 1.29 mg/kg) promotes lipid metabolism and provides antioxidant protection in largemouth bass without adverse effects. Under the conditions of this study, comprehensive evaluation of growth performance, plasma biochemistry, tissue antioxidant indices, and liver histology suggests that the tolerance dose of selenium-yeast for largemouth bass is 0.5 mg/kg (as Se) when dietary background selenium is 0.76 mg/kg, equivalent to the maximum recommended level with a safety margin of 1. Animal protein sources such as fish meal and krill meal contain substantial selenium, warranting caution when supplementing selenium in high-fish-meal aquafeeds.

**Keywords:** largemouth bass (*Micropterus salmoides*); selenium-yeast; tolerance; growth; antioxidant; histology

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Largemouth bass (*Micropterus salmoides*), commonly known as California bass, belongs to Perciformes, Centrarchidae, *Micropterus*. Introduced from California, USA in the 1980s, it has become an important freshwater economic fish species in China. As a eurythermal carnivorous fish, largemouth bass is highly sensitive to dietary oxidation. Selenium (Se), discovered by Swedish chemist Berzelius in 1817, is an essential trace element that serves as a critical component of glutathione peroxidase (GPX), protecting organisms from oxidative damage by peroxides and free radicals while maintaining cellular structural integrity and function. Selenium also regulates inflammatory and immune responses, thyroid hormone secretion, and exhibits anti-tumor activity. Dietary selenium is supplemented as either inorganic forms (sodium selenite and selenate) or organic forms (selenium-yeast and selenomethionine), with organic selenium showing lower toxicity and superior bioavailability compared to inorganic forms. Selenium deficiency inhibits fish growth, while excessive levels are toxic. Previous studies demonstrated growth-promoting effects of 0.24–0.32 mg/kg selenium-yeast in common carp, 0.4 mg/kg in Japanese seabass, and 0.6 mg/kg in juvenile *Elopichthys bambusa*. Conversely, dietary selenium exceeding 3.0 and 4.6 mg/kg increased mortality in rainbow trout and suckers, respectively. Despite widespread application of selenium-yeast, its safety threshold and risk assessment in aquafeeds remain undefined. Therefore, this study evaluated selenium-yeast tolerance in largemouth bass using the maximum recommended dose (0.5 mg/kg) from Ministry of Agriculture Announcement No. 1224 and EU regulations to establish safe limits in aquaculture feeds.

## Materials and Methods

### Experimental Fish

Juvenile largemouth bass were purchased from Foshan Sanshui Baijin Seed Company in June 2015. Prior to the experiment, fish were acclimated for two weeks in the culture system and fed the basal diet without selenium-yeast supplementation.

### Experimental Diets

Following the Ministry of Agriculture' s "Guidelines for Aquatic Target Animal Tolerance Evaluation of Feed Ingredients and Additives (Trial)," four experimental diets were prepared by supplementing a basal diet with selenium-yeast (provided by Lallemand, France, containing 2 g/kg Se) at 0, 0.5, 2.5, and 5.0 mg/kg (as Se), designated Y0, Y0.5, Y2.5, and Y5.0, respectively. The 0.5 mg/kg level represented the maximum recommended dose (Ministry of Agriculture Announcement No. 1224), with 2.5 and 5.0 mg/kg as 5- and 10-fold multiples. Diets were extruded into 2 mm pellets (soybean lecithin dissolved in fish oil before pelleting), air-dried, and stored at -20 °C. The analyzed selenium contents were 0.76, 1.29, 3.50, and 6.35 mg/kg for Y0, Y0.5, Y2.5, and Y5.0, respectively. To simulate practical conditions, diets were stored at room temperature under dark conditions after the trial began. Dietary composition and nutrient levels are presented in Table 1 .

### Experimental Design and Husbandry

The trial was conducted at the National Aquafeed Safety Assessment Station (Beijing Nankou) using an indoor recirculating aquaculture system. Healthy, uniform largemouth bass [average initial weight $(12.99\$\pm0.01)g]were randomly stocked into 24 conical tanks(0.26$ volume) at 20 fish per tank. Four dietary groups were established (n=6 replicates per group). The 10-week feeding trial involved apparent satiation feeding twice daily at 08:00 and 16:00. Water quality parameters were maintained as follows: dissolved oxygen >7.0 mg/L, total ammonia nitrogen <0.3 mg/L, pH 7.5–8.5, and temperature 23–25 °C. At trial termination, fish were fasted for 24 h before final weighing and sampling.

### Sample Collection and Analysis

**Growth Performance** Growth parameters were calculated using the following formulas:

- Survival rate (SR, %) = 100 × Nt/N0
- Weight gain rate (WGR, %) = 100 × (Wt - W0 + Wd)/W0
- Specific growth rate (SGR, %/day) = 100 × (lnWt - lnW0)/t
- Feed conversion ratio (FCR) = C/(Wt - W0 + Wd)
- Feeding rate [FR, %/(kg BW · d)] = 100 × C/[(W0 + Wt + Wd)/2]/t
- Daily selenium intake [mg/(kg BW · d)] = FR × B

Where N0 = initial fish number, Nt = final fish number, W0 = initial total weight (g), Wt = final total weight (g), Wd = weight of dead fish (g), C = feed intake (g), BW = body weight, B = dietary selenium content, and t = experimental days.

**Morphometric Indices** Three fish per tank were randomly selected to measure body length, body weight, liver weight, and viscera weight for calculating:

- Condition factor (CF, g/cm$^3$) = 100 × average body weight/average body length$^3$
- Hepatosomatic index (HSI, %) = 100 × liver weight/body weight
- Viscerasomatic index (VSI, %) = 100 × viscera weight/body weight

**Chemical Analysis** Dietary crude protein, crude lipid, moisture, ash, and gross energy were determined by Kjeldahl, acid hydrolysis, 105 °C drying, 550 °C incineration, and bomb calorimetry methods, respectively. Selenium content in diets and liver tissue was analyzed according to GB/T 13883–2008.

**Plasma Biochemistry and Tissue Antioxidant Indices** Six fish per tank were anesthetized with 80 mg/L chlorobutanol, and blood was collected from the caudal vein using sodium fluoride-potassium oxalate anticoagulant. Plasma was separated by centrifugation (4 °C, 4,000 r/min, 10 min) and stored at -80 °C for analysis of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total protein (TPRO), albumin (ALB), glucose (GLU), urea nitrogen (UN), total bile acid (TBA), total bilirubin (TBILI), immunoglobulin M (IgM), alkaline phosphatase (AKP), aspartate transaminase (AST), and alanine transaminase (ALT) using commercial kits (Nanjing Jiancheng Bioengineering Institute).

Four fish per tank were randomly selected for tissue antioxidant analysis. Liver, heart, and muscle samples were stored at -80 °C for determination of malondialdehyde (MDA), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST) using commercial kits (Nanjing Jiancheng Bioengineering Institute).

**Histological Examination** Two fish per tank were randomly selected, and liver tissue samples (0.5 cm × 0.5 cm × 0.5 cm) were fixed in 4% paraformaldehyde for 24 h after rinsing with 0.7% saline. Following dehydration, clearing, and paraffin embedding, 7  m sections were prepared, stained with hematoxylin-eosin (HE), and examined under a light microscope (Leica DM2500, Germany).

**Statistical Analysis** Data were analyzed by one-way ANOVA using SPSS 17.0 software, with Duncan' s multiple range test for post-hoc comparisons. Significance was set at $P<0.05$. Results are presented as mean ± standard error (SE).

## Results

### Effects of Selenium-Yeast on Growth Performance and Morphometric Indices

As shown in Table 2 , although weight gain rates were relatively higher in selenium-yeast supplemented groups, no significant differences were observed in final body weight, weight gain rate, or specific growth rate among groups ($P>0.05$). However, feeding rate and feed conversion ratio in all selenium-yeast groups were significantly higher than in Y0 ($P<0.05$), with no significant differences among supplemented groups ($P>0.05$). No significant differences were found in condition factor, hepatosomatic index, or viscerasomatic index ($P>0.05$), though HSI tended to decrease in selenium-yeast groups.

### Effects of Selenium-Yeast on Plasma Biochemical Indices

Table 3 presents plasma biochemical parameters. No significant differences were observed among groups in LDL-C, TBA, GLU, ALB, TBILI, AST, ALT, HDL-C/TC, or LDL-C/TC ($P>0.05$). The Y0.5 group exhibited significantly higher TG and TC contents than other groups ($P<0.05$), though values remained within reference ranges. Plasma HDL-C content was also significantly higher in Y0.5 than in other groups ($P<0.05$). Plasma AKP activity decreased with increasing selenium-yeast supplementation, showing significant differences between most groups ($P<0.05$) except between Y2.5 vs. Y0.5 and Y5.0 ($P>0.05$). TPRO content in Y5.0 was significantly higher than in Y0 ($P<0.05$) but similar to Y0.5 and Y2.5 ($P>0.05$). UN content in Y2.5 was significantly higher than in other groups ($P<0.05$), though all values fell within reference ranges. IgM content in Y2.5 and Y5.0 was significantly higher than in Y0 and Y0.5 ($P<0.05$).

### Effects of Selenium-Yeast on Antioxidant Indices

**Liver** Table 4 shows that liver MDA content in Y2.5 was significantly higher than in other groups ($P<0.05$), though all values remained below reference ranges. No significant differences in MDA were observed among Y0, Y0.5, and Y5.0 ($P>0.05$). Liver T-AOC and activities of SOD and GST in Y0.5, as well as T-AOC and activities of SOD, CAT, GST, and GPX in Y2.5, were significantly higher than in Y0 ($P<0.05$). However, GPX and GST activities in Y5.0 were significantly lower than in Y0 ($P<0.05$).

**Muscle** Table 5 indicates no significant differences among groups in muscle SOD and CAT activities or MDA content ($P>0.05$). Muscle T-AOC in Y2.5 was significantly higher than in Y5.0 ($P<0.05$) but similar to Y0 and Y0.5 ($P>0.05$). GPX and GST were not detected in muscle tissue.

**Heart** Table 6 reveals no significant differences among groups in heart T-AOC or activities of SOD, CAT, GPX, or MDA content ($P>0.05$). GST was not detected in heart tissue.

**Plasma** Table 7 demonstrates that plasma CAT activity did not differ significantly among groups (P>0.05). Plasma MDA content in Y0 was significantly higher than in other groups (P<0.05). Plasma T-AOC in Y5.0 was significantly lower than in other groups (P<0.05), while SOD activity was significantly higher (P<0.05). GPX and GST activities in Y2.5 were significantly higher than in Y0 and Y0.5 (P<0.05) but similar to Y5.0 (P>0.05).

### Effects of Selenium-Yeast on Daily Selenium Intake and Liver Selenium Content

Considering the background selenium content (0.76 mg/kg), total selenium content was used to calculate daily intake. Table 8 shows no significant differences in daily selenium intake among groups (P>0.05). Liver selenium content in Y5.0 was significantly higher than in Y0 and Y0.5 (P<0.05) but not significantly different from Y2.5 (P>0.05). Linear regression analysis revealed a significant positive correlation between daily selenium intake and liver selenium content (P<0.05), with liver selenium increasing linearly with intake (Figure 1 [Figure 1: see original paper]).

### Hepatic Histology

Figure 2 [Figure 2: see original paper] illustrates varying degrees of liver damage across groups. In the control group (Y0), 6 of 12 fish showed normal histology while 6 exhibited hepatocyte membrane dissolution, unclear intercellular spaces, and cellular disintegration. In Y0.5, 10 of 12 fish appeared normal with only 2 showing intercellular space abnormalities. Y2.5 showed 10 normal and 2 damaged fish with membrane dissolution. Y5.0 exhibited the most severe damage, with only 4 normal fish and 8 showing membrane disappearance and cellular disintegration.

## Discussion

### Effects on Growth Performance

Although no statistical differences in weight gain were observed, Y0 (without selenium-yeast) showed the lowest weight gain rate, specific growth rate, and final body weight. Selenium-yeast supplementation at 0.5, 2.5, and 5.0 mg/kg marginally improved growth performance but increased feed conversion ratio. These results contrast with previous studies reporting improved growth in mandarin fish, Chinese shrimp, and grouper with selenium supplementation, suggesting species-specific differences in selenium absorption and antioxidant capacity. Research in rats demonstrated that 4–5 mg/kg selenium from various sources significantly decreased body weight, with more pronounced effects at 15 mg/kg. The basal diet' s selenium content (0.76 mg/kg) already exceeded the maximum recommended level (0.5 mg/kg), potentially meeting the basal requirements of largemouth bass and explaining the lack of significant growth improvement. Animal protein sources like fish meal and krill meal contain substantial selenium,

necessitating caution when supplementing selenium in high-fish-meal diets.

### Effects on Plasma Biochemistry

Blood biochemical parameters provide crucial information for assessing nutritional status, metabolism, and disease in fish. TPRO comprises ALB and globulins, with ALB synthesized by the liver to maintain colloidal osmotic pressure and support tissue repair. While selenium-yeast did not significantly affect ALB, it increased TPRO content, indicating enhanced protein deposition and tissue growth. TC and TG reflect lipid absorption and metabolism. HDL-C transports cholesterol to the liver for metabolism; reduced HDL-C impairs cholesterol clearance and hepatic lipid metabolism. At 2.5 and 5.0 mg/kg supplementation, HDL-C fell below reference ranges while TC and TG remained within normal limits, suggesting inhibited cholesterol metabolism. Conversely, 0.5 mg/kg supplementation increased HDL-C significantly while maintaining TC and TG within reference ranges, indicating improved lipid transport and metabolism similar to findings in loach and rats.

AKP, AST, and ALT activities reflect hepatic damage, with elevated plasma activities indicating hepatocyte injury. The significant reduction in AKP activity with selenium-yeast supplementation suggests hepatoprotective effects, possibly through enhanced bile acid circulation, though TBA content remained unchanged. Plasma IgM is a key indicator of specific humoral immunity. The highest IgM levels at 2.5 and 5.0 mg/kg supplementation suggest that excessive selenium intake may activate the immune system and trigger inflammatory responses, indicating potential risks at high doses.

### Effects on Antioxidant Capacity

Selenium-yeast at 0.5 and 2.5 mg/kg enhanced liver T-AOC and activities of SOD, CAT, GPX, and GST. The antioxidant defense system, comprising SOD, CAT, and GPX, is essential for scavenging reactive oxygen species (ROS). As the active center of GPX, selenium exerts its antioxidant effects primarily through this enzyme. SOD catalyzes superoxide anion dismutation, while CAT converts hydrogen peroxide to water and oxygen, collectively protecting tissues from oxidative damage. The significant increase in liver MDA content at 2.5 mg/kg, despite all values remaining below reference ranges, and the elevation of T-AOC and antioxidant enzyme activities at 0.5 mg/kg indicate enhanced antioxidant capacity and hepatic protection. However, 5.0 mg/kg supplementation significantly reduced GPX and GST activities, suggesting that excessive selenium induced oxidative stress and over-activated the antioxidant system.

Heart and muscle tissues showed less sensitivity to selenium, with no significant differences in antioxidant parameters among groups. The absence of GST in heart tissue may reflect tissue-specific expression patterns or insensitivity to selenium-induced stress.

Plasma GPX and SOD activities increased while MDA content decreased with

selenium-yeast supplementation, consistent with studies in crucian carp and broiler chickens. The elevation of SOD activity indicates enhanced free radical scavenging capacity. However, the significant reduction in plasma T-AOC at 5.0 mg/kg suggests that excessive selenium may generate ROS and cause cellular damage in various organs, indicating toxic effects.

### Effects on Liver Histology

Under intensive aquaculture, largemouth bass primarily consume fresh fish, with complete formulated diets still under development. The main pathological response to artificial diets is hepatobiliary damage, associated with dietary carbohydrate levels and lipid oxidation. Histological analysis revealed liver damage in all groups, particularly in Y0 and Y5.0, which showed high proportions of hepatocyte membrane disappearance, intercellular space abnormalities, and cellular disintegration. Supplementation at 0.5 mg/kg alleviated these lesions, though 2.5 mg/kg increased liver MDA and plasma IgM, suggesting that excessive selenium induced lipid peroxidation and immune activation. The trial simulated field feeding conditions with diets stored under high temperature, humidity, and natural light for 10 weeks, causing some oxidative deterioration. Although fish oil was supplemented with 200 mg/kg TBHQ as an antioxidant, liver damage may also relate to high dietary carbohydrate levels. Previous studies reported hepatic lesions when dietary starch exceeded 10% in largemouth bass. Additionally, high selenium levels exhibit hepatotoxicity in fish. The results indicate that 0.5 mg/kg selenium-yeast (total Se 1.29 mg/kg) effectively mitigated hepatic damage, though selenium supplementation alone could not completely prevent oxidative tissue injury.

## Conclusions

1. Dietary supplementation with 0.5 mg/kg selenium-yeast (total Se content 1.29 mg/kg) promotes lipid metabolism and provides antioxidant protection in largemouth bass without adverse effects.
2. Under the conditions of this study, comprehensive evaluation of growth performance, plasma biochemistry, tissue antioxidant indices, and liver histology indicates that the tolerance dose of selenium-yeast for largemouth bass is 0.5 mg/kg (as Se) when dietary background selenium is 0.76 mg/kg, equivalent to the maximum recommended level with a safety margin of 1.
3. Animal protein sources such as fish meal and krill meal contain substantial selenium, necessitating caution when supplementing selenium in high-fish-meal aquafeeds.

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