Effects of Dietary Glutathione Supplementation on Growth Performance, Body Composition, Serum Biochemical Parameters, and Ammonia Nitrogen Stress Resistance in Juvenile Yellow Catfish (Pelteobagrus fulvidraco) Postprint

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Abstract

This study aimed to investigate the effects of dietary glutathione supplementation on growth performance, body composition, serum biochemical indices, and resistance to ammonia nitrogen stress in yellow catfish (Pelteobagrus fulvidraco). Eight hundred yellow catfish with an initial body weight of (1.32$±$0.01) g were randomly allocated into five groups with four replicates per group and 40 fish per replicate. The control group was fed a basal diet, while the experimental groups were fed test diets supplemented with 100, 300, 500, or 700 mg/kg reduced glutathione. After 56 days of feeding, an ammonia nitrogen stress test was conducted using ammonium chloride. The results showed: 1) With increasing dietary glutathione levels, weight gain, specific growth rate, and protein efficiency of yellow catfish exhibited a trend of initially increasing then decreasing, reaching maximum values at 300 mg/kg supplementation, and the 100–500 mg/kg groups were significantly higher than the control group ($P<0.05$). No significant differences were observed in feed conversion ratio, condition factor, or hepatosomatic index among groups ($P>0.05$). 2) Compared with the control group, whole-body crude protein in the 300–700 mg/kg groups and whole-body crude lipid in the 100–700 mg/kg groups were significantly increased ($P<0.05$). 3) Dietary glutathione supplementation had no significant effects on serum total protein, cholesterol, triglyceride, glucose, urea nitrogen concentrations, or aspartate aminotransferase and alanine aminotransferase activities in yellow catfish ($P>0.05$). 4) Following ammonia nitrogen stress, the time to death in experimental groups was delayed compared with the control group, and at 96 h, cumulative mortality in all experimental groups was lower than that in the control group, with significant reductions in the 100 and 300 mg/kg groups ($P<0.05$).

In conclusion, dietary glutathione supplementation can enhance growth performance, whole-body crude protein and crude lipid contents, and resistance to ammonia nitrogen stress in juvenile yellow catfish. By fitting a quadratic regression equation to specific growth rate and glutathione supplementation level, the optimal dietary glutathione supplementation level for juvenile yellow catfish was determined to be 357.69 mg/kg.

Full Text

Effects of Dietary Glutathione on Growth Performance, Body Composition, Serum Biochemical Indices and Anti-Ammonia-Nitrogen Stress Ability of Juvenile Yellow Catfish (Pelteobagrus fulvidraco)

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Abstract

This experiment was conducted to investigate the effects of dietary glutathione on growth performance, body composition, serum biochemical indices and anti-ammonia-nitrogen stress ability of juvenile yellow catfish (*Pelteobagrus fulvidraco*). A total of 800 juvenile yellow catfish with an initial body weight of (1.32$±$0.01) g were randomly divided into 5 groups with 4 replicates per group and 40 fish per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 100, 300, 500, or 700 mg/kg reduced glutathione. After 56 days of feeding, an ammonia-nitrogen stress test was conducted using ammonium chloride. The results showed that: 1) With increasing dietary glutathione supplementation, weight gain, specific growth rate and protein efficiency ratio of yellow catfish increased initially and then decreased, reaching maximum values at 300 mg/kg supplementation, and the 100–500 mg/kg groups were significantly higher than the control group ($P<0.05$). No significant differences were observed in feed conversion ratio, condition factor or hepatosomatic index among all groups ($P>0.05$). 2) Compared with the control group, whole-body crude protein content in the 300–700 mg/kg groups and whole-body crude lipid content in the 100–700 mg/kg groups were significantly increased ($P<0.05$). 3) Dietary glutathione had no significant effects on serum total protein, cholesterol,

triglycerides, glucose, urea nitrogen contents or aspartate aminotransferase and alanine aminotransferase activities (P>0.05). 4) Following ammonia-nitrogen stress, the time to death in experimental groups was delayed compared with the control group. At 96 h, the cumulative mortality rates in all experimental groups were lower than in the control group, with the 100 and 300 mg/kg groups showing significant reductions (P<0.05). In conclusion, dietary glutathione supplementation can improve growth performance, whole-body crude protein and crude lipid contents, and anti-ammonia-nitrogen stress ability in juvenile yellow catfish. Quadratic regression analysis of specific growth rate against dietary glutathione level indicated that the optimal supplementation level was 357.69 mg/kg.

Keywords: glutathione; yellow catfish (*Pelteobagrus fulvidraco*); growth performance; body composition; serum biochemical indices; ammonia-nitrogen stress

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Yellow catfish (*Pelteobagrus fulvidraco*), also known as yellow stingfish, belongs to Siluriformes, Bagridae, and *Pelteobagrus* genus. It is a widely distributed bottom-dwelling economic omnivorous fish in China' s freshwater bodies, with a high flesh content averaging 67.53%. Its meat contains 15.37% protein and only 1.61% fat, making it highly popular among consumers due to its extremely low fat content [1]. With steadily increasing market demand, intensive and large-scale aquaculture has developed rapidly. However, in recent years, expanding stocking densities have introduced numerous stress factors into the culture environment, leading to reduced growth performance, increased oxidative stress, and severely constrained production improvements [2]. Therefore, applying nutritional immunomodulation by adding antioxidant substances to feed to enhance growth performance and antioxidant stress capacity in yellow catfish will positively impact healthy aquaculture practices.

Glutathione (GSH) is a biologically active peptide widely present in cells that plays important physiological functions in organisms. Research has shown that glutathione participates in free radical scavenging, detoxification, nutrient absorption, cell growth, cellular immunity, and DNA biosynthesis, and exerts significant growth-promoting and antioxidant stress-enhancing effects in fish [3]. Studies have found that dietary glutathione supplementation significantly improves growth and stress resistance in Nile tilapia (*Oreochromis niloticus* × *O. aureus*) [4], GIFT tilapia [5], and Pacific white shrimp (*Litopenaeus vannamei*) [6-7], but shows no significant growth-promoting effect in abalone (*Haliotis discus hannai*) [8]. Other studies have reported that different glutathione supplementation levels produce varying effects on growth and antioxidant performance in Nile tilapia [9] and Japanese flounder (*Paralichthys olivaceus*) [10], with appropriate levels significantly improving growth and antioxidant capacity while excessive levels cause negative effects. This indicates that glutathione has good growth-promoting and antioxidant stress effects on aquatic animals, but its efficacy depends on species and dosage. Currently, no reports exist on the nutritional physiological effects of glutathione on yellow catfish. Therefore, this

study used yellow catfish as the research object to investigate the effects of different glutathione supplementation levels in basal diets on growth performance, body composition, serum biochemical indices, and anti-ammonia-nitrogen stress ability, aiming to provide a theoretical basis for glutathione application in yellow catfish culture.

1.1 Experimental Diets

Referring to the feed formulation of Chen et al. [11], a basal diet was formulated using fish meal and soybean meal as the main protein sources, high-gluten wheat flour as the main carbohydrate source, and fish oil and soybean oil as the main lipid sources. The composition and nutrient levels are shown in Table 1 . Four experimental diets were prepared by supplementing the basal diet with 100, 300, 500, and 700 mg/kg glutathione, respectively. The crude protein levels of the four experimental diets were 41.17%, 41.43%, 41.44%, and 41.50%, and the crude lipid levels were 7.02%, 7.35%, 6.97%, and 7.03%, respectively.

Glutathione (reduced form, purity >98.0%) was purchased from AMRESCO (USA). All ingredients were passed through a 60-mesh sieve, and micro-ingredients were added following the principle of stepwise amplification. Glutathione was dissolved in water before being added to each diet. After thorough mixing, the diets were processed into 1.5 mm diameter pellets using an SLX-80 twin-screw extruder, dried at 55°C, cooled, sealed in bags, and stored at -20°C until use.

1.2 Experimental Design and Management

Juvenile yellow catfish were purchased from Huangsha Fishery Base in Qingyuan City, Guangdong Province. The fish were temporarily reared in outdoor cement tanks to approximately 1.3 g, being fed commercial feed twice daily. The feeding trial was conducted in indoor recirculating aquaculture barrels (approximately 300 L) at the Aquatic Research Laboratory of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. Eight hundred juvenile yellow catfish with a body weight of (1.32$±$0.02) g were randomly divided into 5 groups with 4 replicates per group and 40 fish per replicate. The control group was fed the basal diet, and the experimental groups were fed the four experimental diets, designated as G0, G100, G300, G500, and G700 groups. Fish were fed twice daily at 08:30 and 18:30 at 4–6% of body weight, with feeding amounts adjusted according to consumption. Waste was removed daily, and water was exchanged every 2 days at approximately 1/3 of the total volume. During the trial, water temperature was 28–32°C, ammonia nitrogen concentration was <0.2 mg/L, nitrite concentration was <0.01 mg/L, dissolved oxygen content was >6.0 mg/L, and pH was 7.5–8.0. The feeding trial lasted 56 days.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 h. The number of fish in each replicate was recorded and total weight was measured to calculate weight gain and specific growth rate. From each replicate, 15 fish were randomly selected and anesthetized with MS-222. Three fish were stored at -20°C for whole-body composition analysis, six fish were used to measure body weight, body length, liver weight, and viscera weight, and six fish were used for caudal vein blood collection. Blood samples were left at room temperature for 4 h, then centrifuged at 3,500 r/min for 10 min. The supernatant was collected as serum and stored at -80°C for later analysis.

1.4.1 Growth Performance Calculations

Survival rate (SR, %) = [(final fish number - initial fish number)/initial fish number] × 100
Weight gain (WG, g) = final mean weight - initial mean weight
Specific growth rate (SGR, %/d) = 100 × [ln(final mean weight) - ln(initial mean weight)]/feeding days
Feed conversion ratio (FCR) = total feed intake/(final body weight - initial body weight)
Protein efficiency ratio (PER, %) = (final total weight - initial total weight)/(total feed intake × dietary protein content) × 100
Protein deposition rate (PDR, %) = (body tissue protein deposition/protein intake) × 100
Condition factor (CF, g/cm^3) = (body weight/body length^3) × 100
Hepatosomatic index (HSI, %) = (liver weight/body weight) × 100

1.4.2 Proximate Composition Analysis

Crude protein content was determined by the Kjeldahl method using a semi-automatic Kjeldahl analyzer (GB/T 6432-1994). Crude lipid content was determined by ether extraction (GB/T 6433-1994). Crude ash content was determined by incineration at 550°C to constant weight (GB/T 6438-1992). Moisture content was determined by drying in an oven at 105°C to constant weight (GB/T 6435-1986) [12].

1.4.3 Serum Biochemical Indices Analysis

Serum total protein, cholesterol, triglycerides, glucose, urea nitrogen contents, and aspartate aminotransferase and alanine aminotransferase activities were measured by Guangzhou Kingmed Center for Clinical Laboratory (Guangzhou) using a Hitachi 7600 automatic biochemical analyzer.

1.5 Ammonia-Nitrogen Stress Test

After the feeding trial, 20 fish were randomly selected from each replicate for the stress test using ammonium chloride as the stressor. Ammonium chloride stock solution (10 g/L) was added to the water (approximately 150 L) to achieve a total ammonia nitrogen concentration of 105 mg/L, corresponding to a non-ionized ammonia concentration of 2.58 mg/L (at pH 7.5 and temperature (27$±$1)°C). Non-ionized ammonia concentration was calculated according to Li et al. [13]. During the test, water temperature, pH, and ammonia nitrogen concentration were monitored every 6 h and adjusted with ammonium chloride stock solution to maintain non-ionized ammonia concentration. The onset of disease and mortality of yellow catfish were observed and recorded, with dead fish removed promptly. Cumulative mortality rates at 72 and 96 h were calculated as:

Cumulative mortality rate (CMR, %) = 100 × number of dead yellow catfish at the end of stress/total number of stressed yellow catfish.

1.6 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (mean±SD, n=4). One-way ANOVA was performed using SPSS 20.0 software. If significant differences were detected among groups, Duncan' s multiple comparison test was applied. The significance level was set at $P<0.05$. Cumulative mortality rates were arcsine-transformed before one-way ANOVA.

2.1 Effects of Dietary Glutathione on Growth Performance of Yellow Catfish

As shown in Table 2 , with increasing dietary glutathione supplementation, weight gain, specific growth rate, and protein efficiency ratio of yellow catfish increased initially and then decreased, reaching maximum values in the G300 group. The G100, G300, and G500 groups were significantly higher than the control group ($P<0.05$). Compared with the control group, condition factor and hepatosomatic index in experimental groups increased, but the differences were not significant ($P>0.05$). Using specific growth rate as the evaluation index, a quadratic regression equation was established between specific growth rate (y) and dietary glutathione level (x) ($R^2=0.7222$) (Figure 1 [Figure 1: see original paper]). The calculated optimal dietary glutathione supplementation level for juvenile yellow catfish was 357.69 mg/kg.

2.2 Effects of Dietary Glutathione on Body Composition of Yellow Catfish

As shown in Table 3 , whole-body crude protein and crude lipid contents in all experimental groups were higher than in the control group. Whole-body crude protein content in the G300, G500, and G700 groups, and whole-body crude lipid content in the G100, G300, G500, and G700 groups were significantly higher

than in the control group ($P<0.05$). No significant differences were observed in whole-body dry matter or crude ash contents among all groups ($P>0.05$).

2.3 Effects of Dietary Glutathione on Serum Biochemical Indices of Yellow Catfish

As shown in Table 4 , dietary glutathione supplementation had no significant effects on serum total protein, cholesterol, triglycerides, glucose, urea nitrogen contents, or aspartate aminotransferase and alanine aminotransferase activities ($P>0.05$).

2.4 Effects of Dietary Glutathione on Anti-Ammonia-Nitrogen Stress Ability of Yellow Catfish

Mass mortality of yellow catfish occurred at 72 h after ammonia-nitrogen stress. As shown in Table 5 , cumulative mortality rates in all experimental groups were lower than in the control group at both 72 and 96 h, showing a trend of initial decrease followed by increase with rising glutathione supplementation, with the lowest values in the G300 group. At 96 h, the G100 and G300 groups were significantly lower than the control group ($P<0.05$). Comparing cumulative mortality rates at the two time points revealed that the control group reached 45.0% cumulative mortality at 72 h, while experimental groups did not reach cumulative mortality above 40% until 96 h.

3.1 Effects of Glutathione on Growth Performance of Yellow Catfish

The present results demonstrate that dietary glutathione supplementation at appropriate levels significantly improved weight gain, specific growth rate, and protein efficiency ratio in juvenile yellow catfish, indicating that glutathione can enhance growth performance. These findings are consistent with studies on Nile tilapia [4], GIFT tilapia [14], grass carp (*Ctenopharyngodon idellus*) [15], Japanese flounder [10], gibel carp (*Carassius auratus gibelio*) [16], and sea bass (*Lateolabrax japonicus*) [17]. The growth-promoting mechanisms of glutathione may be multifaceted. Zhao et al. [15] in grass carp and Wang et al. [10] in Japanese flounder suggested that the growth-promoting mechanism may be related to its molecular structure, as cysteine in glutathione is a component of coenzyme A that can break disulfide bonds in somatostatin molecules, relieving somatostatin' s inhibition of growth hormone (GH) and other hormones, ultimately exerting growth-promoting effects. Additionally, studies in pigs and sheep have found that glutathione can promote pituitary GH secretion, which upregulates hepatic growth hormone receptor (GHR) gene transcription and increases hepatic and semitendinosus insulin-like growth factor 1 (IGF-1) gene transcription, elevating serum IGF-1 levels and thereby promoting protein synthesis and improving nutrient utilization [18-19]. Zhou et al. [5] in GIFT tilapia found that dietary glutathione supplementation increased serum GH and IGF-1 contents and hepatic GH and IGF-1 mRNA expression, speculating that glutathione promotes GH secretion by consuming somatostatin, which

acts on GHR to produce IGF-1, which then acts on target organs to ultimately promote animal growth. Reports also indicate that glutathione can protect intestinal mucosa from toxin and peroxide damage in the intestinal lumen, which may facilitate nutrient absorption, as confirmed in Pacific white shrimp and Japanese flounder. The growth-promoting mechanisms of glutathione require further investigation.

Using specific growth rate as the evaluation index, the optimal dietary glutathione supplementation level for juvenile yellow catfish was calculated to be 357.69 mg/kg. This result is similar to findings in grass carp (350.00 mg/kg) [15] and GIFT tilapia (355.13 mg/kg) [14], but lower than that in Japanese flounder (368.92 mg/kg) [10] and higher than in Pacific white shrimp (174.13 mg/kg) [21], suggesting that optimal glutathione supplementation levels may vary among animal species. This study also found that yellow catfish growth performance did not show a linear relationship with dietary glutathione level; rather, growth promotion was evident within an appropriate range, while excessive supplementation reduced growth performance. This pattern is similar to results in Pacific white shrimp [20] and Japanese flounder [10], possibly because excessive glutathione exerts toxic effects. Research indicates that although glutathione is an excellent antioxidant, excessive glutathione can combine with various compounds (such as aldehydes, quinones, and haloalkenes) to produce toxic metabolites that can covalently bind to DNA and generate oxygen free radicals, with long-term accumulation of these toxic metabolites adversely affecting the organism [21]. Sah et al. [22] and Gao et al. [23] also reported that glutathione, as an oxide precursor, can be toxic at high concentrations and cause DNA damage.

Studies suggest that hepatosomatic index is a sensitive indicator of short-term and long-term nutrition in fish. Researchers have found that plant protein can increase hepatosomatic index in rainbow trout [24] and black sea bream [25]. In this study, hepatosomatic index in all experimental groups increased to varying degrees compared with the control group, possibly because glutathione promoted hepatic RNA expression and thus enhanced protein synthesis in the liver. Whole-body composition analysis showed that glutathione significantly increased whole-body crude protein content, further demonstrating that glutathione enhances protein synthesis in yellow catfish, consistent with findings in GIFT tilapia [14].

3.2 Effects of Glutathione on Whole-Body Composition and Serum Biochemical Indices of Yellow Catfish

The present results showed that whole-body crude protein and crude lipid contents in all experimental groups were higher than in the control group, with crude protein content in the G300–G700 groups and crude lipid content in the G100–G700 groups reaching significant levels. Since dietary crude protein (41.17%–41.50%) and crude lipid levels (6.97%–7.50%) were essentially consistent across all groups during the culture period, these results indicate that

dietary glutathione supplementation significantly increased whole-body crude protein and crude lipid contents in yellow catfish. These findings are similar to results in grass carp [15], GIFT tilapia [14], and Pacific white shrimp [26], but differ from those in Nile tilapia [9] where no significant differences were observed. Further research is needed on glutathione' s effects on body composition in yellow catfish.

Glucose is the primary energy substrate, and dietary nutrients affect glucose levels. Total protein is an important indicator of protein metabolism and nutritional status. Kaushik et al. [27] demonstrated that dietary protein source quality can significantly affect serum total protein content and thus protein metabolism. In this study, dietary glutathione supplementation did not significantly affect serum glucose or total protein contents in yellow catfish, consistent with findings in grass carp [15] and GIFT tilapia [14], possibly because dietary ingredients and nutrient levels were essentially consistent across all groups. Cholesterol and triglycerides are important lipid substances; cholesterol synthesizes bile and various steroid hormones, while triglycerides are important metabolic energy substrates for cells. Their levels reflect lipid absorption status and hepatic lipid metabolism. In this study, serum cholesterol and triglyceride contents in the G100–G500 groups increased to varying degrees compared with the control group, consistent with results in grass carp [15] and GIFT tilapia [14]. This may be because glutathione promoted GH secretion, enhanced lipid metabolism, and thus increased serum cholesterol and triglyceride levels. Transaminases play important roles in the conversion of the three major nutrients and synthesis of non-essential amino acids; alanine aminotransferase reflects liver function, while aspartate aminotransferase reflects heart and muscle damage [28]. In this study, dietary glutathione had no significant effects on either transaminase, but both transaminases in the G300–G700 groups were higher than in the control group, which may facilitate transamination and enhance non-essential amino acid synthesis capacity. Urea nitrogen is related to protein metabolism as the end product of purine metabolism, and its content can reflect protein metabolism and amino acid balance. Studies in grass carp [15], tilapia [14], and common carp [29] found that bioactive peptides can significantly reduce serum urea nitrogen content, but no significant effects were observed in weaned piglets [30]. The present results are similar to the latter, possibly related to experimental subjects and dietary protein levels.

3.3 Effects of Glutathione on Anti-Ammonia-Nitrogen Stress Ability of Yellow Catfish

Organisms possess a complete antioxidant defense system. Under normal conditions, free radicals are cleared by the antioxidant system, maintaining relative dynamic balance. When organisms encounter environmental stress, large amounts of reactive oxygen species attack nearby cells, causing oxidative stress that leads to DNA fragmentation, enzyme inactivation, lipid peroxidation, and apoptosis [31]. In intensive aquaculture, residual bait and excreta from aquatic

animals produce large amounts of ammonia nitrogen through ammonification, which interferes with the antioxidant defense system and induces disease [32]. Ammonia nitrogen comprises ionized and non-ionized ammonia, with toxicity primarily from non-ionized ammonia [33]. Studies have shown that ammonia nitrogen stress inhibits antioxidant enzyme activity, preventing clearance of large amounts of free radicals and causing toxic effects [34]. Long-term ammonia nitrogen stress also causes growth inhibition, reduced immunity, and even death [33]. The present results showed that cumulative mortality rates in glutathione-supplemented groups were lower than in the control group at both 72 and 96 h after ammonia nitrogen stress, with the G300 group significantly lower than the control group at both time points. As supplementation increased, cumulative mortality increased. Moreover, the control group reached 45.0% cumulative mortality at 72 h, while experimental groups did not reach cumulative mortality above 40% until 96 h. These results demonstrate that glutathione can improve anti-ammonia-nitrogen stress ability in juvenile yellow catfish, not only reducing mortality within a certain period after stress but also delaying the onset of death. The improvement in anti-ammonia-nitrogen stress ability is dosage-dependent, with excessive supplementation potentially reducing this capacity, similar to the negative effects of high supplementation on growth performance. This pattern is consistent with findings in Pacific white shrimp [6]. The enhanced anti-ammonia-nitrogen stress ability may be related to the detoxification function of glutathione' s active groups and its activation of enzyme systems (superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, etc.) [6,35]. As an antioxidant, glutathione can improve antioxidant capacity by increasing antioxidant enzyme activity [36]. The reduced mortality under ammonia nitrogen stress may result from glutathione increasing various antioxidant enzyme activities, improving antioxidant capacity, reducing oxidative damage from ammonia nitrogen stress, and ultimately decreasing mortality. Additionally, glutathione S-transferase can catalyze the conjugation of glutathione's thiol group with various electrophilic compounds, increasing their solubility and facilitating cellular excretion [37]. Current research on glutathione' s detoxification mechanisms remains insufficiently systematic, and studies on glutathione' s effects on anti-ammonia-nitrogen stress ability in yellow catfish are lacking, warranting further investigation.

In conclusion, dietary glutathione supplementation can improve growth performance, whole-body crude protein and crude lipid contents, and anti-ammonia-nitrogen stress ability in juvenile yellow catfish. Quadratic regression analysis of specific growth rate against dietary glutathione level indicated that the optimal supplementation level was 357.69 mg/kg.

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