# Effects of Glutamine on Growth Performance, Intestinal Morphology and Non-Specific Immunity Related Gene Expression in Juvenile Yellow Catfish (Pelteobagrus fulvidraco) Postprint

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## Abstract

This experiment aimed to investigate the effects of glutamine on growth performance, intestinal morphology, and expression of nonspecific immunity-related genes in juvenile yellow catfish. A total of 240 juvenile yellow catfish with a body weight of (2.49$±$0.04) g were selected and randomly divided into 4 groups, with 3 replicates per group and 20 fish per replicate. Four experimental diets containing 0 (control), 0.10%, 0.20%, and 0.40% glutamine were formulated, with each diet randomly fed to one group of experimental fish for a feeding period of 70 d. The results showed that increasing dietary glutamine had no significant effect on growth performance indices of juvenile yellow catfish (P>0.05). Compared with the control group, dietary Gln supplementation promoted the development of intestinal villi, with increased villus height and fold depth but decreased muscular layer thickness; specifically, the 0.20% and 0.40% groups showed significantly increased intestinal villus height and fold depth (P<0.05) and significantly reduced muscular layer thickness (P<0.05). Within the glutamine supplementation range of 0.10%~0.20%, the relative mRNA expression of metallothionein (MT) showed the most pronounced change in muscle, with all supplementation groups being significantly higher than the control group (P<0.05); the relative mRNA expression of glutathione S-transferase (GST) showed the most pronounced change in intestine, with the 0.20% group being significantly higher than the control group (P<0.05); the relative mRNA expression of hepcidin (Hepc) and constitutive heat shock protein 70 (Hsc70) showed the most pronounced changes in liver, with Hepc mRNA relative expression in the 0.10% group reaching 73 times that of the control group (P<0.05), and Hsc70 mRNA relative expression in the 0.20% group reaching 3 times that of the control group (P<0.05). It can be concluded that supplementation of basal diets with a certain amount of glutamine can enhance the nonspecific immunity

and antioxidant capacity of juvenile yellow catfish.

## Full Text

# Effects of Glutamine on Growth Performance, Intestinal Morphology and Non-Specific Immune Related Gene Expression of Juvenile Yellow Catfish (Pelteobagrus fulvidraco)

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**Abstract:** This study investigated the effects of dietary glutamine (Gln) supplementation on growth performance, intestinal morphology, and expression of non-specific immune-related genes in juvenile yellow catfish (*Pelteobagrus fulvidraco*). A total of 240 juvenile yellow catfish with an initial body weight of (2.49$±$0.04) g were randomly divided into four groups with three replicates each (20 fish per replicate). Four experimental diets were formulated to contain 0% (control), 0.10%, 0.20%, and 0.40% Gln, with each diet randomly assigned to one group for a 70-day feeding trial. The results showed that dietary Gln supplementation did not significantly affect growth performance indices ($P>0.05$). Compared with the control group, Gln supplementation promoted intestinal villus development, increasing villus height and fold depth while decreasing muscle thickness. Specifically, the 0.20% and 0.40% Gln groups exhibited significantly increased intestinal villus height and fold depth ($P<0.05$) and significantly reduced muscle thickness ($P<0.05$). Within the 0.10%–0.20% Gln supplementation range, the most pronounced changes in mRNA relative expression were observed for metallothionein (MT) in muscle, which was significantly upregulated in all supplemented groups compared to the control ($P<0.05$); for glutathione-S-transferase (GST) in intestine, which was significantly elevated in the 0.20% group ($P<0.05$); and for hepcidin (Hepc) and heat shock cognate protein 70 (Hsc70) in liver, where Hepc mRNA expression in the 0.10% group reached 73 times that of the control ($P<0.05$) and Hsc70 mRNA expression in the 0.20% group reached 3 times that of the control ($P<0.05$). These findings indicate that dietary Gln supplementation at appropriate levels can enhance the non-specific immunity and antioxidant capacity of juvenile yellow catfish.

**Keywords:** yellow catfish (*Pelteobagrus fulvidraco*); glutamine; non-specific immunity; intestinal morphology

Glutamine (Gln) is the most abundant conditionally essential amino acid in animals and serves as a primary energy source for rapidly dividing cells, including those involved in essential metabolic processes. It plays multiple roles in maintaining intestinal mucosal barrier integrity, improving nutritional status, and enhancing immune function. Previous studies have demonstrated that appropriate Gln supplementation can increase intestinal villus height, reduce mucosal permeability, enhance intestinal immune function, and prevent bacterial and toxin translocation in rats, thereby preserving intestinal barrier function. In animal nutrition, research has shown that 1% Gln supplementation can increase jejunal villus height and reduce muscle thickness in weaned piglets, preventing intestinal mucosal atrophy and maintaining intestinal structure and function. Additionally, Gln can upregulate antimicrobial peptide mRNA expression in porcine intestinal mucosa, improve disease resistance in piglets, and reduce free radical production during stress, thereby minimizing stress-induced tissue damage.

Yellow catfish (*Pelteobagrus fulvidraco*), belonging to the order Siluriformes, family Bagridae, and genus *Pelteobagrus*, is highly valued in China, Korea, and Japan for its delicious taste, high flesh content, and nutritional value. As yellow catfish aquaculture has expanded, disease prevention strategies have become increasingly important. However, research on Gln' s immunological and nutritional effects has primarily focused on livestock and poultry, with only sporadic reports on aquatic animals and no previous studies on its application in yellow catfish diets. Therefore, this study aimed to investigate the effects of dietary Gln supplementation on intestinal morphology and non-specific immune-related gene expression in juvenile yellow catfish, determine the optimal supplementation level, and provide a reference for Gln application in aquafeed production.

### 1.1 Experimental Diets

Experimental diets were formulated to contain Gln (99% purity, Zhengzhou Xinwei Nutrition Technology Company) at four levels: 0% (control), 0.10%, 0.20%, and 0.40%. Feed ingredients were ground to pass through a 60-mesh sieve, weighed according to formulation requirements, and mixed thoroughly. Micronutrients were premixed using a stepwise expansion method before being combined with macro-ingredients. Liquid ingredients were added and passed through a 60-mesh sieve to ensure uniform mixing. After mixing, 30% water was added and the mixture was extruded into two pellet sizes (2.5 mm and 4.0 mm) using a twin-screw pelletizer (G-250, South China University of Technology Science and Technology Industry General Factory). The pellets were cooked in a 90°C oven for 0.5 hours, dried, and stored at -20°C until use. Proximate composition analysis followed AOAC (1995) methods: moisture content was determined by drying at 105°C under normal pressure, crude protein by the Kjeldahl method, and crude fat by Soxhlet extraction. The composition and nutrient levels of the experimental diets are presented in Table 1 .

### 1.2 Feeding Management

Juvenile yellow catfish were purchased from a fish farm in Huzhou, Zhejiang Province, and acclimated under experimental conditions for two weeks prior to the trial. After a 24-hour fasting period, healthy fish of similar body weight were selected and randomly distributed into four groups (240 fish total, initial weight (2.49$±$0.04) g), with three replicates per group (20 fish per replicate) in 300-L blue fiberglass tanks. The experimental period lasted 10 weeks. Body weight was measured every two weeks. Fish were fed twice daily at 07:00 and 17:00 at a rate of 6%–8% of body weight, with feeding amounts adjusted based on consumption observed one hour after feeding. The culture water was aerated tap water, continuously aerated (1 L/min) throughout the experiment to maintain dissolved oxygen at or near saturation. Waste was removed daily, and water was exchanged every other day during the first two weeks, then 40%–60% daily thereafter depending on water quality. Water temperature ranged from 19–29°C, pH was 7.5–7.8, and ammonia nitrogen concentration did not exceed 0.05 mg/L.

### 1.3 Sample Collection and Index Determination

At the end of the trial, fish were fasted for 24 hours, surface moisture and mucus were removed, and fish were weighed to calculate survival rate. Three fish were randomly selected from each tank for body weight measurement. Growth performance indices were calculated as follows:

Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0
Specific growth rate (SGR, %/d) = 100 × (ln Wt - ln W0) / t
Feed efficiency (FE) = (Wt - W0) / F
Survival rate (SR, %) = 100 × (Nt - N0) / N0

where Wt is final body weight (g), W0 is initial body weight (g), t is experimental duration (days), F is feed intake (g), Nt is final fish number, and N0 is initial fish number.

Three additional fish were randomly selected from each replicate and dissected on ice. Liver, muscle, and intestinal tissues were collected in centrifuge tubes containing RNA later and immediately frozen in liquid nitrogen. Approximately 1 cm of the anterior intestine was rinsed with physiological saline, fixed in 10% neutral formalin, embedded in paraffin, and sectioned transversely at 7 m thickness for routine hematoxylin-eosin (HE) staining. Using NIS-Elements D imaging software, two sections per intestine were analyzed, with three measurements each of villus height, fold depth, and muscle thickness taken from each section.

### 1.4 Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from liver, muscle, and intestinal tissues using the Omega R6934-01 Total RNA Kit II according to the manufacturer's instructions. RNA concentration and purity were determined using a micro-volume UV-Vis spectrophotometer. One microgram of total RNA was reverse-transcribed to

cDNA using the TaKaRa PrimeScript® RT Reagent Kit with gDNA Eraser following the kit protocol.

### 1.5 Primer Design and Synthesis

Primers for yellow catfish metallothionein (MT), glutathione-S-transferase (GST), hepcidin (Hepc), heat shock cognate protein 70 (Hsc70), and $\beta$-actin (internal reference gene) were designed using Primer 5.0 software based on sequences from GenBank (Table 2 ) and synthesized by Invitrogen (Shanghai).

Real-time quantitative PCR was performed using a Roche LightCycler 96 system with SYBR® Green I chemistry. Diluted cDNA served as the template in a 20 L reaction mixture containing: SYBR® Premix Ex Taq II (Tli RNase Plus) (2×) 10.0 L, forward primer (10 mol/L) 0.8 L, reverse primer (10 mol/L) 0.8 L, DNA template 2.0 L, and ddH2O 6.4 L. The thermal cycling conditions for MT, Hsc70, and Hepc were: 95°C for 180 s (1 cycle); 94°C for 45 s, 55°C for 45 s, 72°C for 60 s (45 cycles). For GST: 95°C for 30 s (1 cycle); 95°C for 5 s, 60°C for 30 s (45 cycles). Relative mRNA expression levels were quantified using the 2^(-ΔΔCt) method with LightCycler 96 software.

### 1.6 Statistical Analysis

Data were analyzed using SPSS 20.0 software. One-way ANOVA followed by Duncan's multiple range test was used to determine significant differences among groups, with $P<0.05$ considered statistically significant.

### 2.1 Effects of Gln on Growth Performance of Juvenile Yellow Catfish

As shown in Table 3 , survival rate tended to increase with dietary Gln supplementation, though differences among groups were not significant ($P>0.05$). Final body weight, weight gain rate, specific growth rate, and feed efficiency also did not differ significantly among groups ($P>0.05$).

### 2.2 Effects of Gln on Intestinal Morphology of Juvenile Yellow Catfish

Figure 1 [Figure 1: see original paper] shows that compared with the control group, dietary Gln supplementation promoted intestinal villus development, increasing villus height and fold depth while decreasing muscle thickness. Table 4 reveals that intestinal villus length increased with Gln supplementation, with significant differences observed in the 0.20% and 0.40% groups compared to the control ($P<0.05$), while the 0.10% group showed no significant difference ($P>0.05$). Fold depth was significantly increased in the 0.20% and 0.40% groups ($P<0.05$) but not in the 0.10% group ($P>0.05$). Dietary supplementation with 0.10% Gln did not significantly affect intestinal muscle thickness ($P>0.05$), whereas 0.20% and 0.40% Gln significantly reduced muscle thickness ($P<0.05$).

### 2.3 Effects of Gln on MT mRNA Relative Expression in Juvenile Yellow Catfish

As shown in Figure 2 [Figure 2: see original paper], the most pronounced changes in MT mRNA expression occurred in muscle, where all supplemented groups showed significant upregulation compared to the control ($P<0.05$). The 0.40% group reached 3 times the control level, though no dose-dependent effect was observed, with the 0.20% group showing the smallest increase. In intestine, MT mRNA expression was significantly elevated in the 0.20% group (28.42% increase, $P<0.05$) but significantly decreased in the 0.10% and 0.40% groups ($P<0.05$). In liver, MT mRNA expression decreased significantly with increasing Gln levels ($P<0.05$) and remained significantly lower than the control in all supplemented groups ($P<0.05$).

### 2.4 Effects of Gln on GST mRNA Relative Expression in Juvenile Yellow Catfish

Figure 3 [Figure 3: see original paper] shows that intestinal GST mRNA expression increased in the 0.10% and 0.20% groups, with a significant 32.42% elevation in the 0.20% group ($P<0.05$), but decreased in the 0.40% group without reaching statistical significance ($P>0.05$). Hepatic GST mRNA expression decreased slightly in the 0.20% group ($P>0.05$) but was significantly reduced in the 0.10% and 0.40% groups ($P<0.05$). Muscle GST mRNA expression was significantly downregulated by dietary Gln supplementation ($P<0.05$).

### 2.5 Effects of Gln on Hepc mRNA Relative Expression in Juvenile Yellow Catfish

As shown in Figure 4 [Figure 4: see original paper], the most dramatic effects on Hepc mRNA expression were observed in liver, where the 0.10% group showed a 73-fold increase compared to the control ($P<0.05$). Although expression remained elevated at 0.20% and 0.40% supplementation levels, these increases were not statistically significant ($P>0.05$). In muscle, only the 0.20% group exhibited a significant 2-fold increase in Hepc mRNA expression ($P<0.05$), while the other groups showed slight decreases ($P>0.05$). Intestinal Hepc mRNA expression was significantly downregulated by dietary Gln supplementation ($P<0.05$).

### 2.6 Effects of Gln on Hsc70 mRNA Relative Expression in Juvenile Yellow Catfish

Figure 5 [Figure 5: see original paper] shows that muscle Hsc70 mRNA expression increased with dietary Gln levels, with significant upregulation in the 0.20% and 0.40% groups ($P<0.05$). Hepatic Hsc70 mRNA expression was significantly elevated in the 0.20% group (3-fold increase, $P<0.05$) but remained unchanged in the 0.10% and 0.40% groups ($P>0.05$). Intestinal Hsc70 mRNA expression

showed slight increases in the 0.20% group and slight decreases in the 0.10% and 0.40% groups, though none of these differences were significant (P>0.05).

Gln serves as the primary energy source for intestinal mucosa and other rapidly proliferating cells such as immune cells, rather than glucose. The intestine is the major organ for Gln consumption, and the intestinal mucosa acts as the first line of defense, playing a crucial role in non-specific immunity. Appropriate dietary Gln supplementation can maintain intestinal mucosal structural integrity and prevent harmful substances such as bacteria and toxins from translocating across the intestinal barrier into systemic circulation. Previous studies have demonstrated that 0.5% Gln supplementation significantly increased villus height in broiler chickens, 1.2% Gln significantly increased fold depth in Jian carp (*Cyprinus carpio* var. Jian), and 0.5% Gln reduced muscle thickness in weaned piglets. In the present study, dietary Gln supplementation tended to improve weight gain rate, specific growth rate, and survival rate in juvenile yellow catfish, indicating positive effects on health and growth. Although the effects on growth performance were not statistically significant, Gln' s primary role in immune function enhancement may outweigh its impact on growth rate. The finding that 0.20% Gln increased intestinal villus height and fold depth while decreasing muscle thickness suggests this supplementation level can effectively prevent intestinal mucosal atrophy, maintain intestinal structure and function, and enhance non-specific immunity to varying degrees.

Hepc and Hsc70 are closely associated with non-specific immunity. Hepc exhibits strong antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, fungi, spirochetes, and viruses, representing an important component of the fish non-specific immune system. Previous research has shown that under normal conditions, Hepc mRNA is most highly expressed in liver, and infection with *Aeromonas hydrophila* and *Bacillus cereus* induces the most significant changes in hepatic Hepc mRNA expression in yellow catfish. Hsc70, a member of the heat shock protein family, has been shown to present antigens on cell surfaces and enhance endocytosis following pathogen infection. Studies have demonstrated that Hsc70 mRNA is most highly expressed in liver under normal conditions, with the most significant changes occurring in liver following *Vibrio harveyi* infection in turbot (*Scophthalmus maximus*). The current results indicate that dietary Gln supplementation can enhance non-specific immunity, with the most pronounced changes in Hepc and Hsc70 mRNA expression occurring in liver at 0.10%–0.20% supplementation levels.

MT and GST are primarily involved in antioxidant mechanisms of non-specific immunity. MT, rich in reduced sulfhydryl groups and nucleophilic properties with kinetically unstable metal ions, exhibits strong free radical scavenging capacity and provides protection against oxidative damage induced by metals, chemicals, and radiation, while also protecting and enhancing macrophage function. GST is an important redox metabolic enzyme with detoxification functions that can improve organismal defense capabilities. The present results demonstrate that dietary Gln supplementation enhanced both antioxidant capacity

and non-specific immunity, with the most pronounced changes in MT mRNA expression occurring in muscle and GST mRNA expression in intestine at 0.10%–0.20% supplementation levels.

In conclusion, dietary Gln supplementation at appropriate levels can enhance the non-specific immunity and antioxidant capacity of juvenile yellow catfish, thereby promoting healthy growth.

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