Effects of Glutamine on Antioxidant Capacity and Non-Specific Immunity in Juvenile Yellow Catfish (Postprint)

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Abstract

This experiment aimed to investigate the effects of glutamine on the antioxidant capacity and non-specific immunity of juvenile yellow catfish. A total of 240 juvenile yellow catfish with an average 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. The four groups of experimental fish were fed isonitrogenous and isoenergetic experimental diets with glutamine supplementation levels of 0 (control), 0.1%, 0.2%, and 0.4%, respectively. The experimental period lasted for 10 weeks. The results showed that: dietary supplementation with 0.2% glutamine significantly increased the serum total protein, globulin, triglyceride, high-density lipoprotein, and low-density lipoprotein contents of juvenile yellow catfish ($P<0.05$); there were no significant differences in serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities among the groups ($P>0.05$); dietary supplementation with 0.1% glutamine significantly increased the activities of catalase and glutathione peroxidase in the liver, as well as superoxide dismutase and glutathione peroxidase in the muscle of juvenile yellow catfish ($P<0.05$); with the increase of dietary glutamine supplementation, the phagocytic index of head kidney macrophages showed an upward trend, and the 0.2% and 0.4% groups were significantly higher than the control group ($P<0.05$), but there was no significant difference between the 0.2% and 0.4% groups ($P>0.05$). It can be concluded that dietary supplementation with 0.1%-0.2% glutamine can enhance the antioxidant capacity and non-specific immunity of the organism.

Full Text

Effect of Glutamine on Antioxidant Capacity and Non-Specific Immunity of Juvenile Yellow Catfish (Pelteoobagrus fulvidraco)

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Abstract

A 10-week feeding trial was conducted to investigate the effects of dietary glutamine (Gln) on antioxidant capacity and non-specific immunity of juvenile yellow catfish (*Pelteoobagrus fulvidraco*). A total of 240 juvenile yellow catfish with an average body weight of (2.49±0.04) g were randomly divided into 4 groups with 3 replicates per group and 20 fish per replicate. The fish were fed four iso-nitrogenous and iso-energetic diets containing 0% (control), 0.1%, 0.2%, and 0.4% Gln, respectively. The results showed that dietary supplementation with 0.2% Gln significantly increased serum total protein, globulin, triglyceride, high-density lipoprotein, and low-density lipoprotein contents ($P<0.05$). No significant differences were observed in serum alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase activities among groups ($P>0.05$). Supplementation with 0.1% Gln significantly enhanced catalase and glutathione peroxidase activities in liver, as well as superoxide dismutase and glutathione peroxidase activities in muscle ($P<0.05$). The phagocytic index of head-kidney macrophages showed an increasing trend with increasing dietary Gln levels, with values in the 0.2% and 0.4% groups significantly higher than in the control group ($P<0.05$), though no significant difference was found between the 0.2% and 0.4% groups ($P>0.05$). These findings indicate that dietary supplementation with 0.1%-0.2% Gln can improve antioxidant capacity and non-specific immunity in juvenile yellow catfish.

Keywords: yellow catfish (*Pelteoobagrus fulvidraco*); glutamine; hematological characteristics; antioxidant function; non-specific immune

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Glutamine (Gln) has gradually become a research focus in nutrition, physiology, and immunology due to its unique physiological functions. Numerous studies have demonstrated that glutamine is a "conditionally essential amino acid" that must be obtained from the diet to maintain stable Gln levels in animals when endogenous synthesis is insufficient [1]. As a conditionally essential amino acid, glutamine can improve immune function, enhance disease resistance, promote protein synthesis, and protect intestinal function [2]. In addition to serving as

the primary energy source for intestinal mucosal cells, glutamine participates in glutathione (GSH) synthesis, which scavenges various free radicals to reduce peroxide damage to cells and plays a critical role in maintaining homeostasis. Lu et al. [3] reported that dietary supplementation with 0.8% glutamine significantly increased serum total protein in heat-stressed broilers. Huang et al. [4] found that dietary glutamine significantly enhanced glutathione peroxidase (GSH-Px) activity and reduced malondialdehyde (MDA) content in blood, thereby improving antioxidant capacity in yellow-feathered broilers under high temperature conditions. Studies in aquatic animals have shown that dietary glutamine supplementation significantly enhances leukocyte phagocytosis, respiratory burst activity, and non-specific immunity in grouper [5], loach [6], and juvenile Jian carp [7].

Yellow catfish, commonly known as "yellow spicy fish" or "goby," is a high-quality freshwater species widely distributed in southeastern Asia and holds significant commercial value in aquaculture [8]. In recent years, yellow catfish has gained increasing popularity due to its high protein content, low fat, rich nutrition, and tender flesh [9]. This study investigated the effects of dietary glutamine supplementation on antioxidant capacity and non-specific immunity in juvenile yellow catfish, aiming to determine the optimal supplementation level and provide a reference for practical application of glutamine in aquaculture.

1.1 Experimental Diets

Four iso-nitrogenous and iso-energetic experimental diets were formulated with glutamine supplementation levels of 0% (control), 0.1%, 0.2%, and 0.4%. The composition and nutrient levels are shown in Table 1 . Feed ingredients were ground to pass through a 60-mesh sieve, weighed accurately according to the formula, and mixed thoroughly. Micro-ingredients were premixed using the stepwise expansion method before being combined with the bulk 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. The pellets were cooked in a 90°C oven for 0.5 h, dried, and stored at -20°C until use. Proximate composition analysis of the diets followed AOAC (1995) [10] methods: moisture content was determined by oven drying at 105°C, crude protein by the Kjeldahl method, and crude fat by the Soxhlet extraction method.

1.2 Feeding Management

Juvenile yellow catfish were purchased from a fish farm in Huzhou, Zhejiang Province, and acclimated for 2 weeks before the trial. Prior to the experiment, healthy fish of similar size were fasted for 24 h and randomly distributed into 4 groups with 3 replicates each (20 fish per replicate). Fish were stocked in 300 L blue fiberglass tanks. The experimental period lasted 10 weeks. Aerated tap water was used, with temperature maintained at 19–29°C and pH at 7.5–7.8. Continuous aeration (1 L/min) was provided throughout the culture period,

with dissolved oxygen 6 mg/L and ammonia nitrogen 0.05 mg/L. Body weight was measured every 2 weeks. Fish were fed twice daily at 07:00 and 17:00 at 6–8% of body weight, with feeding rates adjusted based on observed consumption. Tanks were cleaned daily, and water was changed every other day during the first 2 weeks, then 40–60% daily depending on water quality.

1.3 Serum Biochemical Indices

At the end of the trial, fish were fasted for 12 h. Three fish per tank were randomly selected and anesthetized. Blood was collected from the heart using non-heparinized syringes, allowed to clot at 4°C overnight, and centrifuged at 5,000 r/min to obtain serum. Serum samples were immediately sent to the First Affiliated Hospital of Ningbo University for biochemical analysis.

1.4 Antioxidant Indices

After blood collection, fish were dissected on ice. Liver, muscle, and intestine tissues were collected and homogenized in ice-cold physiological saline at a 1:9 (w/v) ratio using an electric homogenizer. The homogenates were centrifuged at 3,000 r/min for 20 min at 4°C, and the supernatants were used to determine superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, malondialdehyde (MDA) content, and protein concentration using commercial assay kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Non-Specific Immune Indices

Head-kidney cell isolation: L-15 medium was supplemented with 100 IU/mL penicillin, 100 g/mL streptomycin, 10 IU/mL heparin, and 2% fetal bovine serum. After dissection, head-kidney tissue was immediately placed in the medium, minced, and passed through a 100 m metal mesh. The cell suspension was layered over 51% Percoll solution, and 34% Percoll was gently added on top. Macrophages were isolated by discontinuous density gradient centrifugation (600×g, 5 min, 4°C). The intermediate cell band was collected and resuspended at 1×10 cells/mL. Viability exceeded 95% as determined by 0.01% trypan blue exclusion.

Phagocytic index assay: 100 L yeast suspension (1×10 cells/mL) was added to 100 L head-kidney cell suspension and incubated at 23.5°C for 40 min. The number of yeast cells phagocytosed by macrophages was counted under a microscope.

Respiratory burst activity assay: 100 L of 1 mg/mL nitroblue tetrazolium (NBT) containing 1 g/mL phorbol myristate acetate (PMA) was added to 100 L head-kidney cell suspension and incubated at 25°C for 45 min. The reaction was terminated with absolute methanol, washed twice with 70% methanol, and air-dried. After adding 120 L of 2 mol/L KOH and 140 L dimethyl sulfoxide (DMSO), absorbance was measured at 630 nm using a microplate reader with

KOH/DMSO as blank. Respiratory burst activity was expressed as optical density (OD) values.

1.6 Data Processing

Data are presented as mean ± standard error (SE). One-way ANOVA was performed using SPSS 17.0 software, followed by Duncan' s multiple comparison test when significant differences were detected ($P<0.05$).

2.1 Effects of Glutamine on Serum Biochemical Indices of Juvenile Yellow Catfish

As shown in Table 2 , serum total protein and globulin contents in the 0.2% group were significantly higher than those in the control, 0.1%, and 0.4% groups ($P<0.05$), while no significant differences were observed among the control, 0.1%, and 0.4% groups ($P>0.05$). Dietary glutamine supplementation had no significant effect on alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase activities ($P>0.05$), though maximum values were observed at 0.2% supplementation. Serum glucose content increased initially and then decreased with increasing glutamine levels, peaking at 0.1% supplementation, but differences among groups were not significant ($P>0.05$). Serum triglyceride, cholesterol, high-density lipoprotein, and low-density lipoprotein contents increased with glutamine supplementation, with values in the 0.2% group significantly higher than in the control group ($P<0.05$).

2.2 Effects of Glutamine on Antioxidant Indices in Different Tissues

The effects of glutamine on antioxidant indices in different tissues are presented in Table 3 . In liver, dietary glutamine increased catalase activity, which reached its maximum at 0.2% supplementation, being 129 U/mg prot higher than the control group ($P<0.05$). Glutathione peroxidase activity peaked in the 0.1% group, significantly higher than in other groups ($P<0.05$). In muscle, superoxide dismutase and glutathione peroxidase activities were highest in the 0.1% group, significantly exceeding other groups ($P<0.05$), while glutathione peroxidase activity in the 0.2% group was also significantly higher than in the control group ($P<0.05$). In intestine, dietary glutamine supplementation had no significant effect on any antioxidant indices ($P>0.05$).

2.4 Effects of Glutamine on Head-Kidney Non-Specific Immune Indices

As shown in Table 4 , the phagocytic index of head-kidney macrophages increased with dietary glutamine supplementation, with values in the 0.2% and 0.4% groups significantly higher than in the control group ($P<0.05$), though no significant difference was observed between the 0.2% and 0.4% groups ($P>0.05$). Dietary glutamine supplementation had no significant effect on respiratory burst activity of head-kidney macrophages ($P>0.05$).

Serum biochemical indices reflect the health and physiological status of fish and serve as important diagnostic tools [11]. Serum total protein and albumin contents accurately reflect protein absorption and metabolism, while higher globulin content indicates stronger disease prevention and immune response capabilities [12-13]. Serum cholesterol and triglyceride contents reflect lipid metabolism status. In this study, serum total protein, globulin, triglyceride, high-density lipoprotein, and low-density lipoprotein contents in the 0.2% group were significantly higher than in the control group. This may be because glutamine can synthesize various amino acids through deamination and transamination, reducing protein catabolism and compensating for insufficient protein synthesis capacity in juvenile fish [14-15]. These results are consistent with findings in juvenile sturgeon [16], Japanese tiger prawn [17], tilapia [18], and salmon [19], but differ from studies by Wang et al. [20] and Zhou [21] in weaned piglets, which reported that glutamine supplementation had limited effect on serum total protein. These discrepancies may be attributed to species differences. Overall, dietary glutamine supplementation can improve defense capabilities and non-specific immunity in yellow catfish.

The primary antioxidant enzymes in the protective system include superoxide dismutase, catalase, and glutathione peroxidase, while malondialdehyde is a major product of lipid peroxidation [22]. This study demonstrated that 0.1% dietary glutamine significantly enhanced catalase and glutathione peroxidase activities in liver and superoxide dismutase and glutathione peroxidase activities in muscle, consistent with results in loach [6], carp [23], half-smooth tongue sole [24], and hybrid sturgeon [25]. The effects of glutamine on different antioxidant enzymes in liver, muscle, and intestine varied, with some increasing and others decreasing. The underlying mechanisms remain debated. Zhang et al. [26] hypothesized that a dynamic balance mechanism may exist within the antioxidant system, where activation of one pathway may suppress another as a self-protective mechanism. Overall, glutamine can enhance the antioxidant capacity of juvenile yellow catfish.

The head-kidney is an important lymphoid tissue in fish, rich in macrophages whose phagocytic activity is crucial for resisting pathogen invasion [27]. The enhanced phagocytosis of brewer' s yeast by head-kidney macrophages observed in this study indicates that dietary glutamine can improve the phagocytic capacity of head-kidney macrophages and thereby enhance non-specific immunity. In conclusion, dietary glutamine supplementation effectively improves antioxidant capacity and non-specific immunity in juvenile yellow catfish, with an optimal supplementation level of 0.1%–0.2% based on comprehensive evaluation of all indices.

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