Effects of glutamine and its precursors on tissue antioxidant capacity and serum biochemical indices in Songpu mirror carp (Postprint)

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Abstract

This study aimed to investigate the effects of glutamine and its precursors on tissue antioxidant capacity and serum biochemical indices in juvenile Songpu mirror carp. A total of 1,050 juvenile Songpu mirror carp with an average body weight of (40.27$±$3.96) g were randomly allocated into 7 groups, each consisting of 5 replicates with 30 fish per replicate. Seven isonitrogenous and isoenergetic experimental diets were prepared by supplementing the basal diet with 1.5% glucose (control), glutamine (Gln), glutamic acid (Glu), α-ketoglutarate (AKG), L-ornithine-α-ketoglutarate (OKG), L-arginine-α-ketoglutarate (AAKG), and sodium α-ketoglutarate (2Na-AKG), and were fed to the respective experimental groups. The feeding trial lasted for 8 weeks. The results showed: In the intestine, superoxide dismutase (SOD) activities in the Gln and Glu groups were significantly higher than that in the control group ($P<0.05$), glutathione (GSH) content in the Gln group was significantly higher than that in the control group ($P<0.05$), and malondialdehyde (MDA) contents in the Glu, AKG, OKG, and 2Na-AKG groups were significantly lower than that in the control group ($P<0.05$). In the liver, SOD activities in the Gln and Glu groups were significantly higher than those in all other groups ($P<0.05$), no significant differences were observed in GSH content among all groups ($P>0.05$), and MDA contents in the Gln, Glu, AKG, 2Na-AKG, and OKG groups were significantly lower than that in the control group ($P<0.05$). In serum, SOD activities in the OKG and 2Na-AKG groups were significantly higher than that in the control group ($P<0.05$), GSH content in the 2Na-AKG group was significantly lower than that in the control group ($P<0.05$), and no significant differences were observed in MDA content among all groups ($P>0.05$). Compared with the control group, serum total protein (TP) contents in the Gln, Glu, and AKG groups were significantly increased ($P<0.05$), while that in the 2Na-AKG group was significantly decreased ($P<0.05$). Serum alanine aminotransferase (ALT) activity in the AAKG group was significantly

increased (P<0.05), serum aspartate aminotransferase (AST) activities in the OKG and 2Na-AKG groups were significantly increased (P<0.05), while serum AST activity in the AKG group was significantly decreased (P<0.05). Serum total cholesterol (TCHO) content in the AAKG group was significantly decreased (P<0.05). Serum glucose content in the AKG group was significantly decreased (P<0.05). In conclusion, glutamine and its precursors exerted certain influences on tissue antioxidant capacity in Songpu mirror carp. Regarding the scavenging capacity for lipid peroxidation products, Glu, AKG, OKG, and 2Na-AKG demonstrated superior effects. Gln, Glu, and AKG could enhance protein utilization efficiency and immunity, and AKG could also significantly reduce serum glucose content.

Full Text

Effects of Glutamine and Its Precursors on Tissue Antioxidant Capacity and Serum Biochemical Indices of Songpu Mirror Carp

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Abstract: This experiment investigated the effects of glutamine (Gln) and its precursors on tissue antioxidant capacity and serum biochemical indices in juvenile Songpu mirror carp. A total of 1,050 healthy juvenile Songpu mirror carp with an average body weight of (40.27$±$3.96) g were randomly allocated into seven groups, each consisting of five replicates of 30 fish. Seven isonitrogenous and isocaloric experimental diets were formulated by supplementing the basal diet with 1.5% glucose (control), glutamine (Gln), glutamate (Glu), α-ketoglutarate (AKG), L-ornithine-α-ketoglutarate (OKG), L-arginine-α-ketoglutarate (AAKG), or sodium α-ketoglutarate dibasic (2Na-AKG). The feeding trial lasted for eight weeks. In the intestine, superoxide dismutase (SOD) activity was significantly higher in the Gln and Glu groups compared to the control (P<0.05), glutathione (GSH) content was significantly elevated in the Gln group (P<0.05), and malondialdehyde (MDA) content was significantly lower in the Glu, AKG, OKG, and 2Na-AKG groups (P<0.05). In the liver, SOD activity in the Gln and Glu groups significantly exceeded all other groups (P<0.05), while GSH content showed no significant differences among treatments (P>0.05); MDA content was significantly reduced in the Gln, Glu, AKG, OKG, and 2Na-AKG groups compared to the control (P<0.05). In serum, SOD activity was significantly higher in the OKG and 2Na-AKG groups (P<0.05), GSH content was significantly lower in the 2Na-AKG group (P<0.05), and no significant differences were observed in MDA content among groups (P>0.05). Serum total protein (TP) was significantly elevated in the Gln, Glu, and AKG groups but decreased in the 2Na-AKG group (P<0.05). Serum alanine amino-

transferase (ALT) activity increased significantly in the AAKG group ($P<0.05$), whereas aspartate aminotransferase (AST) activity rose significantly in the OKG and 2Na-AKG groups but declined markedly in the AKG group ($P<0.05$). Total cholesterol (TCHO) was significantly reduced in the AAKG group ($P<0.05$), and serum glucose decreased significantly in the AKG group ($P<0.05$). These results demonstrate that Gln and its precursors modulate tissue antioxidant capacity, with Glu, AKG, OKG, and 2Na-AKG showing superior efficacy in clearing lipid peroxidation products. Furthermore, Gln, Glu, and AKG enhanced protein utilization and immune function, while AKG notably reduced serum glucose levels.

Keywords: Songpu mirror carp; glutamine; precursors; antioxidant capacity; serum biochemical indices

Introduction

Glutamine (Gln) is the most abundant amino acid in animal blood and serves as a crucial precursor for protein synthesis and the formation of pyrimidine and purine nucleotides, nicotinamide adenine dinucleotide, and amino sugars. It functions as a primary energy source for rapidly dividing cells such as lymphocytes and plays vital roles in transamination and nitrogen donation during metabolism. Under normal conditions, Gln can be obtained from exogenous sources and endogenous synthesis. However, during stress or pathological states, endogenous production becomes insufficient, leading to a relative deficiency. Previous research has shown that dietary Gln supplementation promotes intestinal development, improves intestinal structure and function, and protects against hydrogen peroxide-induced oxidative stress in Jian carp. Nevertheless, exogenous Gln is highly unstable and readily decomposes into toxic pyroglutamic acid and ammonia when exposed to heat. Studies in rainbow trout have reported that dietary Gln supplementation at 1.0% or 2.0% significantly reduced villus diameter, height, mucosal thickness, and intestinal gland depth, likely due to Gln degradation into harmful compounds during feed processing.

To address these limitations, researchers have investigated Gln alternatives, particularly glutamine dipeptides. Studies have demonstrated that alanyl-glutamine (Ala-Gln) can inhibit *Clostridium difficile* toxin-induced apoptosis and damage in intestinal epithelial cells and enhance in vitro maturation of porcine oocytes and embryonic development. From a metabolic perspective, Gln has several precursors, including α-ketoglutarate (AKG), glutamate (Glu), L-ornithine-α-ketoglutarate (OKG), L-arginine-α-ketoglutarate (AAKG), and sodium α-ketoglutarate dibasic (2Na-AKG). AKG, an intermediate in the tricarboxylic acid cycle formed through glutamate decarboxylation, can serve as a Gln precursor and performs nitrogen-carrying and storage functions equally important for intestinal growth and development. Glu represents a major energy substrate for animal mucosa and participates in Gln synthesis as

a key amino acid for intestinal mucosal growth and metabolism. OKG inhibits bacterial translocation, promotes small intestinal epithelial cell proliferation, and supports damaged mucosal repair while maintaining protein homeostasis. AAKG, an arginine double salt, facilitates hepatocellular nutrient and energy absorption and maintains normal liver function. 2Na-AKG provides AKG as an organic intermediate that may reduce absorption rate, allowing more time for conversion to other forms. Previous studies have shown that Gln and its precursors (AKG, OKG, Glu) significantly improve amylase and lipase activities in the anterior intestine of Songpu mirror carp, with AKG notably reducing feed conversion ratio and increasing weight gain by 5.96%, demonstrating slightly superior effects on intestinal development compared to other alternatives. Additionally, AKG supplementation has been found to increase crude protein content in muscle, enhance protein absorption and utilization, and improve protein metabolism in this species.

Songpu mirror carp, a new strain developed through hybridization between the fourth generation of selected German mirror carp (F4) and scattered-scale mirror carp, offers advantages including superior body conformation, higher flesh yield, faster growth rate, and greater economic benefits compared to conventional carp varieties. This study selected representative Gln precursors (Glu, OKG, AAKG, and 2Na-AKG) to investigate their effects on tissue antioxidant capacity and serum biochemical indices in Songpu mirror carp.

Materials and Methods

Experimental Materials L-Glu, L-Gln, OKG (L-ornithine to AKG mass ratio of 1:1), 2Na-AKG, and AAKG (L-arginine to AKG mass ratio of 2:1) were purchased from Shanghai Guchen Biotechnology Co., Ltd., while AKG was obtained from Sigma-Aldrich (purity $ $98.0% for all compounds). Experimental fish were obtained from the Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Experimental Design and Feeding Management A basal diet was formulated according to *Compound Feed for Common Carp* (SC/T 1026-2002) and NRC (2011) guidelines, using fish meal and soybean meal as protein sources and soybean oil, fish oil, and phospholipids as lipid sources. Six experimental diets were prepared by replacing 1.5% glucose in the basal diet with Gln, Glu, AKG, OKG, AAKG, or 2Na-AKG, respectively, creating seven isonitrogenous and isocaloric diets (including the glucose control). The basal diet composition and nutrient levels are presented in . Feed ingredients were ground through an 80-mesh sieve, weighed, and mixed progressively before adding water and processing into 2 mm pellets using a small pellet mill according to juvenile fish (10–100 g) requirements. Pellets were air-dried at room temperature and stored at 4°C.

Three thousand Songpu mirror carp were initially selected from the Songpu Experimental Station of the Heilongjiang River Fisheries Research Institute. After

one week of acclimation feeding with the basal diet, 1,050 fish with an average weight of (40.27$±$3.96) g were randomly distributed into seven groups (five replicates per group, 30 fish per replicate). The control group received the basal diet, while six treatment groups were randomly assigned to one of the experimental diets. Fish were reared in a modular recirculating aquaculture system at the Hulan Experimental Station, with cylindrical tanks (1.2 m diameter × 0.6 m height), a biofilter treatment capacity of 8–12 t/h, and total water volume of 30 t. Fish were hand-fed to satiation three times daily (07:30, 12:30, and 16:30) for eight weeks. Tanks were cleaned regularly, with 20% water exchange daily. Water temperature was maintained at (25.0$±$2.0)°C, dissolved oxygen $ $5.0 mg/L, and ammonia nitrogen $ $1.0 mg/L.

Sample Collection and Analysis

Sample Collection At the end of the feeding trial, fish were fasted for 24 h before sampling. Two fish per tank were anesthetized with eugenol, and blood was collected from the caudal vein using heparinized syringes. Serum was separated by centrifugation (3,500 r/min, 15 min) and stored at -20°C. After blood collection, fish were dissected on ice to obtain liver and intestinal tissues. Intestinal samples were rinsed with ice-cold 0.86% saline and blotted dry. Tissue samples were accurately weighed and homogenized in ice-cold 0.86% saline at a 1:9 ratio (w/v). Homogenates were centrifuged according to assay kit requirements, and supernatants were stored at -20°C pending analysis.

Index Determination MDA content, SOD activity, and GSH levels in serum, liver, and intestine were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute. Serum biochemical indices were analyzed using an automatic biochemical analyzer (Beckman ProCX4, Germany), including total protein (TP), albumin (ALB), globulin (GLB), glucose, urea nitrogen (UN), triglycerides (TG), total cholesterol (TCHO), ALT, and AST.

Statistical Analysis Data are presented as means ± standard error. One-way ANOVA was performed using SPSS 19.0 software, followed by Duncan' s multiple range test for post-hoc comparisons. Significance was set at $P<0.05$.

Results

Effects of Gln and Its Precursors on Intestinal Antioxidant Indices The effects of Gln and its precursors on intestinal antioxidant indices are shown in . SOD activity in the Gln and Glu groups was significantly higher than in the control, OKG, and 2Na-AKG groups ($P<0.05$), with no significant differences among other groups ($P>0.05$). GSH content in the Gln group was significantly higher than in the control, AAKG, and 2Na-AKG groups ($P<0.05$), while other groups showed no significant differences ($P>0.05$). MDA content was significantly lower in the 2Na-AKG and OKG groups compared to all other groups

($P<0.05$), and the AKG and Gln groups also exhibited significantly lower MDA than the control ($P<0.05$).

Effects of Gln and Its Precursors on Hepatic Antioxidant Indices As presented in , hepatic SOD activity in the Gln and Glu groups was significantly higher than in all other groups ($P<0.05$), with no significant differences among remaining groups ($P>0.05$). GSH content was significantly higher in the Glu group compared to the AKG, OKG, AAKG, and 2Na-AKG groups ($P<0.05$), while other comparisons showed no significant differences ($P>0.05$). Hepatic MDA content was significantly lower in the Gln, Glu, AKG, OKG, and 2Na-AKG groups relative to the control ($P<0.05$), whereas the AAKG group did not differ significantly from the control ($P>0.05$).

Effects of Gln and Its Precursors on Serum Antioxidant Indices summarizes the effects on serum antioxidant indices. Serum SOD activity was significantly elevated in the OKG and 2Na-AKG groups compared to the control ($P<0.05$), with no significant differences among other groups ($P>0.05$). GSH content was significantly lower in the 2Na-AKG group than in the control, Gln, Glu, AKG, and OKG groups ($P<0.05$), while the Gln group showed significantly higher GSH than the AAKG and 2Na-AKG groups ($P<0.05$). No significant differences were observed in serum MDA content among all groups ($P>0.05$).

Effects of Gln and Its Precursors on Serum Biochemical Indices The effects on serum biochemical indices are presented in . Serum TP content was significantly higher in the Gln, Glu, and AKG groups than in other groups ($P<0.05$), with the 2Na-AKG group showing the lowest value. ALB content was significantly elevated in the Glu group compared to all other groups ($P<0.05$), while GLB content was significantly higher in the Gln, Glu, and AKG groups ($P<0.05$), again with the lowest value in the 2Na-AKG group. ALT activity was significantly increased only in the AAKG group ($P<0.05$). AST activity was significantly higher in the OKG and 2Na-AKG groups ($P<0.05$) but significantly lower in the AKG group compared to all others ($P<0.05$). No significant differences were detected in serum TG or UN content among groups ($P>0.05$). TCHO content was significantly reduced in the AAKG group compared to the control, Gln, and Glu groups ($P<0.05$). Serum glucose was highest in the control group, which was significantly higher than the AKG group ($P<0.05$).

Discussion

Effects on Tissue Antioxidant Capacity Animal organisms maintain a dynamic equilibrium that remains stable under normal conditions but becomes disrupted by external stimuli. The balance between oxidants and antioxidants is crucial for preserving the function of biological macromolecules and cells; any disturbance to this balance can cause cellular or tissue damage, triggering oxidative stress. Gln modulates intestinal epithelial antioxidant capacity primarily

through antioxidant enzyme systems and non-enzymatic pathways. This study evaluated antioxidant capacity in intestine, liver, and serum by measuring SOD activity and levels of GSH and MDA.

Previous research has established a negative correlation between SOD activity and lipid peroxidation products. MDA, the end product of lipid peroxidation, severely damages biological membranes, impairing their function and potentially inducing apoptosis. GSH, a low-molecular-weight thiol peptide composed of glutamate, glycine, and cysteine, constitutes the primary antioxidant defense system that protects proteins and lipids from oxidation. Gln participates in GSH synthesis, thereby enhancing antioxidant capacity. Studies have demonstrated that Gln inhibits lipid peroxidation in carp intestinal epithelial cells and improves clearance of lipid peroxidation products. Dietary Gln supplementation has been shown to enhance antioxidant capacity in intestine, serum, and liver of juvenile Jian carp, and significantly reduce plasma MDA in Chinese soft-shelled turtles infected with *Aeromonas hydrophila*.

In the current study, AKG, OKG, and 2Na-AKG groups exhibited significantly lower MDA content in liver and intestine compared to the control, consistent with findings in weaned piglets. However, these treatments did not significantly affect SOD activity or GSH content, suggesting that AKG may influence antioxidant capacity through alternative mechanisms beyond SOD and GSH regulation. AKG participates in non-enzymatic oxidative decarboxylation that promotes hydrogen peroxide decomposition and serves as a TCA cycle intermediate that utilizes and converts ammonia, reducing ammonia toxicity while providing additional ATP to enhance metabolic capacity and inhibit reactive oxygen species formation, thereby preventing lipid peroxidation. The Gln and Glu groups showed significantly higher hepatic SOD activity and lower MDA content without affecting GSH levels. In the intestine, the Gln group exhibited elevated SOD activity and GSH content, while the Glu group only increased SOD activity significantly, though neither reduced MDA content compared to the control. In serum, although OKG and 2Na-AKG groups showed increased SOD activity and GSH content, no significant differences in MDA were observed among groups. These discrepancies from mammalian studies may reflect species-specific differences in antioxidant regulation mechanisms, possibly involving homeostatic balancing systems that maintain normal physiological functions—a mechanism requiring further investigation.

Effects on Serum Biochemical Indices Blood composition reflects organismal health status, as physiological and pathological changes alter blood constituents, making biochemical parameters valuable diagnostic tools. Blood contains essential nutrients including proteins, lipids, and carbohydrates, and performs transport, humoral regulation, homeostatic maintenance, and defense functions. Serum proteins maintain colloidal osmotic pressure, immunity, transport, tissue repair, and buffering capacity, reflecting metabolic status, immune function, protein synthesis, and nitrogen deposition. Serum TP, ALB, and GLB

levels indicate protein metabolism and absorption, with GLB reflecting immune status and UN reflecting protein catabolism. The present results show that Gln, Glu, and AKG groups had significantly higher serum TP and GLB contents, while all treatment groups showed reduced UN compared to the control, indicating enhanced protein utilization and immune function—findings consistent with previous studies in sturgeon and Songpu mirror carp.

ALT and AST are important transaminases localized in mitochondria that play crucial roles in protein metabolism. Their activity serves as a sensitive indicator of liver damage; under normal conditions, serum transaminase activities remain low, but increase when hepatocytes are damaged and release these enzymes. The significantly elevated ALT activity in the AAKG group and increased AST activity in OKG and 2Na-AKG groups suggest potential hepatocellular stress. Conversely, reduced ALT activity in Gln, Glu, and AKG groups and significantly lower AST activity in the AKG group indicate hepatoprotective effects, particularly for AKG. Serum TCHO and TG reflect fatty acid metabolism. While TCHO showed no significant differences among groups, the AAKG group exhibited significantly lower TG, suggesting enhanced fatty acid utilization. Elevated serum glucose triggers adverse physiological responses and typically increases with dietary carbohydrate levels; fish are considered congenital diabetics due to poor glucose regulation. The significantly reduced serum glucose in the AKG group, with modest decreases in other treatment groups, suggests these compounds may provide alternative energy substrates, with AKG showing the most pronounced effect.

Conclusions

1. Gln and its precursors modulated tissue antioxidant capacity in Songpu mirror carp, with Glu, AKG, OKG, and 2Na-AKG demonstrating superior efficacy in clearing lipid peroxidation products.
2. Gln and its precursors affected serum biochemical indices, with Gln, Glu, and AKG showing optimal performance in improving protein utilization and immune function; notably, AKG significantly reduced serum glucose content.

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