Effects of α-Ketoglutarate Supplementation in Different Protein Source Diets on Liver Glutamine Content, Antioxidant Capacity, and Growth Hormone and Insulin-Like Growth Factor-I Gene Expression in Juvenile Hybrid Sturgeon (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary α-ketoglutarate supplementation in different protein sources on hepatic glutamine (Gln) content, antioxidant capacity, and the expression of growth hormone (GH) and insulin-like growth factor-I (IGF-I) genes in juvenile hybrid sturgeon (Acipenser schrenckii ×Acipenser baeri). Four experimental diets were formulated using soy protein concentrate and soy protein concentrate+fish meal as protein sources, with two α-ketoglutarate (AKG) supplementation levels (0 and 1%). Five hundred juvenile hybrid sturgeon with an average body weight of (7.65$±$0.04) g were selected and randomly divided into 4 groups, with 5 replicates per group and 25 fish per replicate, for an 8-week culture period. The results showed that dietary 1% AKG supplementation significantly increased hepatic Gln content and glutamine synthetase (GS) activity ($P<0.05$), while having no significant effect on hepatic alkaline phosphatase (ALP) activity ($P>0.05$). Protein source had no significant effect on hepatic Gln content, GS activity, and ALP activity ($P>0.05$), and there was no significant interaction between protein source and AKG supplementation level on hepatic Gln content, GS activity, and ALP activity ($P>0.05$). Dietary 1% AKG supplementation significantly decreased hepatic malondialdehyde (MDA) content and significantly increased hepatic glutathione (GSH) content ($P<0.05$). Compared with soy protein concentrate+fish meal as the protein source, soy protein concentrate as the protein source significantly increased hepatic GSH content ($P<0.05$). Protein source and AKG supplementation level had no significant effects on hepatic superoxide dismutase (SOD) and catalase (CAT) activities ($P>0.05$),

and there was no significant interaction between protein source and AKG supplementation level on any hepatic antioxidant indices (P>0.05). Dietary 1% AKG supplementation significantly increased the relative expression levels of hepatic GH and IGF-I genes (P<0.05). Compared with the SPC+FM protein source, the SPC protein source significantly increased the relative expression levels of hepatic IGF-I and GH genes (P<0.05). There was no significant interaction between protein source and AKG supplementation level on the relative expression levels of hepatic GH and IGF-I genes (P>0.05). In conclusion, dietary 1% AKG supplementation in juvenile hybrid sturgeon can increase Gln content by enhancing GS activity in the liver, decrease MDA content by increasing GSH content in the liver, and enhance the expression of growth-related genes GH and IGF-I in the liver.

Full Text

Abstract

This experiment aimed to investigate the effects of dietary α-ketoglutarate (AKG) supplementation on liver glutamine (Gln) content, antioxidant capacity, and the expression of growth hormone (GH) and insulin-like growth factor- (IGF-) genes in juvenile hybrid sturgeon (Acipenser schrenckii ×Acipenser baeri). Four experimental diets were formulated using either soybean protein concentrate (SPC) or SPC+fish meal (FM) as protein sources, each with 0 or 1% AKG supplementation. Five hundred juvenile hybrid sturgeon with an average initial body weight of (7.65$±$0.04) g were randomly allocated into four groups, with five replicates per group and 25 fish per replicate. The feeding trial lasted for eight weeks. The results showed that dietary supplementation with 1% AKG significantly increased liver Gln content and glutamine synthetase (GS) activity (P<0.05), while having no significant effect on liver alkaline phosphatase (ALP) activity (P>0.05). Protein source showed no significant effects on liver Gln content, GS activity, or ALP activity (P>0.05), and no significant interaction was observed between protein source and AKG supplementation level for these parameters (P>0.05). Dietary 1% AKG supplementation significantly decreased liver malondialdehyde (MDA) content and increased liver glutathione (GSH) content (P<0.05). Compared with the SPC+FM protein source, SPC alone significantly increased liver GSH content (P<0.05). Neither protein source nor AKG supplementation level significantly affected liver superoxide dismutase (SOD) or catalase (CAT) activities (P>0.05), and no significant interaction was found between these factors for any antioxidant indices (P>0.05). Dietary 1% AKG supplementation significantly increased the relative expression levels of liver GH and IGF- genes (P<0.05). Compared with the SPC+FM protein source, SPC alone significantly increased the relative expression levels of liver IGF- and GH genes (P<0.05). No significant interaction was observed between protein source and AKG supplementation level on the relative expression of liver GH and IGF- genes (P>0.05). In conclusion, dietary supplementation with 1% AKG in juvenile hybrid sturgeon can increase Gln content by enhancing GS

activity, reduce MDA content by increasing GSH content, and upregulate the expression of growth-related genes GH and IGF- in the liver.

Keywords: juvenile hybrid sturgeon; α-ketoglutarate; glutamine; antioxidation; GH gene; IGF-

Introduction

Glutamine (Gln), as a semi-essential amino acid, is the most abundant amino acid in fish muscle and serum, accounting for approximately 25% of total amino acids in extracellular fluid and over 60% in skeletal muscle. Gln promotes protein deposition and provides nitrogen for the synthesis of purines, pyrimidines, nucleotides, and amino sugars. Previous studies have demonstrated that dietary Gln supplementation enhances growth in Jian carp (Cyprinus carpio var. Jian), hybrid sturgeon (Acipenser schrenckii ×Acipenser baeri), red drum (Sciaenops ocellatus), and hybrid striped bass (Morone chrysops×Morone saxatilis). However, the poor stability and low water solubility of Gln limit its application in animal nutrition.

α-ketoglutarate (AKG), a precursor of Gln and an intermediate metabolite of the tricarboxylic acid cycle, can combine with ammonia to form glutamate and Gln. AKG effectively promotes nitrogen metabolism and reduces ammonia toxicity. It activates the mammalian target of rapamycin signaling pathway, thereby promoting protein deposition in intestinal epithelial cells. Dietary AKG supplementation in common carp significantly increases GS gene expression and consequently elevates Gln content. Our laboratory has previously investigated the effects of AKG on growth, ammonia stress response, intestinal health, and antioxidant function in hybrid sturgeon and Songpu mirror carp. These studies consistently demonstrated growth-promoting effects under various dietary protein levels and ingredients, alleviation of stress through increased heat shock proteins under ammonia stress, and improvements in intestinal morphology and antioxidant capacity. As the liver is the primary site of amino acid metabolism, this experiment was designed to investigate the effects of AKG supplementation in diets with different protein sources on liver function in juvenile hybrid sturgeon, with particular focus on the expression of growth-related genes GH and IGF- , thereby providing further theoretical basis for Gln application in hybrid sturgeon feeds.

Materials and Methods

1.1 Experimental Materials

Juvenile hybrid sturgeon were purchased from the Fangshan Sturgeon Farm in Beijing. AKG was purchased from Sigma-Aldrich with a purity of $ $98.5%.

1.2 Experimental Diets

Four experimental diets were formulated to contain 44% crude protein using either soybean protein concentrate (SPC) or SPC+fish meal (FM, imported) as protein sources, with 0 or 1% AKG supplementation. The composition and nutrient levels of the experimental diets are shown in . All ingredients were ground to pass through a 40-mesh sieve, mixed thoroughly in a stepwise manner, and processed into 2.0 mm pellets. The pellets were air-dried at room temperature and stored at -20 °C.

1.1.3 Experimental Design and Feeding Management

Juvenile hybrid sturgeon were disinfected with saline solution (5%) and acclimated for two weeks prior to the experiment. Five hundred healthy fish with uniform size and an average body weight of $(7.65\$\pm0.04)gwererandomlydistributedintofourgroupswithfivereplicateseachcontaining25fish.Theculturec$ °C with dissolved oxygen >5 mg/L, pH 7.8, and natural photoperiod. Fish were fed to satiation three times daily at 08:00, 13:00, and 17:00 at approximately 5% of body weight. Residual feed and feces were removed after each feeding. Water quality was monitored regularly, with one-third of the water in each tank replaced daily with aerated water to ensure optimal conditions. The feeding trial lasted for eight weeks.

1.3 Sample Collection and Analysis

At the end of the feeding trial, fish were fasted for 24 h. Four fish were randomly selected from each replicate, anesthetized with MS-222 (tricaine methanesulfonate), and their livers were collected. Liver samples were homogenized with ice-cold physiological saline at a ratio of 1:9 (w/v) using an FJ-200CL high-speed tissue homogenizer (15,000 r/min, 3 min) and centrifuged at 4,000 r/min for 10 min at 4 °C. The supernatant was collected in 1.5 mL centrifuge tubes and stored at -40 °C for analysis of Gln content, GS activity, ALP activity, and antioxidant indices. Commercial kits from Nanjing Jiancheng Bioengineering Institute were used to determine Gln content, GS activity, ALP activity, reduced glutathione (GSH) content, superoxide dismutase (SOD) activity, catalase (CAT) activity, and malondialdehyde (MDA) content according to the manufacturer' s instructions.

Additionally, three fish per replicate were randomly selected for gene expression analysis. Their livers were wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80 °C. Total RNA was extracted using the SV Total RNA Isolation System (Promega) following the manufacturer's protocol. Complementary DNA was synthesized using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) and stored at -20 °C. Primers for GH, IGF- , and 18S rRNA were designed using Primer Premier 5.0 software based on sturgeon mRNA sequences: IGF- (F: 5 -TCATCGCCCTGACAGTCTACAT-3 , R: 5 -GGTCGCCTGCTGAAATAAAAG-3), GH (F: 5 -AGATGAGCAGCGTCACTCCAGC-

3 , R: 5 -AGAGCCACAATACCTTCCTCCA-3), and 18S rRNA (F: 5 -CCGCTTTGGTGACTCTGGAT-3 , R: 5 -CTTGGATGTGGTAGCCGTTTC-3). All primers were synthesized by Sangon Biotech (Shanghai). Real-time PCR was performed using an Applied Biosystems 7500 Real-Time PCR System with a 20 µL reaction volume. Relative gene expression was calculated using the 2-ΔΔCT method, with data representing three replicates.

1.4 Data Analysis

Data were analyzed using SPSS 19.0 software with two-way ANOVA, using protein source and AKG supplementation level as factors. Significance was set at $P<0.05$. Results are presented as means ± standard deviation.

Results

2.1 Effects on Liver Gln Content, GS Activity, and ALP Activity

The effects of dietary AKG supplementation on liver Gln content, GS activity, and ALP activity in juvenile hybrid sturgeon fed different protein source diets are presented in . Two-way ANOVA revealed that AKG supplementation level significantly affected liver Gln content and GS activity ($P<0.05$). Dietary supplementation with 1% AKG significantly increased liver Gln content and GS activity ($P<0.05$) but had no significant effect on ALP activity ($P>0.05$). Protein source showed no significant effects on liver Gln content, GS activity, or ALP activity ($P>0.05$), and no significant interaction was observed between protein source and AKG supplementation level for these parameters ($P>0.05$).

2.2 Effects on Liver Antioxidant Indices

The effects of dietary AKG supplementation on liver antioxidant indices are shown in . Two-way ANOVA indicated that AKG supplementation level significantly affected liver MDA and GSH contents ($P<0.05$). Dietary supplementation with 1% AKG significantly decreased liver MDA content and increased liver GSH content ($P<0.05$). Protein source significantly affected liver GSH content ($P<0.05$), with SPC alone resulting in significantly higher liver GSH content compared with the SPC+FM combination ($P<0.05$). Neither protein source nor AKG supplementation level significantly affected liver SOD or CAT activities ($P>0.05$), and no significant interaction was found between these factors for any antioxidant indices ($P>0.05$).

2.3 Effects on Liver IGF- and GH Gene Expression

The relative expression levels of liver IGF- and GH genes are presented in . Two-way ANOVA showed that AKG supplementation level significantly affected the relative expression of both liver IGF- and GH genes ($P<0.05$). Dietary supplementation with 1% AKG significantly increased the relative expression levels of liver IGF- and GH genes ($P<0.05$). Protein source also significantly affected

the relative expression of liver IGF- and GH genes ($P<0.05$), with SPC alone resulting in significantly higher expression levels compared with the SPC+FM combination ($P<0.05$). No significant interaction was observed between protein source and AKG supplementation level on the relative expression of liver IGF- and GH genes ($P>0.05$).

Discussion

3.1 Effects on Liver Gln Content, GS Activity, and ALP Activity

The liver exhibits vigorous amino acid metabolism, during which harmful ammonia is produced and detoxified through urea synthesis. As an intermediate metabolite of the tricarboxylic acid cycle, AKG can be converted to glutamate via glutamate dehydrogenase and subsequently to Gln through the amination action of GS, represented by the following reactions: AKG + ammonium ion (NH4+) + NADH → glutamate + NAD+; glutamate + NH4+ + ATP → glutamine + ADP + Pi. GS is the key enzyme catalyzing the conversion of AKG to Gln and is distributed across various species and tissues. In common carp, GS gene expression in the liver is lower than in the intestine, brain, and eye but significantly higher than in the kidney, spleen, gills, and muscle, indicating active AKG metabolism in the liver. Dietary AKG supplementation has been shown to increase liver Gln content, GS activity, and GS gene expression, which is consistent with our findings. Under ammonia stress conditions, dietary AKG significantly reduces blood ammonia levels while increasing liver Gln content and GS activity in hybrid sturgeon. In the present study, dietary supplementation with 1% AKG significantly increased liver Gln content and GS activity, consistent with previous findings on intestinal effects, demonstrating that dietary AKG can be effectively converted to Gln in fish.

3.2 Effects on Liver Antioxidant Capacity

The antioxidant system comprises enzymatic and non-enzymatic components. The enzymatic system includes SOD, glutathione peroxidase (GSH-Px), and CAT, while the non-enzymatic system includes GSH, vitamin E, and vitamin C. Malondialdehyde can cross-link with nucleic acids, proteins, and phospholipids, causing loss of biological activity, making MDA content a reliable indicator of lipid peroxidation and cellular damage. Glutathione maintains redox homeostasis and serves as a primary defense against reactive oxygen species, providing a comprehensive reflection of cellular antioxidant capacity and oxidative damage. Beyond its role in the tricarboxylic acid cycle, AKG participates in various physiological and biochemical processes and functions as an antioxidant that scavenges reactive oxygen radicals. In vitro studies have demonstrated that AKG can inhibit hydrogen peroxide-induced oxidative stress in human erythrocytes and neurons. Under ammonia stress conditions, dietary AKG significantly increases SOD, GSH-Px, and CAT activities while decreasing MDA content in hybrid sturgeon. Similar results in rats indicate that AKG enhances SOD and

CAT activities by promoting lipid metabolism and inhibiting free radical generation, consistent with our findings. Furthermore, the increased liver Gln content resulting from 1% AKG supplementation may contribute to enhanced antioxidant enzyme activity, as Gln has been shown to improve antioxidant capacity in hybrid sturgeon.

3.3 Effects on Liver IGF- and GH Gene Expression

Growth hormone plays a decisive role in promoting animal growth by stimulating the synthesis and release of IGF- from the liver, which then acts on target cells. Insulin-like growth factor- is primarily produced in the liver in response to GH stimulation and positively influences growth, immune function, bone development, and reproduction. Positive correlations between plasma IGF- levels and weight gain have been observed in piglets. Studies have shown that AKG ingestion increases plasma levels of hormones including insulin, GH, and IGF- in humans, and dietary AKG ameliorates the negative effects of lipopolysaccharide on serum IGF- levels in weaned piglets. These findings collectively demonstrate the positive role of IGF- in growth promotion, with synergistic effects between IGF- and GH. Our results show that dietary supplementation with 1% AKG significantly increased the relative expression levels of liver IGF- and GH genes, thereby promoting growth.

In conclusion, dietary supplementation with 1% AKG in juvenile hybrid sturgeon can increase liver Gln content by enhancing GS activity, reduce MDA content by increasing GSH content, and upregulate the expression of growth-related genes GH and IGF- in the liver.

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