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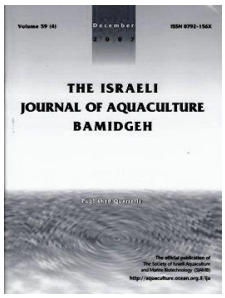

Effects of Dietary Alpha-ketoglutarate Supplementation on Growth and Serum Biochemical Parameters of Grass Carp (*Ctenopharyngodon idella*) Fingerlings

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Key words: *Ctenopharyngodon idella*, Alpha-ketoglutarate, growth performance, serum biochemical parameters

Abstract

An 8-week growth trial was conducted to evaluate the effects of alpha-ketoglutarate (AKG) on growth performance and serum biochemical parameters of grass carp (*Ctenopharyngodon idella*), and to determine the optimal amount of AKG in feeds. A total of 450 grass carp (mean initial body weight, 35.64±1.39 g) were randomly divided into five treatment groups, with three replicates each. Treatments were, basal diet supplemented with 0% (the control group), 0.25%, 0.5%, 0.75% and 1% AKG, respectively. The results showed that fish fed the 0.75% AKG diet had significantly higher final body weight, weight gain rate (WGR), specific growth rate (SGR), serum superoxide dismutase (SOD) activity, lysozyme (LSZ) activity, and significantly lower feed conversion ratio (FCR), viscerasomatic index (VSI), serum malondialdehyde (MDA), and UREA content than fish fed the control diet (p<0.05). Fish fed diets containing AKG had significantly higher serum glucose (GLU) content than fish fed the control diet (p<0.05). No significant differences in survival rate (SR), muscle proximate composition, serum total protein (TP) and albumin (ALB) content were observed among the groups (p>0.05). The present study suggests that feed supplementation with 0.75% AKG can promote growth of *Ctenopharyngodon idella* fingerlings, enhance antioxidant capacity and their non-specific immunity.

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Introduction

The intensive rearing of aquaculture fish species generates a potentially stressful environment, possible suppression of the immune system, and increased susceptibility to different pathogenic organisms. Many studies have looked into the modulation of the immune system in fish as a means to prevent disease outbreaks, and the possibility of altering nutrition to enhance growth and fish health.

AKG is a key intermediate in the tricarboxylic acid cycle as well as a precursor of glutamate and glutamine (Sena et al., 1995), and is of physiological and nutritional importance for neonates, particularly under stressful conditions (Wu, 2010). Exogenous AKG can be converted to glutamate and glutamine in many tissues (Kristensen et al., 2002) and has ve a sparing effect on glutamate and aspartate in cells by serving as a fuel source (Hou et al., 2011a). Studies have demonstrated that dietary supplementation with 1% AKG alleviates intestinal injury (Hou et al., 2010), is beneficial in improving the energy status of the intestinal mucosa of LPS-challenged pigs (Hou et al., 2011b), and effectively maintains the balance of total nitrogen and promotes protein synthesis (Huang et al., 2012; Yao et al., 2012; Wei, 2013), up-regulated gene expression of stress related protein (Liang et al., 2014), increased the level of insulin, growth hormone and insulin like growth factor-1 in plasma (Wang et al., 2010). However, these studies mainly focus on animal husbandry.

Grass carp (*Ctenopharyngodon idella*) is an important economic freshwater species that is widely cultured (FAO, 2015). So far, no study has been conducted to evaluate the effect of dietary AKG supplementation on grass carp. The present study was conducted to investigate the effects of dietary AKG supplementation on growth performance and serum biochemical index, and to determine optimum dietary AKG supplementation level for young grass carp, which could provide partial theoretical evidence for developing AKG as a new green feed additive in grass carp and other aquatic animals.

Materials and Methods

Experimental diets and design. The basal diet was made according to the standard for nutrients in formula diet for grass carp (SC/T 1024-2002). The formulation and proximate composition are shown in Table 1. Five experimental diets were prepared by respectively replacing equivalent wheat middling in the basal diet with 0% (the control group), 0.25%, 0.5%, 0.75% and 1% AKG (Shanghai Haiquchem Co. Ltd., purity of 99%). All ingredients were crushed, mixed, and pelleted into 2-mm diameter granules with a laboratory pelleting machine with an outlet temperature of 85 ± 2 °C. These were then air-dried and stored in plastic bags at -20 °C until use.

Feeding management. The procedures used in this study were approved by the University of Hunan Agricultural Animal Care Advisory Committee. Grass carp were obtained from Fisheries Science Research Institute (Changsha, China). Before the beginning of the experiment, fish were acclimated with the basal diet for 2 weeks, after which 450 grass carp with an average initial weight 35.64±1.39 g were randomly distributed into 15 experimental net cages (1.5×1.5×1.5 m^3). Each experimental diet was randomly assigned to five cages with three replications. The fish were fed with their respective experimental diets to apparent satiation twice a day for 8 weeks. During this period, dissolved oxygen was maintained at >5 mg/L. Water temperature and pH were maintained at 27±3 °C and 7.0±0.5, respectively, under a natural light cycle.

Sample collection and analysis. Fish in each cage were weighed and counted at initiation and termination of the feeding trial. At the end of the experiment, the fish were fasted for 24 h, and five fish per cage were randomly sampled. Three of them were individually weighed and dissected, samples of their viscera were collected and weighed and their viscerosomatic index (VSI) was calculated. The dorsal muscles were scraped off the fish, pooled, chopped, and stored frozen (-20 °C) until analysis of proximate chemical composition. At the same time, blood samples were individually collected from the caudal vein of the other two fish using a 1 ml syringe, and centrifuged at 3500 ×g at 4 °C for 10 min to separate the serum. The supernatant was removed and stored at -80°C for subsequent serum biochemical measurement.

Proximate composition analysis. Moisture, crude protein, and crude lipid content of the diets and fish muscle were determined by standard methods (AOAC, 1997). Moisture was determined by oven drying until constant weight (105°C), crude protein (nitrogen × 6.25) by the Kjeldahl method with an Auto Kjeldahl System (FOSS KT260, Switzerland), crude lipid by ether extraction with the Soxtec System HT6 (FOSS, Tecator, Sweden), and crude ash by combustion at 560°C for 5 h. Gross energy was determined by adiabatic bomb calorimetry (Parr 6300 adiabatic bomb calorimeter, Parr Instrument Co., Moline, Illinois).

Biochemical measurements. The contents of serum total protein (TP), albumin (ALB), glucose (GLU) and UREA were measured by colorimetric method, using Mindray Auto Biochemical Analyzer (BS-200, Mindray, P.R. China) and test kit from Mindray Bio Medical Co., Ltd. in China. The superoxide dismutase (SOD) activity, lysozyme (LSZ) activity, and malondialdehyde (MDA) content were measured by colorimetric method, using 722 spectrophtometer (Shanghai optical instrument factory, China) and test kit from Nanjing Jiancheng Bioengineering Institute in China.

Calculations and Statistical analysis. The parameters for assessment of growth performance were calculated thus:

Weight gain rate (WGR %) = 100×(final body weight - initial body weight)/initial body weight,

Specific growth rate (SGR %/d) = 100×(ln final body weight - ln initial body weight)/feeding days

Feed conversion ratio (FCR) = dry feed intake/fish wet weight gain

Survival rate (SR %) = 100 × Final number/Initial number

Viscerasomatic index (VSI %) = 100×(weight of viscera/total body weight)

Results are presented as the means ± standard deviation (SD). All data were subjected to a one-way analysis of variance (ANOVA), which was followed by Duncan's multiple-range test, to determine significant differences among treatment groups at the level of $p<0.05$ using the software SPSS18.0 (SPSS Inc., Chicago, IL, USA).

Table 1. Composition and nutrient contents of the basal diet (air-dry basis)

Ingredients	*(%)*	*Nutrition levels*	
Corn	2.0	Dry matter (%)	89.21
Soybean oil	1.0	Crude protein (%)	29.51
Soybean meal	18.0	Crude lipid (%)	4.45
Cottonseed meal	20.0	Crude ash (%)	7.62
Rapeseed meal	20.0	Gross energy(kJ/g)	15.78
Oil bran	10.0		
Wheat middling	20.0		
Distillers dried grains with solubles	4.0		
Calcium dihydrogen phosphate	1.9		
Choline chloride (50%)	0.1		
Premix*	3.0		

*Premix provided the following amounts of vitamins and trace minerals/kg of the complete diet: VA 2000 IU, VB_1 5 mg, VB_2 10 mg, VB_6 10 mg, VB_{12} 0.02 mg, VD_3 2000 IU, VE 100 IU, VK_3 10 mg, VC 300 mg, biotin 1mg, folic acid 5 mg, calcium pantothenate 40 mg, nicotinic acid 100 mg, Cu (as copper sulfate) 3 mg, Fe (as ferrous sulfate) 150 mg, Mn (as manganese sulfate) 13 mg, Zn (as zinc sulfate) 34 mg, I (as potassium iodide) 5.5 mg, Se (as sodium selenite) 0.5 mg.

Results

The effects of dietary AKG supplementation on growth parameters and muscle composition respectively in young grass carp are provided in Tables 2 & 3. SR of grass carp was not significantly affected ($p>0.05$) by dietary AKG supplementation (Table2). Fish fed a diet supplemented with 0.75% AKG exhibited significant ($p<0.05$) increases in the FBW, SGR and WGR and significant ($p<0.05$) decreases in FCR compared to those fed the basal diet, while no significant ($p>0.05$) differences were found in the other group. The VSI of grass carp fed diets supplemented with 0.75% and 1.0% AKG decreased significantly compared to the control group ($p<0.05$). The contents of moisture, crude protein and crude lipid in the dorsal muscle did not differ significantly ($p>0.05$) among the groups (Table 3).

Table 2. Growth performance of grass carp fed diets with graded levels of AKG

	Dietary AKG level (%)				
	0 (control)	*0.25%*	*0.5%*	*0.75%*	*1.0%*
IBW (g)	35.37±0.42	35.43±0.38	35.57±0.21	36.1±0.20	35.7±0.44
FBW (g)	121.23±16.16^{b}	125.25±10.45^{b}	119.47±19.32^{b}	161.83±27.66^{a}	131.93±8.97^{ab}
WGR (%)	242.54±44.83^{b}	253.52±23.70^{b}	235.94±55.05^{b}	348.27±76.37^{a}	269.72±29.32^{ab}
SGR (%/d)	2.04±0.21^{b}	2.10±0.11^{ab}	2.00±0.27^{b}	2.49±0.27^{a}	2.18±0.13^{ab}
FCR	1.82±0.12^{a}	1.79±0.30^{a}	1.72±0.16^{ab}	1.68±0.18^{b}	1.71±0.13^{ab}
SR (%)	92.20±1.91	93.33±3.35	94.43±5.10	95.53±3.87	94.43±1.96
VSI (%)	11.54±1.74^{a}	11.06±1.28^{ab}	11.07±0.72^{ab}	10.29±0.99^{b}	10.55±0.99^{b}

Values are mean ± SD. Values in the same row with different superscripts are significantly different (p<0.05); IBW, initial body weight; FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate ; VSI, viscerasomatic index.

Table 3. Muscle composition of grass carp fed diets with graded levels of AKG（%）

	Dietary AKG level (%)				
	0 (control)	*0.25%*	*0.5%*	*0.75%*	*1.0%*
Moisture	78.54±1.94	79.37±1.04	77.75±2.28	79.61±0.67	77.63±1.71
Crude protein	17.47±0.40	17.75±0.87	18.07±0.25	17.54±0.63	18.47±0.35
Crude lipid	2.51±0.17	2.54±0.29	2.78±0.22	2.66±0.51	2.94±0.10

Table 4. Serum biochemical parameters of grass carp fed diets with graded levels of AKG

	Dietary AKG level (%)				
	0 (control)	*0.25%*	*0.5%*	*0.75%*	*1.0%*
TP (g/l)	30.00±3.10	29.80±1.90	31.50±2.90	30.00±5.60	32.20±3.10
ALB (g/l)	14.78±1.59	12.73±1.75	14.62±1.48	12.93±2.91	14.03±1.62
GLU (mmol/l)	4.83±1.18^{c}	8.44±0.47^{b}	8.47±0.76^{b}	7.61±0.45^{b}	11.89±2.05^{a}
UREA (mmol/l)	1.43±0.10^{a}	1.39±0.24^{ab}	1.34±0.12^{ab}	1.15±0.21^{b}	1.26±0.19^{ab}
MDA (nmol/l)	11.06±0.67^{a}	10.82±0.79^{a}	11.71±0.62^{a}	9.68±0.53^{b}	11.27±0.85^{a}
SOD (U/l)	207.26±4.67^{c}	211.28±7.94^{bc}	217.26±8.95^{ab}	224.14±2.49^{a}	215.30±5.40^{ab}
LSZ (U/l)	312.53±8.03^{b}	319.41±7.47^{b}	336.18±21.49^{b}	373.63±53.38^{a}	327.74±9.10^{b}

Serum biochemical parameters. The present study illustrated that the levels of serum TP and ALB did not differ significantly (p>0.05) among all experimental groups. Compared to the control group, GLU level was significantly (p<0.05) increased in serum of fish fed diets containing AKG. The level of serum UREA was decreased with increasing dietary AKG supplementation up to 0.75%, and increased thereafter (p<0.05). The level of MDA in serum of fish fed diet supplemented with 0.75% AKG was significantly (p<0.05) lower than in the other groups. The activity of SOD and LSZ increased with increasing dietary AKG supplementation up to 0.75%, and decreased thereafter (p<0.05) (Table 4).

Discussion

Growth performance is a major criterion in animal production. The present study showed that WGR and SGR improved significantly in grass carp fed a diet supplemented with 0.75% AKG. Similar results were observed in Songpu mirror carp fed the 34% protein diet containing 0.75% AKG (Wei, 2013). Previous studies have shown that dietary supplementation with 1.0% AKG tended to increase average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) in weanling pigs, but did not affect growth performance in lipopolysaccharide (LPS)-challenged pigs when feed intake was decreased (Hou et al., 2011a). It has also been reported that dietary AKG supplementation could relieve growth depression in weaned piglets chronically challenged by LPS when their feed intake was improved (Liu et al., 2009). This suggests that the enhancement of animal growth may be attributed to the fact that FI and FE were improved with appropriate dietary AKG supplementation. The improvement of FE may possibly be attributed to the integrity of the intestinal structure and the improvement of the enzyme activity of the gut (Hou et al., 2011a; Wei, 2013). The present study showed that dietary AKG supplementation did not affect the moisture, lipid, and protein contents

in dorsal muscle and SR values of young grass carp, which were consistent with the results for Songpu mirror carp (Wei, 2013).

Blood testing in fish can be useful in monitoring general fish health. In this study GLU level was lowest in serum of fish fed the control diet, 0% AKG supplementation. UREA levels significantly decreased in serum of grass carp fed a diet supplemented with 0.75% AKG. This reduction is thought to be associated with reduced protein breakdown in the body (Ajeniyi et al., 2014). Our study showed that UREA levels significantly decreased in serum of grass carp fed a diet supplemented with 0.75% AKG. This was also consistent with results for Songpu mirror carp fed the 31% protein diet with supplementation of 0.75% AKG (Wei, 2013).

Increased total protein, albumin, and globulin levels are thought to play a significant role in enhanced immune response in fish (Wiegertjes et al., 1996). TP were significantly higher in the serum of Songpu mirror carp fed the 34% protein diet supplemented with 0.75% AKG, and ALB was significantly higher in the serum of Songpu mirror carp fed the 31% protein diet supplemented with 1.5% AKG (Wei, 2013). However, in the present study, there were no significant differences ($p>0.05$) in the serum TP and ALB values in any of the experimental groups.

Lysozyme splits the β-1,4 glycosidic bonds between N-acetyl glucosamine and N-acetyl muramic acid of peptidoglycan of bacterial cell walls, causing bacteriolysis which controls infection. The increase of lysozyme activity within a certain range stimulates the immune response of fish which may contribute to increased resistance to infection (Ye et al., 2011; Sun et al., 2012). In the present study, grass carp fed a diet containing 0.75% AKG for 8 weeks showed significantly enhanced serum lysozyme activity, suggesting that supplemented AKG may have increased lysozyme activity contributing to the non-specific innate immunity of this fish.

Usually, MDA is an important indicator to reflect the extent in the accumulation of free radicals in the body caused by oxidative damage (Tokur et al., 2007). Superoxide dismutase functions to remove the superoxide anion and is part of free radical scavenging systems. Earlier reports showed that AKG offers protection against oxidative damage of human erythrocytes induced by hydrogen peroxide by participating in the non-enzymatic oxidative decarboxylation in the hydrogen peroxide decomposition process (Sokolowska et al., 2000). Reports have also shown that AKG enhances the intestinal antioxidative capacity by partly increasing the activity of SOD in weaned piglets challenged with LPS (Hou et al., 2011a). In the present study, MDA content and SOD activity were respectively the lowest and the highest in serum of fish fed a diet supplemented with 0.75% AKG, suggesting that AKG may improve the growth performance of grass carp by suppressing oxidative stress.

In conclusion, results of the present study suggest that feed supplementation with 0.75% AKG can promote growth of *C. idella* fingerlings, enhance both antioxidant capacity and their non-specific immunity.

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