Aquaculture International (2020) 28:511–524
https://doi.org/10.1007/s10499-019-00475-2

Effects of diet α-ketoglutarate (AKG) supplementation on the growth performance, antioxidant defense system, intestinal digestive enzymes, and immune response of grass carp (*Ctenopharyngodon idellus*)

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Received: 22 May 2019 / Accepted: 1 October 2019 / Published online: 5 November 2019

Abstract
This study was conducted to investigate the effects of diet 7.5 g/kg α-ketoglutarate (AKG) on the growth performance, antioxidant defense system, digestive enzymes, and immune response of grass carp (*Ctenopharyngodon idellus*). A total of 400 grass carp with an average body weight 10.81 ± 0.68 g was randomly allocated into 2 groups with 4 replicates of 50 fish respectively. The experiment was conducted in net cages (1.5 m × 1.5 m × 1.5 m) suspended in an indoor cement pool. Fish were fed a basic diet containing either 0 (control) or 7.5 g/kg AKG (supplemented diet). The experiment lasted for 8 weeks (56 days). Results indicated that compared with the control group, the final weight (FW), weight gain rate (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) in the AKG group were increased significantly ($P < 0.05$). However, the feed conversion ratio (FCR) was decreased significantly ($P < 0.05$). The 7.5 g/kg AKG supplementation significantly increased the activities of glutamine synthetase (GS), glutathione peroxidase (GSH-Px), catalase (CAT), total superoxide dismutase (T-SOD), and hexokinase (HK), as well as the concentrations of glutathione (GSH), total antioxidant capacity (T-AOC), and complement 3 (C3) in blood ($P < 0.05$), while significantly decreased the concentrations of malondialdehyde (MDA) and hemoglobin (Hb) ($P < 0.05$). The GS activity and GSH concentration in hepatopancreas were increased significantly ($P < 0.05$), whereas the glycogen concentration in hepatopancreas, and the glycogen concentration and GS activity in the muscle were significantly decreased ($P < 0.05$). In addition, 7.5 g/kg AKG supplementation significantly increased the concentration of GSH and the activities of amylase, protease, and lipase in fore-gut, alkaline phosphates (ALP) in the mid-gut, and Na-ATP and Ca-ATP in the gill ($P < 0.05$), as well as γ-glutamyl transpeptidase (γ-GT) both in fore-gut and mid-gut ($P < 0.05$), whereas the activity of acid phosphatase (ACP) in the mid-gut was decreased significantly ($P < 0.05$). In conclusion, diet 7.5 g/kg AKG supplementation in grass carp may improve the growth performance and immune response and play crucial roles in regulating the activities of GS, antioxidant defense system, and digestive enzymes.

Keywords α-Ketoglutarate · Growth performance · Antioxidant defense system · Digestive enzyme · Immune response · *Ctenopharyngodon idellus*

Abbreviations

ROS	Reactive oxygen species
ACP	Acid phosphatase
ALP	Alkaline phosphates
Gln	Glutamine
GS	Glutamine synthetase
AKG	α-Ketoglutarate
TCA	Tricarboxylic acid cycle
IW	Initial weight
FW	Final weight
WGR	Weight gain rate
SGR	Specific growth rate
FCR	Feed conversion ratio
PER	Protein efficiency ratio
TP	Total protein
ALB	Albumin
LSZ	Lysozyme
C3	Complement 3
γ-GT	γ-Glutamyl transpeptidase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GSH	Glutathione
GST	Glutathione-S-transferase
GSH-Px	Glutathione peroxidase
SOD	Superoxide dismutase
CAT	Catalase
T-AOC	Total antioxidant capacity
MDA	Malondialdehyde
NO	Nitric oxide
Hb	Hemoglobin
HK	Hexokinase
INS	Insulin
ADA	Adenosine deaminase

Introduction

Current intensive fish farming usually leads to cultured fish in a sub-healthy status (Bondad-Reantaso et al. 2005; Cock et al. 2009; Mian and Siddiqui 2014). It is now widely accepted that nutritional approaches are essential to alleviate cultured fish sub-health. A large number of feed additives (e.g., organic and inorganic acids, feed enzymes, pre and probiotics, and essential oils) have been used in the aquiculture industry due to the beneficial influences for stimulating digestive function and improving immune response and physical barrier function (Lange et al. 2010). Previous studies indicate that fish intestine, because of the high polyunsaturated fatty acids content (up to 24.9% of total fatty acid composition), is susceptible to be attacked by reactive oxygen species (ROS) (Deng et al. 2014). ROS may trigger apoptosis in fish erythrocytes (Li et al. 2017). ROS production also may result to oxidative damage, which

may be a significant toxicity in aquatic organisms (Livingstone 2003). Oxidative stress has been associated with the development of pathological conditions in tissue such as inflammation due to the high consumption of oxygen and high quantities of polyunsaturated fatty acids in the tissue (Almeida et al. 2008).

α-Ketoglutarate (AKG) is an important intermediate in the citric acid cycle (Krebs cycle) and a key node in the intracellular carbon–nitrogen metabolism. More importantly, AKG is a precursor of some important free amino acids (FAAs) in vivo, such as glutamic acid, glutamine, proline, and arginine. For instance, AKG can be rapidly transaminated to glutamic acid by glutamate dehydrogenase and then further aminated to glutamine by glutamine synthetase (Yao et al. 2012; Wang et al. 2016a). AKG is also considered as a crucial molecule in transmembrane amino acid transport, protein metabolism, and cellular redox regulation (Wang et al. 2017a, Hou et al. 2011). Study on common carp (*Cyprinus carpio*) shows that the fish might be ureagenic or use glutamate to detoxify ammonia (Hoseini et al. 2019). Due to the important role of AKG as an energy donor, it was assumed that AKG may compensate the energy consumption in the process of ureogenesis. The intracellular AKG level may be contributed to the maintenance of cellular identity and play mechanistic roles in the transcriptional and epigenetic status of stem cell (Carey et al. 2015). AKG exerts positive effects on immunological responses and fillet yield of juvenile red drum fed adequate or low-phosphorus diets (Xu and Gatlin 2018). And it also indicated that appropriate levels of AKG to the low-P feed will improve the growth performance of juvenile mirror carp (*Cyprinus carpio*) and promote the digestion and absorption of nutrients (Ai et al. 2019). Previous study also finds that AKG supplementation can promote the growth performance of grass carp, a commercially important freshwater fish in China, and suggests that 0.75% AKG supplementation in diet will enhance the antioxidant capacity and non-specific immunity basing on serum biochemical parameters (Wang et al. 2016a). AKG is also considered as an antioxidant and plays key roles in the detoxification of ROS (Mailloux et al. 2009). The aim of this study was to reconfirm the effects of dietary 0.75 % AKG supplementation in grass carp (*Ctenopharyngodon idellus*) on the growth performance and investigate the antioxidant capacity in the body including serum, hepatopancreas, and intestine biochemical index.

Materials and methods

Diet preparation

Two diets were designed in the experiment, that is, the basal diet and 7.5 g/kg AKG-supplemented diet. The ingredient composition of the basal diet was shown in Table 1 which is in accordance with the grass carp feed nutrition standard (GBT36205-2018). The diet supplemented with AKG was prepared by replacing equivalent wheat middling in the basal diet with 0.75% AKG (Shanghai haiquchem Co. Ltd., with the concentration 99%) according to previous study (Wang et al. 2016a). All ingredients were crushed, mixed, and pelleted into 2-mm-diameter granules with a laboratory pellet machine. Diets were air dried and stored in plastic bags at − 20 °C until use.

Fish and experimental conditions

Experiment was carried out in accordance with the ethical guidelines of Hunan Agricultural University for the care and use of laboratory animals.

Table 1 Composition and nutrient levels of basal diet (%)

Ingredients	Content	Nutrient levels	Content
Corn	2	Crude protein	29.5
Soybean oil	1	Crude fat	4.4
Soybean meal	18	Ash	7.6
Cottonseed meal	20	Gross energy/(MJ/kg)	15.1
Rapeseed meal	20		
Rice bran	10		
Wheat middling	20		
Distiller's dried grains with soluble	4		
Ca $(H_2PO_4)_2$	1.9		
Premix*	3.1		
Total	100		

*Premix: VA, 2000 IU; VB, 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, 1 mg; folic acid, 5 mg; calcium pantothenate, 40 mg; nicotinic acid, 100 mg; antioxidant, 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

Grass carps were obtained from a fish farm in Xiangyin (Hunan, China). Before the experiment, fish were reared for 2 weeks in indoor cement pool (8 m diameter, 2 m height) and fed the basal diet to acclimate to experimental conditions. At the beginning of the experiment, the healthy fish with an average initial weight 10.49 ± 0.58 g were randomly assigned to 8 net cages (1.5 m × 1.5 m × 1.5 m). The initial stocking density was 50 fish per cage. Cages were suspended in an indoor cement pool. Each diet was fed to four randomly assigned cages. All groups of fish were fed their respective diets at a rate of 3.0% body weight per day, divided into equal portions at 9:00 and 17:00. The feeding experiment lasted for 8 weeks with a 12-h light/12-h dark photoperiod (light: 7:00–19:00). Water was continuously aerated to maintain the dissolved oxygen level above saturation, and 1/3 water in the pool was exchanged with fresh water every morning before feeding. During the experiment period, water temperature and pH averaged 28.00 ± 2 °C and 7.0 ± 0.3 respectively. Dissolved oxygen concentrations were not less than 5 mg/L. The ammonia-N concentrations were not in excess of 0.5 mg/L.

Growth performance

At the end of 8 weeks, 56 days of feeding trial, approximately 24 h after the last feeding, grass carps were individually counted and weighed per replicate at the beginning and end of the experiment. The fish weights were measured for calculation of the final body weight. During the experiment, both of two treatments received 100% survival rate. And the weight gain rates (WGR), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were determined as follows:

$$\mathrm{WGR} = \frac{final \quad total \ weight - initial \ total \ weight}{initial \ total \ weight} \times 100\%,$$

$$\mathrm{SGR} = \left[\frac{\mathrm{Ln}(final \quad total \ weight) - \mathrm{Ln}(initial \ total \ weight)}{time}\right] \times 100\%,$$

$$\mathrm{FCR} = \frac{\text{dry } feed\ intake\ (\mathrm{g})}{\text{wet}\ \ weight\ gain\ (\mathrm{g})},$$

$$\mathrm{PER} = \frac{weight\ gain\ (\mathrm{g})}{protein\ intake\ (\mathrm{g})} \times 100\%,$$

Sample collection and analysis

Four fish were randomly selected from each cage to obtain blood samples from the caudal vein. The separated blood sample from each fish was centrifuged at 3000×*g* for 10 min at 4 °C and stored at − 80 °C for the subsequent assays respectively. And then the fish were disserted, and dorsal muscle, hepatopancreas, gill, and intestine were removed, rinsed in ice-cold saline, and processed respectively. Tissues were homogenized in 10 volumes (*w*/*v*) of ice-cold physiological saline and centrifuged at 4000×*g* for 10 min at 4 °C, and the supernatant was conserved at − 80 °C until analyzed. The glutamine synthetase (GS) was measured according to the GS kit protocol (No. A047). The contents of serum total protein (TP), albumin (ALB), and glucose (GLU) 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 glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), catalase (CAT) activity, acid phosphatase (ACP), alkaline phosphates (ALP), lysozyme (LSZ) activity, complement 3 (C3), γ-glutamyl transpeptidase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), adenosine deaminase (ADA), hexokinase (HK), insulin (INS), hemoglobin (Hb), nitric oxide (NO), glycogen, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) contents were measured by colorimetric method, using the 722 spectrophotometer (Shanghai optical instrument factory, China) and test kit from Nanjing Jiancheng Bioengineering Institute Nanjing, China (Wang et al. 2016a).

Statistical analyses

Student's *t* test was used for evaluation all data. All analyses were performed using SPSS 17.0 software (Chicago, IL, USA), with $P < 0.05$ considered statistically significant. The least squares means and standard error mean (SEM) are presented.

Results

Effects of AKG on growth performance of grass carp

During feeding, it was observed that there was no difference when grass carp consumed the diets in AKG group and the CON group. The effects of dietary 7.5 g/kg AKG on the growth performance of grass carp are shown in Table 2. Compared with the CON group, the FW, WGR, SGR, and PER in the AKG group were increased significantly ($P < 0.05$), while the FCR was significantly decreased ($P < 0.05$).

Table 2 Effects of AKG supplementation on growth performance of grass carp for 8 weeks ($n = 4$)

Items	CON (0.00)	AKG (7.50)	SEM[1]	*P* value
IW (g)[2]	11.45	11.08	0.17	0.214
FW (g)[3]	29.98[b]	33.95[a]	0.50	0.031
WGR (%)[4]	162.14[b]	206.52[a]	7.58	0.015
SGR (%)[5]	1.72[b]	2.00[a]	0.05	0.013
FCR[6]	1.81[a]	1.42[b]	0.07	0.015
PER (%)[7]	1.88[b]	2.39[a]	0.09	0.014

Values in the same row with different small letter superscripts indicate significant difference ($P < 0.05$) while with no or the same small letter superscripts mean no difference ($P > 0.05$).

[1] *SEM*, standard error mean; *n*, no. of observations

[2] *IW*, initial weight

[3] *FW*, final weight

[4] *WGR*, weight gain rate

[5] *SGR*, specific growth rate

[6] *FCR*, feed conversion ratio

[7] *PER*, protein efficiency ratio

Effects of AKG on blood biochemical parameters of grass carp

The effects of dietary AKG on the blood biochemical parameters of grass carp are presented in Table 3. Feeding 7.5 g/kg AKG diet had no effects on the LSZ, NO, ADA, ACP, ALB, TP, ALT, AST:ALT ratio, GLU, and INS in the blood of grass carp compared with the basal diet without AKG supplementation ($P > 0.05$), whereas NO showed a trend of decrease ($P = 0.050$). The 7.5 g/kg AKG diet increased significantly GS, GSH, GST, GSH-Px, CAT, T-SOD, T-AOC, AST, C3, and HK in the blood of grass carp ($P < 0.05$), whereas decreased significantly in the MDA and Hb ($P < 0.05$).

Effects of AKG on muscle physiological and biochemical indices of grass carp

The effects of dietary 7.5 g/kg AKG supplementation on muscle physiological and biochemical indices of grass carp are presented in Table 4. Feeding the 7.5 g/kg AKG diet had no effects on the activity of GS in the muscle of grass carp compared with the basal diet ($P > 0.05$). However, the 7.5 g/kg AKG diet decreased significantly the activity of ADA and concentration of glycogen in the muscle of grass carp ($P < 0.05$).

Effects of AKG on gill biochemical parameters of grass carp

The effects of dietary 7.5 g/kg AKG on the gill biochemical parameters of grass carp are shown in Table 5. Feeding the 7.5 g/kg AKG diet had no effects on the activity of T-ATP in the gill of grass carp compared with the basal diet without AKG supplementation ($P > 0.05$). However, the 7.5 g/kg AKG diet increased significantly the activities of Na-ATP and Ca-ATP in the gill ($P < 0.05$).

Effects of AKG on intestinal biochemical parameters of grass carp

The effects of dietary 7.5 g/kg AKG on the intestinal biochemical parameters of grass carp are shown in Table 6. Feeding the 7.5 g/kg AKG diet increased significantly the activity of GSH,

Table 3 Effects of AKG on blood biochemical parameters of grass carp for 8 weeks ($n = 4$)

Items	CON (0.00)	AKG (7.50)	SEM[1]	*P* value
GS (U/mgprot)	7.67[b]	43.17[a]	0.34	< 0.001
GSH (mg/l)	164.78[b]	210.56[a]	1.94	< 0.001
GST (U/ml)	25.07[b]	26.75[a]	0.32	0.008
GSH-Px (U)	1062.37[b]	1434.12[a]	26.91	< 0.001
CAT (U/ml)	8.50[b]	9.58[a]	0.16	0.001
T-SOD (U/ml)	98.74[b]	107.96[a]	0.31	< 0.001
MDA (nmol/ml)	10.68[a]	4.74[b]	0.29	< 0.001
T-AOC (U/ml)	6.48[b]	14.98[a]	0.25	< 0.001
C3 (g/l)	0.69[b]	0.77[a]	0.01	0.002
LSZ (U/ml)	150.27	152.97	7.41	0.824
NO (μmol/l)	17.50	15.20	0.69	0.050
Hb (g/l)	55.20[b]	52.74[a]	0.51	0.021
HK (U/l)	231.36[b]	252.14[a]	2.44	< 0.001
ADA (U/ml)	25.89	25.08	0.80	0.495
ACP (U/100 ml)	19.63	19.59	0.39	0.945
ALB (g/l)	15.85	15.97	1.18	0.944
AST (U/l)	36.20[b]	76.58[a]	8.33	0.016
ALT (U/l)	4.96	7.40	1.77	0.377
AST:ALT ratio	7.93	11.84	1.64	0.158
TP (g/l)	25.44	26.47	1.17	0.548
Glucose (mmol/l)	5.25	5.97	0.38	0.215
INS (mIU/l)	2.80	2.79	0.03	0.935

GS, glutamine synthetase; *GSH*, glutathione; *GST*, glutathione-S-transferase; *GSH-Px*, glutathione peroxidase; *CAT*, catalase; *T-SOD*, total-superoxide dismutase; *MDA*, malondialdehyde; *T-AOC*, total antioxidant capacity; *C3*, complement 3; *LSZ*, lysozyme; *NO*, nitric oxide; *Hb*, hemoglobin; *HK*, hexokinase; *ADA*, adenosine deaminase; *ACP*, acid phosphatase; *ALB*, albumin; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *TP*, total protein; *INS*, insulin. Values in the same row with different small letter superscripts indicate significant difference ($P < 0.05$) while with no or the same small letter superscripts mean no difference ($P > 0.05$)

[1] *SEM*, standard error mean; *n*, no. of observations

amylase, protease, and lipase in the fore-gut of grass, as well as ALP activity in the mid-gut of grass carp, when compared with the basal diet without AKG supplementation ($P < 0.05$), whereas decreased significantly the concentration of NO and the activity of ACP in the mid-gut ($P < 0.05$). Additionally, 7.5 g/kg AKG diet increased significantly the γ-GT activity in the intestine of grass carp including fore-gut and mid-gut ($P < 0.05$).

Table 4 Effects of AKG on muscle physiological and biochemical indices of grass carp for 8 weeks ($n = 4$)

Items	CON (0.00)	AKG (7.50)	SEM[1]	*P* value
ADA (U/mgprot)	14.56[a]	7.97[b]	1.31	0.008
GS (U/mgprot)	9.27	9.47	0.33	0.680
Glycogen (mg/g)	2.28[a]	2.06[b]	0.05	0.012

ADA, adenosine deaminase; *GS*, glutamine synthetase. Values in the same row with different small letter superscripts indicate significant difference ($P < 0.05$) while with no or the same small letter superscripts mean no difference ($P > 0.05$)

[1] *SEM*, standard error mean; *n*, no. of observations

Table 5 Effects of AKG on gill biochemical parameters of grass carp for 8 weeks ($n = 4$)

Items	CON (0.00)	AKG (7.50)	SEM[1]	*P* value
Na-ATP (U/mgprot)	7.38[b]	8.45[a]	0.20	0.006
Ca-ATP (U/mgprot)	19.98[b]	21.27[a]	0.38	0.045
T-ATP (U/mgprot)	31.40	32.91	0.58	0.104

T-ATP, total-ATP

[1] *SEM*, standard error mean; *n*, no. of observations. Values in the same row with different small letter superscripts indicate significant difference (P < 0.05) while with no or the same small letter superscripts mean no difference (P > 0.05)

Effects of AKG on hepatopancreas physiological and biochemical parameters of grass carp

The effects of dietary 7.5 g/kg AKG on the hepatopancreas physiological and biochemical parameters of grass carp are presented in Table 7. Feeding the 7.5 g/kg AKG diet increased significantly the GS activity and GSH concentration in the hepatopancreas compared with the basal diet without AKG supplementation ($P < 0.05$), whereas decreased significantly the concentration of glycogen ($P < 0.05$).

Discussion

In the present study, feeding the 7.5 g/kg AKG diet increased FW, WGR, SGR, and PER of grass carp and reduced FCR compared with the basal diet without AKG supplementation. This was consistent with the result observed in previous research (Wang et al. 2016a). Similarly, Wang et al. (2016b) reported that AKG diet has positive effects on growth performance of juvenile hybrid sturgeon (*Acipenser schrenckii* ♀ × *Acipenser baerii* ♂). However, the present result was contrary to

Table 6 Effects of AKG on intestinal biochemical of grass carp for 8 weeks ($n = 4$)

Items		CON (0.00)	AKG (7.50)	SEM[1]	*P* value
NO (μmol/gprot)	Fore-gut	70.17	68.86	2.45	0.371
	Mid-gut	33.60[a]	25.61[b]	1.19	0.002
GSH (mg/gprot)	Fore-gut	18.32[b]	26.04[a]	0.65	< 0.001
	Mid-gut	8.79	8.24	0.85	0.671
ACP (U/mgprot)	Fore-gut	2.69	2.96	0.11	0.176
	Mid-gut	1.44[a]	1.27[b]	0.04	0.026
ALP (U/mgprot)	Fore-gut	339.79	349.66	11.31	0.587
	Mid-gut	327.97[b]	456.91[a]	12.46	< 0.001
Amylase (U/mgprot)	Fore-gut	362.06[b]	447.21[a]	18.08	0.014
	Mid-gut	432.92	456.81	11.02	0.178
Protease (U/mgprot)	Fore-gut	1062.71[b]	1264.08[a]	25.48	0.001
	Mid-gut	1306.60	1338.27	28.37	0.490
Lipase (U/gprot)	Fore-gut	241.52[b]	409.15[a]	22.04	0.001
	Mid-gut	572.40	649.77	28.46	0.099
γ-GT (U/gprot)	Fore-gut	39.52[b]	53.86[a]	1.39	< 0.001
	Mid-gut	52.56[b]	77.86[a]	1.84	< 0.001

NO, nitric oxide; *GSH*, glutathione; *ACP*, acid phosphatase; *ALP*, alkaline phosphates; *γ-GT*, γ-glutamyl transpeptidase. Values in the same row with different small letter superscripts indicate significant difference (P < 0.05) while with no or the same small letter superscripts mean no difference (P > 0.05)

Table 7 Effects of AKG on hepatopancreas physiological and biochemical parameters of grass carp ($n = 4$)

Items	CON (0.00)	AKG (7.50)	SEM[1]	*P* value
GS (U/mgprot)	12.57[b]	16.49[a]	0.12	< 0.001
GSH (mg/gprot)	22.79[b]	24.38[a]	0.38	0.022
Glycogen (mg/g)	11.53[a]	9.18[b]	0.40	0.004

GS, glutamine synthetase; *GSH*, glutathione. Values in the same row with different small letter superscripts indicate significant difference ($P < 0.05$) while with no or the same small letter superscripts mean no difference ($P > 0.05$)

the report that there is no effect of 1.0% AKG diet on the weight gain of juvenile red drum (Xu and Gatlin 2018). This difference may be caused by different experimental processes including the different fish species or different dosages. The research shows that 1.0% AKG enhances intestinal absorption and increases the synthesis of intestinal mucosal proteins in piglets (Hou et al. 2010). The study has shown that 1.0% AKG also can promote nitrogen (N), calcium (Ca), and phosphorate (P) utilization efficiency and promote the growth performance of pigs (Chen et al. 2017). Therefore, optimal diet AKG level may be related to its effect on the growth performance of different animals. Furthermore, the research also suggests that adding appropriate levels of AKG to the low-P feed can improve the growth performance of hat juvenile Songpu mirror carp (*Cyprinus carpio*) (Ai et al. 2019). The present results indicated that the enhancement of grass carp growth may be attributed to the diet with 7.5 g/kg AKG supplementation. This also reconfirmed that 7.5 g/kg AKG supplementation in grass carp may be an optimal level, which is suggested by previous study (Wang et al. 2016a).

Under usual conditions, the production of ROS in tissues and their elimination are in a dynamic equilibrium. The excessive levels of ROS lead to the damaged of DNA, lipids, and proteins, finally resulting in the impaired cellular physical barrier function of fish intestine (Ko et al. 2014). To protect cells from oxidative damage during the oxygen metabolism, an antioxidative defense system has presumably evolved in aerobic organisms (Zhang et al. 2010). The antioxidant system of the body includes a series of antioxidant enzymes and antioxidants. The antioxidant enzymes such as SOD, CAT, and GSH-Px have a cellular protective action against oxidative stress (Putker and O'Neill 2016; Zhang et al. 2004; Ransberry et al. 2015; Loro et al. 2012; Glasauer and Chandel 2014; Martínez-Álvarez, Morales, and Sanz 2005). GSH is the major endogenous antioxidant scavenger that protects cells from oxidative stress (Sies 1999). The GSH level in fish is sensitive to the changes in external conditions under oxidative stress (Lin et al. 2018). In present study, 7.5 g/kg AKG supplementation diet increased significantly the activities of GST, GSH-Px, CAT, and T-SOD and the concentrations of GSH and T-AOC in the blood. It also improved the concentration of GSH in the fore-gut and hepatopancreas of grass carp, whereas decreased significantly the concentration of MDA. This was consistent with the previous studies that dietary AKG has a positive effect on the neutrophil oxidative radical production (Xu and Gatlin 2018). Supplementation with AKG also enhanced activities of the antioxidant defense system in hybrid sturgeon (*Acipenser schrenckii* ♀ × *A. baerii* ♂) (Wang et al. 2017b). Specially, proline, as conditionally essential amino acids in fish, can be produced from AKG and plays a key role in protein synthesis, wound healing, and antioxidative reaction (Wu et al. 2011). Glutamine (Gln) plays a protective role against apoptosis and oxidative damage by preventing against the generation of ROS and the oxidation of lipid (Li et al. 2013), and eliminating free radicals because it acts as a precursor for synthesis of the antioxidant glutathione (Wang et al. 2016b). This may be contributed to improve the antioxidant defense system of grass carp in the present study. Previous study has shown that γ-GT help to increase the transport

of glutathione precursors into the cell to maintain the intracellular glutathione (Hegazi et al. 2010a). As a precursor of glutathione (GSH), glutamate exerts alleviative effects on oxidative stress (Shaojuan et al. 2018). The increase of γ-GT activity in the intestine of grass carp in the present study indicated that 7.5 g/kg AKG supplementation diet was beneficial to improving the antioxidant function of grass carp intestinal tissue. Hb possesses intrinsic peroxidase activity and is used to transport and store oxygen (Wicher and Fries 2006). Its significant decrease in the present study may be beneficial to the improvement in antioxidant defense system in the grass carp. In MDA, as the final production of lipid peroxidation, the decreasing level is an index of lower lipid peroxidation (Liu et al. 2015), and the present decreasing level was thought that the response of antioxidant system is to protect the cell against the oxidative stress. Above results confirmed that dietary supplementation with 7.5 g/kg AKG contributed to enhance the activities of the antioxidant defense system enzymes in grass carp. Diet 7.5 g/kg AKG may play beneficial role in maintaining ROS equilibrium with free radical scavenging. It was speculated that AKG exerted antioxidative defense by enzymatic systems, while improving the activities of SOD and CAT, and nonenzymatic GSH level, while reducing the levels of MDA.

In teleost, the increased glutamine content was due to Gln formed from glutamate and NH_4^+ generally (Hegazi et al. 2010a, b; Anderson et al. 2002). The reaction of Gln formed is catalyzed by the enzyme GS, which is a detoxification of ammonia (Wang et al. 2017b; Coutinho et al. 2016; Peh et al. 2010). This is considered a master enzyme to catalyze ATP-dependent biosynthesis of Gln from glutamate (Hu et al. 2017). In the present study, the activity of GS in the blood and hepatopancreas of grass carp increased significantly. This was consistent with previous reported results that dietary AKG supplementation will increase the concentration of Gln and the activity of GS in juvenile hybrid sturgeon (Wang et al. 2016b) and improve the GS activity and Gln concentration in common carp (Dong et al. 2014). Also, it can increase the GS activity in the fore-gut of mirror carp (Wang et al. 2017a). It was suggested that dietary supplemented with AKG may increase directly GS activity (Xu and Gatlin 2018).

In addition, the increase of AST enzyme in the hepatopancreas of grass carp in the present study may have aided in the entry of glutamate into the TCA cycle and its re-synthesis from TCA cycle intermediates (Hegazi et al. 2010a, b). Glutamate supplementation improves hepatic glucose metabolism and facilitates protein replacement by carbohydrates in fish feed (Caballero-Solares et al. 2015). Digestive amylase localizes in the entire gastrointestinal tract of many fish species, but less is known about the regulation of amylase activity, secretion, or biosynthesis (Krogdahl et al. 2005). In present study, diet AKG increased significantly the amylase activity in the fore-gut of grass carp, as well as protease and lipase activities. The levels of activities of digestive enzymes are used as comparative indicators of growth rate and digestive capacity of the fish (Suzer et al. 2007). Fish may change and adapt their metabolic functions and induction of the enzymes (Abhijith et al. 2016). It was speculated that AKG, as an intermediate of TCA, also an intermediate of glutamate and Gln, may contribute to ATP homeostasis in the small intestine (Hou et al. 2011). This may take place through enhancing the digestive enzymes activities, and dominate effective site may be in the fore-gut of grass carp.

AKG is also the main source of energy for cells of the gastrointestinal tract (Sliwa et al. 2006). In the present study, the HK activity in the blood and the Na-ATP and Ca-ATP activities in the gill increased significantly. Glucose should be phosphorylated to glucose-6-phosphate by HK to ensure favorable glucose gradients for transport (Moon 2001), and HK play important roles in intermediary metabolism (Enes et al. 2009). Furthermore, the decrease of liver glycogen of grass carp in current study may be due to the AKG supplementation directly blocking glucose uptake (Doucette et al. 2011).

Deficient or excess levels of certain nutrients will influence immune defense mechanisms. Fish largely relies on antibacterial substances such as LSZ, ACP, complement factors, and antimicrobial peptides to play the immune functions (Xu and Gatlin 2018). Gln may not only promote growth performance but also have an array of desirable immunological attributes in different animal species (Pohlenz et al. 2012). In present study, diet AKG increased significantly the concentration of C3 in the blood and the activity of ALP in the mid-gut of the grass carp. Normally, ALP activities are involved in the membrane transport activities (Molina et al. 2005). In brief, the data acquired from the present study revealed that AKG supplementation may contribute to the production of antibacterial substances, so as to improve the grass carp's health. This may be through enhancing the activities of the antioxidant defense system and digestive enzymes, which are beneficial to improving the growth performance of grass carp. However, further studies need to be carried out to investigate the physiology action mechanism in grass carp.

Conclusion

In conclusion, diet supplemented with 7.5 g/kg AKG in grass carp will improve the growth performance and immune response and may be through regulating the activities of glutamine synthetase enzyme, antioxidant defense enzymes, and digestive enzymes.

Funding information This study was funded by National Key R&D Program of China (No. 2018YFD0900302), National Natural Science Foundation of China (31470132), and Natural Science Foundation of Hunan Province (14JJ4039).

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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