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ORIGINAL ARTICLE

Dietary supplementation with glutamate precursor α-ketoglutarate attenuates lipopolysaccharide-induced liver injury in young pigs

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Abstract There is growing interest in glutamate as a functional amino acid in nutrition and health. This study was conducted to determine whether glutamate precursor α-ketoglutarate (AKG) could alleviate lipopolysaccharide (LPS)-induced liver injury in young pigs. Twenty-four piglets were randomly assigned to the control, LPS, or LPS + AKG group. Piglets in the control and LPS groups were fed a basal diet, whereas piglets in the NAC group were fed the basal diet supplemented with 1 % AKG. On days 10, 12, 14, and 16 of the trial, piglets in the LPS and LPS + AKG groups received intraperitoneal administration of LPS (80 μg/kg BW), whereas piglets in the control group received the same volume of saline. On day 16 of the trial, blood samples were collected 3 h after LPS or saline injection. Twenty-four hours post-administration of LPS or saline (on day 17 of the trial), piglets were killed to obtain liver for analysis. Dietary AKG supplementation alleviated LPS-induced histomorphological abnormalities and mitigated LPS-induced increases in aspartate aminotransferase (AST) activity and AST/ALT ratio ($P < 0.05$). Compared with the LPS group, dietary supplementation with AKG decreased plasma glutamate concentration, while increasing hepatic concentrations of glutamate, glutamine, leucine, asparagine, lysine, alanine, serine, threonine, valine, and phenylalanine ($P < 0.05$). LPS challenge dramatically increased concentrations of malondialdehyde and decreased glutathione peroxidase activity in the liver. Additionally, LPS challenge enhanced concentrations of AMP and total protein, as well as RNA/DNA and total protein/DNA ratios, while decreasing hepatic ADP concentrations. These adverse effects of LPS challenge were ameliorated by AKG supplementation. Collectively, dietary AKG supplementation provides a new means to ameliorate LPS-induced liver injury by increasing anti-oxidative capacity and improving energy metabolism in young pigs.

Keywords α-Ketoglutarate · Piglets · Lipopolysaccharide · Liver injury

Abbreviations

AEC	Adenylate energy charge
AKG	α-Ketoglutarate
ADA	Adenosine deaminase
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BW	Body weight
CAT	Catalase
CHE	Cholinesterase
GSH-Px	Glutathione peroxidase
LPS	Lipopolysaccharide
MDA	Malondialdehyde
PBS	Phosphate-buffered saline
SD	Standard deviation
SOD	Superoxide dismutase
TAN	Total adenine nucleotide

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Introduction

Glutamate displays remarkable metabolic versatility in animals (Brosnan and Brosnan 2013). There is growing interest in glutamate as a functional amino acid in nutrition and health (Wu 2013; Chen et al. 2014a). Emerging evidence from studies with animals (including pigs) shows that, in contrast to the traditional view of glutamate as a nutritionally nonessential amino acid, humans, livestock, and poultry have dietary requirements for glutamate to maintain their gut function and sustain their maximal growth (Rezaei et al. 2013a; San Gabriel and Uneyama 2013; Wu et al. 2013; Zhang et al. 2013). Because almost all of dietary glutamate is utilized by the pig small intestine in the first pass (Burrin and Stoll 2009; Wu 2009), it is a practical challenge to increase the availability of glutamate in extra-intestinal tissues (including the liver) directly through supplementation with glutamate to the enteral diet (Rezaei et al. 2013b). In this regard, α-ketoglutarate, which is an effective precursor of glutamate (Hou et al. 2011a) and can be absorbed in a significant quantity from the lumen of the gut into the portal circulation (Lambert et al. 2002, 2006), may provide a useful means to increase glutamate concentrations in extra intestinal tissues.

The liver is a major metabolic organ and the primary detoxification site in the body (Chen et al. 2013; Li et al. 2012). It also plays a critical role in the defense of body against bacteria and their toxic products, such as lipopolysaccharide (LPS) (Yi et al. 2014). Therefore, maintaining liver health is vital for human and animals. LPS is a component of the outer membrane of gram-negative bacteria. LPS-induced effects in the liver are initiated by stimulating Kupffer cells, followed by the subsequent release of pro-inflammatory cytokines (He et al. 2001) and radical oxygen and nitrogen species (Su 2002). These substances result in the injury of tissues, including parenchymal liver damage and liver dysfunction (Li et al. 2012; Yi et al. 2014; Bellezzo et al. 1998). Although some nutrients and chemicals (e.g., *N*-acetylcysteine, arginine, and fish oil) can be effective in attenuating liver damage induced by LPS challenge (Yi et al. 2014; Li et al. 2012; Chen et al. 2013), more low-cost curatives are preferable for doctors and animal producers under certain conditions.

AKG is a key intermediate in the tricarboxylic acid cycle as well as a precursor of glutamate and glutamine (Blachier et al. 2009; Hou et al. 2011a; Yao et al. 2012). Exogenous AKG can be converted to glutamate and glutamine in many tissues (Pierzynowski and Sjodin 1998; Kristensen et al. 2002) and may have a sparing effect on glutamate and aspartate in cells by serving as a fuel source (Hou et al. 2011a; Junghans et al. 2006; Lambert et al. 2006). When AKG enters the tricarboxylic acid cycle, it is oxidized by AKG dehydrogenase (Blachier et al. 2009; Junghans et al. 2006). As a precursor of glutamine, AKG is of physiological and nutritional importance for neonates, particularly under stressful or inflammatory conditions (Baker 2009; Wu 2010). Previous studies have demonstrated that dietary supplementation with 1 % AKG alleviates intestinal injury (Hou et al. 2010) and beneficially improves the energy status of the intestinal mucosa of LPS-challenged pigs (Hou et al. 2011a, b), which is a useful animal model to study human nutrition (Wang et al. 2015; Wu et al. 2014). Because AKG exerts its nutritional benefits in young mammals, we hypothesized that AKG may also afford beneficial effects on the liver of LPS-challenged piglets.

Materials and methods

Experimental animals and design

Twenty-four crossbred healthy piglets (duroc × landrace × yorkshire) were weaned at 21 days of age. After weaning, piglets had free access to the basal diet between days 21 and 24 of age (days 0–3 postweaning) for adapting to solid foods. At 24 days of age, piglets (7.25 ± 1.13 kg body weight) were assigned randomly into one of the three treatment groups: (1) non-challenged control (control group), piglets fed the basal diet and receiving intraperitoneal administration of sterile saline; (2) LPS-challenged control (LPS group), piglets fed the basal diet and receiving intraperitoneal administration of *Escherichia coli* LPS; and (3) LPS+1.0 % AKG (LPS + AKG) group, piglets fed the basal diet supplemented with 1.0 % AKG and receiving intraperitoneal administration of LPS. Each piglet was individually housed in a 1.20 × 1.10 m^2 steel metabolic cage with eight replicate cages per treatment (Hou et al. 2010).

The control and LPS groups were fed the basal diet + 1 % starch, and the LPS + AKG group was fed the basal diet +1 % AKG (purity ≥99.8 %). All diets were isocaloric (Hou et al. 2010). On days 10, 12, 14, and 16 of the trial, the piglets in the LPS and LPS + AKG groups received intraperitoneal administration of LPS (80 μg/kg body weight; *E. coli* serotype 055:B5; Sigma), and the control group was injected with the same volume of physiological saline. During days 0–10 of the trial (pre-challenge), all the piglets had free access to food and drinking water. To exclude possible effects of LPS-induced reduction in food intake on the piglet liver, the control and LPS + AKG piglets were pair-fed during days 10–16 of the trial (post-challenge with LPS) the same amount of feed per kg body weight as piglets in the LPS group. On day 16, 3 h after administration of LPS or physiological saline, blood samples were collected from the precava vein into 10 mL heparinized vacuum tubes (Becton–Dickinson Vacutainer System, Franklin Lakes, NJ, USA) and centrifuged (3500*g*

for 10 min at 4 °C) to separate plasma (Hou et al. 2010). Plasma samples were stored at 80 °C until analysis.

Twenty-four hours post-injection of LPS (on day 17), all of the pigs were anesthetized via intraperitoneal administration of sodium pentobarbital at 50 μg/kg body weight and then euthanized. A liver sample (~5 g) was collected from the left lobe and rinsed thoroughly with ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove blood. Liver samples were then rapidly frozen in liquid nitrogen and stored at −80 °C for further analysis. All samples were collected within 15 min after killing (Yi et al. 2014).

Measurement of plasma enzyme activity

Plasma was used for the determination of enzyme activities by colorimetric methods using commercial kits (Nanjing Jiancheng Biological Product, Nanjing, China) according to the manufacturer's recommendations (Yi et al. 2014). The enzymes included alanine aminotransferase (ALT), aspartate aminotransferase (AST), ADA (adenosine deaminase), and CHE (cholinesterase). Assays were performed in triplicate.

Liver morphology

Morphometric measurements of the liver were performed according to Yi et al. (2014). Briefly, the 0.5 cm^3 segments were cut off the liver, flushed with ice-cold PBS, and then fixed in fresh 4 % paraformaldehyde/phosphate-buffered saline. After 24 h, the liver segments were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE) (Liu et al. 2008; Li et al. 2012). Histomorphological examination was performed with a light microscope (American Optical Co., Scientific Instrument Div., Buffalo, NY, USA).

Analysis of free amino acids in plasma and liver

Free amino acids in plasma and liver were analyzed by HPLC (Hou et al. 2010; Wu and Meininger 2008). Briefly, the frozen liver sample (~100 mg) was homogenized with a tissue homogenizer in 1 mL of 1.5 mol/L ice-cold perchloric acid, followed by addition of 0.5 mL of 2 mol/L potassium carbonate. Sample tubes were vortexed and centrifuged at 3000×*g* for 5 min to obtain the supernatant fluid for analysis. The chromatographic system consisted of Waters Breeze HPLC system (Waters Corporation, Milford, MA, USA), including 1525 binary HPLC pumps, 2487 Dual-λ Absorbance Detector, 717 plus autosampler and Breeze system software); Waters XTerra MS C18 column (5 μm × 4.6 mm × 150 mm); mobile phase A (0.1 mol/L sodium acetate, pH 7.2); and mobile phase B (100 % methanol). Concentrations of amino acids in plasma and liver samples were calculated on the basis of the external standard calibration, as described by Dai et al. (2014).

Measurement of liver DNA, RNA, and protein

The DNA, RNA, and protein were extracted from the liver using the TRI Reagent-RNA/DNA/protein isolation reagent (Hou et al. 2012; Wang et al. 2013). Liver DNA was analyzed fluorimetrically using the method of Prasad et al. (1972). RNA was determined by spectrophotometry using a modified Schmidt-Tannhauser method as described by Munro and Fleck (1969). Protein was analyzed according to the method of Lowry et al. (1951). For measurements of liver DNA and RNA, the liver was homogenized (~2 min) in a 100-fold volume of ice-cold saline (0.9 %) and the homogenate was centrifuged at 1800×*g* for 10 min at 4 °C to obtain the supernatant fluid for analysis. For the analysis of liver protein, liver samples (~0.1 g) were homogenized with a tissue homogenizer in 1 mL of ice-cold PBS-EDTA buffer (0.05 mol/L Na_3PO_4, 2.0 mol/L NaCl, 2 mmol/L EDTA; pH 7.4) and the homogenates were centrifuged at 12,000×*g* for 10 min at 4 °C to obtain the supernatant fluid for assays.

Liver antioxidant enzymes and related products

Activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT), as well as the concentrations of malondialdehyde (MDA) in the liver were determined by commercially available kits (Nanjing Jiancheng Biological Product) according to the manufacturer's recommendations. Assays were performed in triplicate.

Determination of ATP, ADP, and AMP in liver

Concentrations of ATP, ADP, and AMP in liver were analyzed using HPLC as previously described (Hou et al. 2011b; Yi et al. 2014). Briefly, liver samples were homogenized with 1.5 M perchloric acid in ice bath and then centrifuged to obtain the supernatant fluid. An aliquot of the supernatant fluid was neutralized with 2 M potassium carbonate, and then centrifuged to obtain the supernatant fluid for analysis using the Waters Breeze HPLC system (Waters Corporation, Milford, MA, USA) and an analytical column (Waters XBridge C18; 5 μm, 4.6 mm × 150 mm). The detection wavelength was 260 nm, the pump flow rate was 1.0 mL/min, and the column temperature was 35 °C. Each thawed sample was filtered through a 0.20-μm filter membrane and an injection volume was 10 μL. Peaks were identified by their retention time using authentic standards (Sigma Chemical Inc., St. Louis, MO, USA). Total adenine nucleotide (TAN) and adenylate energy charges (AEC) were calculated according to the following equation (Atkinson 1968):

$$\begin{aligned} \text{TAN} &= \text{ATP} + \text{ADP} + \text{AMP, AEC} \\ &= (\text{ATP} + 0.5\ \text{ADP})(\text{ATP} + \text{ADP} + \text{AMP}). \end{aligned}$$

Statistical analysis

Results are expressed as means with SD and analyzed by one-way analysis of variance (ANOVA). Differences among treatment means were determined by the Duncan's multiple range test. All statistical analyses were performed using SPSS 13.0 software (SPSS Inc. Chicago, IL, USA). Probability values ≤ 0.05 were taken to indicate significance (Assaad et al. 2014a).

Results

Liver morphology

The morphological structure of the liver is shown in Fig. 1. The livers of piglets in the control group (Fig. 1, Panel a) were normal. However, in the LPS group, histopathological changes associated with liver injury were observed, which include the infiltration of inflammatory leukocytes, as well as heptatocyte caryolysis, karyopycnosis, vacuolation, and hemorrhage of hepatocytes, and a moderately disordered arrangement of hepatic cell cords (Fig. 1, Panel b). The LPS-induced liver injury was attenuated by dietary AKG supplementation, as indicated by normal liver cell cords, the mild vacuolation of hepatic cells, the limited infiltration of inflammatory leukocytes, and the normal structure of the liver (Fig. 1, Panel c).

Plasma enzyme activities

The data on plasma enzyme activities are presented in Table 1. LPS administration increased ($P < 0.05$) plasma AST and ADA activity, as well as the AST/ALT ratio, compared with the control group. Relative to LPS piglets, dietary supplementation with AKG decreased ($P < 0.05$) the concentrations of AST and the AST/ALT ratio, and tended to decrease ADA activity in plasma ($P = 0.063$) (Table 1).

Concentrations of amino acids in plasma and liver

The data on concentrations of free amino acids in the plasma and liver are summarized in Table 2. LPS administration increased ($P < 0.05$) plasma concentrations of lysine and alanine, compared with the control group. Relative to LPS piglets, dietary supplementation with AKG decreased ($P < 0.05$) the concentrations of glutamate and tended to decreased arginine ($P = 0.065$) in the plasma (Table 2). Concentrations of other amino acids in plasma did not differ ($P > 0.10$) among the three groups of pigs (data not shown).

Compared to the control piglets, LPS piglets exhibited a decrease ($P < 0.05$) in hepatic concentrations of isoleucine, lysine, asparagine, and threonine, and tended to decrease the concentrations of glutamate ($P = 0.071$) and glutamine ($P = 0.076$). In comparison with the LPS piglets, AKG supplementation increased ($P < 0.05$) the concentrations of glutamate, glutamine, leucine, asparagine, lysine, alanine, serine, threonine, valine, and phenylalanine, and tended to increase the concentrations of isoleucine in the liver ($P = 0.067$) (Table 2). Concentrations of other amino acids in the liver did not differ ($P > 0.10$) among the three groups of pigs (data not shown).

Concentrations of DNA, RNA, and protein in liver

The data on concentrations of DNA, RNA, and protein in the liver are summarized in Table 3. Compared to the control group, LPS challenge increased ($P < 0.05$) the concentration of total protein, as well as RNA/DNA and total protein/DNA ratios. In comparison with the LPS group, dietary supplementation of 1.0 % AKG decreased the RNA/DNA and total protein/DNA ratios ($P < 0.05$), and tended to decrease the concentration of total protein in the liver ($P = 0.095$) (Table 3).

Liver antioxidant enzymes and MDA

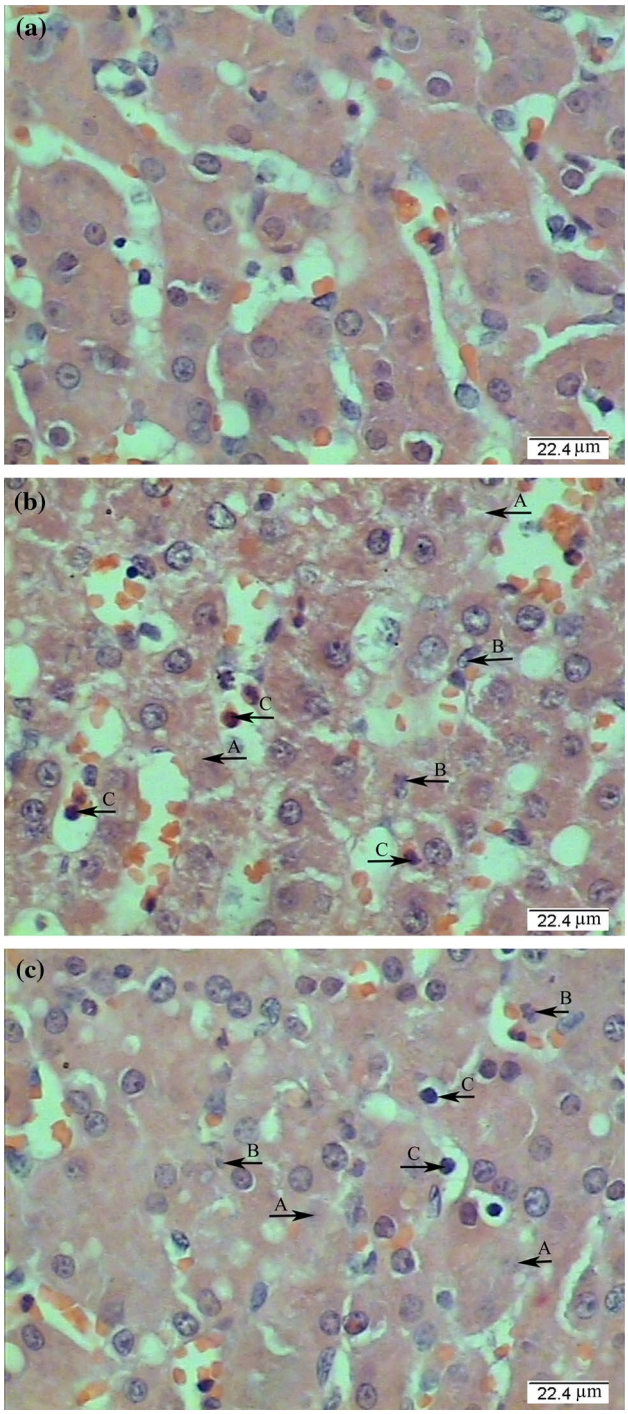
The data for liver antioxidant enzymes and MDA are summarized in Table 4. Compared to the control piglets, LPS piglets exhibited a decrease ($P < 0.05$) in GSH-Px activity and an increase ($P < 0.05$) in MDA content in the liver. In comparison with the LPS piglets, AKG supplementation increased ($P < 0.05$) the activity of GSH-Px and tended to decrease the concentrations of MDA in the liver ($P = 0.083$) (Table 4).

Concentrations of ATP, ADP, and AMP in liver

Data on concentrations of ATP, ADP, and AMP in the liver are summarized in Table 5. Compared with the control group, LPS piglets exhibited a decrease in ADP ($P < 0.05$), and an increase ($P < 0.05$) in AMP in the liver. In comparison with piglets in the LPS group, dietary supplementation with 1 % AKG tended to increase ADP ($P = 0.097$), but decrease AMP ($P = 0.064$) in the liver. Neither LPS nor AKG affected TAN or AEC levels in the liver (Table 5).

Discussion

A well-established porcine model of liver injury induced by administration of LPS (Yi et al. 2014) was employed in the present study to investigate protective effects of AKG



◀**Fig. 1** Photomicrographs of liver sections stained with hematoxylin and eosin. Sections were stained with hematoxylin and eosin (×400). **a** Control group (piglets which were fed the basal diet and received administration of sterile saline). No obvious pathological changes were found. **b** LPS group (piglets which were fed the basal diet and challenged with LPS). Significant morphologic changes associated with liver injury: hepatocyte caryolysis (*A*), hepatocyte karyopycnosis (*B*), and infiltration of inflammatory leukocytes (*C*). Vacuolation of hepatocytes, as well as a moderately disordered arrangement of hepatic cell cords were also observed. **c** LPS + AKG group (piglets which were fed the basal diet supplemented with 1 % AKG and challenged with LPS). Normal liver cell cords, mild vacuolation of hepatocytes, limited infiltration of inflammatory leukocytes, and the normal structure of hepatocytes were observed in the LPS + AKG group

on anti-oxidative capacity and energy metabolism in young mammals. Although the liver plays an important physiological role in LPS detoxification and, in particular, hepatocytes are involved in the clearance of the intestine-derived endotoxin (Jirillo et al. 2002), multiple lines of evidence show that LPS contributes to liver injury in many cases (Nolan 1975; Li et al. 2012; Yi et al. 2014).

Our previous study showed that supplementing 1 % AKG for 16 days alleviated the loss of body weight in weaned pigs receiving LPS challenge, indicating the importance of exogenous AKG supply in piglets under immunological stress (Hou et al. 2010, 2011a, b). In the present study, intraperitoneal injection of LPS caused morphologic change in the liver, including hepatocyte caryolysis, hepatocyte karyopycnosis, infiltration of inflammatory leukocytes, vacuolation, and hemorrhage of hepatocytes, and a moderately disordered arrangement of hepatic cell cords (Fig. 1b). Of note, the LPS-induced liver injury was attenuated by dietary AKG supplementation, as indicated by normal liver cell cords, the mild vacuolation of hepatic cells, the limited infiltration of inflammatory leukocytes, and the normal structure of the liver (Fig. 1c). Similarly, Li et al. (2012) reported that LPS caused morphologic change in the livers of pigs, including hepatocyte caryolysis, karyopycnosis, and fibroblast proliferation, whereas supplementing 0.5 % arginine could attenuate the liver injury. AST and ALT are abundant intracellular enzymes in the liver, which are considered to be nonspecific and specific markers for hepatic injury (Yi et al. 2014; He et al. 2001; Matsuzaki et al. 2001). Elevation in the activities of these enzymes and AST/ALT ratio in the serum indicates their leakage from injured hepatocytes and is considered to be a sensitive indicator of injury in tissues, including the liver (Hou et al. 2015; Nyblom et al. 2004). Plasma adenosine deaminase (ADA) activity has also been described as a valuable biomarker for hepatic injury and dysfunction. In the current study, LPS increased the activities of AST and ADA, and AST/ALT ratio, indicating that LPS induced liver injury. Importantly, AKG alleviated the increases in plasma AST activity and the AST/ALT ratio and tended to decrease plasma ADA activity (Table 1), indicating a beneficial effect of AKG in attenuating liver injury and ameliorating liver dysfunction.

There is growing evidence that AKG displays remarkable metabolic and regulatory versatility in pigs (Hou et al. 2011a). Although AKG has been reported to be extensively metabolized by the pig intestine (Hou et al. 2010), little is known about its nutritional significance in the liver, which is an organ with a high rate of oxygen consumption

Table 1 Effects of AKG on plasma enzyme activities in LPS-challenged piglets

Items	Control group	LPS group	LPS + AKG group
ALT (U/L)	58.7 ± 10.6	50.2 ± 10.7	55.3 ± 8.6
AST (U/L)	42.3 ± 9.6[a]	55.4 ± 12.4[b]	41.3 ± 4.4[a]
AST/ALT	0.73 ± 0.09[a]	1.07 ± 0.16[b]	0.74 ± 0.12[a]
ADA (U/mL)	22.0 ± 1.3[a]	25.4 ± 1.8[b]	23.6 ± 1.8[a,b]
CHE (U/mL)	4237 ± 152	4206 ± 43	4204 ± 160

Data are means ± SD, $n = 8$

Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + AKG (LPS + 1.0 % AKG) = piglets fed the basal diet supplemented with 1.0 % AKG and challenged with LPS

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *ADA* adenosine deaminase, *CHE* cholinesterase

[a, b] Values within a row with different letters differ ($P < 0.05$)

Table 2 Effects of dietary AKG supplementation on concentrations of amino acids in the plasma and liver of piglets after LPS challenge

Items	Control group	LPS group	LPS + AKG group
Plasma (nmol/mL)			
Citrulline	108 ± 42[b]	177 ± 58[a]	112 ± 57[b]
Glutamate	110 ± 20[a,b]	128 ± 25[a]	96 ± 15[b]
Lysine	102 ± 16[b]	131 ± 31[a]	118 ± 27[a,b]
Alanine	447 ± 34[b]	523 ± 34[a]	476 ± 33[a,b]
Liver (nmol/g tissue)			
Glutamate	3722 ± 460[a,b]	3136 ± 177[b]	3869 ± 437[a]
Glutamine	3304 ± 688[a,b]	2705 ± 459[b]	3768 ± 739[a]
Isoleucine	277 ± 41[a]	228 ± 31[b]	248 ± 40[a,b]
Leucine	566 ± 57[a]	460 ± 100[b]	559 ± 112[a]
Asparagine	792 ± 112[a]	572 ± 121[b]	885 ± 199[a]
Lysine	481 ± 56[a]	375 ± 49[b]	465 ± 75[a]
Alanine	3852 ± 852[b]	3376 ± 584[b]	4960 ± 736[a]
Serine	999 ± 165[a,b]	890 ± 77[b]	1064 ± 151[a]
Threonine	852 ± 176[a]	619 ± 88[b]	920 ± 146[a]
Valine	594 ± 187[b]	536 ± 121[b]	785 ± 191[a]
Phenylalanine	181 ± 31[a,b]	147 ± 26[b]	218 ± 64[a]

Data are means ± SD, $n = 8$

Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + AKG (LPS + 1.0 % AKG) = piglets fed the basal diet supplemented with 1.0 % AKG and challenged with LPS

[a, b] Values within a row with different letters differ ($P < 0.05$)

(Assaad et al. 2014b). At the supplemental dose, dietary AKG can enter the portal circulation and then the liver, as indicated by changes in hepatic concentrations of amino acids (Table 2). Interestingly, we found that dietary supplementation with AKG decreased the concentrations of glutamate in the plasma of LPS-challenged pigs, likely due to reduced released glutamate from the liver and other tissues as a result of improved tissue integrity. In agreement with our findings, Kristensen et al. (2002) reported that the plasma concentration glutamine in growing pigs decreased in response to constant infusion of AKG into the mesenteric vein in an amount equivalent to 5 % of feed intake. In addition, it is noteworthy that dietary supplementation with AKG increased the concentrations of glutamate, glutamine, leucine, asparagine, lysine, alanine, serine, threonine, valine, and phenylalanine in the liver of LPS-challenged piglets. These amino acids have important nutritional and regulatory functions in animals, including pigs (Chen et al. 2014b; He et al. 2013; Ren et al. 2014a, b; Suryawan et al. 2013; Wang et al. 2014; Wu 2014). Our results indicated a net increase in the formation of these amino acids in the liver of AKG-supplemented piglets. Thus, our current findings provided a new basis to explain the beneficial effects of AKG on the liver. As a precursor of glutamate and glutamine, AKG is a key intermediate in the tricarboxylic acid cycle (Yao et al. 2012; Hou et al. 2010; Wu 2009). AKG is first converted into glutamate, the contribution of AKG to whole-body glutamine synthesis can be quantitatively important because 10 % of the intraduodenally infused AKG is absorbed into the portal circulation (Kristensen et al. 2002; Lambert et al. 2002, 2006).

Another novel and important finding from the current study is that dietary supplementation with AKG attenuated the increases in the concentration of total protein, as well as RNA/DNA and total protein/DNA ratios in the liver of LPS-challenged piglets (Table 3). DNA concentration reflects the rate of mitosis to produce new cells, RNA/DNA ratio indicates cellular efficiency, and protein/DNA ratio implicates the efficiency of protein synthesis in cells (Hou et al. 2012; Fasina et al. 2007). Infection, sepsis or trauma can induce marked changes in protein metabolism (Jepson et al. 1986). Williams et al. (1997) reported that administration of LPS increased liver and heart protein synthesis. Similarly, Jepson et al. (1986) reported that liver total protein mass was increased in the rats by 16 % at 24 h after LPS treatment, and Raina et al. (2000) found that the liver protein content in rats receiving total parenteral nutrition (TPN) + LPS was significantly higher than that in the respective controls. These results support the notion that dietary AKG can improve the nutritional status of the liver in response to endotoxin treatment.

Oxidative stress is one of major factors contributing to tissue injury (Li et al. 2012; Liu et al. 2009; Lin et al. 2006). Oxidative stress is a common mechanism of liver injury (Kang and Kim 2013). GSH-Px, SOD, and CAT are regarded as the first line of the anti-oxidant enzyme system against reactive oxygen species (ROS) generated during oxidative stress (Li et al. 2012). These anti-oxidative

Table 3 Effects of AKG supplementation on total protein, DNA and RNA in the liver of LPS-challenged piglets

Items	Control group	LPS group	LPS + AKG group
Total protein (g/L)	1.22 ± 0.06^{a}	1.32 ± 0.06^{b}	$1.27 \pm 0.10^{a,b}$
DNA (mg/g)	30.7 ± 2.1	29.0 ± 1.7	31.4 ± 3.7
RNA (mg/g)	2.99 ± 0.17	3.15 ± 0.11	3.08 ± 0.08
RNA/DNA	0.095 ± 0.005^{a}	0.107 ± 0.004^{b}	0.091 ± 0.009^{a}
Total protein/DNA	40.0 ± 2.8^{a}	45.9 ± 3.3^{b}	40.1 ± 3.2^{a}

Data are means ± SD, $n = 8$

Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + AKG (LPS + 1.0 % AKG) = piglets fed the basal diet supplemented with 1.0 % AKG and challenged with LPS

[a, b] Values within a row with different letters differ ($P < 0.05$)

Table 4 Effects of AKG on liver antioxidant enzymes and MDA in LPS-challenged piglets

Items	Control group	LPS group	LPS + AKG group
GSH-Px (U/mg protein)	119 ± 15^{b}	96 ± 11^{a}	120 ± 16^{b}
SOD (U/mg protein)	418 ± 18	395 ± 25	403 ± 25
CAT (U/g protein)	726 ± 95	647 ± 94	671 ± 81
MDA (μmol/g protein)	0.52 ± 0.19^{a}	0.83 ± 0.23^{b}	$0.73 \pm 0.17^{a,b}$

Data are means ± SD, $n = 8$

Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + AKG (LPS + 1.0 % AKG) = piglets fed the basal diet supplemented with 1.0 % AKG and challenged with LPS

GSH-Px glutathione peroxidase, *SOD* superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde

[a, b] Values within a row with different letters differ ($P < 0.05$)

enzymes can cooperatively convert ROS into water and O_2 (Yi et al. 2014; Yu 1994; Zafarullah et al. 2003). MDA is an end-product of lipid peroxidation resulting from reactions with free radicals (Liu et al. 2009), and serves as a useful bio-marker of in vivo oxidative stress (Hou et al. 2013; Fang et al. 2002). In the current study, AKG supplementation to LPS-challenged piglets tended to alleviate the increase in hepatic MDA content. Additionally, we observed that LPS challenge decreased liver GSH-Px activity (Table 4). In agreement with results of the present study, Yi et al. (2014) reported that the activities of GSH-Px in the liver were substantially lower in the LPS group than in the control. Notably, AKG supplementation could alleviate the LPS-induced decrease of GSH-Px, indicating that the enhancement of tissue antioxidant enzymes during endotoxemia may be a preventive measure in the host to handle the superoxide anion load after LPS administration. Therefore, it is possible that supplementing 1 % AKG reduced liver injury partially via enhancing anti-oxidative capacity. Additionally, AKG may regulate expression of anti-inflammatory genes in the liver of endotoxin-challenged pigs.

The energy charge of the adenyl pool is a better measure of the energy state of a tissue than the level of a single nucleotide (Hou et al. 2011a; Atkinson 1968; McKnight et al. 2010). ATP hydrolysis can increase the cellular concentration of ADP, which is converted by adenylate kinase (2 ADP ↔ ATP + AMP) to ATP and AMP (Hardie 2003). Another important finding of the present study is the modulation of liver energy status by dietary AKG supplementation. Previous studies indicated that LPS resulted in mitochondrial dysfunction and impaired oxidative metabolism in the liver and the whole body (Yi et al. 2014; Menguy 1981; Sugino et al. 1987). Specially, LPS administration reduced the hepatic concentration of ATP and increased the lipid peroxide levels in rodents (Lancaster et al. 1989). In line with these observations, the current study showed that ADP concentrations were decreased, but AMP levels were elevated in the liver of LPS-challenged piglets (Table 5). Similarly, Yi et al. (2014) reported LPS-induced alterations in the cellular energy status in the liver of piglets. Of interest in the current study is the fact that dietary supplementation with 1 % AKG tended to increase ADP but decrease AMP in the liver after LPS challenge. This result may be due to the oxidation of AKG through the tricarboxylic acid cycle for ATP production (Yao et al. 2012; Kristensen et al. 2002). Consistent with these reports, our previous study showed that AKG beneficially prevented LPS-induced alterations in cellular energy status in the piglet small intestine (Hou et al. 2011a). There is evidence that 80 % of dietary AKG was oxidized by the small intestine in young pigs and 10 % of dietary AKG was utilized by other splanchnic tissues (Lambert et al. 2002) and possibly intestinal bacteria (Dai et al. 2013). Thus, it is possible that the portal AKG could modulate the liver energy status and improve liver mitochondrial function. Furthermore, LPS directly or indirectly inhibits the mitochondrial function of hepatocytes (Lancaster et al. 1989). Likewise, some oxygen radical scavengers (e.g., *N*-acetylcysteine) could prevent the inhibitory effects of LPS on mitochondrial function and maintain energy metabolism in the liver (Yi et al. 2014; Sugino et al. 1987). Since AKG can be used to synthesize glutathione via the formation of glutamate (Brosnan and Brosnan 2013), these beneficial effects of AKG on hepatic energy metabolism are possibly associated with

Table 5 Effects of AKG on ATP, ADP and AMP in the liver of LPS-challenged piglets

Items	Control group	LPS group	LPS + AKG group
ATP (μg/g)	128 ± 24	122 ± 11	120 ± 21
ADP (μg/g)	134 ± 17[a]	110 ± 3.4[b]	121 ± 13[a,b]
AMP (μg/g)	584 ± 105[b]	729 ± 123[a]	674 ± 54[a,b]
TAN (μg/g)	847 ± 107	951 ± 112	916 ± 87
AEC	0.23 ± 0.05	0.21 ± 0.05	0.20 ± 0.04

Data are means ± SD, $n = 8$

Control (non-challenged control) = piglets fed the basal diet and injected with saline; LPS (LPS challenged control) = piglets fed the basal diet and challenged with *Escherichia coli* LPS; LPS + AKG (LPS + 1.0 % AKG) = piglets fed the basal diet supplemented with 1.0 % AKG and challenged with LPS

TAN = ATP + ADP + AMP

AEC = (ATP + 0.5ADP)/(ATP + ADP + AMP)

AEC adenylate energy charge, *TAN* total adenine nucleotide

[a, b] Values within a row with different letters differ ($P < 0.05$)

its capacity of scavenging ROS. Further investigation is clearly warranted.

In conclusion, dietary supplementation with 1 % AKG effectively increases the hepatic concentrations of glutamate and related amino acids and beneficially ameliorates the abnormal morphological structure and abnormal physiological function of the liver of LPS-challenged piglets. The underlying mechanisms involve the enhancement of anti-oxidative capacity and improvement of energy metabolism in the liver. These findings have important implications for development of new interventions to alleviate hepatic injury and dysfunction in animals and humans with exposure to endotoxin.

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

Ethical statement The use of animals for this research was approved by the Animal Care and Use Committee of Hubei Province.

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