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Alpha-Ketoglutarate and intestinal function

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1. ABSTRACT

Alpha-ketoglutarate (AKG) is an intermediate of the Krebs cycle which bridges amino acid metabolism with glucose oxidation in animals. Of particular interest is the conversion of AKG into glutamate by mitochondrial glutamate dehydrogenase in the gastrointestinal tract where glutamate has multiple physiological functions (including regulation of cell function, neurotransmission, and gastric emptying). Additionally, AKG stimulates the initiation of catabolism of branched-chain amino acids (BCAA) via BCAA transaminase in enterocytes. Oxidation of AKG also provides large amounts of ATP and modulates cellular redox state in the small intestine. Translating the basic research into practice, results of recent studies indicate that dietary supplementation with AKG alleviates oxidative stress and injury in intestinal mucosal cells, while improving intestinal mucosal integrity and absorption of nutrients in endotoxin-challenged pigs. The beneficial effects of AKG are associated with increased activation of the mTOR signaling pathway and net protein synthesis. Thus, AKG is a novel and promising supplement in diets to improve intestinal health in animals and possibly humans.

2. INTRODUCTION

The small intestine is not only the terminal organ for digestion and absorption of dietary nutrients, but is also crucial for preventing the entry of exogenous pathogens into the systemic circulation (1,2). Thus, intestinal integrity is vital to survival, growth, and health of both animals and humans (1-3). Extensive studies with experimental animals, including pigs, have demonstrated that a number of stressful factors, such as early-weaning, infection and inflammation can result in gut mucosal injury and dysfunction (3-5). The consequences are the occurrence of diarrhea, reduced growth, and even deaths, leading to a great deal of economic loss (3,5). Recent work has also identified that nutritional modulation can ameliorate damage of the small intestine in compromised neonates (6-8). Additionally, much evidence shows that glutamate and glutamine (products of AKG metabolism in animals) are main energy sources for intestinal mucosal cells under practical feeding conditions (9). At present, little attention has been paid to enteral or parenteral administration of glutamate to animals because of concerns over potential adverse effects of this amino acid on the

Table 1. Effect of different AKG levels on growth performance of weaned piglets

Items	Control	0.5% AKG	1.0% AKG	2.0% AKG	Pooled SEM	P-value
Average daily weight gain between days 0 and 14 postweaning (g/day)	441	426	478	418	9.06	0.103
Average daily feed intake between days 0 and 14 postweaning (g/day)	669	664	693	610	16.0	0.164
The ratio of feed to weight gain (F/G)	1.52	1.56	1.45	1.46	0.02	0.237
Diarrhea incidence between days 0 and 14 postweaning (%)	23	13	10	16	3	0.637

Adapted from Hu (17). SEM, standard error of the mean. n = 6.

Table 2. Effects of dietary 1% AKG on feed intake and growth performance of piglets after LPS challenge

Variables	Control	LPS	LPS+AKG	Pooled SEM	P-value
Body weight at day 16 of the trial (kg)	14.5	14.1	15.1	1.34	0.756
Average daily feed intake between days 10 and 16 of the trial (g/day)	579	581	578	55	0.974
Average daily weight gain between days 10 and 16 of the trial (g/day)	320	275	316	47	0.280

SEM, standard error of the mean. n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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, c] Values within a row with different letters differ (P<0.05). Adapted from Hou *et al.* (3).

brain. In contrast, much work has been conducted to determine the efficacy of glutamine supplementation on intestinal and whole-body homeostasis (9). However, the cost for chemical synthesis of glutamine is relatively high, which limits its use in swine production. This provides an impetus for us to identify cheaper replacements of glutamine in swine production, and one of them is α-ketoglutarate (AKG) (3,10,11).

Alpha-ketoglutarate (AKG) is a central molecule in the citric acid cycle (Krebs cycle) and an intermediate in the oxidation of key metabolic fuels. Emerging evidence shows that AKG plays a key role in systemic, intestinal and gut bacterial metabolism. Exogenous AKG can be converted to glutamate and glutamine in many tissues and be regarded as an energy substrate (12-14). Of particular interest, biochemical studies have led to the growing recognition that AKG displays remarkable metabolic and regulatory versatility in cells (3,15,16). Additionally, dietary AKG supplementation has been reported to improve mucosal morphology and function of the small intestine in endotoxin-challenged piglets (3,15,17-19). AKG can also maintain intestinal barrier integrity and attenuate gut injury through an anti-inflammatory role in weaned pigs (3). Because of ethical concerns over invasive studies with humans, the pig is often used as an animal model to study AKG metabolism in neonates and adults. Thus, much data included in this review are derived from studies with the porcine model.

3. GROWTH PERFORMANCE

Growth performance is a major criterion in animal production. Dietary supplementation with 1.0% AKG tended to increase ADG, ADFI and feed efficiency in weanling pigs, compared with the control group, 0.5% AKG group or 2.0% AKG group (Table 1). In contrast, dietary supplementation of 1.0% AKG did not affect growth performance in lipopolysaccharide (LPS)-challenged pigs likely due to low feed intake (Table 2) (3). However, AKG could relieve growth depression in weaned piglets chronically challenged by LPS when their food intake was improved (11). In the absence of stress, acute administration of AKG may not affect the growth of young pigs, as reported for rats receiving 0.5% AKG in the regular diet (20).

4. AKG AND GUT FUNCTION

4.1. Intestinal morphology

A high value of the villus: crypt ratio is a useful indicator of a high capacity for digestion and absorption (3, 21). There is evidence that dietary supplementation of AKG could improve intestinal histological morphology. For example, AKG supplementation resulted in: (1) increased villus height; (2) reduced crypt depth; and (3) an increased ratio of villus height to crypt depth in LPS-challenged pigs (Table 4) (3). These findings support the notion that AKG beneficially alleviates the LPS-induced damage of the intestinal structure. Similarly, dietary supplementation with 1% AKG increased the ratio of villus height to crypt depth in the gut of healthy pigs (Table 3) (10,15,17).

4.2. Absorptive and barrier function

Absorption of D-xylose from the intestinal lumen into plasma is a useful marker of *in vivo* intestinal function in animals. On day 14 postweaning, compared with the control group, circulating levels of D-xylose were elevated in AKG-supplemented pigs (17). Indeed, D-xylose content in plasma was the highest (quadratic, P<0.05) when the level of AKG was 1.0 %. In addition, with the increasing dose of supplemental AKG, the activity of diamine oxidase (DAO) in plasma was decreased (P<0.01). The activity of DAO was the lowest (quadratic, P<0.05) with 1% AKG in the diet (Table 5) (17).

AKG is converted into glutamate by mitochondrial glutamate dehydrogenase in the gastrointestinal tract where glutamate has multiple physiological functions (including regulation of cell function, neurotransmission, and gastric emptying) (12). AKG may spare the oxidation ofglutamate and glutamine by enterocytes, thereby increasing

Table 3. Effects of 1% AKG on intestinal mucosal morphogy in weaned piglets

Items	Control	1% AKG	P-value
Villus height, μm			
Duodenum	282±20	310±10	0.65
Jejunum	279±13	280±14	0.74
Ileum	272±168	275±118	0.89
Crypt depth, μm			
Duodenum	230±7 [b]	141±8 [a]	0.01
Jejunum	260±9[b]	132±10[a]	0.02
Ileum	234±16[b]	147±13[a]	0.04
Villus height / Crypt depth			
Duodenum	1.15±0.09[a]	2.22±0.09[b]	0.04
Jejunum	1.07±0.04[a]	2.17±0.14[b]	0.03
Ileum	1.19±0.11[a]	1.9±0.17[b]	0.01

Data are means ± SEM, n = 6. Control = piglets fed the basal diet; 1%AKG= piglets fed the basal diet supplemented with 1.0% AKG. Values within a row with different letters differ (P<0.05). Adapted from Hu *et al.* (17).

Table 4. Effects of AKG on the intestinal mucosal morphology of piglets after LPS challenge

Items	Control	LPS	LPS+AKG	Pooled SEM	P-value
Villus height, μm					
Duodenum	290[ab]	268[a]	327[b]	25	0.038
Jejunum	336	318	327	16	0.541
Ileum	388[b]	329[a]	333[a]	12	0.026
Crypt depth, μm					
Duodenum	61[a]	83[b]	73[ab]	6.7	0.013
Jejunum	74[a]	107[b]	59[a]	7.8	0.013
Ileum	68[a]	81[b]	65[a]	5.7	0.022
Villus height / Crypt depth					
Duodenum	4.91[b]	3.43[a]	4.62[b]	0.57	0.039
Jejunum	4.59[b]	2.96[a]	6.17[c]	0.54	0.001
Ileum	5.57[b]	4.00[a]	4.96[b]	0.49	0.016

SEM, standard error of the mean. n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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. Values within a row with different letters differ (P<0.05). Adapted from Hou *et al.* (3).

the availability of these two amino acids to other pathways (e.g., synthesis of protein, citrulline, arginine, and glutathione). Additionally, AKG is a metabolic fuel to provide the required energy and improve the efficiency of nutrient absorption, as indicated by the entry of luminal D-xylose into the blood circulation (Table 6). This is a simple, specific, and sensitive measure of intestinal absorption ability. Similarly, Yang *et al.* (2005) reported that supplementation with 1% glutamine significantly increased D-xylose concentration in the plasma of pigs on days 7, 14 and 21 postweaning (22).

Diamine oxidase is present in the mammalian intestinal mucosa or the small intestine villi. This enzyme is particularly abundant in rapidly dividing cells (23). When the intestinal mucosa is damaged or replaced, DAO is released into the intestinal lymphatic and vascular space, resulting in increased DAO activity in blood but decreased enzyme activity in enterocytes (24). This occurs when intestinal permeability increases in response to LPS and inflammatory cytokines (25). Consistent with this view, dietary supplementation with 1% AKG increased DAO activity in jejunal mucosa, while decreasing DAO activity in blood (Table 6). These results indicate that AKG can alleviate intestinal injury caused by LPS challenge. When dietaty AKG enters the portal vein, it can be utilized for the synthesis of glutamine which is then released into the circulation and taken up by the small intestine. Like AKG, glutamine can serve as an energy substrate for gastrointestinal cells to promote intestinal mucosal cell proliferation (12) and decrease the expression of intestinal inflammatory factors. Taken together, these data support the notion that AKG improves the ability of the small intestine to absorb dietary nutrients under inflammatory conditions.

4.3. Antioxidative capacity

AKG enhances the intestinal antioxidative capacity by partly increasing the activity of superoxide dismutase (SOD). This effect of AKG is consistent with the decreased content of MDA content in the jejunal mucosa (Table 7). LPS can result in tissue ischemia and hypoxia, changes in cell oxidative metabolism of the microsomal enzyme system, and production of a large number of free radicals (26). Whole-body homeostasis is maintained in the oxidative stress-antioxidant balance. Usually, MDA is an important indicator to reflect the extent in the accumulation of free radicals in the body caused by oxidative damage. Superoxide dismutase functions to remove superoxide anion and is part of free radical scavenging systems. Glutathione (GSH), an antioxidant, helps protect cells from reactive oxygen species such as free radicals and peroxides (16,27). AKG can also act to detoxify excess ammonia, participate in the non-enzymatic oxidative decarboxylation during hydrogen peroxide decomposition, and facilitate the proper metabolism of fats, leading to reduced generation of oxygen radicals and preventing lipid peroxidative damages (28).

Table 5. Effects of different AKG levels on the concentrations of D-xylose, Diamine oxidase (DAO) activities and endotoxin content in plasma of piglets

Items	Control	0.5% AKG	1.0% AKG	2.0% AKG
D-xylose, mmol/L	0.39 ± 0.04^{b}	0.46 ± 0.09^{ab}	0.55 ± 0.07^{a}	0.45 ± 0.04^{ab}
DAO, U/L	18.21 ± 3.15^{a}	14.16 ± 1.66^{bc}	11.35 ± 0.45^{c}	15.02 ± 1.33^{ab}
Endotoxin, Eu/mL	0.48 ± 0.11	0.41 ± 008	0.24 ± 0.07	0.35 ± 0.09

Data are means ± SEM, n = 8. Control = piglets fed the basal diet; 1%AKG= piglets fed the basal diet supplemented with 1.0% AKG. Values within a row with different letters differ (P<0.05). Adapted from Hu *et al.* (17).

Table 6. Effects of dietary supplementation with 1% AKG on D-xylose concentrations in plasma, DAO activities in plasma and intestinal mucosa in piglets after LPS challenge

Items	Control	LPS	LPS+AKG
D-xylose in plasma (mmol/L)	2.22 ± 0.11^{ab}	2.17 ± 0.14^{b}	2.38 ± 0.20^{a}
DAO in plasma (U/mL)	2.54 ± 0.25^{b}	3.56 ± 0.30^{a}	3.19 ± 0.38^{ab}
DAO in jejunal mucosa (U/mg protein)	0.21 ± 0.03^{ab}	0.19 ± 0.03^{b}	0.24 ± 0.03^{a}

SEM, standard error of the mean. n = 8. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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. Values within a row with different letters differ (P<0.05). Adapted from Liu (16).

Table 7. Effects of dietary 1% AKG on antioxidative capacity of jejunal mucosa in piglets after LPS challenge

Items	Control	LPS	LPS+AKG
MDA (nmol/mg protein)	0.60 ± 0.12^{b}	0.82 ± 0.12^{a}	0.72 ± 0.16^{ab}
SOD (U/mg protein)	45.50 ± 4.20^{a}	40.82 ± 4.98^{b}	43.73 ± 2.77^{ab}
GSH (mg/g protein)	15.36 ± 2.37^{a}	10.67 ± 1.45^{b}	9.45 ± 1.89^{b}

SEM, standard error of the mean. n = 8. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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. Values within a row with different letters differ (P<0.05). Adapted from Liu *et al.* (19).

4.4. Concentrations of amino acids and protein in the jejunal mucosa

Hou *et al.* (3) provided a new biochemical basis to explain the beneficial effects of AKG on the intestine (3). These authors reported that dietary supplementation with AKG increased citrulline and glutamate concentration in the intestinal mucosa (Table 4), suggesting a net increase in the formation of these two amino acids in the gut of AKG-supplemented pigs. Citrulline is the immediate precursor for the endogenous synthesis of arginine, which is an essential amino acid for young mammals (3,8) and protects intestinal cells from LPS-induced injury (29). It is possible that AKG is firstly converted into glutamate, which is subsequently utilized for citrulline synthesis via pyrroline-5-carboxylate synthase (30). Indeed, it was found that 64% of the AKG disappeared from the small intestinal lumen within 1 h (31), suggesting rapid utilization of AKG by the mucosa. Alternatively, glutamine (formed from AKG in extraintestinal tissues) is a substrate for intestinal citrulline generation (32), because glutamine in arterial blood is actively taken up by the pig small intestine (33). The contribution of AKG to whole-body glutamine synthesis may be quantitatively important because 10% of the intraduodenally infused AKG is absorbed into the portal circulation (13,31,34).

4.5. Concentrations of ATP, ADP, and AMP in the intestinal mucosa

Mitochondria are key cellular organelles that regulate events related to energy production and apoptosis (35). Most of the ATP is generated by the proton gradient that develops across the inner mitochondrial membrane. ATP is a complex *nanomachine* that serves as the primary energy currency of the cell (36). In contrast to the traditional view that glucose is the primary fuel for the intestine, it is now known that glutamate, glutamine and aspartate are the major sources of ATP in mammalian enterocytes via mitochondrial oxidation.

AMP is a good indicator of cellular stress because an increased rate of ATP hydrolysis leads to a rapid accumulation of AMP in the cell (37). The energy charge of the adenylate pool is a better measure of the energy status in a tissue than the level of a single nucleotide. ATP hydrolysis first increases the cellular ADP concentration. The ADP is then converted by the adenylate kinase reaction (2 ADP↔ATP+AMP) to ATP and AMP (38). Therefore, during increased ATP use, AMP accumulates well before any changes in cellular ATP or ADP concentration occur (37).

As an intermediate in the citric acid cycle and an intermediate in the oxidation of glutamate and glutamine, AKG contributes substantially to ATP homeostasis and mucosal integrity in the small intestine (31). This is in keeping with our findings that AKG supplementation resulted in: 1) increased ATP concentrations; 2) reduced AMP/ATP ratio; and 3) increased AEC (Table 9) (3). These three lines of evidence indicate that dietary supplementation with AKG could modulate the adenine nucleotide pool and support the notion that AKG beneficially alleviates the LPS-induced damage of the intestinal energy metabolism. The results reveal a hitherto unrecognized role for AKG in ameliorating oxidative stress and improving energy status in the small intestine. As an intermediate in the oxidation of key gut fuels, AKG is extensively metabolized in first-pass by the intestinal mucosa (39). Thus, we suggest that AKG exerts its nutritional benefits primarily at the small-intestinal level in weanling piglets.

Table 8. Effects of dietary AKG supplementation on concentrations of amino acids and protein in the intestinal mucosa of piglets after LPS challenge

Items	Control	LPS	LPS+AKG	Pooled SEM	P-value
Amino acids (μmol/L)					
Citrulline	83[ab]	63[a]	124[b]	30	0.050
Ornithine	329[a]	433[b]	325[a]	45	0.031
Glutamate	1404[a]	1365[a]	1744[b]	126	0.016
Arginine	359	362	379	41	0.867
Aspartate	545	474	590	65	0.227
Methionine	109	108	107	14	0.99
Asparagine	311	327	302	41	0.822
Lysine	341	353	339	29	0.86
Alanine	885	1009	882	79	0.216
Protein (%)					
Duodenum	11.6[b]	9.38[a]	10.5[ab]	0.98	0.049
Jejunum	10.8[b]	10.0[a]	11.2[b]	0.30	0.003
Ileum	8.64[b]	7.83[a]	8.16[ab]	0.32	0.046

SEM, standard error of the mean. n = 6. [1] Control (non-challenged control)= piglets fed the basal diet and injected with sterile 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, c] Values within a row with different letters differ (P<0.05). Adapted from Hou *et al.* (3).

Table 9. Effects of dietary supplementation with 1% AKG on adenylate purines in the intestinal mucosa of weaned pigs challenged with LPS

Items	Control	LPS	LPS+AKG
Duodenum			
ATP (μg/g wet wt)	77.1 ± 13.8[a]	43.5 ± 8.3[b]	62.8 ± 10.9[ab]
ADP (μg/g wet wt)	111.2 ± 11.6[a]	52.4 ± 12.3[c]	82.6 ± 8.7[b]
AMP (μg/g wet wt)	196.2 ± 31.8[a]	113.8 ± 33.5[b]	134.4 ± 27.4[ab]
TAN (μg/g wet wt)	377.6 ± 75.8[a]	193.8 ± 57.9[b]	278.7 ± 73.4[ab]
AEC	0.33 ± 0.06	0.36 ± 0.05	0.39 ± 0.09
AMP/ATP	2.55 ± 0.33	2.63 ± 0.22	1.93 ± 0.35
Jejunum			
ATP (μg/g wet wt)	51.2 ± 13.2[a]	32.8 ± 6.4[b]	40.9 ± 6.8[ab]
ADP (μg/g wet wt)	81.0 ± 10.5	71.2 ± 5.1	77.4 ± 13.0
AMP (μg/g wet wt)	118.2 ± 28.6	115.0 ± 14.3	109.7 ± 24.2
TAN (μg/g wet wt)	256.4 ± 35.2	219.4 ± 17.7	232.8 ± 23.9
AEC	0.35 ± 0.04[a]	0.28 ± 0.03[b]	0.31 ± 0.04[ab]
AMP/ATP	2.42 ± 0.16[b]	3.45 ± 0.20[a]	2.76 ± 0.17[b]
Ileum			
ATP (μg/g wet wt)	55.0 ± 2.4	51.9 ± 0.92	56.8 ± 3.9
ADP (μg/g wet wt)	72.2 ± 3.9	58.4 ± 5.9	71.2 ± 9.3
AMP (μg/g wet wt)	114.5 ± 9.6	140.6 ± 10.0	103.9 ± 15.3
TAN (μg/g wet wt)	247.1 ± 23.4	236.7 ± 20.0	227.4 ± 21.4
AEC	0.37 ± 0.02[a]	0.33 ± 0.01[b]	0.35 ± 0.04[ab]
AMP/ATP	2.08 ± 0.08[ab]	2.45 ± 0.11[a]	1.81 ± 0.22[b]

Data are means ± SEM, n = 6. AEC, adenylate energy charge. Control (non-challenged control)= piglets fed a control diet and injected with sterile physiological saline; LPS (LPS challenged control) = piglets fed the same control diet and challenged with *Escherichia coli* LPS; LPS+AKG (LPS+1.0% AKG) = piglets fed a 1.0% AKG diet and challenged with LPS. TAN = ATP+ADP+AMP AEC = (ATP+0.5ADP)/ (ATP+ADP+AMP) [a, b, c] Means in the same row with different superscripts differ significantly (P<0.05). Adapted from Liu *et al.* (18).

4.6. Concentrations of nitric oxide (NO) and nitric oxide synthase (NOS) in the intestinal mucosa

Physiological concentrations of NO are essential to the maintenance of intestinal mucosal integrity, but pathological levels are deleterious to the gut (6-9). AKG could maintain the balance of NO-NOS system in the jejunal mucosa, whereas the content of NO decreased (P<0.05) and NOS activity (P<0.05) increased in LPS group (Table 10). Nitric oxide is synthesized from L-arginine by NO synthase (NOS) in the presence of several cofactors, including NADPH, calcium, and tetrahydrobiopterin (40). The NOS has several isoforms, including inducible NOS (iNOS) and constitutive NOS (cNOS) (41). iNOS is expressed in response to inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β, and interferon-γ, and endotoxin. iNOS generates a relatively large amounts of NO compared with the constitutive isoforms of NOS (40). Liu (16) reported that LPS increased the mucosal NOS activity and this effect of LPS was ameliorated by AKG (16). Dietary supplementation with 1% AKG was also beneficial for maintaining the intestinal barrier function under stressful conditions. Similarly, glutamine supplementation decreased iNOS in an experimental model of colitis in the rat (42). These results may indicate that either AKG or its metabolite (e.g., glutamine) affects expression or turnover of iNOS, thereby attenuating the intestinal injury induced by LPS. Studies indicate that iNOS-derived NO mediates LPS-induced intestinal injury and bacterial translocation from the intestinal lumen to the blood circulation (43). Thus, pathological levels of NO can be toxic and pro-inflammatory. In contrast, physiological levels of NO inhibit adhesion molecule expression, synthesis of

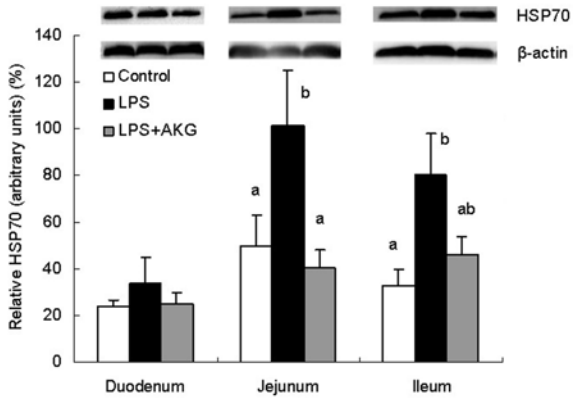

Figure 1. Relative levels of heat shock protein 70 (HSP70) expressed in small intestinal mucosa of piglets. Mucosal extracts (50 μg protein/sample) were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of HSP70 and β-actin. Values for relative HSP70 were normalized for β-actin. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the bsasl diet and injected with sterile saline; LPS (LPS challenged control) = piglets fed the same control 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, c] Within the same intestinal segment, means with different superscripts differ (P<0.05). Adapted from Hou *et al.* (3).

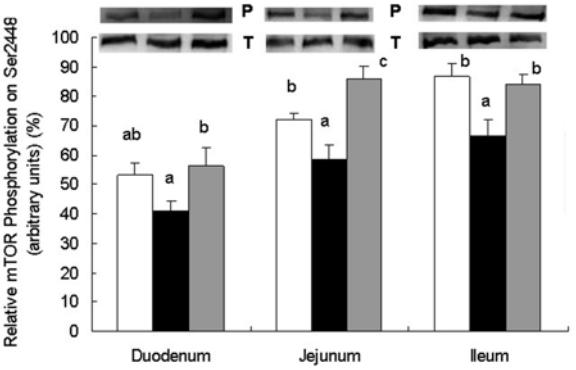

Figure 2. The phosphorylation state of mTOR in the small intestine mucosa of piglets. Mucosal extracts (150 μg protein/sample) were separated by 6% SDS-polyacrylamide gel electrophoresis for determination of phosphorylation of mTOR (P) at Ser2448 and total mTOR (T). Values for phosphorylated mTOR were normalized for total mTOR. Data are means ± SEM, n = 6. Control (non-challenged control)= piglets fed the basal diet and injected with sterile 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, c Within the same intestinal segment, means with different superscripts differ (P<0.05). Adapted from Hou *et al.* (3).

cytokines and chemokines, as well as leukocyte adhesion and transmigration. Additionally, actions of NO are dependent on the distance of the target protein from NO sources and the initial priming of immune cells (44), as well as the activation of guanylate cyclase (45).

4.7. Heat shock protein 70 (HSP70) expression

Oral administration of glutamine, a product of AKG metabolism, can protect intestinal cells from LPS-induced damage (46). AKG may impose effects through many mechanisms. One of these putative mechanisms may involve expression of HSP70 (47). A high concentration of HSP70 is indicative of oxidative stress in intestinal cells (48), which is in agreement with our observations (Figure 1) (3). Notably, dietary supplementation with AKG reduced the elevated concentrations of the HSP70 protein in the mucosae of LPS-challenged piglets (Figure 1), indicating an important role for AKG in ameliorating oxidative stress (3). In response to stress, HSP70 is expressed at elevated levels to promote refolding and prevent aggregation of partially-denatured proteins, thereby protecting cells from injury (49). This is an adaptive mechanism to allow organisms to survive under heat shock stress.

4.8. Phosphorylation levels for mammalian target of rapamycin (mTOR)

AKG can affect expression of key proteins involved in anti-inflammatory responses via the mTOR signaling (Figure 2) (3), a major mechanism for the regulation of protein synthesis in cells (50-51), therefore supporting intestinal growth under septic conditions (11,16). In addition, mTOR plays a crucial role in the control of cell growth and proliferation (52,53). Compelling evidence shows that the mTOR signaling pathway includes the 70-kDa ribosomal protein S6 kinase-1 (S6K1) and eukaryotic initiation factor (eIF) 4E-binding protein-1 (4EBP1) (54). We found that dietary supplementation with AKG increased the phosphorylated level of mTOR (the active state of mTOR) in the intestinal mucosa of piglets (Figure 2) (3). It was postulated that such an effect of AKG contributes to increased protein synthesis in the intestinal mucosa of endotoxin-treated piglets. Accordingly, mucosal protein concentration was greater in AKG-supplemented piglets than in non-supplemented piglets when they were challenged with LPS (Table 8) (3).

4.9. Phosphorylation levels for adenosine monophosphate (AMP)-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC)

AMPK activity in mammalian cells can be regulated by stimuli that affect cellular ATP levels (55). For example, hypoxia leads to activation of AMPK *via* an increase in the AMP/ATP ratio (56, 57). When activated, AMPK switches on catabolic pathways for ATP regeneration, such as glucose and fatty acid β-oxidation, while switching off ATP-requiring pathways, such as fatty acid and triglyceride synthesis (58;). Our finding that AKG regulated AMPK signaling in the intestinal mucosa is novel and important (Fig 3). However, emerging evidence shows that AMPK is a target protein of mTOR in cells (59). mTOR signaling is inhibited under conditions of low nutrients, such as amino acids and low intracellular ATP

Table 10 . The effects of AKG on the levels of NO/NOS of jejunal mucosa

Items	Control	LPS	LPS+AKG
NO (μmol/g protein)	0.80 ± 0.16^{a}	0.54 ± 0.12^{b}	0.65 ± 0.09^{ab}
NOS (Unit/mg protein)	1.25 ± 0.21^{ab}	1.45 ± 0.27^{a}	1.15 ± 0.21^{b}

SEM, standard error of the mean. n = 8. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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. Values within a row with different letters differ (P<0.05). Adapted from Liu (16).

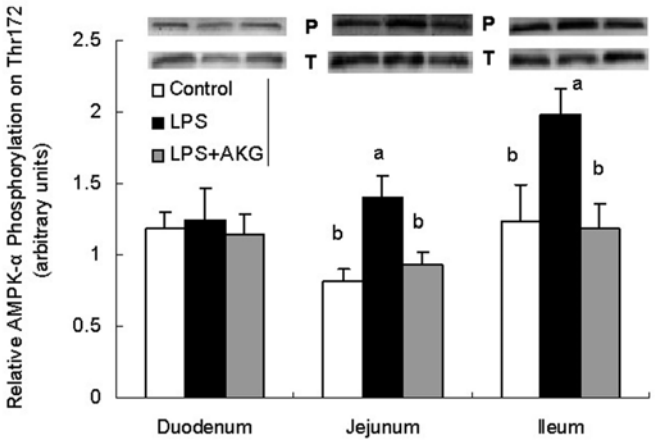

Figure 3. The phosphorylation state of AMPK-α in the small intestine mucosa of piglets. Mucosal protein extracts were separated by 10% SDS-polyacrylamide gel electrophoresis for determination of phosphorylation of AMPK-α (P) at Thr^{72} and total AMPK-α (T). Values for phosphorylated AMPK-α were normalized for total AMPK-α. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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] Within the same intestinal segment, means with different superscripts differ ($P < 0.05$).

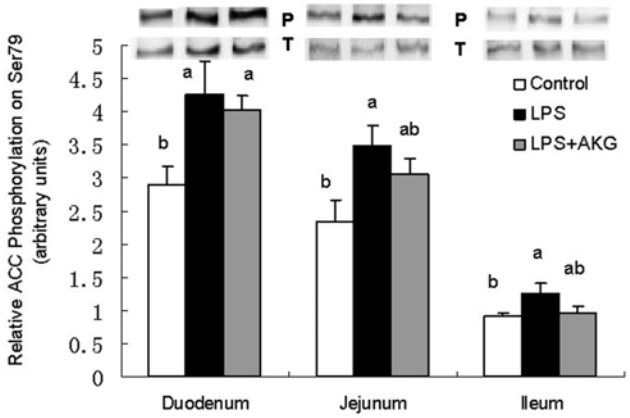

Figure 4. The phosphorylation state of ACC in the small intestine mucosa of piglets. Mucosal protein extracts were separated by 6% SDS-polyacrylamide gel electrophoresis for determination of phosphorylation of ACC-β (P) at Ser^{79} and total ACC (T). Values for phosphorylated ACC were normalized for total ACC. Data are means ± SEM, n = 6. Control (non-challenged control) = piglets fed the basal diet and injected with sterile 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] Within the same intestinal segment, means with different superscripts differ ($P < 0.05$).

levels (60). Whereas mTOR was presumed to serve as the direct cellular sensor for ATP levels (61), some evidence from *in vitro* cell culture studies has implicated AMPK in the regulation of mTOR activity (62-64). Thus, possibly through mTOR activation (3), AKG stimulates AMPK phosphorylation and oxidation of energy substrates (e.g., amino acids, fatty acids, and glucose) in the intestinal mucosa (65,66), thereby enhancing ATP supply and supporting cell function.

Besides activation of ATP-generating pathways, AMPK could also modulate ACC activity in cells by

phosphorylating ACC-β (67-69). ACC is a rate-controlling enzyme in the conversion of acetyl-CoA to malonyl CoA (70). Malonyl CoA inhibits carnitine: palmitoyl-CoA transferase-1 (CPT1), which is a rate-limiting step for the entry of long-chain fatty acyl-CoA into mitochondria to be oxidated (71). Thus, a fall in malonyl CoA levels increases fatty acid oxidation in mitochondria (72). In cells exposed to an energy-depleting stress (such as LPS challenge), AMPK is considered to be as an energy sensor that monitor the level of total cellular ATP (namely inhibiting ATP-consuming processes and stimulating ATP-producing processes) (38). On the other hand, malonyl-CoA is the substrate for fatty acid synthesis, which is an ATP-dependent process (71). Therefore, the phosphorylation of ACC by AMPK (Figure 4) would decrease malonyl CoA levels and favors elevation of energy status in the intestinal mucosa of AKG-supplemented pigs. This novel action of AKG is nutritionally and physiologically relevant for animal growth and health.

5. SUMMARY AND PERSPECTIVE

Dietary supplementation with AKG alleviates intestinal injury and dysfunction in piglets. The actions of AKG are associated with reduced oxidative stress and increased activation of the mTOR signaling. In addition, dietary supplementation with AKG beneficially improves the energy status of the intestinal mucosa as well as AMPK activation and ACC inactivation in the intestinal mucosa of LPS-challenged pigs. AKG may directly activate mTOR and associated proteins or indirectly exert its effects via glutamate formation. Alternatively, through regulating the intestinal metabolism of branched-chain amino acids (73), including leucine (an activator of mTOR), AKG may modulate intracellular protein turnover and functions of the small intestine. Besides the small intestine, AKG may also benefit the vascular system by relieving an inhibitory action of leucine (a branched-chain amino acid) on NO synthesis by endothelial cells (74). Future studies are required to test this novel and important hypothesis.

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Abbreviations: AKG, α-ketoglutarate; ACC, acetyl-CoA carboxylase; ADFI, average daily feed intake; ADG, average daily gain; AEC, adenylate energy charge; AMPK, adenosine monophosphate (AMP)-activated protein kinase; DAO, diamine oxidase; HSP 70, Heat shock protein 70; LPS, lipopolysaccharide; mTOR, mammalian target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; SEM, standard error of the mean. TAN, total adenine nucleotide

Key Words: Alpha-Ketoglutarate, Intestine, Gut function, Piglets, Lipopolysaccharide, Review

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