Physiological Functions of -Ketoglutaric Acid and Its Applications in Animal Production (Postprint)

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Abstract

-ketoglutaric acid (AKG) is a central metabolite in the tricarboxylic acid (TCA) cycle and serves as a bridge between amino acid and carbohydrate metabolism. As an ammonium ion scavenger, AKG serves as a source for glutamine provision, promotes muscle protein synthesis, inhibits protein degradation, and provides fuel for energy metabolism in gastrointestinal tract cells. AKG can generate proline via prolyl-4-hydroxylase, thereby increasing collagen synthesis and promoting skeletal system development. Furthermore, AKG can inhibit ATP synthase and the target of rapamycin (TOR), extending the lifespan of nematodes. AKG not only extends lifespan but also delays age-related diseases. Additionally, AKG plays a critical role in detoxification, enhancing cold tolerance, alleviating oxidative stress damage, and promoting the differentiation of human pluripotent stem cells. This article aims to review the physiological functions of AKG and its applications in the field of animal production, thereby enhancing understanding of AKG and providing a theoretical basis for its application in animal nutrition regulation and production practice.

Full Text

Physiological Functions of Alpha-Ketoglutarate and Its Application in Animal Production

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Abstract

Alpha-ketoglutarate (AKG) is a central metabolite in the tricarboxylic acid (TCA) cycle and serves as a critical bridge between amino acid and carbohydrate metabolism. As an ammonium ion scavenger and a source of glutamine, AKG promotes muscle protein synthesis while inhibiting protein degradation, and provides metabolic fuel for gastrointestinal cells. AKG can generate proline via prolyl-4-hydroxylase, thereby increasing collagen synthesis and promoting skeletal system development. Additionally, AKG extends the lifespan of *C. elegans* by inhibiting ATP synthase and the mammalian target of rapamycin (mTOR), not only prolonging longevity but also delaying age-related pathologies. Furthermore, AKG plays key roles in detoxification, enhancing cold tolerance, alleviating oxidative stress damage, and promoting the differentiation of human pluripotent stem cells. This review aims to summarize the physiological functions of AKG and its applications in animal production to enhance understanding of AKG and provide a theoretical basis for its use in animal nutrition regulation and production practice.

Keywords: alpha-ketoglutarate; protein synthesis; skeletal development; immune system; physiological function; animal production

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Research on key nutrients that may influence metabolic processes has traditionally been limited, primarily focusing on fatty acids, vitamins, trace elements, nucleic acids, and specific amino acids. Moreover, studies in animal nutrition have begun exploring tissue- and organ-specific effects through metabolic regulation rather than simple nutritional improvement. Alpha-ketoglutarate (AKG) is a central metabolite in the tricarboxylic acid (TCA) cycle and plays a critical role in cellular energy metabolism. The production and catabolism of AKG in cellular metabolism involve various metabolic pathways. In the TCA cycle, isocitrate is oxidatively decarboxylated to AKG by isocitrate dehydrogenase, and AKG can be further decarboxylated to succinyl-CoA and carbon dioxide (CO) by AKG dehydrogenase, a key enzyme in the TCA cycle. Glutamate can also be converted to AKG through deamination by glutamate dehydrogenase or via transamination. AKG exhibits good stability and solubility in solution and shows no toxic properties.

While adults have adequate AKG intake, aging individuals cannot meet their physiological needs through dietary intake alone. In cellular metabolism, it is

impossible to utilize AKG from the TCA cycle for amino acid synthesis, necessitating the provision of AKG as a pure dietary supplement. Studies have shown that AKG absorption is higher in the proximal than distal small intestine, and that low pH, ferrous ions (Fe^{2}), or sulfate ions (SO^{2}) can enhance AKG absorption. AKG exists only transiently in the body, likely due to rapid metabolism in intestinal epithelial cells and the liver. More than 60% of enteral AKG passes through the intestine in various forms without being completely oxidized. In enterocytes, AKG can be converted to proline, leucine, and other amino acids. As a dietary supplement, AKG significantly increases blood levels of hormones such as insulin, growth hormone (GH), and insulin-like growth factor-1 (IGF-1). All derivatives of AKG (e.g., glutamine or glutamate) are immediately converted to CO when crossing the intestinal epithelium. Given its critical role in cellular energy metabolism and participation in multiple metabolic pathways, this review aims to summarize the physiological functions of AKG and its applications in animal production to promote understanding of AKG and provide a theoretical basis for its application in animal nutrition regulation and production practice.

1.1 Promoting Protein Synthesis

AKG and related compounds such as glutamine, glutamate, and ornithine-AKG have been applied in nutritional support for geriatric conditions and post-surgical recovery. AKG helps improve negative nitrogen balance in burn patients and restores damaged small intestine function in patients with transient ischemia and radiation therapy, while also influencing amino acid metabolism in dialysis patients. In cellular metabolism, AKG is an important source of glutamine and glutamate that stimulates muscle protein synthesis, inhibits protein degradation, and serves as a crucial metabolic fuel for gastrointestinal cells. Dietary AKG supplementation after surgery can improve negative nitrogen balance and increase protein synthesis in muscle. Glutamine is an energy source for all cell types in living organisms, accounting for over 60% of the total amino acid pool. Therefore, as a glutamine precursor, AKG is the primary energy source for intestinal cells and a preferred substrate for rapidly dividing cells including those in the immune system. Additionally, glutamate released from nerve fibers in bone tissue is synthesized from AKG through reductive amination in periportal stem cells of the liver and can promote proline synthesis, which plays a key role in collagen synthesis. In the liver, glutamine serves as a precursor for urea synthesis, gluconeogenesis, and acute-phase protein synthesis, playing an important role in nitrogen and carbon exchange between tissues and organs. Although glutamine has traditionally been considered a non-essential amino acid, it serves as an important fuel source for gastrointestinal cells and rapidly dividing leukocytes and macrophages in the immune system during catabolic states and stress, and can be rapidly depleted despite significant release from muscle tissue. Studies have shown that AKG can increase Fe^{2} absorption, making AKG and its derivatives important for promoting Fe^{2} absorption in rapidly growing animals and humans with Fe^{2} deficiency. Furthermore, AKG, ascorbic acid, and Fe^{2} can hydroxylate peptide-bound proline to hydroxyproline via

prolyl hydroxylase, increasing the conversion of collagen protein to collagen and bone matrix. Additionally, Yao et al. demonstrated that AKG can inhibit glutamine degradation in porcine intestinal epithelial cells and promote protein synthesis by activating the mTOR signaling pathway. Therefore, AKG plays an important role in protein synthesis in cells and organisms.

1.2 Promoting Skeletal Development

Previous studies have shown that AKG participates in collagen metabolism through various mechanisms, with the primary mechanisms illustrated in Figure 1 [Figure 1: see original paper]. First, AKG is a cofactor for prolyl-4-hydroxylase (P4H). P4H is located in the endoplasmic reticulum (ER) and catalyzes the formation of 4-hydroxyproline, which is essential for the production of the collagen triple helix. Without hydroxylated proline, the repeated amino acid sequence leads to incomplete formation of the collagen triple helix, and misfolded triple helix proteins cannot be secreted into the cytoplasm and are subsequently degraded in the ER. Second, AKG can increase the proline residue pool through glutamate to promote collagen synthesis, with approximately 25% of dietary AKG being converted to proline in intestinal epithelial cells. Proline is the main substrate for collagen synthesis and plays a central role in collagen metabolism. As shown in Figure 1, proline is formed through the conversion of pyrroline-5-carboxylate (P5C), an intermediate in the interconversion of proline, ornithine, and glutamate. Reports indicate that besides serving as a source of proline residues through the P5C pathway, P5C can also activate collagen production via prolidase. In fact, the P5C pathway is a minor contributor to the proline pool during collagen synthesis, with the main source of proline being derived from collagen degradation products. Therefore, AKG, as a precursor of P5C, is also closely related to proline metabolism in cells and organisms.

In a study with growing pigs, enteral AKG administration increased proline levels in portal vein and arterial blood by 45% and 20%, respectively, compared to the control group. Therefore, enteral AKG is considered to increase bone tissue formation. Furthermore, the effect of AKG on bone tissue may be related to its influence on the endocrine system. Glutamine and glutamate are converted to ornithine, which further synthesizes arginine. Both ornithine and arginine can stimulate the secretion of GH and IGF-1. The GH-IGF-1 axis is known to be the main regulatory factor promoting animal skeletal growth and development. AKG may also affect bone structure through glutamate-glutamate receptor (GluR) interactions. GluR have been demonstrated in osteoblasts and osteoclasts, and Spencer et al. confirmed their significance in skeletal system metabolism. Additionally, preliminary evidence suggests that dietary AKG can eliminate bone matrix reduction induced by ovariectomy and orchiectomy in rats. Based on these studies, we can speculate that AKG may play an important role in collagen metabolism, although whether AKG directly affects collagen synthesis remains to be investigated.

1.3 Regulating the Immune System

AKG is also known as an immunonutrient factor that plays an important role in immune metabolism. Xia et al. demonstrated in weaned rats that the combination of Chinese herbal medicine and AKG significantly increased T-lymphocyte counts, improved spleen coefficient, promoted spleen hyperplasia, and enhanced spleen immune function. As a feed additive, AKG can increase small intestinal epithelial lymph node area and intestinal secretory immunoglobulin A (IgA) secretion, and the combination of AKG with Chinese herbal medicine can also improve humoral immunity, cellular immunity, and non-specific immunity. As mentioned previously, AKG is an important source of glutamine and glutamate and is considered a homolog and derivative of glutamine. Glutamine is an important fuel for lymphocytes and macrophages, which participate in early non-specific host defense responses. Dietary glutamine supplementation increases the in vitro bactericidal activity of neutrophils in burn or post-surgical patients. Therefore, as a glutamine homolog, AKG can enhance immune characteristics and improve the activity and phagocytosis of immune cells and neutrophils.

1.4 Regulating Aging

A recent study showed that AKG can delay aging and extend the lifespan of *C. elegans* by approximately 50% by inhibiting ATP synthase and mTOR (Figure 2 [Figure 2: see original paper]). The study also found that AKG not only extended lifespan but also delayed age-related phenotypes such as rapid decline in coordinated limb movement, suggesting the potential application value of AKG in organismal aging. However, the mechanism by which AKG inhibits ATP synthase and mTOR to extend lifespan has not yet been reported.

Mitochondrial ATP synthase is a key enzyme in energy metabolism and a naturally occurring molecular rotary motor that provides energy for cellular life activities and plays an important role in living organisms. Chin et al. found that lifespan extension by AKG requires the ATP synthase subunit and depends on downstream targets of mTOR. Lomenick et al. used a small molecule target identification strategy called drug affinity responsive target stability (DARTS) and found that ATP synthase subunit is a novel binding protein for AKG. AKG inhibits ATP synthase, leading to decreased ATP content, reduced oxygen consumption, and increased autophagy in mitochondria and mammalian cells, similar to the inactivation of ATP synthase 2. It is speculated that AKG may primarily extend lifespan by targeting ATP synthase 2. Previous studies have also shown that complete loss of mitochondrial function is harmful, but partial inhibition of the electron transport chain extends the lifespan of *C. elegans*. Therefore, it is entirely possible that AKG extends lifespan by inhibiting ATP synthase.

mTOR is a serine/threonine protein kinase belonging to the phosphoinositide-related kinase family and is the target of rapamycin, regulating the growth and metabolism of all eukaryotic cells. Previous studies have demonstrated that in-

hibiting mTOR activity can delay the aging process in yeast, worms, fruit flies, and genetically mutated mice. AKG does not directly interact with mTOR but mainly reduces mTOR pathway activity by inhibiting ATP synthase (Figure 2). AKG-mediated lifespan regulation partially depends on AMP-activated protein kinase (AMPK) and forkhead transcription factor (FoxO). AMPK is an evolutionarily conserved cellular energy sensor that plays a critical role in organismal aging and lifespan. When the AMP/ATP ratio increases, AMPK is activated, which subsequently inhibits mTOR signaling by activating the mTOR inhibitor TSC2 phosphorylation, thereby regulating cellular energy metabolism. FoxO proteins are subunits of the forkhead transcription factor family that mediate the effects of insulin and growth factors on various physiological functions including cell proliferation, apoptosis, and metabolism. Like mTOR, FoxO is a transcription factor required for lifespan extension in response to reduced mTOR signaling, which is also important for AKG-regulated lifespan. Additionally, autophagy is significantly increased in AKG-treated worms through mTOR activity inhibition and dietary restriction. Therefore, AKG treatment and mTOR inactivation extend lifespan through the same pathway (AKG acting upstream or through mTOR) and also through independent mechanisms or parallel pathways converging on downstream effectors.

Studies on starved yeast and bacteria, starved pigeon liver, and exercising humans have shown increased physiological levels of AKG. The biochemical basis for this increase can be explained by starvation-induced gluconeogenesis that activates glutamate-linked transaminases in the liver to generate carbon from amino acid catabolism. Chin et al. showed that AKG levels are elevated in starved *C. elegans* and that AKG does not extend the lifespan of dietary-restricted animals. These results suggest that AKG is a key metabolic regulator mediating lifespan extension through starvation or dietary restriction (Figure 2). This also indicates a new molecular link between general metabolites, general cellular energy generators, and dietary restriction that regulates organismal lifespan, providing a novel strategy for preventing and treating age-related diseases.

1.5 Other Functions

Current research on AKG has primarily focused on protein synthesis, skeletal development, and medical applications (e.g., trauma, post-surgical patients, and hip replacement patients). In recent years, deeper investigations have revealed several novel functions. In a rat toxicology study, Bhattacharya et al. found that AKG treatment significantly eliminated cyanide toxicity, suggesting that AKG can serve as a cyanide antidote. Bayliak et al. demonstrated that AKG improves cold tolerance in *Drosophila melanogaster* by enhancing antioxidant capacity and amino acid synthesis. Yang et al. found that AKG can regulate DNA demethylation of the PR domain-containing protein 16 promoter. Additionally, AKG plays an important role in alleviating oxidative stress damage and can promote the proliferation and differentiation of human pluripotent stem cells

and embryonic stem cells.

2 Application of AKG in Animal Production

Numerous studies have demonstrated that AKG plays an important role in maintaining total nitrogen balance, reducing nitrogen loss, and promoting protein synthesis. In rats, dietary supplementation with 215 mol AKG significantly reduced nitrogen loss and increased nitrogen deposition. This finding was further confirmed by Piva et al., who showed that adding AKG (3 and 6 g/kg) to a nitrogen-free diet reduced urinary nitrogen content by 18%. Prandini et al. found that dietary AKG supplementation (3–6 g/kg) significantly reduced endogenous urinary nitrogen loss and tended to decrease endogenous fecal nitrogen. Chen et al. showed that adding 1% AKG to growing pig diets improved growth performance, increased nitrogen utilization, and reduced nitrogen excretion. Wang et al. demonstrated that 1% dietary AKG improved growth performance, promoted glutamine synthesis, and enhanced amino acid metabolism in hybrid sturgeon. Wei et al. found that adding AKG (7.5 and 15.0 g/kg) to low-protein diets promoted amino acid metabolism in the liver and pancreas of Songpu mirror carp, improved protein utilization, and promoted protein synthesis. AKG supplementation effectively promoted collagen synthesis in piglets before and after weaning. Additionally, dietary AKG (2 g/kg) effectively improved negative nitrogen balance and promoted muscle protein synthesis in post-surgical and burn patients.

AKG plays an important role in skeletal development and bone mineral deposition. Oral administration of AKG [0.1 g/(kg BW · d)] to lambs from two weeks after birth increased bone mineralization and bone mineral density in trabecular and cortical bone. Long-term (146 d) feeding of AKG [0.1 g/(kg BW · d)] to newborn male lambs increased tibial weight and length and cortical bone mineral density, while maximum elastic strength and ultimate strength of the tibia increased by 10% and 8%, respectively. In piglets, feeding AKG [0.4 g/(kg BW · d)] after birth increased cortical bone density and improved femoral geometric and mechanical properties. Andersen et al. showed that long-term feeding of AKG [0.1 g/(kg BW · d)] to piglets aged 21–24 days significantly increased femoral mineral density and plasma estrogen levels. Additionally, combined dietary supplementation of AKG and -hydroxy- -methylbutyrate significantly improved piglet growth performance, blood amino acid levels, bone weight, and cortical bone mineral density. In turkeys, Tatara et al. demonstrated that long-term (14 weeks) feeding of AKG [0.4 g/(kg BW · d)] significantly increased femoral and tibial bone mineral density, cross-sectional area, maximum elastic strength, and ultimate strength. Further studies showed that feeding AKG [0.4 g/(kg BW · d)] also significantly increased radial bone weight, bone length, bone mineral density, cross-sectional area, maximum elastic strength, and ultimate strength, while increasing blood concentrations of glutamine, proline, and leucine. In human studies, dietary AKG had similar effects on bone tissue, with potential benefits in maintaining bone mass and reducing bone turnover

in postmenopausal women. These studies indicate that AKG has positive effects on bone metabolism regulation and suggest its potential use in treating osteoporosis.

Furthermore, Schlegel et al. showed that dietary AKG supplementation can limit bacterial dissemination and metabolic changes after injury in rats, suggesting its use in protecting intestinal mucosa. Hou et al. demonstrated that dietary AKG supplementation (1%) alleviated lipopolysaccharide-induced intestinal mucosal injury and promoted small intestinal absorption in piglets. Hu reported that dietary AKG (1%) increased average daily gain in weaned piglets. Wang et al. found that the combination of Chinese herbal medicine and AKG as feed additives had more pronounced growth-promoting effects and higher feed returns than either used alone. Liu et al. also showed that combined supplementation of 1% AKG and 1% allicin in antibiotic-free diets improved growth performance in growing pigs by improving intestinal morphology, enhancing nutrient digestibility of crude protein, calcium, and phosphorus, and promoting intestinal health. Chen et al. found that dietary AKG (1%) promoted the growth of beneficial bacteria, improved intestinal microbial flora, regulated volatile fatty acid production, and reduced intestinal ammonia levels, thereby improving growth performance in growing pigs. In broiler chickens, Yu et al. showed that dietary AKG (0.7%) significantly increased body weight at 2 weeks of age and average daily gain during weeks 1–2, with better growth-promoting effects than 0.7% glutamine. Dietary AKG (0.5%) promoted intestinal mucosal cell growth, improved intestinal antioxidant capacity, and enhanced intestinal energy metabolism in heat-stressed broilers. Therefore, numerous studies have demonstrated that AKG plays an important role in maintaining animal and human health.

To date, the application of AKG in animal production remains in the preliminary exploration stage. To achieve ideal effects, further research is necessary, focusing on: (1) AKG absorption and metabolism in the animal intestine; (2) effects of exogenous AKG on AKG and amino acid metabolism in body tissues; (3) synergistic effects and mechanisms of AKG with other substances (Chinese herbal extracts, organic acids, phytase, probiotics, and vitamins); and (4) evaluation of AKG' s potential as an antibiotic substitute when used as an acidifier.

3 Summary

In summary, the physiological significance of AKG is multifaceted, and not all metabolic pathways have been established. The mechanism by which AKG promotes skeletal system development is related to glutamate receptor activation, proline production for bone collagen, and the possible anti-catabolic and anabolic effects of 17 -estradiol, and these effects are likely multifactorial. Additionally, the positive effects of AKG may improve chest function and visceral organ protection in premature and low-birth-weight neonates. Current research suggests that AKG may play an important role in preventing and treating metabolic bone diseases in humans and animals. Therefore, further research is needed to investigate AKG' s functions, elucidate its mechanisms of action,

and explore its application potential in human society and other fields. Regarding aging, exciting findings have shown that mTOR complex 1 is involved in numerous human diseases including diabetes, obesity, heart disease, and cancer. Aging is a common risk factor for these diseases, and the mechanism linking cellular senescence, disease, and organismal aging has been revealed to be mediated through mTOR. Therefore, the function of AKG metabolism in inhibiting mTOR signaling suggests that AKG may play an important role in tumor suppression.

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