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Study on the Effects of AKG on Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

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Abstract: To observe the effects of different doses of α-ketoglutarate (AKG) on amino acid metabolism in the tissues of growing pigs fed a high-fat diet. Forty growing pigs were selected and divided into 4 groups with 10 pigs in each group. The control group was fed only a basal diet, while the experimental groups were fed a high-fat diet: Experimental Group I (0.5% AKG + high-fat diet), Experimental Group II (1% AKG + high-fat diet), and Experimental Group III (1.5% AKG + high-fat diet). After the feeding period, the small intestine, cecum, colon, serum, liver, and leg muscle were collected to detect amino acid composition. Compared with the control group, the addition of AKG significantly increased the serum content of some glucogenic amino acids, the branched-chain amino acid Ile, and the aromatic amino acid Trp ($p<0.05$); in the liver, AKG significantly decreased the content of some glucogenic and aromatic amino acids ($p<0.05$); in the leg muscle, the addition of AKG extremely significantly decreased the content of branched-chain and aromatic amino acids ($p<0.01$). The addition of AKG delayed weight gain in growing pigs by reducing the content of glucogenic amino acids in the liver and muscles, and had a protective effect on the intestinal mucosal morphology of growing pigs fed a high-fat diet..

Keywords: High-fat diet; Growing pigs; Intestinal mucosa; Amino acid metabolism

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1. Introduction

The intestine not only undertakes the task of absorbing nutrients in mammals but also plays a role in resisting exogenous harmful substances. The intestinal barrier is mainly composed of the normal intestinal flora, mucus layer, intestinal epithelial cells, and the intestinal immune system. In the process of amino acid metabolism, α-ketoglutarate (AKG), as a precursor of glutamate and glutamine, not only directly enters the TCA cycle as an energy substance to produce energy but also combines with ammonium ions produced during glutamine metabolism to form glutamate[1]. Current results from metabolic experiments indicate that approximately 40% of AKG disappears in the intestine, suggesting that AKG can be utilized by cells of various intestinal systems and exert biological functions[2]. Currently, there are relatively few reports on the effects of AKG on amino acid metabolism and its relationship with intestinal absorption in obese model growing pigs. Therefore, this experiment investigated the effects of adding different doses of AKG to the diet of growing pigs fed a high-fat diet on the amino acid metabolomics of some tissues and intestinal mucosal morphology, aiming to reveal the mechanism of action of AKG from the perspective of energy metabolism, and to provide an experimental basis for the development and utilization of AKG as a new feed additive and its application as a lipid-lowering regulator.

2. Materials and Methods

2.1. Experimental Animals and Design

A single-factor experimental design was used. Forty healthy Duroc × Landrace × Yorkshire crossbred growing barrows with similar body weight (approx. 25-30 kg) were selected. They were randomly divided into 4 groups with 10 replicates per group and 1 pig per replicate.

Control group (CON group): Fed a basal diet.

High-fat group (HF group): Fed a high-fat diet.

High-fat + 0.5% AKG group (HF+0.5A group): Fed a high-fat diet + 0.5% AKG.

High-fat + 1.0% AKG group (HF+1.0A group): Fed a high-fat diet + 1.0% AKG.

High-fat + 1.5% AKG group (HF+1.5A group): Fed a high-fat diet + 1.5% AKG.

High-fat diet formulation: 10% lard + 10% egg yolk powder + 4% cholesterol + 1% cholic acid + 75% ordinary feed.

2.2. Measurement Indicators and Methods.

2.2.1. Sample Collection and Preparation

Serum collection: At eight weeks, 10 mL of blood was collected from the anterior vena cava of the experimental pigs into clean centrifuge tubes. After standing at room temperature for 1 hour, it was centrifuged at 4 °C, 3500 r/min for 15 min. The serum was collected, aliquoted into 500 μL sterile centrifuge tubes, and stored at -80°C.

2.2.2. Measurement Methods

Serum biochemical indicators: Serum-related biochemical indicators were measured using a Shenzhen Mindray BS-190 automatic biochemical analyzer, with reagent kits from Shenzhen Mindray.

Amino acid indicators: The method for measuring serum amino acids was as follows: take 300 μL of serum, add an equal volume of 8% sulfosalicylic acid, vortex, let stand at 4°C for about 8 hours, centrifuge at 10000 rpm for 10 min, take the supernatant, filter through a 0.45 μm membrane into the inner tube of an amino acid sample vial, and detect the contents of Glutamate (Glu), Serine (Ser), Histidine (His), Glycine (Gly), Threonine (Thr), Alanine (Ala), Arginine (Arg), Tyrosine (Tyr), Cysteine (Cys), Valine (Val), Methionine (Met), Phenylalanine (Phe), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Proline (Pro), etc., using an L-8800 automatic amino acid analyzer (Beckman). For liver and leg muscle amino acid measurement: take tissue samples, homogenize with physiological saline, centrifuge at 3000 r/min for 20 min, take the supernatant according to the acetonitrile deproteinization method, filter through a 0.45 μm membrane into the inner tube of an amino acid sample vial, and detect with the amino acid analyzer.

2.3. Data Processing

Experimental data were preliminarily sorted using Excel 2010, and then one-way T-test in SPSS 19.0 statistical software was used to compare the significance of differences between treatment groups. $P<0.05$ was considered a significant difference, and $0.05<P<0.10$ was considered a trend towards significance. Results are expressed as mean ± standard error (x ± SE).

3. Results and Analysis

3.1. Effects of AKG on Serum Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

As shown in **Table 1**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) significantly increased the serum content of glucogenic amino acids such as Asp, Ser, and Gly ($P<0.05$), and also significantly increased the content of the aromatic amino acid Trp ($P<0.05$). These results suggest that the glutamate precursor AKG can significantly increase the content of some glucogenic amino acids, branched-chain amino acids, and aromatic amino acids in the serum of obese model pigs.

Table 1. Effects of AKG on Serum Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	51.27±20.85a	69.09±6.87b	88.69±1.58b	72.19±11.97b
	Glu	137.68±10.09	211.27±20.32	80.97±16.85	141.08±39.43
	Asn	13.98±2.39	18.59±5.85	16.28±0.98	22.98±3.68
	Ser	115.23±14.29a	176.39±21.59b	115.26±7.64a	205.97±48.69b
	Gln	497.68±76.38	398.67±5.29	351.27±20.85	428.14±2.49
	Gly	279.69±54.39a	350.61±47.23b	297.77±11.79b	400.39±8.76b
	Thr	215.69±39.85a	249.78±19.42b	297.39±11.95b	303.58±12.66b
	Arg	57.69±21.36	86.95±18.64	79.69±12.09	100.27±20.06
	Ala	259.27±78.49	296.27±72.51	248.75±8.20	324.70±44.29
	Val	41.39±18.69a	66.78±8.36b	51.37±8.69b	54.78±13.94b
Branched-Chain AA	Val	41.39±18.69a	66.78±8.36b	51.37±8.69b	54.78±13.94b
	Ile	35.10±2.89	39.67±11.96	38.74±10.02	69.68±11.88
	Leu	36.66±5.68	35.74±10.85	30.89±9.85	44.48±9.72
Aromatic AA	Tyr	307.25±90.56	278.69±20.09	319.59±10.29	306.42±10.57
	Trp	19.36±1.77a	30.96±7.85	39.40±7.99	41.26±7.68
	Phe	40.56±10.25	31.27±4.25	33.60±2.21	51.17±6.99

3.2. Effects of AKG on Liver Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

As shown in **Table 2**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) extremely significantly decreased the liver content of glucogenic amino acids such as Asn, Gly, Thr, and Ala ($P<0.01$), and also significantly decreased the liver content of aromatic amino acids Tyr, Trp, and Phe ($P<0.05$). However, for the content of branched-chain amino acids, no significant changes occurred in the three groups ($P>0.05$). These results suggest that the glutamate precursor AKG can significantly reduce the content of some glucogenic and aromatic amino acids in the liver of obese model pigs.

Table 2. Effects of AKG on Liver Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	366.82±58.56	136.87±29.57	254.98±32.24	153.57±38.09
	Glu	363.93±19.44	262.27±40.68	280.78±55.28	392.59±52.43
	Asn	84.69±9.87A	43.36±5.97B	56.28±0.98B	38.67±6.27B
	Ser	415.98±25.46	176.39±9.48	252.69±15.9	200.78±16.62
	Gln	264.67±11.38	303.127±18.26	323.36±20.85	196.39±12.49
	Gly	789.23±23.39A	485.69±7.70B	576.31±3.35B	398.69±11.57B
	Thr	80.08±39.85A	101.59±25.36B	152.39±26.87B	98.39±11.29B
	Arg	147.89±21.36	115.66±18.64	179.69±12.09	179.58±20.06
	Ala	1497.36±98.49A	1176.27±99.51B	1348.75±128.20B	324.70±44.29B
	Val	178.26±5.68a	58.36±4.59	79.59±5.72	69.89±3.25

Table 1 (Continued)

AA Classification		Control Group	Group I	Group II	Group III
Branched-Chain AA	Val	178.26±5.68a	58.36±4.59	79.59±5.72	69.89±3.25
	Ile	152.10±2.89	39.67±11.96	41.74±10.02	39.68±11.88
	Leu	232.66±5.68	60.74±10.85	96.89±9.85	85.48±9.72
Aromatic AA	Tyr	907.25±90.56a	1078.69±120.09	1019.59±1.29	896.42±10.57
	Trp	29.36±1.77a	78.96±7.85b	55.82±7.99b	43.26±7.68b
	Phe	144.56±10.25a	39.27±4.25b	68.60±2.21b	54.17±6.99b

3.3. Effects of AKG on Leg Muscle Amino Acid Metabolism in Growing Pigs Fed a High-Fat Die

As shown in **Table 3**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) significantly decreased the muscle content of amino acids such as Asn, Glu, Asp, Ser, Gly, Val, and Thr ($P<0.05$), and also significantly decreased the muscle content of branched-chain amino acids Val, Ile, and Leu ($P<0.05$), and extremely significantly decreased the content of phenylalanine Phe ($P<0.01$). These results suggest that the glutamate precursor AKG can significantly reduce the content of some glucogenic and branched-chain amino acids in the leg muscle of obese model pigs, inducing the catabolism of blood sugar.

Table 3. Effects of AKG on Leg Muscle Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	356.49±44.93a	312.62±27.41b	264.28±32.2b	269.68±38.09b
	Glu	354.93±19.44a	297.27±40.68b	329.78±55.28b	287.59±52.43b
	Asn	52.69±9.87Aa	27.85±5.97Bb	21.97±0.98Bb	24.67±6.27Bb
	Ser	215.98±25.46a	176.39±9.48b	179.69±15.9b	198.78±16.62b
	Gln	233.67±11.38	225.127±18.26	219.36±20.85	152.39±12.49
	Gly	149.23±23.39a	85.69±7.70b	76.31±3.35b	98.69±11.57b
	Thr	380.08±39.85a	310.59±25.36b	258.39±26.87b	178.39±11.29b
	Arg	147.89±21.36	100.66±18.64	79.69±12.09	79.58±20.06
	Ala	497.36±8.49A	396.27±72.51B	348.75±8.20B	324.70±44.29B
	Val	99.26±5.68a	42.36±4.59b	42.59±5.72b	37.89±3.25b
Branched-Chain AA	Val	99.26±5.68a	42.36±4.59b	42.59±5.72b	37.89±3.25b
	Ile	52.10±2.89a	39.67±11.96b	41.74±10.02b	39.68±11.88b
	Leu	236.66±5.68a	135.74±10.85b	130.89±9.85b	144.48±9.72b
Aromatic AA	Tyr	507.25±90.56	378.69±20.09	319.59±10.29	406.42±10.57
	Trp	219.36±71.09	178.96±7.96	155.82±8.99	141.26±7.07
	Phe	63.56±10.08A	34.27±4.96B	41.60±2.87B	38.17±6.45B

4. Discussion

Blood is an important amino acid pool in the body and a hub connecting nutrient absorption and metabolic utilization.

Because blood amino acid composition is easily affected by diet and body health status, measuring blood amino acid composition can be a valuable reference for disease diagnosis[3]. Our experimental results confirm that the glutamate precursor AKG can significantly increase the serum content of some glucogenic amino acids, branched-chain amino acids, and aromatic amino acids in obese model pigs. Measurements of amino acid metabolism in the liver and muscles determined that adding AKG to the diet of SD obese model pigs reduces the aromatic amino acid content in the liver and muscle amino acid pools.

As an energy donor, AKG can serve as a substitute for amino acids in the small intestine. When the body's energy supply is severely insufficient, glucogenic amino acids in the body can be gluconeogenized into glucose in the liver through specific metabolic pathways to serve as an energy supply for the body[4]. The results of this study show that in the high-fat diet groups treated with different concentrations of AKG, the serum Asp, Ser, and Gly content in growing pigs was significantly higher than in the high-fat diet control group, but different doses of AKG treatment significantly reduced the content of most glucogenic amino acids in the liver and leg muscle. Therefore, we speculate that under physiological conditions with sufficient energy to meet the body's energy demands, adding AKG to a high-fat diet may primarily regulate glucogenic amino acids to enhance body protein synthesis, but the specific biological mechanism requires further in-depth study. Muscle tissue not only uses carbohydrates and lipids as metabolic substrates but is also the main site for branched-chain amino acid (BCAA) metabolism in the body[5]. Studies in obese humans and obese animal models have found that BCAA levels in serum are very high, indicating impaired BCAA catabolism. Simultaneously, elevated ceramide levels were found in muscle tissue; this lipid acts as a signaling molecule inhibiting insulin receptor activity[6]. In this study, AKG addition significantly increased the content of the branched-chain amino acid Ile and extremely significantly reduced BCAA metabolism in the leg muscle, consistent with the above research results. Therefore, we speculate that AKG can accelerate BCAA decomposition in the intestine during its metabolism, reducing the concentration of BCAAs in circulation, thus potentially playing a role in remodeling metabolic pathways in muscle.

AKG is an intermediate of the TCA cycle and a precursor for glutamate family amino acids. Endogenous AKG is mainly generated by the oxidative decomposition of glucose. When circulating AKG is insufficient, glutamate acts as an anaplerotic substrate to participate in the cycle[7]. One study using 24 pregnant sows as subjects found that dietary supplementation with AKG during the peripartum period significantly increased the birth weight of newborn piglets and the serum concentrations of aspartate, serine, glutamine, glycine, valine, ornithine, lysine, and arginine compared to the control saline group[8]. Glutamate is a major energy source for upper intestinal epithelial cells and intestinal microorganisms. Even when providing more than 4 times the required amount of Glu, most Glu is oxidized for energy or converted into other non-essential amino acids in intestinal epithelial cells[9]. The mechanism may be that the preliminary digestion of nutrients begins in the stomach, but the main site of nutrient absorption is the small intestine. As a precursor of glutamate, AKG is converted to Glu upon ingestion, while fat digestion begins in the small intestine. Because the dwell time of chyme in the duodenum is relatively short, fat does not exert its effect, while the massively increased monosodium glutamate causes the body to reduce Glu absorption by downregulating the expression of the EAAC1 gene[10] to maintain amino acid balance in duodenal epithelial cells.

In this experiment, adding AKG to pigs fed a high-fat diet significantly reduced the muscle content of amino acids such as Asn, Glu, Asp, Ser, Gly, Val, and Thr. Under energy restriction, dietary fat does not have a significant effect on the preservation of body protein and branched-chain amino acids (including Leu, Ile, and Val)[11]. However, under normal physiological conditions, fat intake can reduce the proportion of energy supplied by nutrients other than fat. The results of this experiment also show that adding high fat to the diet can reduce the level of glucogenic amino acids in the blood. Blood Val is a good predictor of amino acid preservation; studies have found that dietary fat supplementation can increase blood Val concentration, indicating that dietary fat can promote the preservation of body amino acids[12].

5. Conclusion

In obese model pigs, the addition of AKG delayed weight gain by reducing the content of glucogenic amino acids in the liver and muscles and had a protective effect on the intestinal mucosal morphology of growing pigs fed a high-fat diet.

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Disclosure statement

The author declares no conflict of interest.

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