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Effects of α-ketoglutaric acid and allicin on growth, development, and apparent nutrient digestibility in growing pigs: Postprint

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Abstract

This study aimed to investigate the effects of α-ketoglutarate (AKG) and allicin on growth performance, nutrient apparent digestibility, serum biochemical indices, and intestinal morphological structure in growing pigs. Forty healthy crossbred growing pigs (Duroc × Landrace × Yorkshire) with an initial average body weight of (30.64$±$1.35) kg were selected and randomly divided into 5 groups according to the principle of similar body weight and same sex ratio, with 8 replicates per group and 1 pig per replicate. The five groups of experimental pigs were fed antibiotic-free basal diet (control group), antibiotic-free basal diet + 25 mg/kg antibiotics (Group), antibiotic-free basal diet + 1% AKG (Group), antibiotic-free basal diet + 0.5% allicin (Group), and antibiotic-free basal diet + 1% AKG + 0.5% allicin (Group), respectively. The pre-trial period was 7 days, and the formal trial period was 21 days. The results showed that: 1) Compared with the control group, the average daily gain of growing pigs in Group and Group increased by 10.80% and 9.91%, respectively, but the differences were not significant (P>0.05). 2) Compared with the control group, the apparent digestibility of calcium in Group was significantly increased (P<0.05), the apparent digestibility of crude protein, calcium, and phosphorus in Group was significantly increased (P<0.05), and Group significantly increased the apparent digestibility of phosphorus compared with Group (P<0.05). 3) Compared with the control group, serum immunoglobulin G (IgG) content in Group was significantly increased (P<0.05); serum triglyceride (TG) content in Group was significantly decreased (P<0.05); serum total cholesterol (TCHO) and TG contents in Group were significantly decreased (P<0.05), while serum alkaline phosphatase (AKP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and serum immunoglobulin A (IgA) and IgG contents were significantly increased (P<0.05). Compared with Group , serum TCHO and TG contents in Group showed a decreasing trend (P>0.05), while serum

AST and ALT activities were significantly increased (P<0.05). 4) Compared with the control group, ileal villus height and villus height/crypt depth ratio in jejunum and ileum in Group were significantly increased (P<0.05). 5) Compared with the control group, the numbers of goblet cells and lymphocytes in the ileum in Group were significantly increased (P<0.05). In conclusion, the combined supplementation of 1% AKG and 0.5% allicin in antibiotic-free diet could improve growth performance of growing pigs by improving intestinal morphological structure, enhancing the digestion and absorption capacity of nutrients such as crude protein, calcium, and phosphorus, and promoting intestinal health.

Full Text

Effects of Dietary α-Ketoglutarate and Allicin on Growth Performance and Nutrient Apparent Digestibility of Growing Pigs

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Abstract

This experiment investigated the effects of dietary α-ketoglutarate (AKG) and allicin on growth performance, nutrient apparent digestibility, serum biochemical parameters, and intestinal morphological structure of growing pigs. Forty healthy crossbred (Duroc $\times$ Landrace $\times$ Large White) growing pigs with an initial average body weight of (30.64 $\pm$ 1.35) kg were randomly allocated into five groups according to similar body weight and gender ratio, with eight replicates per group and one pig per replicate. The five experimental groups were fed: 1) a basal diet without antibiotics (control group), 2) basal diet + 25 mg/kg antibiotics (Group I), 3) basal diet + 1% AKG (Group II), 4) basal diet + 0.5% allicin (Group III), and 5) basal diet + 1% AKG + 0.5% allicin (Group IV). The experiment consisted of a 7-day pre-feeding period followed by a 21-day formal feeding period. The results showed: 1) Compared with the control group, the average daily gain (ADG) of pigs in Groups I and IV increased by 10.80% and 9.91%, respectively, though the differences were not significant ($P > 0.05$). 2) The apparent digestibility of calcium was significantly higher in Group II than in the control group ($P < 0.05$), while Group IV showed significantly improved apparent digestibility of crude protein, calcium, and phosphorus ($P < 0.05$). Moreover, Group IV exhibited significantly higher phosphorus digestibility compared with Group I ($P < 0.05$). 3) Serum immunoglobulin G (IgG) content was significantly elevated in Group II ($P < 0.05$), whereas serum triglyceride (TG) content was significantly reduced in Group III ($P < 0.05$). Group IV demonstrated significantly lower serum total cholesterol (TCHO) and TG contents ($P < 0.05$), but significantly higher activities of alkaline phosphatase (AKP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), along with increased serum immunoglobulin A (IgA) and IgG contents ($P < 0.05$). Compared with Group I, Group IV showed a tendency toward reduced serum TCHO and TG contents ($P > 0.05$) and significantly increased AST and ALT activities ($P < 0.05$). 4) Group IV exhibited significantly greater ileal villus height and villus height-to-crypt depth ratio in both jejunum and ileum compared with the control group ($P < 0.05$). 5) The numbers of goblet cells and lymphocytes in the ileum were significantly higher in Group IV than in the control group ($P < 0.05$). In conclusion, dietary supplementation with 1% AKG combined with 0.5% allicin in antibiotic-free diets can improve intestinal morphological structure, enhance nutrient digestibility and absorption of crude protein, calcium, and phosphorus, promote intestinal health, and thereby improve the growth performance of growing pigs.

Keywords: α-ketoglutarate; growing pigs; allicin; growth performance; nutrient apparent digestibility; serum biochemical parameters; intestinal morphological structure

Introduction

Antibiotics have been widely used as feed additives in livestock production. However, the abuse of antibiotics has led to serious problems including bacterial resistance, drug residues, and environmental pollution. In recent years, frequent food safety incidents have drawn significant attention from governments and consumers worldwide regarding antibiotic usage. Finding effective alternatives to antibiotics has become an important approach to address these issues. Allicin, a bioactive compound extracted from garlic (*Allium sativum*), has been studied as a potential antibiotic substitute in animal production and exhibits multiple biological functions such as antibacterial and anti-inflammatory effects, anticancer and antitumor properties, lipid-lowering effects, and immune enhancement [1-4]. α-Ketoglutarate (AKG) is a crucial intermediate in the tricarboxylic acid (TCA) cycle and a precursor of glutamine and glutamic acid. Its excellent stability and beneficial properties, including improved animal growth performance, promoted intestinal development, regulated energy metabolism, and enhanced immunity and antioxidant capacity, have attracted considerable research attention [5-7]. Wang et al. [8] reported that dietary supplementation with Chinese herbal medicine combined with AKG improved growth performance and digestive function in weaned piglets. Both AKG and allicin may promote animal growth, immune function, and intestinal development due to their unique biological activities and nutritional roles [9-12]. However, limited research has been conducted on the individual or combined effects of dietary AKG and allicin on growth performance, serum biochemical parameters, and intestinal development in growing pigs. Therefore, this study aimed to investigate the effects of AKG and allicin supplementation in antibiotic-free diets on growth development and nutrient apparent digestibility in growing pigs, providing a theoretical basis for their scientific application in antibiotic-free pig feed.

Materials and Methods

1.1 Experimental Materials

AKG was purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd. with an effective content of 99.5%. Allicin was obtained from Henan Kaifeng Mubo Biotechnology Co., Ltd. with an effective content $\geq$ 25%. The antibiotic used was chlortetracycline, a commercial product added at 25 mg/kg in the diet.

1.2 Experimental Diets

The basal diet was formulated using corn as the main energy source and soybean meal as the primary protein source, following the nutrient requirements for 30–60 kg pigs according to NRC (2012). The composition and nutrient levels of the basal diet are presented in Table 1 . All dietary ingredients were ground, mixed progressively, and prepared as powder, then stored in a ventilated and dry place.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Item	Content
Ingredients	
Soybean meal	
Corn	
Soybean oil	
Limestone	
Calcium hydrogen phosphate ($CaHPO_4$)	
L-Lysine	
DL-Methionine	
L-Threonine	
L-Tryptophan	
Salt (NaCl)	
Sodium bicarbonate ($NaHCO_3$)	
Premix[1])	

Item	Content
Total	
Nutrient levels[2])	
Digestible energy (DE) / (MJ/kg)	
Crude protein (CP)	
Lysine	
Methionine	
Methionine + Cysteine	
Threonine	
Tryptophan	
Available phosphorus (AP)	

[1]) The premix provided the following per kg of diet: VA 4,000 IU, VD_3 400 IU, VE 50 IU, VK 1 mg, VB_1 1.5 mg, VB_2 4 mg, VB_6 2 mg, VB_{12} 0.02 mg, VC 300 mg, nicotinic acid 23 mg, D-pantothenic acid 15 mg, biotin 0.08 mg, folic acid 1.3 mg, choline chloride (50%) 600 mg, high-temperature resistant phytase 150 mg, antioxidant 800 mg, mildew preventive 500 mg, zeolite powder 7,442 mg, Cu 15 mg, Mn 30 mg, Fe 100 mg, Zn 50 mg, I 0.3 mg, Se 0.3 mg.

[2]) DE was a calculated value, while the others were measured values.

1.3 Experimental Animals and Design

Forty healthy crossbred (Duroc × Landrace × Large White) growing pigs with an initial average body weight of (30.64 ± 1.35) kg were randomly divided into five groups according to similar body weight and gender ratio, with eight replicates per group and one pig per replicate. The five groups were fed: 1) basal diet without antibiotics (control group), 2) basal diet + 25 mg/kg antibiotics (Group I), 3) basal diet + 1% AKG (Group II), 4) basal diet + 0.5% allicin (Group III), and 5) basal diet + 1% AKG + 0.5% allicin (Group IV). The experiment included a 7-day pre-feeding period followed by a 21-day formal feeding period.

1.4 Animal Management

The experiment was conducted at the animal experimental building of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Growing pigs were housed individually in cages and fed at 08:30 and 16:30 daily with free access to feed and water. Feed allowance and residual feed were recorded each morning to accurately measure actual feed intake, monitor feeding behavior and health status. Regular cleaning and disinfection of pig houses were performed to maintain hygiene.

1.5 Measurements and Methods

1.5.1 Growth Performance Pigs were weighed on the first and last day of the experiment at 08:00 after overnight fasting to obtain initial and final body weights for calculating average daily gain (ADG). Daily feed intake was recorded, and total feed consumption was calculated at the end of the experiment to determine average daily feed intake (ADFI) and feed-to-gain ratio (F/G). The formulas were as follows:

- ADFI = Total feed consumption / (experimental days × number of pigs)
- ADG = (average final body weight -average initial body weight) / (experimental days × number of pigs)
- F/G = ADFI / ADG

1.5.2 Nutrient Apparent Digestibility During the final five days of the experiment, fresh feces were collected continuously from each pig using the incomplete collection method. Feces were weighed and mixed with 10 mL of 10% dilute sulfuric acid per 15 g of fresh feces, then stored at -80 °C. Prior to analysis, fecal samples were oven-dried at 105 °C to constant weight and ground into powder. The 4 mol/L hydrochloric acid-insoluble ash method was used to determine the contents of crude protein, crude ash, energy, calcium, and phosphorus in both feces and diets. National standard methods were employed to measure crude protein (GB/T 6432—1994), crude ash (GB/T 6438—2007), calcium (GB/T 6436—2002), and total phosphorus (GB/T 6437—2002). Energy content was measured using a GR-3500 oxygen bomb calorimeter. Nutrient apparent digestibility was calculated using the following formula:

Nutrient apparent digestibility (%) = 100 × [1 -(A/B) × (C/D)]

Where: A = nutrient content in feces; B = nutrient content in diet; C = acid-insoluble ash content in diet; D = acid-insoluble ash content in feces.

1.5.3 Serum Biochemical Parameters On the final day of the experiment at 08:00, 10 mL of blood was collected aseptically from the anterior vena cava of each pig after overnight fasting. Serum was separated by centrifugation at 3,000 r/min for 10 min and stored at -80 °C for subsequent analysis. Serum contents of triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TCHO), urea nitrogen (UN), total protein (TP), albumin (ALB), immunoglobulin (Ig) A, IgM, and IgG, as well as activities of alkaline phosphatase (AKP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s instructions.

1.5.4 Intestinal Morphological Structure At the end of the experiment, pigs were slaughtered after overnight fasting. The abdominal cavity was opened immediately to remove the intestines. Middle segments of the jejunum and ileum were isolated, rinsed with phosphate-buffered saline to remove digesta, and fixed in 4% paraformaldehyde for 24 h before paraffin embedding and sectioning. Hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining were performed to observe intestinal mucosal structure and the morphology and distribution of intraepithelial lymphocytes and goblet cells under light microscopy. For each intestinal segment, five slices were prepared, and 2–5 fields of view were selected per slice. Using Moditec imaging software, five villus heights (measured from the crypt-villus junction to the villus tip) and crypt depths (measured from the crypt-villus junction to the crypt base) were measured per cross-section. Additionally, the numbers of lymphocytes and goblet cells per 100 intestinal mucosal epithelial columnar cells were counted along five of the longest and most regularly arranged villi.

1.6 Data Processing and Statistical Analysis

Results are expressed as means ± standard error. One-way ANOVA was performed using SPSS 20.0. Duncan' s multiple comparison test was applied when the model was significant. Differences were considered significant at $P < 0.05$, extremely significant at $P < 0.01$, and trends were identified at $0.05 \leq P < 0.10$.

Results

2.1 Effects of Dietary AKG and Allicin on Growth Performance of Growing Pigs

As shown in Table 2 , compared with the control group, Groups I and IV showed tendencies for improved ADG, increasing by 10.80% ($P = 0.059$) and 9.91% ($P = 0.067$), respectively. All treatment groups exhibited reduced F/G, though no significant differences were observed ($P > 0.05$). No significant differences were detected among groups in ADG, ADFI, or F/G ($P > 0.05$).

Table 2 Effects of dietary AKG and allicin on growth performance of growing pigs

Item	Control Group	Group I	Group II	Group III	Group IV
Initial weight (kg)	30.00 ± 0.79	30.17 ± 1.04	30.52 ± 0.47	31.22 ± 0.47	31.20 ± 1.10
Final weight (kg)	48.92 ± 1.41	51.14 ± 1.53	50.96 ± 0.60	51.37 ± 0.49	52.00 ± 1.70
ADG (g/d)	676 ± 18	749 ± 33	730 ± 24	720 ± 12	743 ± 27
ADFI (g/d)	1,488 ± 39	1,600 ± 86	1,578 ± 57	1,563 ± 24	1,595 ± 82

Item	Control Group	Group I	Group II	Group III	Group IV
F/G	2.20 ± 0.04	2.13 ± 0.02	2.16 ± 0.02	2.17 ± 0.01	2.14 ± 0.04

In the same row, values with different small letter superscripts indicate significant difference (P < 0.05), different capital letter superscripts indicate extremely significant difference (P < 0.01), and same or no letter superscripts indicate no significant difference (P > 0.05). The same applies below.

2.2 Effects of Dietary AKG and Allicin on Nutrient Apparent Digestibility of Growing Pigs

As presented in Table 3 , Group IV showed significantly higher apparent digestibility of crude protein, calcium, and phosphorus compared with the control group, increasing by 5.45%, 5.20%, and 4.26%, respectively ($P < 0.05$), while crude ash digestibility tended to improve ($P = 0.065$). Group II exhibited significantly enhanced calcium digestibility ($P < 0.05$), with a tendency for improved crude protein digestibility ($P = 0.052$) and a 3.43% increase in phosphorus digestibility ($P > 0.05$). Compared with Group I, Group IV demonstrated significantly greater phosphorus digestibility ($P < 0.05$). No significant differences were observed among other groups for any nutrient digestibility parameters ($P > 0.05$).

Table 3 Effects of dietary AKG and allicin on nutrient apparent digestibility of growing pigs (%)

Item	Control Group	Group I	Group II	Group III	Group IV
Energy	82.45 ± 0.23	83.33 ± 0.90	84.15 ± 0.58	83.72 ± 0.95	84.23 ± 0.47
Crude protein	77.82 ± 0.92	79.01 ± 1.28	81.51 ± 1.49	80.59 ± 1.11	82.06 ± 1.42
Crude ash	61.03 ± 0.62	61.51 ± 0.57	62.98 ± 1.11	62.10 ± 1.01	63.40 ± 0.82
Calcium	62.31 ± 0.71	63.60 ± 0.68	64.95 ± 0.52	64.08 ± 0.87	65.55 ± 1.18
Phosphorus	51.92 ± 0.33	52.06 ± 0.35	53.70 ± 0.76	52.96 ± 0.46	54.13 ± 0.99

2.3 Effects of Dietary AKG and Allicin on Serum Biochemical Parameters of Growing Pigs

As shown in Table 4 , Group IV exhibited significantly reduced serum TG and TCHO contents ($P < 0.05$), significantly increased activities of AKP, ALT, and AST (P < 0.05), and significantly elevated serum IgA and IgG contents (P < 0.05) compared with the control group. Group II showed significantly higher serum IgG content (P < 0.05), while Group III had significantly lower serum TG content (P < 0.05) and a tendency for reduced TCHO content (P = 0.066). Compared with Group I, Group IV showed tendencies for decreased serum TCHO (P = 0.068) and TG (P = 0.063) contents, significantly increased AST and ALT activities (P < 0.05), and a tendency for elevated IgG content (P = 0.057). No significant differences were observed among groups for other serum biochemical parameters (P > 0.05).

Table 4 Effects of dietary AKG and allicin on serum biochemical parameters of growing pigs

Item	Control Group	Group I	Group II	Group III	Group IV
TG (mmol/L)	0.56 ± 0.12	0.51 ± 0.07	0.48 ± 0.08	0.31 ± 0.02	0.28 ± 0.03
HDL (mmol/L)	1.65 ± 0.16	1.72 ± 0.39	1.81 ± 0.17	2.06 ± 0.048	2.37 ± 0.31
LDL (mmol/L)	4.84 ± 0.52	3.83 ± 0.62	3.40 ± 0.90	3.05 ± 0.34	2.99 ± 0.39
TCHO (mmol/L)	3.81 ± 0.56	3.54 ± 0.27	3.26 ± 0.22	2.85 ± 0.25	2.75 ± 0.12
UN (mmol/L)	5.80 ± 0.37	5.67 ± 0.22	5.25 ± 0.26	5.46 ± 0.17	5.01 ± 0.45
AKP (U/L)	138.38 ± 10.42	155.00 ± 8.68	168.95 ± 27.10	156.86 ± 13.05	203.62 ± 1.21
AST (U/L)	4.25 ± 0.62	4.46 ± 0.28	5.59 ± 0.21	4.89 ± 0.44	5.80 ± 0.52
ALT (U/L)	6.09 ± 0.28	6.21 ± 0.44	7.47 ± 0.38	7.39 ± 0.58	7.88 ± 0.50
TP (g/L)	55.98 ± 1.77	56.97 ± 3.42	60.67 ± 2.85	59.81 ± 2.86	62.72 ± 2.17
ALB (g/L)	24.26 ± 0.54	24.41 ± 0.57	25.28 ± 0.35	25.23 ± 0.30	25.43 ± 0.28
IgA (mg/mL)	0.21 ± 0.00	0.22 ± 0.02	0.23 ± 0.01	0.23 ± 0.00	0.24 ± 0.01
IgG (mg/mL)	5.06 ± 0.17	5.36 ± 0.04	6.12 ± 0.32	5.61 ± 0.55	6.58 ± 0.73
IgM (mg/mL)	0.61 ± 0.04	0.67 ± 0.02	0.78 ± 0.09	0.70 ± 0.07	0.78 ± 0.02

2.4 Effects of Dietary AKG and Allicin on Intestinal Morphological Structure of Growing Pigs

As shown in Table 5 , Group IV exhibited significantly higher villus height-to-crypt depth (V/C) ratios in both jejunum and ileum (P < 0.05) and significantly greater ileal villus height ($P < 0.05$) compared with the control group. Group IV also showed a tendency for reduced ileal crypt depth ($P = 0.052$) and increased jejunal villus height ($P = 0.059$). The V/C ratios in jejunum ($P = 0.058$) and ileum ($P = 0.052$) tended to be higher in Group IV than in Group I. No significant differences were observed among other groups ($P > 0.05$).

Table 5 Effects of dietary AKG and allicin on intestinal morphological structure of growing pigs

Item	Control Group	Group I	Group II	Group III	Group IV
Jejunum					
Villus height (µm)	346 ± 21	383 ± 39	411 ± 25	404 ± 32	425 ± 24
Crypt depth (µm)	294 ± 15	291 ± 17	284 ± 16	286 ± 37	232 ± 20
V/C ratio	1.17 ± 0.09	1.31 ± 0.12	1.44 ± 0.07	1.41 ± 0.10	1.83 ± 0.33
Ileum					
Villus height (µm)	375 ± 14	387 ± 97	407 ± 13	396 ± 16	420 ± 18
Crypt depth (µm)	305 ± 20	284 ± 17	276 ± 22	278 ± 19	251 ± 12
V/C ratio	1.22 ± 0.16	1.35 ± 0.11	1.47 ± 0.07	1.42 ± 0.08	1.67 ± 0.14

2.5 Effects of Dietary AKG and Allicin on Intestinal Goblet Cells and Lymphocytes in Growing Pigs

As shown in Table 6 , Group IV exhibited significantly increased numbers of goblet cells and lymphocytes in the ileum ($P < 0.05$) and a tendency for increased lymphocyte numbers in the jejunum ($P = 0.056$) compared with the control group. Group II showed a tendency for increased lymphocyte numbers in the ileum ($P = 0.057$). Compared with Group I, Group IV tended to have more lymphocytes in the ileum ($P = 0.066$). No significant differences were observed among other treatment groups ($P > 0.05$).

Table 6 Effects of dietary AKG and allicin on intestinal goblet cells and lymphocytes of growing pigs

Item	Control Group	Group I	Group II	Group III	Group IV
Jejunum					
Goblet cells	13.3 ± 3.7	14.0 ± 3.0	18.0 ± 2.4	15.0 ± 2.5	21.3 ± 2.9
Lymphocytes	63.3 ± 8.4	69.6 ± 5.5	87.0 ± 12.0	77.3 ± 6.3	93.0 ± 13.5
Ileum					
Goblet cells	28.0 ± 2.0	35.6 ± 2.3	39.0 ± 4.1	37.6 ± 6.1	42.3 ± 4.4
Lymphocytes	70.3 ± 6.0	78.0 ± 5.5	87.0 ± 2.6	83.3 ± 4.3	95.3 ± 8.4

Discussion

3.1 Effects of AKG and Allicin on Growth Performance of Growing Pigs

Antibiotics are secondary metabolites produced by microorganisms (including bacteria, fungi, and actinomycetes) or higher plants and animals during their life processes. These chemical substances can interfere with the developmental functions of other living cells and have been widely applied in livestock production and clinical medicine in recent years, primarily by enhancing animal immunity to promote growth. In this study, the antibiotic group increased ADG by 10.80% and reduced F/G by 3.18% compared with the control group, indicating that antibiotics exerted a certain growth-promoting effect on growing pigs. AKG, as a precursor of glutamine, exhibits good stability and plays a vital role in improving animal growth performance. Hu [10] reported that dietary AKG supplementation in 25-day-old weaned piglets improved growth performance, with 1% AKG increasing ADG by 9% as the optimal effect. Yu et al. [13] found that dietary AKG supplementation at 0.7% significantly increased body weight at 2 weeks of age and ADG during weeks 1–2 in broilers. Numerous studies have demonstrated that allicin extracted from Chinese herbal medicine promotes animal growth. Liu [11] reported that dietary allicin supplementation increased ADG by 14.4% and reduced F/G by 16.8% in weaned piglets, indicating its growth-promoting effects. Tu [14] showed that allicin supplementation significantly increased ADG and reduced F/G in finishing pigs (65–110 kg), with optimal levels of 150–300 mg/kg. The current study further confirmed these findings, demonstrating that the combination of 1% AKG and 0.5% allicin in antibiotic-free diets tended to increase ADG compared with the control group, with no significant difference from the antibiotic group. The underlying mechanisms may include: 1) AKG, as a glutamine precursor, provides energy and nitrogen sources for intestinal epithelial and immune cells, reduces intestinal glutamine catabolism, and supplies energy for gastrointestinal cell metabolism, thereby maintaining intestinal barrier integrity and normal absorptive function [15-16]; and 2) The diallyl trisulfide in allicin can inhibit the growth of nitrite-reducing bacteria in gastric juice, protecting the gastrointestinal tract and promoting growth in growing pigs [17].

3.2 Effects of AKG and Allicin on Nutrient Apparent Digestibility in Growing Pigs

Nutrient apparent digestibility is a crucial indicator of feed digestion and absorption capacity in animals, with higher digestibility being more conducive to growth. Fu et al. [18] reported that dietary supplementation with Chinese herbal medicine and AKG improved digestive function in weaned piglets. Liu [11] found that allicin enhanced the apparent digestibility of calcium and phosphorus in weaned piglets. In this study, dietary supplementation with 1% AKG combined with 0.5% allicin significantly improved the apparent digestibility of crude protein, calcium, and phosphorus compared with the control group, consistent with previous findings. This may be attributed to: 1) synergistic interactions between AKG and allicin that enhance digestive and absorptive capacity; 2) AKG' s ability to promote intestinal mucosal growth, thereby increasing the contact area between nutrients and the intestine and expanding absorptive surface area [8]; and 3) allicin' s distinctive odor improving diet palatability, stimulating feed intake, gastric secretion, and gastrointestinal motility, thereby promoting nutrient digestion and absorption [19].

3.3 Effects of AKG and Allicin on Serum Biochemical Parameters in Growing Pigs

Serum biochemical parameters reflect metabolic status and can indicate the functional condition of specific tissues or organs. Serum ALT and AST are important enzymes involved in transamination, primarily distributed in hepatocytes and other tissues. Their activities reflect protein and lipid metabolism efficiency and liver/heart function. Enhanced ALT and AST activities, in the absence of liver damage, indicate increased protein synthesis and catabolism [20]. AKP is a genetically marked isoenzyme whose activity reflects growth rate, performance, bone growth, and calcium-phosphorus and lipid metabolism. Elevated AKP activity indicates vigorous bone growth and increased calcium-phosphorus deposition [21]. Wei et al. [22] reported that dietary AKG supplementation in Songpu mirror carp significantly increased serum ALT and AST activities, promoting hepatic amino acid metabolism and protein synthesis. Wei et al. [23] confirmed that AKG enhanced protein metabolism in fish. In this study, 1% AKG + 0.5% allicin significantly increased AKP, ALT, and AST activities compared with the control group, while 1% AKG alone tended to increase AST and ALT activities. Compared with the antibiotic group, the combination treatment significantly increased AST and ALT activities, suggesting that dietary supplementation with 1% AKG and 0.5% allicin in antibiotic-free diets enhances protein synthesis and lipid metabolism efficiency, likely related to AKG' s role in promoting protein synthesis and inhibiting protein degradation [24].

Serum TCHO and TG contents reflect liver function and are associated with fat deposition. Reduced TCHO and TG levels indicate enhanced lipid metabolism and anti-fatty liver effects [25]. This study demonstrated that 1% AKG + 0.5% allicin significantly decreased serum TCHO and TG contents, while 0.5% allicin alone only significantly reduced TG content and tended to lower TCHO content. These findings suggest that both AKG and allicin enhance lipid metabolism and reduce fat deposition [24,26-27], with synergistic effects exceeding those of antibiotics or individual supplementation.

Serum TP content reflects protein absorption and humoral immunity, comprising primarily ALB and globulin, which indirectly indicate disease resistance. Elevated serum IgA, IgG, and IgM contents signify enhanced immune function [28]. In this study, 1% AKG + 0.5% allicin significantly increased serum IgA and IgG contents and tended to elevate ALB content, while 1% AKG alone significantly increased IgG content. These results indicate that AKG and allicin enhance immune capacity and hepatic protein synthesis [9,12].

3.4 Effects of AKG and Allicin on Intestinal Morphological Structure in Growing Pigs

The small intestine is the primary site for nutrient digestion, absorption, and transport, and intact intestinal mucosal structure is essential for normal nutrient utilization and growth. Villus height and crypt depth reflect intestinal functional status: villus height indicates absorptive capacity (reduced height impairs nutrient absorption), while crypt depth reflects mucosal cell renewal rate (shallower crypts indicate faster renewal and enhanced secretory function) [29]. Hu [10] reported that 1% AKG supplementation significantly reduced crypt depth and increased V/C ratio in piglet small intestine, improving histomorphology and absorptive function. Chen et al. [30] found that AKG significantly increased posterior intestinal villus height in hybrid sturgeon, promoting intestinal development. This study showed that 1% AKG + 0.5% allicin significantly increased V/C ratios in jejunum and ileum and ileal villus height. The V/C ratios in both segments were also higher than those in the antibiotic group. This may be because AKG, as a TCA cycle intermediate, can be converted to glutamate and glutamine, providing energy and nitrogen for gastrointestinal cells, promoting cell proliferation and mucosal repair, maintaining gastrointestinal health, and facilitating intestinal development [31].

3.5 Effects of AKG and Allicin on Intestinal Goblet Cells and Lymphocytes in Growing Pigs

Intestinal lymphocytes and goblet cells are essential components of the intestinal barrier, serving as the first line of defense against harmful microbial adhesion and invasion [32]. Goblet cells are mucus-secreting cells in the intestinal epithelium that maintain mucosal integrity and stability and are involved in various intestinal diseases including infection, inflammatory bowel disease, and necrotizing enteritis [33]. Intraepithelial lymphocytes are a specialized component of gut-associated lymphoid tissue that first contacts foreign antigens and microorganisms and initiates immune responses; their numbers reflect the integrity of local mucosal immune barriers and the effectiveness of immune defense [34]. Studies have shown that AKG, like glutamine, regulates mucosal protein synthesis via the mTOR signaling pathway, provides energy for gastrointestinal epithelial and immune cells, promotes cell proliferation, and maintains intestinal absorptive and structural integrity [16]. Zhang et al. [35] reported that dietary allicin solution supplementation at 1% significantly increased goblet cell numbers and enhanced mucus secretion in mice, thereby strengthening the intestinal mucosal immune barrier.

This study found that 1% AKG + 0.5% allicin significantly increased goblet cell and lymphocyte numbers in the ileum, while 1% AKG alone tended to increase ileal lymphocyte numbers. In the ileum, the combination treatment yielded higher lymphocyte counts than the antibiotic group, consistent with previous reports and indicating synergistic enhancement of individual effects.

In conclusion, dietary supplementation with 1% AKG combined with 0.5% allicin in antibiotic-free diets improves intestinal morphological structure, enhances nutrient digestibility and absorption of crude protein, calcium, and phosphorus, strengthens immune capacity and disease resistance, promotes intestinal health, and thereby improves growth performance in growing pigs.

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