Effects of Guanidinoacetic Acid on Growth Performance, Antioxidant Capacity, and Key Enzymes of Glucose Metabolism in Nursery Pigs: Postprint

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Abstract

This study aimed to investigate the effects of guanidinoacetic acid on growth performance, antioxidant capacity, and key enzymes of glucose metabolism in nursery pigs. A total of 64 healthy three-way crossbred (Duroc × Landrace × Yorkshire) piglets with a body weight of (19.03$±$1.30) kg were randomly allocated into 2 groups, with 4 replicates per group and 8 pigs per replicate. The control group was fed a basal diet, whereas the guanidinoacetic acid group was fed the basal diet supplemented with 600 mg/kg guanidinoacetic acid. The trial consisted of a 7-day adaptation period followed by a 14-day experimental period. The results demonstrated that: 1) Compared with the control group, the guanidinoacetic acid group exhibited significantly increased average daily gain ($P<0.05$) and significantly decreased feed conversion ratio ($P<0.05$), while no significant differences were observed in final body weight or average daily feed intake between the two groups ($P>0.05$). 2) The guanidinoacetic acid group displayed significantly higher plasma superoxide dismutase inhibition rate, glutathione content, and total antioxidant capacity relative to the control group ($P<0.05$), with no significant difference in plasma malondialdehyde content between groups ($P>0.05$). 3) Plasma activities of phosphofructokinase, pyruvate kinase, isocitrate dehydrogenase, malate dehydrogenase, NADH-coenzyme Q reductase, and creatine kinase in the guanidinoacetic acid group were all significantly higher than those in the control group ($P<0.01$), while plasma hexokinase activity tended to be lower ($P<0.10$) and plasma adenosine triphosphate synthase activity tended to be higher ($P<0.10$). These results indicate that dietary supplementation with guanidinoacetic acid can improve growth performance and enhance antioxidant capacity in nursery pigs, and may promote catabolism and elevate systemic ATP levels by increasing the activities of key glycolytic enzymes (PFK, PK, IDH, MDH) and mitochondrial respiratory chain-related

enzymes (NADH-CoQ, ATPase), thereby potentially facilitating the synthesis of energy storage tissues (muscle and fat).

Full Text

Effects of Guanidine Acetic Acid on Growth Performance, Antioxidant Capacity and Glycometabolism Key Enzymes of Nursery Pigs

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Abstract

This experiment was conducted to investigate the effects of guanidine acetic acid on growth performance, antioxidant capacity and glycometabolism key enzymes of nursery pigs. Sixty-four healthy "Duroc×Landrace×Yorkshire" hybrid piglets with body weight of (19.03$±$1.30) kg were randomly divided into 2 groups, with 4 replicates per group and 8 piglets per replicate. The control group was fed a basal diet, while the guanidine acetic acid group was fed the basal diet supplemented with 600 mg/kg guanidine acetic acid. The pre-test period lasted for 7 days, and the formal test period lasted for 14 days. The results showed: 1) Compared with the control group, the average daily gain of nursery pigs in the guanidine acetic acid group was significantly increased ($P<0.05$), and the feed-to-gain ratio was significantly decreased ($P<0.05$); there were no significant differences in final body weight and average daily feed intake between the two groups ($P>0.05$). 2) The plasma superoxide dismutase (SOD) inhibition rate, glutathione (GSH) content and total antioxidant capacity (T-AOC) in the guanidine acetic acid group were significantly higher than those in the control group ($P<0.05$), while there was no significant difference in plasma malondialdehyde (MDA) content between the two groups ($P>0.05$). 3) The activities of fructose-6-phosphate kinase (PFK), pyruvate kinase (PK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), nicotinamide adenine dinucleotide-coenzyme Q reductase (NADH-CoQ) and creatine kinase (CK) in plasma of nursery pigs in the guanidine acetic acid group were all extremely significantly higher than those in the control group ($P<0.01$), the activity of plasma hexokinase (HK) tended to be lower than that in the control group ($P<0.10$), and the activity of plasma adenosine triphosphate (ATP) synthase (ATPase) tended to be higher than that in the control group ($P<0.10$). It can be concluded that dietary supplementation with guanidine acetic acid can improve the growth performance of nursery pigs, enhance their antioxidant capacity, and promote catabolism in vivo by increasing the activities of glycometabolism key enzymes (PFK, PK, IDH, MDH) and mitochondrial respiratory chain related enzymes (NADH-CoQ, ATPase), thereby increasing body ATP levels and potentially promoting the synthesis of energy storage tissues (muscle and fat).

Keywords: guanidine acetic acid; nursery pigs; growth performance; antioxidant capacity; glycometabolism

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Guanidine acetic acid (GAA), also known as guanidinoacetic acid, glycocyamine, or N-guanylglycine, was first isolated from human and dog urine [1]. Guanidine acetic acid is a precursor of creatine, which can be synthesized from L-arginine and glycine and then methylated to form creatine in the liver of vertebrates. Creatine is an important substance in energy metabolism and serves as a site for energy storage [2-3]. Creatine exists in two forms in the body: free creatine and phosphocreatine, which together constitute the phosphagen system. When the body has excess adenosine triphosphate (ATP), phosphocreatine can store energy, and when ATP is insufficient, phosphocreatine releases ATP [4].

As an important substance in energy metabolism, creatine is unstable during feed supplementation [5-7], whereas its precursor guanidine acetic acid is stable under various conditions [8]. Therefore, guanidine acetic acid is now widely used as a substitute for creatine. Previous studies have shown that guanidine acetic acid can improve animal production and reproductive performance, enhance meat quality, and also possesses certain antioxidant functions [9-13]. Guanidine acetic acid can improve energy metabolism efficiency by increasing creatine and ATP content in muscle [14-16]. However, there have been few studies on the effects of guanidine acetic acid on the growth performance and glycometabolism of nursery pigs. This experiment was designed to investigate the effects of guanidine acetic acid on growth performance, antioxidant capacity and glycometabolism key enzymes of nursery pigs, providing a basis for the scientific application of guanidine acetic acid.

Materials and Methods

1.1 Experimental Materials

Guanidine acetic acid: purity >98%, purchased from Beijing Junde Tongchuang Animal Husbandry Technology Co., Ltd.

1.2 Experimental Design

Sixty-four healthy "Duroc×Landrace×Yorkshire" hybrid piglets with body weight of (19.03$±$1.30) kg were randomly divided into 2 groups, with 4 replicates per group and 8 piglets per replicate. The control group (CON group) was fed a basal diet, while the guanidine acetic acid group (GAA group) was fed the basal diet supplemented with 600 mg/kg guanidine acetic acid. The pre-test period lasted for 7 days, and the formal test period lasted for 14 days. The basal diet was formulated according to NRC (2012). The composition and nutrient levels of the basal diet are shown in Table 1 .

1.3 Management

The experiment was conducted at a pig farm in Lüliang City, Shanxi Province. Throughout the experimental period, pigs had free access to feed and water, and were fed three times daily, with each feeding providing slightly more feed than needed to ensure some remained in the trough after satiation. Feces were cleaned once daily, with attention paid to ventilation, and the pigs' feeding behavior, temperature, humidity, and mental state were observed. Regular disinfection was performed, and any diseased pigs were treated promptly.

1.4 Sample Collection and Processing

On day 14 of the experiment, all experimental pigs were collected via anterior vena cava puncture into heparin sodium-containing tubes. Plasma was obtained by centrifugation (3,000 r/min, 10 min), aliquoted into 200 μL centrifuge tubes, and stored at -80 °C for subsequent analysis.

1.5.1 Growth Performance

At the beginning and end of the experiment, individual pigs were weighed after fasting, and feed consumption was recorded. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated.

1.5.2 Plasma Antioxidant Indices

Plasma superoxide dismutase (SOD) inhibition rate was determined using a kit from Tongren Chemical Research Institute by colorimetric method, where SOD inhibition rate is proportional to SOD activity. Plasma glutathione (GSH) content and total antioxidant capacity (T-AOC) were determined using kits from Nanjing Jiancheng Bioengineering Institute by colorimetric method. Plasma malondialdehyde (MDA) content was determined using a kit from Nanjing Jiancheng Bioengineering Institute by thiobarbituric acid (TBA) method.

1.5.3 Plasma Glycometabolism Key Enzymes and Respiratory Chain Related Enzyme Activities

Plasma hexokinase (HK), fructose-6-phosphate kinase (PFK), pyruvate kinase (PK), malate dehydrogenase (MDH), and creatine kinase (CK) activities were determined using kits from Nanjing Jiancheng Bioengineering Institute by colorimetric method. Isocitrate dehydrogenase (IDH), nicotinamide adenine dinucleotide (NADH)-coenzyme Q reductase (NADH-CoQ), and ATP synthase (ATPase) activities were determined using kits from Shanghai Jiemei Gene Medicine Technology Co., Ltd. by colorimetric method. All measurements were performed according to the corresponding kit instructions.

1.6 Statistical Analysis

Experimental data were analyzed by t-test using SAS 9.2 software. $P<0.05$ was considered significant, and $P<0.01$ was considered extremely significant. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects of Guanidine Acetic Acid on Growth Performance of Nursery Pigs

As shown in Table 2 , compared with the control group, the average daily gain of nursery pigs in the guanidine acetic acid group was significantly increased ($P<0.05$), and the feed-to-gain ratio was significantly decreased ($P<0.05$). There were no significant differences in final body weight and average daily feed intake between the two groups ($P>0.05$).

2.2 Effects of Guanidine Acetic Acid on Plasma Antioxidant Indices of Nursery Pigs

As shown in Table 3 , the plasma SOD inhibition rate and T-AOC in the guanidine acetic acid group were extremely significantly higher than those in the control group ($P<0.01$), and plasma GSH content was significantly higher than that in the control group ($P<0.05$). There was no significant difference in plasma MDA content between the two groups ($P>0.05$).

2.3 Effects of Guanidine Acetic Acid on Activities of Glycometabolism Key Enzymes and Respiratory Chain Related Enzymes in Plasma of Nursery Pigs

As shown in Table 4 , compared with the control group, the activities of plasma PFK, PK, IDH, MDH, NADH-CoQ, and CK in the guanidine acetic acid group were all extremely significantly increased ($P<0.01$). The activity of plasma HK tended to be lower than that in the control group ($P<0.10$), and the activity of plasma ATPase tended to be higher than that in the control group ($P<0.10$).

2.4 Effects of Guanidine Acetic Acid on ATP Synthesis and Decomposition in Nursery Pigs

As shown in Table 4 and Figure 1 [Figure 1: see original paper], dietary supplementation with guanidine acetic acid increased the activities of ATP-producing enzymes PK, IDH, MDH, NADH-CoQ, and ATPase by 2.53, 3.12, 1.51, 4.92, and 1.83 times, respectively, and increased the activities of ATP-consuming enzymes PFK and CK by 2.52 and 2.02 times, respectively.

Discussion

3.1 Effects of Guanidine Acetic Acid on Growth Performance of Nursery Pigs

Guanidine acetic acid is the main endogenous substance for creatine synthesis in animal organisms and can be either synthesized by the body itself or obtained from food. This study showed that dietary supplementation with guanidine acetic acid significantly increased the average daily gain and decreased the feed-to-gain ratio of nursery pigs, but had no significant effect on average daily feed intake. These results are consistent with the findings of Wang et al. [17], Qi et al. [18], and Pan et al. [9]. Jiang [19] found that dietary supplementation with 600 mg/kg guanidine acetic acid significantly increased the average daily gain and decreased the feed-to-gain ratio of broilers, while significantly increasing the dressing percentage. Michiels et al. [20] reported that dietary supplementation with 600 mg/kg guanidine acetic acid improved the average daily gain and feed utilization of Ross broilers. Zhang et al. [21] found that dietary supplementation with 300-600 mg/kg guanidine acetic acid improved the growth performance of growing-finishing pigs.

Dietary supplementation with guanidine acetic acid can promote creatine synthesis in the body, thereby increasing the storage or utilization efficiency of high-energy substances such as phosphocreatine and ATP, which promotes muscle energy metabolism, reduces the catabolism of carbohydrates, proteins, and fats for energy supply, and consequently accelerates animal growth [22-23].

3.2 Effects of Guanidine Acetic Acid on Antioxidant Capacity of Nursery Pigs

Animals maintain free radical balance in the body through the antioxidant system, which timely removes excess free radicals. MDA is the final product of lipid peroxidation and can reflect the degree of peroxidation in the body. SOD is one of the antioxidant enzymes in the body that can dismutate superoxide anions into hydrogen peroxide (H2O2) to eliminate their toxicity [24]. GSH can scavenge free radicals in the body, while T-AOC is an indicator reflecting the overall antioxidant function of the body. This study found that dietary supplementation with guanidine acetic acid significantly increased the plasma SOD inhibition rate, GSH content, and T-AOC of nursery pigs, but had no

significant effect on MDA content. Wang et al. [25] and Wang et al. [26] found that dietary supplementation with guanidine acetic acid significantly increased SOD and catalase (CAT) activities and T-AOC in muscle, as well as glutathione peroxidase (GSH-Px), CAT activity, and T-AOC in blood, while extremely significantly decreasing MDA content in muscle and blood. These findings indicate that guanidine acetic acid can improve the antioxidant capacity of the body by increasing antioxidant enzyme activities to a certain extent.

The improvement of antioxidant capacity by guanidine acetic acid may be related to its ability to increase creatine content. Lawler et al. [27] demonstrated in vitro that high concentrations of creatine could scavenge free radicals. Zhao et al. [28] found that phosphocreatine could protect myocardium by reducing MDA content and increasing the function of antioxidant enzyme systems such as SOD and CAT, thereby alleviating doxorubicin-induced oxidative stress. Maddock et al. [29] showed that creatine could improve DNA activity after oxidative stress damage, ensuring its normal function.

3.3 Effects of Guanidine Acetic Acid on Glycometabolism Key Enzymes and Respiratory Chain Related Enzymes of Nursery Pigs

The red arrows represent promoting effects. Pyruvate kinase, isocitrate dehydrogenase, malate dehydrogenase, NADH-Q reductase, and ATP synthase are enzymes that produce ATP, while hexokinase and phosphofructokinase are enzymes that consume ATP.

NADH: nicotinamide adenine dinucleotide; ATP: adenosine triphosphate.

Figure 1 [Figure 1: see original paper] Effects of guanidine acetic acid on glycometabolism

HK catalyzes the phosphorylation of glucose to glucose-6-phosphate, consuming one ATP in this process. PFK catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, consuming one ATP. PK catalyzes the conversion of phosphoenolpyruvate to pyruvate, accompanied by ATP production. The tricarboxylic acid cycle occurs in mitochondria, where isocitrate undergoes oxidative decarboxylation to form α-ketoglutarate. This is the first decarboxylation reaction in the tricarboxylic acid cycle, catalyzed by IDH and accompanied by NADH generation. MDH catalyzes the dehydrogenation of malate to oxaloacetate, accompanied by NADH generation [30].

Changes in blood enzyme activities can reflect the physiological state of the body to a certain extent [31]. In this experiment, compared with the control group, the activities of plasma PFK, PK, IDH, and MDH in the guanidine acetic acid group were all extremely significantly increased. As shown in Figure 1, glucose catabolism in plasma was enhanced. However, Fu et al. [32] reported that 250 mg/kg guanidine acetic acid significantly decreased PK and succinate dehydrogenase activities but significantly increased muscle glycogen content in Jian carp, which may be due to species differences.

NADH generated by the tricarboxylic acid cycle enzyme system can directly enter the respiratory chain to react with oxygen, accompanied by the generation of 2.5 ATP. The increase in NADH-CoQ and ATPase activities induced by guanidine acetic acid may be related to its ability to increase creatine levels in the body. Sun [33] found that phosphocreatine could increase the activities of mitochondrial respiratory chain complex enzymes I, II, III, IV, and V to varying degrees in lipopolysaccharide-induced human venous cells. Studies have also shown that phosphocreatine can promote ATP generation in mitochondria and maintain normal energy production and transport [15,34-36].

Guanidine acetic acid forms creatine with S-adenosylmethionine (SAM) in the liver, and creatine reacts with ATP under the catalysis of CK to generate phosphocreatine and adenosine diphosphate (ADP). In this experiment, dietary supplementation with guanidine acetic acid increased the activities of ATP-producing enzymes PK, IDH, MDH, NADH-CoQ, and ATPase by 2.53, 3.12, 1.51, 4.92, and 1.83 times, respectively, and increased the activities of ATP-consuming enzymes PFK and CK by 2.52 and 2.02 times, respectively. The relative amount of ATP synthesis far exceeded ATP consumption; therefore, dietary supplementation with guanidine acetic acid can increase body ATP levels.

Conclusion

Dietary supplementation with 600 mg/kg guanidine acetic acid can improve the growth performance and antioxidant capacity of nursery pigs, and can promote catabolism in vivo by increasing the activities of plasma PFK, PK, IDH, MDH, NADH-CoQ, and ATPase, thereby increasing body ATP levels and potentially promoting the synthesis of energy storage tissues (muscle and fat).

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Note: Figure translations are in progress. See original paper for figures.