

## Effects of Guanidinoacetic Acid on Growth Performance, Meat Quality and Antioxidation in Growing-Finishing Pigs

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**Abstract:** An experiment was conducted to evaluate the effects of Guanidinoacetic Acid (GAA) on growth performance, meat quality and antioxidation in growing-finishing pigs. In total, 144 crossbred pigs with an initial weight at approximately 45 kg were randomly assigned to 4 treatments with 6 pens per treatment and 6 pigs per pen. The pigs were given a basal maize-soybean meal diet supplemented with 0, 0.8, 1.2 or 2.0 GAA g kg<sup>-1</sup>, respectively. The addition of GAA to their diet did not affect the feed to gain ratio ( $p = 0.253$ ), average daily gain ( $p = 0.153$ ) and average daily feed intake ( $p = 0.212$ ) of the pigs. However, increasing the amount of GAA linearly increased the pH ( $p = 0.009$ ) and quadratically reduced the drip loss ( $p = 0.018$ ), shear force ( $p = 0.030$ ) and b\* value ( $p < 0.001$ ) of the pigs. A higher dosage of GAA quadratically increased the activity of Glutathione Peroxidase (GPx,  $p = 0.012$ ), Catalase (CAT,  $p = 0.005$ ) and Total Antioxidant Capability (TAOC,  $p = 0.035$ ) and quadratically decreased the concentration of Malondialdehyde (MDA,  $p < 0.001$ ) in pig plasma. Raising the dosage of supplemental GAA even further quadratically increased the activity of Superoxide Dismutase (SOD,  $p = 0.020$ ), CAT ( $p = 0.026$ ) and TAOC ( $p = 0.043$ ) and quadratically decreased MDA ( $p < 0.001$ ) in the pig muscle tissue. This indicates that dietary GAA does not improve growth performance in pigs but increases the pH value and reduces the drip loss and yellow colour of the meat by decreasing lipid peroxidation and increasing the activities of some enzymes associated with free radical metabolism in growing-finishing pigs.

**Key words:** Guanidinoacetic acid, growth performance of pigs, meat quality, antioxidation, pig, China

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### INTRODUCTION

Guanidinoacetic Acid (GAA) is a metabolic intermediary product in animal tissues that plays a major role in energy metabolism as a precursor of creatine. Guanidinoacetic acid is formed mainly in kidney and pancreas from the Amino Acids (AAs) arginine and glycine. The enzyme L-arginine: glycine amidinotransferase catalyzes the transfer of a guanidino group from arginine and glycine to form ornithine and guanidinoacetic acid (Guthmiller *et al.*, 1994; Walker, 1979). Guanidinoacetic acid is then transferred to the liver where it receives the methyl group from S-adenosyl methionine. This step is catalyzed by guanidinoacetate methyltransferase and results in the formation of creatine (Daly, 1985; Komoto *et al.*, 2003). Creatine is an AA derivative occurring predominantly in skeletal muscle (Balsom *et al.*, 1994) whose main function is the maintenance of cellular Adenosine Triphosphate (ATP) homeostasis (Harris *et al.*, 1992). It is often used by athletes as a dietary supplement to maintain increased levels of intracellular ATP (Toler, 1997; Williams and Branch, 1998). Greenhaff (1996) showed that supplementation with Creatine monohydrate (CRE) to

humans at a rate of 20 g day<sup>-1</sup> for 5 days increased intramuscular creatine by 20%. In finishing pigs, CRE supplementing in finishing pigs diets as a means of improving growth performance and meat quality (Berg and Allee, 2001; Stahl *et al.*, 2001; Maddock *et al.*, 2002; Young *et al.*, 2005) because CRE monohydrate provides ATP for muscle contraction and metabolism. Increasing the amount of available energy for ATP production that does not involve glycolysis or the production of lactic acid may improve pork quality by slowing pH decline immediately post-mortem.

Reactive Oxygen Species (ROS) are a family of oxygen derivatives that include the superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide. Excessive ROS *in vivo* may oxidize lipids, proteins, DNA or carbohydrates and cause a variety of impairments to tissue (Zhao and Shen, 2005). The GAA precursor arginine is a substrate for the nitric oxide synthase family and can increase the production of nitric oxide, a free radical that modulates metabolism, contractility and glucose uptake in skeletal muscle (Lawler and Powers, 1998; Reid, 2001). Additional data indicate that arginine may be able to quench free radicals such as superoxide

anions (O<sub>2</sub><sup>-</sup>) (Wu and Meininger, 2000). Matthews *et al.* (1998) proposed that creatine supplementation provides direct or indirect antioxidant protection against metabolic damage from Huntington's disease. Given that oxidants such as free radicals can affect muscle fatigue, protein turnover, atrophy and growth (Reid, 2001) determining whether GAA has the ability to improve antioxidant capacity is of some importance.

The experiment was carried out to test whether dietary supplementation of all vegetable pigs diets with guanidinoacetic acid improves growth performance, meat quality and antioxidation in growing-finishing pigs.

## MATERIALS AND METHODS

**Animals and experimental design:** In total, 144 (Landrace x Yorkshire x Duroc) pigs with an initial Body Weight (BW) of approximately 45 kg was used. The pigs were randomly allocated into 4 treatments with 6 pens per treatment and 6 pigs per pen. The barn was mechanically ventilated to provide an ambient temperature of 18°C. Each pen was equipped with a feeder and a nipple drinker and the pigs had free access to feeds and water all the experimental period. The experimental period lasted 54 days. The proximate composition of basal diet is shown in Table 1. The basal diet formulated to meet the requirements recommended of the NRC (1998) supplemented with 0, 0.8, 1.2 or 2.0 GAA g kg<sup>-1</sup>, respectively. BW and feed intake were measured at the

end of experiment to calculate Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed to Gain Ratio (FCR) basis on per pen.

**Sampling:** At the end of the experiment, 6 pigs from each treatment (1 pig/pen) were slaughtered under general anaesthesia. Pigs were individually slapped with an identification tattoo to enable carcass identification at the factory. Blood was collected (5 mL) by vena auricularis posterior puncture using a 10 mL anticoagulant free Vacutainer tube, centrifuged at 3,000×g for 15 min to obtain the serum and stored at -20°C until analysis. Samples of Longissimus Muscle (LM) were collected from the left side of carcass and immediately stored at -80°C for measuring the activities of Superoxide Dismutase (SOD), Glutathione Peroxidase (Gpx), Total Antioxidant Capability (TAOC), Catalase (CAT) and the contents of Malondialdehyde (MDA). A section of 300±25 g of LM was excised at the level of the last rib and stored in individual plastic bags and frozen at -20°C for analysis of meat quality.

**Analysis of antioxidant enzymes and MDA:** About 40 mg of frozen LM tissues in 4 mL of homogenization buffer (0.05 M Tris-HCL, pH 7.4, 1 mM EDTA, 0.25 M sucrose) was homogenized on ice with an Ultra-Turrax homogenizer (T8, IKA-Labortechnik, Staufen, Germany) for 5 sec at 13,500×g. The homogenate was centrifuged at 3,000×g for 10 min. The TAOC was measured by the method of ferric reducing antioxidant power assay (Benzie and Strain, 1996) and detected at 520 nm with the spectrophotometer. The SOD was measured by the inhibition of cytochrome c reduction mediated via superoxide anions generated by xanthine oxidase and monitored at 550 nm as described by Flohe and Otting (1984). Activity of GPx was detected with 5, 5-dithiobis-p-nitrobenzoic acid and the change of absorbance at 412 nm was monitored using a spectrophotometer as described by Hafeman *et al.* (1974). The activity of CAT was determined according to the routine colorimetric method (Sinha, 1972). The MDA level was analyzed with 2-thiobarbituric acid, monitoring the change of absorbance at 532 nm with the spectrophotometer (Placer *et al.*, 1996). Enzyme activity was expressed as units per mg of protein for tissues and units per mL for plasma. The assays were conducted using the assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China) and the instructions of the kits were followed accordingly.

**Analysis of meat quality traits:** The pH value of the LM was measured using a pH meter by inserting the probe directly into the samples (HI 8424, Beijing Hanna Instruments Science and Technology Co., Ltd., Beijing,

Table 1: Ingredient composition and nutrient content of experimental diets

Ingredients	Composition (g kg <sup>-1</sup> )
Maize	631.0
Soybean meal, 43% CP	180.0
Rice bran	120.0
Wheat bran	30.0
Limestone	10.0
Soybean oil	8.0
Dicalcium phosphate	8.0
Vitamin and mineral premixa	5.0
Salt	3.0
L-Lysine	3.0
Choline Chloride (500 g kg <sup>-1</sup> )	2.0
<b>Nutrient composition<sup>b</sup></b>	
CP	150.0
Metabolizable energy (MJ kg <sup>-1</sup> )	13.2
Ash	62.8
Calcium	6.3
Phosphorus available	2.6
Lysine	9.1
Methionine	3.2
Tryptophan	2.4
threonine	4.9

<sup>a</sup>The vitamin premix provided the following per kg of complete diet: vitamin A, 7500 IU; vitamin D, 2500 IU; vitamin E, 35 IU; vitamin K<sub>3</sub>, 2.0 mg; vitamin B<sub>1</sub>, 2.0 mg; vitamin B<sub>6</sub>, 4.0 mg; vitamin B<sub>2</sub>, 2.0 mg; vitamin B<sub>12</sub>, 35 µg; pantothenic acid, 18 mg; niacin, 25 mg; Zn, 90 mg; Fe, 110 mg; Cu, 50 mg; Mn, 35 mg; I, 0.4 mg; Se, 0.25 mg; <sup>b</sup>Calculated values

China). Instrumental colour measurements were recorded for L\* (lightness; 0: black, 100: white) a\* (redness/greenness; positive values: red, negative values: green) and b\* (yellowness/blueness; positive values: yellow, negative values: blue) using a Minolta Chroma Meter  $\alpha$  (Japan). The drip loss of loin samples were determined by the procedure of Kauffman *et al.* (1986). Duplicate loin samples were trimmed to 3×3×3 cm size, blotted to remove the surface water and the initial loin weight was determined. Samples were vertically hung in the freezer at 4°C for 24 h before the final loin weight was determined. Percentage of drip loss was calculated by 100×(initial loin weight-final loin weight)/initial weight (James *et al.*, 2002). Shear force was determined (internal temperature 75°C) using a Warner-Bratzler apparatus (G.R. Electric manufacturing Co., Manhattan, KS). Samples, 1.0 cm (width×0.5 cm thickness×2.5 cm length) parallel to the muscle fiber were prepared from the LM and sheared vertically (Molette *et al.*, 2003). Peak force values in Newtons were recorded.

**Statistical analysis:** Data analysis was performed using the General Linear Model (GLM) procedure of the SPSS Software (Version 17.0 for windows). Linear or quadratic relationships were used to determine effects of increasing guanidinoacetic acid levels on growth performance, meat quality and the antioxidation in pigs. The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

- Y<sub>ij</sub> = Dependent variables
- μ = Represents the general mean
- T<sub>i</sub> = Stands for the effect of the i<sup>th</sup> treatment
- e<sub>ij</sub> = Error effect

The pens were considered as the experimental unit.

## RESULTS AND DISCUSSION

**Growth performance:** The addition of GAA to the diet did not affected the average daily gain (p = 0.675), average daily feed intake (p = 0.396) and the feed to gain ratio (p = 0.501) (Table 2).

Table 2: Effects of Guanidinoacetic Acid (GAA) on performance of growing-finishing pigs

Items <sup>c</sup>	GAA (g kg <sup>-1</sup> )				SEM <sup>a</sup>	p-value <sup>b</sup>	
	0	0.8	1.2	2.0		L	Q
ADG (g)	807.00	849.00	827.00	825.00	8.724	0.675	0.253
ADFI (kg)	2.44	2.38	2.34	2.39	0.025	0.396	0.298
FGR	3.03	2.86	2.88	2.89	0.058	0.501	0.279

<sup>a</sup>Standard error of the mean (n = 6 per treatment), <sup>b</sup>Probability levels of the Linear (L) and Quadratic (Q) effects of increasing guanidinoacetic acid level; <sup>c</sup>ADG = Average Daily Gain; ADFI = Average Daily Feed Intake; FGR = Feed to Gain Ratio

**Meat quality:** Increasing addition of GAA to the diet linearly increased (p = 0.009) pH. As the level of supplemental GAA increased it quadratically reduced drip loss (p = 0.018), shear force (p = 0.030) and b\* value (p<0.001). Whereas L\* value and a\* values were not different among the diets (Table 3).

**Lipid peroxidation and antioxidant enzyme activities:**

Table 4 shows the activities of SOD, CAT, GPx, TAOC and the content of MDA in plasma and LM. Increasing the addition of GAA quadratically increased the activity of GPx (p = 0.012), CAT (p = 0.005) and TAOC (p = 0.035) in plasma. The concentration of MDA quadratically decreased (p<0.001) whereas different concentrations of GAA supplementation did not influence the activity of SOD in plasma. In muscle tissue, as the level of supplemental GAA increased quadratically increased the activity of SOD (p = 0.020), CAT (p = 0.026) and TAOC (p = 0.043). Increasing the addition of GAA quadratically increased (p<0.001) the activity of GPx and decreased MDA quadratically (p<0.001).

Previous research conducted by Berg and Allee (2001), Berg *et al.* (2003), James *et al.* (2002), Stahl *et al.* (2001) and Stahl and Berg (2003) suggested that where no significant weight gain was obtained with 20-25 g day<sup>-1</sup> of GRE supplementation for 5-15 days. Creatine was suggested to be an intracellular signal coupling increased muscle activity and increased muscle growth (Ingwall, 1976). Guanidinoacetic acid is the only immediate precursor for creatine in the body, dietary supplementation with GAA at 0.8, 1.2 or 2.0 g kg<sup>-1</sup> to growing-finishing pigs for 54 days did not affected on average daily gain, average daily feed intake and the FCR. However, another study found increased weight gain after 5 days of 25 g GRE supplementation per day (Maddock *et al.*, 2002). This would subsequently explain the lack of improvement in the growth performance of pigs fed with GAA within the study.

Table 3: Effects of Guanidinoacetic Acid (GAA) on meat quality of growing-finishing pigs

Items <sup>c</sup>	GAA (g kg <sup>-1</sup> )					p-value <sup>b</sup>	
	0	0.8	1.2	2.0	SEM <sup>a</sup>	L	Q
pH	5.78	6.22	5.810	5.99	0.056	0.0090	0.097
L*	48.04	47.14	47.320	47.48	0.208	0.4480	0.249
a*	7.52	7.95	7.540	7.23	0.121	0.2240	0.128
b*	4.09	3.38	2.980	3.81	0.136	0.0450	<0.001
Drip loss (%)	2.42	1.48	1.79	2.20	0.001	0.7270	0.018
Shear force (N)	52.67	45.46	46.650	49.31	1.146	0.3420	0.030

<sup>a</sup>Standard error of the mean (n = 6 per treatment), <sup>b</sup>Probability levels of the Linear (L) and Quadratic (Q) effects of increasing guanidinoacetic acid level; <sup>c</sup>L\*, Lightness; a\*, Redness; b\*, Yellowness

Table 4: Effects of Guanidinoacetic Acid (GAA) on Malondialdehyde (MDA) and antioxidants of plasma and longissimus muscle in pigs

Items <sup>a</sup>	GAA (g kg <sup>-1</sup> )				SEM <sup>a</sup>	p-value <sup>b</sup>	
	0	0.8	1.2	2.0		L	Q
<b>Plasma</b>							
SOD (U mL <sup>-1</sup> )	42.28	43.88	43.51	42.62	0.838	0.938	0.512
CAT (U mL <sup>-1</sup> )	2.80	3.23	3.03	2.99	0.047	0.285	0.005
MDA (nmol mL <sup>-1</sup> )	4.93	3.87	4.15	4.67	0.109	0.449	<0.001
T-AOC (U mL <sup>-1</sup> )	5.15	5.72	5.87	5.62	0.105	0.070	0.035
GPx (U mL <sup>-1</sup> )	868.93	1122.30	1042.72	1011.23	30.582	0.145	0.012
<b>Longissimus muscle</b>							
SOD (U mg <sup>-1</sup> protein)	75.52	86.23	85.78	77.17	2.048	0.795	0.020
CAT (U mg <sup>-1</sup> protein)	12.14	14.47	14.35	13.13	0.398	0.394	0.026
MDA (nmol mg <sup>-1</sup> protein)	5.93	4.15	4.64	6.08	0.212	0.436	<0.001
T-AOC (U mg <sup>-1</sup> protein)	11.28	12.14	12.72	11.13	0.298	0.956	0.043
GPx (U mg <sup>-1</sup> protein)	405.26	516.50	496.66	420.06	13.197	0.771	<0.001

<sup>a</sup>Standard error of the mean (n = 6 per treatment); <sup>b</sup>Probability levels of the Linear (L) and Quadratic (Q) effects of increasing guanidinoacetic acid level; <sup>c</sup>SOD = Superoxide Dismutase; TAOC = Total Antioxidant Capability; GPx = Glutathione Peroxidase; MDA = Malondialdehyde; CAT = Catalase

After slaughter when muscle is converted to meat, the oxygen supply to the muscle tissue is depleted and the energy requirements are fulfilled through anaerobic processes. The increased creatine phosphate (CrP+ADP⇌ATP) in the muscle may delay the conversion of glycogen to lactate H<sup>+</sup> formation and consequently postpone the pH decline post-mortem. The higher loin pH would lead to reduced protein denaturation and consequently reduced drip loss (Henckel *et al.*, 2002; Huff-Lonergan and Lonergan, 2005). In the present study, the supplementation with GAA delayed the pH decline and decreased drip loss or shear force in the early postmortem period. The colour of meat is influenced by the pigment content, the myoglobin forms and the internal reflectance of the meat (Lindahl *et al.*, 2001) and in newly cut fresh meat the ability to bloom, oxygenate deoxymyoglobin (Mb) to oxymyoglobin (MbO<sub>2</sub>) is critical. In the present study showed that lower b\* values may due to enhanced protein denaturation, increasing the reflectance and reducing the activity of the oxygen consuming enzymes at high pH values.

To deal with the excess free radicals produced during oxidative stress, mammals have developed sophisticated mechanisms. Maintenance of redox homeostasis involves many antioxidant enzymes what must be precisely coordinated. GPx, SOD and CAT play important roles in protecting tissues from ROS reactions (Krajcovicova *et al.*, 2003; Mishra and Delivoria-Papadopoulos, 1988; Sohal *et al.*, 1990). CAT and GPx convert H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and the SOD catalyzes the dismutation of the superoxide anion and prevents the subsequent formation of hydroxyl radicals (Imlay and Linn, 1988). This study demonstrated that the addition of GAA improved the ability to detoxify H<sub>2</sub>O<sub>2</sub> and the superoxide anion in both plasma and meat. TAOC used to reflect the total capacity of antioxidant systems in the

body in recent years is an integrative index. GAA is synthesised from arginine and glycine. Arginine is a substrate of the nitric oxide synthase family and can increase the production of nitric oxide a free radical that modulates metabolism, contractility and glucose uptake in skeletal muscle (Lawler and Powers, 1998; Reid, 2001). Wu and Meininger (2000) indicated that arginine may be able to quench free radicals such as superoxide anions. Matthews *et al.* (1998) proposed that creatine supplementation provides direct or indirect antioxidant protection against metabolic damage with Huntington's disease. The results indicated that GAA improved the antioxidative status in growing-finishing by elevating TAOC and the activities of antioxidant enzymes.

Lipid peroxidation is a natural phenomenon involved in peroxidative loss of unsaturated lipids thus bringing about lipid degradation and membrane disordering. MDA is a terminal product of lipid peroxidation so, the content of MDA can be used to estimate the extent of lipid peroxidation. The volume of drip loss is related to the lipid peroxides contents in the muscle (Macit *et al.*, 2003). In the present study with the increased pH value, the significantly elevated activity of GPx and decreased MDA in GAA groups all might contributed to the significantly decreased drip loss in the loin muscle when the exposed time was extended.

### CONCLUSION

In this study the antioxidative status of pigs, determined as T-AOC, antioxidative enzymes and the oxidative stability of lipids increased with the supplementation of GAA to the diet. Increased antioxidative status obtained through GAA supplementation increased meat pH and decreased b\* value, drip loss and shear force.

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