



## Original Research Article

# Dietary guanidinoacetic acid supplementation improves water holding capacity and lowers free amino acid concentration of fresh meat in finishing pigs fed with various dietary protein levels



Lu Wang<sup>a</sup>, Yubo Wang<sup>a</sup>, Doudou Xu<sup>a</sup>, Linjuan He<sup>a</sup>, Xiaoyan Zhu<sup>b</sup>, Jingdong Yin<sup>a,\*</sup>

<sup>a</sup> State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China

<sup>b</sup> College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, 450046, China

## ARTICLE INFO

## Article history:

Received 29 November 2021

Received in revised form

27 May 2022

Accepted 30 June 2022

Available online 14 July 2022

## Keywords:

Guanidinoacetic acid

Dietary crude protein level

Meat quality

Water holding capacity

Amino acid composition

Pork

## ABSTRACT

The current study was carried out to detect the effect of dietary guanidinoacetic acid (GAA) supplementation on carcass characteristics and meat quality in finishing pigs fed different dietary crude protein (CP) levels. Sixty-four barrows with an initial body weight of  $73.05 \pm 2.34$  kg were randomly allocated into 1 of 4 dietary treatments in a  $2$  (100% vs. 125% NRC CP level)  $\times 2$  (0 vs. 300 mg/kg GAA) factorial arrangement ( $n = 7$ ). The feeding trial lasted for 49 d. GAA supplementation significantly reduced drip loss ( $P = 0.01$ ), free water distribution ( $T_{23}$  peak area ratio) ( $P = 0.05$ ) and the concentrations of free alanine, threonine, methionine and isoleucine ( $P < 0.05$ ); but increased total glycine content ( $P = 0.03$ ) in the longissimus dorsi muscle of finishing pigs regardless of the dietary CP levels. Furthermore, primary myogenic cell differentiation system was employed to investigate the influence of inclusion of GAA on free amino acid concentrations in myotubes ( $n = 4$ ) and validate the finding in the animal feeding trial. We found that GAA inclusion in culture medium also decreased intracellular concentrations of free alanine, threonine, methionine, isoleucine, valine and proline in differentiated primary myogenic cells in vitro ( $P < 0.05$ ). Meanwhile, relative to diets with 100% NRC CP level, the intake of diets with 125% NRC CP level improved sarcoplasmic protein solubility, increased the contents of carnosine and total free amino acids as well as flavor amino acids in the longissimus dorsi muscle and decreased backfat thickness at the 6–7th ribs in pigs ( $P < 0.05$ ). In addition, we observed that the impact of dietary GAA supplementation on the last rib fat thickness, shear force, and free lysine content in the longissimus dorsi muscle was dependent on dietary CP levels ( $P < 0.05$ ). Collectively, dietary GAA supplementation can reduce drip loss, decrease the concentrations of free amino acids and flavor amino acids of fresh meat independent of dietary CP levels.

© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Creatine exerts an essential role in energy metabolism in the skeletal muscle (Brosnan et al., 2009). As a natural precursor of

creatine, guanidinoacetic acid (GAA) is mainly produced in the kidney and transferred to the liver via blood circulation for conversion to creatine, catalyzed by arginine: glycine amidinotransferase and guanidinoacetate N-methyltransferase in turn (Michiels et al., 2012; Liu et al., 2015). Dietary supplementation of GAA at 300 mg/kg has been recommended for grower-finishing pigs to obtain optimal Gain: Feed (He et al., 2018). It was hypothesized that dietary supplementation of GAA could increase serum creatine and improve metabolic utilization of arginine (Baker, 2009; McBreaity et al., 2015). GAA supplementation raises creatine and phosphocreatine content in skeletal muscle (Ostojic et al., 2016; J.L. Li et al., 2018), and promotes cellular bioenergetic efficiency in skeletal muscle (McBreaity et al., 2015). Muscular

\* Corresponding author.

E-mail address: [yinjd@cau.edu.cn](mailto:yinjd@cau.edu.cn) (J. Yin).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



bioenergetic efficiency is supposed to be associated with the meat quality of finishing pigs (Wang et al., 2012; Liu et al., 2015; J.L. Li et al., 2018). Therefore, GAA may be implemented as a dietary additive to improve skeletal muscle development and health as well as meat quality in farm animals.

Regarding meat quality, water holding capacity (WHC), represented conventionally by drip loss, is a vital quality attribute. Nuclear magnetic resonance (NMR) relaxation measurement is considered as a useful approach in detecting water mobility and distribution inside muscle according to the information of NMR transverse relaxation ( $T_2$ ) of water (Bertram and Aaslyng, 2007). Moreover, protein solubility—especially sarcoplasmic protein solubility (SPS)—representing the degree of protein denaturation, is supposed to be closely related to WHC of muscle (Joo et al., 1999). Additionally, muscular free amino acid content is also of great interest in recent years, because it is an important index for nutritional value and flavor of meat (Brzostowski and Tański, 2006). Furthermore, myofiber type composition is also responsible for meat quality owing to the different activities of myosin adenosine triphosphatase (mATPase), glycolytic enzymes and corresponding contents of glycogen and lipids (Joo et al., 2013). According to the isoforms of myosin heavy chain (MyHC), muscle fibers are classified into 4 types, including: MyHC-I, MyHC-IIa, MyHC-IIb and MyHC-IIx. It is generally considered that the higher proportion of MyHC-IIb myofiber is negatively correlated with WHC of meat (Choe et al., 2008).

In commercial pig farms, the crude protein level of diets of growing-finishing pigs are generally higher than the recommendation by the NRC (2012) (Y.M. Wang et al., 2018). Dietary crude protein (CP) level has a limited effect on meat quality of finishing pigs as long as dietary standard ileal digestible essential amino acid (SID EAA) meet the requirements of pigs (Qin et al., 2015). However, endogenous GAA synthesis may be limited by the abundance of methyl donors within bodies (McBreairty et al., 2015). Whether dietary CP levels influence GAA function in the skeletal muscle remains obscure. Therefore, it is worth exploring the effect of GAA supplementation on meat quality in finishing pigs fed various dietary CP levels. In the present study, we investigated the interaction of dietary CP level and GAA supplementation on carcass characteristics, meat quality and free amino acid content of fresh meat in finishing pigs.

## 2. Materials and methods

### 2.1. Animal ethics statement

All procedures conducted in the present study were approved by the Institutional Animal Care and Use Committee of China Agriculture University and carried out in accordance with the Chinese Guidelines for Animal Welfare and Experimental Protocol (ID: SKLAB-B-2010-003).

### 2.2. Experiment design and sample collection

A 2 × 2 factorial feeding trial was arranged with or without GAA supplementation (300 mg/kg) at 2 levels of dietary CP (100% vs. 125% NRC CP), constituting 4 dietary treatments. A total of 64 Duroc × Landrace × Large White crossbred barrows, with the initial body weight (BW) of 73.05 ± 2.34 kg, were allocated randomly by BW and litter into one of 4 dietary treatments within a complete randomized block design. Pigs were fed with 2-phase experimental diets as per NRC (2012) recommendations, including Phase 1 (75 to 100 kg) for 30 d and Phase 2 (100 kg to slaughter) for 19 d before slaughtering. Pigs were housed in an environmentally controlled room in FengNing Swine Research Unit of China Agricultural

University (Academician Workstation in Chengdejiuyun Agricultural and Livestock Co., Ltd., Fengning County, Hebei Province, China). Pigs were fed ad libitum, had free access to water and were weighed individually at the beginning and the end of the trial. Feed intake per pen ( $n = 4$ ) was recorded weekly throughout the entire period of the experiment. The GAA used in the present study was provided by Gendone Agriculture Technology Co., Ltd. (Beijing, China) with a purity of more than 98.0%. Ingredient composition and nutrient content of experimental diets are presented in Table 1.

At the end of the trial, after overnight fasting, 7 pigs close to the average BW of a pen from each treatment were selected and humanely slaughtered by electrical stunning, exsanguinated and eviscerated according to the standard commercial procedure. Subsequently, each carcass was split down the center of the vertebral column. Samples of the longissimus dorsi muscle between the 10th and 12th ribs were rapidly separated from the left side carcass and kept at −80 °C until for further analysis.

### 2.3. Carcass characteristics and meat quality

All the parameters were measured on the left side of each carcass. Backfat thickness was measured at the thickest shoulder, the 6th to 7th rib, 10th rib, last rib and last lumbar vertebra. The loin eye area was calculated by a formula of loin eye area (cm<sup>2</sup>) = loin eye height (cm) × width (cm) × 0.7.

Muscle color, including L\* (lightness), a\* (redness), and b\* (yellowness), was measured at both 45 min and 24 h postmortem according to the standard method of CIE lab system by a Minolta

**Table 1**  
Ingredients and nutrient composition of the basal diets provided for finishing pigs (as fed basis, %).<sup>1</sup>

Item	75 to 100 kg BW		100 to 130 kg BW	
	100% CP	125% CP	100% CP	125% CP
<b>Ingredients</b>				
Corn	76.68	70.02	85.50	77.16
Soybean meal	8.28	16.74	4.50	11.26
Wheat bran	9.50	8.35	5.00	7.00
Soybean oil	2.11	1.53	1.69	1.38
Limestone	0.94	0.92	0.88	0.82
Calcium hydrogen phosphate	0.60	0.50	0.58	0.50
Salt	0.35	0.35	0.35	0.35
L-Lysine HCl	0.46	0.46	0.44	0.44
L-Threonine	0.16	0.19	0.15	0.18
L-Tryptophan	0.04	0.04	0.06	0.04
L-Valine	0.10	0.10	0.10	0.10
DL-Methionine	0.09	0.12	0.06	0.09
L-Isoleucine	0.02	0.01	0.02	0.01
Sweetener	0.08	0.08	0.08	0.08
Phytase	0.01	0.01	0.01	0.01
Choline chloride (50%)	0.08	0.08	0.08	0.08
Premix <sup>2</sup>	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
<b>Calculated nutrient levels</b>				
Metabolizable energy, MJ/kg	13.79	13.79	13.79	13.79
Crude protein	12.20	15.20	10.49	13.11
SID Lysine	0.75	0.94	0.63	0.80
SID Methionine + Cysteine	0.43	0.54	0.36	0.46
SID Threonine	0.46	0.59	0.41	0.52
SID Tryptophan	0.14	0.18	0.13	0.15
SID Valine	0.49	0.61	0.43	0.53
SID Isoleucine	0.40	0.52	0.34	0.43

SID = standardized ileal digestibility.

<sup>1</sup> 100% CP, 100% NRC crude protein level; 125% CP, 125% NRC crude protein level.  
<sup>2</sup> The premix provided the following per kilogram of diets: vitamin A, 6,000 IU; vitamin D<sub>3</sub>, 2,400 IU; vitamin E, 20 IU; vitamin K<sub>3</sub>, 2 mg; vitamin B<sub>1</sub>, 0.96 mg; vitamin B<sub>2</sub>, 4 mg; vitamin B<sub>6</sub>, 2 mg; vitamin B<sub>12</sub>, 0.012 mg; biotin, 0.04 mg; folic acid 0.40 mg; pantothenic acid 11.2 mg; nicotinic acid 22 mg; Cu, 120 mg; Fe, 76 mg; Mn, 12 mg; Zn, 76 mg; I, 0.24 mg; Se, 0.40 mg.

chromameter (CR-410, Konica minota, Tokyo, Japan). Substituting  $a^*$  and  $b^*$  into the formula to calculate saturation ( $C^*$ ) and hue ( $h^\circ$ ) ( $C^* = \sqrt{a^{*2} + b^{*2}}$ , Hue =  $\arctan(b^*/a^*)$ ) (Bekhit et al., 2001).

Values of  $\text{pH}_{45 \text{ min}}$  and  $\text{pH}_{24 \text{ h}}$  of the longissimus dorsi muscle were measured with a glass penetration pH electrode (pH-star, Matthäus, Germany). Each chop was measured 3 times in different areas and the average value was obtained. Drip loss was determined as described by a previous study (Xu et al., 2020). Briefly, meat samples were weighed, suspended in a plastic bag at 4 °C for 24 h. Drip loss was calculated by  $100 \times (\text{initial weight} - \text{final weight of meat sample})/\text{initial weight}$ . Cooking loss was determined as described previously (Luo et al., 2018). Briefly, meat was weighted and cooked in a water bath at 70 °C for 20 min. Then, meat samples were cooled to room temperature and reweighed to calculate cooking loss. Meat shear force was measured according to Fortin et al. (2005). Ten cylindrical samples (1.0 cm in diameter) parallel to the muscle fiber direction were obtained from the cooked samples. Peak shear force was measured using a digital display muscle tenderness meter (C-LM3B, Tenovo, Harbin, China).

#### 2.4. Protein solubility

The protein solubility was measured according to the previous study (Joo et al., 1999). In brief, a frozen muscle sample (1 g) was homogenized in 20 mL ice-cold 0.1 mol/L potassium phosphate buffer containing 1.1 mol/L potassium iodide (pH 7.2). After overnight extraction on a shaker at 4 °C, the homogenates were centrifuged at  $1,500 \times g$  at 4 °C for 20 min. Then, protein concentration of the supernatant was determined by bicinchoninic acid method to calculate total protein solubility (TPS). Similarly, a frozen muscle sample (1 g) was homogenized in 10 mL ice-cold 0.025 mol/L potassium phosphate buffer (pH 7.2), and extracted, homogenized and centrifuged to determine SPS. Myofibrillar protein solubility (MPS) was calculated from the difference between TPS and SPS.

#### 2.5. Nuclear magnetic resonance transverse relaxation ( $T_2$ ) measurement

Low field NMR relaxation was measured as described previously (Bertram and Aaslyng, 2007; X. Li et al., 2018). A frozen muscle sample (3 g) was placed in the center of the radio frequency coil of the permanent magnetic field of the nuclear magnetic resonance imager for magnetic resonance spectroscopy measurement. The transverse relaxation time  $T_2$  was measured by Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence in NMR software. The  $T_2$  measurements were performed with a  $\tau$ -value (time between 90° pulse and 180° pulse) of 230  $\mu\text{s}$  using a repetition delay of 4 s. Data from 3,000 echoes were acquired as 4 scan repetitions.

#### 2.6. Intramuscular fat content

Intramuscular fat (IMF) content in the longissimus dorsi muscle was measured by Soxhlet petroleum-ether extraction (Budwi Extraction System B-11; Budwi, Lausanne, Switzerland) as previously described (Zhang et al., 2016).

#### 2.7. Muscle amino acid composition

Muscular amino acid profiles were determined by High Performance Liquid Chromatography with an Automatic Amino Acid Analyzer (L-8800 Amino Acids Analyzer, Hitachi, Tokyo, Japan) as previously described (Qin et al., 2015).

#### 2.8. Cell culture and myogenic induction

To validate the effect of GAA supplementation on muscular free amino acid concentrations of fresh meat, we performed an in vitro study using primary myogenic precursor cells. Porcine primary myogenic precursor cells from our lab (Sun et al., 2017; Qiu et al., 2018) were cultured in growth medium (DMEM/F12 medium supplemented with 10% FBS [Gibco-BRL, Carlsbad, CA, USA], 2 mmol/L glutamine, 100 U/mL penicillin, 100  $\mu\text{g}/\text{mL}$  streptomycin and 5 ng/mL basic fibroblast growth factor [PepTech, Burlington, MA, USA]) on collagen I-coated dishes (Sigma–Aldrich) in an incubator at 37 °C, 5%  $\text{CO}_2$ . Cells at 80% confluence were switched to myogenic differentiation medium contained 2% heat-inactivated horse serum in DMEM for 4-days. Combining the physiological dose of GAA in serum (12.5 mmol/L) and the influence of GAA inclusion on cell viability, we selected 20 mmol/L as GAA dose in following in vitro study (Y.J. Wang et al., 2018). Subsequently, cells were cultured in myogenic differentiation medium contained GAA for 48 h. As myotubes appeared, cells were collected to measure intracellular concentrations of free amino acids.

#### 2.9. Free amino acid concentrations of fresh meat or differentiated cells

Free amino acid concentrations of fresh meat and differentiated cells were measured by Ultra performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) as described previously (Yin et al., 2016). Briefly, approximately 0.5 g of muscle sample or  $4 \times 10^6$  collected cells added with 8  $\mu\text{L}$  2.5 mmol/L norleucine as internal standard were homogenized and extracted with 5 mL or 1 mL methanol–water solution (8:2, vol:vol), respectively, stayed at 4 °C for 2 h, and centrifuged at  $13,000 \times g$  at 4 °C for 10 min. After spin drying in vacuum at 45 °C, the supernatant dissolved in 100  $\mu\text{L}$  boric acid buffer. Then, 10  $\mu\text{L}$  re-dissolved solution was mixed with 50  $\mu\text{L}$  boric acid buffer and 20  $\mu\text{L}$  derivatizing reagent, and subjected to vortex immediately. Subsequently, the mixture was heated in 55 °C oven for 10 min, and filtered through 0.1  $\mu\text{m}$  filter membrane for UPLC-HRMS determination.

#### 2.10. RNA extraction and real-time quantitative PCR

Total RNA was extracted using HiPure Universal RNA Kit (R4130, Magen, Guangzhou, China). The quality and integrity of RNA samples were assessed by electrophoresis on 1% agarose gel, and the purity of the RNA was estimated by nucleic acid analyzer (Nano-Drop 1000; Thermo Fisher, Waltham, MA, USA). cDNA synthesis was performed using reagent kit (PrimeScript RT reagent Kit with gDNA Eraser, Takara, Tokyo, Japan) according to the manufacturer's instructions. Primers for MyHC-I, MyHC-IIa, MyHC-IIb and MyHC-IIx were listed in Table 2. Real-time PCR was performed in a qTOWER 2.2 thermocycler (Analytik Jena AG, Germany), using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the internal reference gene. The relative mRNA expression was analyzed by the  $2^{-\Delta\Delta\text{CT}}$  method (Pfaffl et al., 2002).

#### 2.11. Statistical analysis

Data are presented means  $\pm$  pooled SEM, and were analyzed using the  $2 \times 2$  factorial generalized linear model (GLM) procedure of Statistical Analysis System software (SAS, Version 9.2; SAS Institute, Cary, USA) followed with Tukey's test. The model included the fixed effect of dietary CP level, GAA supplementation,

**Table 2**  
Information of the primers used for real-time quantitative PCR analysis.

Gene	Primer sequence (5' to 3')	Size, bp	Temperature, °C	Accession no.
<i>MyHC-I</i>	F: CGTGGACTACAACATCATAGGC R: CTTTGCCTTCTCAACAGGT	152	64	NM_213855.2
<i>MyHC-IIa</i>	F: GGAGATCGACGACCTTGCTA R: CTCCTGGATTTCAGCTCGC	112	58	NM_214136.1
<i>MyHC-IIx</i>	F: GAAACCGTCAAGGGTCTACG R: CGCTTCCTCAGCTTGCTCT	153	64	NM_001104951.2
<i>MyHC-IIb</i>	F: GTTCTGAAGAGGGTGGTAC R: AGATGCGGATGCCCTCCA	234	63	NM_001123141.1
<i>GAPDH</i>	F: TCGGAGTGAACGGATTG R: CCTGGAAGATGGTGATGG	219	60	NM_001206359.1

*MyHC* = myosin heavy chain; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

associated 2-way interactions and the random errors with the experimental unit of an individual pig. Differences between treatments were considered statistically significant as  $P < 0.05$ . Pearson's correlation was applied to estimate relationships among drip loss, muscle pH, muscle water distribution forms (NMR Relaxation  $T_2$ ) and postmortem protein solubility.

### 3. Results

#### 3.1. Growth performance, carcass traits and meat quality

The average daily feed intake (ADFI), the average daily gain (ADG) and the feed-to-gain ratio (F/G) were not altered by dietary treatment during the period of the study, and no interaction between dietary CP level and GAA supplementation on growth performance was observed (Table 3). Regarding carcass traits, pigs offered higher CP diets had significantly decreased backfat thickness at the 6th to 7th rib compared with the control ( $P = 0.04$ ), while no changes were observed in backfat thickness at the thickest shoulder, the 10th rib, last rib, and last lumbar vertebra, as well as the loin eye area ( $P > 0.10$ ). Furthermore, GAA supplementation did not alter carcass characteristics ( $P > 0.10$ ). In addition, the significant interaction between dietary CP level and GAA supplementation on the back-fat thickness at the last rib was observed ( $P = 0.02$ ) (Table 3). That is, pigs received GAA supplementation in the diet with 125% NRC CP level had the lowest backfat thickness at last rib among all dietary treatments.

**Table 3**  
Effects of dietary crude protein level and GAA supplementation on growth performance ( $n = 4$ ) and carcass traits in finishing pigs ( $n = 7$ ).<sup>1,2</sup>

Item	CP (100% NRC)		CP (125% NRC)		SEM	P-value		
	0	GAA	0	GAA		CP	GAA	CP × GAA
Growth performance								
ADG, g	805	758	780	790	29.41	0.90	0.56	0.35
ADFI, kg	2.69	2.74	2.92	2.73	0.18	0.54	0.70	0.50
F/G	3.33	3.63	3.74	3.46	0.20	0.56	0.96	0.17
Initial body weight, kg	73.03	73.13	72.98	73.04	2.34	0.98	0.97	0.99
Slaughter weight, kg	114.77	114.93	114.24	114.86	1.35	0.83	0.78	0.87
Subcutaneous backfat depth, mm								
Shoulder fat thickness	36.84	37.67	36.55	36.26	1.68	0.62	0.87	0.74
The 6th to 7th rib fat thickness	27.68	28.86	23.87	25.99	1.57	0.04	0.31	0.77
The 10th rib fat thickness	24.34	26.45	25.60	22.69	1.81	0.50	0.83	0.18
The last rib fat thickness	20.54	23.30	21.68	18.97	1.13	0.17	0.98	0.02
Lumbosacral fat thickness	17.10	20.94	18.03	17.08	1.42	0.31	0.32	0.11
Loin eye area, <sup>3</sup> cm <sup>2</sup>	44.65	49.34	50.42	49.03	2.77	0.37	0.47	0.22

GAA = guanidinoacetic acid; SEM = standard error; ADG = average daily gain; ADFI = average daily feed intake; F/G = feed-to-gain ratio.

<sup>1</sup> At the end of the trial, 7 pigs close to the average body weight of pen from each treatment were selected and humanely slaughtered to measure carcass traits.

<sup>2</sup> Diets were supplemented with GAA at 0 or 300 mg/kg according to the experiment design. Data were shown as the means ± SEM. Differences were considered as statistically significant when  $P < 0.05$ .

<sup>3</sup> Loin eye area was measured at the 10th rib of the right-side carcass, and calculated by the following equation: loin eye area (cm<sup>2</sup>) = loin eye height (cm) × width (cm) × 0.7.

Also, GAA supplementation significantly decreased drip loss of fresh pork compared with the control ( $P = 0.01$ ). Neither dietary CP level nor GAA supplementation impacted pH<sub>45 min</sub> and pH<sub>24 h</sub> values, cooking loss and meat color (L\*, a\*, b\*, C\* and h°) at both 45 min and 24 h postmortem ( $P > 0.10$ ). Except for shear force ( $P = 0.05$ ), there was no interaction between dietary CP level and GAA supplementation on meat quality (Table 4).

#### 3.2. Nuclear magnetic resonance transverse relaxation ( $T_2$ ) and protein solubility

The distribution of transverse relaxation time of muscle moisture among 4 dietary treatments is shown in Fig. 1. Three peaks  $T_{21}$ ,  $T_{22}$  and  $T_{23}$  represent bound water, immobilized water and free water, respectively. Dietary supplementation of GAA significantly decreased the  $T_{23}$  peak area ratio compared with the control ( $P = 0.05$ ), while did not alter  $T_{21}$  and  $T_{22}$  peak area ratio (Table 5). Dietary CP level did not alter water distribution forms in fresh meat, meanwhile, no interaction between dietary CP level and GAA supplementation was observed ( $P > 0.10$ ).

We found that the higher CP diet significantly increased SPS compared with the control ( $P = 0.03$ ), but did not alter the solubility of total protein and myofibrillar protein. In addition, GAA supplementation tended to increase SPS ( $P = 0.09$ ) and decrease MPS ( $P = 0.07$ ). No interaction between dietary CP level and GAA supplementation was found on the sarcoplasmic and myofibrillar protein solubilities of fresh meat ( $P > 0.10$ ).



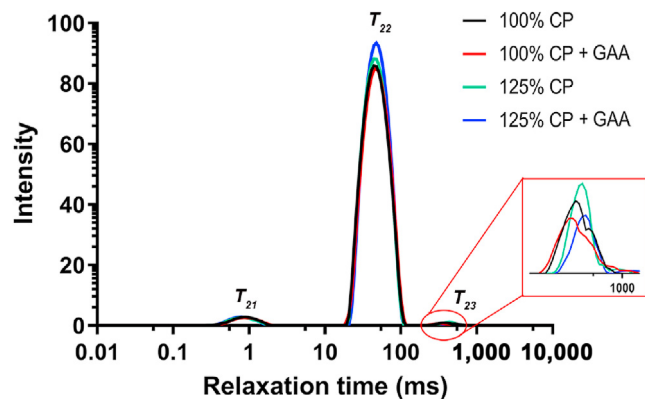
**Table 4**  
Effects of dietary crude protein level and GAA supplementation on the meat quality of the longissimus dorsi muscle in finishing pigs ( $n = 7$ ).<sup>1,2</sup>

Item	CP (100% NRC)		CP (125% NRC)		SEM	P-value		
	0	GAA	0	GAA		CP	GAA	CP × GAA
pH <sub>45 min</sub>	6.00	6.05	5.87	5.85	0.11	0.16	0.92	0.74
pH <sub>24 h</sub>	5.41	5.41	5.45	5.45	0.04	0.28	0.99	0.99
Meat color (45 min)								
L*	49.30	48.36	50.26	49.85	1.53	0.43	0.66	0.86
a*	16.52	16.58	16.27	16.57	0.40	0.75	0.67	0.77
b*	3.67	3.45	3.68	3.33	0.37	0.89	0.47	0.87
C*	16.96	17.06	16.36	16.93	0.40	0.38	0.41	0.57
h°	12.54	11.67	13.04	11.47	1.45	0.91	0.38	0.80
Meat color (24 h)								
L*	55.22	55.35	54.68	53.92	1.05	0.34	0.76	0.67
a*	16.22	16.82	16.55	16.45	0.31	0.94	0.45	0.30
b*	6.48	6.88	6.58	6.26	0.38	0.49	0.94	0.34
C*	17.49	18.12	17.71	17.20	0.47	0.46	0.89	0.24
h°	21.79	22.51	21.45	21.23	1.31	0.52	0.84	0.70
Drip loss, %	4.91	2.91	5.18	3.23	0.75	0.70	0.01	0.98
Cooking loss, %	27.34	27.16	26.36	27.09	1.12	0.61	0.79	0.66
Shear force, N	46.11	35.22	35.58	37.24	3.05	0.18	0.14	0.05
IMF, %	2.29	2.81	3.31	2.44	0.37	0.40	0.64	0.08

GAA = guanidinoacetic acid; SEM = standard error; IMF = intramuscular fat.

<sup>1</sup> GAA was supplemented in diets with or without 300 mg/kg according to the experiment design. Data were shown as the means ± SEM. Differences were considered as significant when  $P < 0.05$ .

<sup>2</sup> L\*, lightness; a\*, redness; b\*, yellowness; C\*, saturation (chroma); h°, hue.



**Fig. 1.** Relaxometry time of low-field nuclear magnetic resonance ( $n = 7$ ). GAA = guanidinoacetic acid.  $T_{21}$ ,  $T_{21}$  peak area ratio;  $T_{22}$ ,  $T_{22}$  peak area ratio;  $T_{23}$ ,  $T_{23}$  peak area ratio.

**Table 5**

Effects of dietary crude protein level and GAA supplementation on the  $T_2$  peak area ratio and 24 h postmortem protein solubility of the longissimus dorsi muscle in finishing pigs ( $n = 7$ ).<sup>1,2</sup>

Item	CP (100% NRC)		CP (125% NRC)		SEM	P-value		
	0	GAA	0	GAA		CP	GAA	CP × GAA
$T_2$ peak area ratio, <sup>2</sup> %								
$T_{21}$	2.81	3.05	2.91	2.96	0.11	0.98	0.18	0.35
$T_{22}$	96.45	96.60	96.40	96.51	0.15	0.65	0.37	0.92
$T_{23}$	0.75	0.35	0.70	0.53	0.13	0.61	0.05	0.39
Protein solubility, mg/g								
Total protein solubility	148.81	137.72	158.73	149.61	9.77	0.17	0.24	0.91
Sarcoplasmic protein solubility	59.90 <sup>b</sup>	76.87 <sup>ab</sup>	80.38 <sup>ab</sup>	86.29 <sup>a</sup>	7.43	0.03	0.09	0.40
Myofibrillar protein solubility	88.92	60.85	78.35	63.33	12.96	0.72	0.07	0.56

GAA = guanidinoacetic acid; SEM = standard error.

<sup>a, b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> GAA was supplemented in diets with or without 300 mg/kg according to the experiment design. Data were shown as the means ± SEM. Differences were considered as significant when  $P < 0.05$ .

<sup>2</sup>  $T_{21}$ ,  $T_{21}$  peak area ratio;  $T_{22}$ ,  $T_{22}$  peak area ratio;  $T_{23}$ ,  $T_{23}$  peak area ratio.

### 3.3. Skeletal muscle amino acid composition

Both dietary CP level and GAA supplementation significantly altered the free amino acid composition of the longissimus dorsi muscle (Table 6). For example, compared with the control, the higher protein diet significantly increased concentrations of carnosine ( $P < 0.01$ ), total free amino acids ( $P < 0.01$ ) and non-essential amino acids (NEAA) ( $P = 0.02$ ), as well as flavor amino acids ( $P = 0.03$ ). Meanwhile, dietary GAA supplementation significantly decreased the concentrations of several free amino acids, including alanine ( $P = 0.01$ ), threonine ( $P = 0.03$ ), isoleucine ( $P = 0.05$ ), methionine ( $P = 0.04$ ) and NEAA ( $P = 0.01$ ), as well as flavor amino acids ( $P = 0.01$ ) in fresh meat compared with the control. Additionally, a significant interaction between dietary CP level and GAA supplementation was observed on the concentration of free lysine ( $P = 0.05$ ) and flavor amino acids ( $P = 0.05$ ) as well as the ratio of free EAA/NEAA ( $P < 0.01$ ) in fresh meat. Pigs in the dietary treatment of GAA supplementation in the diet with 125% NRC CP level had the highest muscular free lysine concentration among four dietary treatments, while pigs in the dietary treatment of GAA supplementation in the diet with 100% NRC CP level had the highest free EAA/NEAA ratio but the lowest flavor amino acid concentrations.

In the present study, dietary CP level did not alter total amino acid content in the longissimus dorsi muscle; whereas, GAA supplementation significantly increased total glycine content ( $P = 0.03$ ). No interaction between dietary CP level and GAA supplementation was observed on total amino acid content ( $P > 0.10$ ) (Table 6).

### 3.4. Free amino acid composition of differentiated myogenic cells in vitro

As shown in Fig. 2, GAA supplementation significantly decreased the intracellular concentrations of free amino acids of differentiated cells, including alanine ( $P = 0.02$ ), valine ( $P = 0.02$ ), threonine ( $P < 0.01$ ), isoleucine ( $P = 0.01$ ), methionine ( $P = 0.02$ ), proline ( $P < 0.01$ ) compared with the control, which validates results of in vivo study.

### 3.5. mRNA expression of myosin heavy-chain isoform genes in the longissimus dorsi muscle

As shown in Fig. 3, an intake of 125% NRC CP diet tended to increase mRNA abundance of *MyHC-IIb* relative to the control ( $P = 0.06$ ), while no changes were observed on the mRNA abundance of *MyHC-IIx*, *MyHC-IIa* and *MyHC-I* ( $P > 0.10$ ). GAA

**Table 6**  
Effects of dietary crude protein level and GAA supplementation on free/total amino acid composition of the longissimus dorsi muscle in finishing pigs ( $n = 7$ ).<sup>1</sup>

Item	CP (100% NRC)		CP (125% NRC)		SEM	P-value		
	0	GAA	0	GAA		CP	GAA	CP × GAA
Free amino acid composition of the longissimus dorsi muscle, nmol/g								
Ala	572.56	438.90	616.00	522.97	40.82	0.13	0.01	0.62
Gly	379.61	315.41	366.03	364.80	24.94	0.48	0.20	0.22
Ser	77.01	67.76	79.91	73.79	5.31	0.41	0.16	0.77
Val	198.68	200.17	218.88	204.25	7.21	0.11	0.37	0.27
Thr	70.97	61.45	70.20	62.71	3.80	0.95	0.03	0.79
Leu	93.80	94.06	115.31	92.82	6.86	0.15	0.12	0.11
Ile	59.65	58.26	72.72	57.61	4.00	0.13	0.05	0.10
Met	17.34	16.01	19.15	15.82	1.05	0.45	0.04	0.35
Phe	68.30	70.36	73.28	68.64	3.84	0.67	0.74	0.39
Lys	62.84	56.25	53.84	64.88	4.31	0.97	0.61	0.05
Arg	102.52	101.70	100.39	97.39	5.83	0.57	0.73	0.84
Tyr	83.72	91.70	87.19	83.93	5.86	0.72	0.69	0.35
Trp	62.43	60.20	63.67	53.95	4.11	0.55	0.16	0.37
Glu	83.84	79.00	102.88	84.38	11.35	0.29	0.31	0.55
Asp	12.31	11.69	11.88	11.14	0.70	0.49	0.34	0.93
Pro	80.30	69.98	84.74	82.91	4.82	0.08	0.22	0.39
His	22.24	23.15	31.21	28.78	4.22	0.10	0.86	0.70
Car	5,885 <sup>b</sup>	6,094 <sup>b</sup>	7,886 <sup>a</sup>	6,998 <sup>ab</sup>	361	<0.01	0.36	0.14
Total	7,672 <sup>b</sup>	7,635 <sup>b</sup>	9,796 <sup>a</sup>	8,720 <sup>ab</sup>	426.46	<0.01	0.20	0.24
EAA <sup>2</sup>	546.88	550.04	608.32	546.82	31.52	0.34	0.34	0.29
NEAA <sup>3</sup>	1239 <sup>ab</sup>	1043 <sup>b</sup>	1301 <sup>a</sup>	1231 <sup>ab</sup>	52.2	0.02	0.01	0.22
EAA/NEAA	0.44 <sup>b</sup>	0.50 <sup>a</sup>	0.49 <sup>ab</sup>	0.47 <sup>ab</sup>	0.01	0.62	0.14	<0.01
Flavor amino acids <sup>4</sup>	1089 <sup>a</sup>	885 <sup>b</sup>	1098 <sup>a</sup>	1074 <sup>a</sup>	39.36	0.02	<0.01	0.03
Total amino acid composition of the longissimus dorsi muscle, % freeze-dried meat								
Ala	3.42	3.43	3.44	3.47	0.09	0.72	0.81	0.88
Gly	3.37	3.46	3.38	3.81	0.11	0.12	0.03	0.14
Ser	3.25	3.28	3.20	3.21	0.11	0.55	0.87	0.95
Val	4.40	4.38	4.27	4.35	0.14	0.55	0.84	0.74
Thr	3.91	3.91	3.81	3.85	0.13	0.56	0.89	0.90
Leu	6.45	6.49	6.41	6.32	0.21	0.61	0.90	0.75
Ile	4.05	4.12	3.95	3.95	0.15	0.39	0.82	0.80
Met	2.65	2.59	2.45	2.45	0.11	0.29	0.43	0.80
Phe	3.36	3.37	3.44	3.39	0.11	0.63	0.82	0.80
Lys	7.50	7.58	7.29	7.29	0.25	0.43	0.94	0.70
Arg	5.17	5.29	5.11	5.16	0.14	0.53	0.57	0.81
Tyr	3.94	3.99	3.98	3.91	0.15	0.91	0.92	0.71
Trp	0.95	0.99	0.94	0.90	0.03	0.15	0.98	0.33
Glu	12.17	12.13	11.76	11.88	0.40	0.42	0.92	0.84
Asp	7.83	7.85	7.58	7.66	0.25	0.40	0.84	0.89
Pro	3.51	3.60	3.73	3.73	0.08	0.17	0.19	0.81
His	3.63	3.64	3.64	3.64	0.13	0.92	0.88	0.97

GAA = guanidinoacetic acid; SEM = standard error; EAA = essential amino acids; NEAA = non-essential amino acids.

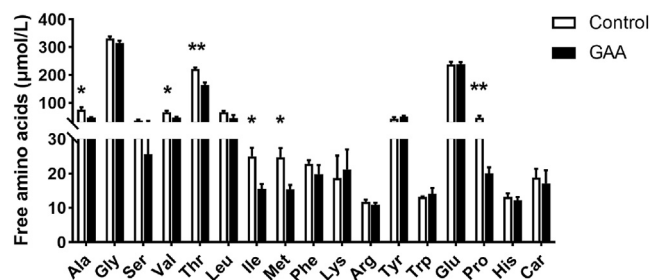
<sup>a, b</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> GAA was supplemented in diets with or without 300 mg/kg according to the experiment design. Data were shown as the means ± SEM. Differences were considered as significant when  $P < 0.05$ .

<sup>2</sup> EAA = Val + Thr + Leu + Ile + Met + Phe + Lys + Arg + Trp + His.

<sup>3</sup> NEAA = Ala + Gly + Ser + Tyr + Pro + Glu.

<sup>4</sup> Flavor amino acids = Ala + Gly + Arg + Glu + Asp.



**Fig. 2.** Effects of guanidinoacetic acid (GAA) supplementation (20 mmol/L) on intracellular free amino acid concentration of the differentiated myogenic cells ( $n = 4$ ). Data were shown as the means ± SEM. Differences were considered as significant when  $P < 0.05$ . \* $P < 0.05$ , \*\* $P < 0.01$ .

supplementation did not affect the mRNA expression of myosin heavy-chain isoforms ( $P > 0.10$ ). There was no interaction between the dietary CP level and GAA supplementation on the

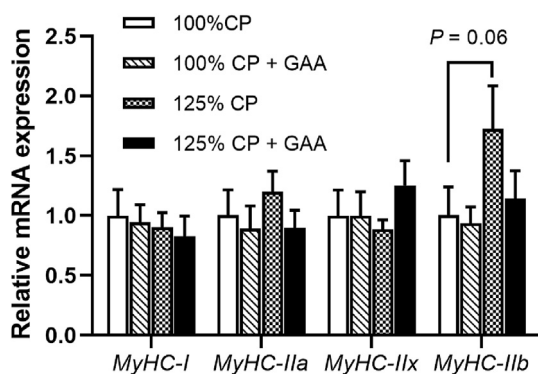
mRNA abundance of MyHC-I, MyHC-IIa, MyHC-IIb and MyHC-IIx ( $P > 0.10$ ).

### 3.6. Relationship among muscle physiological-biochemical traits

As shown in Table 7, drip loss negatively correlated with pH<sub>45 min</sub> value ( $r = -0.54$ ,  $P < 0.01$ ) and pH<sub>24 h</sub> value ( $r = -0.55$ ,  $P = 0.01$ ), while positively correlated with TPS ( $r = 0.43$ ,  $P = 0.02$ ) and MPS ( $r = 0.54$ ,  $P < 0.01$ ). The amount of bound water ( $T_{21}$ ) negatively correlated with immobilized water ( $T_{22}$ ) ( $r = -0.42$ ,  $P = 0.03$ ), and the amount of immobilized water ( $T_{22}$ ) negatively correlated with free water ( $T_{23}$ ) ( $r = -0.73$ ,  $P < 0.01$ ). Additionally, TPS was highly correlated with MPS ( $r = 0.92$ ,  $P < 0.01$ ).

## 4. Discussion

Although the effect of dietary GAA supplementation on meat quality has been studied previously, it remains controversial (J.L. Li



**Fig. 3.** Effects of dietary crude protein level and guanidinoacetic acid (GAA) supplementation on the relative mRNA expression of myosin heavy-chain (MyHC) isoform genes of the longissimus dorsi muscle in finishing pigs. Diets were supplemented with or without GAA at 300 mg/kg according to the experiment design. Data were shown as the means ± SEM (*n* = 7). Differences were considered as significant when *P* < 0.05.

et al., 2018; Jayaraman et al., 2018). It has been supposed that inadequate dietary protein supply failed to support the function of GAA supplementation for promoting body protein synthesis. To the best of our knowledge, there was no study exploring the effect of GAA supplementation on meat quality with various dietary CP levels yet. Therefore, the present study is first to investigate the cooperation between dietary CP level and GAA supplementation on meat quality in finishing pigs.

In the present study, we demonstrated that GAA supplementation did not alter carcass characteristics of finishing pigs. Furthermore, we found dietary CP level did not affect carcass characteristics and meat quality, except that back-fat thickness of the 6th to 7th rib was decreased in pigs offered the 125% NRC CP diet. Previous studies demonstrated that dietary CP level has limited effects on growth and carcass characteristics (Liu et al., 2014) and the meat quality of finishing pigs; as long as dietary available energy and SID EAA levels meet requirements (Madrid et al., 2013; Qin et al., 2015; Grassi et al., 2017). In addition, GAA supplementation in the diet with 125% NRC CP level led to the lowest backfat thickness at the last rib among dietary treatments, which implies an interaction between dietary CP level and GAA supplementation.

Meat quality, meat color (*L*<sup>\*</sup>, *a*<sup>\*</sup>, *b*<sup>\*</sup>, *C*<sup>\*</sup> and *h*<sup>o</sup>) at 45 min and 24 h postmortem, and cooking loss were not altered by dietary GAA supplementation, which was consistent with the previous study (J.L. Li et al., 2018).

We demonstrated that GAA supplementation reduced drip loss in a different way from a previous study (J.L. Li et al., 2018). In the previous study, GAA supplementation increased pH<sub>45 min</sub> and MPS of meat. Nevertheless, we found that pH<sub>45 min</sub> and pH<sub>24 h</sub> values

were not altered and MPS tended to decrease, while free water proportion of fresh meat was significantly decreased by GAA supplementation. In addition, GAA supplementation in the diet with 100% NRC CP level, rather than 125% NRC CP level, decreased shear force and tended to increase IMF content; which was similar to a previous study (Wang et al., 2012). This implies that the diet with 100% NRC CP level is adequate for GAA supplementation to improve drip loss and shear force of fresh pork.

In comparison with immobilized water and bound water, free water only accounts for a minor part of total water in meat (Xu et al., 2020). However, in certain cases, its loss might trigger severe drip loss (Bertram et al., 2002). It has been shown that meat with lower drip loss displays a corresponding decreased *T*<sub>23</sub> peak area (Li et al., 2017). Similarly, in the present study, GAA supplementation reduced the amount of free water (*T*<sub>23</sub> peak area), and lowered drip loss compared with the control. It indicated that the GAA supplementation altered water distribution in muscle, and reduced free water content may partly account for the reduction of drip loss.

In addition, TPS, MPS and SPS that reflect the degree of protein denaturation have been implicated in WHC of fresh meat (Joo et al., 1999). SPS is of importance to WHC, and the relationship of drip loss with SPS was closer than with TPS or MPS (Joo et al., 1999; Ryu et al., 2005). Notably, in the present study, closer relationships between drip loss with MPS (*r* = 0.544) and TPS (*r* = 0.427), than with SPS (*r* = -0.31), revealed that TPS and MPS account more for drip loss than SPS did upon dietary GAA supplementation. It implied that complicated mechanisms might be involved in the causal relationship between muscle protein solubility and drip loss, and GAA supplementation might improve WHC in different ways under various conditions.

Guanidinoacetic acid is synthesized from arginine and glycine via the arginine: glycine amidinotransferase in vivo. Presently, GAA supplementation significantly increased the proportion of total glycine content in the muscle protein, indicating GAA supplementation economizes glycine in the body. Furthermore, as the largest reservoir of free amino acid in the body, skeletal muscle contains approximately 75% of the entire free amino acid pool (Graham et al., 2000). There are very limited data concerning effects of GAA supplementation on muscular free amino acid contents under the condition of various dietary CP levels in finishing pigs. In the present study, we found that the higher-protein diet significantly increased the concentrations of carnosine, total free amino acids and flavor amino acids of fresh meat. The possible reason may be owing to increased dietary CP level relieving the histidine restriction in muscle, and subsequently increase the synthesis of carnosine.

Moreover, we discovered that GAA supplementation might promote the protein anabolism in muscle, demonstrated by decreased the content of free alanine, threonine, isoleucine and methionine both in fresh meat and in myogenic differentiated cells.

**Table 7**  
Relationship among drip loss, muscle pH, muscle water distribution forms (NMR relaxation *T*<sub>2</sub>) and postmortem protein solubility.<sup>1,2</sup>

Item	Drip loss	pH <sub>45 min</sub>	pH <sub>24 h</sub>	<i>T</i> <sub>21</sub>	<i>T</i> <sub>22</sub>	<i>T</i> <sub>23</sub>	TPS	SPS	MPS
Drip loss	1.00								
pH <sub>45 min</sub>	-0.543**	1.00							
pH <sub>24 h</sub>	-0.550**	0.426*	1.00						
<i>T</i> <sub>21</sub>	-0.04	0.23	0.13	1.00					
<i>T</i> <sub>22</sub>	-0.21	-0.26	-0.20	-0.418*	1.00				
<i>T</i> <sub>23</sub>	0.25	0.10	0.11	-0.31	-0.731**	1.00			
TPS	0.427*	-0.12	-0.15	0.17	-0.08	-0.04	1.00		
SPS	-0.31	-0.11	0.396*	-0.06	0.16	-0.12	0.17	1.00	
MPS	0.544**	-0.07	-0.30	0.19	-0.14	0.00	0.923**	-0.22	1.00

NMR = nuclear magnetic resonance; TPS = total protein solubility; SPS = sarcoplasmic protein solubility; MPS = myofibrillar protein solubility.

<sup>1</sup> Differences were considered as statistically significant when *P* < 0.05. \**P* < 0.05, \*\**P* < 0.01.

<sup>2</sup> *T*<sub>21</sub>, *T*<sub>21</sub> peak area ratio; *T*<sub>22</sub>, *T*<sub>22</sub> peak area ratio; *T*<sub>23</sub>, *T*<sub>23</sub> peak area ratio.

In addition, considering that methionine is a main methyl donor, decreased free methionine may be due to the consumption of a large amount of methyl during the synthesis of creatine (Brosnan and Brosnan, 2006; Stead et al., 2006; McBreairey et al., 2015).

The ratio of EAA to NEAA determines the nutritional value of pork protein (Polidori et al., 2015). In the present study, GAA supplementation increased the ratio of EAA to NEAA at 100% NRC CP level rather than 125% NRC CP level, suggesting that GAA supplementation could ameliorate the protein nutritional value of pork depending on dietary levels of protein and amino acids. However, GAA supplementation decreased the sum of flavor amino acids of fresh meat, which warrants further study.

The content of carnosine in muscle is high, especially in the longissimus dorsi muscle (Boldyrev et al., 2013). In the present study, the intake of the high protein diet significantly increased the content of total free amino acids in the longissimus dorsi, mainly due to the increase in the content of carnosine. The longissimus dorsi muscle belongs to glycolytic fiber of fast muscle and increased carnosine content can enhance the glycolysis of muscle (Cornet and Bousset, 1999). It has been shown that carnosine content positively correlates with the proportion of type II muscle fiber (Sewell et al., 1992). Because carnosine may alter muscle glycolysis ability, and lead to the change in muscle fiber type composition, we quantitatively analyzed the mRNA expression of *MyHC* genes in muscle. Consistently, we found that the intake of the diet with 125% NRC CP level tended to increase the mRNA expression level of *MyHC-IIb*, accompanied by increased muscular carnosine content. The higher proportion of *MyHC-IIb* is generally considered to lead to greater drip loss in pork (Joo et al., 2013; Choe et al., 2008). Fortunately, in the present study, the intake of the higher CP diet did not significantly affect drip loss of fresh meat.

## 5. Conclusions

In conclusion, GAA supplementation could reduce drip loss mainly by decreasing free water proportion, while reduced the concentrations of free methionine, isoleucine and threonine and flavor amino acids of fresh meat independent of dietary CP level. Moreover, the intake of diets with 125% NRC CP level increased sarcoplasmic protein solubility, contents of carnosine and total free amino acids, as well as flavor amino acids in the fresh meat, while decreased backfat thickness at the 6–7th ribs in pigs.

## Author contributions

**Lu Wang:** Conceptualization, Investigation, Formal analysis, Data Curation, Writing - Original Draft; **Yubo Wang:** Investigation, Software; **Doudou Xu:** Investigation, Software; **Linjuan He:** Investigation; **Xiaoyan Zhu:** Software; **Jingdong Yin:** Conceptualization, Data Curation, Supervision, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

## Acknowledgments

This study was financially supported by the National Key R&D Program of China (Grant no. 2018YFD0500402) and S&T Program of Hebei (199A7310H).

## References

- Baker DH. Advances in protein-amino acid nutrition of poultry. *Amino Acids* 2009;37:29–41.
- Bekhit AED, Geesink GH, Morton JD, Bickerstaffe R. Metmyoglobin reducing activity and colour stability of ovine longissimus muscle. *Meat Sci* 2001;57:427–35.
- Bertram HC, Aaslyng MD. Pelvic suspension and fast post-mortem chilling: effects on technological and sensory quality of pork—a combined NMR and sensory study. *Meat Sci* 2007;76:524–35.
- Bertram HC, Dønstrup S, Karlsson AH, Andersen HJ. Continuous distribution analysis of T2 relaxation in meat—an approach in the determination of water-holding capacity. *Meat Sci* 2002;60:279–85.
- Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev* 2013;43:1803–45.
- Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. *J Nutr* 2006;136:1636S–40S.
- Brosnan JT, Wijekoon EP, Warford-Woolgar L, Trotter NL, Brosnan ME, Brunton JA, et al. Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. *J Nutr* 2009;139:1292–7.
- Brzostowski H, Tański Z. Nutritional value of the meat of Pomeranian breed lambs and crossbreeds of Blackheaded and Texel rams. *Arch Tierz* 2006;49:345–52.
- Choe JH, Choi YM, Lee SH, Shin HG, Ryu YC, Hong KC, et al. The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. *Meat Sci* 2008;80:355–62.
- Cornet M, Bousset J. Free amino acids and dipeptides in porcine muscles: differences between 'red' and 'white' muscles. *Meat Sci* 1999;51:215–9.
- Fortin A, Robertson WM, Tong AKW. The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Sci* 2005;69:297–305.
- Graham TE, Sgro V, Friars D, Gibala MJ. Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans. *Am J Physiol Endocrinol Metab* 2000;278:E83–9.
- Grassi S, Casiraghi E, Benedetti S, Alamprese C. Effect of low-protein diets in heavy pigs on dry-cured ham quality characteristics. *Meat Sci* 2017;131:152–7.
- He DT, Gai XR, Yang LB, Li JT, Lai WQ. Effects of guanidinoacetic acid on growth performance, creatine and energy metabolism, and carcass characteristics in growing-finishing pigs. *J Anim Sci* 2018;96:3264–73.
- Jayaraman B, Kinh L, Huyen LTT, Vinh D. Supplementation of guanidinoacetic acid to pig diets: effects on performance, carcass characteristics and meat quality. *J Anim Sci* 2018;96:2332–41.
- Joo ST, Kauffman RG, Kim BC, Park GB. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Sci* 1999;52:291–7.
- Joo ST, Kim GD, Hwang YH, Ryu YC. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Sci* 2013;95:828–36.
- Li X, Xie X, Zhang C, Zhang S, Jia W. Role of mid- and far-infrared for improving dehydration efficiency in beef jerky drying. *Dry Technol* 2018;36:283–93.
- Li JL, Zhang L, Fu Y, Li YJ, Jiang Y, Zhou GH, et al. Creatine monohydrate and guanidinoacetic acid supplementation affects the growth performance, meat quality, and creatine metabolism of finishing pigs. *J Agric Food Chem* 2018;66:9952–9.
- Li YJ, Li JL, Zhang L, Gao F, Zhou GH. Effects of dietary starch types on growth performance, meat quality and myofibre type of finishing pigs. *Meat Sci* 2017;131:60–7.
- Liu LY, Wang J, Huang Y, Pan HB, Zhang X, Huang ZX, et al. The effect of dietary protein levels on the expression of genes coding for four selected protein translation initiation factors in muscle tissue of Wujin pig. *J Anim Physiol Anim Nutr* 2014;98:310–7.
- Liu Y, Li JL, Li YJ, Gao T, Zhang L, Gao F, et al. Effects of dietary supplementation of guanidinoacetic acid and combination of guanidinoacetic acid and betaine on postmortem glycolysis and meat quality of finishing pigs. *Anim Feed Sci Technol* 2015;205:82–9.
- Luo Y, Zhang X, Zhu Z, Jiao N, Qiu K, Yin J. Surplus dietary isoleucine intake enhanced monounsaturated fatty acid synthesis and fat accumulation in skeletal muscle of finishing pigs. *J Anim Sci Biotechnol* 2018;9:88.
- Madrid J, Martínez S, López C, Orengo J, López MJ, Hernández F. Effects of low protein diets on growth performance, carcass traits and ammonia emission of barrows and gilts. *Anim Prod Sci* 2013;53:146–53.
- McBreairey LE, Robinson JL, Furlong KR, Brunton JA, Bertolo RF. Guanidinoacetate is more effective than creatine at enhancing tissue creatine stores while consequently limiting methionine availability in Yucatan miniature pigs. *PLoS One* 2015;10:e0131563.
- Michiels J, Maertens L, Buyse J, Lemme A, Rademacher M, Dierick NA, et al. Supplementation of guanidinoacetic acid to broiler diets: effects on performance, carcass characteristics, meat quality and energy metabolism. *Poult Sci* 2012;91:402–12.
- Ostojic SM, Drid P, Ostojic J. Guanidinoacetic acid increases skeletal muscle creatine stores in healthy men. *Nutrition* 2016;32:723–4.
- Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 2002;30:e36.
- Polidori P, Pucciarelli S, Ariani A, Polzonetti V, Vincenzetti S. A comparison of the carcass and meat quality of Martina Franca donkey foals aged 8 or 12 months. *Meat Sci* 2015;106:6–10.



- Qin CF, Huang P, Qiu K, Sun WJ, Xu L, Zhang X, et al. Influences of dietary protein sources and crude protein levels on intracellular free amino acid profile in the *longissimus dorsi* muscle of finishing gilts. *J Anim Sci Biotechnol* 2015;6:52.
- Qiu K, Zhang X, Wang LQ, Jiao N, Xu DD, Yin JD. Protein expression landscape defines the differentiation potential specificity of adipogenic and myogenic precursors in the skeletal muscle. *J Proteome Res* 2018;17:3853–65.
- Ryu YC, Choi YM, Kim BC. Variations in metabolite contents and protein denaturation of the *longissimus dorsi* muscle in various porcine quality classifications and metabolic rates. *Meat Sci* 2005;71:522–9.
- Sewell DA, Harris RC, Marlin DJ, Dunnett M. Estimation of the carnosine content of different fibre types in the middle gluteal muscle of the thoroughbred horse. *J Physiol* 1992;455:447–53.
- Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL. Is it time to reevaluate methyl balance in humans? *Am J Clin Nutr* 2006;83:5–10.
- Sun WJ, He T, Qin CF, Qiu K, Zhang X, Luo YH, et al. A potential regulatory network underlying distinct fate commitment of myogenic and adipogenic cells in skeletal muscle. *Sci Rep* 2017;1:14.
- Wang LS, Shi BM, Shan AS, Shang YY. Effects of guanidinoacetic acid on growth performance, meat quality and antioxidation in growing-finishing pigs. *J Anim Vet Adv* 2012;11:631–6.
- Wang YJ, Ma JD, Qiu WL, Zhang JW, Feng SY, Zhou XK, et al. Guanidinoacetic acid regulates myogenic differentiation and muscle growth through miR-133a-3p and miR-1a-3p co-mediated Akt/mTOR/S6K signaling pathway. *Int J Mol Sci* 2018;19:2837.
- Wang YM, Zhou JY, Wang G, Cai S, Zeng XF, Qiao SY. Advances in low-protein diets for swine. *J Anim Sci Biotechnol* 2018;9:60.
- Xu DD, Wang YB, Jiao N, Qiu K, Zhang X, Wang LQ, et al. The coordination of dietary valine and isoleucine on water holding capacity, pH value and protein solubility of fresh meat in finishing pigs. *Meat Sci* 2020;163:108074.
- Yin BJ, Li TT, Zhang SR, Li Z, He PL. Sensitive analysis of 33 free amino acids in serum, milk, and muscle by ultra-high performance liquid chromatography–quadrupole–orbitrap high resolution mass spectrometry. *Food Anal Method* 2016;9:2814–23.
- Zhang S, Chu L, Qiao S, Mao X, Zeng X. Effects of dietary leucine supplementation in low crude protein diets on performance, nitrogen balance, whole-body protein turnover, carcass characteristics and meat quality of finishing pigs. *J Anim Sci* 2016;87:911–20.