Dietary guanidinoacetic acid increases the *longissimus dorsi* muscle depth of finishing pigs without requiring a higher standardised ileal digestible methionine + cysteine concentration

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**ABSTRACT**

The objective of this study was to evaluate the interaction of guanidinoacetic acid (GAA) with standardised ileal digestible methionine + cysteine (SID Met + Cys) levels in finishing pig diets on the performance, carcass characteristics, pork quality, blood variables, gene expression of creatine transporter (*SLC6A8*) and oxidant and antioxidant action. The experimental design (*n* = 32) was in randomised blocks arranged in a 2 × 2 factorial scheme, consisting of two levels of guanidinoacetic acid (0.00% and 0.05%) and two levels of SID Met + Cys (0.44% and 0.50%). There was no interaction of SID Met + Cys and GAA levels on the performance variables, carcass characteristics, pork quality and creatine transporter gene expression (*p* > .05), except for plasma lactate dehydrogenase (LDH) (*p* = .016), wherein at the highest SID Met + Cys level the GAA supplementation (0.05%) showed lower plasma LDH concentration in relation to non-supplementation. Longissimus dorsi muscle depth (MD) (*p* = .001) showed an isolated effect for the evaluated GAA levels, in which the supplementation increased the MD. Blood urea presented an isolated effect (*p* = .040) for SID Met + Cys levels, showing lower plasma concentration for the highest dietary level. It was concluded that GAA does not require a higher intake of SID Met + Cys in finishing pig diets and its supplementation improved the depth of *Longissimus dorsi* muscle, without changing the performance, meat quality and gene expression of the *SLC6A8* transporter in the liver.

**HIGHLIGHTS**

- Higher levels of SID Met + Cys reduced homocysteine concentration, and GAA supplementation reduced plasma lactate dehydrogenase concentration at higher dietary SID Met + Cys level.
- GAA supplementation improves the depth of *Longissimus dorsi* muscle and did not affect the post-mortem glycolysis.
- Higher concentration of SID Met + Cys in finishing pig diets do not affect the gene expression of the creatine transporter (*SLC6A8*) in the liver.

**Introduction**

Methionine (Met), together with cysteine (Met + Cys) or simply sulphur amino acids (SAA), stand out as one of the main amino acid to be considered in the feed formulation, and are also the second limiting amino acids in a corn and soybean meal-based diet for finishing pigs (Oliveira et al. 2004). Among methionine's functions, the main one is to act as substrate for protein synthesis, being a precursor of other amino acids, notably cysteine that like methionine is also used for body protein synthesis (Brosnan and Brosnan 2006).

Cysteine is also used to synthesise glutathione peroxidase, an important antioxidant of the cells (Boler et al. 2009; Lu, 2013), preventing the oxidation of proteins such as myoglobin and myofibrillar proteins, preserving the meat colour and water holding capacity (Huff-Lonergan and Steven 2005; Mancini and Hunt 2005; Wang et al. 2009).

Methionine provides S-adenosylmethionine (SAM) in the metabolism that is a donor of methyl groups (CH$_3$) to a multitude of body substances, such as creatine, and is also involved in polyamine synthesis
(Nelson and Cox 2014). In this way, guanidinoacetic acid (GAA) is an immediate creatine precursor, requiring a methyl group from SAM to synthesise creatine (Baker 2009). The GAA synthesis begins with a reversible transfer of an amine group from arginine to glycine, providing ornithine and GAA (Persky and Brazeau 2010), in a reaction catalysed by the aminotransferase enzyme. The formed ornithine returns to the urea cycle and will be converted back to arginine, and the GAA is transported to the liver, where it will continue the process of creatine synthesis (Wyss and Kaddurah-Daouk 2000).

However, some tissues are not able to produce creatine and there is a need of a protein to transport it from extracellular medium into cells, occurring against a concentration gradient. The creatine transporter gene (SLC6A8) is one of the creatine and GAA transporters, which is expressed in most tissues but is most relevant in skeletal muscle, kidneys and brain (Wyss and Kaddurah-Daouk 2000).

As aforementioned, dietary GAA supplementation may generate metabolic effects, since methylation of GAA into creatine increase the demand for methyl groups (Stead et al.2006), and may also induce to homocysteine accumulation in the blood (Setoue et al. 2008). In this sense, dietary GAA supplementation maybe associated with higher SID Met + Cys levels to provide good performance, carcass traits and pork meat quality, but this need maybe also related with dietary GAA concentration to change SID Met + Cys level in pig diets. Additionally, dietary GAA contributing to creatine formation may also spare arginine in the metabolism that could be used by the body for other functions.

It has been shown that GAA supplementation in piglet diets requires high levels of SID Met + Cys to facilitate creatine transmethylation, and positive results are verified only when there are sufficient SID Met + Cys to provide methyl groups (Bray et al. 2016). In general, it has been noted that studies conducted so far have evaluated GAA supplementation in pig diets, but have not evaluated its interaction with SID Met + Cys concentration in the diet, since there is a metabolic interaction between GAA and methionine (i.e. methyl groups donated from SAM) to synthesise creatine.

Considering that GAA requires SAM for creatine synthesis, we hypothesise that a high SID Met + Cys level in diets containing GAA could help creatine synthesis and so, improve some aspects of pig production, mainly pork quality and protein deposition.

Thus, this study aimed to evaluate the dietary interaction of GAA and SID Met + Cys for finishing pigs on the performance, carcass traits, pork quality, blood biochemical parameters, creatine transporter gene expression and antioxidant action on the muscle.

Materials and methods

Animals, housing, experimental design and diets

The experimental procedures were previously approved by the Animal Use Ethics Committee (CEUA) of the State University of Maringá (UEM) (Process n° 7665090217). Thirty-two crossbred gilts (Landrace × Large White × Duroc) with an average initial weight of 75.2 ± 0.91 kg, were distributed in a randomised blocks design in a 2 × 2 factorial scheme, consisting of two levels of GAA supplementation (0.00% and 0.05%) and two levels of digestible SID Met + Cys (0.44% and 0.50%). The concentration of 0.44% SID Met + Cys is the requirement proposed by the NRC (2012), and the level 0.50% SID Met + Cys was used to evaluate if there is a need for a higher concentration when supplementing GAA in finishing pig diets. Treatments also consisted of eight replicates and one animal per experimental unit.

The animals were distributed in the treatments based on the initial weight and were housed in concrete-floor stalls with a semi-automatic front feeder and a nipple type drinker at the rear, located in a masonry barn equipped with fans and nebulisers.

The experimental diets were formulated based on corn, soybean meal, minerals, vitamins, amino acids and additives. The diets were isonutritive and met the nutritional recommendations proposed by NRC (2012) (Table 1). Corn and soybean meal were submitted to amino acid determination at Evonik Industries, previously to diets formulation. After that, the standardised ileal digestible (SID) coefficients proposed by Rostagno et al. (2017) were applied to the determined total amino acids content, in order to obtain the SID amino acids concentration of corn and soybean meal.

Growth performance

The animals were weighed at the beginning and at the end of the experiment in order to determine the average daily gain (ADG). The total amount of diets were also weighed for each experimental unit to determine the daily feed intake (DFI) and to calculate the feed:gain (F:G).
Blood biochemical compounds

At the end of the experiment, the blood collection was performed from all animals, to determine plasma concentrations of urea, creatinine, lactate dehydrogenase (LDH), glucose and homocysteine. Blood samples were obtained by puncture in the jugular vein with the help of 100 mm long needles. It was collected approximately 15 mL of blood into glass tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant to determine the plasma concentrations of urea, creatinine and lactate and another tube containing anticoagulant potassium oxalate was used for plasma glucose determination. After the collection, the blood was immediately sent to the Swine Laboratory located at the Experimental Farm of Iguatemi (FEI)/State University of Maringá (UEM), where the samples were centrifuged at 3000 xg during 15 min to obtain the plasma, which was extracted with the aid of an automatic pipette and placed into Eppendorf type tubes.

After that, plasma samples were submitted to the biochemical analysis using colorimetric kits following the manufacturer’s recommendations (Glucose - MS 80022230067, Lactate dehydrogenase - MS 800222300 84, Urea - MS 80022230075 and Creatinine - MS 8002 2230143, Gold Analisa, Brazil and Biochin, Brazil), and the absorbance reading was performed on Biochemical Analyser (Bioplus 2000, Brazil). Additionally, blood samples were also collected in glass tubes containing a gel without physical/chemical properties to perform homocysteine analyzes. Serum homocysteine concentration was determined by the IMMULITE unit (Siemens) in the Sào Camilo Laboratory (Maringá, Paraná, Brazil) by means of chemiluminescence method.

Carass traits

At the end of the experiment the animals were directed to the slaughterhouse of FEI/UEM, considering a total fasting of 24 h. The animals were previously submitted to electric numbness (200 watts) and subsequently slaughtered for bleeding, depilated and eviscerated, according to techniques described by Pacheco and Yamanaka (2008). The carcasses were cooled (1–2 °C) for 24 h and subsequently submitted to quantitative evaluation.

The carcasses were individually evaluated according to Bridi and Silva (2009) guidelines, for carcasses length, hot carcasses yield (HCY), carcasses weight loss (WL), backfat thickness (BT), Longissimus dorsi muscle depth (MD), fat thickness measured at 3 points (P1, P2 and P3), lean meat yield (LMY). The BT and MD were measured in the left half carcass, 24 h post-mortem, with the aid of a digital pachymeter, in the insertion of the last thoracic vertebra with the first lumbar, six centimetres from the midline of the carcass. The pH of the Longissimus dorsi muscle was measured in the hot carcass 45 min post mortem (pH45) and also in the cold carcass maintained in the cold chamber (1–2 °C) for 24 h (pH24), using a Portable Digital pH Meter (HI 99163, Hanna Instruments, Italy), following the recommendations of Bridi and Silva (2009).

Pork quality

Samples (2.5 cm thick) of the Longissimus dorsi were taken from the site of 8th and 10th vertebrae to measure the drip loss (DL) and the determination of thawing loss (TL), cooking loss (CL) and shear force (SF).
The Longissimus dorsi muscle colour was measured 24 h after the slaughter, as described by Bridi and Silva (2009). In the muscle surface were performed the luminosity measurements (L*, a* and b*) using a portable colorimeter (CR-400, Konica Minolta's, Japan). The components L* (luminosity), a* (red-green component) and b* (yellow-blue component) were expressed in the CIELAB colour system.

One of the Longissimus dorsi samples was frozen and then used for the analysis of thawing loss and cooking loss. The thawing loss was obtained by weight difference of frozen sample and after storage at 4 °C. The cooking loss was obtained by the weight difference of the thawed sample and after cooking in a preheated oven at 170 °C, until reaching the internal temperature of 71 °C (Bridi and Silva 2009).

The previously cooked Longissimus dorsi samples were used to determine the shear force (SF, kgf). In each sample, considering the longitudinal direction, towards the muscle fibres, five cylindrical subsamples were taken, according to the recommendations of Ramos and Gomide (2007). Analyzes were performed using a texturimeter (Stable Micro Sytem TA-XT2i, coupled with the manufacturer’s standards and quantifyed using nanodrop 2000-c spectrophotometer. RNA samples were treated with DNase I (Invitrogen, Carlsbad, CA, USA) to remove possible genomic DNA residues according to the manufacturer’s recommendations. Complementary DNA synthesis (cDNA) was performed with the SuperScript III First-Strand Synthesis Super Mix kit (Invitrogen Corporation, Brazil) as per manufacturer’s specifications. The cDNA samples were diluted to 80 ng/µL and stored at −20 °C until further use as template in the amplification reaction.

Creatine transporter gene expression (SLC6A8) was measured by real-time quantitative polymerase chain reaction (qPCR) using SYBR® Green PCR Master Mix (Applied Biosystems, USA) fluorescent dye and the StepOne Real-Time PCR System version 2.3 equipment (Applied Biosystems, Lithuania) in duplicate. The amplification reaction consisted of 5 µL of diluted cDNA for 40 ng/µL, 0.5 µL of each primer (forward and reverse) at 10 µM (final concentration: 200 nM), 12.5 µL of SYBR® Green PCR Master Mix, and ultrapure water to a final volume of 25 µL. To measure the efficiency of each primer/gene set, a series of 25-µL reactions was analysed as described above using 5 µL of a serial dilution of pooled cDNA as the template. The thermal cycling parameters were as follows: hot-start at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min, and ending with a melt curve from 65 to 95 °C.

The primer pairs (forward and reverse) used in the amplification reactions for endogenous control (β-actin - Forward: GGTTTATTTTGAGTGCCAGC and Reverse: CTGGTCTCAAGTCAGTGTACAG; accession number: AY550069.1) and creatine transporter target gene (SLC6A8 - Forward: TTTACCGCTACATCCCCC and Reverse: GGCATCTATCCACACCTGAG; accession number: NM_001177327.1) were constructed based on the available sequences in the NCBI GenBank database (www.ncbi.nlm.nih.gov) for specific swine (Sus scrofa) using the www.idtdna.com program.

The amplification efficiencies (90% to 110%) were similar for the genes of interest. Analysis of the Oxidant and antioxidant action

The determination of lipid oxidation (TBARS), through determining the value of thiobarbituric acid reactive substances, was performed on Longissimus dorsi samples, after the slaughter storage (4 °C) during 24, 48 and 72 h. The procedures were performed according to the methodology described by Vyncke (1975). Absorbance was read at a wavelength of 532 nm and it was used a standard curve of 1.1.3.3 tetraethoxypropane.

To evaluate the inhibition of 2,2-diphenyl-1-picril-hydrazole (DPPH) radical were used methyl alcohol and DPPH (Sigma, USA), following the methodology of Li et al. (2005) and Brand-Williams et al. (1995). The absorbance reading was performed in a cuvette spectrophotometer (Bioespectro SP22, Brazil) at 515 nm absorbance, after 30 min. The blank test was performed with 150 µL of the solvent used for extraction with 2.85 mL of methyl alcohol.

The creatinine content of the Longissimus dorsi was determined with adaptations from the methodology of Willian et al. (1927), using a commercial kit (Gold Analisa, Brazil) and performing a subsequent absorbance reading.

Gene expression of the creatine transporter (SLC6A8)

Immediately after the slaughter, liver tissue samples (left medial lobe) were collected for gene expression analysis. All materials used in the collection were previously treated with RNase inhibitor (RNase Zap®, Life Technologies, Brazil). The samples were packaged in liquid nitrogen and sent to be stored in a freezer at −80 °C until RNA extraction. Total RNA was extracted using TRizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s specifications. The cDNA samples were diluted to 80 ng/µL and stored at −20 °C until further use as template in the amplification reaction.

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dissociation curves did not reveal any non-specific PCR products, such as the formation of primer dimers, thus demonstrating the reliability of the data for estimating the mRNA expression of the evaluated genes. The endogenous control, β-actin, did not show any significant differences among treatments, which confirmed its suitability as a control. The results were expressed as arbitrary units (AU), and the 2−ΔACT method (Livak and Schmittgen 2001) was used for relative quantification of gene expression.

Statistical procedures

The statistical analysis was performed using the computer program R (2015). Data regarding to the performance, blood variables, gene expression, carcass traits, pork quality and oxidation and antioxidant action were submitted to ANOVA, considering SID Met + Cys levels, GAA supplementation and the interaction between these factors in the mathematical model. When interaction between SID Methionine + Cysteine levels and GAA supplementation effects were significant, means were compared by F test (p ≤ .05).

For TBARS test, the degrees of freedom regarding GAA levels and/or storage period were deployed in orthogonal polynomials to fit regression equations. For the isolated significant effect of SID Methionine + Cysteine levels or GAA supplementation, the means were also compared by F test (p ≤ .05).

Results

Table 2 presents the performance results (final weight - FW; daily weight gain - DWG; daily feed intake - DFI; feed:gain - F:G) of finishing pigs intaking diets with different concentrations of SID MET + Cys and GAA. There was no significant effect of interaction (SID Met + Cys × GAA; FW p = .723, DWG p = .094, DFI p = .293, F:G p = .279) and isolated effects of treatments (SID Met + Cys FW p = .583, DWG p = .921, DFI p = .219, F:G p = .615; and GAA FW p = .643, DWG p = .094, DFI p = 1.000, F:G p = .085) on any performance variables evaluated.

The results of blood variables (glucose, LDH, urea, creatinine and homocysteine) of finishing pigs that consumed diets with different concentrations of SID MET + Cys and GAA are presented in Table 3. There was no interaction between treatments on the concentrations of glucose (p = .560), urea (p = .359), creatinine (p = .134) and homocysteine (p = .584) in the blood of the animals. An interaction between SID Met + Cys and GAA levels were observed on plasma

Table 2. Performance of finishing gilts fed diets with different concentrations of standardised ileal digestible methionine + cysteine (SID Met + Cys) and supplemented with guanidinoacetic acid (GAA).

| SID Met + Cys, % | GAA, % | FW, kg | DWG, kg | DFI, kg | F:G, kg/kg |
|-----------------|--------|--------|---------|---------|------------|
| Main effects    |        |        |         |         |            |
| 0.44            | 0.00   | 100.70 | 1.07    | 2.85    | 2.78       |
| 0.50            | 0.00   | 100.66 | 1.06    | 2.89    | 2.84       |
| 0.50            | 0.05   | 99.81  | 1.08    | 2.86    | 2.74       |
| Interaction effects |      |        |         |         |            |
| 0.44            | 0.00   | 100.80 | 1.08    | 2.76    | 2.67       |
| 0.44            | 0.05   | 100.59 | 1.05    | 2.93    | 2.89       |
| 0.50            | 0.00   | 100.51 | 1.08    | 2.95    | 2.81       |
| 0.50            | 0.05   | 99.10  | 1.03    | 2.83    | 2.87       |
| Standard error of means |     | 3.87   | 0.14    | 0.29    | 0.26       |
| p-Value         |        |        |         |         |            |
| SID Met + Cys   | 0.583  | 0.921  | 0.219   | 0.615   |            |
| GAA             | 0.643  | 0.094  | 1.000   | 0.085   |            |
| SID Met + Cys × GAA | 0.723 | 0.094  | 0.293   | 0.279   |            |

Table 3. Blood parameters of finishing gilts fed diets with different concentrations of standardised ileal digestible methionine + cysteine (SID Met + Cys) and supplemented with guanidinoacetic acid (GAA).

| SID Met + Cys, % | GAA, % | Glucose, mg/dL | LDH, mg/dL | Urea, mg/dL | Creatinine, mg/dL | Hcy, mg/dL |
|-----------------|--------|----------------|------------|-------------|------------------|------------|
| Main effects    |        |                |            |             |                  |            |
| 0.44            |        | 51.22          | 13.58      | 14.72       | 3.33             | 31.81*     |
| 0.50            | 0.00   | 59.71          | 14.82      | 11.67       | 2.33             | 23.13*     |
| 0.50            | 0.05   | 55.35          | 15.46      | 12.88       | 2.37             | 20.71      |
| Interaction effects |      |                |            |             |                  |            |
| 0.44            | 0.00   | 49.78          | 11.66      | 15.06       | 2.50             | 34.80      |
| 0.44            | 0.05   | 52.66          | 15.50      | 14.38       | 2.15             | 28.82      |
| 0.50            | 0.00   | 60.92          | 19.26      | 10.69       | 2.23             | 24.62      |
| 0.50            | 0.05   | 58.50          | 10.38      | 12.65       | 2.43             | 21.63      |
| Standard error of means |     | 2.07           | 4.50       | 1.07        | 0.36             | 1.52       |
| p-Value         |        |                |            |             |                  |            |
| SID Met + Cys   | 0.058  | 0.170          | 0.040      | 0.040       | 1.000            | 0.052      |
| GAA             | 0.843  | 0.460          | 0.387      | 0.921       | 0.906            |            |
| SID Met + Cys × GAA | 0.560 | 0.016          | 0.359      | 0.134       | 0.584            |            |

*Average followed by different letters in the column differ from each other by the F test (p ≤ .05).
SID Met + Cys: standardised ileal digestible methionine + cysteine; GAA: guanidinoacetic acid; LDH: lactate dehydrogenase; Hcy: homocysteine.
LDH concentration ($p = .016$). A low LDH concentration was observed in the blood of pigs that received diets containing 0.50% of SID Met + CyS and 0.05% of GAA. The urea and homocysteine concentrations in the blood of pigs were affected by the concentration of SID Met + CyS in the diet (urea - $p = .040$ and homocysteine - $p = .052$), where the lowest level of SID Met + CyS (0.44%) showed an increase in urea (14.72 mg/dL - reference value 14.84 – 51.48 mg/dL - Friendship and Henry 1992) and homocysteine (31.81 mg/dL) concentration in the blood of pigs. There was also no significant effect of treatments on the concentration of glucose and creatinine (reference value: 0.87–1.86 mmol/L - Friendship and Henry 1992) ($p \geq .05$).

Table 4 presents the results of quantitative carcass characteristics of finishing pigs that received diets with different concentrations of SID Met + CyS and GAA, which include carcass length, hot carcass yield (HCY), fat carcass weight loss (WL), fat thickness (FT), Longissimus dorsi muscle depth (MD), fat thickness measured at 3 points (P1, P2, P3) and lean meat yield (LMY). There was no significant effect of interaction between treatments on these quantitative carcass variables ($p \geq .05$). However, dietary GAA affected the MD ($p = .001$), observing that pigs receiving diet with 0.05% of GAA had higher MD (67.37 mm) than pigs receiving diet without GAA (63.11 mm). There was no significant isolated effect of the treatments (SID Met + CyS e GAA) on the other carcass traits.

Table 5 presents the pork quality of finishing pigs fed diets with different concentrations of standardised ileal digestible methionine + cysteine (SID Met + CyS) and supplemented with guanidinoacetic acid (GAA).

| pH 45 min | pH 24 h | DL, % | CL, % | SF, N | L$^*$ | a$^*$ | b$^*$ | Creatinine, mg/g | p-Value |
|----------|--------|------|------|------|------|------|------|-------------|---------|
| 6.11     | 5.52   | 4.38 | 24.63| 3.78 | 54.71| 6.80 | 3.53 | 4.41         | 0.08    |
| 6.11     | 5.63   | 3.90 | 24.61| 3.86 | 54.08| 6.38 | 3.23 | 4.63         | 0.12    |
| 6.12     | 5.65   | 4.35 | 24.00| 3.71 | 55.19| 6.33 | 3.16 | 3.16         | 0.54    |
| 6.15     | 5.52   | 4.34 | 23.99| 3.79 | 56.13| 6.70 | 3.87 | 4.06         | 0.35    |
| 6.15     | 5.52   | 4.34 | 23.99| 3.87 | 56.13| 6.70 | 3.87 | 4.06         | 0.35    |
| 6.15     | 5.52   | 4.34 | 23.99| 3.87 | 56.13| 6.70 | 3.87 | 4.06         | 0.35    |
| 6.15     | 5.52   | 4.34 | 23.99| 3.87 | 56.13| 6.70 | 3.87 | 4.06         | 0.35    |

There was no statistically significant effect of the treatments on any performance parameter evaluated ($p > .05$). Results are presented as mean and standard error of the mean (SEM). SID Met + CyS: standardised ileal digestible methionine + cysteine; GAA: guanidinoacetic acid; pH 45 min: pH at 45 minutes after slaughter; pH 24 h: pH at 24 hours after slaughter; DL: drip loss; CL: thawing loss; SF: shear force; L$^*$, a$^*$ and b$^*$: luminosity measurements.
Figure 1 shows the percentage of DPPH radical inhibition (antioxidant capacity) in the Longissimus dorsi muscle of finishing gilts fed diets with different concentrations of standardised ileal digestible methionine + cysteine (SID Met + Cys) and supplemented with guanidinoacetic acid (GAA). There was no significant effect of medium concentrations on the antioxidant capacity in the muscle of these animals (p ≥ .05).

Figure 2 shows the results of creatine transporter gene expression (SLC6A8) in the liver of finishing gilts fed diets with different concentrations of standardised ileal digestible methionine + cysteine (SID Met + Cys) and supplemented with guanidinoacetic acid (GAA). Results are expressed as arbitrary unit (AU). There was no effect of treatments on SLC6A8 gene expression (p ≥ .05).

Discussion

The GAA is an immediate creatine precursor, requiring a methyl group from SAM to synthesise creatine (Baker 2009). According to Jayaraman et al. (2018), the GAA supplementation (0.12%) improved the weight gain of growing and finishing pigs, and the authors associated this improvement with the creatine formation, which may contribute to the increase of muscle protein and water retention in skeletal muscles. In this study, increasing SID Met + Cys concentration in the diet did not affect pig performance, since the lowest studied level (0.44%) is considered the requirement proposed by the NRC (2012), consequently providing an optimum performance and do not improving it above this level. Therefore, these results suggest that an increased dietary SID Met + Cys level is not necessary when supplementing 0.05% GAA in finishing pig diets. However, it is worth noting that the GAA supplemented level evaluated by Jayaraman et al. (2018)
was higher than the one evaluated in this study (0.05%) and no interactions with some methyl group donor was evaluated.

The improved performance was also observed with broilers, in which GAA supplementation increased creatine concentration in the breast muscle, indicating that GAA supplementation can synthesise creatine in this tissue (Tossenberger et al. 2016), without requiring higher dietary SID Met + Cys levels.

By the other side, levels of 0.6%, 0.9% and 1.2% GAA increased the feed conversion of finishing pigs supplied during the growing and finishing phases (from 30 to 100 kg live weight), and the level of 0.3% GAA showed the best response for feed conversion considering the total period of experiment (He et al. 2018). These different results can be attributed to many factors, including the time consuming of the experimental diets, level of GAA supplementation, concentrations of methyl donors in the metabolism, genetic potential for protein deposition, etc.

The GAA supplementation reduced 53% plasma lactate concentration only at higher dietary SID Met + Cys level (0.50%). Lactate is directly associated with pork quality and one of the main causes of quality loss is the rapid and extensive decrease in post-mortem pH due to the accumulation of lactic acid from anaerobic glycolysis, before an efficient carcass chilling (Stead et al. 2001). In the present study, although the GAA supplementation (0.05%) reduced plasma LDH concentration at 0.50% SID Met + Cys, the pH 45 min and pH 24 h did not change, indicating that these concentrations evaluated did not affect the post-mortem glycolysis at a so high rate to provide changes in muscle pH.

The GAA supplementation increases creatine and phosphocreatine, which would make the post-mortem glycolysis to occur more slowly, decreasing lactic acid concentration (Franco et al. 2016; Li et al. 2016). The same response was observed in this study at the highest dietary level of SID Met + Cys, which may indicate that a higher SID Met + Cys amount was required for GAA to provide this effect. However, several factors may increase blood lactate concentration, from stressors to health problems, due to the disruption of organic homeostasis, leading to the occurrence of various physiological and behavioural responses (Ringel et al. 2008).

The plasma urea provides an indirect and inverse measurement of changes in protein synthesis. As the relative proportion of methionine increases towards its optimal value, more protein is synthesised leading to increased nitrogen retention and decreased plasma urea (Qiao et al. 2008). Additionally, the inverse relationship between plasma urea and lean growth was also observed by Coma et al. (1995). In this way, a lower plasma urea concentration was observed for those animals that received the higher SID Met + Cys in the diet (0.50%), that may indicate a better nitrogen retention directed for protein synthesis.

The oral administration of GAA increases plasma creatinine concentration and consequently there is an increase in homocysteine concentration (Stead et al. 2001). In the same way, higher SID Met + Cys levels may cause hyperhomocysteinemia induced by methionine excess (Li et al. 2016). In humans, levels between 5–15, 16–30, 31–100 and >100 μmol/L are reported as clinically normal, moderate, intermediate and severe hyperhomocysteinemia, respectively (Venâncio et al. 2009 and Beard and Bearden 2011), but there is a lack of information about homocysteine blood reference values for pigs. The aforementioned effects were not observed in this study and may be due to the studied levels, since the main effects of GAA and SID Met + Cys did not affect plasma concentrations of creatine and homocysteine, and these responses may be directly related with the intake of GAA and methionine.

However, homocysteine accumulation can be reduced by many mechanisms, such as acting as a co-substrate for the cystathionine β-synthase (CBS) reaction and also providing carbon unit to react with folate in order to provide methyl group for the methionine synthesis. In other words, the CBS acts as a methionine cycle regulator (Nijhout et al. 2006). The high methionine levels also raise the concentration of SAM and inhibiting the activities of enzymes involved in homocysteine remethylation and also stimulates the activity of CBS, responsible for the irreversible conversion of homocysteine (Ohuchi et al. 2008; Ostojic 2017). According to these reports, the use of a high level of SID Met + Cys in this study may have directed the homocysteine to the cysteine synthesis, since the studied SID Met + Cys levels were achieved by adding DL-Methionine in the diet. Additionally, the blood homocysteine concentration maybe also had been reduced due to the higher activity of CBS, that is stimulated by a higher amount of methionine in the cycle.

A high methionine concentration diet, fed to pigs during 30 days, provided an increase in blood homocysteine compared with the control diet (França et al. 2006), but the methionine concentration was increased in 3%. This concentration is higher than the one used in this study, and maybe the methionine
concentration needs to be higher than the used in the current study to provide changes in homocisteine concentration.

In this study muscle depth showed an isolated effect of GAA levels, in which the supplementation with GAA (0.05%) increased muscle depth. This may indicate the GAA promoting properties, as previously suggested by Wang et al. (2012), Jayaraman et al. (2018) and Li et al. (2018); and GAA supplementation also have the ability to save amino acids, especially arginine and glycine, assigning them to the protein synthesis. However, it is important to consider that in these experiments the amino acid levels were in agreement to the requirements (NRC 2012) for each studied phase.

SID Met + Cys supplementation beyond the requirement may increase the carnitine content in the liver and muscles of pigs (Feng et al. 2006) indicating an effect on the reduction of carcass fat percentage (Valente Júnior et al. 2019). However, the studied levels did not affect the fat deposition. Previously studies showed that GAA supplementation increases total creatinine concentration and muscle phosphocreatine levels (Ohuchi et al. 2008) and also improves meat quality through the increased initial pH and a lower water loss and shear force (Wang et al. 2012).

Methionine is also required for phosphatidylcholine biosynthesis, which is a phospholipid located on mammalian cell membranes (Zeisel 2006) being an important component to maintain the membrane entirety and cell fluid rate, because this characteristic is important to avoid excessive water loss from meat. An adequate methionine supply is necessary for the synthesis of important substances to keep the oxidative stability of meat. In this study, no effect of SID Met + Cys levels were observed on variables related to water loss (DL, TL, CL), showing that the dietary level of 0.44% SID Met + Cys was enough for the water loss results.

The required SID Met + Cys for 75 to 100 kg gilts is at least 10.60 g/day (0.370%) for carcass characteristics and meat quality (Sangali et al. 2018). However, higher SID Met + Cys levels may also increase the synthesis of carnitine and phosphatidylcholine, acting on lipid metabolism affecting the absorption and transportation of fatty acids. On the other hand, carnitine plays an important role in the transport of medium and long chain fatty acids into the mitochondria, where the β-oxidation happens (Huang et al. 2013).

The action of post-mortem time tends to oxidise the meat and reduce its quality (Zeola et al. 2007) and pork is highly susceptible to lipidic oxidation. Lipidic oxidation is a process that involves the deterioration of fatty acids through a chain reaction mechanism, forming lipid hydroperoxides and other by products responsible for the development of rancidity and deterioration of food (Shahidi and Zhong 2010).

According to Wang et al. (2012), guanidinoacetic acid was able to reduce lipid peroxidation by increasing the activity of some enzymes associated with free radical metabolism. This fact was not verified in this study, once GAA supplementation (0.05%) did not affect lipid oxidation. However, in general, our results suggest that the inclusion of 0.44% and SID Met + Cys and 0.05% of GAA in swine diets, although they do not inhibit lipid oxidation, tend to delay this process of meat oxidation.

The addition of GAA at 1.2% decreased plasma MDA levels and increased the activity of antioxidant enzymes and creatine kinase in broilers subjected to cold stress, but associated the use of GAA with betaine, which acts as a donor of methyl groups for transmethylation reactions for creatine synthesis, reducing the need for other methyl group donors, such as methionine (Nasiroleslami et al. 2018).

In addition, sulphur amino acids play an important role in oxidative stress, acting as antioxidants processes through taurine and glutathione peroxidase synthesis (Willemsen et al. 2011). Dietary deficiency may result in the decreased of antioxidant enzymes activity and, consequently, it increases the lipid peroxidation, inducing oxidative damage in the body (Ruan et al. 2018). Methionine and S-adenosylmethionine (SAM) play an important role in neutralising reactive oxygen species, because promoting the synthesis of glutathione from cysteine, and also acts directly on the antioxidant activity of the system by eliminating oxygen of the reactive species (Jung et al. 2013). In addition, methionine residues in proteins have antioxidant action by the methionine sulfoxide reductase system. These residues can be oxidised by oxygen reactive species and converted to methionine sulfoxide, being the methionine, after, reduced again. Each time that this cycle is repeated, an equivalent of the oxygen reactive species is eliminated (Métayer et al. 2008).

The results of creatine transporter gene expression demonstrates that the supplemented GAA may not have been used for creatine formation in the liver. However, GAA can also be transported from the bloodstream into cells by other transporters, such as sodium- and chloride-dependent taurine transporter (SLC6A6) and even by the aminobutyric gamma transporter (GAT2) (Ostojic 2017). In another study, it was verified that 0.1% of GAA increased the expression of...
creatine transporter in the muscle (Longissimus dorsi), liver and kidneys (Li et al. 2018), suggesting that GAA can promote creatine transport to cells, in other words, in addition to different transporters; GAA also has the expression of its transporters in other organs besides the liver (Li et al. 2018).

Diets supplemented with GAA may increase the demand for S-adenosylmethionine (Stead et al. 2001) so, it is necessary enough dietary methionine to meet protein needs and creatine formation (Ibrahim et al. 2019). However, it is important to note that, in addition to methionine, the GAA synthesis is also related to arginine.

The formation of GAA is one of the most important pathways of the arginine, especially in young animals, which have more effective growth rate and protein composition (Li et al. 2018) what may explain the lack of more evident results from this work. Probably, diets with lower SID arginine levels supplemented with GAA may show some effects in such variables (Teixeira et al. 2017). In addition, finishing pigs have a high arginine synthesis than piglets, which may also be an important factor to be considered.

Conclusions
It was concluded that 0.05% of guanidinoacetic acid supplementation does not require a higher concentration of SID methionine + cysteine in finishing pig diets and its supplementation improved the Longissimus dorsi muscle depth, without effects on performance, meat quality and gene expression of the creatine transporter (SLC6A8) in the liver.

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Ethical approval
This experiment followed the guidelines of the Committee on Animal Care of the State University of Maringá, Brazil (Process n° 7665090217).

Disclosure statement
The authors declare that there is no conflict of interest.

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Data availability statement
The data that support the findings of this study are available from the corresponding author, PCP, upon reasonable request.

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