UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ÁCIDO GUANIDINOACÉTICO E CREATINA NO APORTE DE METIONINA+CISTEÍNA EM DIETAS PARA SUÍNOS EM TERMINAÇÃO

Autora: Natália Yoko Sitanaka
Orientador: Prof. Dr. Paulo Cesar Pozza

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Estado do Paraná
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Tese apresentada, como parte das exigências para obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de Concentração de Produção de Monogástricos

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 06 de setembro de 2019.

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“Tudo vem a mim com facilidade, alegria e glória.
Gratidão!”

À minha amada família e aos queridos amigos que foram meu alicerce durante essa jornada, com todo meu amor:
Dedico.

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BIOGRAFIA

NATÁLIA YOKO SITANAKA nasceu em Campinas, São Paulo, no dia 08 de dezembro de 1990.

Em julho de 2009 ingressou na Universidade Estadual Júlio de Mesquita Filho (UNESP), e, em dezembro de 2014, obteve o título de bacharel em Zootecnia.

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Em março de 2017, iniciou no Programa de Pós-Graduação em Zootecnia na Universidade Estadual de Maringá (UEM), em nível de Doutorado. Submeteu-se ao Exame Geral de Qualificação em 26 de julho de 2019 e, em 6 de setembro de 2019, submeteu-se à defesa da Tese.

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LISTA DE ABREVIATURAS

a*	Componente vermelho-verde
AAS	Aminoácidos sulfurados
AMT	Aminotransferase
AT	Amidinotransferase
ATP	Trifosfato de adenosina
b*	Componente amarelo-azul
CA	Conversão alimentar
CBS	Cistationina β-sintase
cDNA	DNA complementar
CDR	Consumo diário de ração
CH3	Grupos metil
CK	Creatina quinase
CMH	Creatina monohidratada
DPPH:	2,2-difenil-1-picril-hydrazil
ET	Espessura de toucinho
FC	Força de cisalhamento
GAA	Ácido guanidinoacético
GMT	Guanidinoacético-metiltransferase
GPD	Ganho de peso diário
GPx	Glutationa peroxidase
GSH	Glutationa reduzida
L*	Luminosidade
Met+Cis	Metionina + cistina
MS	Metionina sintase

MT	Metil-transferases
PC	Perda de água por cocção
PD	Perda de água no descongelamento
PM	Profundidade do músculo *Longissimus dorsi*
PR	Perda de peso no resfriamento
PSE	Pale, soft and exudative
PCR	Reação em cadeia da polimerase quantitativa
RCM	Rendimento de carne magra
RCQ	Rendimento de carcaça quente
SAH	S-adenosil-homocisteína
SAM	S-adenosilmetionina

RESUMO

Foram realizados dois experimentos com o objetivo de avaliar os efeitos da interação de níveis de metionina + cistina digestíveis (met+cis) e da suplementação de ácido guanidonoacético (GAA) e creatina monohidratada (CMH) sobre o desempenho, variáveis sanguíneas, características de carcaça, qualidade de carne e a expressão gênica do transportador de creatina (SLC6A8), em fêmeas suínas na fase de terminação (75 aos 100kg). No experimento I, foram utilizadas 32 fêmeas, com peso médio inicial de 75,25 ± 0,91 kg, distribuídas em um delineamento experimental de blocos casualizados, num esquema fatorial 2×2, constituído de dois níveis de met+cis (0,44% e 0,50%) e dois níveis de GAA (0 e 0,05%), com oito repetições e um animal por unidade experimental. Os animais e a ração consumida foram pesados para determinação do ganho de peso (GPD), consumo de ração (CDR) e conversão alimentar (CA). No término do experimento. foi realizada a coleta de sangue de todos os animais para determinação das concentrações plasmáticas de ureia, creatinina, lactato, glicose e homocisteína. Ao atingirem o peso médio de 100±5,85 kg, os animais foram abatidos, após jejum alimentar (24 horas), no abatedouro da Fazenda Experimental de Iguatemi - FEI/UEM. As carcaças foram resfriadas (1-2ºC) por 24h para, posteriormente, serem submetidas à avaliação quantitativa e avaliação da qualidade da carcaça. Imediatamente após o abate, também foram coletadas amostras do fígado para análises de expressão gênica do transportador de creatina, SLC6A8. Os resultados mostraram que não houve interação dos níveis de met+cis e GAA utilizados para as variáveis do desempenho, qualidade de carcaça e da carne e expressão gênica do transportador de creatina e análises sanguíneas, exceto para lactato (p=0,016), em que o maior nível de met+cis (0,50%) com a suplementação de GAA apresentou valores menores em relação a não suplementação. O GAA apresentou efeito significativo para a variável profundidade do músculo (PM) (p=0,001), a qual a suplementação aumentou a PM. Os níveis de met+cis apresentaram efeito significativo na variável ureia (p=0,040), apresentando menores valores para o nível de0,50%. No experimento II, foram utilizadas 40 fêmeas suínas, mestiças, de alto potencial genético e desempenho superior, com peso inicial médio de 75,26±0,87 kg, distribuídas em quatro tratamentos, dez repetições e um animal por unidade experimental. O delineamento experimental foi em blocos ao acaso em um esquema fatorial 2x2, constituídos de dois níveis CMH, 0,0% e 0,10%, e dois níveis de met+cis, 0,40%

e 0,44%. Os animais e a ração consumida foram pesados para determinação do ganho de peso (GPD), consumo de ração (CDR) e conversão alimentar (CA). No término do experimento, foi realizada a coleta de sangue de todos os animais para determinação das concentrações plasmáticas de ureia, creatinina, lactato, glicose e homocisteína. Ao atingirem o peso médio de 100± 3,78 kg, os animais foram abatidos, após jejum alimentar (24 horas), no abatedouro da Fazenda Experimental de Iguatemi - FEI/UEM. As carcaças foram resfriadas (1-2ºC) por 24h para, posteriormente, serem submetidas à avaliação quantitativa e avaliação da qualidade da carcaça. Imediatamente após o abate, também foram coletadas amostras de tecido do músculo *Longissimus dorsi* para análises de expressão gênica do transportador de creatina, SLC6A8. Os resultados obtidos indicam que não houve interação entre os níveis de met+cis no desempenho e expressão gênica do transportador de creatina ($p>0,05$). Porém, houve interação significativa para o ponto P3 de gordura ($p=0,004$), perda de água por gotejamento (PG) ($p=0,018$) e perda de água por descongelamento (PD) ($p=0,04$) e concentração plasmática de creatinina ($p=0,03$). Para o ponto P3, suplementação com CMH, apresentou valores menores de deposição de gordura. Para as variáveis PG e PD, o menor nível de met+cis com a suplementação de CMH apresentou maiores perdas de água. A creatinina plasmática apresentou menores concentrações com a suplementação de 0,10% de CMH, comparada à não suplementação, somente no nível de 0,44% de met+cis. Além disso, os níveis met+cis influenciaram a porcentagem de inibição do radical DPPH ($p=0,022$). Conclui-se que o GAA não demanda um maior aporte de met+cis nas dietas de suínos em terminação e sua suplementação melhorou a profundidade do músculo *Longissimus dorsi*, sem alterar o desempenho, qualidade de carne e expressão gênica do transportador SLC6A8 no fígado. A suplementação de CMH na dieta de suínos em terminação traz respostas evidentes sobre a qualidade da carne ao se utilizar níveis adequados de metionina+cisteína digestíveis, que também auxiliam na prevenção de danos oxidativos dos lipídeos no *Longissimus dorsi*.

Palavras-chave: aminoácidos, metabolismo proteico, nutrição, suinocultura

ABSTRACT

Two experiments were performed with the aim of evaluating the effects in the interaction of methionine levels + digestible cystine (met + cys) and supplementation of the guanidonoacetic acid (GAA) and monohydrate creatine (CMH) on performance, blood variables, carcass characteristics, meat quality and gene expression of creatine transporter (SLC6A8), in swine females at the finishing stage (75 to 100kg). In experiment I, 32 females were used, with initial average weight of 75.25 ± 0.91 kg, they were distributed in a randomized complete block design in a 2 × 2 factorial scheme, consisting of two levels of met + cys (0. 44% and 0.50%) and two GAA levels (0 and 0.05%), with eight replications and one animal per experimental unit. The animals were weighed, and the feed consumed were weighed to the determination of the weight gain (DWG), feed intake (DFI) and feed conversion (FC). At the end of the experiment, it was realized the blood collect from all animals, to determine plasma concentrations of urea, creatinine, lactate, glucose and homocysteine. When they reached the average weight of 100 ± 5.85 kg, the animals were slaughtered, after fasting (24 hours), at the slaughterhouse of the Experimental Farm of Iguatemi - FEI / UEM. The carcasses were cooled (1-2ºC) for 24h and subsequently submitted to quantitative evaluation and carcass quality evaluation. Immediately, after the slaughter, liver samples were also collected for gene expression analysis of the creatine transporter, SLC6A8. The results showed that there was no interaction of the met + cys and GAA levels, used for variables of performance, carcass and meat quality and creatine transporter gene expression and blood analysis, except for lactate ($p = 0.016$), wherein the highest level of met + cys (0.50%) with GAA supplementation presented lower values in relation to the non-supplementation. GAA showed a significant effect on the muscle depth variable (MP) ($p = 0.001$), in which the supplementation increased the MP. Met + cys levels presented a significant effect on the urea variable ($p = 0.040$), showed lower values for the 0.50% level. In experiment II, 40 crossbred swine females with high genetic potential and

superior performance were used, with an average initial weight of 75.26 ± 0.87 kg, distributed in four treatments, ten replications and one animal per experimental unit. The experimental design was in randomized blocks in a 2x2 factorial scheme, consisting of two CMH levels, 0.0% and 0.10%, and two levels of met + cys, 0.40% and 0.44%. The animals were weighed, and the feed consumed were weighed to the determination of the weight gain (DWG), feed intake (DFI) and feed conversion (FC). At the end of the experiment, it was realized the blood collect from all animals, to determine plasma concentrations of urea, creatinine, lactate, glucose and homocysteine. When they reached the average weight of 100± 3.78 kg, the animals were slaughtered, after fasting (24 hours), at the slaughterhouse of the Experimental Farm of Iguatemi - FEI / UEM. The carcasses were cooled (1-2ºC) for 24h and subsequently submitted to quantitative evaluation and carcass quality evaluation. Immediately, after the slaughter, Longissimus dorsi muscle tissue samples were also collected for gene expression analysis of the creatine transporter, SLC6A8. The obtained results indicate that there was no interaction between met + cys levels in performance and gene expression of creatine transporter ($p > 0.05$). However, there was significant interaction for the fat point P3 ($p = 0.004$), drip water loss (DL) ($p = 0.018$) and thawing water loss (TL) ($p = 0.04$) and plasma concentration of creatinine ($p = 0.03$). For the point P3. CMH supplementation presented lower values of fat depositions.For the DL and TL variables, the lowest met + cys level with CMH supplementation presented higher water losses. Plasma creatinine showed lower concentrations with 0.10% CMH supplementation compared to non-supplementation, only at the 0.44% met + cys level. In addition, met + cys levels influenced the percentage inhibition of DPPH radical ($p = 0.022$). It is concluded that GAA does not require a higher intake of met + cys in finishing swine 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. CMH supplementation in the finishing swine diets provides clear answers about the meat quality by

using adequate levels of digestible methionine + cysteine, which also assist in preventing oxidative damage of lipids in *Longissimus dorsi.*

Keywords: amino acids, protein metabolism, nutrition, pig farming

I. Introdução

A produção brasileira de carne suína alcançou, em 2018, seu melhor resultado desde 2006, com 3,7 milhões/ton., de acordo com o Relatório Anual da Associação Brasileira de Proteína Animal (ABPA). Esse crescimento otimista da produção gera cada vez mais interesse na área da nutrição, devido aos altos custos que essa demanda apresenta..

O principal conceito empregado nas pesquisas sobre nutrição de aminoácidos para suínos é o da proteína ideal, segundo o qual é necessário fornecer aos animais o balanço exato de aminoácidos, sem deficiências ou excessos, com o objetivo de satisfazer as exigências de mantença e máximo ganho de proteína corporal (ZAVIEZO, 1998).

Nesse sentido, muitos estudos acerca dos aminoácidos foram conduzidos até o momento, pois consumo de aminoácidos abaixo ou acima das exigências biológicas do animal pode restringir a taxa e a eficiência de crescimento. Por outro lado, o nível adequado de aminoácidos na ração pode melhorar a eficiência alimentar, a taxa de crescimento e, consequentemente, aumentar o rendimento econômico da atividade suinícola.

A metionina, juntamente com a cisteína (met+cis) ou simplesmente aminoácidos sulfurados (AAS), se destaca como um dos principais aminoácidos a ser considerado na formulação das rações para suínos, pois é considerado o segundo aminoácido limitante (OLIVEIRA NETO, 2014) em dietas à base de milho e farelo de soja, para animais em terminação.

Compostos biológicos poupadores de aminoácidos essenciais podem ser incluídos nas dietas desde que sejam viáveis economicamente e não comprometam o desempenho produtivo, a rentabilidade e qualidade da carne. O ácido guanidinoacético (GAA) é um composto poupador de arginina e glicina, pois é precursor direto de creatina, e desta forma, estes aminoácidos podem ser utilizados mais eficientemente para a síntese protéica, o que pode diminuir a concentração destes aminoácidos nas dietas e proporcionar um ótimo desempenho produtivo.

Por outro lado, o GAA utiliza grupamento metil para a formação de creatina, o que pode justificar maiores níveis de met+cis em combinação com a sua suplementação, enquanto que a suplementação de creatina poderia poupar met+cis.

A creatina monohidratada (CMH) já foi anteriormente suplementada em dietas para suínos, mas o objetivo era melhorar a qualidade da carne, devido à sua capacidade de amenizar a queda brusca do pH, evitando o desenvolvimento de carne PSE *(pale, soft and exudative).*

De maneira geral, nota-se que os estudos realizados até o momento avaliaram a suplementação de CMH ou GAA em rações para suínos sobre o desempenho e a qualidade da carne, pois são parâmetros de extrema importância econômica. No entanto, a interação destes compostos com a metionina deve ser considerada pois, tanto a creatina quanto o ácido guanidinoacético podem influenciar o aporte de metionina a ser fornecido na dieta de suínos.

II. Revisão de Literatura

Importância e metabolismo da metionina

A metionina é considerada um aminoácido essencial para suínos em todas as fases de criação (ROSTAGNO et al., 2011), enquanto que a cisteína é considerada um aminoácido nutricionalmente não essencial, pois pode ser sintetizada a partir da metionina. Esta relação metabólica entre a metionina e a cisteína explica o porquê das suas exigências serem expressas em conjunto, como met+cis, ou simplesmente como aminoácidos sulfurados (AAS) (NRC, 2012).

A metionina merece destaque especial, pois é o aminoácido iniciador do processo de tradução da síntese de proteínas eucarióticas (NELSON e COX, 2014) e também promove a formação de produtos especializados de grande importância no metabolismo.

A metionina e os produtos formados nas reações químicas enzimáticas estão envolvidos com a metilação do RNA, DNA e proteínas, assim como participa da glutationa, que é o antioxidante presente no citosol das células. Além disso, está envolvida com a divisão celular, digestão e absorção de lipídeos e ação anti-inflamatória (OLIVEIRA NETO, 2014).

O metabolismo da metionina pode ser dividido em metilação, remetilação e transulfuração (D'MELLO, 2003). Esses processos são explicados a seguir:

A metilação é a primeira etapa e é caracterizada pela transferência, através da enzima metionina adenosil transferase, da adenosina, proveniente de uma molécula de ATP, para a metionina, transformando-a em S- adenosil metionina (SAM).

A remetilação é o "retorno" da homocisteína em metionina, com a participação de duas enzimas: metionina sintase e betaína homocisteína metiltransferase, que catalisam a reação dos produtos 5-metil tetra hidrofolato e dimetilglicina, respectivamente.

A transulfuração possui duas funções principais, que inclui o catabolismo da cadeia de carbono da metionina, que se transformando-a em α-cetoglutarato, o qual vai a propionil CoA e entra no ciclo do ácido cítrico como succinil-CoA. Assim, como a transferência do grupo S (enxofre) da metionina para L- serina formando uma molécula de cisteína. O primeiro passo da transulfuração é a reação irreversível entre homocisteína e L-serina, catalisada pela enzima cistationina ß-sintase, que resulta na formação da cistationa. Posteriormente, a enzima cistationa Y-liase promove a transformação da cistationa em cisteína que, por sua vez, é responsável pela formação de vários compomentes importantes, como a cistina, taurina e glutationa (Figura 1).

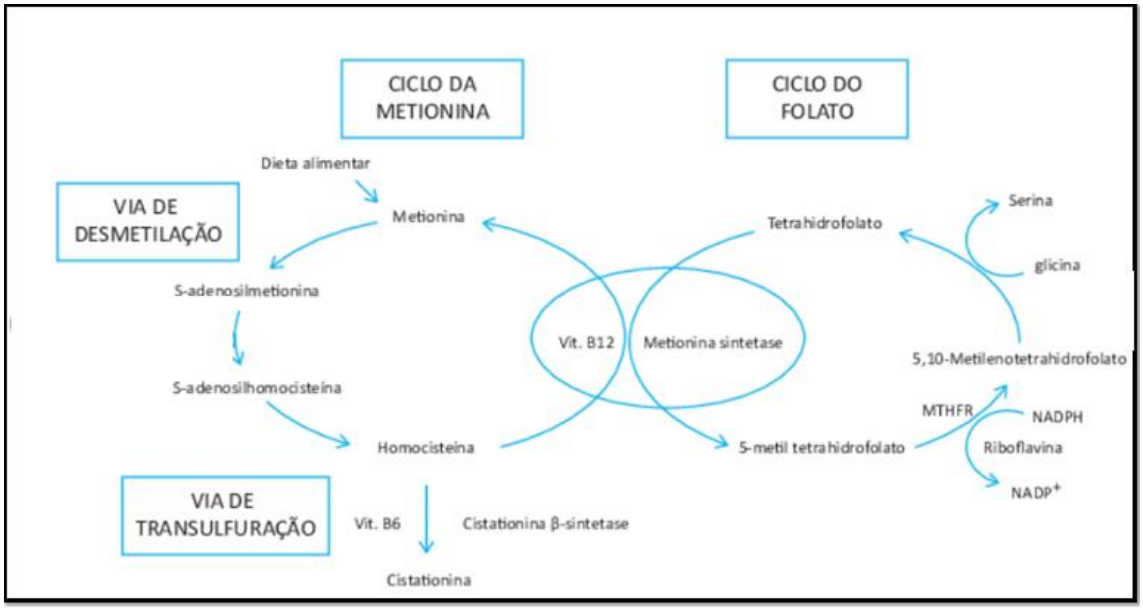

Figura 1: Metabolismo da metionina. Fonte: Amaral (2018).

Quando a metionina é fornecida em quantidades suficientes, a remetilação consome 38% da homocisteína e a transulfuração consome 62% da homocisteína presente na célula (STORCH et al., 1988). A regulação do ciclo da metionina ocorre sempre através da ação das enzimas envolvidas no ciclo. Por exemplo, em caso de doses baixas de metionina, ocorre a inibição das enzimas cistationina ß-sintase e cistationina Y-liase, enquanto que em altas concentrações, a atividades das enzimas N-metil-tetra hidrofolato-redutase e metionina sintase encontram-se reduzidas na célula (PRUDOVA et al., 2006).

Por envolver outros aminoácidos importantes, como a glicina, arginina, e dar origem a produtos importantes no metabolismo, como a creatina, a metionina torna-se um importante aminoácido a ser estudado.

Síntese e metabolismo do GAA

A *European Food Safety Authority* (2009) define o GAA, também conhecido como guanidinoacetato, como um aditivo nutricional sob o grupo funcional aminoácidos e apresenta a fórmula molécula: $C_3H_7N_3O_2$. O GAA pertence à classe dos compostos guanidínicos e é caracterizado pela presença do grupo guanidino, que é extremamente básico.

A síntese do GAA (Figura 2) se inicia com a transferência reversível de um grupo amidino da arginina para a glicina, através da ação da enzima arginina glicina amino transferase (AGAT), proporcionando a obtenção da ornitina e do GAA. Esta reação ocorre nos rins e, após a sua formação, a ornitina retorna para o ciclo da uréia e o GAA é transportado para o fígado, onde dará continuidade à formação da creatina (WALKER, 1979).

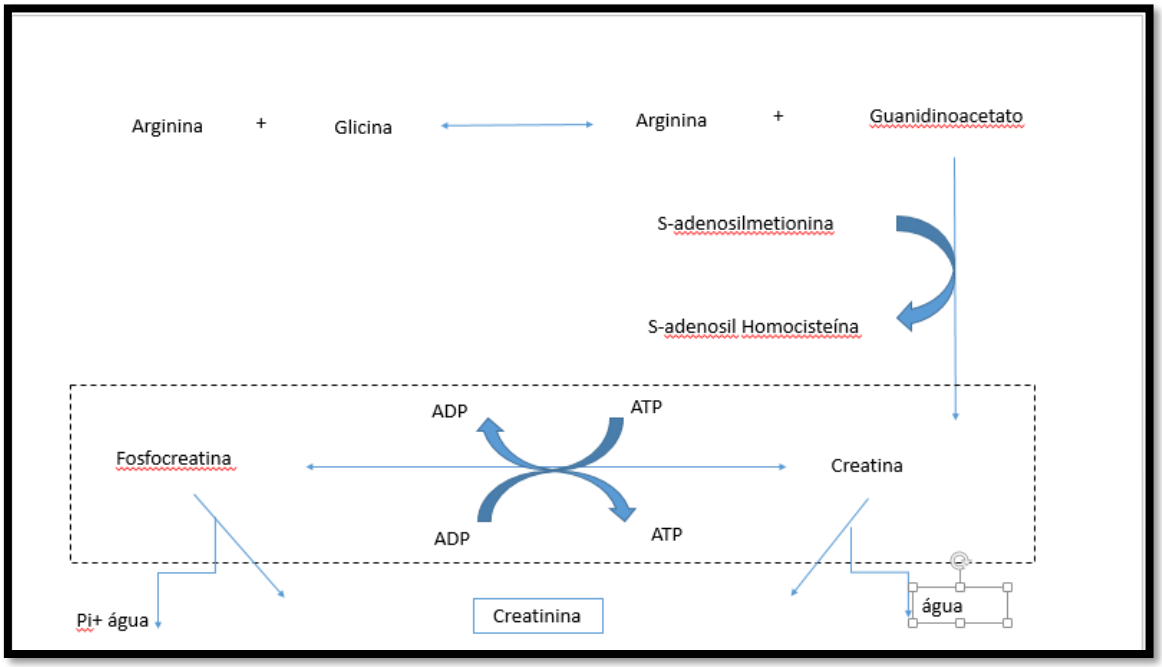

Figura 2: Metabolismo do ácido guanidinoacético

Fonte: Adaptado de Peixoto (2018).

Essa síntese de GAA, a partir de arginina e glicina, representa de 40 a 60% do total de creatina em ratos (GOLDMAN e MOSS, 1959), ou seja, uma grande quantidade de arginina é utilizada na produção de ácido guanidinoacético em mamíferos, indicando, portanto, que a sua suplementação pode poupar arginina (GULANDO et al., 2008).

Desta forma, a arginina é o substrato limitante na produção de GAA, o que pode ser problemático se os animais foram alimentados com dietas deficientes em arginina. Nos mamíferos, isso não é uma preocupação, pois a síntese de arginina é eficiente e contínua a partir do glutamato.

Para aves, a arginina é considerada um aminoácido essencial, pois devido à capacidade insuficiente de produzir arginina, esses animais exigem cerca de duas vezes mais arginina que os suínos. As aves não sintetizarem citrulina e, por isso, não podem fazer a conversão renal de citrulina para arginina (BOORMAN e LEWIS, 1971). Portanto, em aves e outros animais uricotélicos, podem surgir muitas consequências metabólicas, como diminuição do crescimento,

prejuízos em algumas funções metabólicas e menor desenvolvimento muscular (WIETLAKE et al., 1954), sendo necessária a suplementação de arginina nas dietas. Neste contexto, a suplementação com GAA pode funcionar como um "poupador" de arginina.

O GAA pode ser um aditivo mais eficiente em comparação com a creatina porque é menos oneroso e é quimicamente mais estável. O GAA é um precursor imediato da creatina que requer apenas uma transferência de grupo metil da S-adenosil-metionina para que ocorra a sua conversão em creatina (BAKER 2009).

Síntese e metabolismo da Creatina

A creatina ou ácido α-metilguanidinoacético é um composto encontrado naturalmente nos alimentos, principalmente nas carnes e nos peixes. Em humanos, 95% da creatina total são encontrados no músculo esquelético. Os 5% restantes se distribuem entre o encéfalo, fígado, rins e testículos. No entanto, apenas metade da necessidade corporal diária de creatina (~1 g/dia) é obtida na dieta, o restante é obtido pela síntese endógena de creatina (DEMINICE et al, 2009).

A Figura 3 mostra a síntese endógena desse composto nitrogenado que se inicia nos rins, com a formação do GAA, que será transportado para o fígado, onde acontece, de fato, a formação da creatina.

No fígado, ocorre a transferência irreversível de um grupo metil da metionina através da S-adenosilmetionina para o GAA, reação que é catalisada pela enzima guanidinoacetato N-metiltransferase (GAMT), sintetizando, finalmente, a S-adenosil homocisteína e creatina (PERSKY e BRAZEAU, 2001).

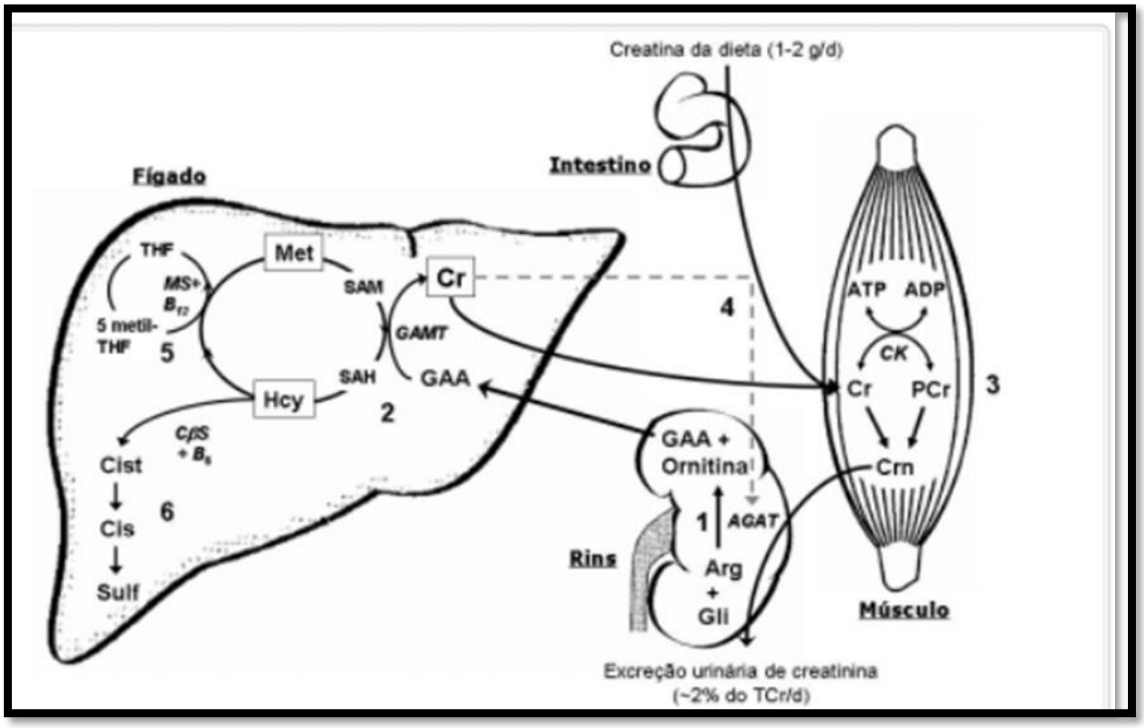

Figura 3: Metabolismo da Creatina. FONTE: Deminice et al., 2007.

Legenda: 1-A creatina inicia sua síntese nos rins com a formação do guanidinoacetato. 2- O GAA é exportado para o fígado onde participa da transmetilação para formação de creatina catalisada pela enzima S-adenosilmetionina:guanidinoacetato N-metil transferase (GAMT). 3- metabolismo energético da creatina 4- Um aumento na ingestão de creatina inibe a ação da enzima arginina:glicina amidinotransferase (AGAT), como consequência, a demanda de metilação é diminuída, reduzindo a formação de S- adenosil homocisteína (SAH) e homocisteína. Sob condições normais (ingestão normal de creatina), a metionina irá interagir com GAA formando a homocisteína. 5-remetilação. 6-transulfuração. SAM: S-adenosilmetionina, THF: Tetrahidrofolato, MS: metionina sintase, CBs: cistationina-B-sintase.

A creatina da dieta é absorvida na sua forma molecular intacta no intestino e após sua absorção intestinal, a creatina é liberada para os vários tecidos do corpo, incluindo o coração, musculatura lisa, cérebro e testículos. Entretanto, a grande maioria dos estoques corporais (cerca de 95%) encontra-se localizada nos músculos esqueléticos (SOUZA et al., 2006). E a maior parte (60 a 70%) está armazenada como fosfocreatina, que é incapaz de passar por membranas (GREENHAFF, 1997).

Em aves, ao contrário do que acontece nos mamíferos, as duas etapas de formação da creatina ocorrem no fígado. Possivelmente, esta seja a razão da creatina dietética induzir maiores respostas positivas em aves que em alguns mamíferos (WALKER, 1979). Em dietas

exclusivamente vegetais, a necessidade de arginina para a síntese de creatina é aumentada, uma vez que os vegetais não se constituem em fontes de creatina. Por outro lado, os subprodutos de origem animal são ricas fontes de creatina, e dietas suplementadas com estes ingredientes podem ser consideradas poupadoras de arginina no metabolismo das espécies uricotélicas.

Síntese e importância da fosfocreatina

A fosfocreatina é a reserva de energia armazenada nos tecidos, principalmente o muscular, e é capaz de realizar a ressíntese de ATP de forma rápida, durante sua contração, pois libera fosfato e creatina quando degradado (GUIMARÃES FERREIRA, 2014). O processo de formação da fosfocreatina é realizado na mitocôndria no período de repouso muscular e se inicia com a formação do ATP. Ao entrar na célula a creatina sofre a ação da enzima creatina quinase, responsável pela sua fosforilação reversível à fosfocreatina (GOMIDE et al., 2013).

A creatina quinase é uma enzima que possui um papel central no metabolismo energético, principalmente para tecidos com alta demanda energética, como cérebro, músculo cardíaco e esquelético. Nesses tecidos ela funciona como um efetivo sistema de tampão para os níveis celulares de ATP, sendo então uma enzima crucial para a homeostase energética (WYSS et al., 2000). Atualmente, são conhecidas cinco isoenzimas da creatina quinase, sendo duas mitocondriais e três citoplasmáticas, entre elas está a creatina quinase-MM, encontrada predominante no músculo esquelético.

A fosfocreatina fica retida no interior da célula até que ocorram alterações no pH celular ou aumento da demanda energética, através do seguinte processo: a fosfocreatina, devido principalmente ao tamanho e facilidade de difusão (YOSHIZAKI et al., 1990), move-se a partir da mitocôndria para o citoplasma, onde é clivada formando creatina e ATP a partir do ADP. O ATP regenerado é convertido novamente em ADP por uma ATPase muscular e o grupo fosfato gerado é utilizado para o trabalho metabólico, como a contração do músculo. A creatina difunde-se de volta para a mitocôndria para ser usada novamente no ciclo (GUIMARÃES-FERREIRA, 2014), esse processo é denominado teoria do “lançamento” (WYSS e KADDURAH-DAOUK,

2000), demonstrada na figura 4. Quando a taxa de utilização de ATP excede sua capacidade de geração por outras vias metabólicas o sistema PCr/CK é importante, pois possui elevada taxa de geração de ATP, sendo assim, essencial nas situações de alta demanda metabólica (GUIMARÃES-FERREIRA, 2014).

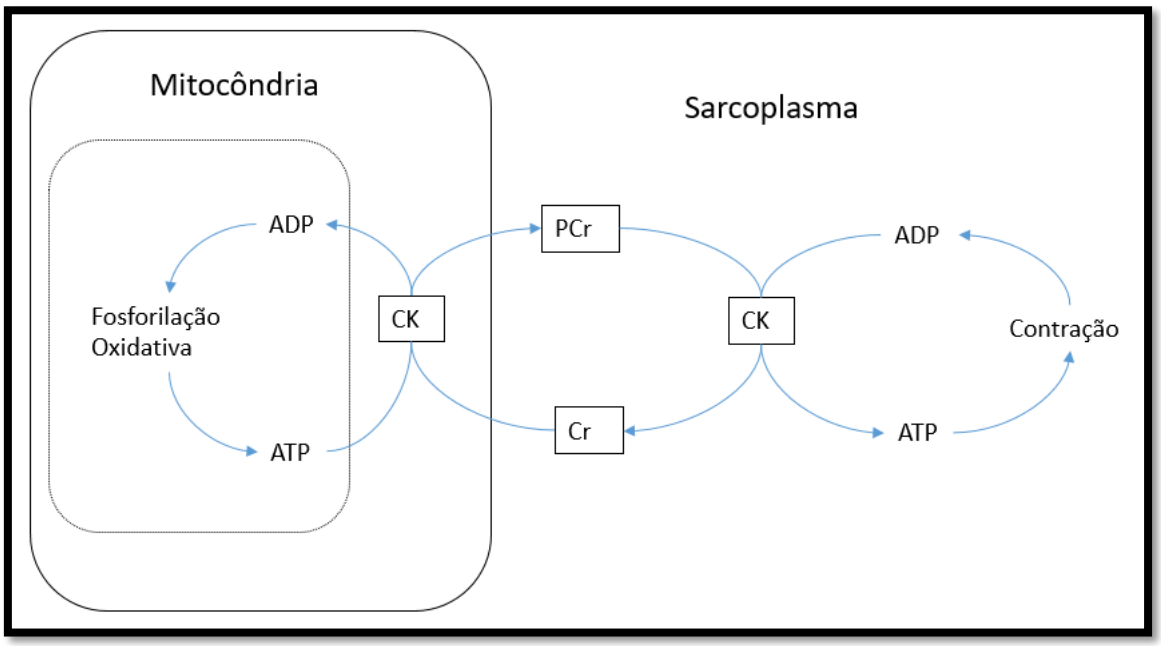

Figura 4: Sistema de "lançadeira" da fosfocreatina.

Fonte: Adaptado de Guimarães Ferreira (2014).

Legenda: ADP: Adenosildifosfato, ATP: Adenosiltrifosfato, CK: Creatina kinase, Cr: Creatina

Homocisteína e sua relação com compostos dietéticos

A homocisteína é um aminoácido sulfurado, não essencial e não proteinogênico, formado a partir da desmetilação da metionina (AMORIN et al., 2011). Em níveis normais, a homocisteína desempenha funções essenciais no organismo animal, incluindo sua remetilação a metionina ou transulfuração a cisteína, disponibilizando estes aminoácidos para a síntese proteica. A homocisteína está envolvida na reciclagem do tetraidrofolato, no metabolismo da colina (via betaína) e na geração da S-adenosil metionina que, por sua vez, pode ser utilizada para a biossíntese de poliaminas ou como cofator para a síntese de outras biomoléculas.

O metabolismo, em geral, mantém as concentrações de homocisteína em níveis baixos, mas a sua elevação no sangue possui causas multifatoriais, como fatores patológicos e fisiológicos, mas principalmente fatores genéticos e nutricionais (AMORIN et al., 2011). Dentre as bases nutricionais, encontram-se a deficiência de vitaminas que estão envolvidas no metabolismo da homocisteína, como as vitaminas B6 (piridoxina), B12 e folato ou a um excesso de metionina na dieta (MARTINEZ-VEGA et al., 2015).

A enzima cistationina ß-sintase (CBS) funciona como reguladora do ciclo da metionina, assim, níveis elevados de metionina elevam a concentração de SAM e reduzem a atividade das enzimas envolvidas na remetilação de homocisteína, e estimulam a atividade da CBS, responsável pela perda irreversível de homocisteína. Por outro lado, quando há níveis baixos de metionina, as concentrações de SAM diminuem e a atividade da CBS retorna ao normal, conservando homocisteína para remetilação. O resultado deste controle é uma conservação de metionina quando as concentrações de metionina são baixas e remoção do excesso de homocisteína quando as concentrações de metionina estão elevadas (SPUTINIK et al., 2004).

Altos níveis de homocisteína nos tecidos de suínos, ratos e humanos têm demonstrado induzir à formação de espécies reativas de oxigênio (HUANG et al., 2013), os quais podem danificar qualquer classe de estrutura celular e promover um stress oxidativo (desequilíbrio entre radicais livres e moléculas antioxidantes). Em humanos, altos índices de homocisteína estão associados a doenças, a exemplo das doenças cardiovasculares (aterosclerose e trombose) doença de Alzheimer, diabetes, osteoporose e doenças renais (THALER et al., 2013).

Evidências experimentais sugerem que o aumento na concentração sanguínea de homocisteína leva à disfunção endotelial e que as lesões resultantes diminuiriam a vasodilatação, podendo também ocorrer dano oxidativo, proliferação de células do músculo liso endotelial, oxidação de lipoproteínas de baixa densidade e redução da produção de óxido nítrico (STEAD et al., 2001).

Acredita-se que as alterações no endotélio vascular, causadas pela hiperhomocisteinemia, são mediadas principalmente pelo efeito tóxico das formas oxidadas deste aminoácido (AMORIM

et al., 2011). Quando liberada para o sangue, a homocisteína é rapidamente auto-oxidada, originando a homocistina e homocisteína-tiolactona, bem como formas ativas de oxigênio consideradas citotóxicas, tais como superóxido, H_2O_2 e radical hidroxila (STEFANELLO et al., 2005).

Além disso, altos níveis de homocisteína reduzem a atividade da glutationa peroxidase (SHARMA et al., 2006, VENÂNCIO et al., 2010), que tem como principais funções: ação antioxidante no citosol, metabolismo de nutrientes, expressão de genes, síntese de proteínas e DNA, proliferação celular e sinalização para controlar apoptose (BAUCHART- THEVRET et al., 2009).

De acordo com Sharma et al. (2006), aproximadamente 135 genes são influenciados, direta ou indiretamente, pelos níveis sanguíneos elevados de homocisteína. Segundo os autores, a maioria destes genes está associada com o metabolismo da glicose, insulina, lactato, cálcio, cloreto, lipídios e também afeta o sistema imune (citocínas e atividade antioxidante), sistema circulatório (coagulação sanguínea, pressão arterial e vasodilatação), sistema nervoso e o ciclo celular.

Em estudos com suínos, França et al. (2006) avaliaram o efeito de uma dieta rica em metionina, por um período de 30 dias, sobre os níveis sanguíneos de homocisteína. Os animais do grupo controle (sem excesso de metionina) não apresentaram alterações nos níveis de homocisteína. Entretanto, os animais que receberam a dieta com excesso de metionina apresentaram um aumento na homocisteína quando comparado aos níveis apresentados no início. Os autores também observaram aterogênese na artéria ilíaca de animais submetidos a dietas ricas em metionina, que está relacionado a hiperhomocisteinemia induzida pelo excesso de metionina.

Em outro estudo, Zhang et al. (2009) avaliaram o efeito da deficiência de vitamina B6, por um período de seis semanas, sobre o metabolismo da homocisteína em suínos na fase inicial e concluíram que o excesso de metionina ou a deficiência de vitamina B6 na dieta podem levar os suínos a apresentarem aumento nos níveis sanguíneos de homocisteína, caracterizando um quadro de hiper-homocisteinemia.

No entanto, Stead et al. (2001) verificaram que animais suplementados com GAA apresentaram nível plasmático de homocisteína 49% maior do que o grupo controle, (animais não suplementados, mas os animais que foram suplementados com creatina apresentaram redução de 27% de homocisteina plasmática. A serina pode afetar o metabolismo da homocisteína por agir como co-substrato para a reação da CBS, fornecendo unidades de um carbono ligado ao folato, que fornece o grupamento metil para a reação de síntese de metionina.

Estudos foram realizados para avaliar os efeitos da suplementação de creatina sobre os níveis de homocisteína em animais. Stead et al. (2006) estudaram a modulação da demanda de metilação pela ingestão de creatina e ácido guanidinoacético sobre a formação de homocisteína, onde utilizaram três grupos de ratos que receberam 0,40% de creatina, 0,36% de ácido guanidinoacético e uma dieta controle. A ingestão de creatina proporcionou uma redução de 25% e a de ácido guanidoacético aumento de 50% nos níveis de homocisteína, sem alteração na metionina plasmática. Esses autores ainda afirmaram que a síntese de creatina, em ratos, é um importante determinante do seu metabolismo e dos níveis plasmáticos.

Muitas vezes, a hiperhomocisteinemia pode ser corrigida, pelo menos em parte, promovendo os mecanismos de remoção da homocisteína através da trans-sulfuração para cisteína, remetilação para metionina, ou em combinação (OSTOJIC et al., 2017).

Transportadores de GAA e creatina

A creatina é transportada, dos tecidos periféricos (músculo esquelético, coração, cérebro e testículos) para dentro das células através de um processo saturável pelo transportador sódio-cloro dependente, chamada de CRT1 ou SLC6A8 (LI et al., 2018).

A constante de Michaelis-Menten do transportador de creatina varia entre 20 e 60 µM, dependendo do local, isso explica as diferenças na concentração de creatina total nos diferentes tecidos (PERSKY & BRAZEAU, 2001).

A administração exógena de GAA pode aumentar efetivamente os níveis de creatina nos tecidos (MCBREAIRTY et al., 2015), sugerindo seu importante papel na bioenergética. Além de transportado através do CRT1 (SLC6A8), o GAA pode ser também transferido para a célula via transportadores de proteína para taurina (SLC6A6) e ácido gama-aminobutírico (GAT2), e via difusão, através de membrana plasmática (OSTOJIC et al., 2016), (Figura 5).

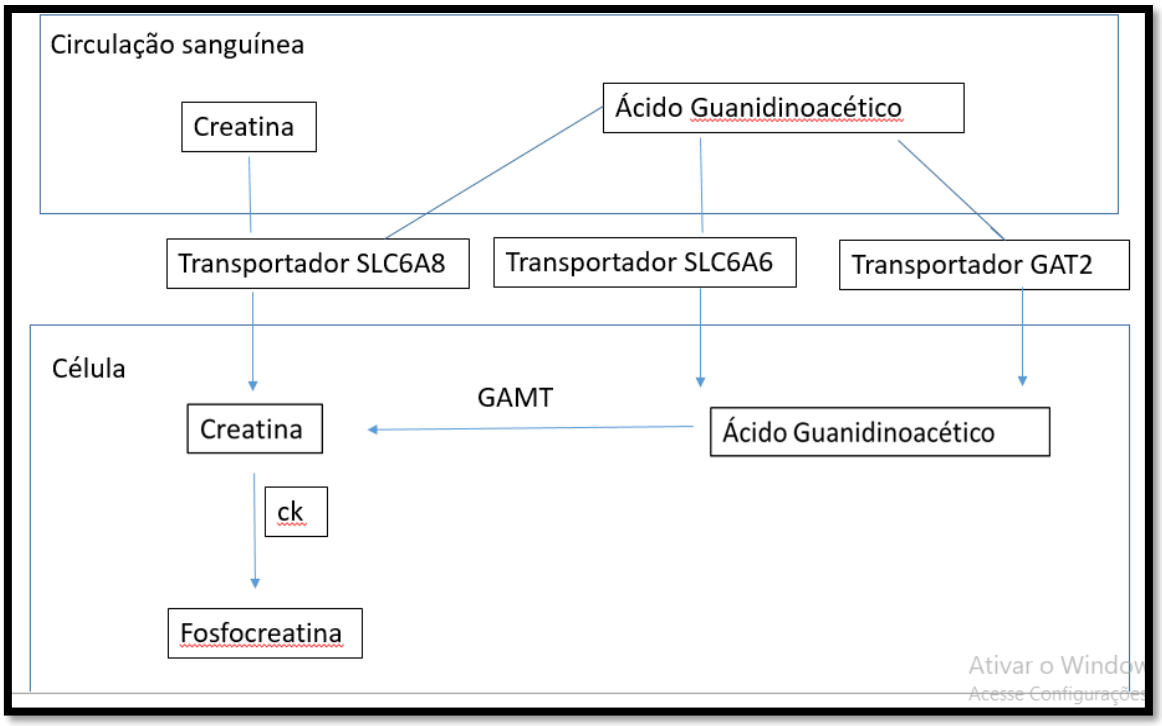

Figura 5: Canais de transporte da creatina e ácido guanidinoacético.

Fonte:Adaptado de Ostojic cbet al. (2017).

Legenda: SCL6A8: transportador de creatina, SLC6A6: transportador de taurina, GAT2: Transportador do ácido gama aminobutírico, GAMT: N-metiltransferase guanidinoacetato, CK: creatina quinase.

A suplementação de creatina pode ocasionar maior expressão do transportador de creatina em animais submetidos a dietas suplementadas com GAA e CMH, o que justifica os melhores resultados no desempenho e qualidade da carne desses animais (Li et al. 2018).

O CRT1 é expresso em grandes quantidades no cérebro, intestino e músculo esquelético, onde desempenha um papel crucial na distribuição de creatina para tecidos alvo. No entanto, o fornecimento extra de creatina pode ser ineficaz para a captação celular (OSTOJIC et al., 2017).

Em relação a creatina, a afinidade de CRT1 para GAA é 10 vezes menor (Km = 269–412 mM), indicando baixo potencial de GAA para ser transportado através deste canal quando a creatina é altamente disponível (TASHIKAWA et al., 2008).

No entanto, quando CRT1 é saturado com creatina, o que pode ocorrer em casos de suplementações de creatina na dieta, a provisão de GAA extra pode ter como alvo outros canais e impulsionar níveis celulares de GAA e creatina, em comparação com a creatina sozinha. Esta estratégia de co-administração pode ser particularmente eficaz em casos de necessidades bioenergéticas avançadas, a exemplo, do exercício físico, densidade limitada de CRT1 em tecidos específicos ou em condições de desordens neurodegenerativas.

Suplementação dietética de GAA e creatina

Suínos

A utilização de GAA (0, 0.05, 0.10, 0.15,e 0.20%) para leitões na fase de creche (21 a 63 dias de idade) foi avaliada por Teixeira et al. (2017) e foi observada uma tendência de aumento no ganho de peso diário (P= 0,069) até o nível de 0,97% de inclusão de GAA, e o nível de creatina quinase aos 42 dias apresentou uma tendência (P=0,077) de aumento que ocorreu de forma quadrática até o nível de 0,085% de inclusão do GAA. No entanto, ao avaliarem níveis de 0,00; 0,08; 1,20 e 2,00 g/kg de GAA em rações para suínos, Wang et al. (2012) verificaram que o desempenho não foi influenciado. Em ambos estudos, os níveis de metionina estavam de acordo com a recomendação para cada fase.

Dietas com nível de metionina de 0,22% e suplementadas com 0,8% de CHM e 0,01% de GAA, para suínos na fase de terminação, foram estudadas por Li et al. (2018) e verificaram que a CMH aumentou o consumo diário de ração, e o GAA, além de aumentar o consumo, aumentou o ganho de peso diário desses animais.

Ao avaliarem a suplementação de CMH (0, 12.5, 25 or 50 g/dia por 5 dias) em dietas que atendiam as exigências nutricionais de suínos (Duroc e Landrace), Young et al. (2005) observaram que a suplementação de creatina proporcionou aumento linear no ganho de peso

diário, sendo mais pronunciado em fêmeas Landrace comparado com as fêmeas Duroc. Esse aumento no ganho diário de peso pode ser devido ao aumento da retenção de água no músculo ou ao aumento da deposição de proteína muscular, podendo, então, inferir que os principais efeitos da suplementação de GAA e CMH estão associados à qualidade da carne, sendo que o desempenho é um resultado secundário.

Além do interesse no desempenho, a suplementação com GAA e creatina visa melhorar a qualidade de carne, pois é considerada uma estratégia nutricional para aumentar a disponibilidade de ATP de outras fontes que não envolva a via glicolítica, o que pode ser uma importante ferramenta para amenizar o declínio do pH *post-mortem*, preservando a qualidade da carne (BERG et al., 2003).

A capacidade de retenção de água é um importante índice de qualidade, pois está diretamente relacionada com as propriedades sensoriais da carne (suculência, textura, sabor) (ZEOLA, 2007). Adicionalmente, quanto maior a capacidade de retenção de água da carne, menores são as perdas por gotejamento e evaporação durante o armazenamento, transporte e comercialização, o que possibilita maior rentabilidade (RAMOS e GOMIDE, 2017).

No metabolismo animal, a metionina, na forma de SAM, atua como doador de grupos metil para a biossíntese de várias substâncias, entre elas a creatina. Quando está na forma de fosfocreatina, a creatina constitui uma importante reserva energética para o músculo, sendo prontamente utilizada na resíntese de ATP (ADP + fosfocreatina = ATP + creatina), à medida que este vai sendo utilizado no metabolismo muscular. A produção de ATP a partir da fosfocreatina é particularmente importante, uma vez que não envolve a via glicolítica e a formação de ácido lático, amenizando a queda de pH durante a conversão do músculo em carne (JANICKI e BUZALA, 2013).

O efeito da creatina em reduzir a velocidade e intensidade de queda do pH post-mortem foi investigado por alguns autores. Trabalhando com uma suplementação diári de 25g de CMH, durante cinco dias antes do abate, Maddock et al. (2002) observaram maior pH inicial (medido 45 minutos após o abate) para os animais suplementados (pH = 6,2), quando comparado ao grupo

não suplementado (pH = 5,9). Berg e Allee (2001), Young et al. (2005) e Machado et al. (2008) também relataram maiores valores de pH inicial do músculo de suínos suplementados com CMH. Além do pH inicial, os autores observaram efeito da suplementação com CMH em outras variáveis de qualidade da carne, como menor queda do pH final, medido 24 horas após o abate (BERG e ALLEE, 2001) e maiores valores de a*, ou seja, carnes mais vermelhas (MACHADO et al., 2008). É válido considerar que nos trabalhos citados houve o fornecimento adequado de metionina, o que permite a síntese de creatina e pode reduzir a velocidade e intensidade de queda do pH post-mortem, melhorando atributos de qualidade da carne.

Níveis de CMH e GAA ainda são extensivamente discutidos entre diferentes autores. De acordo com Maddock et al. (2002), 25 g de creatina por 5 dias antes do abate atenua os efeitos negativos causados pelo estresse animal e aumentam os genes RyR1, que é uma proteína formadora do canal de liberação de Ca^{++} no retículo sarcoplasmático de células musculares que, em casos de funcionamento inadequado, desencadeia um acúmulo intracelular de Ca^{++} e, consequentemente, uma contração muscular permanente (CAMPOS et al., 2014).

No entanto, o período e o nível de suplementação de CMH também podem influenciar as respostas. Berg et al. (2000) suplementaram 20 g/dia/animal, por 15, 10 ou 5 dias antes do abate e verificaram que, enquanto a utlização de CMH por 5 dias antes do abate melhora atributos da carne, a utlização acima de 5 dias pode trazer prejuízos em relação à qualidade. Em outro experimento, Berg et al. (2001) avaliaram o efeito da suplementação de 25 g de creatina/dia/suíno durante 10 ou 5 dias antes do abate e os resultados sugeriram que independentemente do tempo de uso, a suplementação com CMH amortece o pH pós-mortem e reduz a perda de água em 48 horas.

O'Quinn et al. (2000) seguiram um protocolo semelhante com 25 g de creatina/dia/animal durante 10 dias, mas não observaram alterações nas características de carcaça. Berg et al. (2003) sugeriram que a suplementação de creatina em dietas de suínos, por mais de 5 dias, influencia positivamente a qualidade da carne, pois a carne de animais suplementados com CMH apresentou com mais células musculares intactas e maior capacidade de retenção de água

Apesar dos diferentes protocolos utilizados, o uso da creatina traz benefícios. A adição de creatina monohidratada na dieta de suínos pode ser um amortecedor contra o ácido láctico produzido, reduzindo a forte queda no pH de carne PSE, alterando também a sua cor (YOUNG et al., 2005). James et al. (2000) concluíram que a adição de creatina não influenciou o desempenho de suínos em terminação, mas aumentou a firmeza da carne e reduziu a perda de água medida aos14 dias após o abate.

O aumento de ácido guanidinoacético pode aumentar o pH e reduzir a perda de água, a força de cisalhamento e o valor de b* da carne, devido à redução da peroxidação lipídica, através do aumento da atividade de algumas enzimas associadas ao metabolismo de radicais livres (WANG et al., 2012). Foi ainda verificado que o ácido guanidinoacético aumenta a creatina total e níveis de fosfocreatina, melhorando a qualidade da carne, através da melhora na cor, menor perda de água e força de cisalhamento (LIU et al., 2015, WANG et al., 2012).

O efeito da suplementação de CMH e GAA na qualidade da carne de suínos foi recentemente estudado por Li et al. (2018), que verificaram que essas dietas podem aumentar o pH medido 45 minutos após o abate, a concentração de creatina e fosfocreatina muscular e ainda aumentar a expressão do transportador de creatina, com isso, a carne apresentou maior capacidade de retenção de água e maciez.

Anteriormente, Li et al. (2016) e Li et al. (2017) observaram que a suplementação com ácido guanidinoacético e creatina aumenta a creatina e fosfocreatina e atrasou a glicólise post-mortem, o que diminuiu a concentração de ácido lático, favorecendo a qualidade da carne.

Em revisão, Janick e Buzala (2013) demonstraram que suínos alimentados com dietas suplementadas com creatina respondem com aumento no rendimento de carcaça, melhorando a retenção de proteínas musculares e água no esqueleto.

De acordo com estudos realizados por Machado et al. (2008), em que avaliaram o sulfato de magnésio, a creatina monohidratada e a associação de ambos, no período de 5 dias antes do abate, os animais suplementados com creatina monohidratada apresentaram valor de pH, 45 min após o abate, superior ao do grupo controle e os animais suplementados com sulfato de magnésio

com ou sem adição de creatina monohidratada, também apresentaram aumento significativo no valor do pH, medido 24 horas após o abate.

Aves

O GAA pode ter propriedades promotoras sobre o crescimento e eficiência alimentar de frangos de corte quando adicionado em dietas à base de milho e farelo de soja (RINGEL et al., 2008), porém Halle et al. (2006) encontraram efeitos inconsistentes sobre o desempenho produtivo.

A suplementação dietética de creatina ou ácido guanidinoacético em dietas para patos aumentou significativamente ($P < 0,05$) o ganho de peso total e melhorou a taxa de conversão alimentar.A suplementação de GAA associado a níveis de metionina maiores do que o exigido pelos animais também aumentou o rendimento de carcaça de patos, além de melhorar a qualidade da carne, através de maiores valores de pH (IBRAHIN ET AL., 2019).

Um aumento da creatina muscular foi verificado com a utilização de GAA em frangos (MICHIELS et al., 2012). A suplementação de dietas vegetais com GAA, segundo estes autores, melhora o desempenho e as características de carcaça sem comprometer atributos importantes como a capacidade de retenção de água. A suplementação de GAA pode ser particularmente importante em dietas para linhagens de frangos de corte de rápido crescimento inicial, devido à grande demanda de energia para suprir os níveis de creatina muscular (BROSNAN et al., 2009) e ressíntese de ATP a partir da creatina pela fibra muscular cardíaca de frangos dessas linhagens modernas (NAIN et al., 2008), porém Stahl et al. (2003) encontraram apenas melhora na conversão alimentar pelo uso de creatina na dieta das aves.

Em frangos de corte, houve um aumento gradual no conteúdo de creatina muscular, com o uso de GAA. Uma hora *post-mortem*, o conteúdo de ATP muscular foi significativamente maior quando comparado àqueles que não receberam o GAA, provavelmente devido à melhoria no metabolismo energético celular (LEMME et al., 2007). Bryant-Angeloni (2010) suplementou a

dieta de frangos de corte com 1,2% de GAA e encontrou melhor desempenho e o autor concluiu que a razão para esta resposta positiva do GAA pode ser atribuída ao efeito poupador de arginina.

Frangos alimentados com dietas contendo dois níveis de GAA e diferentes níveis de energia metabolizável tiveram a conversão alimentar influenciada, sendo que aqueles que se alimentaram com as dietas suplementadas com GAA, independentemente dos níveis de energia, apresentaram melhores resultados (ABUDABOS et al., 2014). Jayaraman et al. (2018), verificaram que suínos em crescimento e terminação submetidos a dietas com GAA (0,08% e 0,12%) apresentaram melhor desempenho, os níveis de aminoácidos seguiram as recomendações de exigência para cada fase.

Nenhum efeito da suplementação com CMH (0,5 e 0,10%) sobre os atributos de qualidade da carne de frangos foi verificado por Ringel et al. (2008). Já Stahl et al. (2003) e Nissen e Young (2006) observaram alteração do pH e luminosidade da carne do peito de frangos quando suplementados com o GAA. Por outro lado, Ringel et al. (2008) relataram maior rendimento de peito com suplementação dietética de GAA (0,6 e 1,2 g/kg).

Dado o exposto, a importância da metionina para a nutrição animal é evidente, assim como a suplementação de GAA e CMH podem proporcionar respostas positivas em animais monogástricos. Porém, a relação entre esses dois compostos e a metionina não pode ser negligenciada, pois é necessário compreender se ambos influenciam ou não no aporte de metionina. Neste contexto, e através de todo conhecimento já existente acerca do assunto, novas pesquisas podem ser realizadas abrangendo a metionina e a suplementação desses dois compostos.

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III. OBJETIVOS

Objetivos Gerais:

Avaliar a possível interação entre níveis de met+cis digestíveis com o GAA e CMH na dieta de fêmeas suínas, dos 75 aos 100 kg.

Objetivos Específicos:

- Mensurar o consumo diário de ração, ganho de peso diário e a conversão alimentar de fêmeas suínas, dos 75 aos 100 kg, recebendo dietas com diferentes níveis de met+cis digestíveis e suplementação de GAA e CMH,

- Estimar níveis sanguíneos de glicose, ureia, creatinina, lactato e homocisteína de fêmeas suínas, dos 75 aos 100 kg, recebendo dietas com diferentes níveis de met+cis digestíveis e suplementação de GAA e CMH,

- Determinar o efeito de níveis de met+cis digestíveis e suplementação de GAA e CMH sobre o peso de carcaça quente e resfriada, rendimento de carcaça, perda de peso de carcaça no resfriamento, profundidade do músculo *Longissimus dorsi*, espessura de toucinho e rendimento de carne magra de fêmeas suínas, dos 75 aos 100 kg,

- Analisar as variáveis de qualidade de carne (pH, cor, perda de água por gotejamento, no descongelamento e na cocção e força de cisalhamento) de fêmeas suínas, dos 75 aos 100 kg, submetidos a dietas com diferentes níveis de met+cis digestíveis e suplementação GAA e CMH,

- Avaliar a expressão gênica do transportador de creatina, SLC6A8, de fêmeas suínas, dos 75 aos 100 kg, recebendo dietas com diferentes níveis de met+cis digestíveis e suplementação de GAA e CMH,

IV. GUANIDINOACETIC ACID SUPPLEMENTATION INCRESEASES MUSCLE DEPTH IN FINISHING SWINE FEMALE WITHOUT DEMANDING LARGER INTAKE OF METHIONINE + CYSTEINE[1]

Abstract: The objective of this work was to evaluate the interaction of guanidinoacetic acid (GAA) with methionine + cysteine (met + cys) levels in finishing swine diets about the performance, carcass characteristics, meat quality, blood variables, gene expression of creatine transporter and antioxidant action. The experimental design was in randomized blocks in a 2x2 factorial scheme, consisting of two levels of guanidinoacetic acid (0% and 0.05%) and two levels of met + cys (0.44% and 0.50%) and n = 32. There was no interaction of met + cys and GAA levels under performance variables, carcass and meat quality and creatine transporter gene expression, except for plasma lactate (p = 0.016), wherein at the highest met + cys level the GAA supplementation (0.05%) showed lower plasma lactate concentration in relation to non-supplementation. *Longissimus dorsi* muscle depth (DM) (p = 0.001) showed an isolated effect for the evaluated GAA levels, in which the supplementation increased the DM. Urea presented an isolated effect (p = 0.040) for met + cys levels, showing lower plasma concentration for the highest level. It was concluded that GAA does not require a higher intake of met + cys in finishing swine 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.

Keywords: amino acids, creatine, protein metabolism, swine

Resumo: O objetivo do trabalho foi avaliar a interação do ácido guanidinoacético (GAA) com níveis de metionina+cisteína (met+cis) em dietas para suínos na fase de terminação sobre o desempenho, características de carcaça, qualidade de carne, variáveis sanguíneas, expressão gênica do transportador de creatina e ação antioxidante. O delineamento experimental foi em blocos ao acaso em um esquema fatorial 2x2, constituídos de dois níveis de ácido guanidinoacético (0% e 0,05%) e dois níveis de met+cis (0,44% e 0,50%) e n=32. Não houve interação dos níveis de met+cis e GAA sob as variáveis do desempenho, qualidade de carcaça e da carne e expressão gênica do transportador de creatina, exceto para lactato plasmático (p=0,016), em que no maior nível de met+cis a suplementação de GAA(0,05%) apresentou menor concentração plasmática de lactato em relação a não suplementação. A profundidade do músculo *Longissimus dorsi* (PM) (p=0,001) apresentou efeito isolado para os níveis de GAA avaliados,em que a suplementação aumentou a PM. A ureia apresentou efeito isolado (p=0,040) para os níveis de met+cis, apresentando menor concentração plasmática para o maior nível. Conclui-se que o GAA não demanda um maior aporte de met+cis nas dietas de suínos em terminação e sua suplementação melhorou a profundidade do músculo *Longissimus dorsi*, sem alterar o desempenho, qualidade de carne e expressão gênica do transportador SLC6A8 no fígado.

Palavras-chave: aminoácidos, creatina, metabolismo proteico, suínos

Introduction

Methionine, together with cysteine (met + cys) or simply sulfur amino acids (SAA), stands out as one of the main amino acids to be considered in the feed formulation for swine,

[1]Journal of Agricultural and Food Chemistry

being considered the second limiting amino acid for finishing animals[1] in diets based on corn and soybean meal. Among the functions conferred to methionine, the main one is to be substrate for protein synthesis, being a precursor of other amino acids, notably cysteine that, like methionine, is also used for body protein synthesis[2].

In the form of S-adenosylmethionine (SAM), methionine is a donor of methyl groups (CH_3) to a multitude of body substances, such as creatine, and is also involved in polyamine synthesis[3]. Cysteine, in its turn, is involved in the synthesis of fur proteins and other important body components, such as glutathione[4].

Guanidinoacetic acid (GAA) is an immediate creatine precursor that requiring only a methyl group transfer from adenosyl methionine (SAM) to conversion into creatine[5]. GAA synthesis begins with reversible transfer of an amine group from arginine to glycine, forming ornithine and GAA[6]. This reaction is catalyzed by the aminotransferase enzyme and, in mammals, occurs mainly in the kidneys. 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[7]. However, its use generates metabolic effects in the body, since methylation of GAA into creatine increases the demand for methylation[8], which may induce homocysteine accumulation in the blood[9].

The major tissues are not able to produce the creatine, depending on a protein to transport its extracellular medium into cells, which occurs against a concentration gradient. One of the creatine and GAA transporters is the SLC6A8 gene, which is expressed in most tissues but is most relevant in skeletal muscle, kidneys and brain[7].

In the animal metabolism, methionine can be converted into cysteine, in which it is used for the synthesis of glutathione peroxidase, an important antioxidant agent of the cells[10-11]. This system prevents the oxidation of proteins such as myoglobin and myofibrillar proteins, preserving the meat color and water holding capacity[12-13-14].

In this sense, guanidinoacetic acid supplementation in diets associated with higher met + cys levels may influence the performance and the variables related to the carcass and meat of the

animals, because the guanidinoacetic acid contributes to creatine formation, sparing arginine that could be used by the body for other functions. It has been shown in piglets that the GAA supplementation requires excessive levels of met + cys to facilitate creatine transmethylation, and positive results are verified only when there is sufficient met + cys to provide methyl groups[15]. 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 met + cys concentration in the diet.

Thus, the objective of this work was to evaluate the interaction of GAA with met + cys in finishing swine diets about the performance, carcass characteristics, meat quality, blood biochemical parameters, creatine transporter gene expression and antioxidant action on the muscle.

Material and Methods

Animals, facilities and diets. The experiment was approved by the Animal Use Ethics Committee at the Maringá State University - CEUA / UEM (nº7665090217). 32 crossbred swine females with high genetic potential and superior performance were used, with an average initial weight of 75.25 ± 0.91kg, they were distributed in four treatments, eight replications and one animal per experimental unit. The experimental design was in randomized blocks with a 2x2 factorial scheme, consisting of two levels of guanidinoacetic acid (0.00% and 0.05%) and two levels of digestible met + cys (0.44% and 0.50%)., as shown in Table 1. The diets were formulated with corn, soybean meal, minerals, vitamins and amino acids. The diets were isonutritive and met the nutritional recommendations of NRC [16] (Table 1). The animals were distributed in the treatments based on the initial weight. The animals were housed in concrete-floor stalls with a semi-automatic front feeder and a drinking pacifier at the rear, located in a masonry building equipped with fans and nebulizers.

Table 1: Centesimal, chemical and energetic composition of the diets used in the experiment

Met+cys	0.44		0.50	
GAA	0.0	0.05	0.0	0.05
Corn	85.50	85.50	85.50	85.50
soybean meal	10.89	10.89	10.89	10.89
soybean oil	0.84	0.84	0.83	0.83
Limestone	1.01	1.01	1.01	1.01
Dicalcium phosphate	0.45	0.45	0.45	0.45
Salt	0.14	0.14	0.14	0.14
L-Lysine HCl 99%	0.41	0.41	0.41	0.41
Dl-methionine 99%	0.04	0.04	0.10	0.10
L-threonine 98.5%	0.11	0.11	0.11	0.11
L-Tryptophan 98%	0.02	0.02	0.02	0.02
Glutamic acid	0.06	0.06	0.00	0.00
Inert gas [1]	0.11	0.06	0.12	0.07
Antioxidant[2]	0.01	0.01	0.01	0.01
Enradin ®[3]	0.01	0.01	0.01	0.01
Premix min.vit.[4]	0.40	0.40	0.40	0.40
GAA 96% [5]	-	0.05	-	0.05
Calculated Composition (%)				
EM (Mcal/kg)	3.30	3.30	3.30	3.30
N total	2.02	2.02	2.02	2.02
Calcium	0.81	0.81	0.81	0.81
Phosphorus available	0.27	0.27	0.27	0.27
Sodium	0.12	0.12	0.12	0.12
Potassium	1.80	1.80	1.80	1.80
Chlorine	0.09	0.09	0.09	0.09
Lysine dig.	0.770	0.770	0.770	0.770
Met. + cyst. dig.	0.440	0.440	0.500	0.500
Threonine dig.	0.480	0.480	0.480	0.480
Tryptophan dig.	0.130	0.130	0.130	0.130
Valine dig	0.514	0.514	0.514	0.514
Isoleucine dig.	0.427	0.427	0.427	0.427
Leucine dig.	1.129	1.129	1.129	1.129
Histidine dig.	0.308	0.308	0.308	0.308
Phenylalanine dig.	0.541	0.541	0.541	0.541
Arginine dig.	0.650	0.650	0.650	0.650

1.Washed sand 2.BHT. 3. Enramycin. Content/feed kg: iron - 50.00 mg, copper - 5.00 mg, cobalt - 0.50 mg, manganese - 20.00 mg, zinc - 50.00 mg, iodine - 0.75 mg, and selenium - 0 30 mg vit. A – 4400 U.I., vit D3 960 U.I., vit. E – 25.60 U.I., vit B1 – 0.640 mg, vit B2 – 2.13 mg, vit. B6 - 1.58 mg, vit B12 - 0.016 mg, niconic acid - 19.34 mg, pantothenic acid - 12.16 mg, vit. K3 - 1.92 mg, folic acid - 0.192 mg, biotin - 0.064 mg, and choline - 127.31 mg. 5.GAA = guanidinoacetic acid.

Performance. The animals were weighed at the beginning and at the end of the experiment, when they reached 100 ± 3.78 of live weight, to determination of the weight gain (DWG). The diets were weighed every time the animals were fed and the leftovers weighed for the determination of feed intake (DFI) and the calculation of feed conversion (FC).

Biochemical profile. At the end of the experiment, it was realized the blood collect from all animals, to determine plasma concentrations of urea, creatinine, lactate, glucose and homocysteine. Blood samples were obtained by puncture in the jugular vein, according to the indications of Oliveira et al.[17.] with the help of 100mm long needles. It was collected approximately 15 ml of blood, destined to obtain the serum, collection in glass tubes containing EDTA anticoagulant for determination of urea, creatinine and lactate and another tube containing anticoagulant fluoride oxalate was used for glucose determination. After the collection, the blood was immediately sent to the FEI / UEM Pig Laboratory, where the samples were centrifuged at 3000rpm for a period of 15 minutes to obtain the plasma, which was extracted with the aid of an automatic pipette, placed in tubes of *eppendorf*-type and sent for analysis using colorimetric kits (Gold Analyzes, Brazil and Bioclin, Brazil), following the standard operating procedures described in them and the absorbance reading was performed on Biochemical Analyzer (Bioplus 2000. Brazil). Additionally, blood samples were collected in tubes containing gel without physical / chemical properties to perform homocysteine analyzes. Serum homocysteine concentration was determined at São Camilo Laboratory (Maringá, Paraná, Brazil) by means of chemiluminescence method.

Carcass characteristics. When they reached the average weight of 100± 3.78 kg, the animals were slaughtered at the slaughterhouse of the Experimental Farm of Iguatemi - FEI / UEM, after 24 hours of fasting. 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[18]. The carcasses were cooled (1-2ºC) for 24h for, subsequently, being submitted to quantitative evaluation.

Meat quality. The pH of the *Longissimus dorsi* muscle was measured in the hot 45 min *post mortem* carcass (pH45) and in the cold carcass maintained in the cold chamber (1-2ºC) for 24h (pH24) using the Portable Digital pH Meter (HI 99163. Hanna Instruments, Italy), following the recommendations of Bridi and Silva[19]. For carcass qualitative evaluation, samples (2.5 cm thick) were taken from *Longissimus dorsi* in the region of 8th and 10th vertebrae for subsequent

measurement of intramuscular fat (marbling), dripping water loss, thawing and cooking[19] (Bridi and Silva, 2009). *Longissimus dorsi* muscle color was measured 24h after the slaughter, with samples taken between the 8th and 10th thoracic vertebrae, as described by Bridi and Silva (2009) [19]. 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 color system. One of the *Longissimus dorsi* samples was frozen and then used for the analysis of thawing water loss and cooking water loss. The thawing water loss was obtained by weight difference of frozen sample and after storage at 4ºC. The cooking water loss was obtained by the weight difference of the thawed sample and after cooking in a preheated oven at 170ºC, until reaching the internal temperature of 71ºC[19]. Previously cooked *Longissimus dorsi* samples were used to determination of the shear force (kgf). In each sample, they were taken longitudinally, towards the muscle fibers, five cylindrical subsamples, according to the recommendations of Ramos and Gomide[20]. Analyzes were performed using a texturometer (Stable Micro Sytem TA-XT2i, coupled with the Warner-Bratzler Shear Force probe and Texture Expert Exponent software - Stable Micro Systems, USA).

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 hours. The procedures were performed according to the methodology described by Vyncke[21]. Absorbance was read at a wavelength of 532 nm and it was used a standard curve of 1.1.3.3 tetraethoxypropane. In the evaluation of the % inhibition of 2.2-diphenyl-1-picril-hydrazole (DPPH) radical methyl alcohol and DPPH (Sigma, USA) were used, following the methodology of Li et al.[22] and Brand-Williams et al.[23]. The reading was performed in a cuvette spectrophotometer (Bioespectro SP22. Brazil) at 515 nm absorbance after 30 minutes. The blank test was performed with 150 μL of the solvent used for extraction with 2.85 ml of methyl alcohol.

The determination of creatinine concentration contained in *Longissimus dorsi* was adapted from the methodology of Willian et al.[24], and the reading was performed by a commercial kit (Gold Analisa, Brazil).

Gene expression of the SLC6A8 transporter. 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 -80ºC until RNA extraction. Total RNA was extracted using Trizol® reagent (Invitrogen, Carlsbad CA, USA) according to the manufacturer's standards and quantified using nanodrop spectrophotometer. RNA entirety was evaluated on 1% agarose gel, it was ruddied with 10% ethidium bromide and visualized in ultraviolet light. 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 SuperScriptTM III First-Strand Syntesis Super Mix kit (Invitrogen Corporation, Brazil) as per manufacturer's specifications. Creatine transporter gene expression (SLC6A8) was measured by real-time quantitative polymerase chain reaction (qPCR) using SYBR GREEN fluorescent dye (Roche, Basel, Switzerland) and the LightCycler® 96 equipment (Roche, Basel, Switzerland, Switzerland). The primer pairs (forward and reverse) used in the amplification reactions for endogenous ß-actin control (D: GCTACAGCTTCACCACCACA and R: CTCCAGGGAGGAAGAGGAT) and of the creatine transporter gene, SLC6A8. (D: TTTCACCGCTACATTCCCC and R: GGCATCTATCCACACCTGAG) were constructed based on the available sequences in the NCBI GenBank database (www.ncbi.nlm.nih.gov) for swine (*Sus scrofa*) using the www.idtdna.com program.

Statistical analysis. The statistical analysis was performed using the computer program R (2015) [25], in which the data regarding to the performance, blood variables, gene expression, carcass characteristics, meat quality and lipid oxidation were submitted to ANOVA, with two Met

+ Cys digestible levels and two levels of guanidinoacetic acid supplementation and the interaction between the levels included in the mathematical model, as follows:

$$Y_{ijk} = \mu + M_i + V_j + MV_{ij} + B_k + E_{ijk}$$

Where:

Yijk = response variable,

μ = general average common to all observations,

M_i =effect of the ith Met + cys level (i = 0.40% and 0.44%),

V_j = effect of the jth GAA supplementation level (j = 0.0 and 0.05%),

MV_{ij} = effect of the interaction of the ith digestible (met + cys) level with the jth GAA supplementation level,

B_k = effect of the kth block (i = 1 to 8),

E_{ijk} = random error inherent in all observations.

Results and Discussion

There was no significant interaction between met + cys and GAA levels ($p > 0.05$) on animal performance (Table 2). GAA supplementation did not influence the performance ($p > 0.05$), which means that it did not require a higher met + cys intake to obtain greater swine weight gain, neither, this supplementation reduced the performance to the level 0.44% of met + cys, which is the recommendation given by the *National Reserch Concil*[15.] Therefore, these data suggest that is not necessary a met + cys supplementation in the use of GAA in diets for finishing swine, considering performance, as the hypothesis of this work suggests.

Table 2: Performance of female finishing swine submitted to diets with different concentrations of digestible met + cys and supplemented with guanidinoacetic acid (GAA).

Item		IW	FW	DWG	DFI	FC
Met+cys	GAA	Kg	Kg	Kg	Kg	Kg/Kg
			Main effects			
0.44		75.42	100.7	1.09	2.64	2.44
0.50		75.08	100.2	1.10	2.98	2.72
	0.00	75.28	100.6	1.14	2.76	2.44
	0.05	75.22	100.2	1.06	2.87	2.72
			Interaction effects			
0.44	0.00	75.30	100.80	1.14	2.48	2.18
0.44	0.05	75.54	100.59	1.04	2.81	2.70
0.50	0.00	75.26	100.55	1.13	3.04	2.70
0.50	0.05	74.9	99.97	1.07	2.93	2.74
Error		0.091	1.03	0.14	0.35	0.41
			P value			
Met+cys		0.178	0.583	0.921	0.219	0.615
GAA		0.797	0.643	0.094	1.000	0.085
Met+cys *vs.*GAA		0.214	0.723	0.094	0.293	0.279

Met + cys = methionine + cysteine, GAA = guanidonoacetic acid, IW = inicial weight, FW = final weight, DFI = daily feed intake, DWG = daily weight gain and FC = feed conversion. Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

A study by Ibrahim et al., [26] on ducks, showed that the addition of guanidinoacetic acid required more met + cys as a source of methyl groups than that one established as a requirement, which favorably resulted in the birds' performance, as the authors hypothesized and confirmed that GAA supplementation, combined with met + cys supplementation as a methyl group donor, would favor protein synthesis and growth.

Jayaraman et al. [27] performed 0.12% GAA supplementation and verified an improvement in the weight gain of growing and finishing swine and they associated this improvement with creatine formation, which may contribute to the increase of muscle protein and water retention in skeletal muscles, as Michels et al.28 observed, but both worked with recommended methionine levels for the studied phase. This was also observed in an experiment with broilers, in which GAA supplementation increased creatine concentration in the chest muscle, indicating that GAA supplementation can form creatine [29], without requiring higher levels of met + cys in the diets.

However, He et al.[30] verified that the use of GAA increased the feed conversion of swine in the finishing period but considering the total experiment period (from 30 to 100 kg live weight) the level of 0.3% GAA showed the best response for feed conversion.

These different results can be attributed to the evaluation time, because the supplementation period, besides the nutrient levels of the diets, can influence the results. Met + cys levels, for example, may interfere in the swine performance once the amount of met + cys consumed above the requirement level may be used for other physiological functions rather than protein deposition[31]. However, this has not been verified in other studies, wherein methionine supplementation did not influence the performance of swine in the growth and finishing phases [32.] and that, excess met + cys was probably catabolized.

Blood creatinine and homocysteine concentrations did not show interaction ($p > 0.05$) for the studied met + cys and GAA levels (Table 3). According to Stead et al.[33] the oral administration of GAA increases plasma creatinine level and consequently there is an increase in plasma homocysteine concentration.

There was an isolated effect on the met + cys level in the plasma homocysteine concentration, in which the 0.50 met + cys level presented the highest homocysteine concentration. In general, higher met + cys levels may cause hyperhomocysteinemia induced by the excess of methionine [34].

However, homocysteine accumulation can be alleviated by various mechanisms, such as methyl group donors, which can affect homocysteine metabolism by acting as a co-substrate for the cystathionine β-synthase (CBS) enzyme reaction and provide units of one carbon linked to folate, which provides methyl grouping for the methionine synthesis reaction, in other words, the CBS functions as a methionine cycle regulator[35], thus, high methionine levels also raise the concentration of SAM and reduce the amount of methyl group in the cycle, by inhibiting the activities of enzymes involved in homocysteine remethylation and stimulating the activity of CBS, responsible for the irreversible loss of homocysteine of cycle [36. 37.] which may have happened in this work, since the use of higher levels of met + cys reduced homocysteine

concentration and this may have occurred due to higher activity from CBS, that it is stimulated by this higher amount of methionine in the cycle.

Table 3: Blood parameters of female finishing swine submitted to diets with different concentrations of digestible met + cys and supplemented with guanidinoacetic acid (GAA).

Item		Glucose mg/dl =32).	Lactate mg/dl (n=32)	Urea mg/dl (n=32)	Creatinine mg/dl (n=32)	Hcy mg/dl (n=16)
Met+cys	GAA					
			Main effects			
0.44		51.22	13.58	14.72[a]	2.32	31.81[a]
0.50		59.56	14.82	11.67[b]	2.33	23.12[b]
	0.00	55.35	15.46	12.88	2.37	29.71
	0.05	55.58	12.94	13.52	2.29	25.23
			Interaction effects			
0.44	0.00	49.78	11.66[a]	15.06	2.50	34.80
0.44	0.05	52.66	15.50[a]	14.38	2.15	28.82
0.50	0.00	60.92	19.26[a]	10.69	2.23	24.62
0.50	0.05	58.50	10.38[b]	12.65	2.43	21.63
Error		2.07	5	1.07	0.36	1.52
			P value			
Met+cys		0.058	0.170	0.040	1.000	0.052
GAA		0.843	0.460	0.387	0.921	0.906
Met+cys vs. GAA		0.560	0.016	0.359	0.134	0.584

Met + cys = methionine + cysteine, GAA = guanidonoacetic acid, Hcy = homocysteine. Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

There was interaction ($p = 0.016$) between met + cys and GAA levels under blood lactate concentration (Table 3), and the interaction breakdown showed that GAA supplementation reduced plasma lactate concentration (10.38 dl / mh), only at the level of 0.50% met + cys.

Besides that, the increasing of the met + cys level reduced blood lactate concentration. Lactate is directly linked to carcass quality. One of the main causes of pork quality loss is the rapid and extensive decline in *post-mortem* pH due to the accumulation of lactic acid from anaerobic glycolysis before the carcass has cooled efficiently[31]. In this context, one of the functions of creatine is its buffering action. Lactate accumulation in the muscle acidifies the middle and the creatine can maintain normal pH with the use of hydrogen for ATP synthesis[38].

According to studies by Li et al, [39-40] the GAA supplementation increases creatine and phosphocreatine, which would make that the postmortem glycolysis to occur more slowly, decreasing lactic acid concentration. In this study, this behavior was verified with the highest level of met + cys, which may indicate that a greater amount of met + cys was required for GAA to have 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 behavioral responses [41].

There was no interaction (P> 0.05) between met + cys and GAA levels on plasma urea concentration (Table 3), but there was an isolated effect of met + cys levels (p = 0.040) wherein the lower level showed an increase in the blood urea concentration.

Higher levels of met + cys may elevate plasma levels of urea and glucose[3.] because when the amino acids are supplied in excess they need to be catabolized in the body, and excess methionine is deaminated and it produces the succinyl-CoA α-keto acid, that it is converted to pyruvate, which can be employed in the citric acid cycle, generating energy, or be employed in the gluconeogenesis pathway, generating glucose molecules. While the resulting nitrogen can be used for the synthesis of other nitrogen compounds or simply excreted as urea, representing an energy expenditure for the body.

Quantitative carcass evaluations did not show significant interactions between met + cys and GAA levels (p> 0.05). Muscle depth had an isolated effect for GAA levels used (p = 0.001), in which the supplementation with GAA increased muscle depth (Table 4), which may indicate its growth-promoting property, as suggested by Ringel et al. al., [42.] Li et al., [43] and Jayaraman et al.[27.] as these authors have justified GAA supplementation because of its ability to save amino acids, especially methionine and arginine, and assign them to the protein synthesis, however, it is important to consider that in these experiments, the authors used the amino acid levels recommended by the NRC (2012) for each phase that was studied.

The other quantitative carcass variables were not influenced (p> 0.05) by GAA and met + cys levels in finishing swine diet.

Table 4: Carcass characteristics of finishing female swine, submitted to diets with different concentrations of digestible met + cys and supplemented with guanidinoacetic acid (n = 32)

Item Met+cys	GAA	Comp cm	HCY %	WL %	BT mm	MD Mm	P1 Mm	P2 mm	P3 mm	LMY %
Main effects										
0.44		95,94	81.80	3.42	9,65	64,53	33.27	22.47	14.52	61.26
0.50		98.41	81.53	3.51	9,87	65,95	30.02	24,57	14.13	61.01
	0.00	98.31	81.16	3.48	9,99	63.11[a]	30.94	23.61	14.10	60.50
	0.05	96.03	81.52	3.45	9,53	67.37[b]	32.35	23.43	14.55	61.77
0.44	0.00	97.37	81.65	3.58	9.70	63.07	30.20	22.76	13.46	60.67
0.44	0.05	94.50	81.96	3.26	9.60	65.99	36.33	22.19	15.58	61.85
0.50	0.00	99.25	80.67	3.38	10.29	63.16	31.67	24.47	14.75	64.71
0.50	0.05	97.56	82.37	3.64	9.45	68.74	28.36	24.67	13.51	61.69
Error		1.04	0.97	0.13	0.56	0.89	1.36	1.17	1.99	1.96
P value										
met+cys		0.103	0.471	0.071	1.000	0.843	0.419	0.590	0.722	0.284
GAA		0.082	0.534	0.889	0.825	0.001	0.270	0.743	1.000	0.155
Met+cys*GAA		0.921	0.667	0.302	0.615	0.403	0.178	0.693	0.177	0.324

Met+cys= Methionine + cysteine, GAA= guanidinoacetic acid, Lenght= carcass length, HCY= Hot carcass yield, WL= Fat carcass weight loss, FT: thickness, DM= *Longissumus dorsi* muscle depth, P1. P2. P3 = Fat thickness measured at 3 points, LMY = lean meat yield, Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

There was no interaction between met + cys and GAA levels ($p > 0.05$) on the qualitative characteristics of pig meat (Table 6). Studies have shown that guanidinoacetic acid supplementation increases total creatinine concentration and muscle phosphocreatine levels [36.] and also, it improves meat quality through the increased initial pH and lower water loss and shear force[44]. However, in the present study, although the GAA supplementation reduced plasma lactate concentration at 0.50% met + cys, the variables pH 45min and pH 24h did not change (Table 5), indicating that GAA did not influenced for the *post-mortem* glycolysis occur more slowly. In addition, the other meat quality variables were not influenced by GAA and met + cys supplementation.

Table 5: Qualitative carcass characteristics of finishing female swine, submitted to diets with different concentrations of digestible met + cys and supplemented with guanidinoacetic acid (n = 32).

Item		pH	pH	DL	TL	CL	SF	Color			Creatinine
Met+cys	GAA	45 min	24 hrs	%	%	%	N	L	A	B	
Main effects											
0.44		6.11	5,57	4,14	6.92	24,33	4,07	54,40	6.59	3.38	4,52
0.50		6.14	5,58	4,35	6.21	24,29	3.66	55.66	6.52	3.71	3.61
	0.00	6.12	5,59	4,37	6.88	24,93	4,06	54,95	6.57	3.54	3.79
	0.05	6.13	5,58	4,12	6.25	23.68	3.68	55.11	6.54	3.55	4,35
0.44	0.00	6.11	5,52	4,38	3.78	6.9	24,63	54,71	6.80	3.53	4,41
0.44	0.05	6.11	5,63	3.90	3.86	6.54	24,61	54,08	6.38	3.23	4,63
0.50	0.00	6.12	5,65	4,35	3.71	6.58	24,00	55.19	6.33	3.55	3.16
0.50	0.05	6.15	5,52	4,34	3.79	6.23	23.99	56.13	6.70	3.87	4,06
Error		0.08	0.07	0.36	0.32	0.43	0.54	0.44	0.23	0.12	1.47
P value											
Met		0.921	1.000	0.921	0.786	0.450	0.970	0.252	0.722	0.384	0.268
GAA		0.487	0.889	0.667	0.818	0.500	0.335	0.602	0.921	0.387	0.082
Met+cys*GAA		0.780	0.123	1.000	0.749	0.739	0.994	0.356	0.436	0.257	0.499

Met + cys = Methionine + cysteine, GAA = guanidinoacetic acid, pH45 = pH at 45 minutes after slaughter, pH24 = pH at 24 hours after slaughter, PG = Drip water loss, PD = Thawing water loss, PC = cooking water loss, HR = shear force, averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

Methionine is also required for phosphatidylcholine biosynthesis, which is a phospholipid located on mammalian cell membranes[38] being fundamental in maintaining membrane entirety and cell fluid rate. This characteristic is particularly important when it is wanted to avoiding excessive water loss from meat. Although adequate methionine supply is necessary for the synthesis of important substances to maintain the oxidative stability of meat., its excess can have adverse effects and negatively affect the meat quality. However, no influence was found ($p > 0.05$) of met + cys levels on variables related to water loss (DL, TL, CL) in the present study, showing that the dietary level of 0.44% met + cys was enough for the water loss results (Table 5). As well as the 0.50% met + cys level did not negatively affect meat quality characteristics.

According to Sangali et al., [46] the recommended digestible met + cys level for swine females, from 75 to 100 kg, is at least 10.60 g / day (0.370%) for carcass characteristics and meat quality. However, higher levels of met + cys may also increase the synthesis of carnitine and phosphatylcholine, which act directly on lipid metabolism, affecting the absorption and transport of fatty acids, which is present in the intestinal wall. On the other hand, carnitine is responsible for the transport of medium and long chain fatty acids into the mitochondria, where it occurs the β-oxidation [47].

Met + cys supplementation beyond the requirement may increase the carnitine content in the liver and swine muscles[48.] indicating an effect on the reduction of carcass fat percentage [49]. However, in this study, there was no influence of the levels used on the fat deposition (Table 5).

Lipid oxidation that was measured by the amount of malonaldehyde (MDA) by TBARS analysis and DPPH radical inhibition showed no interaction between met + cys and GAA levels for both analyzes (Tables 6 and figure 1). However, the meat storage period (0. 24, 48 and 72 hours) influenced ($p < 0.05$) the TBARS results, because the action of time tends to oxidize the meat and reduce its quality[50].

Table 6: Digestible met + cys levels and guanidinoacetic acid supplementation in diets for finishing swine female and their effects on lipid oxidation by TBARS methodology (mg MDA Eq. Kg-1) in Longissumus dorsi (n = 28)

Item	Met+cys	0.44	0.44	0.50	0.50
Period	GAA	0	0.05	0	0.05
0		0.034	0.029	0.025	0.036
24 hours		0.031	0.025	0.038	0.030
48 hours		0.032	0.032	0.032	0.035
72 hours		0.040	0.037	0.040	0.064
Error		0.016	0.016	0.013	0.033
	Main effects				
0		0.031			
24 hours		0.031			
48 hours		0.033			
72 hours		0.038			
		P-value	Linear	Quadratic	Adjusted Equation
Met+cys		0.681			
GAA		0.276			
Period		0.028	0.002	0.365	$Y=0.0027x+0.0293$[1]
Met+cys*GAA* period		0.362			
Met+cys*GAA		0.109			

Met + cys = Methionine + cysteine, GAA = guanidinoacetic acid, TBARS = thiobarbituric acid reactive substances, Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$). [1]$R^2 = 0.78$

Guanidinoacetic acid was able to reduce lipid peroxidation through the increasing the activity of some enzymes associated with free radical metabolism [44]. This fact was not verified in this study, once GAA supplementation (0.05%) did not influence lipid oxidation. However, the Figure 1 shows a difference in the % inhibition of DPPH radical with and without GAA supplementation at the 0.44% met + cys level, however GAA was not effective in inhibiting DPPH radical ($p > 0.05$).

Nasiroleslami et al. [51] verified that 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.

In addition, sulfur amino acids play an important role in oxidative stress, being involved in antioxidant processes through taurine and glutathione peroxidase synthesis[52]. Dietary deficiency may result in the decreased of antioxidant enzymes activity and, consequently, it increases the lipid peroxidation, inducing oxidative damage in the body[53]. Methionine and S-adenosylmethionine (SAM) play an important role in neutralizing reactive oxygen species. SAM is the main biological donor of the methyl group, 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[54]. In addition, methionine residues in proteins have antioxidant action by the methionine sulfoxide reductase system. These residues can be oxidized 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[55].

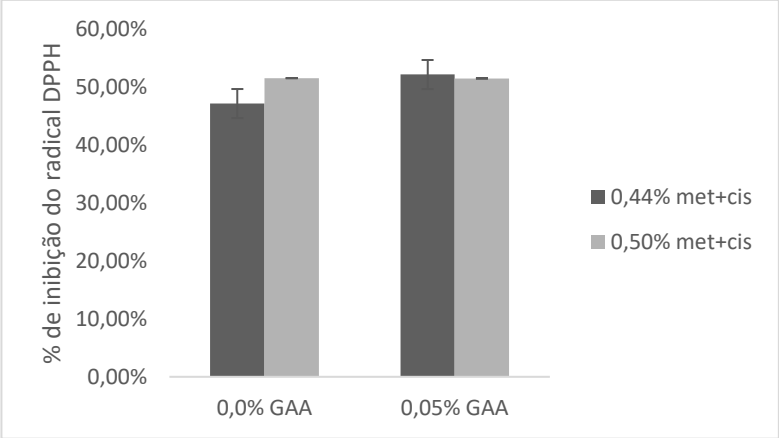

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Figure 1: Diets with different digestible met + cys levels and guanidinoacetic acid supplementation (GAA) in finishing swine female diets and their effects on the % inhibition of DPPH radical in Longissumus dorsi (n = 28)
Subtitle: Met + cys = Methionine + cysteine, GAA = guanidinoacetic acid, DPPH: 2.2-diphenyl-1-picryl-hydrazyl radical. P value: met+cys=0.561; GAA 0.660; met + cys vs. GAA 0.556.

The met + cys and GAA levels evaluated did not influence the relative gene expression of the SLC6A8 gene in the swine liver (Figure 2). This 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 SLC6A6 and even by the

aminobutyric gamma transporter (GAT2) [37]. In another study, it was verified that 0.08% of GAA increased the expression of creatine transporter in the muscle (*Longissimus dorsi*), liver and kidneys, 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 [56].

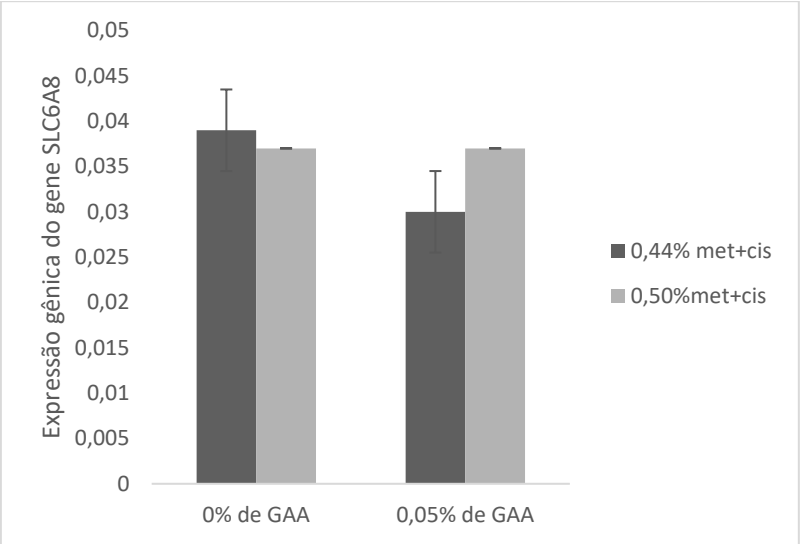

Figure 2: Gene expression of the SLC6A8 in the liver of finishing swine female submitted to diets with different concentrations of digestible met + cys and supplemented with guanidinoacetic acid (GAA), (n=20).

Subtitle: Met + cys = Methionine + cysteine, GAA = guanidinoacetic acid. Standard Error= 0.005. P value: met + cys = 0.561; GAA 0.660; met + cys vs. GAA 0.524.

Diets supplemented with GAA may increase the demand for S-adenosylmethionine [33.] so, it is necessary enough methionine dietary to meet protein needs and creatine formation [26]. However, it is important to note that, in addition to methionine, the synthesis of GAA 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 [43.] what may explain the lack of more evident results from this work. Probably, diets with lower digestible arginine levels, when supplemented with guanidinoacetic acid, may show larger differences in

the studied variables, as also verified by Teixeira et al., [57]. In addition, finishing swine have a high synthesis of arginine, which may also be an important factor.

It was concluded that GAA does not require a higher intake of met + cys in finishing swine diets and its supplementation improved the depth of *Longissimus dorsi* muscle, without effects on performance, meat quality and gene expression of the creatine transporter in the liver.

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VI. THE DIETARY RELATIONSHIP BETWEEN METHIONINE + CYSTEINE AND CREATINE FOR FINISHING SWINE FEMALE (75 TO 100 KG) INFLUENCES IN THE MEAT QUALITY[1]

Abstract: The objective of this work was to evaluate the interaction of methionine + digestible cysteine (met+cys) and creatine monohydrate (CMH) levels in finishing swine diets about the performance, carcass characteristics, meat quality, blood biochemical parameters, creatine transporter gene expression and antioxidant action. 40 swine females with average initial weight of 75.26 ± 0.87 kg, distributed in four treatments, ten replications and one animal per experimental unit.The experimental design was in randomized blocks in a 2x2 factorial scheme, consisting of two CMH levels, (0% and 0.10%), and two levels of met + cys, (0.40% and 0.44%). The animals and the feed consumed were weighed to the determination of the weight gain (DWG), feed intake (DFI) and feed conversion (FC). At the end of the experiment, it was realized the blood collect from all animals to determine the plasma concentrations of urea, creatinine, lactate, glucose and homocysteine. Upon reaching the average weight of 100.00 ± 5.84 kg, the animals were slaughtered, after fasting (24 hours). The carcasses were cooled (1-2ºC) for 24h and subsequently submitted to quantitative evaluation and carcass quality evaluation. Immediately, after the slaughter, *Longissimus dorsi* muscle tissue samples were also collected for gene expression analysis of the creatine transporter, (SLC6A8). The obtained results indicate that there was no interaction between met + cys and CMH levels in performance and gene expression of creatine transporter ($p > 0.05$). However, there was significant interaction for the fat point P3 ($p = 0.004$), drip water loss (DL) ($p = 0.018$) and thawing water loss (TL) ($p = 0.04$) and plasma concentration of creatinine ($p = 0.03$). For the point P3. the supplementation with CMH presented lower values of fat deposition. For the DL and TL variables, the lowest met + cys level with HCM supplementation presented higher water losses. The plasma creatinine showed lower concentrations with 0.10% HCM supplementation in relation to non-supplementation, only at the 0.44% met + cys level. In addition, the higher level of met + cys increased the percentage of inhibition of DPPH radical ($p = 0.022$). The effects of 0.10% creatine supplementation on the diet of finishing swine are evident on meat quality by using adequate levels of digestible methionine + cysteine, which also assist in preventing oxidative lipid damage in *Longissimus dorsi*.

Keywords: guanidinoacetic acid, phosphocreatine, protein metabolism.

Resumo: O objetivo do trabalho foi avaliar a interação de níveis de metionina+cisteína digestível (met+cis) e creatina monohidratada (CMH) em dietas para suínos na fase de terminação sobre o desempenho, características de carcaça, qualidade de carne, parâmetros bioquímicos sanguíneos, expressão gênica do transportador de creatina e ação antioxidante. Foram utilizadas 40 fêmeas suínas com peso inicial médio de 75,26±0,87 kg, distribuídas em quatro tratamentos, dez repetições e um animal por unidade experimental. O delineamento experimental foi em blocos ao acaso em um esquema fatorial 2x2, constituídos de dois níveis CMH, (0% e 0,10%), e dois níveis de met+cis, (0,40% e 0,44%). Os animais e a ração consumida foram pesados para determinação do ganho de peso (GPD), consumo de ração (CDR) e conversão alimentar (CA). No término do experimento, foi realizada a coleta de sangue de todos os animais para determinação das concentrações plasmáticas de ureia, creatinina, lactato, glicose e homocisteína. Ao atingirem o peso médio de 100,00±5,84 kg, os animais foram abatidos, após jejum alimentar (24 horas). As

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carcaças foram resfriadas (1-2ºC) por 24h para, posteriormente, serem submetidas à avaliação quantitativa e avaliação da qualidade da carcaça. Imediatamente após o abate também foram coletadas amostras de tecido do músculo *Longissimus dorsi* para análises de expressão gênica do transportador de creatina (SLC6A8). Os resultados obtidos indicam que não houve interação entre os níveis de met+cis e CMH no desempenho e expressão gênica do transportador de creatina (p>0,05). Porém, houve interação significativa para o ponto P3 de gordura (p=0,004), perda de água por gotejamento (PG) (p=0,018), perda de água por descongelamento (PD) (p=0,04) e concentração plasmática de creatinina (p=0,03). Para o ponto P3, a suplementação com CMH apresentou valores menores de deposição de gordura. Para as variáveis PG e PD, o menor nível de met+cis com a suplementação de CMH apresentaram maiores perdas de água. A concentração plasmática de creatinina foi inferior com a suplementação 0,10% de CMH em relação a não suplementação, apenas para o nível de 0,44% de met+cis. Além disso, o maior nível de met+cis aumentou a porcentagem de inibição do radical DPPH (p=0,022). Os efeitos da suplementação de 0,10% de creatina na dieta de suínos em terminação são evidentes sobre a qualidade da carne ao se utilizar níveis adequados de metionina+cisteína digestíveis, que também auxiliam na prevenção de danos oxidativos dos lipídeos no *Longissimus dorsi.*

Palavras –chave: ácido guanidinoacético, fosfocreatina, metabolismo proteico.

Introduction

Creatine (α-methyl guanidino acetic acid) is involved in energy metabolism through the phosphocreatine system, which provides energy to the muscles. Both dietary and endogenously synthesized creatine enter in the circulation by diffusion and are transported to the intracellular mean by specific transporters. In the intracellular mean, the reatine can be stored as creatine or in its phosphorylated form, the phosphocreatine (Persky et al., 2001). Thus, the main function of creatine in the body is the formation of phosphocreatine in muscle and brain, which will serve as a source of energy. Degradation of creatine and phosphocreatine generates creatinine, which is excreted by the kidneys (Lemme et al., 2007).

Creatine is synthesized in two steps from the glycine and arginine amino acids (Brosnan et al., 2011). Glycine first reacts with arginine to form guanidinoacetic acid (GAA), formed in the kidneys and transported to the liver, where it receives the methyl group of S-adenosyl methionine and forms creatine (Brosnan et al., 2011. Nelson and Cox, 2014). The Creatine synthesis is therefore a process that involves two enzymes, amidinotransferase (AT) and guanidinoacetate methyltransferase (GMT) and three amino acids, arginine, glycine and methionine.

Dietary supplementation of creatine monohydrate (HCM) is still extensively discussed among different authors. According to Maddock et al. 2002), 25 g of HCM for 5 days before slaughter attenuates the negative effects caused by animal stress and increases the expression of

RyR1 genes, which is a calcium release channel forming protein in the sarcoplasmic reticulum of muscle cells, which, in cases of inadequate functioning, it triggers an intracellular calcium accumulation and, consequently, causes a permanent muscle contraction (Campos et al., 2014).

However, the period and the level of CMH supplementation may also influence responses: supplementation of 20 g / day / animal for 15. 10 or 5 days before slaughter and it improves meat attributes when used for 5 days and it brings damage in relation to quality when used for a longer period (Berg et al. 2000). In another experiment, Berg et al. (2001) evaluated the effect of the 25 g creatine supplementation / day / swine during 10 or 5 days before slaughter and the results suggested that regardless of the time of use, the CMH supplementation dampens *postmortem* pH and reduces the loss of water in 48 hours. A similar protocol was suggested by O'Quinn et al. (2000), with 25 g of creatine / day / animal during 10 days, but without effects on carcass characteristics, but with the increased in the daily feed intake and daily weight gain.

In general, the addition of monohydrate creatine in the swine diet can be a buffer against the lactic acid produced, reducing the sharp drop in pH of PSE meat, also changing its color (YOUNG et al., 2005). James et al. (2000) concluded that creatine addition did not influence finishing swine performance, but increased meat firmness and reduced water loss measured at 14 days after slaughter.

However, studies conducted so far have evaluated creatine supplementation in swine feed about meat performance and quality. However, interaction with methionine should also be considered, because the creatine may influence methionine intake in the swine diet. In this sense, the objective of this work was to evaluate the interaction of creatine with digestible methionine + cysteine (met + cys) in finishing swine diets about the performance, carcass characteristics, meat quality, blood biochemical parameters, creatine transporter gene expression and antioxidant action in the muscle.

Material and Methods

Animals, facilities and diets.

The experiment was approved by the Animal Use Ethics Committee at the State University of Maringá - CEUA / UEM under protocol 7665090217. 40 crossbred swine females with high genetic potential and superior performance were used, with an average initial weight of 75.24 ± 0.87 kg, they were distributed in four treatments, 10 replications and one animal per experimental unit. The experimental design was in randomized blocks in a 2x2 factorial scheme, consisting of two CMH levels, (0% and 0.10%), and two levels of met + cys, (0.40% and 0.44%). The rations were formulated based on corn, soybean meal, minerals, vitamins, amino acids and additives. The rations were isonutritive (Table 1) and met the nutritional recommendations of the NRC (2012). The animals were housed in concrete-floor stalls with a semi-automatic front feeder and a drinking pacifier at the rear, located in a masonry building equipped with fans and nebulizers.

Performance.

The animals were weighed at the beginning and at the end of the experiment to determination of the weight gain (DWG). The diets were weighed every time that the animals were fed and the leftovers weighed for the determination of feed intake (DFI) and the calculation of feed conversion (FC).

Table 1: Centesimal, chemical and energetic composition of the diets used in the experiment

Met+cys	0.40%		0.44%	
Met+cys	0.0%	0.10%	0.0%	0.10%
CMH	0.0%	0.10%	86.76	86.76
Soybean meal	10.89	10.89	10.89	10.89
Soy oil	0.30	0.30	0.23	0.23
Limestone	0.39	0.39	0.40	0.40
Dicalcium	0.38	0.38	0.38	0.38
Salt	0.14	0.14	0.14	0.14
L-Lysine HC 99%	0.39	0.39	0.39	0.39
Dl-methionine 99%	0.005	0.005	0.04	0.04
L-threonine 98.5%	0.11	0.11	0.11	0.11
L-Tryptophan	0.02	0.02	0.02	0.02
Inert 1	0.16	0.06	0.19	0.09
Antioxidant2	0.02	0.02	0.02	0.02
Enradin®3	0.015	0.015	0.015	0.015
Premix min.vit.4	0.40	0.40	0.40	0.40
CMH 99% 5	0.00	0.10	0.00	0.10
Calculated Composition (%)				
EM (Mcal/kg)	3.30	3.30	3.30	3.30
Total n	2.02	2.02	2.03	2.03
Calcium	0.56	0.56	0.56	0.56
Available match	0.26	0.26	0.26	0.26
Sodium	0.11	0.11	0.11	0.11
Potassium	1.82	1.82	1.82	1.82
Chlorine	0.097	0.097	0.097	0.097
Lysine dig.	0.770	0.770	0.770	0.770
Met. + cyst dig.	0.400	0.400	0.440	0.440
Threonine dig.	0.482	0.482	0.482	0.482
Tryptophan dig.	0.130	0.130	0.130	0.130
Valine dig	0.522	0.522	0.522	0.522
Isoleucine dig.	0.432	0.432	0.432	0.432
Leucine dig.	1.140	1.140	1.140	1.140
Histidine dig.	0.310	0.310	0.310	0.310
Phenylalanine dig.	0.538	0.520	0.520	0.520
Arginine dig.	0.654	0.654	0.654	0.654

1.Washed sand 2.BHT. 3. Enramycin. 4.Content / kg of feed: iron - 50.00 mg, copper - 5.00 mg, cobalt - 0.50 mg, manganese - 20.00 mg, zinc - 50.00 mg, iodine - 0.75 mg, and selenium - 0.30 mg, vit. A - 4,400 U.I., vit D 3.960 U.I., vit. E - 25.60 U.I., vit B1 - 0.640 mg, vit B2 - 2.13 mg, vit. B6 - 1.58 mg, vit B12 - 0.016 mg, niconic acid - 19.34 mg, pantothenic acid - 12.16 mg, vit. K3 - 1.92 mg, folic acid - 0.192 mg, biotin - 0.064 mg, and choline - 127.31 mg.5.Creatine monohydrate.

Biochemical profile.

At the end of the experiment, it was realized the blood collect from all animals to determine the plasma concentrations of urea, creatinine, lactate, glucose and homocysteine. Blood samples (15 ml) were obtained by puncture in the jugular vein, collected in glass tubes containing anticoagulant EDTA (urea, creatinine and lactate) or oxalate fluoride (glucose). The blood was centrifuged (3000rpm, 15 minutes) for the obtaining of the plasma. Analyzes were performed by colorimetric kits (Gold Analyzes, Brazil and Bioclin, Brazil), following the standard operating procedures described therein.

Blood samples were collected in tubes containing gel without physical / chemical properties to perform homocysteine analyzes. Serum homocysteine concentration was determined at São Camilo Laboratory (Maringá, Paraná, Brazil) by means of chemiluminescence method.

Carcass characteristics.

When they reached the average weight of 100± 5.85 kg, the animals were slaughtered at the slaughterhouse of the Experimental Farm of Iguatemi - FEI / UEM, after fasting of 24 hours. 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 (1-2ºC) during 24h for, subsequently, being submitted to quantitative evaluation, according to the Brazilian Carcass Classification Method (ABCS, 1973).

Meat quality.

Muscle pH was measured in the hot carcass, 45 min *post mortem* (pH45), and in the cold carcass, kept in the cold chamber (1-2ºC) for 24h (pH24), using the Portable Digital pH Meter (HI 99163. Hanna Instruments, Italy). For qualitative evaluation of the carcass samples (2.5 cm thick) were taken from *Longissimus dorsi* in the region of the 8th and 10th vertebrae for subsequent measurement of intramuscular fat (marbling), dripping water loss, thawing and cooking.

Muscle color was measured 24h after slaughter, with samples taken between the 8th and 10th thoracic vertebrae. On the surface of the muscle, lightness measurements were performed (L *, a * and b *) using a portable colorimeter (CR-400. Konica Minolta's, Japan) and the

components L * (lightness), a * (red-green color component) and b * (yellow-blue component) were expressed in the CIELAB color system.

A muscle sample was frozen and then it was used for the analysis of thawing water loss and cooking water loss. The thawing water loss was obtained by weight difference of frozen sample and after storage at 4ºC. Water loss by cooking was obtained by the weight difference of the thawed sample and after cooking in a preheated oven at 170ºC, until reaching the internal temperature of 71ºC, according to the recommendations of Bridi and Silva (2009).

The *Longissimus dorsi* samples, previously cooked, were used to determine shear force (kgf). In each sample, five cylindrical subsamples, measuring 1.27 in diameter were taken longitudinally in the direction of the muscle fibers (Ramos and Gomide, 2007). Analyzes were performed using the texturometer (Stable Micro Sytem TA-XT2i, coupled with the Warner-Bratzler Shear Force probe and Texture Expert Exponent software - Stable Micro Systems, USA).

aThe determination of lipid oxidation through the determination of the value of thiobarbituric acid reactive substances (TBARS), was performed on *Longissimus dorsi* samples, immediately after the slaughter and storage at (4 ° C) during 24, 48 and 72 hours after the slaughter, according to the methodology described by Vyncke (1975). The absorbance was read at a wavelength of 532 nm and a standard curve of 1.1.3.3 tetraethoxypropane was used. For the analysis of inhibition of 2.2-diphenyl-1-picril-hydrazole (DPPH) radical, methyl alcohol and DPPH (Sigma USA) reagents were used, following the recommendations of Li et al. (2005) and Brand-Williams et al. (1995). The reading was performed in a cuvette spectrophotometer (Bioespectro SP22. Brazil) at 515 nm absorbance after 30 minutes. The white test was performed with 150 µL of the solvent used for extraction with 2.85 ml of methyl alcohol at 100%.

The amount of creatinine contained in *Longissimus dorsi* was adapted from the methodology of Willian et al. (1927), using a commercial kit (Gold Analyzes, Brazil) and the absorbance reading was performed on a spectrophotometer (Biospectrum SP22. Brazil).

Gene expression of the SLC6A8 gene

Longissumus dorsi muscle tissue samples were collected immediately after slaughter, to performing gene expression analysis of the SLC6A8 gene. All materials used in the collect 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 -80ºC until the moment of the RNA extraction. Total RNA was extracted using Trizol® reagent (Invitrogen, Carlsbad CA, USA) according to the manufacturer's standards and quantified using spectrophotometer at 260 nm.

RNA entirety was evaluated on 1% agarose gel, it was ruddied with 10% ethidium bromide and visualized in ultraviolet light. 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 SuperScriptTM III First-Strand Syntesis Super Mix kit (Invitrogen Corporation, Brazil) as per manufacturer's specifications, and stored at -80ºC until its use. Creatine transporter gene expression (SLC6A8) was measured by real-time quantitative polymerase chain reaction (qPCR) using SYBR GREEN fluorescent dye (Roche, Basel, Switzerland) and the LightCycler® 96 equipment (Roche, Basel, Switzerland, Switzerland). The primer pairs (forward and reverse) used in the amplification reactions for endogenous ß-actin control (D: GCTACAGCTTCACCACCACA and R: CTCCAGGGAGGAAGAGGAT) and the target gene, SLC6A8. (D: TTTCACCGCTACATTCCCC and R: GGCATCTATCCACACCTGAG) were constructed based on the available sequences in the NCBI GenBank database (www.ncbi.nlm.nih.gov) for swine (*Sus scrofa*) using the www.idtdna.com program (Table 2). The ß-actin was used as an endogenous control for presenting better efficiency and higher reaction specificity.

Statistical analysis.

The statistical analysis was performed using the computer program R (2015) 25, in which the data related to performance, blood variables, gene expression, carcass characteristics, meat quality and lipid oxidation were submitted to ANOVA, being the block effects, met + cys levels,

CMH supplementation, and the interaction of met + cys and CMH included in the mathematical model: $Y_{ijk} = \mu + M_i + V_j + MV_{ij} + Bk + E_{ijk}$, where: Y_{ijk} = response variable, μ = overall mean common to all observations, M_i = effect of i-th level of metione (i = 0.40 and 0.44), V_j = effect of j-th level of creatine supplementation (j = 0.0 and 0.1), MV_{ij} = effect of the interaction of the i-th level of met + cys with the j-th level of CMH, B_k = effect of the k-th block (i = 1 to 10), E_{ijk} = random error inherent in all observations.

Results and Discussion

No interaction was observed between met + cys and CMH ($p > 0.05$) about performance (Table 2). Even using a met + cys level of 0.40%, in other words, below about what it is recommended by the National Research Concil (NRC, 2012), the daily intake of met + cys was 11.20 g / day and 11.16 g. / day for the treatment containing 0.0% or 0.1% CMH in the diet, respectively, that is, still above the requirement suggested by the NRC (2012), which is 10.6 g / day, indicating that the amount daily intake was sufficient to meet the requirements for maximum performance of the animals, explaining the lack of results for treatment with 0.40% met + cys with or without CMH supplementation.

Table 2: Performance of female finishing swine submitted to diets with different concentrations of digestible met + cys (met+cys) and supplemented with creatine monohydrate (CMH), (n = 40).

Item Met+cys	CMH	IW Kg	FW Kg	DWG Kg	DFI Kg	DI Met+cys (g)	FC Kg/Kg
			Main effects				
0.40		75.38	100.61	0.99	2.79	11.18	2.82
0.44		75.14	99.46	0.92	2.75	12.09	2.98
	0.0	75.34	100.96	0.99	2.82	11.83	2.84
	0.1	75.18	99.12	0.92	2.73	11.45	2.97
0.40	0.0	75.47	101.22	1.00	2.80	11.20	2.80
0.40	0.1	75.29	100.01	0.98	2.79	11.16	2.84
0.44	0.0	75.21	100.70	0.98	2.83	12.45	2.88
0.44	0.1	75.08	98.23	0.87	2.67	11.74	3.05
Error		0.14	1.09	0.03	0.10	0.10	0.08
			P value				
Met+cys		0.549	0.063	0.473	0.889	0.155	0.417
CMH		0.384	0.680	0.473	0.808	0.612	0.754
Met+cys vs. CMH		0.934	0.883	0.732	0.864	0.641	0.864

IW = inicial weight, FW = final weight, DFI = daily feed intake, DI met + cys= daily intake of the methionine + cysteine. DWG = daily weight gain and FC = feed conversion. Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

Dietary of CMH supplementation may reduce feed intake, once it can restore the energy body needs through creatine and phosphocreatine (Ibrahim et al., 2019). However, in the present study, there was no change ($p > 0.05$) in feed intake with CMH supplementation.

According to Young et al. (2017), the supplementation of CMH in the swine diet favored muscle energy metabolism, increasing protein synthesis and improving growth and feed efficiency. However, DWG was not influenced by CMH supplementation (Table 2) in the present study. The literature emphasizes that the ingestion of large doses of CMH by humans is usually accompanied by physical exercyses, which leads to muscle hypertrophy (Kraemer et al., 2019). In addition, Greenhaff (1997) noted that 20 to 30% of individuals ingesting creatine did not show response to CMH intake. In the case of confined swine, daily exercyse is minimal.

The effects of creatine supplementation are well reported, in contrast, Gualano et al. (2010) argue that the factors responsible for adaptations are uncertain. The creatine mechanisms to improve muscle performance and protein synthesis may be related to the increase of muscle anaerobic capacity by phosphocreatine restoration, thus preserving the normal physiological role

and delaying the onset of muscle exhaustion, or may be due to the increased of intramuscular absorption phosphocreatine, which results in osmotic fluid extraction in the muscle cell, overhydration process, and increased cell volume. However, the authors still differ whether creatine is able of promoting such effects or whether the combination with strength training is required.

Studies show that met + cys levels also have effects on swine performance responses. Loughmiller et al. (1998) and Knowles et al. (1998) concluded that the optimum relation to the maximum performance is 0.306% met + cys in the diet; however, diets with methionine deficient may slow the swine growth (Humphrey et al., 2018). Since met + cys levels used did not influence animal performance (Table 3), carcass yield (Table 5), such as muscle depth (MD) and lean meat yield (LMY) and concentration of Plasma urea (Table 4), it can be inferred that the amount of met + cys consumed above the recommended level may have been used for other physiological functions rather than protein deposition, as suggested by Vaz et al. (2005). If the excess met + cys was not used by the body, the plasma urea concentration would probably be higher because amino acids offered in excess are deaminated and the resulting nitrogen can be excreted as urea (Nelson and Cox, 2014).

Although there was no interaction ($p > 0.05$) between met + cys levels and CMH levels (Table 3), met + cys levels influenced ($p = 0.022$) the fat thickness (FT), where the lower met + cys level increased the fat thickness, as also it was verified by Humphrey et al. (2018), in which animals were fed with met + cys deficient diet presented higher fat content in proportion to lean tissue. In animal metabolism, methionine is used for carnitine synthesis, which transports long chain fatty acids across the mitochondrial membrane, thereby facilitating oxidation. Thus, higher levels of met + cys in the diet of finishing swine may lead to reduced fat thickness and increased percentage of lean carcasses (Vaz et al., 2005, Pena et al., 2008). However, the variables related to lean meat yield were not influenced by the treatments in this study.

Table 3: Carcass characteristics of finishing female swine, submitted to diets with different concentrations of digestible met + cys and supplemented with CMH (n = 40).

Item Met+cys	CMH	Length Cm	HCY %	WL %	FT Mm	MD mm	P1 mm	P2 Mm	P3 Mm	LMY %
							Main effects			
0.40		98.58	80.70	3.84	12.33[a]	64,80	32.03	23.48	12.50	59,77
0.44		97.31	80.75	3.63	10.36[b]	63.57	34,45	23.46	11.65	60.08
	0	98.47	80.92	3.69	11.06	65,50	34,97	24,53	11.96	60.46
	0.1	97.42	80.53	3.80	12.02	62.86	31.51	22.41	12.19	59,39
0.40	0.0	99.44	81.20	4,05	11.25	63.80	33.38	24,80	11.05[a]	60.59
0.40	0.1	97.72	80.20	3.63	13.41	65,79	30.67	22.15	13.96[a]	58.95
0.44	0.0	97.50	80.64	3.32	10.88	67.21	36.55	24,25	12.87[a]	60.32
0.44	0.1	97.11	80.85	3.96	10.63	59,93	32.35	22.66	10.43[b]	59,83
Error		0.47	0.45	0.16	0.41	1.91	0.94	1.05	0.57	0.34
							P value			
Met+cys		0.218	0.667	0.332	0.022	0.571	0.160	0.470	0.589	0.255
CMH		0.999	0.224	0.889	0.153	0.615	0.055	0.504	0.518	0.234
Met+cys vs. CMH		0.643	0.112	0.106	0.073	0.140	0.767	0.176	0.004	0.453

Met + cys = Methionine + Cystine, CMH = Creatine monohydrate, Length = carcass length, HCY= Hot carcass yield, WL = thawing carcass weight loss, FT: Fat thickness, DP = muscle depth *Longissumus dorsi*, P1. P2. P3 = Fat thickness measured at 3 points, LMY = lean meat yield. Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

There was an interaction (P = 0.004) between met + cys and CMH levels for P3. the third point in which the fat thickness is measured, where CMH supplementation increased fat deposition with only 0.40% met. + cys. Met + cys supplementation from 0.40% to 0.44% reduced fat deposition only by 0.10% CMH (Table).

When creatine is directed to muscle deposition, it is natural that in proportion to the carcass, the amount of fat has decreased. In a similar way, Berg et al. (2011), working with finishing swine, found that CMH supplementation promoted a decrease in fat thickness. In this study, the fat thickness increased in animals that received diets containing 0.40% met + cys and CMH (Table 3).

In this study, the met + cys and CMH levels used did not influence ($p > 0.05$) muscle creatinine (Table 4). However, according to Ibrahim et al. (2019), the mechanism responsible for higher creatinine levels in duck breast muscle may be the result of hepatic creatine transport, endogenous muscle synthesis, or a combination of both.

Table 4: Quantitative carcass characteristics of finishing female swine, submitted to diets with different concentrations of digestible met + cys and supplemented with CMH (n = n=40).

Item		pH45	pH24	DL	TL	CL	SF Color				Creatinin
Met+cys	CMH			%	%	%	Kg/N	L	A	b	e mg/dl
					Main effects						
0.40		6.51	6.10	4,55	3.92	28.10	3.96	55.88	13.54	4,03	3.76
0.44		6.52	6.01	4,14	3.92	28.21	4,12	54,87	12.51	3.86	3.57
	0	6.46	6.09	4,26	4.06	28.96	4,12	55.46	13.12	3.79	3.61
	0.1	6.58	6.02	4,44	3.77	27.35	3.97	55.28	12.94	4,10	3.73
0.40	0.0	6.39	6.14	4,19[a]	3.48[a]	28.91	4,04	56.13	13.28	3.87	3.69
0.40	0.1	6.62	6.06	4,91[a]	4,35[a]	27.30	3.89	55.63	13.81	4,18	3.84
0.44	0.0	6.52	6.04	4,33[a]	4,64[a]	29,02	4,19	54,80	12.96	3.71	3.53
0.44	0.1	6.53	5,98	3.96[b]	3.19[b]	27.40	4,05	54,94	12.06	4,02	3.61
Error		0.03	0.05	0.21	0.19	0.56	0.23	0.42	0.34	0.15	0.33
					P value						
Met+cys		0.809	0.780	0.843	0.833	0.085	0.093	0.255	0.158	0.554	0.139
CMH		0.070	0.132	0.577	0.865	0.934	0.128	0.712	0.462	0.270	0.106
Met+cys vs. CMH		0.086	0.352	0.018	0.042	0.383	0.896	0.843	0.675	0.999	0.938

Met + cys = Methionine + cysteine, CMH = creatine monohydrate, pH45 = pH at 45 minutes after slaughter, pH24 = pH at 24 hours after slaughter, PG = Drip water loss, PD = Thawing water loss, PC = cooking water loss, SF = shear force, averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

There was interaction between met + cys and CMH levels on drip water loss ($p = 0.018$) and thawing water loss ($p = 0.040$), as shown in Table 4.

The myofibrillar actin and myosin proteins are responsible for retaining water in muscle tissue. Most of the muscle water is in the intracellular means and the other fraction is in the extracellular means. Water contained in the extracellular fraction may slowly come out to the surface of the meat. If the *post mortem* pH reduction is too rapid in combination with high carcass temperatures, partial denaturation of the sarcoplasmic and myofibrillar proteins occurs, causing a decrease in meat water retention capacity (Gomide et al., 2013).

Methionine is also required for phosphatidylcholine biosynthesis, phospholipid more abundant in mammalian cell membranes (Zeisel et al., 2006), being fundamental in maintaining membrane entirety and cell fluid rate. Adequate met + cys supply is necessary for the synthesis of these membrane-lining substances and decrease the water loss, and in this work, it was observed that the 0.44% met + cys level associated with 0.01% CMH, reduced water losses (Table 5).

CMH supplementation may induce lower water losses, as it was verified in the study performed by Li et al., (2018), wherein, besides a lower water loss, the pH 24 was higher. It is also important to mention that creatine is an osmotically active substance, that is, it depends on water to perform its functions. Thus, there is an increase in its concentration in the intracellular space that may explain an influx of water of the cells (Williams et al., 2000).

The relationship between CMH supplementation and meat quality is in the fact that phosphocreatine is an important energy reserve for muscle and it is readily used in ATP synthesis (ADP + phosphocreatine = ATP + creatine) as it is used in Muscle metabolism. ATP production from phosphocreatine is particularly important, once it does not involve the glycolytic pathway and lactic acid production (Berg and Allee, 2001; Janicki and Buzala, 2013). Thus, adequate supply of methionine for creatine synthesis, or supplementation of this compound, increases the amount of energy available for ATP production. Consequently, this process, not involving the glycolytic pathway, does not imply in the production of lactic acid, and this may reduce the rate and intensity of *post mortem* pH drops (Li et al., 2016. Li et al., 2017). Nevertheless, CMH

supplementation, associated or not with higher met + cys levels, did not influence (p> 0.005) pH 45 and pH 24. Possibly, 0.1% CMH supplementation may not have been sufficient to provide such an effect.

Blood tests showed no interaction (p> 0.05) for met + cys and MCH levels (Table 5), except for creatinine (p = 0.030), whose plasma concentration was lower than the additional 0.10% of CMH relation to non-supplementation, only for the 0.44 met + cys level. Creatinine is the end product of creatine and phosphocreatine degradation in skeletal muscle, being diffused from the muscle into the bloodstream and subsequently excreted in the urine (Brosnan et al., 2007. Nelson and Cox, 2014), so these results may indicate that creatine was used in other functions and not being degraded to creatinine.

Table 5: Blood biochemical profile of finishing swine female, submitted to diets with different concentrations of digestible met + cys and supplemented with CMH.

Met+cys	CMH	Glucose Mg/dl (n=40)	Lactate Mg/dl (n=40)	Urea Mg/dl (n=40)	Creatinine Mg/dl (n=40)	Homocysteine Mg/dl (n=16)
			Main effects			
0.40		58.93	11.52	8.79	7.75	21.40
0.44		67.16	11.48	8.38	7.68	23.40
	0	67.01	13.44	8.14	8.39	20.00
	0.1	59,08	9,55	9,03	7.05	24,80
0.40	0.0	64.35	13.28	9,23	7.82[a]	19,30
0.40	0.1	53.50	9,75	8.34	7.69[a]	23.50
0.44	0.0	69,67	13.61	7.04	8.96[a]	20.70
0.44	0.1	64,65	9,35	9,72	6.40[b]	26.10
Error		2.69	1.11	0.66	0.49	2.87
			P value			
Met+cys		0.370	0.921	0.825	0.921	0.656
CMH		0.367	0.242	0.999	0.018	0.543
Met+cys *vs.* CMH		0.675	0.825	0.370	0.030	0.104

Met + cys = Methionine + Cysteine, CMH = creatine monohydrate, Averages followed by different letters in the column differ from each other by the F test (P ≤ 0.05).

Plasma homocysteine is inversely related to methyl group supplementation, Ibrahim et al. 2019) verified that it was reduced with CMH supplementation, which may spare methyl groups by negative feedback on arginine: glycine amidinotransferase activity (McGuire et al. 1984), decreasing GAA synthesis and the demand for methyl groups required for creatine production,

reducing homocysteine concentration (Deminice et al., 2009). However, the levels of met + cys and CMH used in this study were not sufficient to alter plasma homocysteine concentrations (Table 5).

Met + cys levels may alter the oxidative capacity of meat and CMH can be used as an agent that improves its quality due to its antioxidant action, however, the MDA concentration in *Longissimus dorsi* was not influenced (p> 0 .05) by the dietary levels of met + cys and CMH (Table 6). MDA is one of the several lipid peroxidation products that alter membrane fluidity and increase its fragility (Naziroglu, 2012). Thus, high levels of MDA indicate the increased lipid peroxidation and an accumulation of lipid peroxides and impaired antioxidant functions.

Table 6: Effects of diets with different digestible met + cys and CMH supplemented concentrations on *Longissumus dorsi* lipid oxidation of finishing swine females by Tbars methodology (mg MDA Eq. Kg-1), (n = 40).

Item	Met+cys	0.40	0.40	0.44	0.44
Period	CMH	0.00	0.10	0.00	0.10
0		0.027	0.028	0.030	0.023
24 hours		0.036	0.040	0.032	0.029
48 hours		0.038	0.030	0.031	0.039
72 hours		0.035	0.037	0.036	0.034
Error		0.013	0.002	0.003	0.003
Medium effect isolated period					
Met + cys		CMH			
0.40				0.036	
0.44				0.035	
		0.00		0.035	
		0.10		0.035	
Medium effect isolated period					
0				0.028	
24 hours				0.036	
48 hours				0.037	
72 hours				0,040	
P value					
Met+cys				0.806	
CMH				0.841	
Period				0.186	
Met+cys *vs*. CMH				0.761	
Met+cys vs. period				0.635	
CMH vs. period				0.436	
Met+cys vs. CMH vs. Period				0.978	

Met + cys = Methionine + Cysteine, CMH = creatine monohydrate, TBARS = thiobarbituric acid reactive substances, Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

There was no interaction between met + cys and CMH levels for % inhibition of DPPH radical (Table 7), but there was an effect of met + cys levels (p = 0.022), wherein the highest level showed an increase in inhibition of DPPH radical. It is known that methionine and S-adenosylmethionine play an important role in neutralizing reactive oxygen species (Jung et al., 2013) and methionine residues can act as catalytic antioxidants, protecting both protein membrane and localized macromolecules (Luo and Levine, 2009).

Met + cys levels below of the requirement may destabilize the oxidative capacity (Zeisel et al., 2006), because met + cys is involved in phosphatidylcholine synthesis and is essential in maintaining membrane integrity and cell fluid rate, which may be associated with the results obtained (Table 7).

Table 7: Effects of diets with different digestible met + cys and CMH supplemented concentrations in % inhibition of DPPH radical in *Longissumus dorsi* of finishing swine female (n = 40) .

Item		DPPH
Met+cys	CMH	%
	Main effects	
0.40		72.05[a]
0.44		75.19[b]
	0.0	76.27
	0.1	70.98
0.40	0.0	74.16
0.40	0.1	69,41
0.44	0.0	77.84
0.44	0.1	72.55
Error		0.388
	P value	
Met+cys		0.022
CMH		0.173
Met+cys *vs.* CMH		0.862

Met + cys = Methionine + Cysteine, CMH = creatine monohydrate, DPPH: 2.2-diphenyl-1-picryl-hydrazyl radical, Averages followed by different letters in the column differ from each other by the F test ($P \leq 0.05$).

CMH supplementation could improve the antioxidant capacity of diets, as verified by Lahucký et al. (2013), the antioxidant capacity of *Longissimus dorsi* muscle was substantially improved by creatine supplementation. However, the supplementation with 0.1% CMH did not influence the oxidative parameters evaluated in the present study.

There was no significant interaction (p> 0.05) of met + cys and CMH levels in relation to gene expression of the creatine transporter gene SLC6A8 (Graph 1). Similarly, CMH supplementation or higher met + cys levels did not influence gene expression of the SLC6A8 transporter.

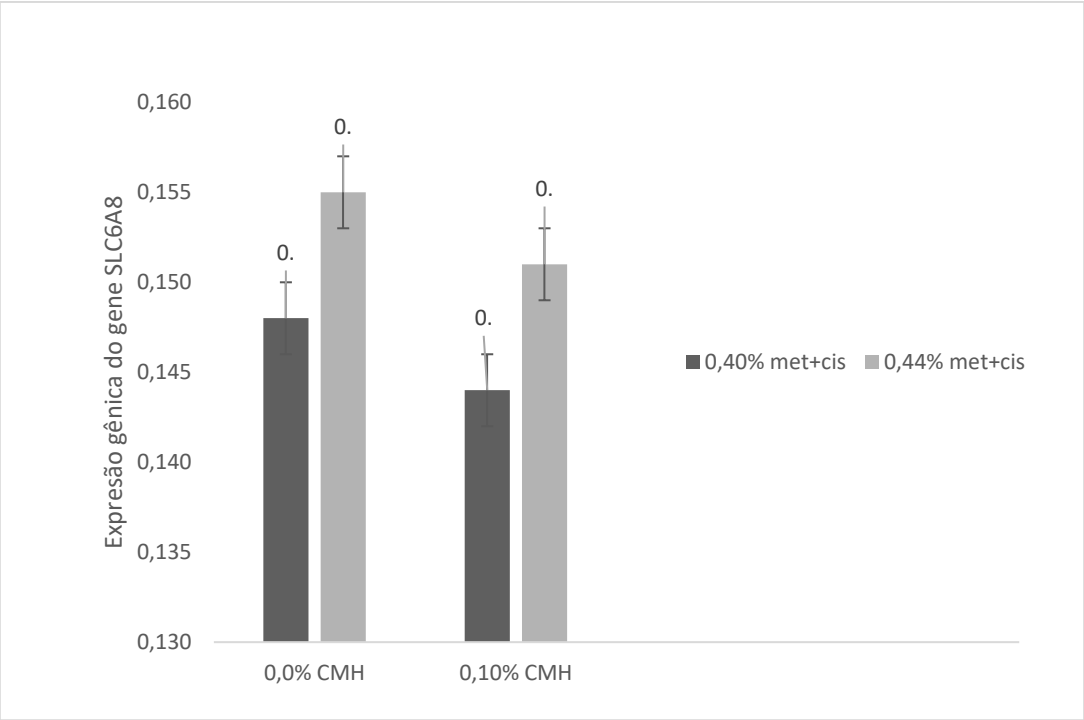

Figure 1: Gene expression of the SLC6A8 in the liver of finishing swine female submitted to diets with different concentrations of digestible met + cys and supplemented with CMH (n=20). Subtitle: Met + cys = Methionine + Cystine, CMH = creatine monohydrate. P value: met+cys=0.773; CMH=0.837; met+cys vs. CMH 0.704

The gene expression of the SLC6A8 transporter was determined in the *Longissumus dorsi* muscle, because when CMH is provided in the diet, it enters in the circulation by diffusion and it is transported to the intracellular means by specific transporters (Ostojic et al., 2017). However, Li et al. (2018) evaluated creatine transporter gene expression in muscle, liver and kidneys after feeding animals with diets supplemented with CMH and concluded that transporter gene expression showed significant difference only in the liver of these animals. Besides that, according to Wang et al. (2017), there was an increase in creatine transporter gene expression in the brain of animals supplemented with CMH. This area of the brain is involved in regulating

food intake, which indicates that creatine is likely to play a crucial role in regulating food intake and, consequently, in body weight. In this study, gene expression analysis was performed only in the muscle, but considering the data in the literature, there may have been a modulation in creatine transporter gene expression in other organs, such as liver and brain.

Conclusion

The effects of 0.10% creatine supplementation on the diet of finishing swine are evident on meat quality by using adequate levels of digestible methionine + cysteine, which also assist in preventing oxidative lipid damage in *Longissimus dorsi*.

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VI. CONSIDERAÇÕES FINAIS

Considerando os os resultados, o ácido guanidinoacético não demanda um maior aporte de met+cis nas dietas de suínos em terminação e sua suplementação melhorou a profundidade do músculo *Longissimus dorsi*, sem efeitos ao desempenho, qualidade de carne e expressão gênica do transportador de creatina no fígado. No entanto, houve efeito positivo do uso do GAA no lactato plasmático.

Os efeitos da suplementação de creatina na dieta de suínos em terminação são evidentes sobre a qualidade da carne ao se utilizar níveis adequados de metionina+cisteína digestíveis, sem alterações no desempenho, mas com efeito na creatinina plasmática.

Logo, esses resultados sugerem que outros níveis de GAA e CMH podem ser explorados e a interação com outros aminoácidos que também estão envolvidos no metabolismo podem ser alvo de novos estudos com a suplementação de GAA e CMH. Além disso, a análiase da expressão gênica das demais enzimas que participam do metabolismo da metionina podem trazer respostas mais consistentes em relação à modulação do metabolismo em casos de suplementação de GAA e CMH.