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Maintaining the same ratio between standardized ileal digestible methionine and cysteine do not affect its requirements for starting pigs

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Abstract

This study aimed to determine the requirements of standardized ileal digestible (SID) methionine (Met) + cysteine (Cys) of 15–30 kg barrows, maintaining or increasing the 1:1 ratio between SID Met and SID Cys. Seventy crossbred barrows averaging 15.2 ± 0.54 kg of live weight were allotted in a randomized block design 2 × 3 + 1 factorial scheme, with five replicates and two animals per pen. Treatments consisted of maintaining or increasing the 1:1 ratio between SID Met and SID Cys, three levels of SID Met + Cys (5.8, 6.4 and 7.0 g/kg) and a basal diet containing the lowest SID Met + Cys level (5.2 g/kg), formulated to provide a 1:1 ratio of SID Met (2.6 g/kg) and SID Cys (2.6 g/kg). Performance, blood parameters, *longissimus dorsi* muscle depth, backfat thickness and S–S linkages in the bristles were evaluated. The best average daily gain was estimated at 6.61 g/kg of SID Met + Cys, without maintaining the 1:1 ratio between sulphur amino acids (SAA). The daily intake of SID Met + Cys increased due to dietary SID Met + Cys levels, maintaining or increasing the same ratio between SAA. Plasma glucose increased and total cholesterol decreased according to SID Met + Cys levels, without maintaining the same ratio between the SAA. The requirement of SID Met + Cys for 15–30 kg barrows was 6.61 g/kg (7.88 g/day) for an optimum average daily gain, with no need to maintain the same ratio between the SAA.

Introduction

An adequate dietary amino acid (AA) supply is often evaluated for pigs and most research has studied the requirements of a single AA; mainly the most limiting essential AAs in pig diets (Kiefer *et al.*, 2005). There is much information about lysine requirements for different genetic groups, categories and sex of pigs (Santos *et al.*, 2011), but there are few studies about the sulphur containing AAs (SAA) methionine (Met) and cysteine (Cys), mainly for starting pigs, since Met is typically the second or third limiting indispensable AA in grain-based swine diets (Yang *et al.*, 2019).

Dietary Met plays a key role in the metabolism of SAA or their derivatives, which include Met, Cys, homocysteine, glutathione (GSH) and taurine (Yang *et al.*, 2020). Cysteine, in its turn, acts in the synthesis of the coat protein and other major body components, such as GSH (Stipanuk and Ueki, 2011). It is known that Cys is a nutritionally non-essential AA and three sources contribute to the L-Cys concentration in the body, like its absorption from the diet, the transsulphuration pathway and breakdown of endogenous proteins (Yin *et al.*, 2016).

In this sense, Met is an AA which aims to meet its own requirement, as well as Cys requirement, through a cycle that also covers homocysteine; but providing an adequate amount of dietary Cys may save dietary Met (Polese *et al.*, 2012), reducing homocysteine synthesis due to a decreased action of the transsulphuration pathway. However, an excess of dietary oxidized Cys provided a reduction in the average weight gain of pigs (Dilger *et al.*, 2007).

Moderate homocysteine levels collaborate with the growth and maintenance of tissues, but its concentration in the blood plasma shows that it is an atherogenic metabolite. In this sense, the metabolism keeps the homocysteine concentrations at normal levels in blood plasma, but its high concentration in blood plasma has multifactorial causes, including a high Met intake (Lima *et al.*, 2007).

Based on such background, diets are formulated to meet the requirements for Met plus Cys (Met + Cys), based on the assumption that Met is converted into Cys. The consequence is that Met may be provided in excess, reducing the efficiency that AAs are used by the organism.

This inconvenient could be easily avoided with the knowledge of the ideal Met:Cys/Total SAA ratio (Pacheco *et al.*, 2018). Additionally, the adequate knowledge of standardized ileal digestible (SID) Met requirements, associated with the ratios with SID Cys, may avoid the synthesis of high amounts of some metabolites (i.e. homocysteine).

Still in this sense, Wu (2013) showed a 1:1 ratio between Met and Cys for 5–110 kg pigs. Rostagno *et al.* (2017) suggested 3.63 g/kg as the SID Met requirement and 7.13 g/kg as the best SID Met + Cys level in the diet of 15–30 kg pigs, providing a Met:Cis ratio close to 1:0.96. However, the estimated Met:Cys ratio of 1:0.89 is obtained considering 3.6 and 6.8 g/kg as the requirements of SID Met and SID Met + Cys respectively, for 11–25 kg pigs (NRC, 2012). Considering the aforementioned researches, the Met: Cys ratios ranged from 1:0.89 to 1:1, with no optimal ratio recommendations because the SAA pathway supplies Cys from Met, but pig diets commonly shows Met:Cys ratios higher than 1:1.

In this sense, there is a need to know the adequate Met + Cys requirements associated with its ratios, according to the different pig production phases, due to the importance of some metabolites produced in the SAA pathways and the study of SAA levels close to that practiced in pig diets may provide important information. Thus, this study aimed to determine the requirements of SID Met + Cys of 15–30 kg barrows, maintaining or increasing the same ratio between the SAA.

Materials and methods

Experimental design, animals, housing and diets

Seventy crossbreed barrows (Topigs 20 × Tybor) averaging 15.2 ± 0.54 kg of initial live weight were distributed in a randomized block design in a 2 × 3 + 1 factorial scheme, with five replicates and two animals per experimental unit. Pigs were housed in suspended nursery cages, with front feeders and nipple drinkers in the opposite side. Diets and water were provided *ad libitum* throughout the experimental period.

The 2 × 3 + 1 factorial arrangement was designed in the direction that one of the factors consisted of maintaining a 1:1 ratio between the SAA in the diets (1:1 SAA), supplementing DL-Met and L-Cys; or increasing the 1:1 SAA (free SAA ratio), supplementing only DL-Met. The other factor consisted of three levels of SID Met + Cys (7.0, 6.4 and 5.8 g/kg). Additionally, a basal diet (5.2 g/kg SID Met + Cys) was formulated to maintain a 1:1 SAA ratio between SID Met (2.6 g/kg) and SID Cys (2.6 g/kg), used as the basal level to fit the regressions models for both situations of SAA ratios.

Experimental diets (Table 1) were based on maize, soybean meal, minerals, vitamins and additives to meet the nutritional requirements proposed by Rostagno *et al.* (2011), excepting the SID Met + Cys levels of the basal diet (5.2 g/kg) and diets with 5.8 g/kg (maintaining and increasing SAA ratios), which the proposed requirement by the aforementioned authors is 6.1 g/kg. Amino acid compositions of maize and soybean meal were determined at Evonik Industries and the standardized ileal digestibility coefficients were applied according to Rostagno *et al.* (2011), in order to estimate the SID AA contents of maize and soybean meal.

Growth performance

Body weight and feed intake were taken during the experimental period to determine the average daily gain (ADG), average daily feed intake (ADFI) and feed:gain ratio (F:G). The ADFI was also used to determine the daily intake of SID Met + Cys.

Backfat thickness and muscle depth

The backfat thickness (BF) and *longissimus dorsi* muscle depth (LD) of the barrows were measured at the end of the experimental period by using a set of equipment consisting of an ecocamera (Aloka, Inc., SSD-500 Vet, Twinsburg, USA) and a 11.5 cm and 3.5 MHz probe (Aloka, Inc., Twinsburg, USA). The measurements were performed in the P2 site, between the last and penultimate thoracic rib, 4 cm from the midline, being previously shaved in the cranial–caudal and dorsal–ventral directions, as described by Dutra Júnior *et al.* (2001). Two images were taken and the measurements were performed using the software Image-Pro® Plus (Media Cybernetics, Inc., Rockville, USA).

Blood sampling and analysis

Blood samples were collected at the end of the trial, after 6 h fasting, and were obtained from the jugular vein by using 40 × 12 mm disposable needles and then were harvested into glass tubes containing EDTA to determine urea, creatinine, total protein, triglycerides and HDL cholesterol; and glass tubes containing fluoride were used to determine plasma glucose concentration.

Glass tubes were centrifuged (3000 rpm for 15 min) and 3 ml of plasma were transferred to properly identified Eppendorf type tubes. Specific biochemical kits (Bioclin, Inc., Belo Horizonte, Brazil) were used and its standard operational procedures were followed. The absorbance was taken by using a spectrophotometer (Bioplus, Ltd., BIO-2000, Barueri, Brazil). Blood samples were also collected into tubes containing a gel without physicochemical properties, chilled and then determined the homocysteine concentration using the Immulite® unit (Siemens AG, Erlangen, Germany) by the chemiluminescence method (Demuth *et al.*, 2004).

Bristles S–S linkages determined by optical Raman spectrometry

The bristles sampling of the pigs was hand sampled performed by using latex gloves and then washed with distilled water and dried at environmental temperature, during 24 h; after that, the spectra were determined referring to the S–S linkages by optical Raman spectrometry.

Raman spectra were collected at room temperature (19°C) at a scattering geometry by means of the confocal Raman microscope (Bruker Corporation, SENTERRA®, Billerica, USA). The spectra were excited by a laser source of 785 nm and recorded in the range of 440 and 1800 cm^{-1}. The laser power was 50 MW, focused on sample through an optical microscope with a 20× objective lens (0.75 NA). The spectral data were processed with the software Opus® (Bruker Corporation, Billerica, USA).

The spatial resolution of 3–5 cm^{-1} was used, the detector integration time was set at 3 s, and each curved end was the result of an average of 100 spectra. Moreover, to improve the signal quality, the temperature of the detector was reduced to −90.15°C. All spectra were collected under the same conditions and the flattest central area of the surface (appearing on the scales better) was selected and photographed to ensure that the following measurements were repeated at the same site.

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Table 1. Composition of experimental diets containing levels of standardized ileal digestible (SID) Methionine (Met) + Cysteine (Cys) for starting barrows, maintaining (1:1 SAA ratio) or increasing (Free SAA ratio) the ratio between SID Met and SID Cys

	Levels (g/kg)						
Item (g/kg)	Basal	1:1 SAA ratio			Free SAA ratio		
SID Met + Cys	5.2	5.8	6.4	7.0	5.8	6.4	7.0
SID Met	2.6	2.9	3.2	3.5	3.2	3.8	4.4
SID Cys	2.6	2.9	3.2	3.5	2.6	2.6	2.6
Met: Cys ratio	1:1	1:1	1:1	1:1	1:1.23	1:1.46	1:1.69
Maize	699.3	699.1	699.1	699.1	699.1	699.1	699.1
Soybean meal	254.0	254.0	254.0	254.0	254.0	254.0	254.0
Dicalcium phosphate	15.25	15.25	15.25	15.25	15.25	15.25	15.25
Limestone	8.29	8.29	8.29	8.29	8.29	8.29	8.29
Sodium bicarbonate	5.28	5.28	5.28	5.28	5.28	5.28	5.28
Salt	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Vegetable oil	7.06	6.92	6.77	6.62	6.96	6.83	6.69
L-Lysine HCl	4.04	4.04	4.04	4.04	4.04	4.04	4.04
DL-Methionine	0.21	0.52	0.82	1.13	0.82	1.43	2.04
L-Cysteine	–	0.33	0.65	0.98	–	–	–
L-Threonine	1.39	1.39	1.39	1.39	1.39	1.39	1.39
L-Tryptophan	0.21	0.21	0.21	0.21	0.21	0.21	0.21
L-Valine	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Glutamic acid	2.00	1.51	1.03	0.54	1.28	0.64	0.01
Inert[a]	–	–	–	–	0.21	0.37	0.53
Antioxidant[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tylosin phosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin supplement[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineral supplement[d]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Composition (g/kg unless indicated otherwise)							
Metabolizable energy (MJ/kg)[e]	13.51	13.51	13.51	13.51	13.51	13.51	13.51
Crude protein[f]	171.0	171.0	171.0	171.0	171.0	171.0	171.0
Calcium[e]	7.68	7.68	7.68	7.68	7.68	7.68	7.68
Available P[e]	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Na[e]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
K[e]	4.67	4.67	4.67	4.67	4.67	4.67	4.67
Cl[e]	1.9	1.9	1.9	1.9	1.9	1.9	1.9
SID Lys[g]	10.93	10.93	10.93	10.93	10.93	10.93	10.93
SID Met + Cys[g]	5.2	5.8	6.4	7.0	5.8	6.4	7.0
SID Met[g]	2.6	2.9	3.2	3.5	3.2	3.8	4.4
SID Cys[g]	2.6	2.9	3.2	3.5	2.6	2.6	2.6
SID Thr[g]	6.89	6.89	6.89	6.89	6.89	6.89	6.89
SID Trp[g]	1.97	1.97	1.97	1.97	1.97	1.97	1.97
SID Arg[g]	10.55	10.55	10.55	10.55	10.55	10.55	10.55
SID Val[g]	7.54	7.54	7.54	7.54	7.54	7.54	7.54

(*Continued*)

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Table 1. (*Continued.*)

Item (g/kg)	Levels (g/kg)						
	Basal	1:1 SAA ratio			Free SAA ratio		
SID Leu[g]	13.84	13.84	13.84	13.84	13.84	13.84	13.84
SID Ile[g]	6.34	6.34	6.34	6.34	6.34	6.34	6.34
DEB (mEq/kg)[h,e]	152.8	152.8	152.8	152.8	152.8	152.8	152.8

[a]Fine sand.
[b]Butylated hydroxytoluene.
[c]Vitamin supplement for starter pigs (content/kg: Vit. A – 1 800 000.00 UI; Vit. D3 – 360 000.00 UI; Vit. E – 4000.00 mg; Vit. K3 – 600.00 mg; Vit. B1 – 280.00 mg; Vit. B2 – 800.00 mg; Vit. B6 – 300.00 mg; Vit. B12 – 3600.00 mcg; Pantothenic acid - 3200.00 mg; Niacin – 6000.00 mg; Folic acid 80.00 mg; Biotin - 20.00 mg; Choline – 31.20 g.
[d]Mineral supplement for initial pigs (content/kg: Cu – 50.00 g; Fe – 20.00 g; Mn – 11.00 g; Co – 120.00 mg; I – 200.00 mg; Zn- 18.00 g; Se – 60.00 mg).
[e]Calculated values.
[f]Determined values.
[g]Total values determined and applied the SID coefficients.
[h]Dietary electrolyte balance.

The stretch results of disulphide linkages (S–S linkages) were evaluated by normalizing the spectra, using phenylalanine methyl ester (1004 cm^{-1}) as a reference peak and a baseline corrected in the software. The area measurements of the spectra were obtained by the band width of 550–480 cm^{-1}.

Statistical analysis

Four models were statistically tested to assess the existence of first and second-degree coefficients, common or specific to treatments that maintained (1:1) or increased the ratio between SID Met and SID Cys. The likelihood ratio test was used to determine the best fitting model for performance, blood parameters, carcass traits (LD and BF), serum homocysteine and bristles S–S linkages. Thus, specific coefficients to treatments pointed out the interaction between factors.

Data were subjected to statistical analysis using the SAS® University Edition software (SAS, Cary, USA) and the Sistema de Análises Estatísticas e Genéticas - SAEG® (Universidade Federal de Viçosa, Viçosa, Brazil). The initial body weight of the pigs was used as a covariate to evaluate the performance and carcass traits. The best level of SID Met + Cys was estimated based on the performance results, blood parameters, carcass characteristics and serum homocysteine levels using the Broken Line model. The minimum confidence level was $P < 0.05$.

Results

Growth performance

There was no effect ($P > 0.05$) of SID Met + Cys levels (Table 2) on the final weight, ADFI, ADG, F:G, LD and BF by maintaining the 1:1 SAA ratio. The ADFI, F:G, LD and BF were also not affected ($P > 0.05$) with a free SAA ratio, but a second-degree model was fitted for the final weight ($P = 0.019$) and ADG ($P = 0.003$), providing an optimum SID Met + Cys levels estimated at 6.63 and 6.61 g/kg for the final weight and ADG, respectively (Figs 1(*a*) and (*b*)).

A linear increase of daily intake of SID Met + Cys was observed by increasing the dietary SID Met + Cys levels, either with the 1:1 SAA ($P = 0.002$) or free SAA ($P < 0.001$) ratios (Table 2), represented by the equations $Y = -0.89744 + 1.18264X$ ($R^2 = 0.57$) and $Y = 0.485 + 1.0541$ X ($R^2 = 0.87$), respectively.

Backfat thickness and muscle depth

LD and BF were not affected ($P > 0.05$) by the SID Met + Cys levels (Table 2), maintaining the 1:1 SAA or with a free SAA ratio.

Blood parameters

There was no effect ($P > 0.05$) of dietary SID Met + Cys levels on plasma urea, triglycerides, total proteins, creatinine, homocysteine and HDL maintaining or increasing the 1:1 SAA ratio (Table 3). Plasma glucose was not affected ($P > 0.05$) by maintaining the 1:1 SAA ratio, but a linear ($P = 0.006$) and a quadratic ($P = 0.006$) models were observed using a free SAA ratio.

The total cholesterol concentration linearly decreased ($P = 0.003$) according to increasing dietary SID Met + Cys levels with a free SAA ratio (Table 3), providing 21.4% reduction, represented by the equation $Y = 105.529–6.500X$ ($R^2 = 0.52$).

Bristles S–S linkages

No effect of dietary SID Met + Cys levels was observed on the wavelength bands of the area ($P > 0.05$) referring to the S–S linkages of the animals' bristles, for maintaining or increasing the 1:1 SAA (Table 2).

Discussion

Cysteine is synthesized from the catabolism of Met through the transsulphuration pathway in the liver (Rezaei *et al.*, 2013), however, increasing the same dietary levels of Met (DL-Met) and Cys (L-Cys), in order to maintain the 1:1 SAA in the diet, did not provide any differences in the performance. On the other hand, increasing SID Met + Cys using a free SAA ratio (i.e. supplementing only DL-Met) provided the fitting of second-degree models for final weight and ADG, estimating the best SID Met + Cys levels at 6.63 and 6.61 g/kg, respectively.

Evaluating dietary SID Met + Cys levels for barrows and gilts (15–30 kg), Moura *et al.* (2006) observed a better ADG at 5.82 g/kg of SID Met + Cys, which is lower than the obtained in this study (6.61 g/kg). The NRC (2012) proposed 6.8 g/kg SID Met + Cys as the requirement for barrows and gilts, that is close to the optimum level obtained for the ADG in the current study (6.61 g/kg). However, the best SID Met + Cys obtained in this study was intermediate compared with the requirements of medium (6.38 g/kg)

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Table 2. Performance, *longissimus dorsi* muscle depth (LD), backfat thickness (BF) and wavelength area of bristles' S–S linkages (S–S linkages) of starting barrows receiving diets containing levels of standardized ileal digestible (SID) Methionine (Met) + Cysteine (Cys), maintaining (1:1 SAA ratio) or increasing (Free SAA ratio) the ratio between SID Met and SID Cys

Item	Basal	1:1 SAA ratio						Free SAA ratio						
SID Met + Cys (g/kg)	5.2	5.8	6.4	7.0	*P* value			5.8	6.4	7.0	*P* value			SEM
SID Met (g/kg)	2.6	2.9	3.2	3.5				3.2	3.8	4.4				
SID Cys (g/kg)	2.6	2.9	3.2	3.5	Lin	Quad	LRP[a]	2.6	2.6	2.6	Lin	Quad	LRP	
Initial weight (kg)	15.45	15.37	15.14	15.11	–	–	–	15.24	15.16	15.24	–	–	–	0.078
Final weight (kg)	30.0	31.8	32.0	31.7	–	–	–	30.7	32.3	32.6	–	–	0.019[b]	0.27
ADFI (g)[c]	1137	1192	1193	1148	–	–	–	1138	1188	1200	–	–	–	11.3
DI SID Met + Cys (g)[d]	5.9	6.9	7.6	8.0	0.002[e]	–	–	6.6	7.6	8.4	0.001[f]	–	–	0.12
ADG (g)[g]	530	573	619	589	–	–	–	564	613	625	–	–	0.003[h]	6.8
F:G (g/g)[i]	2.15	2.10	1.93	1.95	–	–	–	2.02	1.94	1.92	–	–	–	0.019
LD (cm)	2.21	2.38	2.43	2.50	–	–	–	2.37	2.53	2.71	–	–	–	0.030
BF (cm)	0.50	0.59	0.53	0.48	–	–	–	0.46	0.45	0.48	–	–	–	0.009
S–S linkages	44.4	46.2	46.0	45.3	–	–	–	43.3	42.9	44.9	–	–	–	0.67

[a]Linear Response Plateau.
[b]$Y = 19.8317 + 1.925\ X$ ($R^2 = 0.94$).
[c]Average daily feed intake.
[d]Daily intake of SID Met + Cys.
[e]$Y = -0.89744 + 1.18264\ X$ ($R^2 = 0.57$).
[f]$Y = 0.485 + 1.0541\ X$ ($R^2 = 0.87$).
[g]Average daily gain.
[h]$Y = 167.8334 + 69.1667\ X$ ($R^2 = 0.99$).
[i]Feed:gain ratio.

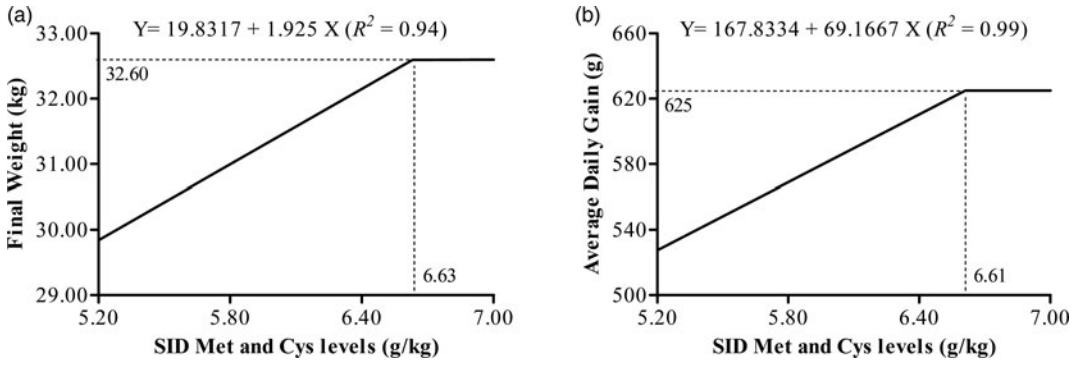

Fig. 1. Final weight (a) and average daily gain (b) of starting barrows receiving diets containing levels of standardized ileal digestible (SID) methionine (Met) + cysteine (Cys), increasing the ratio between SID Met and SID Cys.

and superior (7.13 g/kg) performance barrows (15–30 kg) proposed by Rostagno *et al.* (2017).

The obtained results for ADG may be related to a higher SID Met requirement than SID Cys, since the studied levels of SID Met + Cys were the same, maintaining or increasing the 1:1 ratio between SAA and because ADG was only affected by increasing the 1:1 SAA ratio, i.e. only increasing SID Met in the diets to achieve the studied SID Met + Cys levels. These findings may be related to the importance of Met in the metabolism, since it participates as a building block in the protein synthesis and plays important roles in many metabolic and physiological functions in pigs, including its role as the main methyl donor, also considered an important source of sulphur, participates in endogenous antioxidant processes and acts as a precursor of many bioactive compounds (Zhai *et al.*, 2012; Shen *et al.*, 2014). Other important reason may be due to the proportion of Met + Cys that can be provided by Cys, which is more used for maintenance than for new tissue accretion (Lewis, 2003), impairing the L-Cys supplementation (i.e. to maintain the 1:1 SAA ratio) to show the same ADG obtained by supplementing only DL-Met, in order to provide the free SAA ratio.

The observed results showed no need to maintain the same ratio between the SAA in starting pig diets. In this regard, Wu (2013) showed a 1:1 ratio between Met and Cys for pigs (5–110 kg), but in the current study the best dietary SID Met + Cys level obtained for ADG (6.61 g/kg), increasing the 1:1 SAA (free SAA ratio), provided a 1:0.65 SAA ratio, showing that 60.67% of the pigs' requirement was supplied by SID Met. On the other hand, Rostagno *et al.* (2017) suggested that 50.91% of the requirement of SID Met + Cys should be met by SID Met. The differences between the obtained ratio and that one's reported in the literature suggest that SID Cys requirement needs to be reevaluated for starting pigs, in order to stablish a better ratio between the SAA, because Met can fulfil the need for both SAA but cysteine can only fulfil its own need. Moreover, Roth and Kirchgessner (1989) reported a better ADG for pigs (30–60 kg) when the Met:Cys ratio was about 55%; this ratio was higher (57%) for pigs from 60 to 90 kg.

The L-Cys supplementation may reduce the feed intake and weight gain in young animals (Yin *et al.*, 2016), but in this study the L-Cys supplementation, used to maintain the 1:1 SAA, did not affect the ADFI, maybe due to its low dietary supplementation, even to achieve the highest level of SID Met + Cys evaluated in this trial. Additionally, Dilger *et al.* (2007) evaluating Cys excess in piglet diets reported a decreased feed intake and ADG, showing that the highest SID Cys level used in this study, mainly with the 1:1 SAA, was below the limit considered undesirable for the pigs' feed.

The ADFI was not affected but an increased DI Met + Cys was observed according to the increased levels of dietary SID Met + Cys, maintaining the 1:1 SAA or with a free SAA ratio. This result was expected, since treatments consisted of increasing levels of dietary SAA and the ADFI was not affected by the treatments. Excepting the daily intake Met + Cys, no differences were observed for the other studied parameters by maintaining the 1:1 SAA ratio.

Methionine is an essential AA used in protein synthesis (Frantz *et al.* 2009), but the LD was not affected by SID Met + Cys levels. Additionally, the increased muscle deposition also depends on the genetic potential of the pigs and maybe the studied SAA levels given their genetic make-up for the LD. In this sense, the increased ADG observed for pigs receiving the increasing dietary SID Met + Cys levels until 6.61 g/kg, with a free SAA ratio, is still associated with other lean tissues, because dietary Met increases nitrogen retention and protein accretion due to increasing protein synthesis and also decreasing protein degradation, improving the growth performance of pigs (Kong et al., 2016*a*, *b*).

Methionine also acts donating methyl group (CH_3) to synthesize biomolecules, such as carnitine, that acts in the lipid metabolism and long-chain fatty acid transfer across the mitochondrial membrane. The fatty acyl binds to carnitine providing fatty acyl carnitine, enabling its transport from the cytosol to the mitochondrial matrix to be oxidized (Strijbis *et al.*, 2008; Apple *et al.*, 2011).

Despite of the carnitine effect, and the Met role in its synthesis, the studied SID Met + Cys levels were unable to provide a reduction in the BF, either maintaining the 1:1 SAA ratio or using a free SAA ratio in the diets, mainly in the low studied levels using a 1:1 SAA ratio. On the other side, Castellano *et al.* (2015) observed an increased level of lipogenesis and lipolytic indicators in porcine adipose tissues due to a Met-deficient diet. The aforementioned authors also reported that expression levels of transcriptional regulators of adipogenesis and adipocyte number estimated in adipose tissues did not differ between feeding groups receiving a Met-deficient diet or a control treatment, ruling out the possibility of adipogenic modulation in response to dietary Met deficiency in young pigs. On the opposite, up-regulations in genes coding for the insulin-responsive glucose transporter GLUT4 and lipogenic enzymes elevated activities of NADPH enzyme suppliers in adipose tissues of pigs receiving Met-deficient diets, both argue for an increased rate of de novo fatty acid synthesis in these fat tissues

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Table 3. Blood parameters of starting barrows receiving diets containing levels of standardized ileal digestible (SID) Methionine (Met) + Cysteine (Cys), maintaining (1:1 SAA ratio) or increasing (Free SAA ratio) the ratio between SID Met and SID Cys

Item	Basal	1:1 SAA ratio						Free SAA ratio						
SID Met + Cys (g/kg)	5.2	5.8	6.4	7.0	*P* value			5.8	6.4	7.0	*P* value			SEM
SID Met (g/kg)	2.6	2.9	3.2	3.5				3.2	3.8	4.4				
SID Cys (g/kg)	2.6	2.9	3.2	3.5	Lin	Quad	LRP[a]	2.6	2.6	2.6	Lin	Quad	LRP	
Urea (mg/dl)	26.56	27.12	26.80	26.10	–	–	–	27.30	28.10	21.30	–	–	–	0.661
Triglycerides (mg/dl)	44	45	43	44	–	–	–	45	43	44	–	–	–	1.3
Total Proteins (g/dl)	5.88	5.97	5.95	5.73	–	–	–	6.13	5.96	5.84	–	–	–	0.041
Creatinine (mg/dl)	0.97	1.26	1.26	1.14	–	–	–	1.03	1.09	1.14	–	–	–	0.027
Glucose (mg/dl)	77.1	84.1	85.8	86.2	–	–	–	80.0	83.1	90.3	0.006[b]	0.006[c]	–	0.99
Homocysteine (μmol/l)	53.1	52.6	52.1	51.0	–	–	–	50.0	50.6	50.2	–	–	–	0.27
Total cholesterol (mg/dl)	64	62	60	55	–	–	–	64	61	50	0.003[d]	–	–	1.1
HDL (mg/dl)[e]	22.9	25.7	26.2	25.1	–	–	–	21.5	25.3	24.9	–	–	–	0.51

[a]Linear response plateau.
[b]$Y = 39.2133 + 7.11667\ X$ ($R^2 = 0.32$).
[c]$Y = 148.983–29.3139\ X + 2.98611\ X^2$ ($R^2 = 0.34$).
[d]$Y = 105.529–6.50\ X$ ($R^2 = 0.52$).
[e]High-density lipoprotein.

when pigs are submitted to a Met-deficient diet (Castellano *et al.*, 2015).

Cysteine presents an expressive participation in the bristle's protein synthesis (Stipanuk and Ueki, 2011), because the cells of the bristle fibre cuticle have several layers with different Cys concentrations. More than 30% may be found in layer A, the layer B contains about 15% and the endocuticle about 3% (Velasco *et al.*, 2009). However, no effect of SID Met + Cys levels was observed on the wavelength bands of the area, referring to the S–S linkages of the animals' bristles.

The obtained results showed that increasing SAA in the diets, using L-Cys and/or DL-Met, did not affect the disulphide bonds in the bristles, which are characteristics of Cys bonds observed in the Raman spectrum. The amount of SAA included in the experimental diets were not able to directly affect the S–S linkages in the bristles. Additionally, it should be considered that between 20 and 48% of the total Met intake may be intended for the splanchnic tissue (Stoll and Burrin, 2006) comprising all tissues drained by the venous circulation, including the liver, in which many metabolic reactions involving Met occurs (Riedijk *et al.*, 2007). Furthermore, Stoll and Burrin (2006) reported that about 70% of the dietary Cys is metabolized in the gut, not reaching the bloodstream, limiting the metabolic use (intermediate metabolism) of this AA supplied by the diet. These reports help understanding that the amount of SAA necessary to affect the bristles' S–S linkages can be higher, mainly Cys supplementation.

The increased concentration of plasma glucose due to increasing SID Met + Cys, with a free SAA ratio, is in agreement with Castellano *et al.* (2015), which observed a plasma glucose concentration 8% lower in pigs receiving a Met-deficient diet. However, the determination coefficients (R^2) obtained in this study do not provide an acceptable fit in order to make more inferences about this response.

The depressing effect of Met on cholesterol in the mammalian body may be related to Met's ability to generates the lipid peroxidation of cell membrane components (cholesterol, gangliosides, glycosphingolipids glycerophospholipids and unsaturated), as high rates of lipid peroxidation (TBARS) were observed in the cerebral cortex of rats subjected to Met infusion (Stefanello *et al.*, 2005), but this response needs to be further investigated. By the other side, França *et al.* (2006) evaluating two diets for starting pigs (4.6 and 34.6 g/kg of SID Met + Cys) reported that serum cholesterol was not affected by treatments, but the authors observed higher homocysteine levels when adding synthetic Met in the diet.

Methionine participates in homocysteine metabolism in two biological pathways, i.e. remethylation and transsulphuration. Large amounts of Met or S-adenosyl-methyltransferase, stimulates the transulphuration route, producing Cys in the metabolism. The remethylation is favoured when there are low concentrations of Met or S-adenosyl-methyltransferase. At this stage, homocysteine receives a methyl group N 5-methyltetrahydrofolate, or betaine, to synthesize Met (Finkelstein, 1998).

However, no relationship between plasma homocysteine and increasing dietary SID Met + Cys levels were observed, maintaining the 1:1 SAA ratio or increasing (free SAA ratio). This response may be associated with the amount of dietary Met, since França *et al.* (2006) observed high plasma homocysteine concentration by adding synthetic Met in the experimental diets of pigs to meet 34.6 g/kg of Met + Cys, that is expressively higher than the ones evaluated in the current study. Additionally, these authors also observed that 34.6 g/kg of Met + Cys enhanced HDL levels compared to 4.6 g/kg of Met + Cys in the diet; and this response was not observed in this study, since plasma HDL was not affected for both studied SAA ratio situation. As aforementioned, Met + Cys levels used by the authors were significantly higher compared to the levels assessed in the current study.

The best dietary level of SID Met + Cys obtained in the current study was 6.61 g/kg, based on ADG. Although the Met meets the needs of Cys, many studies differ about the optimum SAA ratios. The results obtained in this study showed the effectiveness of using only Met to meet the requirements of SID Met + Cys for starting barrows, which is a common practice used to formulate diets to swine. As aforementioned, there was not a need to maintain the same ratio between the SAA, and there is further a need to evaluate an ideal ratio between the SAA.

Conclusion

The requirement of SID Met + Cys for starting barrows (15–30 kg) was 6.61 g/kg for an optimum ADG, corresponding to 7.88 g/day, with no need to maintain the same ratio between the SAA.

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Conflict of interest. None.

Ethical standards. All procedures referred to the animals used in the experiment were approved by the Ethical Conduct Committee on the Use of Experimental Animals (003/2013) of the State University of Maringa, Brazil.

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