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Bioavailability of di-peptide DL-methionyl-DL-methionine in comparison to DL-methionine in weaned and growing pigs

L.S. Santos[a], J.K. Htoo[b], C. Fracaroli[a], W.C. Silva[a], J.P. Gobi[a], A.M. Veira[a], N.A.A. Barbosa[c], L. Hauschild[a,*]

[a] São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Animal Science Department, Jaboticabal CEP: 14883-108, Brazil
[b] Evonik Nutrition & Care GmbH, 63457 Hanau-Wolfgang, Germany
[c] Evonik Degussa Brasil Ltda, 07.222-000 São Paulo, Brazil

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ABSTRACT

The relative bioavailability (RBV) of a dipeptide DL-methionyl-DL-methionine (DL-Met-Met) was compared with DL-methionine (DL-Met) in growing pigs (experiment 1; N-balance study) and in weaned pigs (experiment 2; performance study). In experiment 1, 42 barrows with an initial body weight (BW) of 21.0 ± 1.37 kg were assigned to 7 dietary treatments with 6 replicate/pigs per treatment in a nitrogen (N) balance study to evaluate the RBV of DL-Met-Met to DL-Met. A basal diet (BD) was formulated to be adequate for all amino acids with the exception of Met + Cys which was 68% of the requirement [4.7 g/kg standardized ileal digestible (SID) Met + Cys; 11.5 g/kg SID Lys] for 20–25 kg pigs. Three graded levels of DL-Met (0.3, 0.6 and 0.9 g/kg) and DL-Met-Met (0.306, 0.612 and 0.919 g/kg) were supplemented to the BD to create diets 2–7. In experiment 2, a total of 189 weaned pigs (initial BW of 10.2 ± 0.98 kg) were assigned to 7 dietary treatments with 9 replicates/pens of 3 pigs per treatment. The dietary treatments consisted of a Met-deficient BD (5.3 g/kg SID Met + Cys; 13.0 g/kg SID Lys) and the same 3 graded levels of DL-Met and DL-Met-Met as in Exp. 1. In experiment 1, supplementation with DL-Met or DL-Met-Met linearly decreased ($P \leq 0.01$; linear) urinary N excretion and increased ($P \leq 0.02$; linear) N retained (g/day), N retention (% of intake and % of absorbed). However, there was no effect of Met sources on all N balance parameters. Based on the slope-ratio regression the RBV for DL-Met-Met compared to DL-Met was estimated 111% [95% confidence interval (CI): 63-158%] for N retained (g/d), 109% (95% CI: 57-160%) for N retention (% of intake) and 98% (95% CI: 43–154%) for N retention (% of absorbed) on an equi-molar basis. In experiment 2, the overall average daily gain (ADG) and gain:feed ratio (G:F) increased linearly ($P < 0.01$) by supplementation with DL-Met or DL-Met-Met. The average daily feed intake increased by supplementation with DL-Met ($P = 0.02$) and DL-Met-Met ($P = 0.09$). For ADG, the RBV for DL-Met-Met was estimated 104% (95% CI: 66-141%) on an equi-molar basis by the slope-ratio. Based on G:F, the RBV for DL-Met-Met was estimated 117% (95% CI: 61–174%) on an equi-molar basis. The results of both experiments indicate that the bioavailability DL-Met-Met is not different and at least equally bioavailable as DL-Met as a Met source for pigs.

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; BD, basal diet; BW, body weight; CI, confidence interval; DL-Met, DL-methionine; DL-Met-Met, DL-methionyl-DL-methionine; Exp, experiment; G:F, gain:feed ratio; Met, methionine; RBV, relative bioavailability; SID, standardized ileal digestible

* Corresponding author.
E-mail address: lhauschild@fcav.unesp.br (L. Hauschild).

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1. Introduction

Methionine (Met) is the second or third limiting amino acids (AA) in typical swine diets, and supplemental Met sources are commonly used to supply adequate dietary level of Met + Cys. Different supplemental Met products are available which include DL-methionine (DL-Met; 99% purity), L-methionine (99% purity), liquid or calcium salt of methionine hydroxy analogue free acid (MHA-FA, 88% active substance, Kim et al., 2006; MHA-Ca, 84% active substance, Opapeju et al., 2012). A new Met source as a dipeptide DL-methionyl-DL-methionine (DL-Met-Met; 97% purity; AQUAVI® Met-Met produced by Evonik Nutrition & care GmbH) is currently available mainly for shrimp.

It is well accepted that supplemental (crystalline) AA are well utilized by animals including pigs (Nørgaard et al., 2016). Di- and tri-peptides are transported into the enterocyte intact by peptide transporter, PepT1 and then hydrolysed to free amino acids by peptidases (Leibach and Ganapathy, 1996; Gilbert et al., 2008). It has been reported that di- and tri-peptides are absorbed rapidly and efficiently by the intestine without initial pancreatic digestion in sea bass (Infante et al., 1997). Feeding a peptide product (dried hydrolysate of pig intestines) improved growth performance of weaned pigs (Cho et al., 2010). As reported by Sleisinger et al. (1976) the intestinal transport systems of small peptides are different from those of free AA and this might minimize the competition for transport sites. Compared with other Met sources, dipeptide of DL-Met-Met due to its low water solubility (Niu et al., 2018), may presumably be less unstable and better synchronize with protein bound AA in terms of absorption along the small intestine.

Bioavailability is the extent to which an ingested nutrient in a particular source is digested and absorbed in a form that can be utilized in metabolism by the animal and it is commonly determined by a slope-ratio model (Littell et al., 1997). Recently, a greater relative bioavailability (RBV) for DL-Met-Met (AQUAVI® Met-Met) relative to DL-Met has been reported in white shrimp (Niu et al., 2018). However, there is a lack of information about the RBV of DL-Met-Met relative to DL-Met in pigs. Therefore, the RBV of DL-Met-Met in comparison to DL-Met was evaluated in 20–28 kg pigs using a nitrogen (N)-balance assay (Exp. 1) applying N deposition as the basis for RBV comparison. Additionally, a classical dose-response growth trial (Exp. 2) was conducted with 10–22 kg pigs for comparing RBV of DL-Met-Met with DL-Met using average daily gain (ADG) and gain:feed ratio (G:F) to confirm the results obtained in Exp. 1.

2. Materials and methods

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of Sao Paulo State University. Two experiments were conducted using crossbred pigs (Agroceres Pig Improvement Company, Rio Claro, SP, Brazil: AGPIC 337^{TM} x Camborough^{TM}) in a 12-h of natural light program at the facility of the São Paulo State University.

2.1. Experiment 1 (N-balance assay)

2.1.1. Animals, housing and experimental design

Forty-two barrows with an average initial body weight (BW) of 21.0 ± 1.37 kg were used in two batches of 21 pigs each. Each batch of pigs served as a block. Three pigs were randomly allotted to each of 7 experimental diets within each block resulting in a total of 6 replicate pigs per dietary treatment. Pigs were housed individually in adjustable metabolism crates (0.60 × 1.60 m) in a temperature controlled room (22 °C). Each crate was equipped with a single low-pressure drinking nipple and a stainless steel self-feeder.

2.1.2. Dietary treatments

A basal diet was formulated based on corn and soybean meal to be adequate for all AA with the exception of Met + Cys which was 68% of the requirement [4.7 g/kg standardized ileal digestible (SID) Met + Cys; 11.5 g/kg SID Lys] for 20–25 kg pigs (NRC, 2012; Table 1). Three graded levels of DL-Met (0.3, 0.6 and 0.9 g/kg) or DL-Met-Met (0.306, 0.612 and 0.919 g/kg) based on the purity of the products, were added to the basal diet for achieving the target Met dietary levels to create diets 2–7. The Met + Cys inclusion levels ranged from 68 to 80% of the requirement, while the (Met + Cys)/Lys ratio increased from 0.41 to 0.48%, from the basal to the most supplemented diet, respectively. Supplemental DL-Met (MetAMINO®) and DL-Met-Met (AQUAVI® Met-Met) were obtained from a commercial company (Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). The diets were formulated on the basis of analyzed AA contents of ingredients and the SID coefficients of AA (AMINODat 4.0 Platinum version, 2010).

2.1.3. Feeding and collection

Nitrogen balance trial lasted for 12 days consisting of a 7-day adaptation period to the metabolism crates, meal regimen and experimental diets and a 5-day period of quantitative collection of feces and urine. Each metabolism crate had a collection tray for urine collection and a fine-mesh net just above the tray for fecal collection thus, allowing for separate but total collection of feces and urine. Pigs were weighed at the beginning and end of the adaptation period and at the end of collection. Daily feed allowance during the adaptation and collection periods was set at 4% (as-fed basis) of the average BW of all pigs at the beginning and end of adaptation period, respectively, corresponding to approximately 3 times their daily energy requirement for maintenance (i.e., 0.44 MJ ME/kg of $BW^{0.75}$/day) (NRC, 1998) to ensure similar feed intake.

The daily feed allotment was divided into two equal portions and offered at 0800 and 1600 h. Diets were fed as a mash and pigs had unlimited access to water at all times. On the morning of day 8, each pig received 100 g of feed mixed with 3 g of ferric oxide as an indigestible marker to identify the start of fecal collection. Upon complete consumption of the marked diet, the remainder of the

Table 1
Ingredient and nutrient compositions of the basal diets, as-fed basis[a].

	Exp. 1 (20–28 kg)	Exp. 2 (10–22 kg)
Ingredients, g/kg		
Corn	731.6	677.1
Soybean meal	216.2	235.1
Soybean oil	8.4	26.7
Spray-dried plasma	–	20.0
Limestone	7.0	7.8
Dicalcium phosphate	15.4	13.7
Biolys (54.6% L-Lys)	8.3	8.1
L-Threonine	2.3	2.2
L-Valine	1.2	1.0
L-Isoleucine	0.2	0.5
L-Tryptophan	1.1	1.0
Salt	5.3	3.8
Choline chloride (60%)	0.5	0.5
Mineral –vitamin premix[b]	2.5	2.5
Calculated composition, g/kg[c]		
Net energy, MJ/kg	10.3	10.6
SID Lys	11.5	13.0
SID Met	2.4	2.6
SID Met + Cys	4.7	5.3
SID Thr	7.5	8.4
SID Trp	2.6	2.9
Analyzed composition, g/kg		
Crude protein	170.4	188.2
Lys	12.5	14.0
Met	2.6	2.8
Met + Cys	5.3	5.9
Thr	7.8	9.3
Trp	2.7	3.1
Ile	6.7	7.8
Val	8.7	9.9

Exp. = experiment; Lys = lysine; Met = methionine; Cys = cysteine; Thr = threonine; Trp = tryptophan; Ile = isoleucine; Val = valine; SID = standardized ileal digestible.

[a] Graded levels of DL-Met (0.3, 0.6 and 0.9 g/kg) or DL-Met-Met (0.306, 0.612 and 0.919 g/kg) were added to a single common batch of the basal diet to obtain diets 2–7, respectively in both Exp. 1 and 2. The analyzed supplemental Met content was 0.35, 0.62, 0.97, 0.29, 0.63 and 0.80 g/kg in diets 2–7, respectively in Exp. 1. The analyzed supplemental Met content was 0.34, 0.59, 1.00, 0.31, 0.68 and 0.87 g/kg in diets 2–7, respectively in Exp. 2.

[b] Supplied the following per kg of diet: 10,000 IU of vitamin A, 2000 IU of vitamin D3, 15 mg of vitamin E, 1.3 mg of vitamin B1, 3.5 mg of vitamin B2, 0.025 mg of vitamin B12, 1.5 mg of vitamin B6, 10 mg of calcium pantothenate, 15 mg of nicotinic acid, 0.1 mg of biotin, 0.6 mg of folic acid, 2 mg of vitamin K3, 80 mg of Fe as ferrous sulfate (monohydrate), 6 mg of Cu as copper sulfate (pentahydrate), 0.75 mg of Co as cobalt sulfate, 150 mg of Zn as zinc sulfate (monohydrate), 60 mg of Mn as manganese sulfate (monohydrate), 0.75 mg of I as calcium iodate, 0.10 mg of Se as sodium selenite and 150 mg of ethoxiquin.

[c] Values calculated for net energy according to NRC (2012) and for SID AA based on analyzed AA values for individual ingredients and SID coefficients from AMINODat® 4.0. Platinum version, 2010; Evonik Degussa GmbH, Hanau-Wolfgang, Germany.

morning allotment was fed to the pigs.

Feces collection commenced once the marked (red) feces appeared. Feces were collected daily, weighed and stored immediately in a −20 °C freezer. At the end of the collection period, pigs were fed 100 g of feed mixed with 3 g of ferric oxide on the morning of day 13 and this was followed by feeding the remaining of the morning allotment. Fecal collection was terminated immediately when the marked feces appeared. At the end of collection, fecal samples were pooled per pig and then sub-sampled for analysis. Urine weight was determined daily and a 10% aliquot was sub-sampled and stored at a −20 °C freezer for subsequent analysis. The urine collection was initiated and terminated with the feeding of marked diet on the morning of days 9 and 13, respectively. Urine was collected in plastic jugs containing 10 ml of 6 M HCl. Glass wool was placed in the funnel of the collection jugs to trap any fecal materials contained in the urine.

2.2. Experiment 2 (performance trial)

2.2.1. Animals, housing and experimental design

A total of 189 weaned pigs (98 castrated males and 91 females), weaned approximately at 28 day of age (about 8 kg BW) were housed in 63 pens (1.45 × 1.75 m), equipped with semi-automatic feeder (fed ad libitum) and a single drinking nipple. Room temperature was controlled at 24 °C by adjustment of the heaters and management of side curtains.

After arriving to the facility, all pigs were given an adaptation period of about 7 days during which they were fed a commercial pre-starter diet until they reached average initial BW of 10.2 ± 1.0 kg. The initial BW and sex were balanced at the same proportion across pens at the start of the study. Pigs were housed in a nursery room with 63 identical pens, with 3 pigs each (2 males and 1 female or vice versa) and 9 replicate pens for each of the 7 dietary treatments during 21 days. The BW of individual pigs and feed disappearance were recorded on a weekly basis to calculate ADG, ADFI and G:F.

2.2.2. Dietary treatments

The dietary treatments consisted of a Met-deficient basal diet (2.4 g/kg SID Met; 5.3 g/kg SID Met + Cys; 13.0 g/kg SID Lys; diet 1) and the same 3 graded levels of DL-Met and DL-Met-Met used in Exp.1 were supplemented to the basal diets to create diets 2 to 7. The diets were based on corn, soybean meal and spray-dried blood plasma. The dietary levels of AA were balanced to be adequate for all AA with the exception of Met + Cys which was set to be deficient containing about 68% of the requirement for 10 to 25 kg pigs (NRC, 2012; Table 1) up to 80% of the Met + Cys requirement in both highest supplemented diets. The (Met + Cys)/Lys ratio ranged between 0.41 to 0.49%, from the basal to the most supplemented diet. The diets were formulated on the basis of analyzed AA contents of ingredients and the SID coefficients of AA (AMINODat 4.0 Platinum version, 2010).

2.3. Chemical analysis

Fecal and feed samples of 5 days were dried at 55 °C for 72 h and ground in a Wiley mill through a 0.5 mm sieve. The fecal and feed samples were analyzed for dry matter (procedure 4.1.06, AOAC, 2000). A Leco FP 528 Nitrogen Analyzer (LECO Corporation, USA) was used to determine N in feces, feed and urine. Chemical analysis of AA from the ingredients and diet samples were performed as outlined by Kim et al. (2006).

2.4. Statistical analysis

In Exp. 1, data were analyzed as a randomized complete block design using GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The fixed effect of diet and block (batch) were included in the model. The individual pigs were used as the experimental unit. Orthogonal-polynomial contrasts were used to determine the linear and quadratic effects of increasing levels of DL-Met and DL-Met-Met on response criteria, and the effect of Met sources. The N retention data were subjected to the multiple linear regression using the following equation: $y = a + b_1\ x_1 + b_2\ x_2$where y is the response variable, a is the common intercept, b_1 is the slope of the line in response to percent DL-Met in the diet, x_1 DL-Met is the percent supplemental DL-Met in the diet, b_2 is the slope of the line for percent DL-Met-Met in the diet, x_2 DL-Met-Met is the percent supplemental DL-Met-Met in the diet. Based on the N retention data (N retained, g/d; N retention, % of intake; N retention, % of absorbed), the RBV of DL-Met-Met as compared with DL-Met were calculated as the ratio of their linear slopes (i.e. $b_2/b_1 \times 100$) as described by Littell et al. (1997).

In Exp. 2, data were analyzed as a completely randomized design using the GLM procedure of SAS with pen as the experimental unit. The model included diet as the fixed effect. Orthogonal-polynomial contrasts were used to determine linear and quadratic effects of increasing levels of DL-Met and DL-Met-Met on response criteria, and the effect of Met sources. The statistical significance levels were claimed at $P \leq 0.05$ and $0.10 > P > 0.05$ considered a trend. Based on the fitness of the responses according to the lower residual error, the RBV of DL-Met-Met relative to DL-Met were estimated based on ADG using the linear slope ratio. The G:F data, having a better fitness for a nonlinear response, was evaluated by the nonlinear exponential model (at 95% of maximum response) as described by Littell et al. (1997).

3. Results

3.1. Diets and AA levels

The analyzed crude protein and AA contents of experimental diets (Table 1) were close to the formulated levels in both experiments, confirming adequate mixing accuracy. The analyzed content of the supplemental DL-Met and DL-Met-Met were within 10% of the calculated values for the diets, resulting in graded levels.

3.2. Experiment 1 (N-balance assay)

All pigs consumed their daily feed allotment during the experiment. Met supplementations had no effect on fecal N excretion. Supplementation with DL-Met or DL-Met-Met linearly decreased ($P \leq 0.01$; linear) urinary N excretion but did not affect fecal N excretion. As a result, N retained (g/day), N retention (% of intake) and N retention (% of absorbed) increased ($P \leq 0.02$; linear) by graded supplementation with DL-Met or DL-Met-Met. Nitrogen retention (g/d and % of intake) were the lowest for pigs fed the Met-

Table 2
Effects of dietary DL-Met or DL-Met-Met supplementation on nitrogen balance, Exp. 1[a].

	Supplementation level (g/kg of diet; as-fed)									
		DL-Met			DL-Met-Met				Contrast P-value[b,d]	
Items	Basal	0.3	0.6	0.9	0.306	0.612	0.919	SEM	C1	C2
BW, kg										
At initiation of adaptation	20.95	21.10	20.82	20.80	21.05	20.88	20.93	0.32	0.61	0.88
At end of adaptation	23.46	23.89	23.27	23.01	23.39	23.62	23.88	0.41	0.30	0.45
At end of collection	27.02	28.02	27.28	27.04	27.24	27.50	28.10	0.44	0.73	0.10
N intake, g/d[c]	22.64	22.39	22.44	22.37	22.28	22.44	22.51	0.05	< 0.01	0.31
Fecal N, g	3.31	3.35	3.56	3.61	3.48	3.48	3.26	0.30	0.40	0.91
Urine N, g	6.05	6.01	4.34	4.26	4.99	4.91	4.16	0.47	< 0.01	0.01
N absorbed, g/d	19.33	19.04	18.87	18.76	18.80	18.96	19.25	0.31	0.17	0.96
N retained, g/d	13.28	13.03	14.54	14.50	13.81	14.05	15.09	0.49	0.02	0.01
N retention, % of intake	58.81	58.26	64.85	64.78	61.99	62.77	66.83	2.12	0.01	0.01
N retention, % of absorbed	69.12	68.66	77.10	77.32	73.49	74.25	78.32	2.28	< 0.01	0.01

[a] Each least square mean represents 6 pigs (n = 6); DL-Met = DL-methionine; DL-Met-Met = DL-methionyl-DL-methionine; BW = body weight; Exp. = experiment; N = nitrogen.
[b] C1 = Linear effect of DL-Met; C2 = Linear effect of DL-Met-Met.
[c] Quadratic effect of DL-Met-Met (P = 0.07) and quadratic effect of DL-Met (P < 0.01).
[d] The contrast of DL-Met vs. DL-Met-Met was not significant (P > 0.10).

deficient diet and was highest for pigs fed diet supplemented with 0.09% DL-Met and 0.092% DL-Met-Met (Table 2). There was no effect of Met sources on all N balance parameters.

Based on the slope-ratio regression the RBV for DL-Met-Met compared to DL-Met was estimated 111% [95% confidence interval (CI): 63–158%] for N retained (g/d), 109% (95% CI: 57–160%) for N retention (% of intake), and 98% (95% CI: 43–154%) for N retention (% of absorbed), respectively, on an equi-molar basis (Figs. 1–3). However, 95% of CI for the RBV reaches 100% indicating the RBV of DL-Met-Met was statistically not different from that of DL-Met.

3.3. Experiment 2 (performance trial)

The performance results during the 21-day experimental period are presented in Table 3. The average initial and final BW of the pigs was 10.2 ± 1.0 kg and 20.6 ± 2.2 kg. Performance of pigs fed the Met-deficient basal diet was the lowest and graded addition of both Met sources to the basal diet linearly increased (P < 0.01) ADG, final BW and G:F for each individual and the overall period evaluated. The overall ADFI increased (P = 0.02) by supplementation with DL-Met, and during the same phase, ADFI tended to improve (P = 0.09) by DL-Met-Met supplementation. For d 0–7, d 0–14 and overall (d 0–21), ADFI, ADG and G:F were not different (P > 0.40) among pigs fed similar inclusion levels of the two Met sources. For ADG, the RBV for DL-Met-Met was estimated 104% (95% CI: 66–141%) on an equi-molar basis by the slope-ratio. Based on G:F, the RBV for DL-Met-Met was estimated 117% (95% CI: 61-174%) on an equi-molar basis (Figs. 4 and 5). The 95% of CI for the RBV covers 100% which indicate that DL-Met-Met and DL-Met were equally bioavailable for weaned pigs.

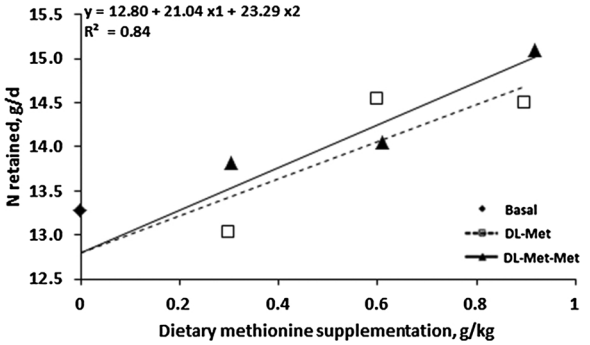

Fig. 1. Slope-ratio comparison of dietary DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (DL-Met-Met) based on N retained (g/d) in Exp. 1. Relative bioavailability of DL-Met-Met was 111% on an equimolar basis (95% confidence interval: 63–158%).

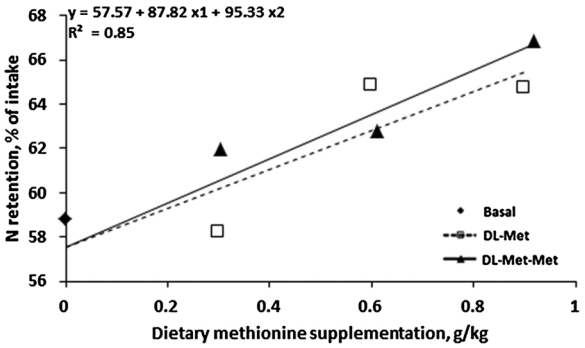

Fig. 2. Slope-ratio comparison of dietary DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (DL-Met-Met) based on N retention (% of intake) in Exp. 1. Relative bioavailability of DL-Met-Met was 109% on an equimolar basis (95% confidence interval: 57–160%).

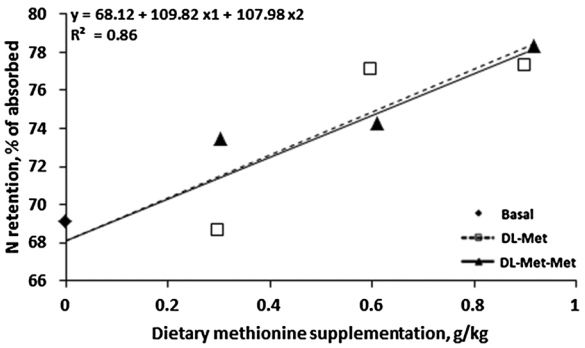

Fig. 3. Slope-ratio comparison of dietary DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (DL-Met-Met) based on N retention (% of absorbed) in Exp. 1. Relative bioavailability of DL-Met-Met was 98% on an equimolar basis (95% confidence interval: 43–154%).

Table 3
Effects of dietary DL-Met and DL-Met-Met supplementation on performance of pigs, Exp. 2[a].

	Supplementation level (g/kg of diet; as-fed)									
		DL-Met			DL-Met-Met				Contrast P-value[b,d]	
	Basal	0.3	0.6	0.9	0.306	0.612	0.919	SEM	C1	C2
Initial BW, kg	10.17	10.34	10.25	10.24	10.18	10.27	10.30	0.180	0.75	0.59
Final BW, kg	19.07	20.30	20.22	21.71	20.16	20.88	21.69	0.703	< 0.01	< 0.01
0–7 days										
ADFI, g/d[c]	662	680	686	781	697	722	759	21.5	< 0.01	< 0.01
ADG, g/d	353	415	419	486	413	440	506	21.0	< 0.01	< 0.01
G:F	0.533	0.609	0.610	0.626	0.592	0.608	0.676	0.022	< 0.01	< 0.01
0–14 days										
ADFI, g/d	751	783	792	879	798	815	885	23.0	< 0.01	< 0.01
ADG, g/d	389	447	451	520	449	469	525	15.8	< 0.01	< 0.01
G:F	0.517	0.573	0.567	0.597	0.563	0.577	0.603	0.013	< 0.01	< 0.01
0–21 days										
ADFI, g/d	873	884	887	978	906	926	941	28.1	0.02	0.09
ADG, g/d	424	475	475	546	475	505	542	14.7	< 0.01	< 0.01
G:F	0.485	0.537	0.536	0.562	0.526	0.547	0.583	0.013	< 0.01	< 0.01

[a] Each least square mean represents 9 pens of 3 pigs per pen. DL-Met = DL-methionine; DL-Met-Met = DL-methionyl-DL-methionine; Exp. = experiment.

[b] C1 = Linear effect of DL-Met; C2 = Linear effect of DL-Met-Met; Quadratic effect of DL-Met: non-significant.

[c] Quadratic effect of DL-Met-Met (P = 0.08).

[d] The contrast DL-Met vs. DL-Met-Met was not significant (P > 0.10).

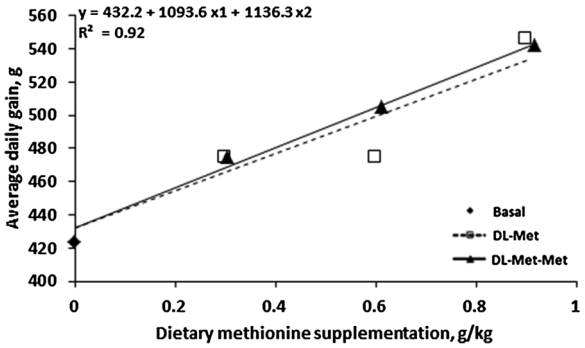

Fig. 4. Slope-ratio comparison of dietary DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (DL-Met-Met) based on average daily gain of pigs from 0 to 21 days (Exp. 2). Relative bioavailability of DL-Met-Met was 104% on an equimolar basis (95% confidence interval: 66 to 141%).

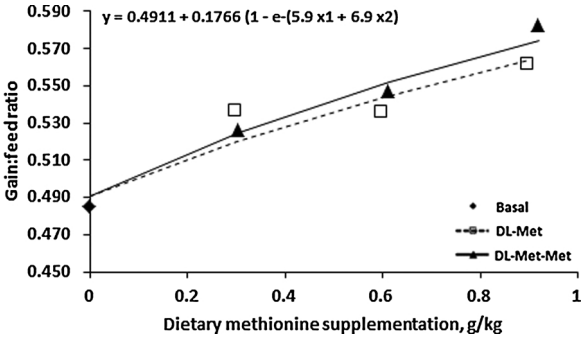

Fig. 5. Slope-ratio comparison of dietary DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (DL-Met-Met) based on gain:feed ratio of pigs from 0 to 21 days (Exp. 2). Relative bioavailability of DL-Met-Met was 117% on an equimolar basis (95% confidence interval: 61–174%).

4. Discussion

4.1. N-balance assay

Because one of the assumptions for validity of the slope-ratio assay is linearity (Littell et al., 1997), the (Met + Cys)/Lys ratio ranged between 0.41 to 0.48%, from the basal to the most supplemented diet. Therefore, an excess of Lys ensured that only Met + Cys was limiting. The N-balance technique allows measurement of N deposition under similar feed intake conditions in a short period. The measured N deposition can be recalculated into protein deposition which is a more sensitive parameter influenced by differences in the bioavailability of AA sources (Figueroa et al., 2002). Therefore, the RBV of DL-Met-Met in comparison to DL-Met was evaluated in 20–28 kg pigs using an N-balance assay in Exp. 1. Nitrogen retention (g/d and % of intake) were the lowest for pigs fed the Met-deficient diet which confirms that the basal diet was indeed deficient in Met and the deficiency of one or more AA caused reduced growth and poor nutrient efficiency. Previous studies also reported poor N retention of pigs fed Met-deficient diet (Zimmermann et al., 2005; Kim et al., 2006). Supplementation with DL-Met and DL-Met-Met decreased urinary N excretion and increased N retention response measures while the highest N retention was achieved with the highest supplemental level of both Met sources which demonstrates improved AA balance and utilization for these diets. Similar improvement in N retention in growing pigs has been reported for DL-Met supplementation to a Met-deficient diet (Zimmermann et al., 2005; Kim et al., 2006).

The presented RBV in both Met sources (DL-Met or DL-Met-Met) for N retained (g/d), N retention (% of intake), N retention (% of absorbed), was consistently greater for DL-Met-Met but not statistically different because the 95% of CI for the RBV includes 100%.

4.2. Performance trial

Herein, as well as in Exp. 1, pigs received from 68% up to 80% of the Met + Cys requirement, from both Met sources. This approach enabled an increased pigs performance according to supplementary Met levels. Hence, the (Met + Cys)/Lys ratio ranged between 0.41 to 0.49%, from the basal to the most supplemented diet, ensuring that only Met + Cys was limiting. Growth performance response can be used as response criteria for AA bioavailability assay (Chung and Baker, 1992; Adeola, 2009; Shen et al., 2014). Therefore, Exp. 2 was conducted as a dose-response growth trial (10–22 kg pigs) for comparing RBV of DL-Met-Met with DL-Met. As expected, the ADFI, ADG and G:F of pigs fed the Met-deficient basal diet was the lowest which confirms that the basal diet was indeed deficient in Met and in good agreement with previous studies (Zimmermann et al., 2005; Kim et al., 2006; Htoo and Morales, 2016). Supplementation with DL-Met and DL-Met-Met increased ADFI, ADG and G:F while the highest performance was

achieved with the highest supplemental level of both Met sources which was due to improved dietary Met supply and utilization by the pigs. Similar improvement in growth performance in growing pigs has been reported for DL-Met supplementation to a Met-deficient diet (Zimmermann et al., 2005; Kim et al., 2006; Htoo and Morales, 2016). In the current study, compared with DL-Met, the RBV of DL-Met-Met was 104 and 117% to optimize ADG and G:F, respectively on an equi-molar basis but the 95% of CI suggests that the RBV was not different between the two Met sources.

Based on results of both studies, the same extent of increase in both N retention and growth performance of pigs was achieved by supplementing a Met-deficient diet with dipeptide DL-Met-Met compared with DL-Met supplementation. This supports the statement that DL-Met-Met can be enzymatically cleaved in vivo into two Met molecules (Friedman and Gumbmann, 1988; EFSA, 2015). Even though different response criteria were used, the RBV of DL-Met-Met derived from Exp. 1 and 2 were similar, i.e. the bioavailability DL-Met-Met seems to be numerically higher, but the RBV was not different because 95% of CI for the RBV also includes 100%.

5. Conclusion

In conclusion, based on the results of both experiments, the bioavailability of DL-Met-Met (AQUAVI® Met-Met) is at least equally bioavailable as DL-Met as a Met source for pigs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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