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Efficacy of Methionine Hydroxy Analog and Dl-Methionine as Methionine Sources for Growing Pigs

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Abstract: The objective of this study was to compare the biological effectiveness of liquid methionine hydroxy analog free acid (MHA-FA) relative to DL-methionine for growing pigs. Two N-balance trials were conducted using a total of 42 barrows (Large White x Landrace). The initial body weight was 11.73±1.05 kg in the first trial and 13.69¡À1.48 kg in the second. In each trial, 21 pigs were randomly assigned to one of seven dietary treatments with three observations per treatment. The diets included a methionine-deficient basal diet with all other essential nutrients meeting the pig's requirements and six diets formulated with graded levels of DL-methionine (0.03, 0.06 and 0.09%) or MHA-FA (0.034, 0.068 and 0.102%) added on an equimolar basis. Each trial lasted 12 days, consisting of an adaptation period of 7 days followed by a 5-day total collection of urine and feces. During the collection period, pigs were fed (630 g d^{-1} for the first trial and 669 g/d for the second) three times (0800, 1500, and 2200 h) daily. Pigs had *ad libitum* access to water after feeding. Pigs receiving the experimental diets achieved higher daily gain and lower feed conversion than those receiving the basal diet. At each inclusion level, the treatment with added DL-methionine was superior to the corresponding treatment with added MHA-FA. The concentration of plasma urea nitrogen for pigs receiving the supplemental methionine sources, regardless of form, was lower than that for pigs receiving the basal diet. For each treatment, N retention and percentage of N retained were higher and urinary N production was lower than that of the basal diet. N retention in the experimental diets increased as the level of methionine equivalents increased. Additionally, at each inclusion rate, all treatments with DL-methionine supplementation showed a higher N retention but a lower plasma urea nitrogen concentration than the corresponding treatments with MHA-FA. An exponential model was used to determine the bioefficacy of MHA-FA relative to DL-methionine, and this model was a good fit for the responses. MHA-FA was estimated to be 73.2% as effective as DL-methionine for N retention and 45.6% for plasma urea nitrogen on an equimolar basis.

Key words: Pigs, MHA-FA, DL-methionine, biological effectiveness, N retention

INTRODUCTION

Methionine is commonly supplemented in animal diets as either methionine hydroxy analog free acid (MHA-FA) or as DL-methionine[1]. Typically, MHA-FA is used in liquid form with a purity of 88%, whereas DL-methionine contains 99% methionine. Many studies with broilers have been conducted to determine the relative bioefficiency of MHA-FA compared with DL-methionine. The relative bioefficiency based on these studies was estimated to be from 62 to 100% on an equimolar basis[2,6].

A recent literature review published by the Dutch Central Bureau for Livestock Feeding[7] reported that on an equimolar basis, the relative bioefficacy of MHA-FA was 77% for broilers and 82% for pigs. In dose-response trials, MHA-FA was reported to supply equimolar amounts of L-methionine compared with DL-methionine in young pigs[8,10]. However, other research in pigs indicated DL-methionine was more efficient compared with MHA-FA[11,13].

The N balance technique is a precise and well-accepted method to determine the biological value of proteins and amino acids fed to swine[14,15]. However, research data are still scarce about the efficacy of liquid MHA-FA in comparison with DL-methionine using N-balance in pigs. Therefore, the present study was conducted to examine the relative bioefficacy of MHA-FA compared with DL-methionine in young pigs fed wheat-corn-soybean meal based diets.

MATERIALS AND METHODS

Animals and diets: Two N-balance trials were conducted in the metabolism laboratory of the Animal Science and Technology College at China Agriculture University

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Table 1: Ingredient composition of the experimental diets (% as fed basis)

	Basal diet	MHA-FA (88%)			DL-methionine (99%)		
Item	0%	0.034%	0.068%	0.102%	0.03%	0.06%	0.09%
Wheat	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Corn	32.79	32.76	32.72	32.69	32.64	32.73	32.73
Soybean meal	10.62	10.62	10.62	10.62	10.62	10.62	10.62
Dried whey	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	1.96	1.96	1.96	1.96	1.96	1.96	1.96
Dicalcium phosphate	1.26	1.26	1.26	1.26	1.26	1.26	1.26
L-LysineHCl	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Calcium carbonate	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Minerals and vitamins[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Threonine	0.31	0.31	0.31	0.31	0.31	0.31	0.31
L-Valine	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Isoleucine	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Choline chloride, 50%	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Leucine	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-Tryptophan	0.07	0.07	0.07	0.07	0.07	0.07	0.07
DL-methionine, 99%	—	—	—	—	0.03	0.06	0.09
MHA-FA[a], 88%	—	0.034	0.068	0.102	—	—	—

[a]Provided per kilogram complete feed:: vitamin A, 5512 IU; vitamin D_3, 2200 IU; vitamin E, 30 IU; vitamin B_{12} 27.6 ug; riboflavin, 5.5 mg; thiamine, 2 mg; D-pantothenic acid,13.8 mg; niacin, 30.3 mg; pyridoxne, 6mg; Mn 100 mg; Fe, 100 mg; Zn, 100 mg; Cu,120 mg; Mn, 20 mg; I, 0.4 mg; Se, 0.3 mg; Co, 1.0 mg

Table 2: Analyzed chemical composition of the experimental diets (% as fed basis)

	Basal diet[a]	MHA-FA (88%)			DL-methionine (99%)		
	0%	0.034%	0.068%	0.102%	0.03%	0.06%	0.09%
Supplemented methionine equivalents, %							
DL-methionine	—	—	—	—	0.026	0.055	0.083
MHA-FA[b]	—	0.025	0.052	0.081	—	—	—
Chemical analysis							
Dry matter	89.97	89.76	89.74	90.47	89.83	89.86	89.48
Ash	4.81	4.81	4.83	4.83	4.79	4.81	4.76
Crude Protein	16.18	16.4	16.30	16.51	16.26	16.15	16.11
Crude Fibre	2.37	2.35	2.32	2.40	2.40	2.29	2.35
Ether Extract	3.92	3.96	3.77	3.91	3.84	3.92	3.86
Amino acids							
Arginine	0.79	0.78	0.80	0.80	0.81	0.79	0.78
Cystine	0.27	0.27	0.26	0.28	0.28	0.27	0.27
Histidine	0.34	0.34	0.35	0.35	0.35	0.34	0.34
Isoleucine	0.78	0.79	0.79	0.79	0.79	0.79	0.79
Leucine	1.19	1.17	1.19	1.19	1.19	1.18	1.16
Lysine	1.31	1.30	1.31	1.32	1.29	1.30	1.29
Methionine	0.25	0.25	0.25	0.25	0.27	0.30	0.32
Threonine	0.80	0.82	0.80	0.80	0.80	0.79	0.80
Valine	0.90	0.92	0.91	0.91	0.91	0.91	0.90

[a]Metabolizable energy in the basal diet was calculated to be 14.02 MJ kg^{-1} [b]The MHA-FA value was converted from the determined content of MFA-FA commercial product. 1 unit of the product contains 0.88 units MHA-FA, which equals 0.88 units methionine equivalent

using 42 crossbred barrows (Large White x Landrace) obtained from the Huadu Group (Beijing, China). Each trial lasted for 12 days consisting of a 7-day adaptation period and a 5-day total collection of urine and feces. The initial average body weight of pigs was 11.73±1.05 kg for the first trial and 13.69±1.48 kg for the second.

In each trial, 21 pigs were randomly assigned to one of seven dietary treatments with three observations per treatment. A methionine-deficient basal diet, formulated to provide all other nutrients according to the values recommended by NRC[16], was used as the negative control. The other six experimental diets consisted of the basal diet supplemented with three levels of either DL-methionine (0.03, 0.06 and 0.09%) or MHA-FA (0.034, 0.068 and 0.102%) on an equimolar basis. Supplementation was made at the expense of corn.

The basal diet was formulated on the basis of true ileal digestible amino acids according to the recommendations of Rademacher *et al.*,[17]. The digestibilities of synthetic lysine, threonine and other amino acids were assumed to be 100%[18]. The true digestible methionine contents in the experimental diets ranged from 0.19% (the basal diet) to 0.28% (the highest supplementation), and the true digestible methionine

+ cysteine contents ranged from 0.42% (the basal diet) to 0.43% (the highest supplementation). The ingredient and chemical composition of all diets is presented in Table 1 and 2, respectively.

Management procedures: Pigs were kept individually in stainless steel metabolism crates (0.6 × 0.3 × 0.5 m) equipped with plastic slotted flooring and a 0.25 m^3 round bottom single feeder at the front of the crate. The room temperature, humidity and ventilation were automatically controlled. Temperature, humidity, ammonia and carbon dioxide levels were checked and recorded every day at approximately 0900 and 2030 h. The temperature ranged from 22 to 25°C, the humidity from 55 to 70%, and the ammonia and carbon dioxide were below recommended levels for the duration of the experiment.

Feed was provided in mash form and supplied three times daily at 0800, 1500 and 2200 h, respectively and water was available *ad libitum*. The daily feed allowance of the experimental animals was adjusted according to their feed intake during the first three days of the acclimation period. From day 4 until the end of the 12-d experimental period, the same amount of feed (630 g d for the first trial and 669 g/d for the second) was fed, which provided 2.6 times the maintenance energy requirement. The pigs typically consumed their rations within 20 min.

At the beginning of each trial and after each collection period, the animal body weights were taken at 0800-0900 with no feed available. Feed refusals for each pig were collected, dried and weighed. These values were used to determine weight gain, feed intake and feed conversion.

Sample collection : During the 5-day collection period, the pigs were fitted with adhesive fecal collection bags that allowed a separate collection of feces and urine[19]. All feces were collected and weighed, twice daily, for each pig. Immediately after collection, the feces were placed in labeled plastic bags, frozen and stored at approximately -20°C. After the fifth day, the feces were thawed at room temperature and pooled into uniform slurries for each pig. A sub-sample of 8-10% was obtained and dried in a forced-air oven at 65°C. The feces were then allowed to equilibrate with atmospheric moisture for 24 h, ground to pass through #40-mesh screen, and stored at 4°C prior to analyses for total N and dry matter.

Total urinary output was collected once daily (at approximately 1600 h) in plastic containers located under funnels placed below the metabolism cages. Prior to collection, 50 mL of 6 N HCL was added to the collection container to limit microbial growth and reduce loss of ammonia. Urine volume was recorded daily, and then filtered through glass wool and a fixed proportion of the urine from each pig was collected in screw-capped polyethylene containers and frozen. After the fifth day, all urine was thawed at room temperature. Each pig's daily output was then pooled Into a single sample. A sub-sample (about 100 mL) was obtained and frozen at approximately -20°C prior to analysis for total nitrogen content.

At the end of each collection period, approximately 7 mL of blood was collected by jugular vena puncture into heparinized tubes (Greiner Bio-One Company) approximately one hour after feeding. The blood samples were stored on ice until blood collection from all pigs was complete. Samples were then centrifuged, within 1 h of collection, at 3,000 × g for 15 min at 4°C. After centrifuging, the plasma was collected and stored in plastic test tubes and stored at -20°C until plasma urea nitrogen was analyzed.

Chemical analyses: Dry matter, ash, crude fiber, crude protein, crude fat, calcium and phosphorus content were analyzed according to the procedures of the Association of Official Analytical Chemists[20]. The amino acids level in both ingredients and diets as well as supplemental DL-methionine were analyzed according to AOAC[20] methods. Supplemental MHA-FA in the diet was analyzed based on a modified method of Naumann *et al.*,[21]. Fecal and urinary nitrogen were analyzed with a semi-automatic analyzer (Kjeltec™ 2100 Distillation Unit) by the Kjeldahl method[20]. Plasma urea nitrogen was measured with an Automatic Biochemical Analyzer (Technicon RA 1000).

Statistical analysis: Data from the two trials was subjected to Analysis of Variance using the GLM procedure of SAS[22]. The factors in the model were trial, methionine source and the level of added methionine equivalents. Pig served as the experimental unit. There was no significant trial × treatment interaction ($P>0.05$) for any of the variables studied. Therefore, the data of two trials were combined for analysis in a 2 (methionine sources) × 4 (added methionine levels) factorial arrangement. The results were considered significant if $P<0.05$. An exponential regression analysis with means of treatments was used to compare the efficacy of the two methionine sources for N retention and plasma urea nitrogen (the NLIN procedure of SAS,[22]).
The applied model was:

$$Y = a \pm b \times (1 - e^{-(c1 \times 1 + c2 \times 2)})$$

Where Y = the response parameter (N retention, plasma

Table 3: Effects of dietary DL-methionine and methionine hydroxy analog free acid on performance and N retention in pigs

		MHA-FA, %			DL-methionine, %			SEM[a]	P value	
	Basal	0.034	0.068	0.102	0.030	0.060	0.090		MHA-FA[b]	DLM[c]
Pig performance										
Feed intake, g d^{-1}	647	647	647	646	647	647	646	9.21	0.946	0.946
Weight gain, g d^{-1}	357	380	403	430	405	417	443	12.21	< 0.001	< 0.001
Feed conversion	1.82	1.71	1.61	1.51	1.61	1.56	1.47	0.064	0.001	< 0.001
Nitrogen balance and PUN										
N intake, g d^{-1}	16.40	16.42	16.18	16.66	16.40	16.68	16.72	0.235	0.599	0.249
Fecal N, g d^{-1}	1.58	1.65	1.84	1.50	1.74	1.79	1.59	0.137	.959	0.869
Urinary N, g d^{-1}	5.43	4.29	3.16	3.65	3.85	3.44	3.49	0.348.	001	< 0.001
Retained N, g/d	9.39	10.48	11.18	11.51	10.82	11.46	11.64	0.294	.001	< 0.001
Retained N, % of intake	57.28	63.97	69.15	69.09	65.85	68.72	69.65	1.75	0.001	< 0.001
N digestibility, %	90.37	89.92	88.57	90.97	89.39	89.23	90.40	0.925	.917	0.987
Plasma urea N, mg dL^{-1}	8.83	8.17	6.83	6.33	6.83	5.83	5.33	0.692	0.007	< 0.001

[a] SEM=standard error of the means Linear effect of liquid MHA-FA, quadratic effects is significant ($P < 0.05$) for urinary nitrogen excretion[c] Linear effect of DL-Methionine, quadratic effect are significant ($P < 0.05$) for urinary nitrogen excretion, nitrogen retention and nitrogen retained as a percentage of intake

Table 4: Characteristics of PUN and N retention responses to analysed methionine equivalents

Variables	Parameter	Estimate	Std Error	95% Confidence Limit	P valu
N retention	B1	9.38	0.03	9.27-9.49	0.0001
	B2	2.43	0.05	2.27-2.59	
	C1	34.59	2.05	28.06-41.12	
	C2	25.32	1.30	21.16-29.48	
	C2/C1	0.732	0.03	0.646-0.823	
PUN	B1	8.94	0.21	8.28-9.60	0.0034
	B2	4.04	0.47	2.55-5.54	
	C1	27.48	8.27	1.17-53.79	
	C2	12.54	2.97	3.09-21.99	
	C2/C1	0.456	0.076	0.215-0.689	

urea nitrogen), a =? intercept (N-retention, plasma urea nitrogen with basal diet), a ±?? b = common asymptote (the maximum N retention, minimum plasma urea nitrogen level), c_1 = curvature steepness coefficient for DL-methionine, c_2 = ?curvature steepness coefficient for liquid MHA-FA, and x_1 =? the dietary level of the added DL-methionine, x2 = the dietary level of the added MHA-FA. The efficacy of liquid MHA-FA compared with DL-methionine was calculated as the ratio of their c-values (c_2/c_1) in the exponential model[23].

RESULTS

Results are presented in Table 3 and 4. With the addition of DL-methionine or MHA-FA to the basal diet, feed conversion rate, plasma urea nitrogen and urinary nitrogen decreased, while average daily gain and retained N in either grams or percent increased significantly. It is also important to note that the average daily gain, feed conversion, N retention and plasma urea nitrogen responded in a dose-response manner to either methionine source. For each source, average daily gain and N retention gradually increased. Meanwhile, the feed conversion and plasma urea nitrogen gradually decreased with increasing dietary methionine level. At each inclusion rate, pigs fed the diet supplemented with DL-methionine had higher daily gain and retained N, and lower feed conversion and plasma u rea nitrogen. DL-methionine was numerically more effective in promoting pig growth and protein deposition than MHA-FA.

In the present study, pigs responded consistently over the whole range of supplemented methionine equivalents. The analysis showed that it was sensitive for the continuous N retention response over three supplemental levels to detect differences between methionine sources and furthermore the N retention data fit well with the exponential model ($P<0.0001$). Therefore, the following regression equation was obtained to determine the relative bioefficacy of MHA-FA to DL-methionine, where Y represents N retention:

$$Y = 9.38 + 2.43 \times (1 - e^{-(34.59\,x1 + 25.32\,x2)})$$

The relative biological efficiency of MHA-FA product was determined to be 73.2% (confidence interval 65- 82%) of DL-methionine on a molar basis (Fig. 1; Table 4), which was significantly lower ($P<0.05$) than for DL-methionine.

The same analysis was conducted on plasma urea nitrogen. An exponential regression equation was obtained as follows ($p<0.0034$):

$$Y = 8.94 - 4.04 \times (1 - e^{-(27.48\,x1 + 12.54\,x2)})$$

The relative biological efficiency of MHA-FA was determined to be 45.6% (confidence interval 21 – 70%) of DL-methionine on a molar basis (Fig. 2 and Table 4),

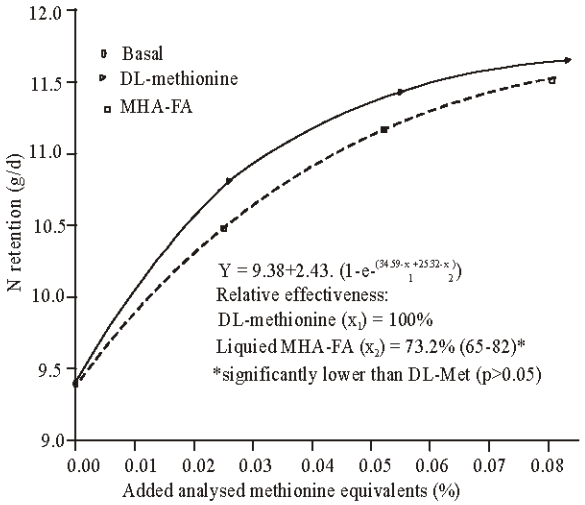

Fig. 1: N retention response to supplemented methionine equivalents

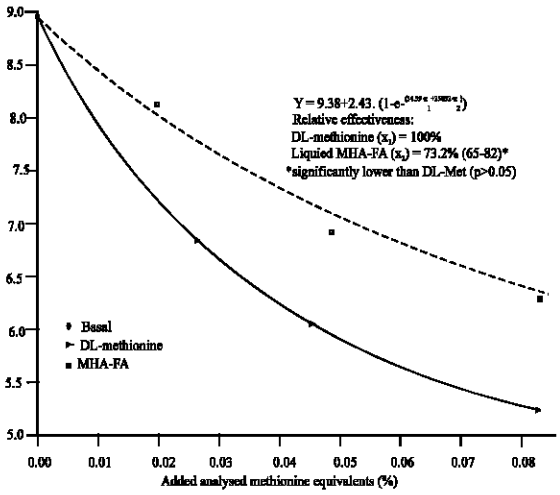

Fig. 2: Plasma urea nitrogen response to supplemented methionine equivalents

which was significantly lower ($p<0.05$) than for DL-methionine.

DISCUSSION

In the present study, N balance, plasma urea nitrogen and pig performance were used as response criteria to estimate the bioefficacy of MHA-FA relative to DL-methionine in 10 to 20 kg pigs. The protein content in the basal diet was reduced by more than four percentage units compared with the basal diet. However, synthetic essential amino acids were supplemented to meet the pig's requirements, including L-lysine, L-threonine, L-tryptophan, L-isoleucine, L-valine and L-leucine.

DL-methionine was superior to MHA-FA at each similar molar amount of inclusion for N retention, and similar trends were observed for plasma urea nitrogen, feed conversion and daily gain (Table 3). This suggests that DL-methionine provides more methionine equivalents than MHA-FA and that DL-methionine is used more effectively by pigs.

Most researchers have used growth trials to determine the bioefficiency of MHA-FA relative to DL-methionine in poultry and swine. Although the N balance technique is a good method for studying amino acids, only a few N balance trials have been conducted comparing DL-methionine and MHA-FA. Figueroa *et al.*, [24]pointed out that N balance was more sensitive than growth to amino acid adequacy in pigs. In the present study, we found step-by-step increases from the basal diet for N retention of 11, 7 and 3% for MHA-FA supplementations and 15, 6 and 2% for DL-methionine, respectively. However, the daily gain was gradually improved by steps of 6, 6 and 7% for MHA-FA and 13, 3 and 7% for DL-methionine, respectively. It should be noted that for the 0.03 and 0.06% level of supplementation, the magnitude of increase for N retention was more than for performance. However, when the level of added methionine equivalents went from 0.06% to 0.09%, closer to the requirement of the growing pigs, the improvements in performance were more than for N retention. This indicates that N retention is a more sensitive response variable than performance to methionine supplementations. Therefore, N retention was considered as the major response parameter to evaluate the bioefficiency of MHA-FA relative to DL-methionine in our study.

An exponential regression analysis has been commonly used to determine the relative bioavailability of MHA-FA in comparison with DL-methionine in growth trials conducted with poultry[2,5]. This analysis describes the response in performance to a limiting nutrient very well[25]. The standard statistical analyses with SAS program for relative bioavailability studies were explained in detail by Littell *et al.*,[21]. In the current study, using the exponential regression model, the bioefficiency of MHA-FA relative to DL-methionine was determined to be 73.2% on a molar basis when using N retention as the response variable. Based on N retention response, Zimmermann *et al.*,[13] found the biological effectiveness of liquid MHA-FA relative to DL-methionine was 62% on an equal weight basis and 70.5% on an equal molar basis in growing pigs. Our results agree well with this estimate.

In a growth assay for broilers, Lemme *et al.*,[5] noted that liquid MHA-FA was 72% (weight gain), 51% (feed conversion), 48% (carcass yield), and 60% (breast yield) as efficacious as DL-methionine on a weight-for-weight basis. Hoehler *et al.*,[6]pooled and analyzed the experimental data from growth trials in turkeyd using the nonlinear model, and found a 65% relative bioefficacy of MHA-FA to DL-methionine on an equal weight basis. These results are in agreement with the previous estimates in broilers. However, in other N balance trials for broiler chickens and pigs, Roemer and Abel[21] revealed an equal utilization of MHA-FA and DL-methionine based on N retention response. Several growth trials in pigs also indicted that MHA-FA was as effective as DL-methionine for daily gain and feed conversion[1,9,27]. Thus, controversy still exists about the availability of two methionine sources in pigs.

The inconsistency in these estimates of MHA-FA bioefficiency could be attributed to poor sensitivity of the experiments and unsuitable evaluation of the data obtained[2]. In a comment from Pack and Hoehler[28], the investigation conducted by Roemer and Abel[27] was considered not suitable to make conclusions about the relative value of methionine sources because of inadequate statistics to estimate relative biological effectiveness of the two sources due to lack of a dose-dependent response and high within-treatment variation.

In a previous amino acid dose-titration trial, Lewis *et al.*,[29] found that as the limiting amino acid increased, the concentration of plasma urea nitrogen decreased continuously until the limiting amino acid reached requirement. In a 7-d N balance trial conducted by Coma *et al.*,[30], plasma urea nitrogen and N retention were used to estimate the lysine requirement of growing pigs, and the result was found to be identical. Therefore, we can conclude that the concentration change in plasma urea nitrogen to added methionine equivalents can be used to evaluate the deficient or excess status of methionine. Although plasma urea nitrogen was not used to evaluate the relative bioefficiency of MHA-FA to DL-methionine in the previous research, an attempt was made to do so in the current study. By the exponential regression analysis, MHA-FA was estimated to be 45.6% on a molar basis as effective as DL-methionine.

Obviously, the determined value based on plasma urea nitrogen response was lower than that based on N retention response.

Contrasting the two analytical strategies, the model describing the response of N retention to increasing supplemental methionine equivalents fits the model better ($p<0.0001$; Fig. 1, Table 4) than plasma urea nitrogen ($p<0.0034$; Fig. 2, Table 4). Considering asymptotic characteristics and asymptote 95% confidence intervals, the model built on N retention response was more accurate than that based on plasma urea nitrogen response (Table 4). In fact, the response of plasma urea nitrogen in pigs to low protein diets supplemented with graded methionine has been inconsistent and varies depending on several factors, including bleeding time, the storage period and conditions of samples, the status of the deficiency, excess, or imbalance of amino acids. Therefore, the bioefficacy of MHA-FA[31] in relation to DL-methionine established on N retention response, 73.2% on a molar basis is more reasonable and acceptable.

Recent studies provide the physiological background for the distinctly inferior bioefficacy of MHA-FA compared with DL-methionine which can be attributed to several factors such as poorly effective dimers and oligomers in MHA-FA, the loss of MHA-FA due to intestinal microbial breakdown[32,33] and a less effective intestinal absorption mechanism for MHA-FA[34,35].

CONCLUSION

In the present N balance trials, N retention and plasma urea nitrogen were more sensitive than feed conversion and daily gain to the different supplemental methionine sources. Furthermore, DL-methionine was superior to MHA-FA at each similar molar inclusion rate when performance, N retention and plasma urea nitrogen were compared. N retention and plasma urea nitrogen data were analyzed to fit with the exponential model, which was usually used to evaluate the bioefficiency of MHA-FA relative to DL-methionine. Based on N retention response, the relative biological efficiency of MHA-FA was determined to be about 73.2 % on a molar basis. When plasma urea nitrogen was the response variable, the bioefficiency of MFA-FA relative to DL-methionine was determined to be 45.6% on a molar basis. However, whether plasma urea nitrogen can be used as a reliable parameter should be validated with further studies.

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