Comparative Studies on the Relative Efficacy of DL-methionine and Liquid Methionine Hydroxy Analogue in Growing Pigs

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ABSTRACT : A study consisting of 3 trials was designed to assess the relative biological efficacy of DL-methionine (DL-Met) in comparison to liquid methionine hydroxy analogue (MHA-FA) in growing pigs. In trial I a basal diet was supplemented with three graded levels of DL-methionine (0.25, 0.50 and 0.75 g/kg) or liquid MHA-FA (0.0285, 0.0570 and 0.0855 g/kg) on equimolar basis. The basal diet contained 18.3% CP, 0.22% Met and 0.51% Met+Cys, which is below the methionine requirement for weaned pigs between 10 and 20 kg BW according to NRC (1998) but adequate in all other essential nutrients and energy. Using an exponential model, the efficacy of the two methionine sources was estimated from nitrogen retention data obtained in 42 piglets with an initial BW between 11.0 kg (Exp. 1) and 11.7 kg (Exp. 2). In trials II and III, with a total of 192 and 96 pigs, and with an initial BW of 10.6 and 21 kg, respectively, growth response criteria were assessed to determine if in agreement with previous studies in pigs and poultry a biological effectiveness of about 65% on average could be confirmed for liquid MHA-FA in comparison to DL-met. Based on N-retention (trial I) the biological efficacy of liquid MHA-FA on a weight-to-weight basis was calculated to be 62% relative to DL-met. Basically, these results were confirmed using growth response criteria as measures; the results of trial II and III revealed no significant differences in growth performance and feed conversion between treatments indicating that 100 parts of liquid MHA-FA can be replaced by 65 parts of DL-met. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 1003-1010)***

Key Words : DL-methionine, Liquid Methionine Hydroxy Analogue, Nitrogen Retention, Growth Performance, Bioefficacy, Pig

INTRODUCTION

Primarily, pigs do not have a requirement for protein, but for the appropriate level and balance of individual amino acids in the diet both for growth and maintenance. In particular, low protein diets for growing pigs are usually limited by lysine first whereas methionine is usually the second limiting amino acid. Methionine-deficient diets for pigs can be supplemented with DL-methionine (DL-Met, 99% purity) or with liquid methionine hydroxy analogue (liquid MHA-FA) containing 88% of a mixture of mono-, di- and oligomers of 2-hydroxy-4-(methylthio) butanoic acid molecules. MHA-FA is a source of methionine in feeds which differs from DL-Met in that the molecule has a hydroxy group instead of an amino group. There is a controversial discussion about the biological effectiveness of these two sources of methionine. In some studies with growing pigs the efficacy of liquid MHA-FA relative to DL-Met ranged between 63 and 78% (Roth and Kirchgessner 1986; Walz and Pallauf, 1996; Schmidt, 2000). These results are in line with those obtained in poultry (Esteve-Garcia and Austic, 1993; Huyghebaert, 1993; Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Lemme et al., 2002; Mandal et al., 2004). Other reports, however, showed no significant differences in bioefficacy between DL-Met and MHA-FA (e.g. Roemer and Abel, 1996; Knight et al., 1998).

A comprehensive study including three trials was carried out to determine the relative effectiveness of liquid MHA-FA compared to DL-Met in starter and grower pigs fed cereal-based diets formulated to be limiting in Met and Cys. In trial I, the N-balance technique was used as a response parameter to test the hypothesis if DL-Met and MHA-FA, based on a MHA-FA content of 88% in the commercial product, provide equimolar levels of methionine activity in weaned pigs. In trials II and III growth response criteria were used to determine the biological effectiveness of liquid MHA-FA in comparison to DL-Met. The findings were then related to previous studies in pigs and poultry, where, on average, the relative bioefficacy for liquid MHA-FA was calculated to be about 65% (e.g. Esteve-Garcia and Austic, 1993; Huyghebaert, 1993; Walz and Pallauf, 1996; Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Schmidt, 2000; Lemme et al., 2002).

MATERIALS AND METHODS

Trial I (University of Hohenheim, Germany)

Animals and dietary treatments : A total of 42 barrows (Piétrain×German Landrace) with an initial BW between 11.0 kg (Exp. 1) and 11.7 kg (Exp. 2) was used in two consecutive experiments with 21 piglets each. The animals were kept individually in stainless steel metabolism crates

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Table 1. Diet formulation, energy and nutrient content of the basal diet in trial I (g/kg)

Ingredients	g/kg
Wheat	280.0
Soybean meal	190.0
Peas	190.0
Barley	150.0
Tapioca	122.4
Vegetable oil	30.0
L-lysine·HCl	4.0
L-threonine	2.0
L-tryptophan	0.4
Dicalcium phosphate	14.0
Calcium carbonate	8.8
NaCl	3.4
Mineral/vitamin premix[1]	5.0
Energy (MJ/kg) and nutrient content (g/kg)	
Digestible energy[2]	13.6
Crude protein	183.0
Calcium	8.9
Phosphorus	6.9
Lysine	12.9
Threonine	7.9
Methionine	2.2
Methionine/cystine	5.1
Tryptophan[2]	2.5

[1] Supplied per kg diet: Vitamin A: 900 µg; Vitamin D_3: 750 mg; Vitamin E: 10 mg; Vitamin B_1: 175 µg; Vitamin B_2: 500 µg; Vitamin B_{12}: 2.25 µg; Vitamin B_6: 0.35 mg; Pantothenic acid: 1.5 mg; Nicotinic acid: 2.75 mg; Folic acid: 75 µg; Vitamin K_3: 150 µg; Fe: 25 mg; Cu: 5 mg; Zn: 25 mg; Mn: 10 mg; I: 0.3 mg; Se: 6.5 µg.

[2] Calculated according to NRC (1998).

to allow separate collection of feces and urine. Room temperature ranged between 24 and 26°C. The experimental protocol was approved by the German Ethical Commission. The animals used in this experiment were cared for in accordance with the guidelines issued by the German regulation for care and treatments of animals (Lorz and Metzger, 1999). Piglets were randomly distributed among 7 treatments, with 6 observations per treatment based on BW and litter origin. Following the assignment to the dietary treatments, the pigs received the experimental diets.

The composition of the basal diet is presented in Table 1. This diet, based on wheat, barley, tapioca, soybean meal and peas was supplemented on an equimolar basis with three graded levels of DL-Met (0.25, 0.50 and 0.75 g/kg) or liquid MHA-FA (0.285, 0.570 and 0.855 g/kg). These supplementation levels of DL-Met and liquid MHA-FA were based on a MHA-FA content of 88% in the commercial product. The diet was formulated to meet or to exceed the nutrient and energy requirement for piglets from 10-20 kg BW according to NRC (1998) standards except for Met and Met+Cys with methionine being approximately 73% of the methionine requirement. For piglets from 10 to 20 kg BW the recommendations for CP, Met and Met+Cys were 209.0, 3.0 and 6.5 g/kg, respectively. Pigs were fed twice daily at 08:30 and 18:00 h 225 g per meal of pelleted feed which corresponds to 1.9% of BW. They had free access to water. The animals were weighed at the beginning and end of the balance periods.

Sample collection : Pigs were acclimated to dietary treatments and cages for a period of 10 d before a 7 d collection period began. Feces were collected quantitatively four times daily and stored at -18°C. During fecal collection, the pigs were fitted with adhesive collection bags that were attached to the anus. Urine was collected in H_2SO_4, (26 ml/d, 12%), recorded daily, and stored at 4°C until the end of the experimental period. In addition, urine was filtered with a sieve to remove any hair or contaminants before analysis of N content. After the conclusion of the first experiment, it was repeated with another set of animals according to the procedures described above.

Chemical analyses : At the end of the balance period, the individual samples of feces and urine were each pooled per pig, weighed and mixed. Proximate analyses of nutrients both in feed samples and in fresh feces and urine were performed according to Naumann et al. (1976). Dietary amino acid contents were determined by using ion-exchange chromatography with postcolumn derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with sodium metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were hydrolyzed by means of 6 N HCl for 24 h at 110°C. Amino acids were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000). Tyrosine was not determined. Supplemented amino acids were determined following the extraction with 0.1 N HCl (Commission Directive, 1998). Supplemented liquid MHA-FA was analyzed using the method described by Naumann et al. (1976).

Statistical analyses : The experimental data were analyzed using the GLM Procedure of SAS (1996). The LSQ-test was used to test differences between treatment means. The statistical significance level was claimed at $p<0.05$. The results were subjected to nonlinear regression analysis. The efficacy of the two methionine sources was estimated from different response data using an exponential model ($Y = a+b\times[1 - e^{-(C_1X_1+C_2X_2)}]$), whereas Y means the response parameter, x_1 the added analyzed DL-Met and x_2 the added analyzed liquid MHA-FA product. The efficacy of liquid MHA-FA as compared to DL-Met was calculated as the ratio of their c-values (c_2/c_1) of the statistical exponential model.

Table 2. Diet formulation, energy and nutrient content of the basal diet in trial II (g/kg)

Ingredients	g/kg
Wheat	200.0
Barley	75.0
Full fat soybeans	160.0
Fish meal	25.0
Dried whey	125.0
Peas	240.0
Soybean oil	25.0
Corn starch	120.0
L-lysine·HCl	2.8
L-threonine	2.0
L-tryptophan	0.5
Dicalcium phosphate	11.0
Calcium carbonate	8.6
NaCl	1.5
Mineral/vitamin premix[1]	3.4
Energy (MJ/kg) and nutrient content (g/kg)	
Digestible energy[2]	14.8
Crude protein	180.0
Calcium[2]	8.1
Phosphorus[2]	6.6
Lysine	13.0
Threonine	9.1
Methionine	2.5
Methionine/cystine	5.3
Tryptophan	2.0

[1] supplied per kg diet: Vitamin A: 1.8 mg; Vitamin D_3: 25 µg; Vitamin E: 100 mg; Vitamin B_1: 2 mg; Vitamin B_2: 2 mg; Vitamin B_{12}: 15 µg; Vitamin B_6: 3 mg; Pantothenic acid: 10 mg; Nicotinic acid: 12 mg; Vitamin K_3: 4 mg; Fe: 140 mg; Cu: 17 mg; Zn: 120 mg; Mn: 47 mg; I: 0.6 mg; Se: 0.3 mg; choline chloride: 250 mg.

[2] Calculated according to CVB (2003).

Trial II (Teagasc, Moorepark Research Centre, Ireland)

Animals and dietary treatments : One hundred and ninety two pigs (96 male, 96 female), with an initial BW of 10.6 kg (s.d. 0.1 kg) were randomly distributed among 3 treatments for an experimental period of 16 d, from d 12 to 28 after weaning. Pigs were crossbred, progeny of meat-line sires out of F1 (i.e. Landrace×Large White sows) and weaned at 26 to 31 d of age. They were housed in groups of two pigs (one male and one female of similar weaning weight and from different litters) per pen with completely slatted floor (plastic slats, FAROEX, Manitoba, Canada, 1.1 m×0.9 m). The animals had free access to water and feed. The trial was carried out in four rooms each holding 24 pairs/pens of pigs. Temperature was maintained at 28°C in the first wk and reduced by 2°C per wk to 22°C in wk four. Temperature control was achieved by regulation of the fan speed and a hot air heating system.

A basal diet was formulated to meet or to exceed the requirements for energy and nutrients according to NRC (1998) standards, except for methionine (Table 2). The basal diet was supplemented with 0.65 g/kg DL-Met or 1 g/kg liquid MHA-FA, i. e. on a weight for weight basis, the ratio between DL-Met and liquid MHA-FA supplementation was 65:100. Feed ingredients were ground by means of a hammer-mill (3 mm screen) prior to mixing (6-8 min) in a horizontal ribbon mixer. The diet was pelleted (5 mm pellets) after steam-heating to 50°C and fed *ad libitum*.

Pigs were fed two times daily at 08:30 and 16:00 h during the first wk. Thereafter, pigs were checked two times daily for health as well as room temperature and availability of feed and water. A commercial starter diet (Startrite 88-SCA Feeds, Monread Industrial Estate, Naas Co. Kildare) was fed until d 11 after weaning; pigs were then weighed and fed the experimental diets from d 12 to 28. At the conclusion of the experiment, the weight of the pigs was recorded again.

Statistical analyses : Statistical analysis was carried out using the GLM Procedure of SAS (1996). The pair/pen was considered as the experimental unit. Treatment effects were analysed as main effects, and the effect of room was included in the model as a covariate with mean weight of the pair on d 11. Single degree of freedom comparisons were carried out to compare the control (unsupplemented) treatment with the mean of the two supplemented treatments and, to compare the liquid MHA-FA diet with the DL-Met supplemented diet.

Trial III (Experimental Station of IRTA, El Prat de Llobregat, Spain)

Animals and dietary treatments : Ninety six pigs from a cross between two Large White lines originally from Brittany, with an initial BW of 21 kg (s.d. 0.08 kg), were randomly distributed in 24 pens, avoiding that two pigs from the same litter went into one pen. The animals were kept under 9 h light/15 h dark cycles at room temperatures declining from 32°C at the beginning to 24°C at the end of the experiment. Pigs were housed in groups of four pigs per pen with completely slatted floor. The stocking density was 0.65 m² per pig. Half of the pigs were boars and the other half gilts. The experimental period lasted 35 d.

There were three dietary treatments. The nutrient composition of the basal diet is given in Table 3. This diet was supplemented with 0.65 g/kg DL-Met or 1 g/kg liquid MHA-FA; on a weight for weight basis, the ratio between DL-Met and liquid MHA-FA supplementation was 65:100. To evaluate the efficiency of the two methionine sources, the basal diet was formulated to meet or to exceed the requirements for energy and essential nutrients provided by NRC (1998), except for methionine which was about 67% of the methionine requirement.

The diets were fed *ad libitum* in pelleted form (4 mm); the animals had free access to water. The experiment comprised in total 5 wk; the BW of the pigs was recorded at

Table 3. Diet formulation, energy and nutrient content of the basal diet in trial III (g/kg)

Ingredients	g/kg
Wheat	316.0
Barley	122.0
Lard	30.0
Manioc	123.0
Peas	230.0
Soybean meal	148.0
L-lysine·HCl	2.44
L-threonine	1.49
L-tryptophan	0.30
Calcium carbonate	8.7
Dicalcium phosphate	11.3
Salt	3.2
Mineral/vitamin premix[1]	4.0
Energy (MJ/kg) and nutrient content (g/kg)	
Metabolisable energy[2]	13.4
Crude protein	174.0
Lysine	10.7
Methionine	2.0
Methionine/cystine	4.7
Threonine	7.1
Tryptophan	2.3

[1] Supplied per kg diet: Vitamin A: 25 µg; Vitamin D_3: 1.75 mg; Vitamin E: 15 mg; Vitamin B_1: 1.3 mg; Vitamin B_2: 3.5 mg; Vitamin B_{12}: 0.025 mg; Vitamin B_6: 1.5 mg; Calcium pantothenate: 10 mg; Nicotinic acid: 15 mg; Biotin: 0.1 mg; Folic acid: 0.6 mg; Vitamin K_3: 2 mg; Fe: 80 mg; Cu: 140 mg; Co: 0.75 mg; Zn: 60 mg; Mn: 30 mg; I: 0.75 mg; Se: 0.10 mg; Ethoxyquin: 0.15 mg.
[2] Calculated according to INRA (1984).

the beginning of the experiment, after 2 wk (penwise) and after 5 wk. Feed consumption was measured at the end of wk 2 and wk 5. Average daily gain, average daily feed intake and feed to gain ratio were determined for each period and for the overall experimental period.

Statistical analyses : The trial was designed as a randomized complete block design with 8 blocks (4 blocks of males and 4 blocks of females) and three dietary treatments. Blocks included sex, weight and location effects. Comparisons between the three treatment groups were made using t-test procedures (SAS 1996).

RESULTS

Trial I

The analyzed chemical composition of the experimental diets of trial I is presented in Table 4. The calculated nutrient contents including the expected levels for DL-Met and liquid MHA-FA in the experimental diets were confirmed by analyses.

Since there was no major variation in growth performance between animals a sound health status of all animals can be assumed, and none of the pigs received medical treatment.

Due to the restricted feeding regimen, there were only minor differences in N intake between the dietary treatments supplemented with either methionine source (Table 5). There were no effects of supplemented DL-Met or liquid MHA-FA on daily total fecal N excretion (2.0-2.2 g) and, as a result, the digestibility of crude protein ranged between 80.6-82.8%. However, with increasing levels of DL-Met or liquid MHA-FA in the basal diet there was a reduction ($p<0.05$) in daily urinary N excretion from 4.7 g in the basal diet without methionine supplementation to 3.9 and 3.6 g ($p>0.05$) at the highest level of DL-Met and liquid MHA-FA supplementation, respectively. Consequently, the utilisation of N was improved, which resulted in an increase ($p<0{\cdot}05$) in N retention from 4.6 g/d in pigs fed the basal diet to 5.9 g/d when the diets with the highest level of supplemental DL-Met or liquid MHA-FA were given. However, at the lowest level of liquid MHA-FA supplementation to the basal diet there was no effect ($p>0.05$) on N retention. Bioefficacy values were determined by regressing nitrogen retention versus supplemental dietary concentrations of both methionine sources. According to non-linear regression analysis increasing levels of dietary methionine caused an elevation in nitrogen retention by 33%. In order to reach the same nitrogen retention, liquid MHA-FA was only 62% as efficient as DL-Met (Figure 1).

Table 4. Analyzed chemical composition of the basal and experimental diets in trial I (g/kg DM)

Treatment	1	2	3	4	5	6	7
Observations (n)	6	6	6	5	5	6	6
DL-Met supplementation (g/kg)	-	0.25	0.50	0.75	-	-	-
MHA-FA supplementation (g/kg)[1]	-	-	-	-	0.285	0.570	0.855
DM (%)[2]	90.4	90.3	90.4	90.3	90.3	90.3	90.4
Ash	67	68	66	62	70	68	66
EE	51	48	50	50	50	49	49
CP	201	203	201	202	202	202	201
CF	39	41	39	42	46	45	41
DL-Met supplementation (g/kg)	-	0.26	0.49	0.72	-	-	-
MHA-FA supplementation (g/kg)	-	-	-	-	0.316	0.574	0.846

[1] g/kg of product; based on a MHA-FA content of 88 % in the commercial product.
[2] Abbreviations: DM = dry matter; EE = ether extract; CP = crude protein; CF = crude fiber.

Table 5. The effect of supplemental DL-Met and liquid MHA-FA on N balance and on N digestibility in starter pigs (LS Means) in trial I

Treatment	1	2	3	4	5	6	7	Pooled SEM
Observations (n)	6	6	6	5	5	6	6	-
DL-Met supplementation (g/kg)	-	0.26	0.49	0.72	-	-	-	-
MHA-FA supplementation (g/kg)	-	-	-	-	0.316	0.574	0.846	-
N intake (g/d)	11.6	11.9	11.7	11.7	11.7	11.7	11.7	-
N excretion in feces (g/d)	2.24	2.24	2.16	2.01	2.18	2.22	2.20	0.208
N excretion in urine (g/d)	4.71^{a}	4.29^{c}	3.68^{d}	3.87^{bcd}	4.46^{ab}	4.01^{bcd}	3.60^{d}	0.157
N retention (g/d)	4.63^{b}	5.39^{a}	5.83^{a}	5.87^{a}	5.03^{ab}	5.49^{a}	5.88^{a}	0.250
N digestibility	0.806	0.812	0.815	0.826	0.828	0.811	0.812	1.795

$^{a, b, c}$ Least squares means in the same row with different superscripts differ significantly ($p<0.05$).

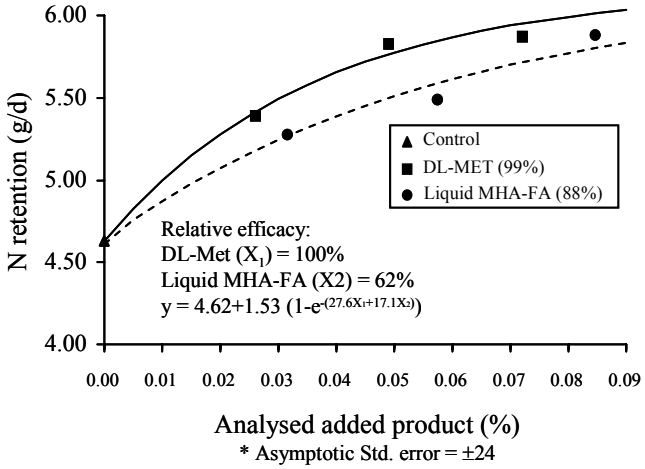

Figure 1. N retention in piglets fed diets with increasing levels of DL-Met or liquid MHA-FA in trial I.

Trial II

Supplementation of either 65 parts of DL-Met or 100 parts of liquid MHA-FA of a methionine-deficient basal diet resulted in a significantly ($p<0.01$) higher daily weight gain (501 and 488 g vs. 432 g) and improved ($p<0.05$) feed conversion (1.48 and 1.48 vs. 1.61) compared with the pigs of the control group. During the growth period from 20 to 30 kg BW no significant differences were found between both methionine sources for the variables that were measured.

Trial III

As presented in Table 6, during the first period (wk 1-2), supplementation of either DL-Met or liquid MHA-FA to a methionine deficient basal diet resulted in significant ($p<0.05$) improvements in feed to gain ratio and daily weight gain in comparison to pigs fed the basal diet. Feed to gain ratio was similar for both methionine sources, while DL-Met supplementation improved ($p>0.05$) daily weight gain by 8% compared with liquid MHA-FA addition (698 vs. 645 g/d). In the second period (wk 3-5), both methionine sources caused an improvement ($p<0.05$) in feed to gain ratio in comparison to the control pigs, which was slightly more pronounced for liquid MHA-FA. Daily weight gain of the liquid MHA-FA treated group was significantly ($p<0.05$) higher when compared to the control group, but only numerically higher compared to the DL-Met treated groups. Overall, i.e. from 20 to 49 kg of BW, there were no significant differences between both methionine sources for any of the variables that were measured when MHA-FA was added at a level of 65% of the methionine activity of DL-Met.

DISCUSSION

There is a controversial discussion about the biological efficacy of DL-Met in relation to liquid MHA-FA. The results of this study support earlier findings by Schmidt (2000) who also used the N balance technique for testing the efficacy of liquid MHA-FA in relation to DL-Met. The author used pigs in the BW range from 20 to 50 kg. Their basal diet, clearly deficient in methionine, was supplemented with three graded levels of DL-Met (0.39, 0.44 and 0.94 g/kg) or liquid MHA-FA (0.55, 0.93 and 1.05 g/kg) on an equimolar basis. Based on the same level of feed intake, the efficacy of liquid MHA-FA on N retention was calculated to be 63% compared with DL-Met. These results are in close agreement with the presented results in trial I and with data for poultry. For example, as a source of methionine for poultry, liquid MHA-FA has been shown to have on average an efficacy of about 65% relative to DL-Met (Esteve-Garcia and Austic, 1993; Huyghebaert, 1993; Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Lemme et al., 2002). Estimates of bioeffectiveness of liquid MHA-FA in these studies and in trial I were based on an appropriate data structure from dose-response trials for exponential or slope ratio regression analysis according to Littell et al. (1997). These models have been successfully applied for comparative evaluation of bioefficacy or bioavailability of essential nutrients such as phosphorus (Zimmermann et al., 2002) and iron (Boling et al., 1998).

Using growth performance criteria as measure for testing the relative effectiveness of MHA-FA compared to DL-Met, several authors could confirm the results obtained with the N-balance technique. For example, Walz and

Table 6. The effect of supplemental DL-Met and liquid MHA-FA on growth performance of pigs in trial III

	Basal diet	Basal diet+0.065% DL-Methionine	Basal diet+0.10% liquid MHA-FA[1]	SE
Period I (20-30 kg BW)				
Feed intake (g/d)	1,127	1,239	1,143	32.9
Weight gain (g/d)	564[b]	698[a]	645[ab]	25.0
Feed:gain ratio	2.03[b]	1.78[a]	1.78[a]	0.045
Period II (30-49 kg BW)				
Feed intake (g/d)	1,911[a]	1,824[b]	1,839[ab]	28.3
Weight gain (g/d)	831[b]	858[ab]	899[a]	13.7
Feed:gain ratio	2.31[b]	2.14[a]	2.05[a]	0.032
Overall (20-49 kg BW)				
Feed intake (g/d)	1,597	1,590	1,561	19.2
Weight gain (g/d)	725[b]	794[a]	797[a]	11.7
Feed:gain ratio	2.21[b]	2.01[a]	1.96[a]	0.024

[1] Alimet®. [a, b] Means in the same row, not sharing a common superscript differ significantly ($p<0.05$).

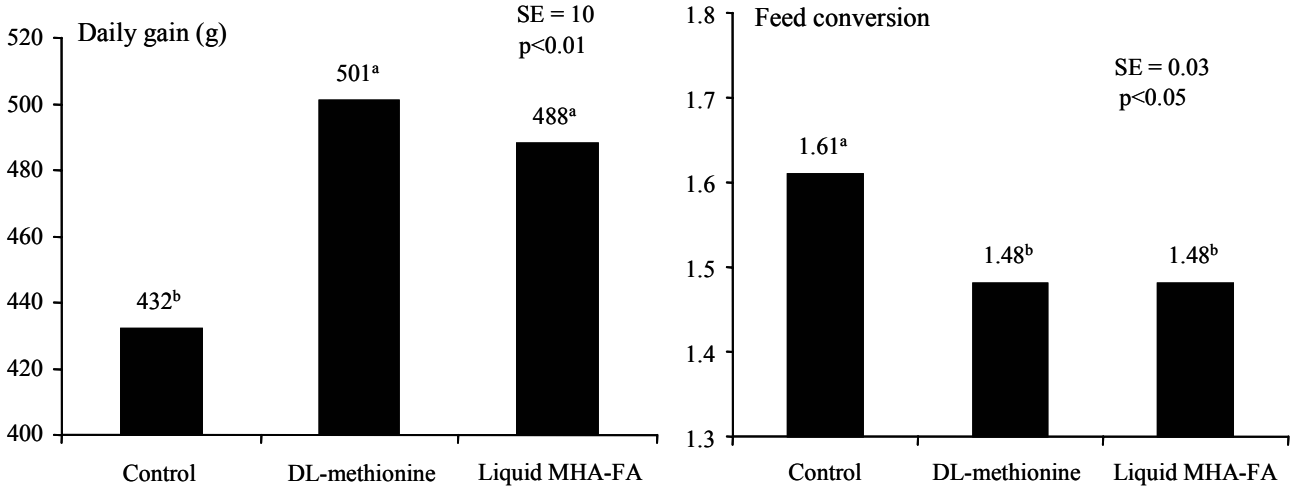

Figure 2. Growth performance in pigs fed diets supplemented with DL-Met or MHA-FA in trial II.

Pallauf (1996) fed a methionine-deficient basal diet to growing pigs in the weight range from 10 to 25 kg BW. Additionally, two other treatment groups received the basal diet either supplemented with 1.5 g/kg DL-Met or 2.3 g/kg liquid MHA-FA. On a weight for weight basis, the ratio between DL-Met and liquid MHA-FA supplementation was 65:100. There was no significant difference ($p>0.05$) in growth response and feed conversion between both methionine sources. The results of this report are in accordance with those obtained in trial II (Figure 2) and trial III (Table 6) of the present study, confirming that 65 parts of DL-Met can replace 100 parts of liquid MHA-FA in diets for growing pigs. In both trials, the basal diet was clearly deficient in methionine, confirmed by a significant response in growth rate and feed efficiency, irrespective of the source of supplemental methionine.

Using the slope-ratio technique in a study with growing pigs from 8 to 55 kg BW, Roth and Kirchgessner (1986) concluded that MHA-FA was only 78% (on equimolar basis) as efficacious as pure DL-Met based on daily gain. These results substantiate earlier findings with piglets which revealed in tendency a lower performance response for the MHA-FA in comparison to the DL-Met treatment (Steinhart and Kirchgessner, 1985).

According to other reports, however, MHA-FA and DL-Met provide equimolar levels of methionine activity in pigs (Chung and Baker, 1992; Stockland et al., 1992; Owen et al., 1995a, b; Roemer and Abel, 1996; Knight et al., 1998; Roemer and Abel, 1999). For example, Roemer and Abel (1996) supplemented a methionine-deficient diet (1.9 g/kg Met) with equimolar levels of 0.8, 1.2 and 1.6 g/kg DL-Met or MHA-FA. The experimental diets were fed to pigs ranging from 29-35 kg in BW. The daily N retention showed no significant differences in response to the supplementation of DL-Met or MHA-FA which is not in agreement with the results of trial I in this study.

As was pointed out by several authors (Fandrejewski and Rymarz, 1986; Imbeah et al., 1988; Kemme et al., 1997), a comparisons of results in different reports is often difficult, because these are confounded by differences in experimental conditions such as feed intake, diet composition, feeding regimen, age and BW of the animals.

For example, to obtain dose dependent differences in the bioavailability of any essential nutrient it is mandatory that the basal diet is clearly deficient in the nutrient to be tested (Huyghebaert, 1993; Zimmermann et al., 2002). This may explain, at least in part, the discrepancies between the results presented by Knight et al. (1998) and those obtained in this study. In one of their dose response experiments, a basal diet formulated to contain 0.45% Met and 0.52% Cys was fed to early weaned pigs compared to corresponding values of 0.22 and 0.29% Met and Cys, respectively, in the present study (trial I). Average supplemented levels for the DL-Met treatment were 0.061 and 0.091% and for the MHA-FA treatment 0.046 and 0.093%. The source of methionine did not result in a different performance response, which would limit the value of slope potency determinations (gain vs. intake of methionine source), with values of 119, 111 and 95% for the relative efficacy of MHA-FA vs. DL-Met for cumulative weekly performance.

Furthermore, differences between reports pertaining to the relative bioefficacy of DL-Met and MHA-FA may also be attributed to variations in diet composition. Studies in broiler chicks revealed that the supplementation of MHA-FA to a synthetic type of diet in comparison to practical based feed mixtures may result in different growth responses (Saunderson, 1985). The same applies if the ratio between mono-, di- and oligomers of the MHA product used in the different studies varies (Boebel and Baker, 1982), with MHA oligomers being poorly absorbed (Saunderson, 1991), and showing in poultry a significantly lower relative effectiveness in comparison to a mixture of mono-, di-, and oligomers (van Weerden et al., 1992). There is also evidence from poultry studies that the intestinal transport mechanisms responsible for the absorption of DL-MHA molecules are less efficient than those for DL-Met (Maenz and Engele-Schaan, 1996a) which explains the significantly lower absorption rates of MHA-FA compared to DL-Met (Lingens and Molnar, 1996; Maenz and Engele-Schaan, 1996b). Moreover, studies in rats suggest that the transport of MHA-FA across the brush border membrane of rat jejunum may be affected depending on the level of methionine being supplemented to the experimental diets, thus explaining discrepancies between the results of different studies (Brachet and Puigserver, 1987).

It is interesting to note that the majority of physiological studies designed to explain the discrepancies in the relative bioefficacy between MHA-FA and DL-Met has been carried out with poultry rather than pigs, and there is a need to validate these results in studies with pigs as well. For example, recent experiments with poultry suggest a considerable microbial degradation of MHA-FA in the small intestine to compounds not being utilisable as a source of Met (Lemme et al., 2001). Since the retention time of digesta in the small intestine of pigs is significantly longer than in poultry, a major impact of microbial fermentation on MHA-FA can be expected which, in turn, might explain partly the differences in relative bioefficacy between MHA-FA and DL-Met.

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