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Bioefficacy Determination of Methionine Hydroxy Analog-Free Acid Relative to DL-Methionine in Laying Hen Diets with Limited Methionine Using Different Regression Models

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Abstract: A study was conducted to compare bioefficacy of liquid DL-methionine hydroxy analogue-free acid (MHA-FA) and DL-methionine (DL-Met) in laying hens. Biological efficacy was determined for egg production, egg mass, and egg weight using five regression models. Four levels of DL-Met (0.012, 0.024, 0.036, and 0.048%) and MHA-FA (0.014, 0.027, 0.041, and 0.054%) were added on an equimolar basis to a basal diet containing 14.97% protein and 0.27% methionine. Twenty week old Hy-Line W-36 hens were used in this trial with 8 replicates per treatment. The bioefficacy of MHA-FA related to DL-Met was 0.77 on a weight basis (or 0.87 on a molar basis) based on egg mass with the best goodness of model fit (average R^2 equal to 83.33%). The bioefficacy was 0.71 on a weight basis (or 0.80 on a molar basis) based on egg production with the goodness of model fit at average R^2 equal to 76.98%. The bioefficacy was 1.03 on a weight basis (or 1.17 on a molar basis) based on egg weight with the goodness of model fit at average R^2 equal to 68.83%.

Key words: Bioavailability, DL-methionine, DL-methionine hydroxyl analogue, laying hens, regression model

Introduction

Methionine (Met) is a limiting amino acid in commercial poultry diets and is commonly supplemented as dry DL-methionine (DL-Met; 99% pure) or as liquid DL-methionine hydroxy analog-free acid (MHA-FA, containing 88% of active substance). Our lab had conducted studies (Roland *et al*., 2000 and 2003; Yadalam *et al*., 2000; Bateman *et al*., 2000), and the results indicated that producers were overfeeding supplemental Met by approximately 75-150%, or by 0.77 kg/ton of feed. We had used dry DL-Met as the source of supplemental Met, so we wanted to be sure of the relative bioefficacy between the two primary sources of supplemental Met for corn-soy diets using limited Met+Cys levels.

There was an ongoing discussion in the literature regarding the relative bioefficacy of MHA-FA related to DL-Met in laying hen diets (Reid *et al*., 1982; van Weerden *et al*., 1984; Scott, 1987; Harms and Russell, 1994; Wideman *et al*., 1994; Dänner and Bessei, 2002; Liu *et al*., 2004a and 2004b; Bateman *et al*., 2005). According to Littell *et al*. (1997), standardization of the statistical analyses would make comparisons of various nutrient sources among different experiments more precise, as well as easier to interpret. Depending on the data structure of the respective dose-response trial, bioefficacy estimates can be obtained by linear or nonlinear models. These models can be used for estimation of comparative bioefficacy of MHA-FA relative to DL-Met (Thomas *et al*., 1991; Wallis, 1999; Lemme *et al*., 2002), and other nutrients such as phosphorus (Potter, 1988; Potter *et al*., 1995; Fernandes *et al*., 1999), iron (Boling *et al*., 1998) or copper (Guo *et al*., 2001) in feed ingredients.

Objective of the present study was to determine the relative bioefficacy of MHA-FA compared to DL-Met in corn-soy diets formulated to have limited Met+Cys levels using different regression models.

Materials and Methods

Supplemental Met sources used were DL-Met (Degussa AG, Hanau, Germany) and MHA-FA (Alimet, Novus International Inc., St. Louis, MO). The basal diet was formulated with limited Met (0.27%, Table 1), and four levels of DL-Met (0.012, 0.024, 0.036, and 0.048%) and MHA-FA (0.014, 0.027, 0.041, and 0.054%) were added on an equimolar basis to a basal diet. Twenty week old Hy-Line® W-36 laying hens (1440) were used. Laying hens were randomly allocated to 360 cages (40.6 cm × 45.7 cm) with 4 birds per cage. Five adjoining cages consisted of a replicate, and then the seventy-two replicates were randomly assigned to 9 dietary treatments. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. Experiments were conducted in a computer regulated, environmentally controlled house under warm conditions with an average daily temperature of approximately 25.6°C (21.1°C during the night and 28.9°C during the day). A standard lighting program (16 h light vs 8 h dark) was followed as stated in the Hy-Line management guide (1998-99). Hens in each replicate shared a feed trough and had access to drinking cups. Feed and water were supplied *ad libitum*. Feed consumption was recorded weekly. Egg production was summarized weekly. Egg weights were determined bi-

Table 1: Ingredients and nutrient composition of experimental basal diet

Ingredients	%
Corn	68.47
Soybean meal, 48%	18.92
Limestone	7.07
Hardshell	2.00
Dicalcium phosphate	1.66
Poultry oil	0.97
Salt	0.42
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
DL-Methionine	0.00
Calculated analysis	
ME (kcal/kg)	2863.00
Protein (%)	14.97
Calcium (%)	4.00
Total phosphorus (%)	0.59
Available phosphorus (%)	0.40
Sodium (%)	0.18
Methionine	0.27
Met + Cys (%)	0.51
Lysine (%)	0.75

[1]Provided per kg of diet: retinol acetate, 8,000 IU; cholecalciferol, 2,200 ICU; dl, a-tocopherol acetate, 8 IU; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; d-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamin, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; menadione sodium bisulfate complex, 2 mg.
[2] Provided per kg of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg; copper, 6 mg; zinc, 55 mg; selenium, 0.15 mg.

weekly using all eggs collected for two consecutive days. Data were analyzed using the GLM procedure of SAS/STAT (SAS Institute, 1986) to determine if a methionine level effect existed. Exponential analysis was used with the nonlinear procedure (PROC NLIN) in SAS/STAT software for the bioefficacy estimation. The statistical model was

$$y = a + b \times (1 - e^{-(c_1 \times x_1 + c_2 \times x_2)}) + e$$

where y = performance criterion, a = intercept, b = asymptotic response (basal performance, a + b = common asymptote (maximum performance), c_1 = steepness coefficient for pure DL-Met, c_2 = steepness coefficient for MHA-FA, e = the random error. Bioefficacy of MHA-FA relative to DL-Met was determined by c_2/c_1, the ratio of regression coefficients. Slope-ratio assays were also performed with the general linear procedure (PROC GLM) in SAS/STAT software for the bioefficacy determination using the following equation:

$$y = a + b_1x_1 + b_2x_2 + e$$

where y is performance criterion, b_1 is the slope for DL-Met, b_2 is the slope for MHA-FA, and e is the random error. The bioefficacy of MHA-FA relative to DL-Met was b_2/b_1, the ratio of regression coefficients.

Results

Feed consumption increased with increasing supplemental Met levels for DL-Met and MHA-FA (Table 2), but there was no difference (P>0.05) in feed consumption between the two Met sources at any supplemental Met level. Feed conversion was improved (P<0.05) with increasing supplemental Met levels for both DL-Met and MHA-FA (Table 2). Feed conversion for the basal diet was 2.09, and the lowest feed conversion was 1.93 for DL-Met at 0.036% supplemental Met level, and was 1.95 for MHA-FA at 0.048% supplemental Met level. Feed conversion (2.03) at 0.036% supplemental Met level was worse than those at 0.024% and 0.048% supplemental Met levels, suggesting large variations existed for feed conversion. When the data for feed conversion was subjected to analysis with five models, some of the regression did not converge. Therefore the average bioefficacy value for feed conversion was not available based on the five models.

Egg production, egg mass and egg weight increased as the supplemental dietary Met levels for DL-Met and MHA-FA increased (Table 3). Using previously mentioned models, it was estimated that the relative bioefficacy of MHA-FA compared to DL-Met based on egg production was 0.80 on a molar basis or 0.71 on a weight basis (Table 4), the bioefficacy based on egg mass was 0.87 on a molar basis or 0.77 on a weight basis (Table 5), and the bioefficacy based on egg weight was 1.17 on a molar basis or 1.03 on a weight basis (Table 6). The bioefficacies based on different criterion and models were summarized in Table 7.

Discussion

Inconsistent bioefficacy values of MHA-FA related to DL-Met were obtained from previous studies. Several researchers (Reid *et al.*, 1982; Scott, 1987; Harms and Russell, 1994; Wideman *et al.*, 1994) have concluded that there was no difference between the activity of DL-Met and MHA-FA, whereas van Weerden *et al.* (1984) found that hens fed MHA-FA produced less egg mass and had poorer feed efficiency than hens fed equivalent amounts of DL-Met. Dänner and Bessei (2002) estimated the relative bioefficacy of MHA-FA as 0.67 (egg mass) and 0.69 (feed conversion) compared with DL-Met. Dänner and Bessei (2002) also recalculated the results of Reid *et al.* (1982), van Weerden *et al.* (1984), and Harms and Russell (1994) using exponential regression analysis. These recalculated figures estimate bioefficacy as 0.52 (egg mass) and 0.69 (feed conversion) for the data of Reid *et al.* (1982), 0.61 (egg mass) and 0.55 (feed conversion) for the data of van Weerden *et al.* (1984), and ranging from 0.75-0.83 for the data of Harms and Russell (1994). Taking all of these figures into account, Dänner and Bessei (2002) estimated the relative bioefficacy of MHA-FA was 0.68 compared to DL-Met.

In this experiment, inconsistent bioefficacies of MHA-FA related to DL-Met were obtained based on egg production, egg mass, and egg weight (Table 7). The bioefficacies based on egg production and egg mass

Table 2: Influence of methionine sources and levels on feed consumption and feed conversion

Level	Feed consumption (g/day)		Feed conversion (g feed/g egg)	
	DL-Met[1]	MHA-FA[2]	DL-Met	MHA-FA
0.000	64.31±0.93^{d}	64.31±0.93^{d}	2.09±0.06^{a}	2.09±0.06^{a}
0.012	66.72±1.83^{cd}	68.11±1.42^{c}	2.05±0.04^{ab}	2.04±0.02^{abc}
0.024	71.75±1.66^{ab}	69.39±0.69^{bc}	2.01±0.03^{abcde}	1.97±0.03^{bcde}
0.036	72.93±0.65^{a}	72.63±1.27^{ab}	1.93±0.01^{e}	2.03±0.05^{abcd}
0.048	72.98±0.30^{a}	74.17±0.79^{a}	1.94±0.01d^{e}	1.95±0.02^{cde}

[1]DL-Met is DL-methionine. [2]MHA-FA is liquid DL-methionine hydroxyl analogue free acid.
^{abcd}Means with different superscripts differ significantly from each other, P<0.05.

Table 3: Influence of methionine sources and levels on egg production, egg mass and egg weight

Level	Egg production (eggs/hen d)		Egg mass (g/day)		Egg weight (g)	
	DL-Met[1]	MHA-FA[2]	DL-Met	MHA-FA	DL-Met	MHA-FA
0.000	0.66±0.01^{e}	0.66±0.01^{e}	31.20±0.57^{e}	31.20±0.57^{e}	47.54±0.39^{c}	47.54±0.39^{c}
0.012	0.69±0.01^{d}	0.71±0.02^{cd}	33.20±0.74^{d}	33.92±0.85^{cd}	48.09±0.28^{c}	47.55±0.13^{c}
0.024	0.75±0.01^{ab}	0.73±0.01^{bc}	36.77±0.80^{ab}	35.52±0.27^{bc}	49.11±0.42^{b}	48.89±0.18^{b}
0.036	0.77±0.01^{a}	0.75±0.01^{ab}	38.37±0.38^{a}	37.08±0.50^{ab}	49.46±0.22^{ab}	49.52±0.25^{ab}
0.048	0.77±0.01^{a}	0.77±0.01^{a}	38.10±0.45^{a}	38.51±0.57^{a}	49.34±0.14^{ab}	50.10±0.26^{a}

[1]DL-Met is DL-methionine. [2]MHA-FA is liquid DL-methionine hydroxyl analogue free acid.
^{abcd}Means with different superscripts differ significantly from each other, P < 0.05.

Table 4: Relative bioefficacy based on egg production

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy	0.73	0.72	0.82	0.76	0.61
Confidence Interval (95%)	(0.49,0.97)	(0.48,0.96)	(0.54,1.09)	(0.58,0.94)	(0.34,0.88)
R^2 (%)	75.31	77.20	77.20	72.17	83.02
Equation[2]	Model A: $Y = 65.68 + 14.90\ (1 - e^{-(34.03x_1 + 24.75x_2)})$		Model B: $Y = 65.68 + 14.31\ (1 - e^{-(51.55x_1 + 37.18x_2)})$		
	Model C: $Y = 65.68 + 14.31\ (1 - e^{-(51.71x_1 + 42.25x_2)})$		Model D: $Y = 67.46 + 322.26x_1 + 244.24x_2$		
	Model E: $Y = 66.83 + 150.68x_1 + 91.84x_2$				

[1]Method A: Exponential model with supplemental methionine level on a weight basis as the independent variables; Method B: Exponential model with supplemental methionine intake on a weight basis as the independent variables; Method C: Exponential model with supplemental methionine intake on a molar basis as the independent variables; Method D: Slope-ratio model with supplemental methionine intake on a weight basis as the independent variables; Method E: Slope-ratio model with methionine intake above basal diet as the independent variables. [2]X_1 refers to DL-Met, and X_2 refers to MHA-FA.

were close with relative better goodness of model fit, and the average R^2 is 76.98% and 83.33% respectively. However, the bioefficacy based on egg weight were 1.17 on a molar basis. Compared to the bioefficacies based on egg production and egg mass, the inconsistent bioefficacy value in egg weight could be explained by large variations since the regression analysis for the data of egg weight has the lowest goodness of model fit (average R^2 equal to 68.83%) compared to egg production (76.98%) and egg mass (83.33%). Therefore, based on the best goodness of model fit of egg mass, the bioefficacy was 0.77 on a weight basis or 0.87 on a molar basis, which is consistent with another study done in our lab (Liu *et al.*, 2004a).

In this study, five different regression models were used to analyze the data. The first three models are exponential models with different independent variables, and the last two are slope-ratio models. The goodness of model fits (R^2 value) for Model A to Model D are close for each performance criteria (Table 4 to Table 6), indicating all these models are appropriate for estimating the bioefficacy of MHA-FA relative to DL-Met in this study. Relative high goodness of model fit (relative high R^2) was obtained for model E, in which methionine intake above basal diet was used as the independent variable. However, it did not mean that the value from this model is more believable, since natural methionine is included in the independent variable, which brings confounding effect of natural methionine into this regression model. Therefore, the average bioefficacies coming from these five models was used to determine the bioefficay of MHA-FA relative to DL-Met.

Questions remain about the physiological reasons for these results. Several studies with broilers using radiolabelled Met sources indicated a lower absorption of the hydroxy analog compared to Met (Lingens and

Table 5: Relative bioefficacy based on egg mass

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy	0.77	0.77	0.87	0.81	0.73
Confidence Interval (95%)	(0.57,0.98)	(0.56,0.97)	(0.64, 1.10)	(0.66,0.97)	(0.50,0.95)
R^2 (%)	81.35	83.33	83.33	78.57	90.08
Equation[2]	Model A: $Y = 30.96 + 10.09 (1 - e^{-(30.56x_1 + 23.65x_2)})$		Model B: $Y = 30.96 + 9.63 (1 - e^{-(46.68x_1 + 35.81x_2)})$		
	Model C: $Y = 30.96 + 9.63 (1 - e^{-(46.82x_1 + 40.70x_2)})$		Model D: $Y = 32.03 + 206.32x_1 + 168.13x_2$		
	Model E: $Y = 31.63 + 92.54x_1 + 67.10x_2$				

[1]Method A: Exponential model with supplemental methionine level on a weight basis as the independent variables; Method B: Exponential model with supplemental methionine intake on a weight basis as the independent variables; Method C: Exponential model with supplemental methionine intake on a molar basis as the independent variables; Method D: Slope-ratio model with supplemental methionine intake on a weight basis as the independent variables; Method E: Slope-ratio model with methionine intake above basal diet as the independent variables. [2]X_1 refers to DL-Met, and X_2 refers to MHA-FA.

Table 6: Relative bioefficacy based on egg weight

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy	0.98	0.98	1.11	1.00	1.19
Confidence Interval (95%)	(0.68,1.29)	(0.67,1.30)	(0.76,1.48)	(0.75,1.26)	(0.52,1.87)
R^2 (%)	67.65	67.65	67.65	67.65	73.53
Equation[2]	Model A: $Y = 47.35 + 5.53 (1 - e^{-(11.80x_1 + 11.61x_2)})$		Model B: $Y = 47.35 + 4.63 (1 - e^{-(20.85x_1 + 20.46x_2)})$		
	Model C: $Y = 47.35 + 4.63 (1 - e^{-(20.91x_1 + 23.25x_2)})$		Model D: $Y = 47.53 + 63.80x_1 + 63.96x_2$		
	Model E: $Y = 47.43 + 24.17x_1 + 28.84x_2$				

[1]Method A: Exponential model with supplemental methionine level on a weight basis as the independent variables; Method B: Exponential model with supplemental methionine intake on a weight basis as the independent variables; Method C: Exponential model with supplemental methionine intake on a molar basis as the independent variables; Method D: Slope-ratio model with supplemental methionine intake on a weight basis as the independent variables; Method E: Slope-ratio model with methionine intake above basal diet as the independent variables. [2]X_1 refers to DL-Met, and X_2 refers to MHA-FA.

Table 7: Summary of relative bioefficacies based on egg production (EP), egg mass (EM) and egg weight (EW)

		Model A	Model B	Model C	Model D	Model E	Average Bioefficacy	Average R^2 (%)
Weight Basis	EP	0.73	0.72	0.72	0.76	0.61	0.71	76.98
	EM	0.77	0.77	0.76	0.81	0.73	0.77	83.33
	EW	0.98	0.98	0.98	1.00	1.19	1.03	68.83
Molar Basis	EP	0.83	0.82	0.82	0.86	0.69	0.80	76.98
	EM	0.88	0.87	0.87	0.93	0.82	0.87	83.33
	EW	1.12	1.12	1.11	1.14	1.36	1.17	68.83

Molnar, 1996). Studies performed by Saunderson (1991) provide strong evidence that the oligomers of liquid MHA-FA are poorly absorbed. Also, the hydroxy analog molecules have to be converted to Met before intermediate use and incorporation into body tissues and in eggs (Saunderson, 1991).

In summary, based on this study, the best bioefficacy of MHA-FA relative to DL-Met was 0.77 on a weight basis or 0.87 on a molar basis in laying hen diets with limited Methionine level, and more research is needed to determine the bioefficacy difference between MHA-FA and DL-Met.

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Abbreviation key: DL-Met = DL-methionine; MHA-FA = liquid DL-methionine hydroxy analogue-free acid.