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Explanation on How to Interpret Properly the Bioefficacy of Methionine Hydroxy Analog-Free Acid Relative to Dl-Methionine Estimated by Regression Models in Laying Hens

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Abstract: Two studies were conducted to compare bioefficacy of liquid DL-methionine hydroxy analogue-free acid (MHA-FA) and DL-methionine (DL-Met). Biological efficacy was determined for egg production, feed consumption, egg mass, and egg weight using linear and nonlinear regression models. In Experiment 1, five levels of DL-Met (0.023, 0.045, 0.068, 0.090 and 0.113%) and MHA-FA (0.026, 0.051, 0.077, 0.102 and 0.128%) were added on an equimolar basis to a basal diet containing 14.97% protein and 0.27% Met. This trial used 1,760 first cycle, Phase II Hy-Line W-36 hens. There was no response above the basal diet in any of the criteria measured, so regression analysis was not performed. In Experiment 2, five levels of DL-Met (0.012, 0.024, 0.036, 0.048 and 0.060%) and MHA-FA (0.014, 0.027, 0.041, 0.054 and 0.068%) were added on an equimolar basis to the basal diet used in Experiment 1. This trial used 1,760 second cycle, Phase I Hy-Line W-36 hens. The average bioefficacy of MHA-FA related to DL-Met was 82.45% on a weight basis (or 93.70% on a molar basis) based on egg production, was 89.23% on a weight basis (or 101.40% on a molar basis) based on egg mass, and was 106.29% on a weight basis (or 120.79% on a molar basis) based on egg weight, more research is needed to improve accuracy of bioefficacy values.

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-Met 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 many producers were overfeeding supplemental Met up to 1 kg/ton. 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.

There was an ongoing discussion in the literature regarding the relative bioefficacy of MHA-FA and 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). The correct statistical explanation to the experimental data for evaluating bioefficacy of MHA-FA relative to DL-Met has also been discussed (Liu *et al.*, 2004c). Depending on the data structure of the respective dose-response trial, bioefficacy estimates can be obtained by different regression models, such as slope-ratio of exponential models (Littell *et al.*, 1997).

Objective of the present studies was to determine the relative bioefficacy of MHA-FA relative to DL-Met with different regression models, and to explain how to interpret the bioefficacy values from these models.

Materials and Methods

The basal diet was formulated with limited Met (0.27%, Table 1). In Experiment 1, five levels of DL-Met (0.023, 0.045, 0.068, 0.090 and 0.113%) and MHA-FA (0.026, 0.051, 0.077, 0.102 and 0.128%) were added on an equimolar basis to the basal diet, and 1,760 first cycle, Phase II Hy-Line W-36 hens were used. In Experiment 2, five levels of DL-Met (0.012, 0.024, 0.036, 0.048 and 0.060%) and MHA-FA (0.014, 0.027, 0.041, 0.054 and 0.068%) were added on an equimolar basis to the same basal diet used in Experiment 1, and 1,760 second cycle, Phase I Hy-Line W-36 hens were used (Table 2). Supplemental Met sources used were DL-Met (Degussa AG, Hanau, Germany) and MHA-FA (Alimet, Novus International Inc., St. Louis, MO). Laying hens were randomly allocated to 440 cages (40.6 cm × 45.7 cm) with 4 birds per cage. Five adjoining cages consisted of a replicate, and then the eighty-eight replicates were randomly assigned to 11 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^{o}C (21.1 during the night and 28.9^{o}C during the day). A standard lighting program (16 h light vs 8 h dark) was followed as

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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.

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-weekly using all eggs collected for two consecutive days. Specific gravity was determined monthly using eggs collected for two consecutive days by the method of Strong (1989), which involved placing eggs in a series of saline solutions ranging from 1.060 to 1.100 in 0.005 unit increments. Mortality was recorded daily.

Data were analyzed using the GLM procedure of SAS/STAT (SAS Institute, 1986) to determine if a methionine level effect existed, and the mean between DL-Met and MHA-FA was separated with Fisher LSD method. If there were some improvements from adding supplemental Met to basal diet, regression analysis would be conducted to determine the bioefficacy. 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

Experiment 1: Since there were no improvements (P > 0.05) from adding supplemental Met to basal diet after the first level (0.023%) in any of the performance criteria, neither the linear nor nonlinear model were fit to data to determine relative bioefficacy of MHA-FA compared to DL-Met in this experiment (Table 3). Our first supplemental level of Met was too high to show a response at higher inclusion levels, so a second experiment was conducted with lower levels of supplemental Met to pick up differences along the response curve.

Experiment 2: Feed consumption increased with increasing supplemental Met levels for DL-Met and MHA-FA (Table 3), but there was no difference (P > 0.05) in feed consumption between these two Met sources at any supplemental Met level. Feed conversion was improved with increasing supplemental Met levels for DL-Met, except for 0.012% supplemental Met level, but the improvement on feed conversion by increasing supplemental Met was inconsistent at different levels (Table 3). When the data for feed conversion was subjected to analysis with five models, some of the regressions did not converge. Therefore, the average bioefficacy values were not available based on all 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 93.70% on a molar basis or 82.45% on a weight basis (Table 4), the bioefficacy based on egg mass was 101.40% on a molar basis or 89.23% on a weight basis (Table 5), and the bioefficacy based on egg weight was 120.79% on a molar basis or 106.29% on a weight basis (Table 6). The bioefficacies based on different criterion and models was summarized in Table 7.

Discussion

In Experiment 1, no improvements (P > 0.05) from adding supplemental Met to basal diet after the first

Table 2: Experimental design

Treatment	Met Source[1]	Experiment 1		Experiment 2	
		Addition of Met source (%)	Addition of Met equivalents (%)	Addition of Met source (%)	Addition of Met equivalents (%)
1	Basal diet	-	-	-	-
2	DL-Met	0.023	0.023	0.012	0.012
3	DL-Met	0.045	0.045	0.024	0.024
4	DL-Met	0.068	0.068	0.036	0.036
5	DL-Met	0.090	0.090	0.048	0.048
6	DL-Met	0.113	0.113	0.060	0.060
7	Liquid MHA-FA[2]	0.026	0.023	0.014	0.012
8	Liquid MHA-FA	0.051	0.045	0.027	0.024
9	Liquid MHA-FA	0.077	0.068	0.041	0.036
10	Liquid MHA-FA	0.102	0.090	0.054	0.048
11	Liquid MHA-FA	0.128	0.113	0.068	0.060

[1]DL-Met = DL-Methionine; liquid MHA-FA = liquid Met hydroxy analog-free acid.
[2]Based on a liquid MHA-FA content of 88% in the commercial product.

Table 3: Influence of Met source and level, Experiment 1

Level	Feed consumption (g/day)		Feed conversion (g feed/g egg)		Egg production (%)	
	DL-Met	MHA-FA	DL-Met	MHA-FA	DL-Met	MHA-FA
Experiment 1						
0.000	86.65±1.03^{e}	86.65±1.03^{e}	1.87±0.01^{a}	1.87±0.01^{a}	80.98±1.62^{b}	80.98±1.62^{b}
0.023	90.20±1.02^{abc}	90.63±0.39^{ab}	1.86±0.02^{abc}	1.86±0.00^{ab}	83.25±0.68^{ab}	83.48±0.42^{ab}
0.045	89.66±0.65^{abcd}	91.84±0.89^{a}	1.84±0.02^{abcd}	1.85±0.01^{abcd}	83.56±1.12^{ab}	83.72±0.57^{a}
0.068	89.22±0.85^{bcd}	88.42±1.10^{bcde}	1.85±0.02^{abcd}	1.81±0.03^{bcd}	81.69±1.08^{ab}	83.92±0.44^{a}
0.080	88.16±0.65^{cde}	88.86±0.33^{bcde}	1.84±0.02^{abcd}	1.83±0.02^{abcd}	82.18±1.06^{ab}	83.26±0.87^{ab}
0.113	90.38±0.78^{abc}	87.67±0.61^{de}	1.81±0.02^{cd}	1.80±0.03^{d}	84.20±0.48^{a}	83.03±0.93^{ab}
Experiment 2						
0.000	73.04±0.81^{a}	73.04±0.81^{a}	2.63±0.13^{ab}	2.63±0.13^{ab}	53.58±1.52^{a}	53.58±1.52^{a}
0.012	75.98±1.17^{ab}	76.81±0.80^{abc}	2.78±0.09^{a}	2.50±0.06^{ab}	54.94±1.23^{ab}	58.01±1.47^{bc}
0.024	78.12±1.29^{bc}	79.24±1.19^{bc}	2.61±0.04^{ab}	2.71±0.10^{ab}	57.61±0.64^{abc}	58.32±1.12^{bc}
0.036	79.46±1.61^{bc}	78.48±1.06^{bc}	2.53±0.04^{ab}	2.60±0.06^{ab}	60.00±1.15^{c}	57.80±0.28^{bc}
0.048	80.90±2.11^{c}	80.23±2.21^{bc}	2.50±0.09^{b}	2.56±0.07^{ab}	60.32±2.16^{c}	61.02±1.82^{c}
0.060	80.99±1.89^{c}	80.27±1.25^{c}	2.50±0.07^{b}	2.58±0.17^{ab}	61.42±1.05^{c}	59.65±2.05^{c}

	Egg mass (g/hen/day)		Egg weight (g)	
	DL-Met	MHA-FA	DL-Met	MHA-FA 1
Experiment 1				
0.000	46.33±0.77^{c}	46.33±0.77^{c}	57.25±0.37^{e}	57.25±0.37^{e}
0.023	48.59±0.66^{ab}	48.74±0.24^{ab}	58.37±0.46^{cd}	58.39±0.28^{cd}
0.045	48.68±0.76^{ab}	49.62±0.44^{ab}	58.26±0.030^{cd}	59.29±0.25^{ab}
0.068	48.28±0.71^{b}	48.82±0.33^{ab}	59.12±0.23^{abc}	58.19±0.15^{d}
0.080	48.00±0.83^{bc}	48.65±0.54^{ab}	58.41±0.26^{bcd}	58.46±0.30^{bcd}
0.113	50.09±0.18^{a}	48.76±0.52^{ab}	59.51±0.45^{a}	58.74±0.15^{abcd}
Experiment 2				
0.000	30.43±1.10^{a}	30.43±1.10^{a}	57.00±0.45^{f}	57.00±0.45^{f}
0.012	32.04±0.90^{ab}	33.82±0.93^{ab}	58.33±0.39d^{e}	58.48±0.33^{d}
0.024	33.72±0.50^{ab}	34.41±0.66^{ab}	58.62±0.40^{cd}	59.13±0.21^{bcd}
0.036	35.91±0.91^{b}	34.89±0.19^{ab}	59.79±0.42^{abc}	60.51±0.50^{a}
0.048	36.51±1.71^{b}	36.77±1.32^{b}	60.61±0.56^{a}	60.32±0.42^{ab}
0.060	36.96±1.20^{b}	36.33±1.43^{b}	60.42±0.83^{ab}	60.91±0.45^{a}

^{abcde} means within a criterion with different superscripts differ significantly from each other, $P < 0.05$.

Table 4: Bioefficacy based on the data of egg production (%) in Experiment 2

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy (%)	89.73	82.94	93.97	78.77	78.14
Confidence Interval	(28, 152)	(37, 149)	(41, 146)	(47, 110)	(43, 113)
R^2 (%)	38.82	41.67	41.67	40.65	67.07
Equation[2]	Model A: $Y = 53.66 + 8.14 (1 - e^{-(32.22x_1 + 28.91x_2)})$				
	Model B: $Y = 53.97 + 9.85 (1 - e^{-(27.01x_1 + 22.40x_2)})$				
	Model C: $Y = 53.97 + 9.85 (1 - e^{-(27.10x_1 + 25.47x_2)})$				
	Model D: $Y = 54.80 + 147.11x_1 + 115.88x_2$				
	Model E: $Y = 53.44 + 98.13x_1 + 76.68x_2$[1]				

[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 5: Bioefficacy based on the data of egg mass (%) in Experiment 2

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy (%)	93.83	89.95	101.91	84.96	87.75
Confidence Interval	(45, 143)	(50, 130)	(57, 148)	(58, 112)	(60, 115)
R^2 (%)	50.97	54.52	54.52	52.90	80.65
Equation[2]	Model A: $Y = 30.46 + 7.58 (1 - e^{-(29.10x_1 + 27.30x_2)})$				
	Model B: $Y = 30.65 + 8.73 (1 - e^{-(27.20x_1 + 24.47x_2)})$				
	Model C: $Y = 30.65 + 8.73 (1 - e^{-(27.29x_1 + 27.81x_2)})$				
	Model D: $Y = 31.43 + 128.57x_1 + 109.23x_2$				
	Model E: $Y = 30.37 + 81.02x_1 + 71.10x_2$				

[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: Bioefficacy based on the data of egg weight (%) in Experiment 2

Method[1]	Method A	Method B	Method C	Method D	Method E
Relative Bioefficacy (%)	107.85	106.54	120.70	98.02	112.84
Confidence Interval	(68, 147)	(71, 142)	(81, 161)	(75, 121)	(81, 144)
R^2 (%)	67.44	69.77	69.77	67.44	84.88
Equation[2]	Model A: $Y = 57.00 + 4.79 (1 - e^{-(23.32x_1 + 25.15x_2)})$				
	Model B: $Y = 57.00 + 5.11 (1 - e^{-(25.59x_1 + 27.26x_2)})$				
	Model C: $Y = 57.06 + 5.11 (1 - e^{-(25.67x_1 + 30.98x_2)})$				
	Model D: $Y = 57.53 + 70.48x_1 + 69.08x_2$				
	Model E: $Y = 57.14 + 38.13x_1 + 43.03x_2$				

[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.

supplemental Met level (0.023%) were obtained in any of the performance criteria, indicating the low sensitivity of laying hens to methionine deficiency. The low sensitivity of laying hens to methionine deficiency is one of important reasons for those inconsistent bioefficacy values of MHA-FA related to DL-Met 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 67% (egg mass) and 69% (feed conversion) on a weight basis. Because the low sensitivity of laying hens to methionine deficiency, it is difficult to detect any potential difference between MHA-FA and DL-Met or to

Table 7: Summary of relative bioefficacies based on egg production (EP), egg mass (EM) and egg weight (EW) in Experiment 2

		Model A (%)	Model B (%)	Model C (%)	Model D (%)	Model E (%)	Average Bioefficacy (%)	Average R^2 (%)
Weight	EP	89.73	82.94	82.69	78.77	78.14	82.45	45.98
Basis	EM	93.83	89.95	89.68	84.96	87.75	89.23	58.71
	EW	107.85	106.54	106.22	98.02	112.84	106.29	71.86
Molar	EP	101.97	94.25	93.97	89.51	88.80	93.70	45.98
Basis	EM	106.63	102.22	101.91	96.55	99.72	101.40	58.71
	EW	122.56	121.07	120.70	111.39	128.23	120.79	71.86

determine an accurate relative bioefficacy value.

In Experiment 2, low graded levels of DL-Met and MHA-FA were added to basal diet in order to get significant response of laying hens to methionine supplementation. The results (Table 3) showed that positive responses were obtained for most of the progressive increase of supplemental methionine. Therefore, five different linear or nonlinear models were used to estimate the bioefficacy of MHA-FA relative to DL-Met. However, due to large variation in performances (egg production, egg mass, and egg weight), it was difficult to give an accurate value for bioefficacy of MHA-FA relative to DL-Met. For instance, the bioefficacy was 89.73% based on egg production with model A, which used supplemental methionine level on a weight basis as the independent variable. However, the 95% confidence interval was from 28 to 152%.

Currently, some researchers (Lemme *et al*., 2002; Dänner and Bessei, 2002) reported that the bioefficacy of MHA-FA relative to DL-Met was as low as 65% on a weight basis (or 74% on a molar basis) in broilers or laying hens, and some researchers (Reid *et al.*, 1982; Scott, 1987; Dibner, 2003) reported that there was no difference of bioefficacy between MHA-FA and DL-Met, indicating the bioefficacy is 88% on a weight basis (or 100% on a molar basis). In this study, all the 95% confidence intervals for the bioefficacies based on egg production, egg mass, and egg weight with different regression models included both 65 and 88% on a weight basis (or 74 and 100% on a molar basis). Therefore, it could not be concluded from this study that the bioefficacy is significantly greater than 65% or less than 88% on a weight basis. More studies are necessary to give a more accurate value of bioefficacy.

In this study, we obtained the highest goodness of fit for model E, which used methionine intake above basal diet 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 into this regression model.

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 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 conclusion, the results of this study suggested that the value for the relative bioefficacy of MHA-FA as compared to DL-Met is 82.45% (egg production), 89.23% (egg mass), or 106.29% (egg weight) on a weight basis. However, it is clear that because of the large range of 95% confidence intervals more research is required to improve the relative bioefficacy values.

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