Evidence for 2-Hydroxy-4(Methylthio) Butanoic Acid and DL-Methionine Having Different Dose Responses in Growing Broilers

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ABSTRACT The objective of this study was to compare the gain-response curve to dietary levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) and DL-Met (DLM) across 4 floor pen trials in which different diets were used. Six replicates of 38 or 41 birds per pen (trials 1 to 2 and 3 to 4, respectively) were used in a 2 × 3 factorial arrangement. A control with 12 replicates was also included. The 2 Met sources were fed at 3 equimolar levels equally spaced, with the highest level added at requirements from 1 to 48, 49, 43, or 49 d for trials 1, 2, 3, and 4, respectively. Commercial-type TSAA-deficient control diets contained sorghum, wheat, corn, or corn plus meat and bone meal for trials 1, 2, 3, and 4, respectively. Performance improved at all times for most parameters after supplementing with HMTBA or DLM ($P < 0.05$). No differences were found in the birds fed HMTBA or DLM at any age and trial ($P > 0.05$), except for trial 1, in which 17-d-old birds performed better when fed HMTBA than DLM ($P < 0.05$). In each trial, linear, quadratic, and exponential regressions were conducted upon the gain response of birds fed HMTBA and DLM separately. Equations with better goodness of fit were used to compare the estimated gain responses to feeding HMTBA vs. DLM. In 3 trials, the shape of the gain-response curve differed when feeding HMTBA vs. DLM. In trials 3 and 4, feeding HMTBA at commercial levels resulted in greater gain responses than DLM ($P < 0.05$), whereas, in trials 2 and 4, at very deficient levels, DLM-fed birds outperformed those fed HMTBA ($P < 0.05$). When the 4 trials were combined, the dose-response curve with the best goodness of fit was linear for HMTBA and quadratic for DLM. It can be concluded that the 2 Met sources have a different dose-response form, HMTBA could outperform DLM at commercial levels, and DLM could outperform HMTBA at deficient levels.

Key words: 2-hydroxy-4(methylthio) butanoic acid, DL-methionine, broiler, dose response

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INTRODUCTION

In poultry diets based on soybean meal, Met is considered to be the first limiting amino acid, and synthetic Met is typically added either as DL-Met (**DLM**) or as 2-hydroxy-4(methylthio) butanoic acid (**HMTBA**). Although extensive research evaluating the relative efficiency of HMTBA and DLM as sources of Met activity in broilers has been conducted during the last 5 decades, this subject remains controversial. Although both compounds provide Met precursors to the broilers, there are substantial differences between them with respect to chemistry, absorption (Knight and Dibner, 1984), transport in the body (Lobley et al., 2006; Wester et al., 2006), and metabolism by the tissues (Dibner, 2003). Because HMTBA and DLM both provide Met activity, it has been assumed that birds fed the Met sources would follow the same performance dose response. This assumption has been accepted in several studies that have compared the relative performance of feeding the 2 Met sources using slope-ratio analysis, where an asymptotic exponential curve with common intercept and plateau was fitted over the mean response to the 2 Met sources (Littell et al., 1997; Jansman et al., 2003). This bioassay technique assumes that HMTBA behaves as DLM, and, therefore, both sources follow the same asymptotic exponential response curves that approach the same plateau. There are several studies in the literature that either demonstrate different dose-response characteristics or assume a common dose response, but the published mean responses do not support common dose response (Thomas et al., 1984; Schutte and de Jong, 1996; Lemme et al., 2002; Vázquez-Añón et al., 2003; González-Esquerra et al., 2004). Given the strong evidence that HMTBA does not function as DLM, it is critical that the methodology to compare the 2 Met sources allows the data from each source to define its own response-curve model and determine relative perfor-

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Table 1. Composition of the diets fed in trial 1

	Age of birds (d)		
	Starter	Grower	Finisher
	1 to 17	18 to 34	35 to 48
Ingredients (%)			
Sorghum, tannin-free (10.75% CP)	59.15	62.99	71.15
Soybean meal (46.66% CP)	35.18	30.8	26.38
Soybean oil	4.13	4.36	3.98
Dicalcium phosphate	1.75	1.61	1.38
Limestone	0.97	0.88	0.84
Salt	0.33	0.33	0.37
Vitamin-mineral premix[1]	0.26	0.264	0.264
L-Lys-HCl	0.10	0.05	0.14
L-Thr	0.06	0.06	0.08
Bio-Cox 60[2]	0.10	0.10	0.10
Choline (60%)	0.04	0.04	0.02
Santoquin[3]	0.02	0.02	0.02
HMTBA or DLM[4]	0, 0.07, 0.14, 0.21	0, 0.05, 0.10, 0.15	0, 0.05, 0.10, 0.15
Nutrients of control diets[5]			
ME (kcal/kg)	3,100	3,150	3,200
CP[6] (%)	21.22	19.54	16.40
Lys[6] (%)	1.22 (1.09)	1.07 (0.95)	0.92 (0.82)
Met + Cys[6] (%)	0.68 (0.58)	0.63 (0.54)	0.54 (0.46)
Met[6] (%)	0.32 (0.29)	0.3 (0.27)	0.26 (0.23)
Ca[6] (%)	0.93	0.89	0.75
Available P (%)	0.43	0.40	0.35

[1]Supplied per kilogram of total diet: vitamin A (vitamin A acetate), 13,236 IU; cholecalciferol, 6,637 IU; vitamin E, 44.1 IU; menadione, 4.5 mg; B_{12}, 24.7 μg; D-biotin, 0.33 mg; niacin, 88.2 mg; riboflavin, 6.62 mg; pantothenic acid, 24.26 mg; folic acid, 1.1 mg; B_6, 3.3 mg; thiamin, 2.2 mg; Zn from Zn sulfate, 276 mg; Mn from Mn sulfate, 275 mg; Fe from Fe sulfate, 111 mg; Se from Na selenite, 0.666 mg; Cu from Cu sulfate, 17 mg.

[2]Alpharma Inc., Fort Lee, NJ.

[3]Novus International Inc., St. Louis, MO.

[4]Equimolar levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) were added (4 levels each) at the expense of soybean meal. Alimet (Novus International Inc.) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

[5]Apparent ileal digestible amino acid values are given in parenthesis.

[6]Analyzed values.

mance of HMTBA and DLM by comparing the predictions of each model.

The objectives of the following trials were to assess the dose response of broilers when fed a variety of commercial-type diets containing HMTBA or DLM and to assess the relative gain response of broilers to these Met sources.

MATERIALS AND METHODS

Experimental Design

Four trials of similar protocol were carried out from January 2002 to March 2003 at the University of Missouri-Columbia in a curtain-sided facility equipped with thermostat controllers and 48 floor pens of 1.8 × 1.2 m each. A tube feeder, 5 nipple drinkers, and a lamp heater were installed in each pen. One-day-old male chicks were obtained from a local hatchery after in ovo vaccination against Marek's disease and posthatch coarse spray vaccination against Newcastle disease and infectious bronchitis.

Male Ross × Ross 305 birds were used in trial 1, whereas male Cobb × Cobb 500 birds were used in all other studies. There were 38 birds per pen in trials 1 and 2 and 41 birds per pen in trials 3 and 4. Birds were randomly distributed to each pen. A 3 × 2 factorial arrangement of treatments was used, with 6 pens randomly assigned to each treatment, in which Alimet feed supplement (88% HMTBA; Novus International Inc., St. Louis, MO) or DLM (99% Met) were fed at 3 equimolar levels of supplementation. A control treatment of 12 pens fed diets devoid of supplemental Met was also included in the experimental design. Oasis nutritional supplement (Novus International Inc.) was fed daily during the first 2 d of the trial at the rate of 1.25 g per bird. The temperature provided to birds was controlled to maximize performance; 33°C during the first 14 d of age, 26°C from 15 to 21 d of age, 24°C from 22 to 28 d of age, and 21°C from 29 d to end of study. The lighting program consisted of 24 h of full light during the first 5 d, 16 h of light from 6 to 13 d of age, 20 h of light from 14 d of age to 2 d before killing, and 24 h of light during the last 2 d before killing. Birds were handled in accordance with the University of Missouri Animal Care and Use Committee.

Diets

Crumbled starter and pelleted grower and finisher diets deficient in TSAA but adequate in other nutrients (National Research Council, 1994) were formulated to reflect

Table 2. Composition of the diets fed in trial 2

	Age of birds (d)		
	Starter	Grower	Finisher
	1 to 17	18 to 35	36 to 48
Ingredients (%)			
Wheat (11.62% CP)	41.38	23.00	15.00
Soybean meal (46.66% CP)	27.77	26.42	18.67
Corn (8.24% CP)	16.25	34.21	47.27
Animal fat	7.60	6.94	6.25
Peameal (22.50% CP)	3.10	6.00	9.66
Dicalcium phosphate	1.81	1.58	1.36
Limestone	0.84	0.73	0.69
Salt	0.33	0.33	0.35
Vitamin-mineral premix[1]	0.35	0.35	0.35
L-Lys	0.161	0.064	0.064
L-Thr	0.087	0.057	0.06
Bio-Cox 60[2]	0.10	0.10	0.10
Propionic acid-based antifungal	0.10	0.10	0.10
Avizyme 1502[3]	0.10	0.075	0.05
Copper sulfate	0.003	0.003	0.003
Santoquin[4]	0.02	0.02	0.02
HMTBA or DLM[5]	0, 0.06, 0.12, 0.18	0, 0.06, 0.12, 0.18	0, 0.047, 0.093, 0.14
Nutrients of control diets[6]			
ME (kcal/kg)	3,100	3,150	3,200
CP[7] (%)	21.15	20.37	17.45
Lys[7] (%)	1.22 (1.10)	1.13 (1.01)	0.96 (0.85)
Met + Cys[7] (%)	0.70 (0.61)	0.67 (0.58)	0.58 (0.50)
Met[7] (%)	0.32 (0.28)	0.31 (0.28)	0.26 (0.24)
Thr[7] (%)	0.84 (0.74)	0.80 (0.71)	0.68 (0.60)
Ca[7] (%)	0.93	0.83	0.74
Available P (%)	0.45	0.40	0.35

[1]Supplied per kilogram of total diet: vitamin A (vitamin A acetate), 13,236 IU; cholecalciferol, 6,637 IU; vitamin E, 44.1 IU; menadione, 4.5 mg; B_{12}, 24.7 μg; D-biotin, 0.33 mg; niacin, 88.2 mg; riboflavin, 6.62 mg; pantothenic acid, 24.26 mg; folic acid, 1.1 mg; B_6, 3.3 mg; thiamin, 2.2 mg; Zn from Zn sulfate, 276 mg; Mn from Mn sulfate, 275 mg; Fe from Fe sulfate, 111 mg; Se from Na selenite, 0.666 mg; Cu from Cu sulfate, 17 mg.

[2]Alpharma Inc., Fort Lee, NJ.

[3]Danisco, Copenhagen, Denmark.

[4]Novus International Inc., St. Louis, MO.

[5]Equimolar levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) were added (4 levels each) at the expense of soybean meal. Alimet (Novus International Inc.) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

[6]Apparent ileal digestible amino acid values are given in parentheses.

[7]Analyzed values.

commercial diets typically used in various areas of the world. Diets were formulated using digestible amino acid values generated from IDEA (Novus International Inc.) or obtained from tables and imposing ideal amino acid ratios relative to Lys (Baker and Han, 1994; Baker et al., 2002). The degree of TSAA deficiency in the control diet devoid of any supplemental Met was defined to provide a significant response to Met supplementation. The 3 equimolar levels of HMTBA or DLM supplemented to the control diet varied depending on the study and feeding phase (Tables 1 to 4). Alimet, with 88% Met activity, was used as source of HMTBA, and DLM, with 99% Met activity, was also used. Across studies, the highest level of supplementation was defined to provide adequate levels of dietary TSAA (Baker and Han, 1994; Baker et al., 2002). The lowest and intermediate levels of HMTBA and DLM were defined to provide an equally spaced dose response. In trial 1, the level of Met supplementation in the starter diets was 0, 0.07, 0.14, and 0.21% and was 0, 0.05, 0.10, and 0.15% in the grower and finisher diets. In trial 2 and 3, the levels of Met supplementation in the starter and grower diets were 0, 0.06, 0.12, and 0.18% and were 0, 0.047, 0.093, and 0.14% in the finisher diets. In trial 4, the levels of Met supplementation in starter and grower diets were 0, 0.07, 0.14, and 0.20% and were 0, 0.04, 0.08, and 0.13% in the finisher diets. The levels of Met addition were verified from blind samples by the analysis of HMTBA (Ontiveros et al., 1987) by Novus International Inc. and free Met using an amino acid analyzer (Beckman Coulter Inc., Fullerton, CA) by the Experiment Station Chemical Laboratories of the University of Missouri in all diets. The nutrient profile and amino acid concentration of major ingredients used in all trials was determined at the Experiment Station Chemical Laboratories before diet formulation and in final diets. All feed samples were ground through a 0.5-mm sieve in a stainless steel Retsch mill (Retsch GmbH, Haan, Germany) and hydrolyzed at 115°C under N for 24 h before amino acid analysis. For Met and Cys analysis, samples were oxidized using performic acid and then hydrolyzed according to the Association of Official Analytical Chemists [1999; method 982.30 E(a,b,c)]. The experimental diets

Table 3. Composition of the diets fed in trial 3

	Age of birds (d)		
	Starter	Grower	Finisher
	1 to 16	17 to 30	30 to 44
Ingredients (%)			
Corn (7.40% CP)	60.65	63.62	70.65
Soybean meal (47.80% CP)	32.24	29.29	22.74
Animal fat	3.53	3.81	3.48
Dicalcium phosphate	1.86	1.61	1.36
Limestone	0.81	0.75	0.79
Salt	0.34	0.33	0.37
Vitamin-mineral premix[1]	0.35	0.34	0.35
L-Lys	0.10	0.10	0.10
L-Thr	0.05	0.07	0.06
Sacox 60[2]	0.05	0.05	0.05
Copper sulfate	0.003	0.003	0.003
Santoquin[3]	0.02	0.02	0.02
HMTBA or DLM[4]	0, 0.06, 0.12, 0.18	0, 0.06, 0.12, 0.18	0, 0.047, 0.093, 0.14
Nutrients of control diets[5]			
ME (kcal/kg)	3,100	3,150	3,200
CP[6] (%)	21.42	20.20	17.00
Lys[6] (%)	1.21(1.09)	1.14 (1.01)	0.95 (0.85)
Met + Cys[6] (%)	0.71 (0.61)	0.67 (0.58)	0.61 (0.52)
Met[6] (%)	0.33 (0.30)	0.31 (0.28)	0.28 (0.25)
Thr[6] (%)	0.82 (0.73)	0.80 (0.71)	0.67 (0.60)
Ca[6] (%)	0.93	0.84	0.78
Available P (%)	0.45	0.40	0.35

[1]Supplied per kilogram of total diet: vitamin A (vitamin A acetate), 13,236 IU; cholecalciferol, 6,637 IU; vitamin E, 44.1 IU; menadione, 4.5 mg; B_{12}, 24.7 μg; D-biotin, 0.33 mg; niacin, 88.2 mg; riboflavin, 6.62 mg; pantothenic acid, 24.26 mg; folic acid, 1.1 mg; B_6, 3.3 mg; thiamin, 2.2 mg; Zn from Zn sulfate, 276 mg; Mn from Mn sulfate, 275 mg; Fe from Fe sulfate; 111 mg, Se from Na selenite, 0.666 mg; Cu from Cu sulfate, 17 mg.

[2]Intervet, Millsboro, DE.

[3]Novus International Inc., St. Louis, MO.

[4]Equimolar levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) were added (4 levels each) at the expense of soybean meal. Alimet (Novus International Inc.) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

[5]Apparent ileal digestible amino acid values are given in parentheses.

[6]Analyzed values.

contained sorghum tannin-free cultivar, wheat, peas, corn, and corn plus meat and bone meal in trials 1 (Table 1), 2 (Table 2), 3 (Table 3), and 4 (Table 4), respectively. These diets are commonly fed in different countries of the world, such as Mexico, Northern Europe, Canada, Brazil, and the United States.

Statistical Analysis and Measurements

Body weight, feed intake (**FI**), and feed conversion corrected for mortality (**FCR**) were recorded for each pen at the end of the starter, grower, and finisher phases. Overall treatment effect was subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2003). Duncan's test was used to compare multiple treatments within each study. Significance differences were declared at $P < 0.05$ and trends at $P \leq 0.1$ and $P > 0.05$. Main effects of Met source and level of supplementation were tested as a 2 × 3 factorial arrangement excluding the control treatment.

Linear (**LIN**), quadratic (**QUAD**), and exponential (**EXP**) regressions were conducted independently to HMTBA and DLM-fed groups using BW gain over that of the controls (**BWGc**) and the Met intake over that of the controls (**MIOC**) as dependent and independent variables, respectively. Body weight gain over that of the control was used instead of BW gain to allow combining the results of the 4 trials and reduce the number of parameter estimates in the regression analysis. Met intake over the controls was used instead of the concentration of the Met equivalent added to the diet to improve the goodness of fit, as described by González-Esquerra et al. (2004) in poultry and Yi et al. (2005) in swine. All possible LIN, QUAD and EXP combinations were tested as detailed in the following equations:

A) HMTBA = LIN; DLM = LIN:
$$BWGc = A1 \times Hin + B1 \times DLin$$

B) HMTBA = LIN; DLM = QUAD:
$$BWGc = A1 \times Hin + B2 \times DLin + B3 \times (Dlin \times DLin)$$

C) HMTBA = QUAD; DLM = LIN:
$$BWGc = A2 \times Hin + A3 \times (Hin \times Hin) + B1 \times DLin$$

D) HMTBA = QUAD; DLM = QUAD:
$$BWGc = A2 \times Hin + A3 \times (Hin \times Hin) + B2 \times DLin + B3 \times (Dlin \times DLin)$$

E) HMTBA = LIN; DLM = EXP:
$$BWGc = A1 \times Hin + B4 \times [1 - EXP(-B5 \times DLin)]$$

Table 4. Composition of the diets fed in trial 4

	Age of birds (d)		
	Starter	Grower	Finisher
	1 to 16	17 to 35	36 to 49
Ingredients (%)			
Corn (7.40% CP)	59.72	60.25	66.38
Soybean meal (49.07% CP)	30.21	29.84	23.24
Meat and bone meal (51.66% CP)	5.08	4.71	4.73
Animal fat	2.39	2.98	3.52
Dicalcium phosphate	1.25	1.08	0.79
Limestone	0.40	0.30	0.27
Salt	0.34	0.30	0.48
Vitamin-mineral premix[1]	0.35	0.34	0.35
L-Lys	0.12	0.09	0.10
L-Thr	0.07	0.07	0.07
Coban 60[2]	0.05	0.05	0.05
Copper sulfate	0.003	0.003	0.003
Santoquin[3]	0.02	0.02	0.02
HMTBA or DLM[4]	0, 0.07, 0.14, 0.20	0, 0.07, 0.14, 0.20	0, 0.04, 0.08, 0.13
Nutrients of control diets[5]			
ME (kcal/kg)	3,055	3,100	3,187
CP[6] (%)	22.98	22.00	19.08
Lys[6] (%)	1.30 (1.15)	1.25 (1.11)	1.05 (0.89)
Met + Cys[6] (%)	0.74 (0.63)	0.74 (0.62)	0.65 (0.54)
Met[6] (%)	0.35 (0.31)	0.35 (0.31)	0.30 (0.27)
Thr[6] (%)	0.88 (0.77)	0.86 (0.76)	0.73 (0.62)
Ca[6] (%)	1.02	0.91	0.80
Available P (%)	0.45	0.41	0.35

[1]Supplied per kilogram of total diet: vitamin A (vitamin A acetate), 13,236 IU; cholecalciferol, 6,637 IU; vitamin E, 44.1 IU; menadione, 4.5 mg; B_{12}, 24.7 μg; D-biotin, 0.33 mg; niacin, 88.2 mg; riboflavin, 6.62 mg; pantothenic acid, 24.26 mg; folic acid, 1.1 mg; B_6, 3.3 mg; thiamin, 2.2 mg; Zn from Zn sulfate, 276 mg; Mn from Mn sulfate, 275 mg; Fe from Fe sulfate, 111 mg; Se from Na selenite, 0.666 mg; Cu from Cu sulfate, 17 mg.

[2]Elanco Animal Health, Indianapolis, IN.

[3]Novus International Inc., St. Louis, MO.

[4]Equimolar levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) were added (4 levels each) at the expense of soybean meal. Alimet (Novus International Inc.) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

[5]Apparent ileal digestible amino acid values are given in parentheses.

[6]Analyzed values.

F) HMTBA = QUAD; DLM = EXP:

$$\text{BWGc} = \text{A2} \times \text{Hin} + \text{A3} \times (\text{Hin} \times \text{Hin}) + \text{B4} \times [1 - \text{EXP}(-\text{B5} \times \text{DLin})]$$

G) HMTBA = EXP; DLM = LIN:

$$\text{BWGc} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B1} \times \text{Dlin}$$

H) HMTBA = EXP; DLM = QUAD:

$$\text{BWGc} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B2} \times \text{DLin} + \text{B3} \times (\text{Dlin} \times \text{DLin})$$

I) HMTBA = EXP; DLM = EXP:

$$\text{BWGc} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B4} \times [1 - \text{EXP}(-\text{B5} \times \text{DLin})]$$

where A1 and B1 = parameter estimates for the LIN term for birds fed HMTBA or DLM, respectively; A2 and B2 = parameter estimates for the LIN term of the QUAD equation for birds fed HMTBA or DLM, respectively; A3 and B3 = parameter estimates for the QUAD term of the QUAD equation for birds fed HMTBA or DLM, respectively; A4 and B4 = asymptote for HMTBA or DLM, respectively; A5 and B5 = steepness coefficient for HMTBA or DLM, respectively; Hin = MIOC in birds fed HMTBA; DLin = MIOC in birds fed DLM.

A measurement that allows the comparison of goodness of fit across equations (from A to I) was obtained by calculating Schwarz's Bayesian information criteria index (**BIC**; Schwarz, 1978) using the NLMIXED procedure of SAS (SAS Institute, 2003). The equations with the best goodness of fit (lowest BIC) were used to calculate the growth responses of birds fed HMTBA or DLM at graded levels. This methodology ensured an unbiased model selection process, and the gain responses were calculated for HMTBA and DLM separately. The ESTIMATE statements were used to test the significance ($P < 0.05$) of the parameter estimates of the selected models and the differences among the predicted responses to the 2 Met sources at a given MIOC as described by Kratzer and Littell (2006). Standard errors of the estimates are reported in parentheses.

To determine the most consistent response curve of HMTBA and DLM across the 4 trials, the individual pen cumulative gain response over control (BWGc) from each trial was combined into 1 analysis using the MIXED procedure of SAS Institute (2003) in which trial was defined

Table 5. Performance response to 2-hydroxy-4(methylthio) butanoic acid (HMTBA) and DL-Met (DLM) supplementation in chickens fed from 0 to 48 d (trial 1)[1]

Source	Level,[2] % (S/G/F)	0 to 17 d			0 to 34 d			0 to 48 d		
		BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)
Control	0.0/0.0/0.0	0.513[c]	1.512[ab]	0.775[b]	1.693[bc]	1.780[bc]	3.013[b]	2.812[bc]	1.970[ab]	5.787
HMTBA	0.07/0.05/0.05	0.592[a]	1.448[bc]	0.858[a]	1.655[c]	1.935[ab]	3.195[a]	2.778[c]	2.032[a]	5.642
HMTBA	0.14/0.10/0.10	0.593[a]	1.397[cd]	0.828[a]	1.843[a]	1.805[abc]	3.322[a]	2.935[abc]	1.915[ab]	5.617
HMTBA	0.21/0.15/0.15	0.603[a]	1.378[d]	0.833[a]	1.828[ab]	1.745[c]	3.187[a]	3.045[a]	1.878[b]	5.727
DLM	0.07/0.05/0.05	0.552[b]	1.470[b]	0.810[ab]	1.655[c]	1.967[a]	3.210[a]	2.847[bc]	2.053[a]	5.803
DLM	0.14/0.10/0.10	0.540[bc]	1.548[a]	0.827[a]	1.758[abc]	1.828[abc]	3.207[a]	2.887[ab]	1.990[ab]	5.737
DLM	0.21/0.15/0.15	0.573[ab]	1.475[b]	0.843[a]	1.852[a]	1.743[c]	3.227[a]	2.983[ab]	1.920[ab]	5.728
SE		0.010	0.020	0.030	0.050	0.050	0.060	0.060	0.050	0.010
P-value										
Overall treatment		0.001	0.001	0.050	0.001	0.001	0.050	0.001	0.001	0.215
Source		0.001	0.001	0.325	0.625	0.725	0.688	0.760	0.268	0.334
Level		0.199	0.120	0.801	0.003	0.007	0.529	0.012	0.026	0.890
Source × level		0.630	0.020	0.185	0.542	0.961	0.399	0.525	0.864	0.781

[a–d]Values within a column with different superscripts differ significantly ($P < 0.05$).

[1]FCR = feed-to-gain ratio corrected for mortality; FI = feed intake per bird.

[2]Levels of Met supplementation during the starter (S), grower (G), and finisher (F) periods. Equimolar levels of HMTBA or DLM were added to the control diet. Alimet (Novus International Inc., St. Louis, MO) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

as random effect to account for the variation among trials not accounted for by other factors considered in the model and to reduce the bias associated with the parameter estimates (Littell et al., 1996). The LIN and QUAD terms of the regression were considered as fixed effects and were conducted independently to HMTBA and DLM groups using BWGc and MIOC as dependent and independent variables, respectively. The EXP terms were not included in the analysis, because they are not available in the MIXED procedure of SAS Institute (2003). All possible LIN and QUAD combinations were tested as described earlier (equations A to D), and the equations with the lowest BIC or best goodness of fit were used to calculate the growth responses of birds fed HMTBA or DLM at graded levels. A random shift on intercept and the LIN and QUAD terms of the regression was modeled using trial as a subject and a simple variance-covariance matrix for the random parameters as described by St-Pierre (2001). The ESTIMATE statements were used to test the significance ($P < 0.05$) of the differences among the predicted responses to the 2 Met sources at a given MIOC, as described by Kratzer and Littell (2006). Standard errors of the estimates are reported in parentheses.

RESULTS

Performance Parameters

Across all 4 trials and feeding phases, addition of HMTBA and DLM elicited a positive response in BW and FCR ($P < 0.05$), indicating that the control diets were deficient in TSAA. The magnitude and shape of the gain

Table 6. Performance response to 2-hydroxy-4(methylthio) butanoic acid (HMTBA) and DL-Met (DLM) supplementation in chickens fed from 0 to 48 d (trial 2)[1]

Source	Level,[2] % (S/G/F)	0 to 17 d			0 to 35 d			0 to 48 d		
		BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)
Control	0.0/0.0/0.0	0.323[b]	1.313[a]	0.372[c]	1.288[b]	1.828[a]	2.280[b]	2.376[b]	1.838[a]	4.277[b]
HMTBA	0.06/0.06/0.047	0.410[a]	1.262[ab]	0.468[b]	1.593[a]	1.652[b]	2.570[a]	2.740[a]	1.742[ab]	4.705[a]
HMTBA	0.12/0.12/0.093	0.438[a]	1.285[ab]	0.513[a]	1.684[a]	1.600[bc]	2.636[a]	2.752[a]	1.732[ab]	4.682[a]
HMTBA	0.18/0.18/0.14	0.430[a]	1.202[b]	0.468[b]	1.703[a]	1.570[c]	2.613[a]	2.893[a]	1.670[b]	4.762[a]
DLM	0.06/0.06/0.047	0.427[a]	1.265[ab]	0.492[ab]	1.678[a]	1.613[bc]	2.645[a]	2.848[a]	1.718[ab]	4.827[a]
DLM	0.12/0.12/0.093	0.432[a]	1.230[ab]	0.482[ab]	1.658[a]	1.590[bc]	2.578[a]	2.795[a]	1.728[ab]	4.762[a]
DLM	0.18/0.18/0.14	0.425[a]	1.270[ab]	0.493[ab]	1.642[a]	1.627[bc]	2.607[a]	2.785[a]	1.738[ab]	4.763[a]
SE		0.009	0.030	0.012	0.030	0.020	0.050	0.060	0.050	0.110
P-value										
Overall treatment		0.001	0.001	0.001	0.001	0.001	0.050	0.01	0.001	0.050
Source		0.810	0.822	0.580	0.977	0.880	0.930	0.779	0.723	0.500
Level		0.168	0.630	0.290	0.470	0.220	0.998	0.565	0.816	0.920
Source × level		0.329	0.139	0.040	0.090	0.130	0.468	0.211	0.589	0.880

[a–c]Values within a column with different superscripts differ significantly ($P < 0.05$).

[1]FCR = feed-to-gain ratio corrected for mortality; FI = feed intake per bird.

[2]Levels of Met supplementation during the starter (S), grower (G), and finisher (F) periods. Equimolar levels of HMTBA or DLM were added to the control diet. Alimet (Novus International Inc., St. Louis, MO) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

Table 7. Performance response to 2-hydroxy-4(methylthio) butanoic acid (HMTBA) and DL-Met (DLM) supplementation in chickens fed from 0 to 43 d (trial 3)[1]

Source	Level,[2] % (S/G/F)	0 to 16 d			0 to 30 d			0 to 43 d		
		BS (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)
Control	0.0/ 0.0/0.0	0.362[c]	1.378[a]	0.448[b]	1.298[c]	1.483[a]	1.873[b]	2.376[c]	1.668[a]	3.908[b]
HMTBA	0.06/0.06/0.047	0.407[b]	1.360[ab]	0.503[a]	1.488[b]	1.353[b]	1.968[ab]	2.587[b]	1.582[b]	4.037[a]
HMTBA	0.12/0.12/0.093	0.425[a]	1.325[b]	0.515[a]	1.540[a]	1.317[c]	1.983[ab]	2.635[ab]	1.555[bc]	4.042[a]
HMTBA	0.18/0.18/0.14	0.417[ab]	1.310[b]	0.498[a]	1.535[a]	1.327[bc]	1.993[a]	2.695[ab]	1.545[c]	4.113[a]
DLM	0.06/0.06/0.047	0.423[ab]	1.315[b]	0.515[a]	1.522[ab]	1.355[b]	2.013[a]	2.615[ab]	1.578[b]	4.068[a]
DLM	0.12/0.12/0.093	0.418[ab]	1.328[b]	0.507[a]	1.538[a]	1.310[c]	1.970[ab]	2.668[ab]	1.544[c]	4.064[a]
DLM	0.18/0.18/0.14	0.425[a]	1.330[b]	0.518[a]	1.547[a]	1.315[c]	1.985[ab]	2.602[b]	1.555[bc]	3.988[ab]
SE		0.006	0.008	0.007	0.012	0.011	0.015	0.030	0.012	0.04
P-value										
Overall treatment		0.005	0.010	0.050	0.001	0.001	0.001	0.001	0.010	0.050
Source		0.220	0.290	0.220	0.310	0.490	0.960	0.660	0.829	0.501
Level		0.490	0.110	0.940	0.070	0.001	0.590	0.170	0.001	0.999
Source × level		0.160	0.001	0.170	0.590	0.790	0.120	0.060	0.438	0.132

[a–c]Values within a column with different superscripts differ significantly ($P < 0.05$).

[1]FCR = feed-to-gain ratio corrected for mortality; FI = feed intake per bird.

[2]Levels of Met supplementation during the starter (S), grower (G), and finisher (F) periods. Equimolar levels of HMTBA or DLM were added to the control diet. Alimet (Novus International Inc., St. Louis, MO) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

response to additional Met varied with diet and age (Tables 5 to 8). The main effect of level of supplementation significantly improved some performance parameters at specific feeding phases in all trials except for trial 2 ($P < 0.05$). The effect of Met source was not significant at any age for any trial, with the exception of BW and FCR for 17-d-old birds fed sorghum-based diets (trial 1; Table 5). In those birds, feeding HMTBA significantly improved performance over feeding DLM ($P < 0.001$).

A significant level by source interaction was observed at several ages and trials. A 2-way interaction ($P < 0.05$) was observed for FCR during the starter period in birds fed sorghum- and corn-based diets (Tables 5 and 7, respectively), such that FCR was optimized when feeding HMTBA at the highest level of supplementation. A significant 2-way interaction was also observed ($P < 0.05$) for FI during the starter period in birds fed wheat- or pea-based diets (trial 2) for which HMTBA optimized this parameter (Table 6). In all other accounts, the interaction between Met source and level of supplementation was not significant.

Relative Growth-Response Curves

Regression analysis was used to evaluate and compare the shape of the dose-response curve of birds fed HMTBA or DLM. The equations that resulted in the best goodness of fit for the gain response of birds to MIOC varied depending on the trial (Table 9). For sorghum-based diets in trial 1, conducting an inverse QUAD for HMTBA BWGc and a LIN equation for DLM BWGc resulted in best goodness of fit (Figure 1). When the predicted BWGc

Table 8. Performance response to 2-hydroxy-4(methylthio) butanoic acid (HMTBA) and DL-Met (DLM) supplementation in chickens fed from 0 to 49 d (trial 4)[1]

Source	Level,[2] % (S/G/F)	0 to 16 d			0 to 35 d			0 to 49 d		
		BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)	BW (kg)	FCR	FI (kg)
Control	0.0/0.0/0.0	0.530[b]	1.302[a]	0.634[b]	1.848[c]	1.449[a]	2.616	3.300[b]	1.833[a]	5.973
HMTBA	0.07/0.07/0.04	0.558[a]	1.260[b]	0.652[ab]	1.900[bc]	1.418[ab]	2.632	3.314[ab]	1.768[b]	5.788
HMTBA	0.14/0.14/0.08	0.567[a]	1.248[b]	0.657[ab]	1.990[a]	1.363[c]	2.653	3.377[ab]	1.752[b]	5.843
HMTBA	0.20/0.20/0.13	0.557[a]	1.268[ab]	0.655[ab]	1.978[a]	1.392[bc]	2.690	3.463[a]	1.772[ab]	6.068
DLM	0.07/0.07/0.04	0.560[a]	1.237[b]	0.642[ab]	1.952[ab]	1.383[bc]	2.640	3.352[ab]	1.793[b]	5.932
DLM	0.14/0.14/0.08	0.570[a]	1.253[b]	0.662[a]	1.983[a]	1.360[c]	2.638	3.375[ab]	1.758[b]	5.868
DLM	0.20/0.20/0.13	0.550[a]	1.266[ab]	0.644[b]	1.960[ab]	1.385[bc]	2.655	3.376[ab]	1.750[b]	5.836
SE		0.008	0.008	0.007	0.017	0.014	0.014	0.03	0.012	0.038
P-value										
Overall treatment		0.001	0.001	0.001	0.001	0.001	0.230	0.001	0.001	0.312
Source		0.947	0.453	0.444	0.640	0.200	0.539	0.699	0.810	0.790
Level		0.192	0.206	0.324	0.035	0.023	0.426	0.298	0.320	0.550
Source × level		0.805	0.425	0.571	0.293	0.470	0.750	0.511	0.420	0.180

[a–c]Values within a column with different superscripts differ significantly ($P < 0.05$).

[1]FCR = feed-to-gain ratio corrected for mortality; FI = feed intake per bird.

[2]Levels of Met supplementation during the starter (S), grower (G), and finisher (F) periods. Equimolar levels of HMTBA or DLM were added to the control diet. Alimet (Novus International Inc., St. Louis, MO) feed supplement, with 88% Met activity, was used as the source of HMTBA, and DL-Met, with 99% Met activity, was also used.

Table 9. Model selection based on goodness of fit as indicated by Schwarz's Bayesian information criteria (BIC)[1]

Regressions		BIC			
					Trial 4
		Trial 1	Trial 2	Trial 3	Corn and meat and
HMTBA	DLM	Sorghum	Wheat	Corn	bone meal
LIN	LIN	−56.1	−19.8	−87.7	−119.5
LIN	QUAD	−52.4	−25.9	−108.3	−122.3
LIN	EXP	−52.4	−29.0	−104.3	−124.4
QUAD	LIN	−57.8	−18.5	−89.4	−116.6
QUAD	QUAD	−54.1	−25.3	−114.3	−119.6
QUAD	EXP	−54.1	NC	−109.4	−121.8
EXP	LIN	NC	−18.9	−89.8	NC
EXP	QUAD	NC	−25.7	−1,15.1	NC
EXP	EXP	NC	−29.1	−1,10.1	NC

[1]Linear (LIN), quadratic (QUAD), and exponential (EXP) equations were fitted to the gain response of birds to dietary intake of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM). Lower BIC values represent better goodness of fit. Taking trial 1 as an example, the lowest BIC value is −57.8, which indicates that the best goodness of fit for that data set is QUAD and LIN for HMTBA and DLM, respectively. NC = no convergence.

responses to HMTBA vs. DLM were compared, no differences were found at any point within the MIOC range.

In trial 2, conducting EXP equations upon both Met sources gain response resulted in improved goodness of fit in wheat- or pea-based diets (Figure 2). None of the estimated coefficients were different among the equations, but the predicted BWGc values were higher for DLM at MIOC less than 3.0 g ($P < 0.05$).

In trial 3, the best goodness of fit was obtained by imposing EXP and QUAD equations to the gain response of birds fed HMTBA or DLM in corn-based diets, respectively (Figure 3). The predicted BWGc values for HMTBA were higher than those for DLM at MIOC greater than 5.2 g, reaching statistical significance at levels greater than 6.4 g ($P < 0.05$).

In trial 4, birds fed corn-based diets with meat and bone meal, better goodness of fit was achieved by conducting a LIN and an EXP equation upon BWGc to HMTBA and DLM, respectively (Figure 4). The predicted BWGc to feeding HMTBA was significantly greater than for DLM at MIOC greater than 6.0 g ($P < 0.05$). Conversely, predicted BWGc values for DLM were greater than those for HMTBA when fed at suboptimal levels (2.7 g; $P < 0.05$). The maximum growth response was significantly greater when birds were fed HMTBA vs. DLM (0.189 ± 0.022 vs. 0.108 ± 0.016 kg, respectively; $P < 0.05$).

When the individual pen data from the 4 trials were combined in 1 analysis, the best goodness of fit was obtained by conducting a LIN and a QUAD equation upon BWGc response to HTMBA and DLM, respectively (Table 10 and Figure 5). The predicted BWGc to HMTBA vs. DLM was not different at any point within the data range.

Table 10. Model selection based on goodness of fit as indicated by Schwarz's Bayesian information criteria (BIC) of the 4 trials[1]

Regressions		
HMTBA	DLM	BIC
LIN	LIN	−237.9
LIN	QUAD	−250.3
QUAD	LIN	−235.5
QUAD	QUAD	−249.3

[1]Linear (LIN) and quadratic (QUAD) equations were fitted to the gain response of birds to dietary intake levels of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM). Lower BIC values represent better goodness of fit. The lowest BIC value is −250.3, which indicates that the best goodness of fit for that data set is LIN and QUAD for HMTBA and DLM, respectively.

DISCUSSION

The improvements in performance observed with increasing levels of supplemental Met indicated that diets were deficient in TSAA (Tables 5 to 8). In most cases, improvements in BW gain were partially associated with improvements in FI. In the current report, the shape of the dose response to increasing MIOC for HMTBA and DLM of each trial was determined by imposing LIN, QUAD, and EXP equations to BWGc and selecting the equation with the best goodness of fit using the BIC index.

The full dose response to HMTBA and DLM over a wide range of supplementation levels follows a QUAD function (Vázquez-Añón et al., 2006). However, in the current trials, the best goodness of fit was obtained with LIN, QUAD, or EXP, depending on trial and Met source (Tables 9 and 10). The use of these equations may be appropriate, depending on the area of the dose response covered within the tested data range. A LIN and an inverse QUAD response may imply when the maximum response was not reached within the data range used; a QUAD indicates that data were within the range where a positive response to MIOC is achieved, followed by a peak and a subsequent decline. An EXP response may indicate that a decline in performance was not observed at the highest MIOC levels. Therefore, the differences in the shape of the growth response to Met supplementation among trials may have been related to the area of the dose response relative to requirements.

The shape of the growth response of birds to Met supplementation was also dependent on the Met source used, such that in 3 out of 4 cases, the dose response to HMTBA differed from that of DLM (Figures 1, 3, and 4). This was confirmed when the 4 trials were combined in 1 analysis (Figure 5). In 2 trials, feeding HMTBA at suboptimal levels resulted in significantly lower BWGc in contrast to DLM (Figures 2 and 4; $P < 0.05$), but in 2 accounts, feeding HMTBA at levels closer to commercial practice yielded significantly higher BWGc relative to DLM (Figures 3 and 4; $P < 0.05$). Invariably, the maximum growth response was achieved by feeding HMTBA instead of DLM. When the data from the 4 trials were combined into 1 analysis, the overall dose response to HMTBA followed a LIN response, whereas DLM followed a QUAD response. Across the 4 trials, the HMTBA birds had not achieved the maximum growth within the range of HMTBA fed, resulting in LIN and not QUAD as the best-fit model.

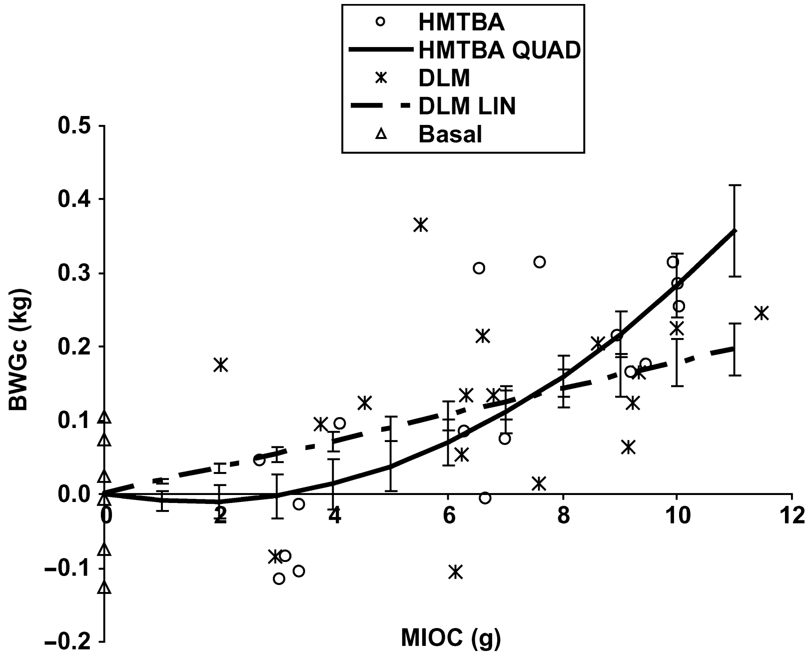

Figure 1. Dose response of broilers fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in sorghum-based diets (TRIAL1). Open circles represent pen data from birds fed HMTBA; stars represent pen data from birds fed DLM; and triangles represent pen data from birds fed a control diet depleted of Met supplementation. Body weight gain over the control (BWGc) and the Met intake over the control (MIOC) were used as independent and dependent variables, respectively. A better goodness of fit was obtained by imposing inverse quadratic (QUAD) and linear (LIN) regressions to birds fed HMTBA and DLM, respectively, following the equation BWGc = –0.0131 ± 0.0149 × Hin + 0.00415 × 0.00173 × Hin^2 + 0.0179 ± 0.0033 × DLin where Hin and DLin represent MIOC for birds fed HMTBA and DLM, respectively (±SE). Error bars represent the SE of predicted BWGc values along various MIOC levels.

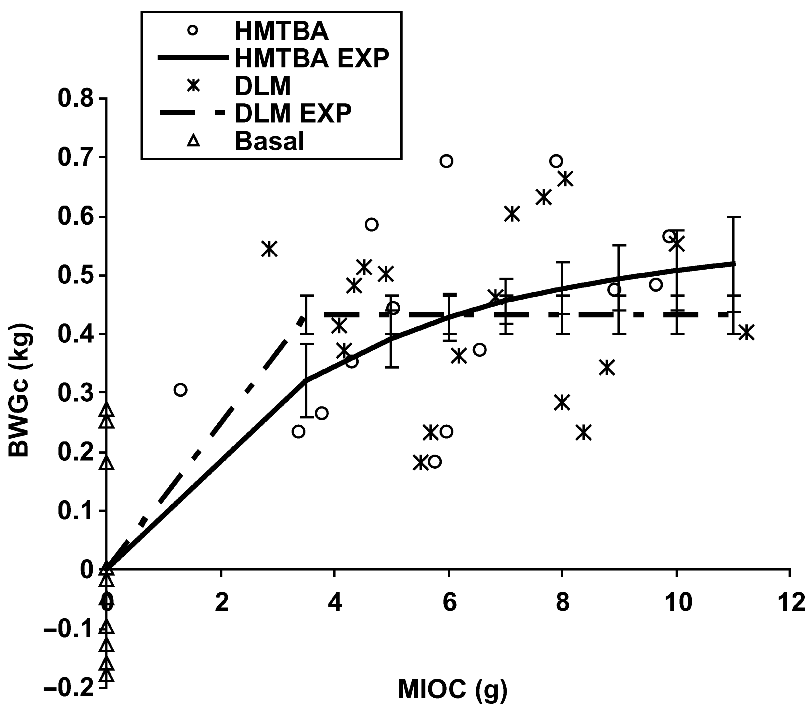

Figure 2. Dose response of broilers fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in wheat- or pea-based diets (trial 2). Open circles represent pen data from birds fed HMTBA; stars represent pen data from birds fed DLM; and triangles represent pen data from birds fed a control diet depleted of Met supplementation. Error bars represent the SE of predicted values of BW gain over the control (BWGc) along various levels of Met intake over control (MIOC). Body weight gain over the control and MIOC were used as independent and dependent variables, respectively. A better goodness of fit was obtained by imposing exponential regressions (EXP) to birds fed both HMTBA (solid line) and DLM (dotted line), which is expressed by the equation BWGc = 0.555 ± 0.158 × [1 – EXP(–0.246 ± 0.176 ± Hin)] + 0.433 ± 0.034 × [1 – Exp(–5.93 ± 1133.86 ± DLin)] where Hin and DLin represent MIOC for birds fed HMTBA and DLM, respectively (±SE). Feeding DLM at MIOC <3 g resulted in significant greater BWGc than feeding HMTBA ($P > 0.05$).

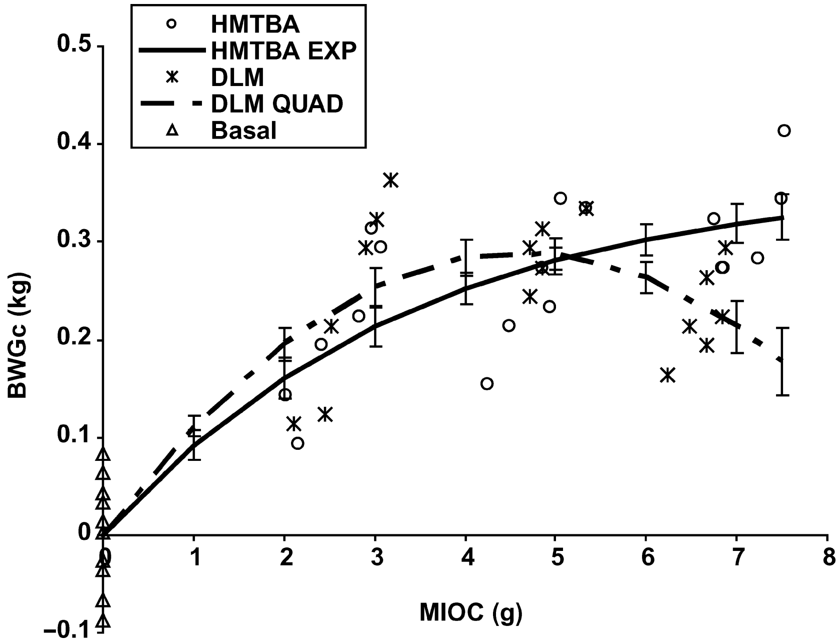

Figure 3. Dose response of broilers fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in corn-based diets (trial 3). Open circles represent pen data from birds fed HMTBA; stars represent pen data from birds fed DLM; and triangles represent pen data from birds fed a control diet depleted of Met supplementation. Error bars represent the SE of predicted values of BW gain over the control (BWGc) along various levels of Met intake over the control (MIOC). Body weight gain over the control and MIOC were used as independent and dependent variables, respectively. A better goodness of fit was obtained by imposing an exponential (EXP) and a quadratic (QUAD) regression to birds fed both HMTBA and DLM, respectively, which is expressed by the equation BWGc = 0.367 ± 0.060 × [1 − EXP(−0.289 ± 0.108 × Hin)] + 0.125 ± 0.013 × DLin −0.0135 ± 0.00212 × Hin^2 where Hin and DLin represent MIOC for birds fed HMTBA and DLM, respectively (±SE). Significantly greater BWGc was obtained when feeding HMTBA instead of DLM at MIOC >6.5 g ($P < 0.05$).

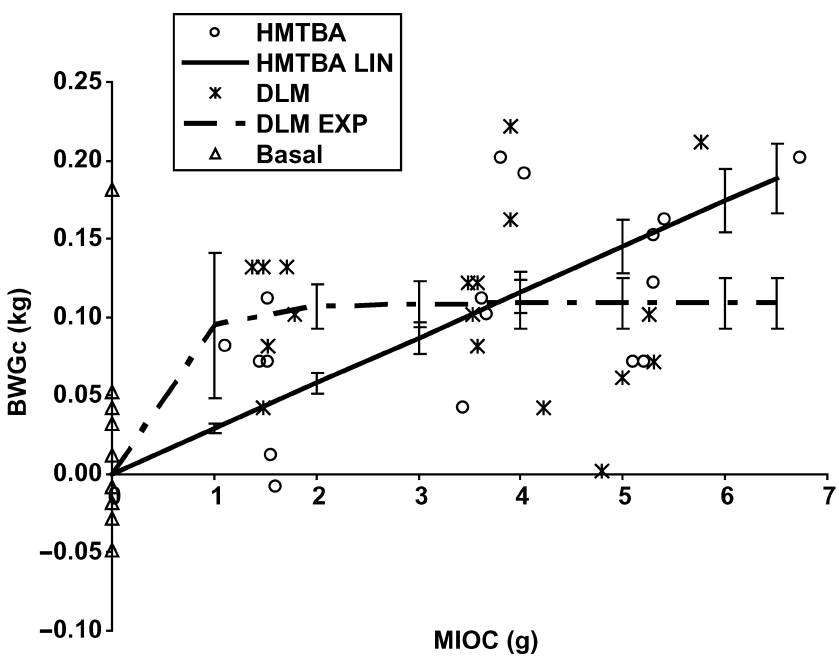

Figure 4. Dose response of broilers fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in corn and meat meal-based diets (trial 4). Open circles represent pen data from birds fed HMTBA; stars represent pen data from birds fed DLM; and triangles represent pen data from birds fed control diet depleted of Met supplementation. Error bars represent the SE of predicted values of BW gain over the control (BWGc) along various levels of Met intake over the control (MIOC). Body weight gain over the control and MIOC were used as independent and dependent variables, respectively. A better goodness of fit was obtained by imposing linear (LIN) and exponential (EXP) regressions to birds fed HMTBA or DLM, respectively. This is expressed by the equation BWGc = 0.029 ± 0.034 × Hin + 0.108 ± 0.016 × [1 − EXP(−2.060 ± 3.794 × DLin)] where Hin and DLin represent MIOC for birds fed HMTBA and DLM, respectively (±SE). The BWGc of birds fed HMTBA was significantly higher than those fed DLM at MIOC >6 g ($P < 0.05$).

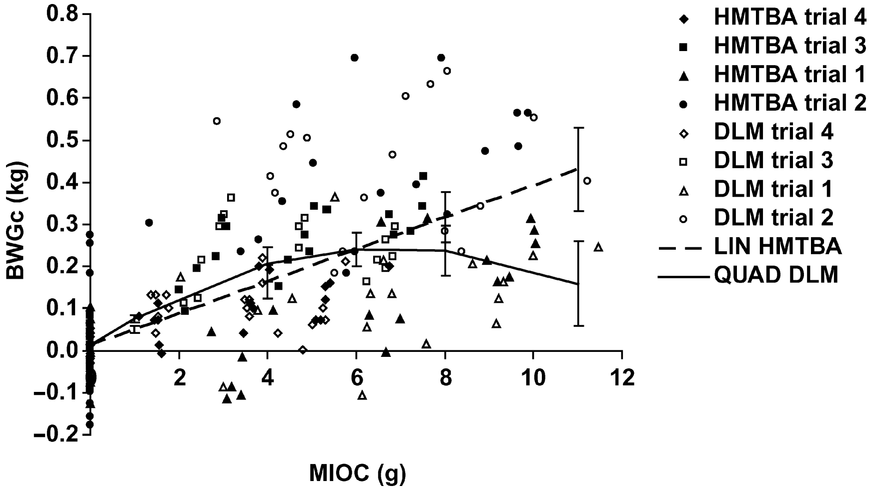

Figure 5. Dose response of broilers fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) across the 4 trials. Solid triangles, circles, squares, and diamonds represent HMTBA pen data from trials 1, 2, 3, and 4, respectively; open triangles, circles, squares, and diamonds represent DLM pen data from trials 1, 2, 3, and 4, respectively. Body weight gain over the control (BWGc) and the Met intake over the control (MIOC) were used as independent and dependent variables, respectively. Best goodness of fit was obtained by imposing linear (LIN) regression to the gain response of birds fed HMTBA and quadratic (QUAD) regression to birds fed DLM. This is expressed by the equation BWGc = 3.797 ± 0.89 × Hin + 6.786 ± 1.322 × DLin − 49.59 ± 13.055 × (Dlin × DLin) where Hin and DLin represent MIOC for birds fed HMTBA and DLM, respectively (±SE).

For the DLM birds, the maximum growth was already achieved and started to decline within the range of DLM fed; therefore, a QUAD was the best-fit model.

These data provide further evidence that feeding HMTBA and DLM results in different dose-responses in which HMTBA may outperform DLM at levels of supplementation near the maximum gain-response, but DLM may outperform HMTBA at suboptimal levels. Higher peak response to HMTBA than to DLM as well as more rapid decline response of DLM was reported in a recent compilation of published results when separate equations to the growth response of the 2 Met sources were imposed (Vázquez-Añón et al., 2006).

The fact that these Met sources show a differential dose response indicates that the relative response to HMTBA and DLM is dose-dependent and that values of relative bioefficacy obtained at very deficient levels of TSAA are not indicators of Met efficacy at the maximum gain response used in commercial practice. Consequently, conducting an EXP curve that assumes a common maximum gain response to assess the bioefficacy of HTMBA vs. DLM as a source of Met, as described by Littell et al. (1997) and Jansman et al. (2003), would not be adequate. Thomas et al. (1984), Schutte and de Jong (1996), and Lemme et al. (2002) reported greater maximum gain response for HMTBA when fed at levels near maximum gain response and higher gain response with DLM when fed at low levels of supplementation. However, the method used to determine the relative bioefficacy of HMTBA vs. DLM in these studies assumed HMTBA had the same form of dose response as DLM and approached a common plateau. As shown in the 4 trials, the gain dose response to either source of Met does not always follow an EXP curve, and each source approaches a different maximum gain response. Therefore, the dose response for each source of Met should be defined independently using an appropriate unbiased test to determine the goodness of fit of the model.

The differences in the dose response of birds to HMTBA and DLM may be partially attributed to the differences in chemistry, mechanism and site of absorption (Knight and Dibner, 1984), transport in the body (Lobley et al., 2006; Wester et al., 2006), and metabolism by the tissues (Dibner, 2003) of the 2 Met sources. The differences in how the 2 molecules are absorbed and metabolized by the tissues are apparent when measuring plasma-free Met concentrations (Lobley et al., 2006; Wester et al., 2006). A higher concentration of plasma-free Met at and above maximum gain response in birds fed DLM has been associated with slower metabolism of the D-isomer (Elkin et al., 1988) and lower feed consumption and gain when compared with HMTBA (Vázquez-Añón et al., 2003; González-Esquerra et al., 2004) and might explain the lower DLM response in FI and BW gain.

It is concluded that HMTBA and DLM elicit different dose responses when fed to broilers; HMTBA can outperform DLM at TSAA levels near the maximun gain response, whereas DLM-fed broilers can result in greater BW gain than HMTBA at low-TSAA levels. Relevant comparisons between HMTBA and DLM are equimolar, at levels of commercial use, with commercial-type diets and under conditions as close as possible to practice.

REFERENCES

Association of Official Analytical Chemists. 1999. Pages 59–60 in Official Methods of Analysis. 6th ed. Vol. II. AOAC Int., Gaithersburg, MD.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485–494.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult. Sci. 73:1441–1447.

Dibner, J. J. 2003. Review of the metabolism of 2-hydroxy-4-(methylthio) butanoic acid. World's Poult. Sci. J. 59:99–109.

Elkin, R. G., M. L. Lyons, and J. C. Rogler. 1988. Comparative utilization of D- and L-methionine by the White Pekin duckling (*Anas platyrhynchos*). Comp. Biochem. Physiol. B. 91:325–329.

González-Esquerra, R., M. Vázquez-Añón, T. Hampton, T. W. York, S. D. Peak, C. W. Wuelling, and C. D. Knight. 2004. Comparison of statistical models to calculate the relative bioefficacy of 2-hydroxy-4(methylthio) butanoic acid (HMB) and DL-methionine (DLM) for turkeys. Poult. Sci. 83(Suppl. 1):32. (Abstr.)

Jansman, A. J. M., C. A. Kan, and J. Wiebenga. 2003. Comparison of the biological efficacy of DL-methionine and hydroxyl-4-methylthiobutanoic acid (HMB) in pigs and poultry. Report no 2209. ID-Lelystad. Anim. Sci. Group, Wageninger Univ. Res. Cent., The Netherlands.

Knight, C. D., and J. J. Dibner. 1984. Comparative absorption of 2-hydroxy-4-methylthio butanoic acid and L-methionine in the broiler chick. J. Nutr. 114:2179–2186.

Kratzer, D. D., and R. C. Littell. 2006. Appropriate statistical methods to compare dose responses of methionine sources. Poult. Sci. 85:947–954.

Lemme, A., D. Hoehler, J. J. Brennan, and R. F. Mannion. 2002. Relative effectiveness of methionine hydroxyl analog compared to DL-methionine in broiler chickens. Poult. Sci. 81:838–845.

Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672–2683.

Littell, R. C., G. A. Milligan, W. W. Stroup, and E. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Inst. Inc., Cary, NC.

Lobley, G. E., T. J. Wester, A. G. Calder, D. S. Parker, J. J. Dibner, and M. Vázquez-Añón. 2006. Absorption of 2-hydroxy-4-methylthiobutyrate and conversion to methionine in lambs. J. Dairy Sci. 89:1072–1080.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Sci., Washington, DC.

Ontiveros, R. R., W. D. Shermer, and R. A. Berner. 1987. An HPLC method for determination of 2-hydroxy-4-(methylthio) butanoic acid (HMB) in supplemented animal feeds. J. Agric. Food Chem. 35:692–694.

SAS Institute. 2003. SAS User's Guide: Statistics. Version 9.0. SAS Institute Inc., Cary, NC.

Schutte, J. B., and J. de Jong. 1996. Biological efficacy of DL-methionine hydroxy analog-free acid compared to DL-methionine in broiler chicks as determined by performance and breast meat yield. Agribiol. Res. 49:74–82.

Schwarz, G. 1978. Estimating the dimension of a model. Ann. Stat. 6:461–464.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741–755.

Thomas, O. P., E. H. Bossard, S. D. Crissey, and K. P. Engler. 1984. Further studies on the evaluation of DL-methionine and related compounds. Pages 41–45 in Proc. MD Nutr. Conf. Feed Manuf., Baltimore.

Vázquez-Añón, M., D. Kratzer, R. González-Esquerra, I. G. Yi, and C. D. Knight. 2006. A multiple regression model approach to contrast the performance of 2-hydroxy-4-methylthio butanoic acid and DL-methionine supplementation tested in broiler trials that are reported in the literature. Poult. Sci. 85:693–705.

Vázquez-Añón, M., M. Wehmeyer, C. W. Wuelling, T. Hampton, C. D. Knight, and J. J. Dibner, 2003. Differential response to 2-hydroxy-4-methylthio-butanoic acid and DL-methionine above requirement on broilers and pig performance and iron metabolism. Pages 725–729 in Progress in Research on Energy and Protein Metabolism. EAAP publication no 109. Rostock-Warnemunde, Germany.

Yi, G. F., A. M. Gaines, B. W. Ratliff, P. Srichana, G. L. Allee, K. R. Perryman, and C. D. Knight. 2005. Estimation of the ideal ratio of true ileal digestible sulfur amino acids: Lysine in 8- to 26-kg nursery pigs. J. Anim. Sci. 83:2527–2534.

Wester, T. J., M. Vázquez-Añón, J. Dibner, D. S. Parker, A. G. Calder, and G. E. Lobley. 2006. Hepatic metabolism of 2-hydroxy-4-methylthiobutyrate in growing lambs. J. Dairy Sci. 89:1062–1071.