Evidence of a Different Dose Response in Turkeys When Fed 2-Hydroxy-4(Methylthio) Butanoic Acid Versus DL-Methionine

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ABSTRACT In broilers, 2-hydroxy-4(methylthio) butanoic acid (HMTBA) can elicit a different dose response relative to DL-Met (DLM) such that birds could have lower gain responses at deficient TSAA concentrations but greater gain responses at maximum response concentrations. Two experiments tested if the 2 Met sources have a different dose response in 1-d-old turkeys using a 2 × 4 factorial plus a control design with 8 replicates of 12 toms per treatment. 2-Hydroxy-4(methylthio) butanoic acid and DLM were supplemented at equimolar concentrations of 0.05, 0.10, 0.15, and 0.20% or 0.04, 0.08, 0.16, and 0.32% for experiments 1 and 2, respectively, in commercial-type TSAA-deficient (0.99 to 1.02%) diets for 21 d. No differences in any performance parameter tested were found between HMTBA and DLM in either trial by ANOVA. Linear (LIN), quadratic (QUAD), and exponential regressions were fitted to the gain response of birds fed HMTBA or DLM. Equations with better goodness of fit as determined by Schwarz's Bayesian information criteria index were used for further calculations of predicted differences between HMTBA and DLM. In both trials, the shape of the dose response differed according to the Met source used, and best-fit equations were obtained when using Met intake over control rather than dietary Met concentration as the dependent variable. In experiment 1, the best-fit equations were an inverse QUAD for HMTBA and a LIN for DLM, and in experiment 2 with higher Met concentrations, the best-fit equations were a QUAD for DLM and a LIN for HMTBA. Feeding HMTBA at deficient TSAA resulted in lower ($P < 0.05$) gains in experiment 1 but greater gains at maximum response concentrations ($P < 0.05$) in both experiments. Plasma-free Met increased at 3 times the rate for DLM than HMTBA ($P < 0.01$) with increasing Met concentration, which may play a role in the evolution of different dose responses at the extremes of the Met dose response. These results demonstrate that Met sources elicit a differential dose response in turkeys such that feeding HMTBA at deficient TSAA concentrations can be lower than DLM and can reach a higher maximum performance than with DLM.

Key words: 2-hydroxy-4(methylthio) butanoic acid, DL-methionine, turkey

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INTRODUCTION

In poultry diets with the primary source of protein as soybean meal, Met is considered to be the first limiting amino acid, and synthetic Met is typically supplemented either as DL-Met (**DLM**) or as 2-hydroxy-4(methylthio) butanoic acid (**HMTBA**). These compounds both provide L-Met activity to avian and mammalian species; however, there are substantial differences between them with respect to chemistry, absorption, and transport to and metabolism by the tissues (Dibner, 2003; Lobley et al., 2006; Wester et al., 2006). These 2 sources of Met activity have been commercially available and used in animal production systems for over 5 decades; however, there remains controversy and confusion with respect to their relative biological efficacy (**RBE**). A recent analysis of the statistical methodology used to compare these Met sources by Kratzer and Littell (2006) concluded that the longstanding controversy was due at least in part to the misapplication of a nonlinear common plateau asymptotic regression (**NLCPAR**) method, sometimes called exponential regression analysis, by which a ratio of the slopes of the 2 lines are used to determine the RBE of the Met sources. This conclusion was based on the determination that the primary assumptions of the NLCPAR technique were not true for HMTBA and DLM when applied to a compilation of previously published broiler studies (Jansman et al., 2003), i.e., that the test compound (HMTBA) had the same form of dose response and the same predicted plateau as the standard (DLM). Rather, the 2 Met sources demonstrated different dose responses, making a single RBE or slope for the entire dose range irrelevant, because the magnitude of performance response to either Met source

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depended upon what portion of the TSAA dose range the experimental diets were formulated to compare.

Given the strong evidence that HMTBA and DLM provide in vivo Met activity differently, it is critical that the methodology to compare the 2 Met sources permits the data resulting from each source to define its own response-curve model. This type of methodology was used in a recent paper by Vázquez-Añón et al. (2006), in which dose responses of HMTBA and DLM were compared across 4 broiler experiments. Vázquez-Añón et al (2006) concluded that the 2 Met sources demonstrated different forms of dose response, such that HMTBA outperformed DLM at TSAA levels closer to maximum responsiveness, commensurate with levels typically fed in the industry, whereas DLM outperformed HMTBA at TSAA levels that were representative of the more deficient portion of the response curve.

In turkeys, the first reported use of this NLCPAR method for comparing RBE of HMTBA and DLM was by Blair (1983) and Noll et al. (1984). Blair (1983) concluded that the average RBE of HMTBA to DLM for improving BW gain (**BWG**) and feed conversion ratio (**FCR**) was 92.5% with 95% confidence interval of 65 to 125%. Noll et al. (1984) concluded that the average RBE of HMTBA to DLM was 96% with 95% confidence interval of 89 to 103%. Neither Blair (1983) or Noll et al. (1984) reported a significantly different RBE for HMTBA and DLM. Hoehler et al. (2005) concluded the efficacy of liquid Met hydroxy analogue-free acid, the commercial form of 88% HMTBA in 12% water, was 44 to 66% effective or 55 to 74% accounting for the water content. The authors also used the NLCPAR methodology; however, the Met source comparisons were not conducted on an equal *M* isosulfurous basis, i.e., the molecular activity of both products based on 100% conversion to L-Met such that the products were not fed at the same theoretical Met activity.

Given the recent conclusion of Kratzer and Littell (2006) and Vázquez-Añón et al. (2006), the purpose of the work reported herein was to critically evaluate BWG and FCR dose responses of turkey poults to HMTBA and DLM in TSAA-deficient sorghum and soybean meal and corn- and soybean meal-based diets to determine the best-fit prediction equations to describe the dose response of these 2 sources of Met activity and thereby predict the efficacy of the 2 Met sources at various points of the TSAA response curve.

MATERIALS AND METHODS

Experimental Design

Two experiments of similar design were conducted using unvaccinated 1-d-old Nicholas male turkeys kept in wire-floored battery cages equipped with individual feed troughs and nipple drinkers. Battery cages (50.8 cm deep × 68.58 cm long × 36.19 cm high) were arranged in 4 rows of 6 racks with 3 cages per rack. Initial BW ± SEM was 51.82 ± 1.74 and 61.28 ± 3.79 g per bird for experiments 1 and 2, respectively. Room temperature was kept at 32°C at 1 d of age, gradually decreased to 23°C by 18 d of age, and kept at 23°C thereafter. The lighting regimen consisted of 24L:0D at 1 d of age and 14L:10D from d 2 to 21. Eight replicates of 12 toms were randomly assigned to 9 treatments arranged in a factorial 2 × 4 plus a control design, in which the effect of feeding graded equimolar concentrations of HMTBA (molecular weight = 150) and DLM (molecular weight = 149) on performance was tested.

In experiment 1, HMTBA or DLM were added at 0.05, 0.10, 0.15, and 0.20% to a sorghum- and soybean meal-based diet (Table 1), whereas in experiment 2, supplemental concentrations were 0.04, 0.08, 0.16, and 0.32% added to a corn- and soybean meal-based diet. A common basal diet was made for each experiment, and following addition of the Met source premix, diets were pelleted and crumbled. The basal diets were formulated to be adequate in all nutrients except for TSAA, use digestible amino acid coefficients for each ingredient generated from an in vitro digestibility IDEA assay (Novus International Inc.; Schasteen and Wu, 2004) or obtained from ingredient tables (Ajinomoto Heartland LLC, Chicago, IL), and impose ideal amino acid ratios relative to Lys for chickens (Baker and Han, 1994; Baker et al., 2002). The concentrations of Met addition were verified from blind samples by the analysis of HMTBA (Ontiveros et al., 1987) by Novus International Inc. and plasma-free Met (**PFM**) using an amino acid analyzer (Beckman, Moline, IL) by the Experiment Station Chemical Laboratories of the University of Missouri-Columbia in all diets (Table 2). 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. Amino acid composition of the common basal diets was determined after acid hydrolysis, whereas TSAA was determined after performic acid oxidation and Trp content was determined after alkaline hydrolysis (AOAC, 1999). All feed samples were ground through a 0.5-mm sieve in a stainless-steel Retsch mill (Retsch Inc., Newtown, PA) 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 as per AOAC [1999; 982.30 E(a,b,c)].

At the end of experiment 2, blood samples were collected from the wing vein of 1 randomly selected bird per cage. Poults had access to feed for 2 h before taking blood samples. Ten-milliliter EDTA-coated vacuum collection tubes were used. After sample collection, tubes were immediately placed on ice and further centrifuged at 1,286 × *g* for 20 min at 24 C. Plasma was stored at –30°C before shipping. Properly identified plasma tubes were sent to the University of Missouri-Columbia for further PFM analysis of deproteinized plasma (Fekkes, 1996).

Statistical Analysis and Measurements

Body weight, BWG, feed intake (**FI**), and FCR corrected for mortality were recorded for each cage at 21 d of age.

Table 1. Experimental diets (1 to 21 d of age)

Ingredients[1] (%)	Trial 1	Trial 2
Soybean meal (49%)	58.82	56.73
Sorghum	27.72	0.00
Corn	0.00	26.91
Animal fat	7.96	7.27
Meat meal (49%)	0.00	4.97
Dicalcium phosphate	3.52	2.48
Limestone	0.76	0.50
Salt	0.47	0.44
Vitamin-mineral premix[2]	0.35	0.35
L-Lys-HCl	0.21	0.25
L-Thr	0.16	0.08
Santoquin[3]	0.02	0.02
Nutrient composition[4]		
ME[5] (kcal/kg)	2,972	3,040
CP[5] (%)	31.02	31.23
Lys[5] (%)	2.03 (1.74)	2.03 (1.72)
Met[5] (%)	0.48 (0.39)	0.50 (0.40)
Met + Cys[5] (%)	0.99 (0.79)	1.02 (0.82)
Thr[5] (%)	1.22 (1.10)	1.16 (1.04)
Ile[5] (%)	1.36 (1.24)	1.36 (1.17)
Val[5] (%)	1.57 (1.34)	1.52 (1.27)
Arg[6] (%)	2.03 (1.87)	2.06 (1.87)
Trp[6] (%)	0.47 (0.38)	0.38 (0.33)
Ca[6] (%)	1.18	1.32
Total P[6] (%)	1.15	1.19
Na[6] (%)	0.20	0.22

[1]Equimolar concentrations of 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) were added at the expense of sorghum or corn for trials 1 or 2, respectively. Supplemented concentrations of HMTBA and DLM were verified by analysis.

[2]Supplied the following 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; vitamin 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; vitamin B_6, 3.3 mg; thiamin, 2.2 mg; Zn, 276 mg; I, 5 mg; Mn, 275 mg; Fe, 111 mg; Se, 0.666 mg; and Cu, 17 mg.

[3]Novus International Inc.

[4]Calculated apparent ileal digestible amino acid values are given in parentheses.

[5]Analyzed values from the common basal diet.

[6]Calculated values from the common basal diet.

Treatment effects were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2003). The interaction between Met source and concentration of supplementation was tested excluding the basal treatment. Differences among treatment means were established using the least significant difference test obtained using the PDIFF statement of SAS. In addition, PFM values were analyzed by PROC GLM using a model with concentrations of HMTBA and DLM as linear regressions to determine rate of PFM increase for each Met source.

Linear (LIN), quadratic (QUAD), and exponential (EXP) regressions were imposed independently to HMTBA- and DLM-fed groups. The BWG over the mean BWG of the controls (BWGC) was used as the dependent variable, whereas intake of analyzed Met equivalents over the controls (**MIOC**) and analyzed supplemented concentrations of Met equivalents were used as independent variables. The intercept constant for each model was the origin. All possible LIN, QUAD, and EXP combinations were tested as detailed in the following equations:

$$\text{HMTBA} = \text{LIN; DLM} = \text{LIN: BWGC} = \text{A1} \times \text{Hin} + \text{B1} \times \text{DLin} \quad [A]$$

$$\text{HMTBA} = \text{LIN; DLM} = \text{QUAD: BWGC} = \text{A1} \times \text{Hin} + \text{B2} \times \text{DLin} + \text{B3} \times (\text{DLin} \times \text{DLin}) \quad [B]$$

$$\text{HMTBA} = \text{LIN; DLM} = \text{EXP: BWGC} = \text{A1} \times \text{Hin} + \text{B4} \times [1 - \text{EXP}(-\text{B5} \times \text{DLin})] \quad [C]$$

$$\text{HMTBA} = \text{QUAD; DLM} = \text{LIN: BWGC} = \text{A2} \times \text{Hin} + \text{A3} \times (\text{Hin} \times \text{Hin}) + \text{B1} \times \text{DLin} \quad [D]$$

$$\text{HMTBA} = \text{QUAD; DLM} = \text{QUAD: BWGC} = \text{A2} \times \text{Hin} + \text{A3} \times (\text{Hin} \times \text{Hin}) + \text{B2} \times \text{DLin} + \text{B3} \times (\text{DLin} \times \text{DLin}) \quad [E]$$

$$\text{HMTBA} = \text{QUAD; DLM} = \text{EXP: BWGC} = \text{A2} \times \text{Hin} + \text{A3} \times (\text{Hin} \times \text{Hin}) + \text{B4} \times [1 - \text{EXP}(-\text{B5} \times \text{DLin})] \quad [F]$$

$$\text{HMTBA} = \text{EXP; DLM} = \text{LIN: BWGC} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B1} \times \text{DLin} \quad [G]$$

$$\text{HMTBA} = \text{EXP; DLM} = \text{QUAD: BWGC} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B2} \times \text{DLin} + \text{B3} \times (\text{DLin} \times \text{DLin}) \quad [H]$$

$$\text{HMTBA} = \text{EXP; DLM} = \text{EXP: BWGC} = \text{A4} \times [1 - \text{EXP}(-\text{A5} \times \text{Hin})] + \text{B4} \times [1 - \text{EXP}(-\text{B5} \times \text{DLin})] \quad [I]$$

where A1 and B1 = parameter estimates for the linear term for birds fed HMTBA or DLM, respectively; A2 and B2 = parameter estimates for the linear term of the

Table 2. Chemically analyzed values for graded concentrations of Met supplementation from 2 sources

Supplemental Met concentration (%), experiment 1	Met source[1]	Analyzed supplemental Met concentration (%), experiment 1	Supplemental Met concentration (%), experiment 2	Met source[1]	Analyzed supplemental Met concentration (%), experiment 2
0.00	Basal	0.00	0.00	Basal	0.00
0.05	HMTBA	0.051	0.04	HMTBA	0.036
0.10	HMTBA	0.099	0.08	HMTBA	0.07
0.15	HMTBA	0.137	0.16	HMTBA	0.142
0.20	HMTBA	0.180	0.32	HMTBA	0.263
0.05	DLM	0.048	0.04	DLM	0.031
0.10	DLM	0.091	0.08	DLM	0.098
0.15	DLM	0.144	0.16	DLM	0.142
0.20	DLM	0.174	0.32	DLM	0.291

[1]HMTBA = DL-2-hydroxy-4(methylthio) butanoic acid; DLM = DL-Met.

Table 3. Effect of graded concentrations of Met supplementation from 2 sources on the performance of 1- to 21-d-old toms (experiment 1)

Supplemental Met concentration (%)	Met source[1]	BW (g)	Weight gain (g)	Feed intake (g)	Feed:gain (mortality corrected)	Mortality (%)
0.00	Basal	607 ± 10[c]	555 ± 10[b]	786 ± 14[b]	1.417 ± 0.015[a]	3.7 ± 2.8[a]
0.05	HMTBA	608 ± 16[bc]	557 ± 16[b]	791 ± 19[ab]	1.421 ± 0.011[a]	2.2 ± 1.4[a]
0.10	HMTBA	646 ± 9[a]	593 ± 9[a]	818 ± 10[ab]	1.381 ± 0.006[bc]	2.3 ± 1.5[a]
0.15	HMTBA	638 ± 16[abc]	587 ± 16[ab]	808 ± 19[ab]	1.378 ± 0.007[bc]	0.0 ± 0.0[a]
0.20	HMTBA	664 ± 15[a]	612 ± 15[a]	830 ± 15[a]	1.361 ± 0.011[c]	1.1 ± 1.1[a]
0.05	DLM	637 ± 3[abc]	585 ± 3[ab]	882 ± 10[ab]	1.405 ± 0.014[ab]	4.5 ± 2.5[a]
0.10	DLM	639 ± 15[abc]	587 ± 15[ab]	805 ± 15[ab]	1.372 ± 0.010[c]	2.4 ± 1.6[a]
0.15	DLM	642 ± 8[ab]	590 ± 8[ab]	803 ± 8[ab]	1.361 ± 0.004[c]	0.0 ± 0.0[a]
0.20	DLM	662 ± 12[a]	611 ± 12[a]	831 ± 12[a]	1.361 ± 0.008[c]	3.4 ± 1.7[a]
CV		5.4	5.9	5.1	2.1	214.7
Root MS error		34.4	34.5	41.2	0.029	4.69
				P-value		
Concentration		0.021	0.024	0.300	<0.001	0.1469
Source		0.496	0.483	0.757	0.118	0.2558
Concentration × source		0.495	0.535	0.460	0.7719	0.7647

[a–c]Treatment means (±individual SE) with different superscripts differ statistically ($P < 0.05$.)

[1]HMTBA = DL-2-hydroxy-4(methylthio) butanoic acid; DLM = DL-Met.

quadratic equation for birds fed HMTBA or DLM, respectively; A3 and B3 = parameter estimates for the quadratic term of the quadratic equation for birds fed HMTBA or DLM, respectively; A4 and B4 = asymptotes for HMTBA or DLM, respectively; A5 and B5 = steepness coefficient for HMTBA or DLM, respectively; Hin = MIOC in birds fed HMTBA; and DLin = MIOC in birds fed DLM.

The Bayesian information criteria (**BIC**) index (Schwarz, 1978; NLMIXED procedure of SAS; SAS Institute, 2003) was obtained for each equation. This parameter was used as an unbiased indicator of goodness of fit comparable across all equations. The equation with the lowest BIC value, i.e., better goodness of fit, was used for further analyses. To assess goodness of fit when using concentration instead of MIOC as the independent variable, Hin and DLin were replaced by HLev and DLev, respectively (where HLev = concentration in birds fed HMTBA and DLev = concentration in birds fed DLM), in equations A to I.

Using the selected equation, estimates of relative growth response were compared between toms fed HMTBA vs. DLM at various points of the dose-response curve using *t*-test and 95% confidence limits by PROC NLMIXED. Standard errors of the estimated relative performance were reported in parentheses. Significance was declared at $P < 0.05$.

RESULTS

Performance Parameters

In both experiments, main effects of Met concentration were observed for BW, BWG, and FCR ($P < 0.05$; Tables 3 and 4). Feed intake responses to Met source for both experiments were relatively lower than that of other performance parameters, however, generally increased with increasing Met concentration. For experiment 1, the least significant difference indicated that FI for poults fed the 0.20% diets for both HMTBA and DLM was greater than the basal ($P < 0.05$). In experiment 2, FI for the basal, 0.04, and 0.08% HMTBA diets were less than that of 0.16 and 0.32% HMTBA ($P < 0.05$), whereas all supplemental DLM concentrations were neither different from the basal or themselves. No differences between Met sources were observed using this analysis at any concentration for any production parameter.

Relative Growth Response

In both experiments, using MIOC instead of level as the independent variable improved goodness of fit for all equations (Table 5). In experiment 1, the lowest BIC value was obtained when fitting an inverse QUAD and a LIN regression to the BWGC of birds fed HMTBA or DLM, respectively (equation D using MIOC), indicating better goodness of fit (Table 5). The EXP regressions did not converge for either Met source at any time. When comparing the corresponding predicted BWG values of each equation (Figure 1), predicted BWGC at the most deficient part of the curve (MIOC of <1.0 g/bird) was lower for HMTBA-supplemented birds than for DLM-supplemented birds ($P < 0.05$) but higher for HMTBA at MIOC higher than 2.2 g/bird ($P < 0.05$). The best-fit LIN and inverse QUAD prediction equations for the DLM and HMTBA treatments, respectively, indicate that the maximum BWGC response was not achieved in this experiment.

In experiment 2, the LIN and QUAD regression models were the best-fit equations to describe the BWG of birds fed HMTBA and DLM, respectively. (equation B; Table 5). In general EXP regressions resulted in higher BIC values, indicating that EXP models did not fit the data as well as the other options. Predicted values were significantly higher for HMTBA at MIOC higher than 2.7 g/bird ($P < 0.05$), and the maximum growth response was greater when HMTBA was fed in contrast to DLM (Figure 2; $P < 0.05$).

Table 4. Effect of graded concentrations of Met supplementation from 2 sources on the performance of 1- to 21-d-old toms (experiment 2)

Supplemental Met concentration (%)	Met source[1]	BW (g)	Weight gain (g)	Feed intake (g)	Feed:gain (mortality corrected)	Mortality (%)
0.00	Basal	716 ± 7[d]	655 ± 7[d]	864 ± 13[b]	1.318 ± 0.011[a]	6.5 ± 3.4[a]
0.04	HMTBA	735 ± 10[cd]	675 ± 10[cd]	867 ± 12[b]	1.285 ± 0.007[b]	3.4 ± 2.4[a]
0.08	HMTBA	734 ± 9[cd]	671 ± 8[cd]	861 ± 12[b]	1.283 ± 0.004[bc]	3.4 ± 1.7[a]
0.16	HMTBA	781 ± 7[a]	720 ± 7[a]	907 ± 10[a]	1.260 ± 0.007[cd]	1.0 ± 1.0[a]
0.32	HMTBA	781 ± 19[a]	718 ± 18[a]	911 ± 19[a]	1.269 ± 0.011[bcd]	3.7 ± 2.6[a]
0.04	DLM	745 ± 10[bcd]	683 ± 10[bcd]	882 ± 11[ab]	1.291 ± 0.008[b]	8.4 ± 4.0[a]
0.08	DLM	751 ± 19[abc]	689 ± 18[abc]	884 ± 21[ab]	1.284 ± 0.012[bc]	8.0 ± 4.4[a]
0.16	DLM	759 ± 13[abc]	700 ± 12[abc]	881 ± 15[ab]	1.259 ± 0.006[cd]	2.3 ± 1.5[a]
0.32	DLM	771 ± 7[ab]	709 ± 7[ab]	889 ± 11[ab]	1.253 ± 0.009[c]	1.1 ± 1.1[a]
CV		4.6	4.8	4.6	1.9	183.2
Root MS error		34.3	32.9	40.4	0.025	7.681
		P-value				
Concentration		0.008	0.005	0.156	0.001	0.249
Source		0.898	0.925	0.781	0.673	0.270
Concentration × source		0.401	0.384	0.219	0.588	0.453

[a–d]Treatment means (±individual SE) with different superscripts differ statistically ($P < 0.05$).

[1]HMTBA = DL-2-hydroxy-4(methylthio) butanoic acid; DLM = DL-Met.

PFM

There was a significant increase of PFM in response to increasing supplemental Met; however, the magnitude of the increase was dependent on Met source. This resulted in a significant Met source × Met concentration interaction for PFM (Figure 3; $P < 0.001$). The regression slope for HMTBA was 22.0 nmol/mL of PFM per unit of supplemented HMTBA, whereas the slope for DLM was 62.9 nmol/mL of PFM per unit of DLM. The difference between these 2 slopes was highly significant ($P < 0.0001$) and indicated that PFM for DLM increased at nearly 3 times the rate of that for HMTBA over the range of supplemental concentrations of Met included in this experiment.

DISCUSSION

The statistical procedure used herein compared the gain responses of turkeys for each Met source and tested the goodness of fit of 3 basic equations (LIN, QUAD, and EXP) independently for both HMTBA and DLM. The prediction equations that resulted in the best goodness of fit, i.e., lowest BIC, were then used to calculate the gain response for each Met source. This methodology has been fully described (Kratzer and Littell, 2006) and provides an unbiased method for selection of regression models that can then be used to obtain prediction equations such that the responses to the 2 Met sources can be compared at various points of the TSAA response curve.

Although each of the current experiments demonstrated a significant effect of Met concentration on bird performance, ANOVA did not detect differences in response between HMTBA or DLM ($P > 0.05$) nor were significant Met source × concentration interactions detected. These results would suggest that either Met source would support turkey performance equally. However, because the prediction models for each Met source use all the data to determine differences at individual points, in general, they represent a more powerful method for determining differences than pairwise comparisons of the ANOVA procedure (Kratzer and Littell, 2006). Given that within each of the 2 experiments the best-fit regressions for HMTBA and DLM were different, these data collec-

Table 5. The goodness of fit of various equations for the response variable BW gain over the mean BW gain of the controls determined using Schwarz's Bayesian information criteria[1] (BIC)

	Regressions		Trial 1		Trial 2	
Equation	HMTBA	DLM	MIOC	Concentration	MIOC	Concentration
A	LIN	LIN	660.4	705.8	706.6	725.7
B	LIN	QUAD	663.9	709.4	703.1	724.3
C	LIN	EXP	NC	NC	711.7	730.0
D	QUAD	LIN	659.0	710.0	709.6	725.6
E	QUAD	QUAD	662.6	713.6	705.9	723.8
F	QUAD	EXP	NC	NC	714.7	730.0
G	EXP	LIN	NC	NC	756.4	NC
H	EXP	QUAD	NC	NC	760.6	NC
I	EXP	EXP	NC	NC	760.6	NC

[1]Linear (LIN), quadratic (QUAD), and exponential (EXP) equations were fitted to each Met source using Met intake over controls (MIOC) or concentration of supplementation as independent variables. Lower BIC values represent better goodness of fit. NC = no convergence; HMTBA = DL-2-hydroxy-4(methylthio) butanoic acid; DLM = DL-Met.

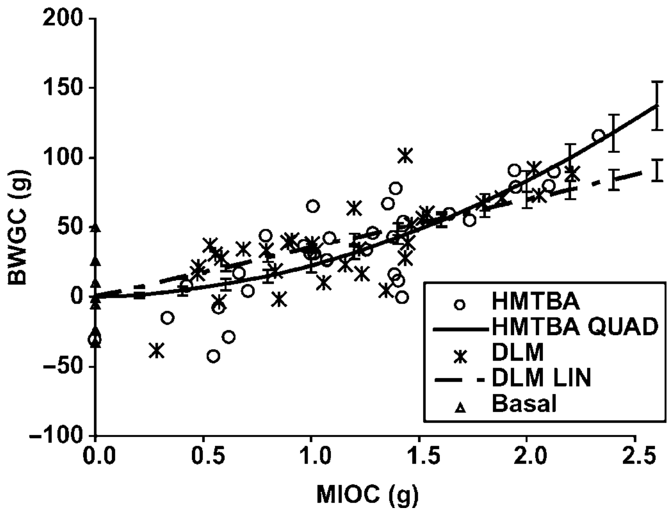

Figure 1. Dose response of 21-d-old toms fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in sorghum-based diets (experiment 1). Body weight gain over the basal (BWGC) and the intake of Met equivalents over the control (MIOC) were used as dependent and independent variables, respectively. A better goodness of fit was obtained by imposing an inverse quadratic (HMTBA QUAD) and a linear (DLM LIN) regression to HMTBA and DLM, respectively. This is expressed by the equation BWGC = 3.84 ± 10.65 × Hin + 18.85 ± 6.27 × Hin^2 + 34.89 ± 3.07 × Din, where Hin and Din = MIOC for birds fed HMTBA and DLM, respectively (±SE). The gain response to HMTBA relative to DLM was greater ($P < 0.05$) at MIOC greater than 2.2 g but lower than 100% ($P < 0.05$) at deficient MIOC levels (<1.0 g).

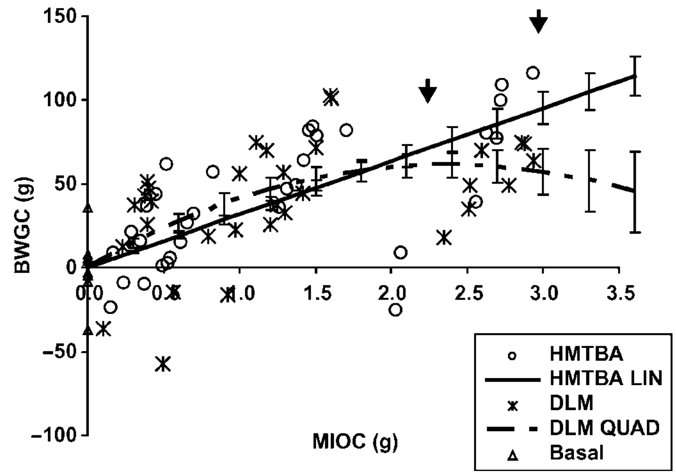

Figure 2. Dose response of 21-d-old toms fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in corn-based diets (experiment 2). Body weight gain over the basal (BWGC) and the intake of Met equivalents over the control (MIOC) were used as dependent and independent variables, respectively. A better goodness of fit was obtained by imposing a linear (HMTBA LIN) and a quadratic (DLM QUAD) regression to HMTBA and DLM, respectively. This is expressed by the equation BWGC = 31.74 ± 3.27 × Hin + 51.88 ± 10.72 × Din − 10.94 ± 4.48 × Din^2, where Hin and Din = MIOC for birds fed HMTBA and DLM, respectively. The maximum response to HMTBA was calculated at the highest Hin level (3.00 g), whereas that of DLM was achieved at 2.37 ± 0.52 g of Din using the corresponding equation (arrows). Maximum growth response was higher ($P < 0.05$) when feeding HMTBA vs. DLM (95.2 ± 9.8 g vs. 61.5 ± 7.4 g, respectively; ±SE). The gain response to HMTBA relative to DLM was greater at MIOC higher than 2.7 g ($P < 0.05$).

tively demonstrate that, as in broilers, young poults respond to these Met sources with different dose responses.

The fact that the best-fit regressions for each of the 2 experiments were different for each Met source is associated with the range of dietary Met and TSAA concentrations tested. Graded concentrations of Met supplementation induce a QUAD gain response if a sufficiently wide range of concentrations are fed (Vázquez-Añón et al., 2003; Dibner et al., 2004). However, if concentrations of supplementation are low relative to peak response, a LIN rather than a QUAD equation may fit the data better. Alternatively, if Met concentrations are approaching peak response but are insufficient to create a decline in performance, EXP equations may provide a better goodness of fit. It is likely that, in experiment 1, birds did not reach the peak response by the highest concentration of supplemental Met (at 1.19% TSAA), because an inverse QUAD and a LIN rather than a QUAD equation resulted in better goodness of fit for HMTBA and DLM, respectively (Figure 1). In experiment 2, in which the highest concentration of supplementation reached 1.34% TSAA, the growth of birds fed DLM was better described by a QUAD equation, whereas that of birds fed HMTBA was better described by a LIN regression. This suggests that the peak growth response was obtained within the range of Met supplementation when feeding DLM but not when HMTBA was fed. Using the corresponding equations for HMTBA and DLM, the maximum gain response for birds fed DLM was lower than for those fed HMTBA (61.5 ± 7.4 g vs. 95.2 ± 9.8 g; $P < 0.05$; Figure 2). This is further evidence that DLM and HMTBA have a different dose response in turkeys and agrees well with data in broilers (Schutte and de Jong, 1996; Kratzer and Littell, 2006; Vázquez-Añon et al., 2006). Therefore, as with broilers, the basic assumptions of the NLCPAR methodology that materials being compared have the same form of dose response and approach a common plateau are not true and as such would result in erroneous conclusions concerning the RBE of HMTBA and DLM in poults. When 2 compounds result in different forms of dose response, there is no single RBE value; rather, the response depends on where in the TSAA dose response curve one is feeding the poults. The results of experiment 1 and 2 demonstrated a greater maximum response to HMTBA than for DLM.

The report of Hoehler et al. (2005) tested the Met sources at dietary TSAA concentrations that were 30 to 40% lower than the current trial (basal TSAA 0.59 to 0.68%). Because they did not account for the 12% water present in the commercial product, HMTBA was also tested at lower concentrations than DLM. Given results of the current trials, these factors in addition to the use of the NLCPAR methodology for RBE determination would predictably conclude that HMTBA had lower efficacy than DLM across the entire dose range. As shown in both experiments 1 and 2, the HMTBA vs. DLM gain response tended to be smaller at lower concentrations of TSAA but caught up (experiment 1, inverse QUAD vs. LIN) and exceeded the response of DLM (experiment 1, experiment 2, LIN vs. QUAD, respectively) at higher concentrations of supplementation. The NLCPAR does not provide for

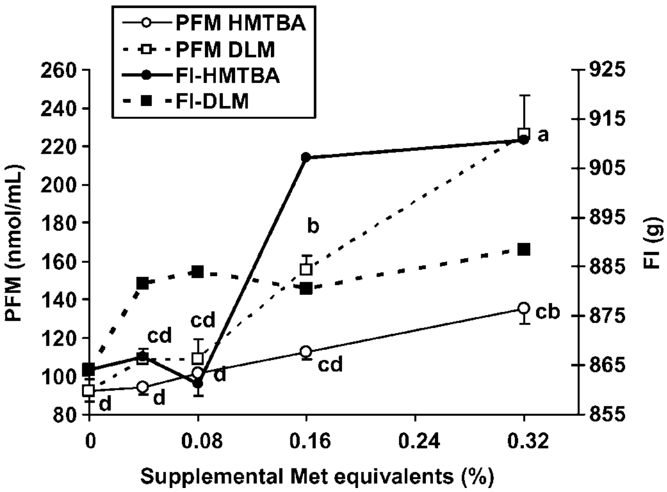

Figure 3. Association between plasma-free Met (PFM) concentrations and feed intake (FI) in turkeys fed 2-hydroxy-4(methylthio) butanoic acid (HMTBA) or DL-Met (DLM) in corn-based diets (experiment 2). This figure demonstrates the association between PFM and FI for HMTBA and DLM for experiment 2 in that the PFM response to increasing DLM was 62.9 nmol/mL and 22.0 nmol/mL (SE 4.7 nmol/mL) for HMTBA. Pairwise comparisons (means ± SE with common letters are not different, $P < 0.05$) between PFM demonstrate greater PFM for DLM at 0.16 and 0.32% Met supplementation rates. There was no Met source × concentration interaction by ANOVA; however, the pairwise comparison of FI for the highest concentration of HMTBA was greater than basal ($P < 0.05$), whereas FI for DLM was not. Previously published data indicate a similar association between different PFM concentrations and different FI for HMTBA and DLM (Vázquez-Añón et al., 2003; Dibner et al., 2004; Knight et al., 2006).

any change in slope from the lowest concentration of supplementation because it employs the assumption that the compounds have the same form of dose response and approach the same plateau (Kratzer and Littell, 2006). Consequently, the NLCPAR will create the erroneous conclusion that HMTBA is less than DLM across the entire response curve.

Although there is ample evidence of different dose responses for HMTBA and DLM, the reasons for the differences are not well defined. Recent isotope dilution infusion studies with sheep (Lobley et al., 2006; Wester et al., 2006) demonstrated that more than 60% of abomasally infused HMTBA is delivered to peripheral tissues as HMTBA, where it is subsequently converted to L-Met in situ and incorporated into protein. These studies also demonstrated that the kidney was the only organ in which significant quantities of HMTBA-derived L-Met was secreted back into the plasma, accounting for approximately 40% of the HMTBA metabolized by the kidney. These data provide the metabolic rationale for a lower PFM increase with HMTBA supplementation than DLM, as observed in the current work and previously reported in broilers and pigs (Vázquez-Añón et al., 2003), while providing comparable quantities of L-Met to the body.

Dietary Met strongly affects FI such that both low and high concentrations of Met depress feed consumption (Harper, 1970; Okumura and Yamaguchi, 1980; Edmonds and Baker; 1987; Sugahara and Kubo, 1992; Picard et al., 1993). In rats, FI reductions due to amino acid imbalances have been shown to be associated with reduced concentrations of the limiting amino acid in plasma and particularly in the brain (Peng et al., 1972, 1973). When Met is supplemented above 1% of the diet, broiler FI and growth rate are significantly reduced; however, the magnitude of FI depression is less with HMTBA (Baker, 1977; Dibner et al., 2004). Although PFM concentrations are elevated for both Met sources at these supplementation rates, DLM-supplemented chickens and pigs demonstrate significantly greater PFM than for HMTBA (Vázquez-Añón et al., 2003; Dibner et al., 2004), indicating a close association of differences in PFM and differences in FI levels for HMTBA and DLM. At the dietary concentrations of TSAA tested in experiment 2, PFM concentrations resulting from increasing DLM doses increased over basal at approximately 3 times the rate of that for HMTBA doses.

Knight et al. (2006) demonstrated that when fed TSAA-deficient (0.45%), purified diets, broilers supplemented with 0.08 or 0.10% HMTBA consumed significantly less feed and grew more slowly than those supplemented with equimolar quantities of DLM. However, DLM-supplemented broilers pair-fed to the HMTBA ad libitum treatments had the same growth rate as the HMTBA treatment. A similar experiment using corn and soybean meal basal diets (0.70% TSAA) at high concentrations of Met supplementation (1%) demonstrated greater ad libitum consumption of HMTBA than DLM; however, HMTBA pair-fed to the DLM treatment produced growth equal to the ad libitum DLM treatment. Thus, these results demonstrated that the differences in gain at the extremes of the TSAA response curve were due to differences in feed consumption, because no differences in gain between the 2 Met sources were observed in pair-fed treatments. Because HMTBA-supplemented poults demonstrated lower PFM in the current TSAA-deficient dietary conditions and PFM has been previously reported to be lower than DLM in broilers supplemented from 0.5 to 2% in corn soy diets (Dibner et al., 2004), it would appear that the in vivo difference between HMTBA and DLM with respect to transport and site of conversion plays a role in the observed difference in dose responses of the 2 Met sources.

In summary, these data demonstrate that HMTBA and DLM elicit a different dose response in young turkey poults in which a lower growth may be obtained when feeding HMTBA vs. DLM at more deficient concentrations, whereas a greater maximum response is observed for HMTBA. These effects are consistent with those recently reported for broiler comparisons of HMTBA and DLM (Vázquez-Añón et al., 2006). This effect may be linked, at least partially, to the differential effect of HMTBA and DLM on PFM and the consequent effect on FI and growth. Regardless of mechanism, because the dose response of birds to HMTBA differs from those fed DLM, not only is it inappropriate to use the NLCPAR methodology to determine RBE, but the concept of a single bioefficacy value for the 2 Met sources is meaningless because relative performance will depend on where in the dose response one is feeding. This understanding leads to a statistical approach in which prediction equations are developed for each Met source independently, and predicted differences are determined along the dose response (Kratzer and Littell, 2006). An important corollary to the understanding that these 2 Met sources demonstrate different dose responses is that the relative performance at such deficient TSAA concentrations is not predictive of performance at intended use rates of maximum performance. Thus, future comparisons should use dietary TSAA levels that include concentrations in which the maximum response to the Met sources can be obtained.

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