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Effect of Supplementing 2-Hydroxy-4-(Methylthio) Butanoic Acid and DL-methionine in Corn-soybean-cottonseed Meal Diets on Growth Performance and Carcass Quality of Broilers

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ABSTRACT : This experiment was conducted to compare the effects of feeding DL-2-hydroxy-4-(methylthio)butanoic acid (HMTBA) and DL-methionine (DLM) supplemented corn-soybean-cottonseed meal diets on growth performance, carcass composition, and muscle color of broilers. The trial was designed as a 2×3×2 factorial experiment, including two methionine (Met) sources (HMTBA and DLM), three equimolar graded levels of Met supplementation (i.e., 0.08, 0.16, and 0.24% in the starter diet and 0.07, 0.14, and 0.21% in the grower and finisher diets, respectively), and two sexes (male and female). Additionally, one basal diet for each sex was formulated to be limiting in Met to test the dosage response of increasing supplemental Met levels. Four hundred and twenty 10-d-old broilers were randomly allotted to 14 treatments (seven each for males and females), with five replicate pens per treatment and six chicks per pen. There was no difference (p>0.05) between the two Met sources in growth performance and muscle deposition of broilers throughout the whole experimental period (d 10 to 49). With the increasing Met supplementation levels, average daily gain was increased (quadratic; p<0.01) during the starter, grower, and overall phases, average daily feed intake was increased (quadratic; p<0.01) during the starter phase, and feed:gain ratio was decreased (quadratic; p<0.05) during the grower and overall phases. At the end of finisher phase, Met supplementation increased breast muscle content (quadratic; p<0.01) and thigh muscle content (linear; p<0.05), and decreased abdominal fat content (quadratic; p<0.02). Compared to the broiler fed DLM, broilers fed HMTBA had superior breast and thigh muscle coloration (p<0.01). Male broilers had higher weight gain and feed intake and better feed conversion than female broilers (p<0.01). The fat content of thigh muscle in female broilers was higher than that of male broilers (p<0.03). The best fit comparison of HMTBA vs. DLM was determined by Schwarz Bayesian Criteria index, which indicated that the average relative bioefficacy of HMTBA vs. DLM was 120% with 95% confidence limit 67 to 172%. These results indicated that Met supplementation improved growth performance and carcass quality of broilers fed corn-soybean-cottonseed meal diets irrespective of Met sources. Compared to DLM, HMTBA has the same molar bioefficacy on improving the growth performance and carcass quality of broilers; however, HMTBA fed birds had superior meat color to DLM fed birds. (**Key Words :** Broilers, Carcass Quality, Growth Performance, DL-methionine, 2-Hydroxy-4-(Methylthio) Butanoic Acid)

INTRODUCTION

Soybean meal is a predominant protein source in formulating poultry diets. However, it is difficult to have a consistent supply of good quality soybean meal in China for poultry production at a reasonable price range. When the price of soybean meal increases, poultry producers seek some unconventional protein sources such as cottonseed meal as a replacement for soybean meal in poultry feeds. Compared with soybean meal, cottonseed meal has similar or slightly lower crude protein, but a much lower market price. When broilers are fed cottonseed meal based diets, the presence of anti-nutritional factors such as gossypol and cyclopropenoid fatty acids impairs the absorption of nutrients and reduces amino acid digestibility, which may increase the need for dietary synthetic amino acid supplementation such as methionine (Met) to optimize the growth performance of poultry.

Supplemental Met is available as DL-Met (DLM; 99% powder or 40% liquid) or 88% aqueous solution of DL-2-

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Table 1. Experimental design

Diets	Met Source[1]	Starter (d 10 to 21)		Grower and finisher (d 21 to 49)	
		Addition of Met equivalents (%)	Addition of Met source (% of product)[2]	Addition of Met equivalents (%)	Addition of Met source (% of product)[2]
1	Basal	0.000	0.000	0.000	0.000
2	DLM	0.080	0.081	0.070	0.071
3	DLM	0.160	0.162	0.140	0.141
4	DLM	0.240	0.242	0.210	0.212
5	HMTBA	0.080	0.092	0.070	0.080
6	HMTBA	0.160	0.183	0.140	0.160
7	HMTBA	0.240	0.275	0.210	0.240

[1] HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid (HMTBA), brand name of Novus International, Inc., St. Louis, MO, USA] and DLM (99% purity) is a 50:50 blend of D- and L-Met.
[2] Based on the contents of 99% DLM or 88% HMTBA in the commercial products, respectively.

Table 2. Ingredient composition of the basal diets (%, as-fed basis)

Ingredients[1]	Starter (d 10 to 21)	Grower (d 21 to 42)	Finisher (d 42 to 49)
Corn	57.97	60.83	64.29
Soybean meal	27.40	20.10	11.50
Cottonseed meal	4.00	7.00	10.00
Fish meal	4.00	2.00	2.00
Corn gluten meal	-	2.00	3.50
Soybean oil	2.50	3.60	4.50
Limestone	1.46	1.46	1.35
Dicalcium phosphate	1.12	1.23	1.05
Salt	0.25	0.30	0.30
L-lysine·HCl	0.20	0.32	0.32
L-threonine	-	0.06	0.09
0.5% mineral premix	0.50	0.50	0.50
0.5% vitamin premix	0.50	0.50	0.50
Choline chloride (50%)	0.10	0.10	0.10
Total	100.00	100.00	100.00
Nutrient levels			
ME (kcal/kg)[2]	2,880	2,970	3,060
Crude protein	20.90	19.10	17.50
Calcium	1.10	0.99	0.92
Available phosphorus[2]	0.50	0.45	0.40
Lysine	1.22	1.11	0.96
Methionine	0.34	0.31	0.29
Methionine +cystine	0.67	0.63	0.61
Tryptophan	0.27	0.22	0.19
Threonine	0.84	0.82	0.74

[1] Vitamin and mineral premixes supplied per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; vitamin D_3, 2,750 IU; vitamin E (α-tocopheryl acetate), 20 IU; menadione, 3.0 mg; thiamin, 2.5 mg; riboflavin, 6.0 mg; pyridoxine, 2.5 mg; vitamin B_{12}, 12 μg; folic acid, 1.5 mg; niacin, 20 mg; Ca-pantothenate, 15 mg; biotin, 80 μg; Co, 300 μg; Cu, 16 mg; Fe, 102 mg; I, 1.2 mg; Mn, 95 mg; Se, 300 μg; Zn, 80 mg.
[2] ME and available phosphorus are calculated; crude protein, calcium, and amino acid profiles are analyzed.

hydroxy-4-(methylthio) butanoic acid (HMTBA; supplied as ALIMET® feed supplement, a product of Novus International, Inc., St. Louis, MO, USA). Many previous reports concluded that HMTBA and DLM supply equimolar amounts of L-Met for broilers (Garlich, 1985; Daenner and Bessei, 2003; Motl et al., 2005a, b). However, these reports were not in agreement with other publications indicating lower bioavailability of HMTBA compared to DLM in broilers (Lemme et al., 2002; Meirelles et al., 2003; Johri et al., 2004) and in pigs (Zimmermann et al., 2005).

The objective of our current research was to compare the relative bioefficacy of HMTBA and DLM supplemented on an equimolar basis on growth performance and carcass quality of broilers fed corn-soybean-cottonseed meal diets limiting in Met concentration.

MATERIALS AND METHODS

Experimental animals

The trial was conducted at the Wuhan Polytechnic University experimental broiler facility in Hubei province, P. R. China. A total of 420 (210 males and 210 females) 1-d-old Arber Acres broilers were obtained from a commercial hatchery (Wuhan Zhengda Arbor Acres Poultry Breeding Co., Ltd., Wuhan, P. R. China) and then wing-banded and raised in 1.00×1.00 m^2 wire-floored brooders in an environmentally controlled electrically-heated room. A commercial broiler starter diet was fed from d 0 to 10 and was formulated according to NRC (1994) nutrient recommendations. On d 10, the birds were transferred to the 0.45×0.45 m^2 wire-floored battery brooders in another environmentally controlled room with electrical heating. The brooders were equipped with nipple drinkers and tube feeder. The male broilers and female broilers were allotted to 14 dietary treatments (seven each for males and females), with five replicate pens per treatment and six birds per pen. The room temperature was maintained at 32°C from d 1 to 7, and then gradually reduced 2°C per week until the temperature reached 25°C. Chicks were exposed to 23 h light and 1 h darkness from d 1 to 4 and 16 h light and 8 h darkness from d 5 to 49. Birds were allowed to consume mash feed and water *ad libitum* for the entire duration of the experiment.

Experimental diets

The experiment was divided into three phases as starter (d 10 to 21), grower (d 22 to 42), and finisher (d 43 to 49) phases. The basal corn-soybean-cottonseed meal diets (Table 2) were formulated to be limiting in Met and total sulfur amino acids. The content of cottonseed meal in the basal diets was 4, 7, and 10% in the starter, grower, and finisher phases, respectively. The cottonseed meal was obtained from a commercial oil processing plant (Hubei Hanchuan Oil Processing Plant, Hubei, P. R. China), which used a prepressing-extraction processing method. The free gossypol of cottonseed meal was analyzed to be 239 mg/kg. The nutrient content of cottonseed meal was 37.7 g crude protein, 0.24 g calcium, 0.97 g total phosphorus, 1.87 g lysine, 0.59 g methionine, and 0.73 g cystine per 100 g.

Experimental design

The trial was designed as a 2×3×2 factorial experiment with two Met sources (HMTBA, Supplied as ALIMET feed supplement, Novus International, Inc., St. Louis, MO, USA; DLM, Degussa AG, Hanau, Germany), three equimolar graded levels of Met supplementation (i.e., 0.08, 0.16, and 0.24% in the starter diet and 0.07, 0.14, and 0.21% in the grower and finisher diets, respectively), and two sexes (male and female). Additionally, one basal diet for each sex was formulated to be limiting in Met to test the dosage response of increasing supplemental Met levels (Table 1). The Met deficient basal diet was used as a common basal diet for both HMTBA and DLM supplementation levels.

Experimental procedures and chemical Analysis

Birds were weighed on d 10, 21, 42, and 49. On d 49, five broilers of each treatment were randomly selected, bled, killed, and dissected for carcass quality evaluation. The pectoral and femoral muscles were dissected, weighed, and their ratios to body weight were calculated. The slaughtering procedure was according to the method of Yang (1994). Fresh color of pectoral and femoral muscles was determined by Model 43 Smoke Stain Reflectometer Diffusion Systems (Diffusion Systems LTD, England). The reflectance was measured in five different spots and the five measurements were averaged. The reflectance value is negatively correlated with the muscular color. Breast muscle and thigh muscle were collected and stored at -70°C prior to analysis.

The concentrations of moisture, crude protein, and crude fat in breast muscle and thigh muscle, and proximate analysis of feedstuffs and diets were performed according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). Amino acid composition of the diets was determined using ion-exchange chromatography on an automatic AA analyzer (L-8800) (Hitachi, Tokyo, Japan) after hydrolyzing with 6 M HCl for 24 h at 110°C. Tryptophan was analyzed following the alkaline hydrolysis method (Andersen, 1991) with reverse-phase HPLC (LC 10) (Shimadzu, Kyoto, Japan). Methionine and cystine were determined by using performic acid protection prior to acid hydrolysis (6 M HCl for 24 h at 110°C). Dietary HMTBA was analyzed at the feed analytical laboratory of Novus International., Inc., MO, USA using the HPLC method adapted from Ontiveros et al. (1987). Free gossypol of cottonseed meal was determined by the procedures of the Association of Official Analytical Chemists (AOAC, 1989).

Statistical analysis

Data were analyzed by ANOVA using the GLM procedures of SAS software (SAS Institute, 2003) appropriate for a factorial arrangement of treatments in a randomized complete block design. The statistical model included the effects of Met source, Met supplementation level, sex, and their interactions. Single degree of freedom orthogonal contrasts were used to compare the treatment effects of birds fed the basal diet deficient in Met vs. those fed diets supplemented with different Met levels. Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing Met supplementation levels on all measurements. Pen was used as an experimental unit for the performance data, whereas individual bird data were used as the experimental unit in carcass quality measurements.

For the entire experimental period, linear, quadratic, and exponential regressions were imposed independently to HMTBA- and DLM-fed groups. The body weight gain over basal was used as the dependent variable whereas Met intake over basal was used as the independent variable. The goodness of fit of the combined models was tested using the Schwarz Bayesian Information Criteria (BIC) index (Schwarz, 1978) generated by the NLMIXED procedure of SAS (SAS Institute, 2003). This methodology ensured an unbiased model selection process for each Met source independently based on the actual results of the experiment rather than assuming a prior dose response relationship between the Met sources (Gonzalez-Esquerra et al., 2004). The model with the best goodness of fit (as indicated by the lowest BIC value) was then used for further calculation of the relative bioefficay of HMTBA vs. DLM on an equimolar basis. An alpha level of $p<0.05$ was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

Growth performance

In the current experiment, the objective was to compare the effect of DLM and HMTBA on an equimolar basis on growth performance and carcass composition of broiler chicks fed low protein diets supplemented with 4, 7, and

Table 3. Performance of broilers fed Met-deficient corn-soybean-cottonseed meal basal diets supplemented with graded levels of DL-Met or HMTBA[1]

Treatment	Met source[2]	Inclusion level[3] (%)	ADG (g)				ADFI (g)				Feed:gain ratio			
			10-21d	21-42 d	42-49 d	10-49 d	10-21 d	21-42 d	42-49 d	10-49 d	10-21 d	21-42 d	42-49 d	10-49 d
Female														
1	Basal	0.00	28.8	54.4	66.7	49.8	52.7	124.4	157.1	110.0	1.83	2.30	2.27	2.21
2	DLM	0.08	30.4	59.0	74.9	53.8	53.4	126.0	159.3	111.5	1.76	2.14	2.13	2.08
3	DLM	0.16	32.4	59.4	69.9	53.7	59.3	127.6	156.3	113.5	1.84	2.15	2.24	2.11
4	DLM	0.24	32.7	59.5	64.8	52.9	61.6	128.5	142.1	112.0	1.89	2.16	2.19	2.12
5	HMTBA	0.08	30.8	57.3	70.9	52.2	56.2	124.1	156.0	110.7	1.83	2.17	2.20	2.12
6	HMTBA	0.16	32.0	63.2	71.7	55.9	58.4	132.2	156.4	115.7	1.82	2.09	2.18	2.07
7	HMTBA	0.24	32.6	60.9	72.1	54.9	59.8	131.6	158.2	116.1	1.84	2.19	2.20	2.12
Male														
8	Basal	0.00	30.9	60.3	78.3	55.2	57.7	128.4	176.1	117.0	1.87	2.13	2.25	2.12
9	DLM	0.08	33.0	63.0	76.7	57.0	59.1	136.3	162.2	119.1	1.79	2.16	2.11	2.09
10	DLM	0.16	33.7	64.1	83.6	59.0	57.8	132.1	174.1	118.7	1.72	2.08	2.09	2.11
11	DLM	0.24	34.0	64.6	75.1	57.8	60.8	135.1	158.1	118.3	1.79	2.09	2.10	2.05
12	HMTBA	0.08	32.8	61.4	82.4	56.5	57.5	133.3	174.2	119.2	1.75	2.18	2.20	2.11
13	HMTBA	0.16	33.6	65.0	80.3	58.9	58.3	132.0	168.7	117.8	1.74	2.03	2.10	2.00
14	HMTBA	0.24	34.2	65.4	78.0	58.9	59.2	132.5	167.9	118.2	1.73	2.05	2.15	2.01
SEM			0.9	1.7	3.4	1.3	1.5	3.2	7.8	2.6	0.04	0.05	0.06	0.03
P-value														
Sex			<0.01	<0.01	<0.01	<0.01	0.44	<0.01	<0.01	<0.01	<0.01	0.09	0.06	<0.01
Source			0.94	0.55	0.39	0.48	0.59	0.99	0.28	0.62	0.64	0.66	0.38	0.76
Sex × source			0.99	0.56	0.99	0.64	0.58	0.30	0.89	0.48	0.46	0.66	0.57	0.79
Level			0.04	0.06	0.22	0.11	<0.01	0.67	0.36	0.76	0.35	0.12	0.95	0.09
Sex × level			0.77	0.82	0.65	0.94	0.07	0.22	0.92	0.42	0.16	0.23	0.44	0.07
Source × level			0.97	0.25	0.48	0.37	0.53	0.58	0.37	0.81	0.37	0.57	0.50	0.33
Sex × source × level			0.92	0.80	0.25	0.65	0.31	0.86	0.55	0.76	0.35	0.82	0.94	0.74
Basal vs. supplemented[4]			<0.01	<0.01	0.72	<0.01	<0.01	0.08	0.42	0.31	0.05	0.04	0.02	<0.01
Met supplementation level[5]														
Linear			<0.01	<0.01	0.70	0.01	<0.01	0.06	0.20	0.29	0.55	0.04	0.12	<0.01
Quadratic			<0.01	<0.01	0.26	0.01	<0.01	0.14	0.40	0.48	0.06	0.04	0.07	<0.01

[1] Values are means for five pens treatment with six birds per pen.
[2] HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid (HMTBA), brand name of Novus International, Inc., St. Louis, MO, USA] and DLM (99% purity) is a 50:50 blend of D- and L-Met.
[3] Met supplementation level: 0.00, 0.08, 0.16, and 0.24% in the starter diets; 0.00, 0.07, 0.14, and 0.21% in the grower and finisher diets.
[4] Orthogonal contrast to test basal diet vs. all Met supplementation diets.
[5] Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

10% cottonseed meal in the starter, grower, and finisher phases, respectively. The low protein level was used to increase dietary sensitivity to Met supplementation. Furthermore, the presence of anti-nutritional factors including gossypol and cyclopropenoid fatty acids in cottonseed meal can lower the utilization of amino acids such as Met (Zhang, 2001), which further increased the magnitude of Met deficiency in the basal diets. As presented in Table 3, no significant interaction among Met source, Met supplementation level, and sex for growth performance was observed throughout the entire experimental period regardless of growth phase ($p>0.05$). There was a significant improvement of average daily gain (ADG) and feed:gain ratio ($p<0.05$) of all Met supplemented diets over the basal treatment in the starter (d 10 to 21) and overall experimental period (d 10 to 49). With the increasing Met supplementation levels, ADG was increased (quadratic; $p<0.01$) during the starter, grower, and overall phases, average daily feed intake (ADFI) was increased (quadratic; $p<0.01$) during the starter phase, and feed:gain ratio was decreased (quadratic; $p<0.05$) during the grower and overall phases. This indicates that the basal diets were deficient in sulfur amino acids, which enabled us to determine the relative bioefficacy of HMTBA vs. DLM.

The improved growth performance is in agreement with many other authors (Garlich, 1985; Daenner and Bessei, 2003; Johri et al., 2004; Motl et al., 2005a, b). However, there was no effect of Met supplementation level observed for ADG, ADFI, and feed:gain ratio during the finisher phase (d 42 to 49) ($p>0.05$). There are several possibilities for this result. Firstly, the young broilers during the starter and grower phases may be more sensitive to the anti-nutritional substances of cottonseed meal (i.e., gossypol and cyclopropenoid fatty acids); however, during the finisher phase broilers may have developed a tolerance to these substances and therefore they were less dependent on

Table 4. Schwarz Bayesian Information Criteria index for body weight gain over basal regressed on dietary Met intake over basal[1]

HMTBA[2]	DLM [2]	BIC (Smaller is better)
Lin	Lin	860.0
Lin	Quad	861.9
Lin	Exp	864.6
Quad	Lin	863.2
Quad	Quad	864.3
Quad	Exp	NA
Exp	Lin	NA
Exp	Quad	864.3
Exp	Exp	875.5

[1] The lower BIC index indicated the better goodness of fit of the statistical model (generated by the SAS Proc NLMIXED model). Lin, linear regression model; Quad, quadratic regression model; Exp, exponential regression model; NA, not applicable.

[2] HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid (HMTBA), brand name of Novus International, Inc., St. Louis, MO, USA] and DLM (99% purity) is a 50:50 blend of D- and L-Met.

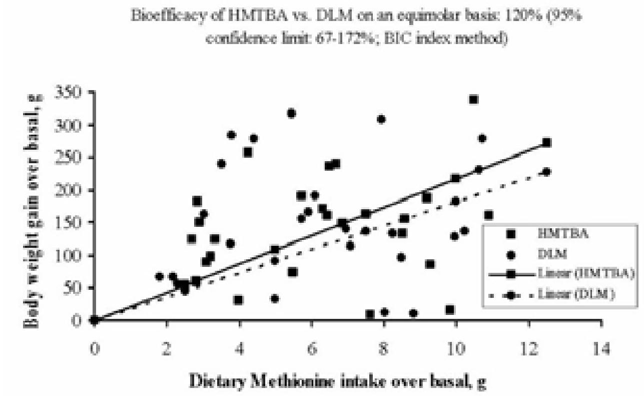

Figure 1. Relative bioefficacy of 2-hydroxy-4-(methylthio) butanoic acid (HMTBA) vs. DLM on an equimolar basis as determined by the linear-linear regression model (the best fit as indicated by the lowest Schwarz Bayesian Information Criteria index generated by SAS Proc NLMIXED model) (body weight gain over basal regressed on dietary Met intake over basal). The relative bioefficacy of HMTBA vs. DLM was 100% with 95% confidence limit 67 to 172% (R^2 = 0.66, observation with five replicate pens per treatment and seven birds per pen). HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of HMTBA, brand name of Novus International, Inc., St. Louis, MO] and DL-Met (99% purity) is a 50:50 blend of D- and L-Met.

dietary amino acid supplementation. Secondly, the Met content in the basal diets may be sufficient to maintain their growth during a short finishing period. Thirdly, the birds with depressed growth due to Met deficiency during the starter and grower phases, may show a compensatory growth phenomenon during the finisher phase (Leeson and Zubair, 1997).

During the whole experimental period (d 10 to 49), there was no Met source (p>0.05) or Met source×Met supplementation level interaction (p>0.05) for growth performance, which demonstrated that there was no bioefficacy difference between HMTBA and DLM on an equimolar basis (Table 3). In the current study, we took a different statistical approach (i.e., the BIC index method) and determined that the linear regression of body weight gain over basal on Met intake over basal was the best fitted regression model to estimate the relative bioefficacy of two Met sources. The results of the current Met source comparison indicated an average relative bioefficacy of HMTBA to DLM of 120% with 95% confidence limit of 67 to 172% (Table 4 and Figure 1). Since 100% bioefficacy was included in the 95% confidence limit, it indicated there was no Met source difference for growth performance, which was consistent with the ANOVA results. The current finding is in agreement with many previous studies comparing the bioefficacy of DLM and HMTBA in broilers (Knight and Dibner, 1984; Garlich, 1985; Han et al., 1990; Daenner and Bessei, 2003; Motl et al., 2005a, b; Liu et al., unpublished data) and in pigs (Knight et al., 1998; Jansman and de Jong, 1999; Gaines et al., 2005a, b) fed various types of diets. In a nitrogen balance study, Römer and Abel (1999) found there was no difference between the equimolar HMTBA and DLM on nitrogen retention of broilers and growing pigs. Our current study was also corroborated in the *in vitro* studies, in which HMTBA and DLM were found to be biochemically equivalent Met sources for protein synthesis in primary cultures of porcine or broiler hepatocytes (Dibner, 1983; Knight et al., 1998). Dibner et al. (1990) reported that HMTBA was a precursor of L-Met and an intermediate in a naturally occurring pathway for the synthesis of L-Met in the chick. In addition, Dibner et al. (1988) observed a superior absorption ability of HMTBA in that HMTBA was absorbed not only by small intestine, but also by large intestine, while DLM was absorbed only by small intestine. Recently, by using an *in vitro* everted intestinal slice model, HMTBA is also demonstrated to be absorbed completely along the gastrointestinal tract, especially the upper intestinal tract, and found to be a highly effective supplemental Met source as DLM in broiler chicks (Richards et al., 2005). These research reports are in agreement with the non-significant growth performance of birds fed different Met sources in the current study. However, some authors reported HMTBA had lower bioavailability than DLM in broilers (Lemme et al., 2002; Meirelles et al., 2003; Johri et al., 2004; Liu et al., 2004) and in pigs (Zimmermann et al., 2005). In addition, some researchers found that HMTBA was absorbed less efficiently compared to DLM (Van Weerden et al., 1992; Drew et al., 2003). The discrepancy might be caused by different experimental models used to measure absorption of Met in the gastrointestinal tract.

As expected, male broilers had significantly higher ADG,

Table 5. Muscle deposition and color of broilers fed Met-deficient corn-soybean-cottonseed meal basal diets supplemented with graded levels of DL-Met or HMTBA[1]

Treatment	Met source[2]	Inclusion level[3] (%)	Breast muscle content (% of live weight)	Thigh muscle content (% of live weight)	Abdominal fat content (% of live weight)	Breast muscle reflectance[4]	Thigh muscle reflectance[4]
Female							
1	Basal	0.00	14.74	14.55	1.90	22.6	29.8
2	DLM	0.08	15.42	14.82	2.06	24.9	34.7
3	DLM	0.16	16.56	15.22	1.43	22.7	36.1
4	DLM	0.24	16.60	15.10	1.96	22.9	31.1
5	HMTBA	0.08	15.69	13.59	2.13	22.2	30.2
6	HMTBA	0.16	16.24	14.61	1.81	21.2	27.8
7	HMTBA	0.24	16.70	14.52	1.69	20.9	30.3
Male							
8	Basal	0.00	14.49	14.14	2.16	22.2	30.7
9	DLM	0.08	15.36	14.86	1.70	24.8	35.3
10	DLM	0.16	16.25	15.41	1.27	23.8	32.4
11	DLM	0.24	15.67	15.19	1.24	22.5	30.6
12	HMTBA	0.08	15.15	15.06	1.65	21.2	30.3
13	HMTBA	0.16	16.88	14.81	1.75	19.3	29.0
14	HMTBA	0.24	15.93	15.79	1.45	23.2	32.2
SEM			0.63	0.59	0.20	1.2	1.8
P-value							
Sex			0.36	0.11	<0.01	0.99	0.95
Source			0.73	0.28	0.24	<0.01	<0.01
Sex × source			0.77	0.20	0.52	0.77	0.33
Level			0.05	0.36	0.05	0.23	0.37
Sex × level			0.52	0.77	0.39	0.62	0.65
Source × level			0.98	0.73	0.21	0.29	0.03
Sex×source × level			0.72	0.66	0.56	0.27	0.57
Basal vs. supplemented[5]			<0.01	0.19	0.04	0.96	0.32
Met supplementation level[6]							
Linear			<0.01	0.05	<0.01	0.50	0.83
Quadratic			<0.01	0.15	0.02	0.79	0.47

[1] Values are means for five birds (one bird per pen).

[2] HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid (HMTBA), brand name of Novus International, Inc., St. Louis, MO, USA] and DLM (99% purity) is a 50:50 blend of D- and L-Met.

[3] Met supplementation level: 0.00, 0.08, 0.16, and 0.24% in the starter diets; 0.00, 0.07, 0.14, and 0.21% in the grower and finisher diets.

[4] Values are expressed as reflectance value, which was determined by Model 43 Smoke Stain Reflectometer Diffusion Systems.

[5] Orthogonal contrast to test basal diet vs. all Met supplemented diets.

[6] Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

ADFI, and better feed conversion efficiency than female broilers ($p<0.05$). This was in agreement with our recent study with corn-soybean-rapeseed meal diets conducted in the same facility (Liu et al., unpublished data) and some other studies (Luo et al., 1994). These findings indicate that male broilers grow faster and consume more feed with better feed efficiency, which is due to the genetic difference of male and female broilers (Yang, 1993).

Muscle deposition

With the increasing preference for white meat, the breast meat yield is becoming one of the most important objectives in the modern poultry industry. This demands higher nutrient supply, especially the limiting amino acid supply to optimize the growth potential and carcass composition toward breast meat production (Liu and Guo, 2001). It is reported that higher Met supplementation increased breast meat yield and decreased abdominal fat yield (Liu and Guo, 2001). In the current study, increasing Met supplementation level resulted in increased breast muscle content (quadratic; $p<0.01$), improved thigh muscle content (linear; $p<0.05$), and reduced abdominal fat content (quadratic; $p<0.02$) (Table 5). These results are consistent with many other researchers (Schutte and Pack, 1995; Liu and Guo, 2001; Johri et al., 2004).

There was no significant interaction among Met source, Met supplementation level, and sex detected for muscle deposition throughout the trial ($p>0.05$). Compared to the basal diet deficient in Met, diet supplemented with 0.21% Met regardless of Met source had 11% increase in breast muscle content, 6% improvement in thigh muscle content, and 22% decrease in the abdominal fat content. These

Table 6. Muscle composition of male and female broilers fed Met-deficient corn-soybean-cottonseed meal basal diets supplemented with graded levels of DL-Met or HMTBA[1]

Treatment	Met source[2]	Inclusion level[3] (%)	Moisture content (%)		Crude protein content (%)		Crude fat content (%)	
			Breast muscle	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle
Female								
1	Basal	0.00	73.30	75.01	24.60	21.13	1.19	4.30
2	DLM	0.08	72.53	73.55	24.61	20.17	1.26	5.48
3	DLM	0.16	73.23	74.29	24.49	20.89	1.14	3.17
4	DLM	0.24	74.00	75.05	24.59	20.89	2.17	4.74
5	HMTBA	0.08	73.16	74.22	24.43	20.44	2.00	3.50
6	HMTBA	0.16	73.34	74.37	24.47	21.66	1.08	3.87
7	HMTBA	0.24	73.79	74.88	24.71	20.95	1.25	3.96
Male								
8	Basal	0.00	74.61	75.17	24.96	21.03	1.00	3.14
9	DLM	0.08	71.75	73.64	24.29	20.50	1.12	2.91
10	DLM	0.16	74.07	75.15	25.32	20.62	1.65	3.55
11	DLM	0.24	74.21	75.26	24.83	20.62	1.44	3.30
12	HMTBA	0.08	73.81	74.88	24.81	21.06	1.23	3.11
13	HMTBA	0.16	72.46	73.49	24.55	20.67	1.09	3.13
14	HMTBA	0.24	74.92	73.19	24.70	20.49	1.84	3.09
SEM			1.02	0.88	0.35	0.36	0.36	0.69
P-value								
Sex			0.74	0.80	0.31	0.41	0.66	0.03
Source			0.63	0.53	0.70	0.21	0.81	0.31
Sex × source			0.85	0.31	0.79	0.62	0.88	0.50
Level			0.14	0.70	0.71	0.26	0.23	0.73
Sex × level			0.85	0.65	0.66	0.08	0.37	0.39
Source × level			0.35	0.20	0.50	0.60	0.28	0.57
Sex × source × level			0.50	0.53	0.32	0.61	0.11	0.26
Basal vs. supplemented[4]			0.52	0.22	0.64	0.21	0.18	0.89
Met supplementation level[5]								
Linear			0.34	0.86	0.85	0.79	0.08	0.95
Quadratic			0.14	0.40	0.85	0.80	0.20	0.90

[1] Values are means for five birds (one bird per pen).
[2] HMTBA is a L-Met precursor supplied as ALIMET feed supplement [an 88% aqueous solution of 2-hydroxy-4-(methylthio)butanoic acid (HMTBA), brand name of Novus International, Inc., St. Louis, MO, USA] and DLM (99% purity) is a 50:50 blend of D- and L-Met.
[3] Met supplementation level: 0.00, 0.08, 0.16, and 0.24% in the starter diets; 0.00, 0.07, 0.14, and 0.21% in the grower and finisher diets.
[4] Orthogonal contrast to test basal diet vs. all Met supplementation diets.
[5] Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

findings support the hypothesis that dietary amino acid fortification can improve live performance, lower fat deposition, and increase muscle yield of modern broilers. A higher abdominal fat content was observed in female rather than male broilers ($p<0.01$), which is in agreement with Yang (1993). This indicates that female broilers grow fatter than male broiler in the current study, which is due to the genetic difference of male and female broilers (Yang, 1993).

No difference was observed for the effects of the two Met sources on muscle deposition ($p>0.05$), which indicates HMTBA has the same molar efficacy for optimizing muscle deposition as DLM. This result is in agreement with the report of Meirelles et al. (2003) and our recent study with corn-soybean-rapeseed meal diets (Liu et al., unpublished data). However, our result was not consistent with some previous publications indicating lower efficacy of HMTBA in promoting muscle deposition of the birds when compared to DLM (Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Lemme et al., 2002; Johri et al., 2004). The discrepancy might be attributive to different diet types, experimental design, and application of statistical methodologies for interpretation of data.

Muscle color

Meat color has an important impact on marketing and meat price of broilers, so it is one of the important parameters for which the broiler producers, sellers, and consumers are concerned (Fletcher, 1999). Many dietary factors including vitamins, amino acids, minerals, and antioxidants may have an effect on broiler meat color (Froning, 1995). Some previous studies found that Met supplementation improved pigment deposition and resulted in superior meat color (Yu and Zeng, 1996; Liu and Feng, 1998). However, in the present experiment, there is no significant effect of Met supplementation level on breast muscle and thigh muscle reflectance ($p>0.05$) (Table 5).

Significant interaction was observed for the reflectance of thigh muscle between Met source and Met supplementation level ($p<0.03$), in which the thigh muscle reflectance of birds fed diets with DLM was increased due to the increasing DLM supplementation level; however, that of birds fed diets with HMTBA did not change with the increasing HMTBA supplementation level. Met sources had a significant effect on breast muscle reflectance, in which broilers fed HMTBA had lower reflectance value ($p<0.01$) than DLM fed birds. The reflectance value is negatively correlated with the muscular color, so broiler fed diets with HMTBA had more fresh reddish color in breast and thigh muscle. HMTBA and DLM have different metabolic pathways in broilers (Dibner and Knight, 1984; Dibner, 2003). Whether meat color difference is related to their different metabolic pathways remains unknown. Further studies are needed to evaluate the possible mechanism(s) that result in the meat color difference between HMTBA and DLM.

Muscular composition

The nutrient composition of broiler meat is closely associated with dietary nutrient composition. It is well documented that dietary amino acid deficiency, especially limiting essential amino acid such as Met and lysine results in decreased protein content and increased fat content of carcass; whereas moderate supplementation of Met can increase carcass protein content and decreased fat content (Guo et al., 1998). However, in the current experiment, there was no significant interaction among Met source, Met supplementation level, and sex detected for moisture, crude protein, and crude fat content of breast muscle and thigh muscle ($p>0.05$) throughout the trial (Table 6). Met supplementation level also had no impact on the moisture, crude protein, and crude fat content of breast muscle and thigh muscle ($p>0.05$). There was no difference observed for the effects of equimolar HMTBA and DLM on nutrient composition of breast muscle and thigh muscle ($p>0.05$), which indicates HMTBA has a similar effect on nutrient deposition in muscle tissues as DLM. However, the fat content of thigh muscle in female broilers was higher than that in male broilers ($p<0.05$), which is consistent with Yang (1993) and Luo et al. (1994). This may be due to the genetic difference between male and female broilers (Yang, 1993).

In conclusion, in the current study, increasing dietary Met supplementation increased growth performance, improved breast and thigh muscle deposition, and reduced fat deposition of broilers fed corn-soybean-cottonseed meal based diets deficient in Met. When supplemented on an equimolar basis, there was no difference between HMTBA and DLM in improving growth performance and carcass quality of broilers. Furthermore, compared to birds fed DLM supplemented diets, broilers fed HMTBA had a superior breast and thigh muscular color, which warrant further research.

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