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Effect of Methionine Source and Dietary Crude Protein Level on Growth Performance, Carcass Traits and Nutrient Retention in Chinese Color-feathered Chicks*

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ABSTRACT : A total of 1,200 LinNan Chinese color-feathered chicks were used to study the effects of methionine source [DL-2-hydroxy-4-methylthio-butanoic acid (HMTBa) or DL-methionine (DLM)] and dietary crude protein (CP) level on growth performance, carcass traits, and whole-body nitrogen and fat retention. The trial was designed as a 2×2 factorial arrangement, including two CP levels (adequate and low) and two methionine sources (HMTBa and DL-methionine). Diets were formulated for three phases, starter (0-21 d), grower (21-42 d), and finisher (42-63 d). Chicks fed HMTBa had higher daily gain and improved feed efficiency than DLM during the grower phase (p<0.05). A significant two-way interaction was observed for growth performance during the finisher phase and overall (0-63 d). Growth performance was greater for chicks fed HMTBa than DLM on adequate-CP diets (p<0.05), but this was not observed at low-CP level (p>0.05). Chicks fed low-CP diets grew slower, used feed less efficiently during the grower, finisher phase and overall. On d 42, regardless of dietary CP levels, birds fed HMTBa had higher carcass weights, breast and thigh weights than DLM-fed birds (p<0.04). Birds fed low-CP diet had lighter carcass weights and less breast muscle, thigh muscle, and dressing percentage at the end of starter, grower and finisher phases (p<0.05). Whole body composition analyses found that birds fed HMTBa tended to contain more protein and less fat compared to those chicks fed DLM at the end of the starter phase (p<0.10). Low-CP diets increased CP concentration in the whole body at the end of the finisher phase (p = 0.05). HMTBa supplementation increased whole-body N retention rate during the finisher phase and overall (p<0.01), and low-CP diets reduced N intake and whole-body fat retention during the finisher phase and overall (p<0.05). In summary, HMTBa was better than DLM on an equimolar basis for growth performance, carcass traits, and N retention in Chinese color-feathered chicks. Low-CP diets lowered growth performance as well as carcass traits in color-feathered birds, probably due to imbalanced AA profiles. (**Key Words :** Chinese Color-feathered Chicks, Methionine Source, Dietary Crude Protein, Growth Performance, Carcass Trait, Nutrient Retention)

INTRODUCTION

Methionine (Met) is a limiting amino acid (AA) in commercial poultry diets and is commonly supplemented as DL-Met (DLM; 99% powder or 40% liquid) or an 88% aqueous solution of DL-2-hydroxy-4-methylthio-butanoic acid (HMTBa. Novus International. Inc.. USA). Although both compounds provide Met precursors to the animals. there are substantial differences with respect to their chemistry, absorption (Knight and Dibner. 1984), transport in the body (Lobley et al., 2006). and metabolism by tissues (Dibner, 2003). Over the last five decades considerable research has been conducted to investigate the effectiveness of HMTBa relative to DLM as a Met source in chicks. A large body of literature indicates that chicks can utilize HMTBa and DLM with the same biological efficiency for either growth or N retention, when both sources were supplemented on an equimolar basis (Emad et al., 2004; Motl et al., 2005a. b; Liu et al. 2006; Vazquez-Añon. et al.. 2006). However, others reported significant differences

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Table 1. Composition and nutrient level of the starter ration (0 to 21 d, as-fed basis)

Item (%)	Adequate CP		Low CP	
	HMTBa	DLM	HMTBa	DLM
Corn	59.29	59.32	63.45	63.47
Soybean meal	30.45	30.45	26.30	26.30
Fish meal	2.50	2.50	2.50	2.50
Corn protein meal	1.50	1.50	1.50	1.50
Soybean oil	2.25	2.25	2.00	2.00
Dicalcium phosphate	1.55	1.55	1.57	1.57
Calcium carbonate	1.09	1.09	1.11	1.11
Salt	0.25	0.25	0.25	0.25
L-lysine·HCl	0.06	0.06	0.18	0.18
HMTBa[1]	0.24	-	0.28	-
DLM[1]	-	0.21	-	0.25
L-threonine	0.02	0.02	0.07	0.07
Premix [2]	0.80	0.80	0.80	0.80
Total	100.00	100.00	100.00	100.00
Nutrient composition[3]				
ME (kcal/kg)	2,950	2,950	2,950	2,950
CP (%)	20.8	20.6	19.2	19.1
Calcium (%)	1.00	0.98	0.99	0.98
Total phosphorus (%)	0.64	0.64	0.64	0.66
Digestible amino acids[4] (%)				
Lysine	0.97	0.94	0.95	0.95
Methionine	0.47	0.46	0.47	0.48
Methionine+cystine	0.76	0.77	0.75	0.77
Threonine	0.77	0.75	0.77	0.76
Tryptophan	0.18	0.19	0.17	0.19

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

[2] Supplied per kilogram of diets: vitamin A (retinyl acetate), 15,000 IU; vitamin D_3, 3,600 IU; vitamin E (DL-α-tocopherol acetate), 33 IU; vitamin K, 6 mg; thiamin, 3 mg; riboflavin, 9 mg; pyridoxine, 6 mg; vitamin B_{12}, 0.03 mg; niacin, 60 mg; D-pantothenate, 18 mg; folic acid, 1.5 mg; biotin, 0.06 mg; choline, 750 mg; iron, 80 mg; copper, 6.4 mg; zinc, 80 mg; manganese, 96 mg; iodine, 0.56 mg; selenium, 0.24 mg.

[3] ME is calculated.

[4] Calculated using digestibility coefficients assayed by NOVUS International, Inc using reagent kit for *in vitro* digestion of amino acid in feed ingredient. Crystalline amino acids were assumed 100% digestible.

Table 2. Composition and nutrient level of the grower ration (21 to 42 d, as-fed basis)

Item (%)	Adequate CP		Low CP	
	HMTBa	DLM	HMTBa	DLM
Corn	65.16	65.16	69.72	69.72
Soybean meal	24.83	24.83	20.62	20.62
Fish meal	1.50	1.50	1.50	1.50
Corn protein meal	2.50	2.50	2.50	2.50
Soybean oil	2.13	2.13	1.57	1.57
Dicalcium phosphate	1.43	1.43	1.45	1.45
Calcium carbonate	1.05	1.05	1.07	1.07
Salt	0.25	0.25	0.25	0.25
L-lysine·HCl	0.13	0.13	0.24	0.24
HMTBa[1]	0.15	-	0.19	-
DLM[1]	-	0.13	-	0.17
L-threonine	0.02	0.02	0.08	0.08
Premix [2]	0.85	0.87	0.81	0.83
Total	100.00	100.00	100.00	100.00
Nutrient composition[3]				
ME (kcal/kg)	3,000	3,000	3,000	3,000
CP (%)	18.4	18.6	17.1	16.8
Calcium (%)	0.88	0.89	0.89	0.89
Total phosphorus (%)	0.58	0.57	0.58	0.59
Digestible amino acids[4] (%)				
Lysine	0.88	0.84	0.83	0.86
Methionine	0.39	0.39	0.41	0.39
Methionine+cystine	0.63	0.62	0.62	0.63
Threonine	0.70	0.68	0.69	0.68
Tryptophan	0.16	0.17	0.16	0.16

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

[2] Supplied per kilogram of diet: vitamin A (retinyl acetate), 12,500 IU; vitamin D_3, 3,000 IU; vitamin E (DL-α-tocopherol acetate), 27.5 IU; vitamin K, 5 mg; thiamin, 2.5 mg; riboflavin, 7.5 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.025 mg; niacin, 50 mg; D-pantothenate, 15 mg; folic acid, 1.25 mg; biotin, 0.05 mg; choline, 500 mg; iron, 72 mg; copper, 5.76 mg; zinc, 72 mg; manganese, 86.4 mg; iodine, 0.50 mg; selenium, 0.22 mg.

[3] ME is calculated.

[4] Calculated using digestibility coefficients assayed by NOVUS International, Inc using reagent kit for *in vitro* digestion of amino acid in feed ingredients. Crystalline amino acids were assumed 100% digestible.

between the two Met sources, and that DL-Met has greater bioefficacy than HMTBa (Mandal et al., 2004; Vieira et al., 2004).

Environmental pressures to reduce N waste as well as variable protein ingredient costs have driven poultry producers to reduce dietary CP levels and to supplement with synthetic AA to achieve both economic and environmental benefits. Previous research indicated that low CP diets could achieve similar growth performance as long as all essential AA are supplemented to requirements (Morris et al., 1999). However, several experiments also have shown that growth performance and carcass composition became inferior when dietary CP content was lowered by more than three to four percentage points (Emmert et al., 2000; Bregendahl et al., 2002).

The Chinese color-feathered chick is a very unique broiler species in China. The chicks grow slower and have lower nutrient density requirements compared to commercial fast-growing broilers. HMTBa instead of DLM is commonly used in feed for Chinese color-feathered chicks. Limited research has been conducted to evaluate the efficiency of HMTBa and DLM on growth performance in color-feathered chicks. The objectives of this study were to evaluate the bioefficay of HMTBa and DLM, and to validate the low-CP diet concept on growth performance, carcass traits, whole-body composition, and nutrient retention in Chinese color-feathered chicks.

Table 3. Composition and nutrient level of the finisher ration (42 to 63 d, as-fed basis)

Item (%)	Adequate CP		Low CP	
	HMTBa	DLM	HMTBa	DLM
Corn	70.10	70.10	74.66	74.66
Soybean meal	20.75	20.75	16.53	16.53
Corn protein meal	3.00	3.00	3.00	3.00
Soybean oil	2.26	2.26	1.70	1.70
Dicalcium phosphate	1.40	1.40	1.41	1.41
Calcium carbonate	1.00	1.00	1.03	1.03
Salt	0.25	0.25	0.25	0.25
L-lysine·HCl	0.20	0.20	0.32	0.32
HMTBa[1]	0.14	-	0.18	-
DLM[1]	-	0.12	-	0.16
L-threonine	0.04	0.04	0.09	0.09
L-tryptophan	-	-	0.02	0.02
Premix[2]	0.86	0.88	0.81	0.83
Total	100.00	100.00	100.00	100.00
Nutrient composition[3]				
ME (kcal/kg)	3,050	3,050	3,050	3,050
CP (%)	16.6	16.7	15.3	15.3
Calcium (%)	0.74	0.76	0.73	0.74
Total phosphorus (%)	0.52	0.51	0.50	0.51
Digestible amino acids[4] (%)				
Lysine	0.80	0.77	0.78	0.78
Methionine	0.34	0.35	0.36	0.38
Methionine+cystine	0.60	0.61	0.60	0.63
Threonine	0.64	0.63	0.63	0.63
Tryptophan	0.15	0.14	0.14	0.14

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

[2] Supplied per kilogram of diet: vitamin A (retinyl acetate), 12,500 IU; vitamin D_3, 3,000 IU; vitamin E (DL-α-tocopherol acetate), 27.5 IU; vitamin K, 5 mg; thiamin, 2.5 mg; riboflavin, 7.5 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.025 mg; niacin, 50 mg; D-pantothenate, 15 mg; folic acid, 1.25 mg; biotin, 0.05 mg; choline, 500 mg; iron, 64 mg; copper, 5.12 mg; zinc, 64 mg; manganese, 76.8 mg; iodine, 0.45 mg; selenium, 0.19 mg, et al.

[3] ME is calculated.

[4] Calculated using digestibility coefficients assayed by NOVUS International, Inc using reagent kit for *in vitro* digestion of amino acid of feed ingredients. Crystalline amino acids were assumed 100% digestible.

MATERIALS AND METHODS

Chicks and housing

A total of 1,200 one-day-old male LinNan Chinese color-feathered chicks (average BW 37.1±0.1 g) were randomly allotted to four dietary treatments consisting of six replicate floor pens with 50 birds per pen. Each pen was 2.73×1.87 m and the floor was covered with wood shavings. Birds were allowed to consume water and mash diets *ad libitum*. House temperature was maintained at 33 to 35°C for the first 3 d, and then decreased by 2 to 3°C every week until 18°C was reached. A 24-h constant lighting schedule was maintained. Animal care was followed by the standard practices of color-feathered chicks recommended by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences.

Experimental diets

The trial was designed as a 2×2 factorial arrangement with two CP levels (adequate and low) and two Met sources (HMTBa and DLM) within the starter, grower, and finisher phases. The adequate level of CP was 20.5%, 18.5%, and 16.5% for the starter, grower, and finisher phases, respectively. The low CP diets were formulated to contain 1.6%, 1.3%, and 1.3% incremental reductions in CP percentage for all three phases (Tables 1, 2, and 3). All diets were formulated to meet or exceed the nutrient requirements recommended by Ministry of Agriculture of the People's Republic of China (2005). All diets were formulated using digestible AA values of each ingredient obtained using the IDEA® kit (Novus International, Inc. St. Louis, USA). HMTBa and DLM were supplemented on an equimolar basis to meet the sulfur AA requirement of 0.78%, 0.66%, and 0.60% for the starter, grower, and finisher diets, respectively.

Measurements

All birds were weighed as a group, and feed consumption was measured per pen at placement and again at 21, 42, and 63 d, when the experiment ended. Mortality was recorded daily and dead birds were weighed to adjust feed efficiency.

At 21, 42 and 63 d of age, two birds per pen were randomly selected and slaughtered by severing the carotid arteries and jugular vein after 16-h of feed withdrawal. Each bird was then defeathered and dissected as previously described (National Poultry Breeding Council, 1984). Carcass weight, dressing percentage, and weight of breast muscle, thigh muscle, and fat pad were obtained.

At the end of each feeding phase, two birds per pen with a BW close to the pen average were selected, and euthanized by cervical dislocation after 24-h of feed withdrawal. Carcasses were dissected to remove gut fill, weighed, and stored in an airtight plastic bag at -20°C for whole-body composition analysis. Ten chicks were sampled at day 1 for determination of the baseline whole-body composition.

Analytical procedures

Proximate analyses of feed ingredients and feeds followed AOAC (1990). AA composition in the diets was determined by ion-exchange chromatography on an automatic AA analyzer (L-8800, Hitachi, Japan). Tryptophan was analyzed following the alkaline hydrolysis method with High Performance Liquid Chromatography (HP1050, Hewlett-Packard Company, USA). The whole

Table 4. Growth performance of Chinese color-feathered chicks (0 to 63 d) [1]

Variable[2]	Adequate CP		Low CP		SEM	p values		
	HMTBa[3]	DLM[3]	HMTBa[3]	DLM[3]		Met[4]	CP[4]	Met×CP[4]
Initial BW (g)	37.11	37.06	37.09	37.22	0.10	0.29	0.19	0.12
Days 0 to 21								
ADG (g/d)	15.4	15.0	15.0	15.4	0.20	0.95	0.88	0.14
ADFI (g/d)	26.1	26.0	26.3	26.9	0.50	0.60	0.23	0.39
FCR (g/g)	1.70	1.73	1.75	1.75	0.02	0.42	0.07	0.48
Days 21 to 42								
ADG (g/d)	33.5	29.6	31.4	29.4	0.60	0.01	0.06	0.12
ADFI (g/d)	75.2	69.4	74.1	70.5	1.10	0.01	0.98	0.32
FCR (g/g)	2.25	2.34	2.36	2.40	0.03	0.04	0.01	0.35
Days 42 to 63								
ADG (g/d)	44.3[a]	40.4[b]	37.3[b]	39.9[b]	1.40	0.66	0.01	0.03
ADFI (g/d)	126.4[a]	118.4[b]	114.9[b]	119.0[b]	2.40	0.42	0.03	0.02
FCR (g/g)	2.86	2.94	3.09	2.98	0.05	0.75	0.02	0.10
Days 0 to 63								
ADG (g/d)	31.0[a]	28.4[b]	27.9[b]	28.3[b]	0.50	0.03	0.01	0.01
ADFI (g/d)	75.9[a]	71.3[b]	71.7[b]	72.1[b]	0.90	0.02	0.07	0.01
FCR (g/g)	2.27[c]	2.34[b]	2.40[a]	2.38[ab]	0.02	0.23	0.01	0.02

[a-c] Means within a row without a common superscript differ significantly ($p<0.05$).

[1] Values are means for six replicate pens per treatment with 50 birds per pen.

[2] BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed:gain ratio.

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

[4] Main effect of methionine source (Met) or CP levels (CP).

bodies of the euthanized birds were sampled according to procedures described by Barker and Sell (1994). The whole body of individual birds (including feathers) was cut into sections, distilled water equivalent to BW added, autoclaved for 8 h, and allowed to cool overnight. Two birds of each pen were blended together and dried at 60°C for 3 d, then ground through a 0.8 mm mesh screen (TECATOR 1093, Foss Tecator AB, Sweden), and stored at -20°C for N and ether extract analyses. Whole-body DM was calculated from the dry weight of the ground birds and losses of water. The whole-body N content was analyzed by the Kjeldahl procedure (AOAC, 1990). Whole-body ether extract content was assayed according to AOAC (1990) using a fat extraction unit (SOXTEC HT2/AVANTi2050, Foss Tecator AB, Sweden). Fat and N retention were calculated based on the difference between the whole-body ether extract and N contents at the end of each phase and whole-body composition of the initial phase.

Statistical analysis

Pen served as the experimental unit for growth performance, whole-body composition, and nutrient retention, whereas individual chick was used as experimental unit for carcass characteristics. Data were subjected to ANOVA analysis using the GLM procedures of SAS (SAS Institute Inc., 2003). The model included main effects of Met source, CP level, and all potential two-way interactions. Statistical significance of differences between dietary treatments were assessed by LSD test. A level of $p<0.05$ was used as the criterion for statistical significance.

RESULTS

Growth performance

No difference between CP levels or Met source was observed in chicks during the first phase of growth ($p>0.05$, Table 4). Chicks fed the adequate CP diets had higher ADG and improved feed efficiency from d 21 to 42 ($p<0.05$). Also, chicks fed HMTBa had greater ADG, ADFI, and improved feed efficiency than chicks fed DLM ($p<0.05$). Significant two-way interactions of Met source and dietary CP level were observed during the finisher phase ($p<0.05$). Chicks fed HMTBa gained 9% more and ate 6% more than those fed DLM on the adequate-CP diets ($p<0.05$), while the benefit of HMTBa was not observed on the low-CP diets. Overall (d 0-63), chicks fed the adequate CP diets had higher ADG and improved FCR, and the response to Met source was found on the adequate CP diets, but not on the low CP diets.

Carcass traits

No two-way interaction of Met source or dietary CP levels was found for carcass traits. Most of the differences were from dietary CP levels (Table 5). Low-CP diets

Table 5. Carcass traits of Chinese color-feathered chicks at 21, 42 and 63 d of age [1]

Variables[2]	Adequate CP		Low CP		SEM	p values		
	HMTBa[3]	DLM[3]	HMTBa[3]	DLM[3]		Met[4]	CP[4]	Met×CP[4]
Day 21								
Carcass weight, g	213.6	214.4	201.9	202.4	3.40	0.85	0.01	0.97
Breast muscle weight, g	16.3	16.2	15.3	14.3	0.53	0.29	0.01	0.42
Thigh muscle weight, g	22.3	21.4	19.7	20.2	0.40	0.68	0.01	0.15
Abdominal fat weight, g	3.7	3.7	3.1	3.6	0.27	0.52	0.32	0.53
Dressing percentage, %	68.2	68.5	67.8	68.6	0.45	0.18	0.70	0.56
Day 42								
Carcass weight, g	667.7	623.4	623.7	565.7	10.77	0.01	0.01	0.53
Breast muscle weight, g	52.2	47.9	46.9	42.2	1.57	0.01	0.01	0.87
Thigh muscle weight, g	73.7	67.4	67.8	60.4	1.72	0.01	0.01	0.75
Abdominal fat weight, g	16.1	12.0	14.4	12.2	1.53	0.04	0.64	0.55
Dressing percentage, %	72.4	71.4	70.6	71.4	0.54	0.84	0.10	0.14
Day 63								
Carcass weight, g	1,298.5	1,262.9	1,165.7	1,156.2	13.54	0.10	0.01	0.34
Breast muscle weight, g	112.1	109.0	95.2	97.3	2.82	0.87	0.01	0.37
Thigh muscle weight, g	148.0	145.5	127.4	130.9	2.31	0.84	0.01	0.20
Abdominal fat weight, g	49.2	37.1	45.7	39.1	4.49	0.04	0.86	0.55
Dressing percentage, %	75.8	75.9	74.4	74.9	0.46	0.52	0.01	0.70

[a-b] Means within a row without a common superscript differ significantly ($p<0.05$).
[1] Values are means for six replicate pens with two birds per pen.
[2] Dressing percentage are expressed on live weight.
[3] HMTBa is a L-Met precursor supplied as ALIMET feed supplement (an 88% aqueous solution of 2-hydroxy-4-methylthio-butanoic acid (HMTBa), Novus International, Inc., St. Louis, MO) and DLM (99% purity) is a 50:50 blend of D- and L-Met.
[4] Main effect of methionine source (Met) or CP levels (CP).

Table 6. Whole body composition of Chinese color-feathered chicks (DM basis)[1]

Item	Adequate CP		Low CP		SEM	p values		
	HMTBa[2]	DLM[2]	HMTBa[2]	DLM[2]		Met[3]	CP[3]	Met×CP[3]
Day 21								
DM (%)	29.24	30.44	30.50	30.52	0.46	0.20	0.16	0.22
CP (%)	62.15	58.44	59.17	59.06	0.99	0.07	0.25	0.09
Fat (%)	23.94	27.39	25.33	27.19	1.50	0.09	0.70	0.60
Day 42								
DM (%)	34.15	34.05	35.18	33.97	0.70	0.36	0.51	0.44
CP (%)	55.94	55.39	55.62	56.52	1.15	0.88	0.73	0.53
Fat (%)	28.83	27.49	28.21	27.67	1.83	0.61	0.91	0.83
Day 63								
DM (%)	38.13	36.52	36.57	36.83	0.61	0.28	0.32	0.14
CP (%)	48.72	51.38	52.60	51.86	1.02	0.36	0.05	0.11
Fat (%)	35.20	31.34	30.85	30.80	1.40	0.18	0.10	0.19

[1] Values are means for six replicate pens with two 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), Novus International, Inc., St. Louis, MO) and DLM (99% purity) is a 50:50 blend of D- and L-Met.
[3] Main effect of methionine source (Met) or CP levels (CP).

reduced carcass, breast muscle and thigh muscle weights at all sampling times and dressing percentage was only affected on d 63 ($p<0.05$). The effects of Met source on carcass traits were observed on d 42. HMTBa increased carcass weight as well as breast and thigh muscle weights ($p<0.05$). However, abdominal fat was also increased in the chicks fed HMTBa at d 42 and 63 compared to those fed the DLM diets ($p<0.05$).

Whole-body composition, nitrogen and fat retention

No two-way interaction of Met source and dietary CP level was found for whole-body composition (Table 6). Chicks fed HMTBa tended to have higher percentage of protein and lower percentage of fat in the carcass at 21 d ($p<0.10$).Chicks fed low-CP diets had higher percentage of protein than those fed adequate-CP diets at 63 d ($p = 0.05$).

Feed intake affected N intake (Table 7), in that low-CP diets had lower N intake, and HMTBa groups had higher N

Table7. Whole body nitrogen and fat retention in Chinese color-feathered chicks (DM basis)[1]

Item	Adequate CP		Low CP		SEM	p values		
	HMTBa[2]	DLM[2]	HMTBa[2]	DLM[2]		Met[3]	CP[3]	Met×CP[3]
Days 0 to 21[4]								
N intake (g/d/chick)	0.87	0.86	0.81	0.82	0.01	0.96	0.01	0.41
N retention (%)	44.66	45.59	46.79	46.17	1.48	0.97	0.06	0.52
Fat retention (g/d/chick)	0.95	1.16	1.03	1.12	0.09	0.09	0.84	0.48
Days 21 to 42[4]								
N intake (g/d/chick)	2.22	2.07	2.03	1.90	0.03	0.01	0.01	0.73
N retention (%)	43.10	42.96	46.21	42.11	1.31	0.12	0.40	0.15
Fat retention (g/d/chick)	3.30	2.97	3.17	2.64	0.38	0.28	0.56	0.79
Days 42 to 63[4]								
N intake (g/d/chick)	3.35[a]	3.17[b]	2.81[c]	2.91[c]	0.06	0.52	0.01	0.03
N retention (%)	38.68	34.97	36.93	33.62	1.30	0.01	0.25	0.88
Fat retention (g/d/chick)	7.98[a]	5.53[b]	4.88[b]	4.85[b]	0.59	0.05	0.01	0.05
Days 0 to 63								
N intake (g/d/chick)	2.15[a]	2.03[b]	1.88[c]	1.88[bc]	0.02	0.01	0.01	0.02
N retention (%)	42.15	41.17	43.31	40.63	0.64	0.01	0.63	0.20
Fat retention (g/d/chick)	4.07	3.22	3.03	2.87	0.21	0.03	0.01	0.11

[a-c] Means within a row without a common superscript differ (p<0.05).

[1] Values are means for six replicate pens with two 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), Novus International, Inc., St. Louis, MO) and DLM is a 50:50 blend of D- and L-Met.

[3] Main effect of methionine source (Met) or CP levels (CP).

[4] N = Nitrogen.

intake on the adequate-CP diets but not on the low-CP diets in the finisher phase and for the whole experimental period (two-way interaction p<0.05). Chicks fed the low-CP diets had higher N retention rate in the starter phase (p = 0.06) and less N excretion (p<0.05) in the grower and finisher phases (data not shown). Similar to effects of low-CP diets, chicks fed HMTBa had higher % N retention compared to those fed DLM in the finisher phase and overall (p<0.05).

For fat retention, results were variable at different phases. Chicks fed HMTBa tended to have less fat retention in the starter phase (p<0.10, Table 7) and more fat in the finisher phase (p = 0.05) and overall (p<0.05) compared to those fed DLM. Significant two-way interactions of Met source and dietary CP levels on fat retention were observed for the finisher phase (p<0.05). Chicks fed the HMTBa diets had higher fat retention than those fed DLM at adequate CP level, but not at the low-CP level. In addition, less fat was deposited in chicks fed the low-CP diets during the finisher phase and the whole experimental period, compared to those chicks fed the adequate CP diets (p<0.01).

DISCUSSION

The concept of low-CP diets in conjunction with synthetic AA supplementation is well known in the poultry industry (Han et al., 1992; Deschepper and De Groote, 1995; Morris et al., 1999). However, lowering dietary CP levels without balancing essential AA profiles reduced growth performance and nutrient retention in chicks (Emmert et al., 2000; Bregendahl et al., 2002). In this study, a 1.5 percentage point of dietary CP reduction impaired growth performance, even with synthetic HMTBa or DLM, L-lysine·HCL, L-threonine, and L-tryptophan supplementation. All diets were formulated to have similar concentrations of methionine, lysine, threonine, and tryptophan. The main differences came from other essential AA, including arginine, valine, and isoleucine, which may lead to an imbalanced AA profile in the low-CP diets. For example, the measured Arg:Lys ratio for adequate-CP and low-CP diets were respectively 1.27 and 1.15 in the starter, 1.22 and 1.08 in the grower, and 1.16 and 1.02 in the finisher phase. In addition, insufficient supply of non-essential AAs in low-CP diets restricted growth and feed efficiency even with all essential AA being balanced (Graber and Baker, 1973; Fancher and Jensen, 1989). The situation gets worse for today's lean-genetic chicks because of their fast growth potential (Alleman et al., 2000). Compared to modern avian broilers, Chinese color-feathered chicks grow slower, and have lower carcass and meat yields, and more fat retention (P. B. Xi, unpublished data). Because of the slower growth rate, the nutrient requirements of Chinese yellow birds are lower than commercial fast-growing broilers. For example, the CP requirement of Chinese color-feathered chicks was lower by one to two percentage points, and AA requirements were lower by 0.01 to 0.06 percentage points at the starter phase, according to the recommendation of Ministry of Agriculture of the People's Republic of China (2005). Interestingly, AA requirements for the grower and finisher phases and the ideal AA profiles for any growing phases were similar for Chinese color-feathered chicks and modern lean broilers.

In general, chicks fed HMTBa had better growth performance than chicks fed DLM, and the benefits were more profound when diets were formulated at adequate-CP levels. Similar results were reported by Derick et al. (1999), and Ribeiro and Penz (2001) who found that chicks consuming HMTBa had better weight gain and feed conversion efficiency than chicks fed DLM on an equimolar basis. HMTBa and DLM are different chemicals; their mechanism and site of absorption (Knight and Dibner, 1984), transport in the body (Lobley et al., 2006) and metabolism by tissues (Dibner, 2003) are different. Birds fed DLM had higher plasma Met concentration than those fed HMTBa which has been associated with lower feed consumption and weight gain (González-Esquerra et al., 2004). In addition, HMTBa is chemically an organic acid and its properties in the gut are similar to lactic acid before its absorption and metabolism as a precursor for Met source (Dibner and Buttin, 2002). As an organic acid, HMTBa has broad antimicrobial activity, which can be particularly effective against acid-intolerant species like *E. coli*, salmonella, and campylobacter (Partanen and Mroz, 1999; Enthoven et al., 2002), and can reduce subclinical infections and immune activation. However, no intestinal or fecal microflora populations were measured in this study. Further research is needed to confirm that improved growth performance by HMTBa was due to an organic acid effect.

Significant two-way interactions of dietary CP level and Met source were observed on growth performance. HMTBa was superior to DLM on weight gain and feed efficiency when chicks were fed adequate-CP diets, but no difference was observed at low-CP diets. The major difference between adequate- and low-CP diets was the Arg:Lys ratio (1.16 and 1.02, respectively at finisher phase). Therefore, the bioefficacy of HMTB and DLM may depend on the Arg:Lys ratio. Thomas et al. (1991) reported an improved growth of HMTBa relative to DLM with increasing Arg:Lys ratio in practical diets. Derick et al. (1999) found that favorable growth responses to HMTBa were evident at higher Arg:Lys ratios (i.e., 1.20 and 1.34), whereas the growth response to DLM was optimized at a lower dietary Arg:Lys ratio (i.e., 1.03). In the experiment of Chen et al. (2003), broilers exhibited increased BW gain with increasing Arg:Lys ratio from 1.04 to 1.35 in the presence of HMTBa but not DLM during acute heat stress. The Arg:Lys ratio may become a critical factor that limits growth and may obscure the difference between methionine sources at low dietary CP level.

In accordance with growth performance, HMTBa increased carcass weight, breast muscle and thigh muscle weights in the grower phase without impacting on dressing percentage. Mandal et al. (2004) also observed that broilers fed HMTBa had higher breast yield than birds fed DLM, while Emad et al. (2004) reported no significant difference in breast muscle and thigh muscle percentage between broilers fed HMTBa and DLM. These inconsistent results may be due to differences in chick breed and TSAA level between HMTBa and DLM supplemented diets. Chinese color-feathered chicks were used in this study and commercial fast-growing broilers were used in the experiments of Mandal et al. (2004) and Emad et al. (2004). In our experiment and the experiment of Emad et al. (2004), HMTBa and DLM were supplemented on an equimolar basis and the TSAA content in all diets were similar, whereas the TSAA content of the HMTBa diet was 1.4 times higher than the DLM diet in the experiment of Mandal et al. (2004). At the grower and finisher phases, the birds fed HMTBa had higher abdominal fat weight than DLM fed birds, which reflected increased BW.

Lowering dietary CP had detrimental effects on carcass weight, breast muscle weight, and thigh muscle weight. This observation was in agreement with the results reported by Alleman et al. (2000) and Wijtten et al. (2004). In the current study, the decreased meat yield of the birds fed the low-CP diets implied an imbalanced essential AA profile and/or insufficient non-essential AA supply that resulted in reduction of protein deposition.

Very limited data have been reported on whole-body composition and nutrient retention of Chinese color-feathered chicks. One report showed whole-body CP, fat contents and N retention rate of Chinese color-feathered chicks were 59.2%, 30.6% and 41.1%, respectively (Zhou et al., 2004), which were similar to our measurements. The only impact of Met source on body composition was observed at the starter phase where birds fed HMTBa tended to have more muscle growth ($p = 0.07$) and have higher CP body composition when chicks were fed adequate-CP levels (two-way interaction $p<0.10$). However, chicks fed the low-CP diets had higher protein percentage in the whole body than birds fed the adequate-CP diet at 63 d of age. Fat deposition was not affected by dietary CP levels ($p>0.10$). These results contrast with previous research reports, in which lower dietary CP reduced whole-body protein concentration, and increased whole-body lipid content (Aletor et al., 2000; Alleman et al., 2000; Garcia et al., 2000; Bregendahl et al., 2002).

There was a Met source by dietary CP level interaction for N intake during the finisher and overall period, which was reflected in feed intake differences. Regardless of dietary CP levels, HMTBa increased N intake during the grower and overall phases and improved whole-body N retention rate during the finisher and overall phases. These results suggest that HMTBa was superior to DLM for enhancing whole-body N retention. In agreement with our results, Ribeiro and Penz (2001) observed that N retention was significantly higher in Cobb×Cobb male broiler chicks supplemented with HMTBa compared to birds fed DLM. However, the N retention was not influenced by Met source during the starter phase, which was consistent with the growth performance. This further confirmed that the response to HMTBa was more pronounced in the grower and finisher phases than in the starter phase.

Due to low feed intake, chicks fed low-CP diets consumed less N, excreted less N and retained less N in the whole-body (data not shown), which was in agreement with previous reports (Fancher and Jensen, 1989; Bregendahl et al., 2002). The efficiency of N utilization tended to be higher in low-CP diets during the starter phase. In the present study, the effect of dietary CP on whole-body fat retention differed from previous studies which indicated that lowering dietary CP concentrations enhanced fat deposition in the carcass (Bregendahl et al., 2002; Wijtten et al., 2004). It is possible that with the low CP diets, although essential AA were present at or above the requirement, some non-essential AA were insufficient for maximal protein deposition leading to the decreased efficiency of protein and energy utilization (Hurwitz et al., 1998). In the current study, carcass and meat yield of Chinese color-feathered chick decreased as dietary CP reduced by 1.5% points. In contrast, previously reported studies in broilers have shown that growth performance and carcass composition become inferior to those fed standard high-CP diets only when the dietary CP content was lowered by more than three to four percentage points (Ferguson et al., 1998; Aletor et al., 2000). It suggests that Chinese color-feathered chicks may be more sensitive to lower dietary CP level, or they may have different requirements for other essential AA and the essential to non-essential AA ratio.

In summary, HMTBa was superior to DLM on an equimolar basis for growth performance and carcass traits at adequate dietary CP level. Moreover, the growth performance and carcass trait responses of Chinese color-feathered chicks to Met source were more profound during the grower and finisher phase. Under the current experimental conditions, low-CP diets resulted in inferior growth performance, carcass and meat yield and whole-body N and fat retention compared to adequate-CP diets. This observation might be attributable to imbalanced essential AA profiles and/or insufficient non-essential AA supply on the low-CP diets.

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