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The Relative Effectiveness of Liquid Methionine Hydroxy Analogue Compared to DL-methionine in Broilers

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ABSTRACT : The present experiment was conducted to assess the efficacy of liquid methionine hydroxy analogue-free acid (MHA-FA) in comparison to DL-methionine (DL-Met) in broilers. 567 day-old Avian chicks were divided into 7 treatments with 5 replicates of 16 birds each. During the 35d (7-42 d) experimental periods chicks were given two basal diets. From 7 to 21d of age, a starting basal diet containing 19.5% protein and 0.33% methionine was supplemented with two graded levels of DL-Met (0.070 and 0.160%) or four levels of MHA-FA (0.118, 0.143, 0.221 and 0.268%). From 22 to 42d of age DL-Met (0.050 and 0.080%) or MHA-FA (0.071, 0.074, 0.112 and 0.140%) were added to a finishing basal diet with 18.0% protein and 0.28% methionine. Chicks fed on supplemental DL-Met or MHA-FA had significantly higher ($p<0.05$) body weight gain and feed conversion ratio (FCR) than the control group from 7-21d of age. During the finishing phase (22-42 d), body weight and weight gain of chicks in DL-Met or MHA-FA treatments were similar to those in the control, but FCR was improved ($p<0.05$) with supplementation of DL-Met or MHA-FA. Breast yield was higher ($p<0.05$) on DL-Met or MHA-FA supplemented than un-supplemented diets. The thigh meat yields emanating from diets with DL-Met or MHA-FA were lower ($p<0.05$) than that in control. Abdominal fat was also higher in broilers fed the control diet than in DL-Met or MHA-FA supplemented treatments. Methionine requirement of broilers was calculated to be 0.44 and 0.35% and cystine requirement was 0.35 and 0.31% for the starting (7-21 d) and finishing phase (22-42 d), respectively. The efficacy of MHA-FA in comparison to DL-Met for weight gain was 64 and 85% and for FCR was 55 and 60% at 7-21 and 22-42 d of age, respectively, while it was 74, 72, 52 and 48% for breast yield, thigh meat production, body energy content and energy deposition ratio at 42 d of age, respectively. In conclusion, in practical diet formulation for broiler chicks the average bioavailability of MHA-FA relative to DL-Met could be considered as 60 and 73% for 7 to 21d and 22 to 42 d of age, respectively. (**Key Words :** DL-Met, MHA-FA, Broiler, Growth Performance, Carcass Traits)

INTRODUCTION

Methionine plays a significant role in poultry production because it is considered to be the first-limiting amino acid, especially in low protein diets. Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing chickens (Schutte and Pack, 1995). This phenomenon indicates that meat retention transferred from thigh or other parts to breast when methionine added to reach some level. Methionine is commonly supplemented as two sources, DL-methionine (DL-Met) or liquid methionine hydroxy analogue-free acid (MHA-FA). The latter is available commercially in liquid form, and can be atomized after pelleting. Hence, dusts from feed were reduced by adding MHA-FA. Both of them allow accurate balancing of the dietary amino acid profile. However, the two products greatly differ regarding their biological effectiveness because MHA-FA: (a) is not an amino acid in biochemical term and has to be converted into methionine or cystine in metabolism (Xie et al., 2004); (b) is not pure but contains 12% water and impurities; (c) is partially subjected to microbial degradation in the small intestine and hence not fully available for absorption; (d) is composed of mono-, di- and oligomers-, the latter being poorly absorbed (Lemme, 2002). Knowing the relative biological value (RBV) of MHA-FA compared to DL-Met is an important precondition to cost-effective purchasing, feed formulation and poultry production (Hoehler and Hooge, 2003).

Numerous studies involving different approaches and various animal species have been carried out in order to establish the efficacy of MHA-FA relative to DL-Met (Huyghebaert, 1993; Esteve-Garcia and Llaurado, 1997). In

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Table 1. Ingredient, energy and nutrient content of the starting and finishing basal diets

Ingredients (%)	Starting diet (7-21 d)	Finishing diet (22-42 d)
Corn (yellow)	63.26	67.71
Soybean oil	1.20	1.40
Soybean meal	19.30	9.20
Cotton seed meal	8.00	12.0
Rapeseed meal	3.00	4.00
Blood meal	0	1.00
Meat-bone meal	3.20	2.70
Limestone	0.80	0.80
Sodium chloride	0.30	0.30
Lysine-HCl	0.44	0.39
Mineral/vitamin premix[1]	0.50	0.50
Energy (MJ/kg) and nutrient content (%)		
Apparent metabolizable energy[2]	12.13	12.26
Crude protein	19.50	18.00
Calcium	1.00	0.90
Total phosphorus	0.67	0.63
Available phosphorus[2]	0.45	0.40
Methionine	0.33	0.28
Lysine	1.10	0.95
Threonine[2]	0.74	0.65
Tryptophan[2]	0.22	0.19
Methionine/cystine	0.64	0.61

[1] Supplied per kg starting and finishing diet: Vitamin A: 20,000 and 15,000 IU; Vitamin D_3: 6,800 and 5,100 IU; Vitamin E: 32 and 24 IU; Vitamin K_3: 6.0 and 4.5 mg; Vitamin B_1: 4.0 and 3.0 mg; Vitamin B_2: 13.6 and 10.2 mg; Vitamin B_6: 8.0 and 6.0 mg; Vitamin B_{12}: 40.0 and 30.0 μg; Nicotinic acid: 40.0 and 30.0 mg; Pantothenic acid: 20.0 and 15.0 mg; Folic acid: 1.6 and 1.2 mg; Biotin: 260.0 and 195.0 μg; Cu: 10.0 and 8.0 mg; Fe: 60.0 and 40.0 mg; Zn: 80.0 and 60.0 mg; Mn: 80.0 and 60.0 mg; I: 600.0 and 400.0 μg; Se: 300.0 and 300.0 μg, respectively.

[2] Calculated according to NRC (1994).

some studies with growing pigs the efficacy of liquid MHA-FA relative to DL-Met ranged between 63 and 78% (Roth and Kirchgessner, 1986; Walz and pallauf, 1996; Schmidt, 2000; Zimmermann et al., 2005). These results are in line with those obtained in broilers (Wallis, 1999; Mandal et al., 2004). Other reports, however, showed no significant differences in biological efficacy between DL-Met and MHA-FA (Knight et al., 1998). Some of the results were inconsistent due to large variation in performances (Harms and Russell, 1994; Danner and Bessei, 2002), age and gender of animals (Jansman et al., 2003). The objective of the present study was to determine the effect of DL-Met or MHA-FA on performance and carcass traits in broilers, and to evaluate the biological efficacy of MHA-FA relative to DL-Met at 7-21 and 22-42 d of age based on different criteria.

MATERIALS AND METHODS

Bird and management

The study was carried out with five hundred and sixty seven-d-old Avian broilers. Chickens were divided into 7 experimental treatments equalizing body weight and variances. Each treatment was applied to 5 replicate pens of 16 birds each. The experiment was conducted over a period of 35 d (7-42 d), during which birds were housed in cages. Temperature, light intensity, immunization and ventilation were typical of settings used under local commercial condition. Water and feed in pellet form were provided *ad libitum*. The whole trial was divided into two phases, 7-21 and 22-42 d of age. In both basal diets nutrients were designed to meet the nutritional requirements with exception to methionine (Table 1). Two levels of DL-Met or four levels of MHA-FA were added to one of them to formulate the experimental diets (Table 2).

Analytical item and methods

Feed intake and body weight of each replicate pen were recorded weekly, and then calculated the average daily feed intake (ADFI) and daily weight gain (DWG). The feed conversion ratio (FCR) was calculated on the basis of unit consumed to unit body weight gain for each replicate separately.

At 42 d of age, five birds were picked up randomly from each treatment (one broiler per replica). They were starved for 12 h (indeed water was supplied *ad libitum*), and were sacrificed as per standard procedure for evaluation of carcass characteristics including the yield of defeathered weight, eviscerated weight and abdominal fat. The carcass

Table 2. Experimental design (%)

Dietary treatment	Methionine source	Starting diet		Finishing diet	
		Addition level[3]	Met+cys	Addition level[3]	Met+cys
1	Basal	0.000	0.640	0.000	0.610
2	DL-Met[1]	0.070	0.710	0.050	0.660
3	DL-Met	0.160	0.800	0.080	0.690
4	MHA-FA[2]	0.118	0.744	0.071	0.672
5	MHA-FA	0.143	0.766	0.074	0.675
6	MHA-FA	0.221	0.834	0.112	0.709
7	MHA-FA	0.268	0.876	0.140	0.733

[1] DLM = DL-methionine. [2] MHA-FA = Liquid methionine hydroxy analogue-free acid. [3] Values which were analyzed.

Table 3. The effect of supplemental DL-Met and MHA-FA on broiler performance from 7 to 21 d

Attributes	Dietary treatment							Statistical	
	1	2	3	4	5	6	7	Pooled SEM	Probability
7-21 d									
BW at 21 d (g)	577[c]	601[ab]	600[ab]	593[bc]	605[ab]	618[a]	593[bc]	8.6	p<0.05
ADFI[1] (g/d)	59.06	54.17	55.21	54.92	55.40	55.77	54.72	1.203	NS
DWG[2] (g/d)	33.24[c]	34.85[b]	34.98[b]	34.53[b]	35.35[ab]	36.60[a]	34.57[ab]	1.056	p<0.05
FCR[3] (g/g)	1.687[a]	1.555[bc]	1.580[bc]	1.591[b]	1.569[bc]	1.524[c]	1.584[bc]	0.042	p<0.05
22-42 d									
BW at 42 d (g)	1,855	1,967	1,972	1,962	1,927	1,947	1,904	24.3	NS
ADFI[1] (g/d)	139.61	140.59	140.84	142.51	141.63	140.71	137.85	3.524	NS
DWG[2] (g/d)	60.50	64.41	65.12	64.65	62.75	62.58	62.16	2.137	NS
FCR[3] (g/g)	2.317[a]	2.184[b]	2.164[b]	2.204[b]	2.258[ab]	2.249[ab]	2.214[b]	0.052	p<0.05

[a-c] Means bearing different superscripts in a row differ significantly (p<0.05).
[1] ADFI = average daily feed intake. [2] DWG = daily weight gain.
[3] FCR = feed conversion ratio (calculated on the basis of unit consumed to unit body weight gain).

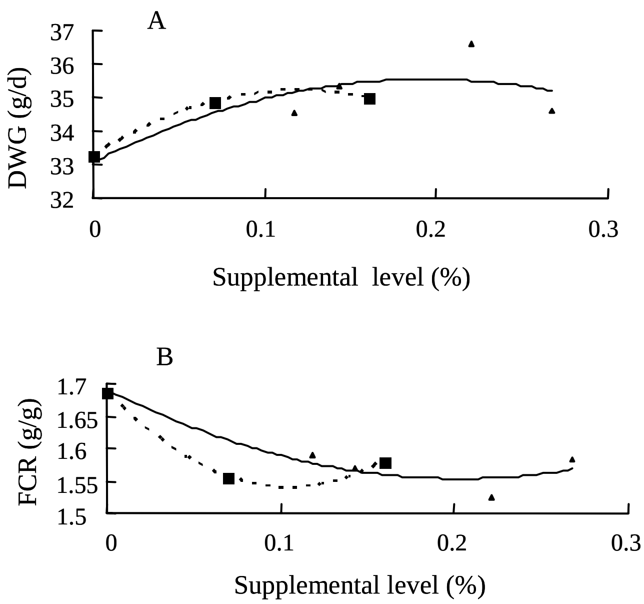

Figure 1. Bioavailability of MHA-FA relative to DL-Met based on DWG and FCR form 7 to 21 d. The dashed line followed by DL-Met and solid line followed by MHA-FA. A: Regression equation for DL-Met is $Y = -142.02X^2+34.71X+33.07$ ($R^2 = 0.68$), for MHA-FA is $Y = -65.12X^2+25.36X+33.07$ ($R^2 = 0.74$). B: Regression equation for DL-Met is $Y = 13.356X^2-2.841X+1.691$ ($R^2 = 0.72$), for MHA-FA is $Y = 3.356X^2-1.362X+1.691$ ($R^2 = 0.78$).

was cut to standard parts that included back, breast, thigh, neck and wings. The various carcass trait recorded, were then expressed in terms of percentage of live weight.

At the beginning of experiment, ten broilers were randomly picked up to slaughter after 24 h starvation with water available, were then beaten into mash, the content of body crude protein (CP) and energy were measured (AOAC, 1996). At the end of experiment, five broilers were taken randomly per treatment (one bird per replica) to determine the body CP or energy content according to the same methods as mentioned above. CP or energy deposition ratio was calculated as following:

CP or energy deposition ratio (%)

$$= \frac{\text{Increment of body CP or energy during experiment}}{\text{Intake of CP or energy during experiment}}$$

Statistical analysis

The experimental data were analyzed using the GLM Procedure of SAS (1996). Difference between means was tested for F-value significance (p<0.05) by using Duncan's Multiple Range Test. Quadratic analysis was used with the nonlinear procedure in SAS/STAT software for the dependent variables (DWG and FCR) to determine the bioavailability of MHA-FA relative to DL-Met. The statistical model was:

$$Y = \alpha+\beta_1X+\beta_2X^2$$

Where Y = response parameter; β_1 and β_2 = the ratio of regression coefficients; X = added analyzed MHA-FA or DL-Met. According to Yang (2000), the RBV of MHA-FA compared with DL-Met was calculated by expressions described as below, whereas $X_{DL\text{-}Met}$ and $X_{MHA\text{-}FA}$ mean supplemental level of DL-Met and MHA-FA for the optimal value of responded parameter ($Y_{DL\text{-}Met}$ or $Y_{MHA\text{-}FA}$), respectively.

$$RBV(DWG) = \frac{Y_{MHA-FA}}{Y_{DLM}} \times \frac{X_{DLM}}{X_{MHA-FA}}$$

$$RBV(FCR) = \frac{Y_{DLM}}{Y_{MHA-FA}} \times \frac{X_{DLM}}{X_{MHA-FA}}$$

To determine the RBV of MHA-FA relative to DL-Met based on breast meat yield, thigh meat percentage, body energy content and energy deposition ratio, slope-ratio analysis was used with the GLM procedure in SAS/STAT software. The statistical model was:

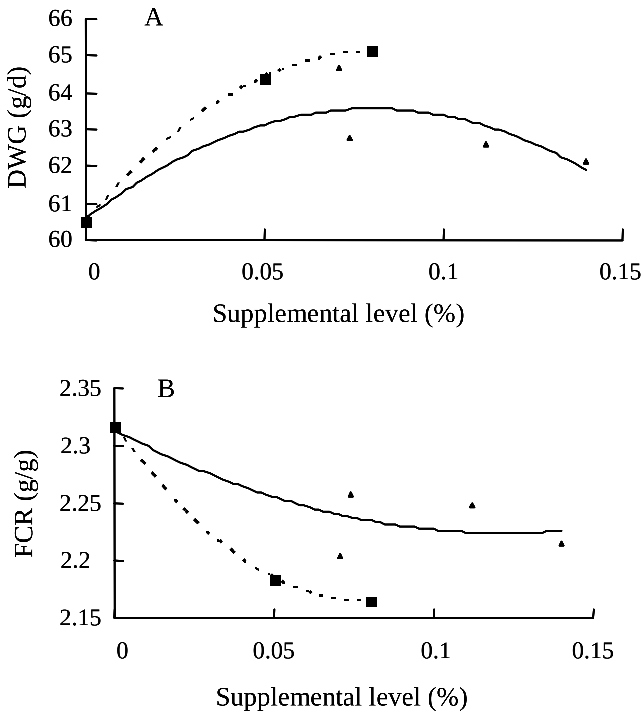

Figure 2. Bioavailability of MHA-FA relative to DL-Met based on DWG and FCR from 22 to 42 d. The dashed line followed by DL-Met and solid line followed by MHA-FA. A: Regression equation for DL-Met is $Y = -868.14X^2+127.77X+60.58$ ($R^2 = 0.77$), for MHA-FA is $Y = -465.49X^2+74.62X+60.58$ ($R^2 = 0.79$). B: Regression equation for DL-Met is $Y = 30.41X^2-4.367X+2.314$ ($R^2 = 0.66$), for MHA-FA is $Y = 6.047X^2-1.473X+2.314$ ($R^2 = 0.71$).

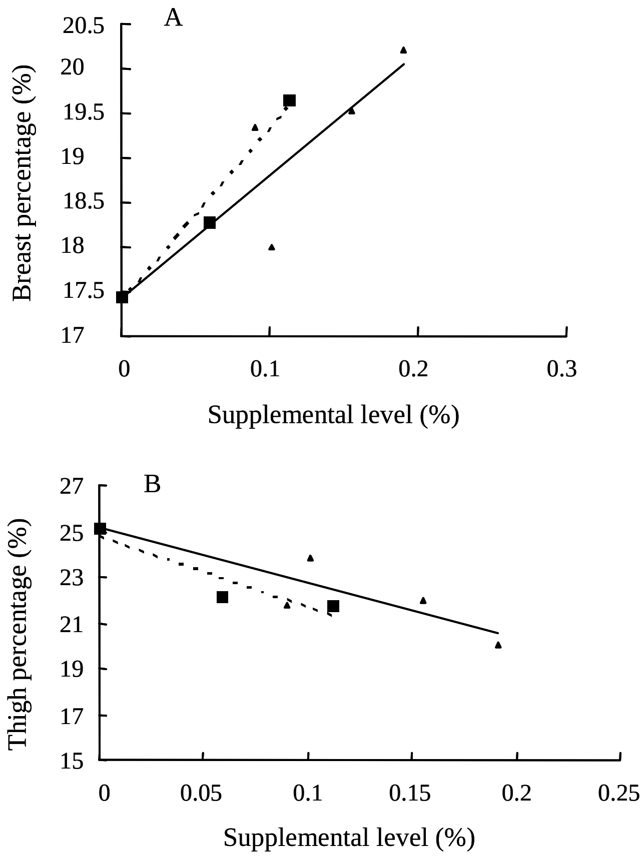

Figure 3. Bioavailability of MHA-FA relative to DL-Met on breast and thigh percentage at 42 d of age. The dashed line followed by DL-Met and solid line followed by MHA-FA. A: Regression equation for DL-Met is $Y = 19.02X+17.39$ ($R^2 = 0.82$), for MHA-FA is $Y = 14.02X+17.39$ ($R^2 = 0.86$). B: Regression equation for DL-Met is $Y = -29.77X+24.75$ ($R^2 = 0.78$), for MHA-FA is $Y = -21.35X+24.75$ ($R^2 = 0.74$).

$$Y = \alpha + \beta X$$

Where Y = response criteria; α = intercept (the minimum performance); β = slope for DL-Met or MHA-FA, X = added analyzed MHA-FA or DL-Met; the RBV of MHA-FA to DL-Met is given by $RBV = \beta_{MHA\text{-}FA}/\beta_{DL\text{-}Met}$

RESULTS

Growth performance and FCR

Data from 7 to 21 d of age were summarized in Table 3. Compared with the control, chickens fed the basal diet supplemented with 0.221% MHA-FA (treatment 6) showed significantly increases ($p<0.05$) in body weight, DWG and improvement in FCR. No significant difference of feed intake was observed among treatments ($p>0.05$). The data was further employed to generate a dose-response curve as shown as Figure 1. Based on regression analysis, the relative effectiveness of MHA-FA to DL-Met from 7 to 21 d of age was determined to be 64% for DWG and 55% for FCR, respectively.

The final body weight, ADFI, DWG and FCR from 22 to 42 d of age were given in Table 3. Despite no significant difference ($p>0.05$) was observed in the BW at 42 d of age, ADFI and DWG between treatments, broilers with diets supplemented with DL-Met or MHA-FA yielded a better ($p<0.05$) FCR compared to the un-supplemented control. By plotting these data in Figure 2, the response data for both DWG and FCR followed quadratic curves. Analysis based on DWG and FCR at 42 d of age indicated that the bioavailability of MHA-FA relative to DL-Met was 85% and 60%, respectively.

Carcass characteristics

Data of carcass characteristics at 42 d of age were presented in Table 4. Dressed weight or dressing percentage did not differ statistically, but dressed weight was numerically higher ($p>0.05$) in MHA-FA and DL-Met added diets. Breast percentage in the control and treatment 5 were significantly lower ($p<0.05$) than those in other treatments but statistically similar to treatment 2. Chickens fed the basal diet had the highest percentage of thigh meat yield and the lowest resulted from treatment 7 ($p<0.05$).

Table 4. Carcass characteristics of broilers in different treatments

Attributes	Dietary treatment							Statistical	
	1	2	3	4	5	6	7	Pooled SEM	Probability
Dressed weight (g)	1,757	1,856	1,862	1,856	1,826	1,858	1,793	10.1	NS
Dressing percentage[1] (%)	94.75	94.38	94.46	94.58	94.75	95.47	94.16	1.054	NS
Breast percentage[2] (%)	17.46[c]	18.30[bc]	19.66[ab]	19.35[ab]	17.99[c]	19.53[ab]	20.20[a]	0.493	p<0.05
Thigh percentage[3] (%)	25.1[a]	22.1[bc]	21.7[bc]	21.7[bc]	23.7[ab]	21.9[bc]	20.0[c]	0.36	p<0.05
Abdominal fat percentage[4] (%)	2.25[a]	2.27[a]	1.81[b]	2.11[ab]	2.20[a]	2.21[a]	2.24[a]	0.143	p<0.05

[a-c] Means bearing different superscripts in a row differ significantly (p<0.05).
[1] Dressing percentage = dressed weight/ live weight. [2] Breast percentage = breast meat weight/carcass weight.
[3] Thigh percentage = thigh meat weight/carcass weight. [4] Abodominal fat percentage = abdominal fat weight/live weight.

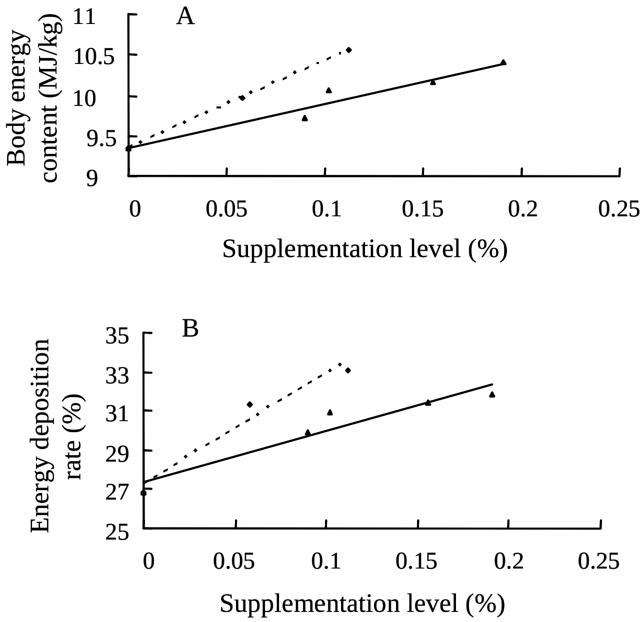

Figure 4. Efficacy of MHA-FA relative to DL-Met based on body energy content and energy deposition ratio. The dashed line followed by DL-Met and solid line followed by MHA-FA. A: Regression equation for DL-Met is Y = 10.20X+9.38 (R^2 = 0.85), for MHA-FA is Y = 5.32X+9.38 (R^2 = 0.91). B: Regression equation for DL-Met is Y = 50.15X+27.67 (R^2 = 0.89), for MHA-FA is Y = 24.01X+27.67 (R^2 = 0.91).

Abdominal fat percentage was not affected by MHA-FA supplementation, but was significantly lower (p<0.05) at 0.16% DL-Met supplementation (treatment 3). Figure 3 showed that breast and thigh meat percentage were linearly responded to DL-Met or MHA-FA supplementation. The efficacy of MHA-FA in comparison to DL-Met was 74 and 72% for breast and thigh meat percentage, respectively.

Energy and protein deposition

Body energy content in treatment 3 was higher than that in control (p<0.05), no significant difference was observed between other treatments (p>0.05). Energy deposition ratio increased significantly (p<0.05) with the addition of DL-Met or MHA-FA in basal diets, the highest value of which was observed in treatment 3 and the lowest was from the un-supplemented control. Though apparently there was reduction of body crude protein content either in DL-Met or MHA-FA added treatments compared with the control, but the differences were not significant except for treatment 5 and 6. The ratio of protein deposition was increased by supplementing DL-Met (treatment 3 and 2), but the effect was not observed when MHA-FA was added (4 to 7 treatments). Figure 4 showed that the body energy content and energy deposition ratio were markedly responded to the dietary supplementation of DL-Met or MHA-FA. Based on the established regression equation, the relative effectiveness of MHA-FA to DL-Met was determined to be 52% and 48% for body energy content and energy deposition ratio, respectively.

DISCUSSION

Growth performance or FCR both in 7-21 or 22-42 d of age was improved by supplementation of either DL-Met or MHA-FA to corn-soybean meal based diet, which agrees with the previous findings (Danner and Bessei, 2002; Mandal et al., 2004). However, in another study (Lu et al., 2003) supplementation of DL-Met or MHA-FA did not improve growth performance and feed efficiency for broilers during 0-3 weeks of age, the contradiction may attribute to the content of methionine or sulfur-containing amino acid in basal diet. In both phase of the experiment, basal diets were clearly deficient in methionine, confirmed by a significant response in growth rate or feed efficiency, irrespective of the source of supplemental methionine. Based on the data obtained from this experiment, 0.45 and 0.35% methionine are required at the aim of optimal DWG and FCR for 7-21 and 22-42 d of age, respectively. According to the proportional relationship between methionine and cystine provided by NRC (1994), requirement for cystine was calculate to be 0.35 and 0.31% for 7-21 and 22-42 d of age.

By employing statistical analysis, we calculated the RBV of MHA-FA compared with DL-Met was 64 and 85% for DWG, 55 and 60% for FCR from 7-21 and 22-42 d of age, respectively. Therefore, it can be pointed out that the broiler chicks responded lesser to MHA-FA than DL-Met in

Table 5. Effect of DL-Met or MHA-FA supplementation on energy and protein retention

Attributes	Dietary treatment							Statistical	
	1	2	3	4	5	6	7	Pooled SEM	Probability
Body energy content (MJ/kg)	9.35[b]	9.97[ab]	10.56[a]	9.72[ab]	10.15[ab]	10.15[ab]	10.40[ab]	0.412	p<0.05
Energy retention ratio (%)	26.81[d]	31.30[bc]	33.10[a]	29.85[c]	30.90[bc]	31.41[abc]	31.83[ab]	1.053	p<0.05
Body protein content[1] (g/kg)	185.0[a]	184.0[ab]	178.2[abc]	182.3[ab]	176.8[bc]	172.9[c]	182.9[ab]	1.23	p<0.05
Protein retention ratio (%)	46.90[b]	51.43[a]	52.11[a]	50.03[ab]	48.77[ab]	46.97[b]	50.39[ab]	1.075	p<0.05

[a-c] Means bearing different superscripts in a row differ significantly (p<0.05).
[1] Bdoy protein content is determined as crude protein.

early age. It may be attributed to the time required for adjustment of the birds to the taste to MHA-FA, MHA-FA absorption efficiency which might be less at early age, lesser conversion efficiency of MHA-FA to DL-Met at an early age due to lesser development of transamination process or inhibition of MHA-FA to the development of gastrointestinal tract. However, it needs further investigation to prove these suppositions. The similar phenomenon is also observed previous researchers (Crespo and Esteve- Garcia, 2002; Mandal et al., 2004).

Sulfur-containing amino acids were easy to be deposited in breast meat (Nitsan et al., 1981), therefore, it is a sensitive parameter to estimate the RBV for different methionine sources. The RBV of MHA-FA compared with DL-Met regarding to breast meat is lower than that regarding to DWG (Esteve-Garcia and Llaurado, 1997; Lemme et al., 2002), which in line with the finding of the present study (74 for breast yield and 85% for DWG). Abdominal fat yield was decreased when sulfur amino acids were added (Moran, 1994). Research (Esteve-Garcia and Llaurado, 1997) showed that, DL-Met was more effective than MHA-FA regarding to decreasing abdominal fat, also, DL-Met supplementation leaded a low feed consumption but this phenomenon was not appeared when MHA-FA was added. The author inferred that less abdominal fat pad by adding DL-Met may result from fewer feed consumption. In this research we found different sources or variable levels of methionine did not affect feed consumption, abdominal fat pad was decreased at higher levels of DL-Met but MHA-FA had no effect. This observation suggested MHA-FA can't instead of DL-Met in some biological functions such as regulation abdominal fat synthesis.

There is still a scarcity of information on energy and protein content in broiler. Body protein retention ratio decreased as dietary protein increasing (Kang et al., 2002; Sklan and Plavnik, 2002; Song, 2003). In this study, we found that content and retention of energy in body was increased, but those of protein was not affected by adding methionine.

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