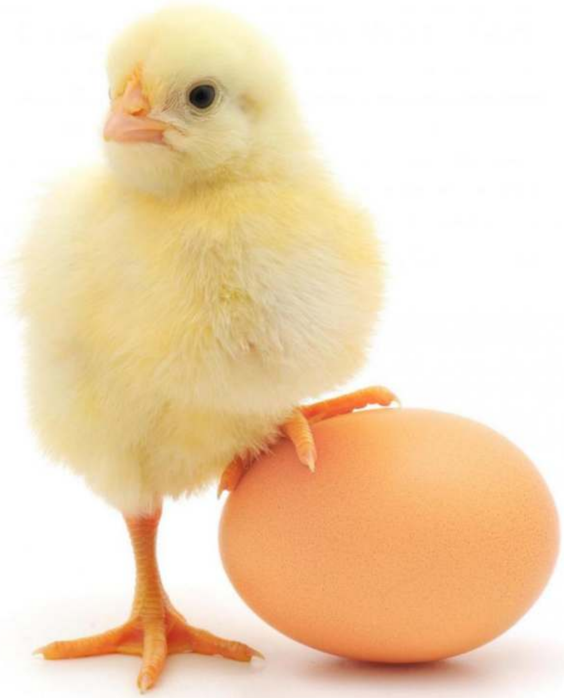
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (8): 744-752, 2006
ISSN 1682-8356

Effect of Adding Methionine Hydroxy Analogue as Methionine Source at the Commercial Requirement Recommendation on Production Performance and Evidence of Ascites Syndrome of Male Broiler Chicks Fed Corn-Soybean Based

Chaiyapoom Bunchasak, Takawan Sooksridang and Ratchadaporn Chaiyapit
Department of Animal Science, Faculty of Agriculture, Kasetsart University,
Bangkhen, Bangkok, 10900, Thailand

Abstract: An experiment was carried out to determine effects of adding liquid DL-methionine hydroxy analog free acid (LMA) as source of dry DL-methionine (DLM) on growth performance, carcass quality and incidence of ascites syndrome during 0-6 weeks of age. Nine hundred commercial male broiler chicks (ROSS strain) were used. The chicks were divided into 6 groups; each group consisted of 6 replications of twenty five chicks each, and the chicks were kept in floor pens. Water and feed were offered *ad libitum* throughout the experiment. The following six experimental diets were provided: 1) corn-soybean based diet deficient in methionine, 2) corn-soybean based diet deficient in methionine added with DLM to meet its requirements 3) the first group added with LMA at 88% bioefficacy compared to DLM of the second group 4) the first group added with LMA at the 84% bioefficacy 5) the first group added with LMA at the 80% bioefficacy and 6) the first group added with LMA at the 76% bioefficacy. The results showed that during the starter period, weight gain of chicks fed LMA or DLM were significantly heavier than chicks received methionine-deficient diet ($P<0.01$), whilst addition of LMA at 88% bioefficacy of DLM slightly improved weight gain higher than the DLM group. Adding DLM or LMA significantly improved feed conversion ratio (FCR) and percentage of uniformity ($P<0.01$). No significant differences were found between supplementing DLM and various LMA on these parameters. During the grower period (3-6 weeks of age), weight gain, FCR, uniformity and feed intake of chicks that received diet supplemented with DLM or LMA were superior to the methionine-deficient group ($P<0.01$). Outer breast meat yields was significantly improved ($P<0.05$) and abdominal fat slightly decreased when methionine sources were added, while adding LMA at 80% bioefficacy of DLM tends to promote edible meat growth more than the other groups. As for incidence of ascites syndrome, significant effects of methionine sources on heart characteristic and hematocrit value were not seen, but adding methionine significantly depressed plasma triiodothyronine (T_3) (at 21 days of age) and seemed to increase plasma uric acid level. This suggests that LMA is an acceptable source of methionine for broiler chicks fed corn-soybean meal diets from day-old to market weight, if maximal growth performances are used as response criteria concerning commercial methionine requirements recommendation. Moreover, antagonistic effect of T_3 and uric acid in plasma due to methionine supplementation may be a key factor to eliminate occurrence of ascites syndrome in high productive chicks.

Key words: Carcass quality, growth performance, ascites, methionine sources

Introduction

Dry DL-methionine (DLM) and liquid DL-2-hydroxy-4-(methylthio) butanoic acid (LMA) are commonly used in poultry diet, particularly based on corn-soybean. However, several investigators have reported that the average relative bioavailability of LMA compared with DLM in various animal species ranged from 65 to 90% (Reid *et al*., 1982; Van Weerden and Schutte, 1984; Scott, 1987; Harms and Russell, 1994; Hoehler and Hooge, 2003 and Bunchasak and Keawarun, 2006).

In broiler chickens, when used as the only source of sulphur amino acids (SAA), Baker and Boebel (1980) reported that the bioavailability of purified form of LMA was 78% of DLM. Similarly, based on the regression coefficients, equimolar bioefficacy of LMA relative to DLM was 80% for daily weight gain and 83% for feed conversion ratio (FCR) (Esteve-Garcia and Liaurado, 1997). Recently, based on the recommendation of NRC (1994), we found that the relative effectiveness of LMA compared to DLM for response in weight gain, FCR and uniformity was certainly not less than 80% (Bunchasak and Keawarun, 2006). Additionally, the relative bioefficacies of LMA and LDM between starter and grower periods may perhaps be different (Bunchasak and Keawarun, 2006). However, as for the commercially recommended level of methionine required to achieve maximal production performance, it is generally accepted that the recommendation is always higher than that of NRC (1994). Based on different growth production targets and levels of methionine recommendation, therefore, the relative bioefficacy of LMA compared to DLM for production performance may be different.

Table 1: Composition of basal diet (%)

Ingredients	Starter period (0-3 wks of age)	Grower period (3-6 wks of age)
Corn (7.15 %CP)	46.42	54.62
Soybean meal (43.5 %CP)	42.79	34.22
Soybean oil	6.39	6.92
L-Lysine	0.07	0.20
L–threonine	0.02	0.02
Monocalcium phosphate (Ca15/P21)	1.93	1.55
Choline chloride (60%)	0.10	0.27
Limestone (38.67%)	1.49	1.45
Sodium bicarbonate (27%)	0.20	0.20
Salt	0.24	0.20
Premix[3]	0.25	0.25
SACOX 120[1]	0.05	0.05
MTB100[2]	0.05	0.05
Total	100	100

[1] SACOX 120: Coccidostate product [2]MTB 100: Mycotoxin binder. [3]Premix: BP 920 Broiler consist of Vitamin A 4.00 MIU; D_3 0.56 MIU; E 4.48 g; K_3 0.68 g; B_1 0.52 g; B_2 2.00 g; B_6 0.68 g; B_{12} 5.60 mg; Pantothenic acid 3.36 g; Niacin 6.80 g; Folic acid 0.17 g; Biotin 0.014 g; Choline chloride 200 g; Se 0.03 g; Fe 17.2 g; Mn 26.40 g Zn 26.40 g; Cu 3.20 g; I 0.32 g; Feed preservative substant 4.80 g and carrier added to 1.00 kg premix.

Nowadays, due to very successful genetic selection techniques and nutritional improvements, 2.5-2.8 kg of body weight of the chicks can be achieved in 42 days, while FCR is just around 1.7-1.8. Conversely, incidence of ascites that is a condition in which excess amounts of fluids accumulation in the body cavity due to high metabolic loads (metabolic disorder) has increased worldwide over the past several years. Enlargement of heart and accumulation of fluid in pericardium due to high activities of heart and lungs to provide enough oxygen cause development of ascites syndrome (Julian, 1990 and Government of Alberta, 2004).

Recently, Keawarun and Bunchasak (2005) have reported that male broiler chicks fed with LMA had smaller heart weight than those fed with DLM supplementation, while production performance was not different. Because of the different chemical structure of LMA from DLM, Danner and Bessei (2002) reported that LMA does not represent a complete amino acid and has to be transformed to methionine by transamination. Keawarun and Bunchasak (2005) hypothesized that different sources of methionine (LMA vs DLM) may have different effects on blood circulation and heart activities of the birds. However, there has been no further report on this aspect.

Therefore, this study was conducted to investigate relative bioefficacy of LMA compared to DLM based on the commercial recommendation of total sulfur amino acids requirement and its effect on incidence of ascetic syndrome of the male broiler chicks from 0 to 6 weeks of age fed with corn-soybean based diet.

Materials and Methods

Management and diets: The study had a completely randomized design. Nine hundred commercial male broiler chicks (ROSS-208 strain) were used during 0-6 weeks of age. The chicks were divided into 6 groups; each group consisted of 6 replications of twenty five chicks each. They were kept, maintained and treated in compliance with the accepted standards for the humane treatment of animals. The chicks were kept in floor pens (100X200 cm) located in an evaporative cooling house system; hence each chick had approximately 800 cm^2 of floor space. The lighting management and vaccinations were provided according to the commercial practice. Diets were fed as a 1-mm and 3-mm pellet in the starter period (1-21 days) and the grower period (22-42 days), respectively. Water and feed were offered *ad libitum* throughout the experiment.

Two sources of methionine, DLM (Sumimet-P, 99% pure, Sumitomo Chemical Co., Ltd., Tokyo, Japan) and LMA (Sumimet-L, containing 88% of active substance, Sumitomo Chemical Co., Ltd.) were used. For experimental diets formulation, the ratio between DLM and LMA ranged between 88, 84, 80 and 76% based on the assumption that 100 units of liquid LMA can be replaced by 88, 84, 80 and 76 units of DLM. Six experimental diets (iso-nitrogenous, 22% crude protein and iso-caloric, 3,100 kcal ME/kg during the starter period; and 20% crude protein with 3,200 kcal ME/kg during the grower period) were provided as following:

1) A corn-soybean based diet deficient in methionine, therefore, the calculated methionine+cysteine (TSAA) contents of the methionine-deficient diet in the starter and grower periods were 0.75 and 0.66%, respectively.
2) A methionine-deficient diet added with DLM to meet commercial requirements recommendation (DLM were added in methionine-deficient diet to reach a TSAA content of 1.03% in the starter period and 0.93% in the grower period).
3) A methionine-deficient diet added with LMA 1.14 times (weight/weight) of DLM of the second group (88% bioefficacy of LMA relative to DLM).
4) A methionine-deficient diet added with LMA 1.19 times of DLM of the second group (84% bioefficacy of LMA relative to DLM).
5) A methionine-deficient diet added with LMA 1.25 times of DLM of the second group (80% bioefficacy of LMA relative to DLM).
6) A methionine-deficient diet added with LMA 1.32 times of DLM of the second group (76% bioefficacy of LMA relative to DLM).

Feed formula and nutrients contained in the basal diet are shown in Tables 1 and 2, respectively. The test substances were added to aliquots of the basal diet at the expense of corn starch (Table 3). Feed samples were collected and ground using a 1-mm screen in

Table 2: Nutrients in basal diet (according to theoretical calculation and chemical analysis)

Nutrients	Starter diet		Grower diet	
	Calculated	Analysis	Calculated	Analysis
Dry matter	-	93.26	-	90.94
Metabolizable energy for poultry (Kcal/kg)	3100	-	3200	-
Crude protein (%)	22	22.32	20	20.40
Crude fat (%)	-	10.69	-	11.61
Methionine	0.35	0.34	0.31	0.27
Cysteine	0.40	0.38	0.35	0.28
Methionine+Cysteine	0.75	0.72	0.66	0.55
Asparagine	2.46	2.35	2.10	2.07
Threonine	0.88	0.83	0.80	0.79
Serine	1.17	0.95	1.01	0.97
Glutamine	4.45	4.18	3.74	3.79
Glycine	1.00	0.96	0.92	0.86
Alanine	1.07	1.07	0.98	0.96
Valine	1.11	0.99	0.99	0.89
Isoleucine	1.03	0.90	0.94	0.81
Leucine	1.84	1.79	1.63	1.66
Tyrosine	0.88	0.71	0.66	0.75
Phenylalanine	1.09	1.06	1.03	1.07
Histidine	0.62	0.66	0.57	0.59
Lysine	1.35	1.32	1.247	1.27
Proline	1.24	1.30	1.15	1.14
Arginine	1.59	1.44	1.50	1.29

grinder. All diets were analyzed for protein, fat, calcium and total phosphorus according to the AOAC (1990) methods. Amino acids composition of the basal diet in both starter and grower periods were determined using Amino Acid Analyzer.

Measurements: The following performance variables were determined: weight gain, feed intake, FCR, mortality rate and uniformity, for each period and for the total period (1 to 42 days). The chicks were weighed at the end of each period, while feed intake was measured daily. All pens were checked daily for mortality. For the determination of feed intake and FCR, the day of death and the weight of the dead birds were included in the calculation.

At the end of experiment, after overnight feed deprivation, 18 chicks from each group of which body weights are close to the group mean were chosen and killed using CO_2 to evaluate carcass quality. For CO_2 killing, birds were killed by asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2) for 1.5-2.0 min. Before the birds were killed, blood from wing vein of each bird was taken in order to investigate hematocrit (% of pack cell volume) and plasma was separated from blood by centrifugation at 3,000 x g at room temperature for 10 min. and stored at -20°C for chemical analysis. Heparin was used in order to prohibit blood clotting. Subsequently, radioactivity was counted for measurement of level of triiodothyronine hormone (T_3) in plasma using a commercial test kit (Diagnostic Products Corporation) and concentration of plasma uric acid was determined by the colorimetric method using phosphotungstate-carbonate (Life science dynamics of arnaparn Co., LTD).

Carcass yield was obtained by weighing the birds before and after fasting for 12 hours, bleeding, scalding, plucking, evisceration, viscera and abdominal fat weighing and eviscerated carcass weighing. According to Cabel *et al.* (1987) the fat surrounding gizzard was also counted as abdominal fat. Heart of each bird was taken and stored at -20°C until parameter for heart disorder such as heart weight, right-left ventricle area, heart area, right-left ventricle weight and right-left ventricle thickness were measured according to McGovern *et al.* (2001).

Statistical analysis: All data were statistically analyzed using the analysis of variance (ANOVA). The differences between the means of groups were separated by Duncan's Multiple Range Test (Duncan, 1955). Statements of statistical significance are based on $P<0.05$. All statistical analyses were done in accordance with the method of Steel and Torrie (1980).

Results and Discussion

Growth performance: Effects of experimental diets on growth performances of broiler chicks during 0-3 and 3-6 weeks of age are presented in Table 4. During 0-3 weeks of age, supplementing methionine sources (DLM or LMA) resulted in significant improvement of growth performance such as body weight gain, feed intake, FCR and uniformity ($P<0.01$). It seems that LMA supplementation at 88% bioefficacy of DLM had slightly heavier body weight than the DLM group, while mortality

Table 3: Methionine sources and corn starch supplements to the basal diet

Groups	Periods	Supplementary methionine (mg/100 g)	Corn starch (mg/100 g) in diet (%)	Methionine contains
Basal diet (deficient in methionine)	Starter period	0	367.0	0.35
	Grower period	0	350.0	0.30
DLM	Starter period	278.0	89.0	0.63
	Grower period	265.0	85.0	0.56
LMA (as 88% bioefficacy of DLM)	Starter period	317.0	50.0	0.67
	Grower period	302.0	48.0	0.60
LMA (as 84% bioefficacy of DLM)	Starter period	331.0	36.0	0.68
	Grower period	315.0	34.0	0.61
LMA (as 80% bioefficacy of DLM)	Starter period	347.5	19.4	0.69
	Grower period	331.0	18.6	0.63
LMA (as 76% bioefficacy of DLM)	Starter period	367.0	0	0.72
	Grower period	350.0	0	0.65

DLM, dry DL-methionine; LMA, DL-methionine hydroxy analog-free acid.

Table 4: The effect of dietary treatments on growth performance of male broiler chickens from 0-3 weeks and 3-6 weeks of age

Item	Basal diet	DLM	Relative bioefficacy of LMA to DLM (%)				P-value
			88%	84%	80%	76%	
Starter period (0-3 weeks)							
Initial weight (g)	38.6	38.6	38.6	38.6	38.6	38.6	
Final weight (g)	774±14.47^{a}	952±11.07^{b}	977±26.96^{b}	967±9.33^{b}	958±11.84^{b}	958±5.28^{b}	<0.0001
Body weight gain (g)	735±14.33^{a}	914±11.21^{b}	938±26.99^{b}	929±9.32^{b}	920±11.76^{b}	919±5.09^{b}	<0.0001
Feed intake (g/b)	965±16.44^{a}	1092 ±8.12^{b}	1,157±33.69^{c}	1,090±18.04^{b}	1,126±8.05^{bc}	1,089±5.62^{b}	<0.0001
Feed conversion ratio	1.31±0.01^{a}	1.20±0.01^{bc}	1.23±0.01^{b}	1.17±0.02^{c}	1.23±0.02^{b}	1.18±0.001^{c}	<0.0001
Mortality (%)	0.00±0.00^{a}	0.00±0.00^{a}	2.67±0.84^{b}	0.00±0.00^{a}	0.67±0.66^{a}	1.33±0.84^{ab}	0.0104
Uniformity (%)	88.08±1.42^{a}	91.26±0.56^{b}	92.52±0.42^{b}	91.48±0.38^{b}	91.70±0.63^{b}	91.33±0.61^{b}	0.0057
Grower period (3-6 weeks)							
Final weight (g)	2,376±52.50^{a}	2,725±37.96^{b}	2,804±65.35^{b}	2,735±35.85^{b}	2,789±29.26^{b}	2,721±25.20^{b}	<0.0001
Body weight gain (g)	1,602±44.06^{a}	1,773±33.74^{b}	1,827±42.12^{b}	1,768±27.89^{b}	1,831±31.61^{b}	1,763±21.36^{b}	0.0007
Feed intake (g/b)	3,067±74.17^{a}	3,191±49.24^{ab}	3,362±99.18^{b}	3,234±45.91^{ab}	3,370±55.64^{b}	3,189±31.75^{ab}	0.0164
Feed conversion ratio	1.92±0.01^{a}	1.80±0.02^{b}	1.84±0.03^{b}	1.83±0.01^{b}	1.84±0.02^{b}	1.81±0.02^{b}	0.0023
Mortality (%)	0.00±0.00	0.00±0.00	0.76±0.76	1.33±1.33	0.00±0.00	0.00±0.00	0.5398
Uniformity (%)	88.07 ±1.04	89.62±1.12	90.07±1.15	89.82±1.07	92.29±0.51	89.06 ±1.41	0.0657

a, b and c Means within row with no common superscripts differ significantly (P<0.05)

rate of this group was significantly higher than those of the other groups (P<0.05). Among LMA-added groups, supplementing LMA at 84 and 76% bioefficacy of DLM had better FCR than the other groups (P<0.01) except DLM group.

Since relative bioefficacy of LMA in this study was calculated as 88, 84, 80 or 76% equivalent to DLM, body weight of chicks fed LMA was slightly better than DLM (of no significance) and FCR was not different from the DLM. It can be said that at commercial methionine requirement recommendation, bioefficacy of LMA relative to DLM for weight gain and FCR during the starter period might be 88% (weight/weight). The findings of the current study disagree with Esteve-Garcia and Llaurado (1997); Baker and Boebel (1980); Hoehler and Hooge (2003), who reported that bioefficacy of LMA relative to DLM for these parameters was around 65-83% only. The result on feed intake is in accord with Bunchasak *et al.* (1996a, 1996b, 1997); Bunchasak and Silapasorn (2005); Bunchasak and Keawarun (2006); and Schutte and Pack (1995), who reported that feed consumption of broiler chicks or laying hen increased due to DLM supplementation. Surprisingly, supplementing LMA as 88% bioefficacy compared to DLM induced a high mortality rate. Only reason that can be given is that the birds may have suffered from too high a metabolic or physiological load since growth rate of this group was dramatically higher than the standard performance of birds' strain (ROSS 208) due to high feed intake.

During 3-6 weeks of age, supplementing both DLM and LMA significantly improved body weight and FCR (P<0.01), and feed intake of the chicks fed with LMA at 80 and 88% bioefficacy of DLM was significantly higher than the methionine-deficient group (P<0.05). On the other hand, varied levels of LMA supplementation did not significantly affect growth performance and mortality rate. During this period, growth performances of chicks which received diet supplemented with DLM or LMA were still superior to the methionine-deficient group. In addition, chicks fed with LMA at 80 or 88% bioefficacy of DLM tend to have better body weight gain than the DLM group. Thus, chicks fed with LMA at 88% bioefficacy of DLM improved weight gain by 14.04% compared to those which ate methionine-deficient diet, while improvement was 27.6% during the starter period. This result is not surprising since young animals are always more

Table 5: The effect of dietary treatments on growth performance of male broiler chickens from 0-6 weeks of age

	Basal diet	DLM	Relative bioefficacy of LMA to DLM (%)				P-value
			88%	84%	80%	76%	
Initial weight (g)	38.6	38.6	38.6	38.6	38.6	38.6	
Final weight (g)	2,376±52.50[a]	2,725±37.96[b]	2,804±65.35[b]	2,735±35.85[b]	2,789±29.26[b]	2,721±25.20[b]	<0.01
Body weight gain (g)	2,338±52.39[a]	2,687±37.90[b]	2,765±65.35[b]	2,697±35.93[b]	2,751±29.36[b]	2,682±24.99[b]	<0.01
Feed intake (g/b)	4,030±86.24[a]	4,277±55.16[b]	4,498±127.56[b]	4,319±54.50[b]	4,491±54.76[b]	4,268±39.71[b]	<0.01
Feed conversion ratio	1.72±0.01[a]	1.59±0.01[b]	1.63±0.02[b]	1.60±0.01[b]	1.63±0.02[b]	1.59±0.01[b]	<0.01
Mortality (%)	0.00±0.00	0.00±0.00	3.33±1.23	1.33±1.33	0.67±0.67	1.33±0.84	0.09
Uniformity (%)	88.08±1.17[a]	90.44±0.69[ab]	91.61±0.67[b]	90.65±0.66[b]	92.31±0.39[b]	90.19±0.91[ab]	<0.05

a and b Means within row with no common superscripts differ significantly (P<0.05)

Table 6: The effect of dietary treatments on carcass components of male broiler chicks at 42 days of age

Carcass quality (% of body weight)	Basal diet	DLM	Relative bioefficacy of LMA to DLM (%)				P-value
			88%	84%	80%	76%	
Carcass yield	79.96±0.37	80.96±0.51	80.19±0.49	80.67±0.30	81.10±0.38	80.03±0.50	0.28
Abdominal fat	2.10±0.12	1.75±0.11	1.69±0.08	1.81±0.12	1.68±0.11	1.78±0.12	0.07
Thigh	12.02±0.15	11.97±0.19	11.95±0.18	11.99±0.20	12.25±0.17	12.02±0.21	0.89
Drumstick	10.38±0.12	10.36±0.13	10.14±0.16	10.20±0.14	10.49±0.12	10.11±0.15	0.30
Inner breast meat	3.19±0.07[a]	3.59±0.06[b]	3.70±0.08[b]	3.67±0.06[b]	3.56±0.07[b]	3.53±0.08[b]	<0.01
Outer breast meat	12.03±0.26[a]	13.83±0.30[b]	13.45±0.26[b]	13.66±0.26[b]	13.65±0.25[b]	13.38±0.22[b]	<0.01
Breast meat[1]	15.21±0.29[a]	17.42±0.34[b]	17.15±0.27[b]	17.33±0.28[b]	17.22±0.29[b]	16.91±0.29[b]	<0.01
Wing	8.07±0.12	8.13±0.08	8.10±0.11	7.99±0.09	8.15±0.11	7.99±0.12	0.85
Edible meat[2]	45.67±0.35[a]	47.88±0.40[b]	47.35±0.35[b]	47.52±0.35[b]	48.11±0.31[b]	47.04±0.29[b]	<0.01

sensitive to nutrients deficiency than older ones. However, better data were obtained than several investigators who reported that increased methionine levels promoted a weight gain of about 10-14% when compared to the methionine-deficient diet (Bunchasak and Keawarun, 2006; Garlich, 1985; Huyghebaert, 1993; Rostagno and Barbosa, 1995). This high growth rate may have been due to high nutrients densities in dietary treatments since the nutrients requirements were based on the commercial recommendation.

Throughout the overall period (0-6 weeks of age) (Table 5), body weight of the broiler chicks increased when methionine sources were added (P<0.01). However, increase of LMA at 1.14, 1.19, 1.25 or 1.32 times DLM (88, 84, 80 or 76% bioefficacy of DLM) did not give any benefit to growth performance, although LMA supplementations seem to have improved percentage of uniformity more than DLM supplementations. This is in agreement with Romoser *et al.* (1976); Waldroup *et al.* (1981); Garlich (1985), who reported that when synthetic sources of methionine were used to meet the requirement for total sulfur amino acids in practical corn-soybean meal diets, chicks were capable of utilizing LMA and DLM at an indistinguishable efficiency. Therefore, it can be concluded that LMA is an acceptable source of methionine for broiler chicks fed with corn-soybean meal diets from day-old to market weight, if maximal growth performance is used as response criteria concerning commercial methionine requirements recommendation.

Carcass yields: The carcass components (% of body weight) at 42 days of age are shown in Table 6. Dressing carcass increased and abdominal fat content decreased when DLM and various LMA were added, although there were no significant differences. Significant improvements of outer breast meat (*pectoralis major*) and inner breast meat (*pectoralis minor*) were shown when both sources of methionine were added (P<0.01). When calculated as total edible meat (including breast meat, leg meat and wing), therefore, methionine supplementation can significantly improve yields. Among various LMA supplementations, it seems that adding LMA at 80% bioefficacy of DLM induced high meat yield of chicks.

Breast meat is considered as the most valuable part of broiler chickens. Moran (1994) reported that dietary SAA had high potential to stimulate breast meat growth compared to other amino acids. The results of present study also confirm the finding of this author. Since Schutte and Pack (1995); Hickling *et al.* (1990) and Meirelles *et al.* (2003) reported that requirement of SAA for optimal FCR and maximal breast meat growth were higher than for the greatest weight gain, this may be a reason why supplementing LMA at 80% bioefficacy of DLM had high meat production yield. This is in agreement with our previous suggestion that adding LMA 1.25 times DLM (weight/weight) or 80% bioefficacy of DLM might be an optimal level for meat production during grower period (Bunchasak and Keawarun, 2006). On the other hand, high LMA supplementation at 76% bioefficacy of DLM did not promote but suppressed meat production. This indicates that, under commercial methionine requirement recommendation, supplementing LMA at this level might be an excess to

Table 7: The effect of dietary treatments on heart characteristic of male broiler chicks at 42 days of age

Item	Basal diet	DLM	Relative bioefficacy of LMA to DLM (%)				P-value
			88%	84%	80%	76%	
Heart wt. (g)	12.39±0.50	12.04±0.37	12.34±0.32	12.43±0.38	12.04±0.47	12.14±0.54	0.97
Heart wt. (%BW)	0.52±0.02[a]	0.44±0.01[b]	0.44±0.01[b]	0.45±0.01[b]	0.43±0.02[b]	0.44±0.02b	<0.01
Right ventricle area $(mm)^2$	77.65±5.57	81.11±6.84	78.61±6.27	78.28±5.96	85.28±6.08	83.89±5.38	0.92
Left ventricle area $(mm)^2$	26.53±1.91	28.71±3.08	25.94±1.85	25.29±1.55	27.94±3.22	25.01±2.00	0.85
Heart area $(mm)^2$	368.24±12.53	381.11±11.39	365.72±15.48	365.17±10.39	370.00±15.10	379.94±16.81	0.93
Right ventricle wt (g)	0.968±0.064	0.982±0.052	0.968±0.062	1.025±0.056	0.963±0.053	1.008±0.067	0.96
Left ventricle wt. (g)	5.328±0.232	5.401±0.132	5.272±0.179	5.469±0.186	5.342±0.174	5.503±0.221	0.96
Right ventricle wt. (%BW)	0.041±0.003[a]	0.035±0.002[b]	0.035±0.002[b]	0.037±0.002[b]	0.034±0.002[b]	0.037±0.002[b]	<0.01
Left ventricle wt. (%BW)	0.222±0.010[a]	0.198±0.005[b]	0.188±0.006[b]	0.199±0.006[b]	0.191±0.009[b]	0.201±0.008[b]	0.03
Right ventricle thickness (cm)	0.232±0.022	0.231±0.017	0.211±0.016	0.204±0.010	0.211±0.015	0.237±0.019	0.66
Left ventricle thickness (cm)	0.446±0.023	0.442±0.024	0.478±0.023	0.420±0.022	0.434±0.024	0.433±0.019	0.44
Right ventricle/Total ventricle	0.155±0.009	0.154±0.006	0.154±0.008	0.158±0.007	0.154±0.007	0.155±0.008	0.99

a, and b means within row with no common superscripts differ significantly (P<0.05). N= 18 chicks per treatment

Table 8: The effect of dietary treatments on triiodothyronine (T3) and uric acid in plasma and hematocrit value of male broiler chicks at 21 and 42 days of age

Item	Basal diet	DLM	Relative bioefficacy of LMA to DLM (%)				P-value
			88%	84%	80%	76%	
At 21 days of age T_3 (ng/dl)	86.75±14.55[a]	52.00±9.98[b]	39.00±4.02[b]	77.83±1917[ab]	43.14±7.08[b]	50.00±5.73[b]	0.01
Uric acid (mg %)	2.90±0.93	5.46±0.67	2.95±0.30	3.79±0.76	5.26±1.24	4.38±1.30	0.23
At 42 days of age							
Hematocrit (% PCV)	31.13±0.74	33.75±0.92	31.63±1.21	30.88±1.07	31.50±1.04	30.63±1.32	0.36
T_3 (ng/dl)	47.14±4.07	47.50±5.77	36.50±4.419	48.75±7.97	41.25±6.05	36.71±4.40	0.39
Uric acid (mg %)	2.90±0.46	6.08±0.52	4.22±0.81	5.35±0.91	5.00±0.75	4.66±0.85	0.07

[a and b] Values with different literals within row show significant differences (p<0.05).

the required amounts needed. In addition, an exceed methionine supplementation may induce toxicity in body (Han and Baker, 1993; Edmonds and Baker, 1987 and Okumura and Yamaguchi, 1980).

In general, fat deposition in broilers is reduced by DLM or LMA supplementation (Bunchasak *et al*., 1996a, 1997; Jensen *et al*., 1989 and Bunchasak and Keawarun, 2006). Similarly, the results of present study also show decrease of abdominal fat due to DLM or LMA supplementation. Although Esteve-Garcia and Liaurado (1997) suggested that DLM and LMA might yield somewhat different effects on fat and protein deposition.

Evidence of ascetic syndrome: Heart characteristics of chicks at 42 days of age are shown in Table 7. Heart weight, right ventricle and left ventricle, expressed as percentage of body weight, significantly decreased when methionine sources were added (P<0.05), while other parameters such as right and left ventricle area, heart area, ventricles' thickness as well as ventricle weight (g/bird) were not affected by supplementation of methionine sources.

The effects of supplementing methionine sources on hematocrit value (% PCV), T_3 hormone and plasma uric acid are presented in Table 8. At 42 days of age, there was no significant effect of dietary treatments on hematocrit value; but, adding DLM seemed to elevate this value. At 21 days of age, deficiency in methionine intake significantly enhanced level of T_3 compared to the other groups (P<0.05), except the supplementing LMA at 84% bioefficacy of DLM group. There was no significant effect of dietary treatments at 42 days of age on the level of T_3, but there was the same pattern as the levels of the 21 days of age. In addition, it seems that adding both DLM and LMA reduced the hormone level, while plasma uric acid tended to increase (P=0.07).

The housing environment, temperature, inhaled dust (small particles of 0.5 to 10 micro m), carbon dioxide/oxygen levels, rapid growth rates, high basal metabolic rate and high energy rations have been indicated as the main factors that induce the ascites syndrome (Schelle *et al*., 1991; Julian, 1993). The etiology of ascites can be classified into the following three categories: 1) pulmonary hypertension, 2) miscellaneous cardiac pathologies, and 3) cellular damage caused by oxidative stress (Currie, 1999). The heart of ascetic chickens is enlarged; the right ventricle is grossly dilated, and can make up as much as 40% of total ventricle weight, compared with the normal 20% (Government of Alberta, 2004).

In the present study, the normal heart characteristics (right ventricle was about 15% of total ventricle) and hematocrit value were found; it was revealed that there had been no occurrence of ascetic chicks due to rapid growth rates or high metabolic rates, although heart weight (expressed as percentage of body weight) of chicks fed with diets sufficient in methionine were smaller than the methionine-deficient groups. Decrease of heart weight may have been due to increase in body weight, because the data are expressed proportionally. Furthermore, heart weigh expressed as g/birds was not significantly affected by dietary treatments.

Luger *et al*. (2001) concluded that a high rate of weight gain did not always predict good ascites development, while hematocrit and thyroid hormones provided a good indication for the last week of life. Accordingly, several investigators have also reported that the ascitic chickens had significantly higher hematocrit values and lower T_3 concentrations than normal chickens during low environmental temperature (Olkowski and Classen, 1998; Luger *et al*. 2002; Luger *et al*. 2001). Moreover, Luger *et al*. (2002) concluded that deficiency in thyroid hormone might play a key deleterious role in the development of the ascites syndrome, in view of its fundamental role in the cardiovascular homeostasis both in physiological and pathological conditions (Pingitore and Iervasi, 2005).

We have found that poor growth performance caused by deficiency in methionine induced high T_3 concentration. This finding is in accord with that of Decuypere *et al.* (1994), who reported that adding 0.5 mg T_3/kg of diet had depressed growth rate, concentration of plasma growth hormone and insulin-like growth factor of broiler chicks. Additionally, Nukreaw (2006) reported that feeding 18% protein diet to laying hen had resulted in an improvement of egg production and decrease of T_3 concentration. It can be said that although no ascites syndrome was found, low concentrations of T_3 due to high growth performance or high metabolic rate were seen. Interestingly, supplementing LMA at 84% bioefficacy of DML significantly enhanced T_3 concentration as high as the level of chicks fed with methionine-deficient diet, while growth performance was not depressed. Therefore, supplementing an appropriate level of LMA may result in decreased susceptibility to ascites development of broilers, even though no causal relationship has been demonstrated. Adding methionine sources results in increase of feed or protein intake, and consequently glycine which is needed for uric acid formation may also be elevated. This hypothesis is supported by Machin *et al.* (2004), who reported that increasing protein intake can increase plasma uric acid concentrations of broiler chicks. In the present study, therefore, plasma uric acid of chicks fed with diet sufficient in methionine was higher than the insufficient methionine consumption groups although significant difference was not found. Hellstein *et al.,* (1997) and Machin *et al*. (2004) proposed that uric acid is a potent scavenger of oxidants in human and animal tissues. So, decrease of uric acid concentration in broiler chick will result in an elevation of oxidative stress which causes metabolic disease (ascites syndrome) (Klandorf *et al*., 2002 and Enkvetchakul *et al*., 1993). Thus, elevation of plasma uric acid due to adding methionine sources may give benefit to the health of chicks.

Plasma uric acid seems to contrast with the level of T_3 concentration, and therefore it is considered that the antagonistic effect between uric acid and T_3 in plasma might be a reason why no significant evidence of ascites syndrome on heart characteristic was there even though rapid growth rates were observed.

Acknowledgements

The authors gratefully acknowledge that DLM and LMA were supplied from Sumitomo Chemical Co., Ltd.

References

AOAC., 1990. Official Method of Analysis. 15th ed. Association of Official Agricultural Chemists, Washington, DC.

Baker, D.H. and K.P. Boebel, 1980. Utilization of the D- and L-isomers of methionine and methionine hydroxy analogue as determined by chick bioassay. J. Nutr., 110: 959-964.

Bunchasak, C., K. Tanaka, S. Ohtani and C.M. Collado. 1996a. Effect of Met+Cys supplementation to a low-protein diet on the growth performance and fat accumulation of broiler chicks at starter period. Anim. Sci. Tech. (Jpn.), 67: 956-966.

Bunchasak, C., G. Kimura, K. Tanaka, S. Ohtani and C.M. Collada, 1996b. The effect of supplementing cystine on the growth performance and liver lipid and phospholipids contents of broiler chicks. Jpn. Poult. Sci., 35: 60-66.

Bunchasak, C., U. Santoso, K. Tanaka, S. Ohtni and C.M. Collado, 1997. The effect of supplementing methionine plus cystine to a low-protein diet on the growth performance and fat accumulation of growing broiler chicks. AJAS., 10: 185-191.

Bunchasak, C. and T. Silapasorn, 2005. Effects of Adding Methionine in Low-protein Diet on Production Performance, Reproductive Organs and Chemical Liver Composition of Laying Hens under Tropical Conditions. Int. J. Poult. Sci., 4: 301-308.

Bunchasak, C. and N. Keawarun, 2006. Effect of methionine hydroxy analogue free acid on growth performance and chemical composition of liver of broiler chicks fed a corn-soybean based diet from 0 to 6 weeks of age. J. Anim. Sci., 77: 95-102.

Cabel, M.C., T.L. Goodwin and P.W. Waldroup, 1987. Reduction in abdominal fat content of broiler chickens by the addition of feather meal to finisher diets. Poult. Sci., 66: 1644-1651.

Currie, R., 1999. Ascites in poultry: recent investigations. Avian Pathol., 28: 313-326.

Danner, E.E. and W. Bessei, 2002. Effectiveness of liquid DL-methionine hydroxy analogue-free acid (DL-MHA-FA) compared to DL-methionine on performance of laying hens. Arch. Geflugelk., 66: 97 -101.

Decuypere, E., C. Vega, T. Bartha, J. Buyse, J. Zoon and G.A. Albers, 1994. Increased sensitivity to triiodothyronine (T3) of broiler lines with a high susceptibility for ascites. Br. Poult. Sci., 35: 287-297.

Duncan, D.B., 1955. Multiple Range Test. Biometrics., 11: 1-42.

Edmonds, M.S. and D.H. Baker, 1987. Comparative effects of individual amino acid excesses when added to a cornsoybean meal diet: Effects on growth and dietary choice in the chick. J. Anim. Sci., 65: 699-705.

Enkvetchakul, B., W. Bottje, N. Anthony and R. Moore, 1993. Compromised antioxidant status asociated with ascites in broilers. Poult. Sci., 72: 2272-2280.

Esteve-Garcia, E. and L.L. Liaurado, 1997. Performance, breast meat yield and abdominal fat deposition of male broiler chickens fed diets supplemented with DL - methionine or DL - methionine hydroxy analogue free acid. Br. Poult. Sci., 38: 397-404.

Garlich, J.D., 1985. Response of broiler to DL-methionine hydroxy analogue free acid, DL-methionine, and L-methionine. Poult. Sci., 64: 1541-1548.

Government of Alberta, 2004. Ascites, Agricuture, food and rural development, http://www1. agic. gov. ab. ca/$ deartment/deptdocs.nsf/all/pou3546?opendo

Han, Y., and D.H. Baker, 1993. Effects of excess methionine or lysine for broilers fed a corn-soybean meal diet. Poult. Sci., 72: 1070-1074.

Harms, R.H. and G.B. Russell, 1994. A comparison of the bioavailability of DL-methionine and methionine hydroxy analogue acid for the commercial laying hen. J. Appl. Poult. Res., 3: 1-6.

Hellstein, Y., P.C. Tullson, E.A. Richter and J. Bangsbo, 1997. Oxidation of urate in human skeletal muscle during exercise. Free. Radic. Biol. Med., 22: 169-174.

Hickling, D., W. Guenter, M.E. Jackson, 1990. The effect of dietary methionine and lysine on broiler chicken performance and breast meat yield. Can. J. Anim. Sci., 70: 673-678.

Hoehler, D. and D.M. Hooge, 2003. Relative effectiveness of methionine sources in turkeys-scientific and new commercial data. Int. J. Poult. Sci., 2: 361-366.

Huyghebaert, G., 1993. Comparison of DL-methionine and methionine hydroxy analogue - free acid in broilers by using multi-exponential regression model. Br. Poult. Sci., 34: 351-359.

Jensen, L.S., C.L. Wyatt and B.I. Francer, 1989. Sulphur amino acid requirement of broiler chickens from 3 to 6 weeks of age. Poult. Sci., 68: 163-168.

Julian, R.J., 1990. Pulmonary hypertension: a cause of right heart failure, ascites in meat-type chickens. Feedstuff, 20.

Julian, R.J., 1993. Ascites in Poultry. Avian Pathol., 22: 419-454.

Klandorf, H., D. Rathore, M. Iqbal, X. Shi, M. Simoyi and K. Van Dyke, 2002. Accelerated tissue aging and increased oxidative stress in broiler chickens fed allopurinol. Comp. Biochem. and Physio., 129: 93-104.

Keawarun, N. and C. Bunchasak, 2005. Effect of DL-methionine and methionine hydroxy analogue on growth performance and mineral retention in body of male broiler chickens. pp.42-43. in Proceedings of Sustainable Livestock Production 5th (animal science and veterinary medicine) Chaing Mai University, Chaing Mai.

Luger, D., D. Shinder and S. Yahav, 2002. Hyper- or hypothyroidism: its association with the development of ascites syndrome in fast-growing chickens. Gen. Comp. Endocrinol., 127: 293-299.

Luger, D., D. Shinder, V. Rzepakovsky, M. Rusal and S. Yahav, 2001. Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in Broiler Chickens1. Poult. Sci., 80: 965-971.

Machin, M., M.F. Simoyi, K.P. Blemings and H. Klandorf, 2004. Increased dietary protein elevates plasma uric acid and is associated with decreased oxidative stress in rapidly-growing broilers. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 137: 383-90.

McGovern, R.H., J.J.R. Feddes, M.J. Zuidhof, J.A. Hanson and F.E. Robinson, 2001. Growth performance, heart characteristics and the incidence of ascites in broilers in response to carbon dioxide and oxygen concentrations. Can. Bio. Eng., 43: 4.1-4.5.

Meirelles, H.T., R. Albuquerque, L.M.O. Borgatti, L.W.O. Souza, N.C. Meister and F.R. Lima, 2003. Performance of broilers fed with different levels of methionine hydroxy analogue and DL-methionine. Rev. Bra. Cie. Avi., 5: 69-74.

Moran, E.Jr.T., 1994. Response of broiler strains differing in body fat to inadequate methionine: live performance and processing yields. Poult. Sci., 73: 1116-1126.

National Research Council, 1994. Nutrition Requirement of Poultry .9th ed. National Academy of Sciencxe. Washington, D.C.

Nukreaw, R., 2006. Factor affecting of dietary protein and methionine levels on lipid metabolism of layers. M.S. thesis, Kasetsart University.

Okumura, J. and K. Yamaguchi, 1980. Effect of excess individual essential amino acids in diets on chicks. Jpn. Poult. Sci., 17: 135-139.

Olkowski, A.A. and H.L. Classen, 1998. Progressive bradycardia, a possible factor in the pathogenesis of ascites in fast growing broiler chickens raised at low altitude. Br. Poult. Sci., 39: 139-146.

Pingitore, A. and G. Lervasi, 2005. Thyroid function and heart failure: from the new clinical evidences to the potential therapeutical implications. Recenti. Prog. Med. 96: 535-541.

Reid, B.L., A. Madrid and R.M. Maiodrino, 1982. Relative biopotency of three methionine sources for laying hens. Poult. Sci., 61: 726-730.

Romoser, G.L., P.L. Wright and R.B. Grainger, 1976. An evaluation of the L-methionine activity of the hydroxyl analogue of methionine. Poult. Sci., 55: 1099-1103.

Rostagno, H.S. and W.A. Barbosa, 1995. Biological efficacy and absorption of DL-methionine hydroxy analogue free acid compared to DL-methionine in chickens as affected by heat stress. Br. Poult. Sci., 36: 303-312.

Schelle, C.W., W. Dewit, M.T. Frankenhuis and P.F.G. Vereijken, 1991. Ascites in broilers. 1. Experimental factors evoking symptoms related to ascites. Poult. Sci., 70: 1069-1083.

Schutte, J.B. and M. Pack, 1995. Sulfur amino acid requirement of broiler chick from fourteen to thirty eight days of age. 1. Performance and carcass yield. Poult. Sci., 74: 480-487.

Scott, M.L., 1987. Studies on the comparative utilization of synthetic sources of methionine activity in laying pullets. Nutr. Rep. Int., 36: 1043-1052.

Steel, R.G.D. and J.H. Torrie, 1980. Principle and procedures of statistics. Mc Graw Hill Publishers, New York. 2nd edn.

Van Weerden, E.J. and J.B. Schutte, 1984. Comparison of DL-methionine, DL-methionine-Na, DL-methionine hydroxy analogue-Ca, and DL-methionine hydroxy analogue-free acid with layers. Poult. Sci., 63: 1793-1799.

Waldroup, P.W., C.J. Mabray, J.R. Blackman, P.J. Slagter, R.J. Short and Z.B. Johnson, 1981. Effectiveness of the free acid of methionine hydroxy analogue as a methionine supplement in broiler diets. Poult. Sci., 60: 438-443.