Comparing Bio-efficacy of Liquid DL-methionine Hydroxy Analogue Free Acid with DL-methionine in Broiler Chickens

A. B. Mandal*, A.V. Elangovan and T. S. Johri

Central Avian Research Institute, Izatnagar 243 122, UP, India

ABSTRACT : The present experiment was conducted to assess the efficacy of methionine hydroxy analogue free fatty acid (MHA-FA) in comparison to DL-methionine (DL-Met) utilizing day-old commercial broiler chicks (n=300). The chicks were randomly distributed into 30 groups of 10 chicks each. Three dietary treatments, viz. D1-maize-soybean meal based basal diet (Control), D2-control diet supplemented with DL- methionine to meet its requirements and D3-control supplemented with MHA-FA @ 1.54 times of DL-methionine used in D2, were formulated. Each dietary treatment was offered to 10 replicated groups of chicks following completely random design (CRD). The chicks fed on supplemental DL-Met had significantly higher ($p<0.01$) gain in body weight, followed by MHA-FA group and control during 0-3 weeks of age. During overall growth period (0-6 weeks), chicks in DL-Met and MHA-FA groups grew better ($p<0.01$) than those in control. Feed conversion ratio (FCR) improved ($p<0.01$) on supplementation of either DL- Met or MHA-FA in the basal (Control) diet during 0-3 weeks of age. The FCR for overall period, however, did not differ statistically ($p>0.05$) amongst the treatments. The eviscerated yields emanated from diets with DL-Met or MHA-FA were higher ($p<0.01$) than that in Control. Abdominal fat pad was also more ($p<0.01$) in broilers fed control diet than in DL-Met or MHA-FA supplemented group. Breast yield was higher ($p<0.05$) in MHA-FA fed broilers than those fed DL-methionine supplemented or un-supplemented diets. The efficacy of MHA-FA in comparison to DL-Met for growth was 62.11, 64.82 and 63.88% and for feed efficiency was 62.98, 67.73 and 64.01% at 0-3, 3-6 and 0-6 weeks of age, respectively, while it was 65.85, 71.40 and 67.49% for eviscerated yield, abdominal fat pad reduction and breast yield at 6 weeks of age, respectively. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 1 : 102-108)***

Key Words : MHA-FA, DL-methionine, Broiler, Growth Performance, Carcass Traits

INTRODUCTION

Traditionally, groundnut meal and fish meal have been used as major protein supplements in India. However, because of the problems of mycotoxins and inferior protein quality of groundnut meal and non availability of quality fish meal, use of these ingredients has been limited. As a result, soybean meal is being used as a major protein supplement in poultry diet along with cereal maize. Moreover, the production of soybean meal has also increased in India in recent past. The amino acid methionine is the most limiting one in maize-soybean meal based diet. Supplementation with limiting amino acids allows the level of protein-rich feedstuffs to be reduced, while maintaining performance, and thus help reduce nitrogen excretion through its better utilization for protein synthesis. Methionine requirement is met either by supplementing synthetic DL-methionine or DL-2-hydroxy 4-methylthiobutanoic acid (DL-HMB), more commonly referred to and known as methionine hydroxy analogue (MHA-FA). The DL-MHA-FA is available commercially as salt of calcium (MHA Ca^{2+}) and in liquid form (Alimet). The liquid contains 88% a mixture of mono, di and oligomers of hydroxy analogue molecules. Numerous studies have been conducted to compare the efficacy of DL-methionine with that of liquid MHA. Results from these studies have often been either inconclusive or inconsistent. Several workers (Greger et al., 1968; Romoser et al., 1976; Waldroup et al., 1981; Reid et al., 1982; Noll et al., 1984. Harms and Buresh, 1985) stated that DL-methionine and MHA-FA were equivalent in promoting growth of birds. Other researchers (Boebel and Baker, 1982; Van Weerden et al., 1982; 1983; Thomas et al., 1983; Kirchgessner and Steinhart, 1984; Uzu, 1986; Larbier, 1988; Thomas et al., 1991) concluded that the analogue products were biologically less active than DL-methionine. The quite wide variation in reported relative biological efficacy values of MHA-FA have often been difficult to interpret because of differences in the type of basal diet or age and strain of chicks used (Van Weerden et al., 1982), relative amounts of DL-methionine or MHA supplemented (Christensen and Anderson, 1980), chemical structure of MHA (degree of di- or polymerization-Huyghebaert and Van Schagen, 1989), or lack of information about the dietary content of cystine (methionine/cystine-ratio- Christensen and Anderson, 1980) and other amino acids. Hence, the present study was undertaken to evaluate the comparative efficacy of liquid DL-MHA-FA with DL-methionine in maize-soybean meal diet on meat production performance.

MATERIALS AND METHODS

Selection of chicks and experimental design

Day-old three hundred unsexed, commercial Hubbard chicks were selected, wing banded, weighed and randomly

* Corresponding Author: A. B. Mandal. Tel: +91-581-2300204, Fax: +91-0581-447321, E-Mail: abmcari@rediffmail.com
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Table 1. Ingredient (%) and chemical composition of starting and finishing diets

	Starting diet (0-3 weeks of age)			Finishing diet (3-6 weeks of age)		
	D1-Control	D2-Met	D3-MHA	D1-Control	D2-Met	D3-MHA
Maize (yellow)	63.07	62.88	62.64	65.06	64.89	64.67
Soybean meal (solv. ext.)	29.8	29.8	29.8	27.3	27.3	27.3
MHA-FA premix[1]	0	0	0.436	0	0	0.39
Soybean oil	3.5	3.5	3.5	4	4	4
Constants [2]	3.5	3.5	3.5	3.5	3.5	3.5
L-lysine	0.13	0.13	0.13	0.14	0.14	0.14
DL-methionine	0	0.19	0	0	0.17	0
Total	100	100	100	100	100	100
Nutrient composition, % (analyzed, on DM basis)						
CP	21.36	21.39	21.18	20.20	20.32	20.14
Methionine	0.35	0.59	0.35	0.36	0.53	0.36
Cystine	0.38	0.40	0.37	0.39	0.40	0.38
Methionine+cystine	0.73	0.99	0.73	0.76	0.93	0.74
Lysine	1.18	1.25	1.27	1.17	1.15	1.18
Threonine	0.86	0.80	0.86	0.86	0.89	0.85
Arginine	1.44	1.53	1.53	1.39	1.39	1.41
Isoleucine	0.92	1.03	0.98	0.97	0.81	0.94
Leucine	1.93	2.07	1.94	2.06	2.09	2.07
Valine	1.02	1.15	1.09	1.07	1.01	1.04
Histidine	0.64	0.87	0.67	0.66	0.65	0.64
Phenylalanine	1.13	1.21	1.15	1.15	1.16	1.16
Glycine	0.94	1.00	0.95	0.83	0.94	0.92
Serine	1.15	1.17	1.12	1.12	1.22	1.16
Proline	1.33	1.35	1.31	1.32	1.34	1.32
Alanine	1.16	1.24	1.17	1.22	1.26	1.22
Aspartic acid	2.22	2.36	2.32	2.19	2.25	2.20
Glutamic acid	3.73	3.89	3.73	3.72	3.80	3.78

[1] MHA-FA premix contained 66.955% MHA-FA in silica base.

[2] Constant includes: lime stone. 1.5: DCP. 1.5: trace mineral premix 0.1%; vitamin premix, 0.1% and salt, 0.3%. Trace mineral premix supplied mg/kg diatom, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit. A, 8,250 IU; Vit. D_3, 1,200 ICU; Cit., 1 mg; Vote. 40 IU; Vit. B_1, 2 mg; Vit. B_2 4 mg; Vit. B_{12},10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

* Calculated values. ** Analysed values.

distributed into 30 groups of 10 chicks each. The experiment was conducted following completely randomized design having 3 dietary treatments with 10 observations (replicates) in each.

Housing and brooding

The day-old broiler chicks were housed in battery cages. The cages were fitted with feeder, waterer and dropping trays along with electrical heating arrangement. Standard managemental practices were followed for vaccination, brooding, feeding and watering of the birds. Light was provided 24 h to encourage feed intake. Temperature and lighting in the experimental shed were according to the local commercial environment conditions.

Feeds and feeding

During the entire growth period extra care was exercised to ensure efficient feeding and watering of chicks. They were supplied fresh drinking water daily *ad lib*. Three diets, 21% crude protein and iso-caloric, 3,150 kcal ME/kg for starting phase (0-3 weeks) and 20% crude protein 3,200 kcal ME/kg for finishing phase (3-6 weeks of age) were formulated following standard specifications (Table 1). The diets were D1 (Control, maize-soya based basal diet without methionine supplementation), D2 (maize-soya based basal diet with methionine supplementation). D3 (maize-soya based basal diet supplemented with MHA-FA @ 1.54 times of DL-methionine of D2). Each diet was formulated and mixed separately for starting and (0-3 weeks of age) and finishing phase (3-6 weeks of age) and offered to 10 replicated groups in both the phases of production i.e. starting and finishing phases. As addition of liquid MHA is slightly difficult, it was added as silica carrier, which was analyzed to contain 66.955% ALIMET. The MHA silica carrier was premixed with maize and then in whole lot.

Body weight changes and feed intake

Body weight of individual broiler chicks was recorded weekly, while the feed intake of chicks, allotted in replicates, was recorded at weekly interval up to 6 weeks of age. The mortality of birds was recorded as and when it occurred, weighed and sent for postmortem examination.

Table 2. Body weight gain, feed intake and conversion efficiencies in treatments

Attributes/growth phase	Dietary treatments			Statistical	
	D1-control	D2-met	D3-MHA	SEM	Probability
Live weight gain, g/bird					
0-3 wk	421[c]	472[a]	451[b]	4.31	p<0.01
3-6 wk	980[b]	1,095[a]	1,092[a]	8.98	p<0.01
0-6 wk	1,400[b]	1,570[a]	1,543[a]	11.12	p<0.01
Feed intake, g/bird					
0-3 wk	700	732	714	5.85	NS
3-6 wk	1,963[c]	2,189[a]	2,083[b]	25.01	p<0.01
0-6 wk	2,664[c]	2,922[a]	2,797[b]	27.62	p<0.01
Feed conversion ratio (feed: gain)					
0-3 wk	1.69[a]	1.55[b]	1.60[c]	0.01	p<0.01
3-6 wk	2.01[a]	2.00[a]	1.92[b]	0.02	p<0.05
0-6 wk	1.98	1.91	1.94	0.02	NS
Energy efficiency (ME intake, kcal: gain, g)					
0-3 wk	4.79[a]	4.37[c]	4.51[b]	0.04	p<0.01
3-6 wk	5.79	5.74	5.57	0.05	NS
0-6 wk	5.66	5.47	5.59	0.06	NS
Protein efficiency (CP intake, g: gain, g)					
0-3 wk	0.321[a]	0.293[c]	0.305[b]	0.003	p<0.01
3-6 wk	0.363	0.361	0.351	0.003	NS
0-6 wk	0.362	0.349	0.359	0.004	NS

Means bearing different superscripts in a row differ significantly.

The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately.

Carcass traits

At the end of 6th week of age, two birds were picked up randomly from each replicate (20 birds per treatment). They were starved for 12 h (indeed drinking water was supplied *ad lib*), and were sacrificed as per standard procedure for evaluation of carcass characteristics including the yield of defeathered weight, eviscerated weight, different organs viz., liver, heart, spleen, bursa, thymus, proventriculus and gizzard, and abdominal fat. The weight and length of small intestine, large intestine and caecum were also recorded. The carcass was cut to standard parts that included back, breast, thigh, drum stick, neck and wings. The various carcass traits recorded, were then expressed in terms of percentage of live weight.

Laboratory analyses

The representative samples of feed ingredients were analyzed for proximate composition, calcium and phosphorus following standard techniques (AOAC, 1990). The amino acid contents of the ingredients and the mixed feed were analyzed following standard techniques (Llames and Fontaine, 1994). The supplemented concentration of MHA-FA in diets D3 for starting and finishing phases, was further confirmed through chemical analysis (Naumann et al., 1997). The analysed nutrient composition for the starting and finishing feeds is given in Table 1.

Statistical analysis

Data were subjected to analyses of variance following one-way classification of completely randomized design (Snedecor and Cochran, 1989). Due to unequal number of observations for BW in different dietary treatments, least square analysis of variance technique as per Harvey (1975) was used for analysis of data. The means of different dietary treatments were tested for statistical significance at 5% level using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Live weight gain

The live weight gains at 0-3, 3-6 and 0-6 weeks of age are given in Table 2. The broilers responded markedly to the dietary supplementation of DL-methionine or MHA-FA. The data of 0-3 weeks period revealed that broiler chickens received supplemental DL-methionine (D2) had significantly higher ($p<0.01$) body weight which was followed by MHA supplemented group and un-supplemented Control. The live weight gain recorded in DL-Met supplemented group during 3-6 weeks of age were similar to that of MHA groups but the gains in both the treatments were significantly higher than the un-supplemented Control group. During overall growth phase though highest gain in body weight was recorded in DL-Met group but it was statistically similar to MHA group. Supplementation of either DL-methionine or MHA to maize-soybean meal based basal diet improved the body weight gain significantly ($p<0.01$) at any growth phase. The efficacy of DL-methionine hydroxy analogue in comparison to DL-methionine was 62.11, 64.82 and 63.88% at 0-3, 3-6 and 0-6weeks of age, respectively.

Response to DL-methionine or MHA supplementation in maize-soybean, maize-barley-soybean and sorghum-wheat-soybean meal has been observed by earlier workers also (Bertram et al., 1991; Brennan 1998; Lemme et al., 2002; Damner and Bessei, 2002). In earlier experiment (Bertram et al., 1991), with the highest level of DL-methionine supplementation (0.15%), the growth was improved by 8.3% than the un-supplemented control group. However in the present experiment the improvements were 12.14 and 10.21% in DL-Met or MHA group with the addition of 0.19% DL-methionine or equivalent amount of MHA. The bio-efficacy (62.11, 64.82 and 63.88% at 0-3, 3-6 and 0-6 weeks of age) of MHA-FA emanated from broilers in the present study was closer to the reported values of 65% in maize-soybean (Brennan 1998) and 72% in sorghum-wheat-soybean meal based diets (Lemme et al., 2002) on 42 day old broilers.

Feed intake and feed conversion ratio

The feed intake during 0-3 weeks of age (Table 2) in the dietary treatment D2 differed from control diet, but remained similar to that recorded in MHA group. During 3-6 weeks and overall period (0-6 wk) the feed intake differed significantly due to treatments being highest in D2, followed by in D3 and D1

However, in another study (Lu et al., 2003) Met and MHA supplementation did not improve growth and feed efficiency for broilers during 03- weeks of age.

Feed conversion efficiency (Table 2) improved significantly ($p<0.01$) on supplementation of either DL-methionine or MHA in the basal diet containing maize and soybean meal during 0-3 weeks of age. During this period the improvement was significantly higher in DL-methionine-supplemented group than MHA group. In contrary, the broilers kept on finisher diet supplemented with MHA had better FCR than DL-methionine supplemented or un-supplemented group. The feed conversion efficiency calculated for overall period however did not differ significantly. The efficacy of DL-methionine hydroxy analogue in comparison to DL-methionine was 62.98, 67.73 and 64.01% at 0-3, 3-6 and 0-6 weeks of age, respectively for feed efficiency.

As a single basal diet was used in all dietary treatments, the differences in feed intake might be attributed to the body weights of the birds in different dietary treatments as feed intake is a function of body weight. Deficiency of DL-methionine in dietary treatment D1 resulted in poor growth and thus less feed intake. The birds received MHA-FA supplementation in diet consumed less feed in comparison to those received DL-methionine supplementation both at 3-6 and 0-6 weeks of growth phases. This is in contrary to the reports of Bertram et al. (1991) who reported similar feed intake in the birds received DL-methionine or DL-MHA-FA. Feed efficiency improved by about 3.7 and 3.4% in DL-methionine and MHA supplemental groups on 38 days of age in earlier study (Bertram et al., 1991). However in the present study feed conversion efficiency improved by 8.28 and 5.33% at 21 day of age and 3.53 and 2.02% at 42 day of age over control in DL-methionine and MHA supplemented groups, respectively. A much lower bio-efficacy (51%) for feed conversion efficiency was observed by Brennan. (1998) and Lemme et al. (2002) than the present values accrued at different ages i.e. 62.98, 67.73 and 64.01% at 0-3, 3-6 and 0-6 weeks of age, respectively. However, the bio-efficacy emanated in the present experiment was similar to the value of 68% as reported by Mannion (1999).

Critical analysis of the data revealed that there was significantly lower body weight gain and poorer feed conversion efficiency in MHA treated group at 0-3 weeks of age. Thereafter (during 3-6 weeks of age), the difference in gain in body weight was not significant between these two groups, while a better feed conversion ratio was recorded in MHA treated group. When overall period was considered, body weight gain and feed conversion efficiency remained similar. Therefore, it can be pointed out that the broiler chicks responded lesser to MHA than DL-methionine in early age. It may be attributed to the time required for adjustment of the birds to the taste of MHA. MHA absorption efficiency which might be less at early age, lesser conversion efficiency of MHA to DL-methionine at an early age due to lesser development of transamination process or inhibition of MHA to the development of gastrointestinal tract. However, it needs further investigation to prove these suppositions.

Efficiency of energy and protein utilization

Efficiency of energy and protein utilization was calculated and is presented in Table 2. Energetic efficiency (kcal intake/g gain in live wt) differed significantly ($p<0.01$) during 0-3 weeks of age. The least amount of energy was consumed per unit gain by the broilers received DL-methionine supplementation which was followed by those received MHA or control diet without supplementation during 0-3 weeks of age. Thereafter or for overall phase efficiency of energy utilization did not differ due to dietary treatments.

Similarly protein efficiency (protein intake in g/g gain in live wt) was different ($p<0.01$) in dietary treatments being the best in DL-methionine supplemented group which followed by MHA and control group. However during 3-6 and overall period, the broilers of all the dietary treatments utilized protein with similar efficiency.

Table 3. Live weight and carcass characteristics (% of live weight) of broilers in different dietary treatments

Attributes	Dietary treatments			Statistical	
	D1-Control	D2-Met	D3-MHA	SEM	Probability
Live wt., g	1,444[b]	1,614[a]	1,587[a]	11.26	p<0.01
Feather loss	6.16[a]	5.20[b]	4.75[b]	0.20	p<0.05
Blood loss	4.07	4.63	4.28	0.11	NS
Evisc. yield	70.09[b]	71.75[a]	72.69[a]	0.33	p<0.01
Giblet	4.60	4.73	4.64	0.07	NS
Heart	0.45	0.45	0.49	0.01	NS
Abdominal Fat	2.58[a]	2.23[b]	2.03[b]	0.07	p<0.01
Cut-of body parts					
Wings	8.44[b]	8.81[a]	8.70[ab]	0.07	p<0.01
Breast	16.14[b]	16.70[ab]	17.34[a]	0.20	p<0.05
Keel/Back	15.42	16.19	16.26	0.19	NS
Neck	5.88	5.56	5.94	0.10	NS
Drum stick	9.66	9.95	9.84	0.09	NS
Thigh	9.96	9.80	9.97	0.09	NS

Means bearing different superscripts in a row differ significantly.

Table 4. Yield (% of live weight) of immune and digestive organs of broilers at 6th week of age

Attributes	Dietary treatments			Statistical	
	D1-Control	D2-Met	D3-MHA	SEM	Probability
Immune organs					
Thymus	0.087	0.077	0.075	0.004	NS
Spleen	0.13[b]	0.17[a]	0.17[a]	0.01	p<0.05
Bursa	0.07	0.06	0.06	0.002	NS
Digestive organs					
Proventriculus	0.54	0.51	0.47	0.01	NS
Small intestine length, cm	8.71[a]	8.25[a]	7.59[b]	0.12	p<0.01
Small intestine wt., g	2.82	2.85	2.57	0.06	NS
Large intestine length, cm	2.02[a]	1.86[b]	1.79[b]	0.03	p<0.01
Large intestine wt., g	0.61	0.65	0.58	0.01	NS
Caecal length, cm	1.13[a]	1.06[b]	1.04[b]	0.02	NS
Caecal wt.	0.34	0.34	0.34	0.007	NS
Pancreas	0.23	0.25	0.23	0.0061	NS
Liver	2.42	2.58	2.32	0.07	NS
Gizzard	1.72	1.70	1.83	0.03	NS

Means bearing different superscripts in a row differ significantly.

Mortality

A total of 10 birds died during the whole experimental period, which is equivalent to 3.33%, an insignificant event. The mortality of birds was 3, 3 and 4 in dietary treatments control, DL-Met and MHA supplemented, respectively. The pathological changes observed on post-mortem examination of dead birds did not attribute to the dietary treatments.

Carcass characteristics

The carcass characteristics (Table 3) in terms of feather loss ($p<0.05$), eviscerated yield ($p<0.01$), abdominal fat pad ($p<0.01$), and breast yield ($p<0.05$) were significantly different among the treatments. The loss feather or weight of feather, as percent of live weight, was significantly higher in un-supplemented control than DL-Met or MHA supplemented group. Feather development did not differ statistically, but it was numerically lower in MHA groups than DL-Met group. The eviscerated yields emanated from diets D3 or D2 were statistically higher ($p<0.01$) to that of control. Abdominal fat pad was also significantly higher in broilers fed control diet than DL-Met or MHA supplemented group. Breast yield was higher in MHA fed broilers than those fed DL-methionine supplemented or un-supplemented diets. Blood loss, yields of giblet and heart were however statistically similar. The efficacy of DL-methionine hydroxy analogue in comparison to DL-methionine was 65.85, 71.40 and 67.49% for eviscerated yield, abdominal fat pad reduction and breast yield, respectively. The efficacy of MHA for carcass yield was reported to be 49% (Brennan, 1998) or 48% (Lemme et al., 2002) against the value of 65.85% in the present experiment. Similarly, the efficacy of MHA for breast yield observed in the present experiment was also higher than the values of 64% (Brennan, 1998) or 60% (Lemme et al., 2002).

Yield of immune and digestive organs

The weight of spleen (Table 4) as per unit body weight

Table 5. Composition (%) of breast and thigh muscles in different dietary treatments

Attributes	Dietary treatments			Statistical	
	D1-Control	D2-Met	D3-MHA	SEM	Probability
Breast muscle					
Moisture	74.436	74.017	73.771	0.10	NS
Crude protein	22.78[b]	23.34[a]	23.46[a]	0.10	p<0.01
Ether extract	1.52	1.40	1.47	0.09	NS
Total ash	1.27	1.25	1.30	0.02	NS
Thigh muscle					
Moisture	76.495	76.204	76.122	0.12	NS
Crude protein	21.30	21.21	20.75	0.12	NS
Ether extract	2.19	2.39	2.91	0.15	NS
Total ash	1.22	1.20	1.23	0.01	NS

Means bearing different superscripts in a row differ significantly.

was significantly higher in DL-Met or MHA supplemented diets than Control diet. Blood loss, yields of giblet, heart, liver, gizzard, pancreas and some immune organs (thymus and bursa) were however statistically similar. The weight of proventriculus was apparently lower in DL-methionine or MHA supplemented groups in comparison to control. Similarly, the length of small intestine decreased significantly in MHA supplemented group. The length of large intestine was also lower in DL-methionine or MHA supplemented groups. Similar trends were also observed in caecal length and large intestine weight.

Composition of meat

The composition of breast and thigh muscles is given in Table 5. Crude protein content in breast muscle increased significantly ($p<0.01$) with the addition of DL-methionine or MHA in diet. Though apparently there was reduction in ether extract contents in breast muscle in DL-Met or MHA group than un-supplemented control but the differences were not significant. In thigh muscle, however, CP, EE or total ash did not differ due to dietary treatments.

It can be concluded that supplementation of DL-methionine or methionine hydroxy analogue improved growth, efficiency of feed utilization, eviscerated yield and breast yield. The supposition that methionine hydroxy analogue-free acid (MHA-FA) is about 65% efficient to that of DL-methionine for meat production performance of broiler chickens at 6 weeks of age is valid.

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