Relative Performance and Immune Response in White Leghorn Layers Fed Liquid DL-methionine Hydroxy Analogue and DL-methionine

A. K. Panda*, S. V. Rama Rao, M. V. L. N. Raju and S. K. Bhanja

Project Directorate on Poultry, Rajendranagar, Hyderabad, 500 030, India

ABSTRACT : The relative performance and immune response was evaluated in White Leghorn layers fed liquid DL-methionine hydroxyl analogue-free acid (MHA-FA) relative to dry DL-methionine (DLM) in maize-soybean-sunflower based diets. Three graded levels of methionine (Met) from DLM or MHA-FA were added to the basal diet containing 0.27% Met on an equimolar basis to achieve 0.30, 0.36 and 0.42% Met in the diet. Each diet was fed *ad libitum* to 25 replicates of one bird (individual feeding) each, from 24 to 40 weeks of age. A regime of 16 h light was provided and all the layers were kept under uniform management throughout the experimental period. None of the parameters studied were influenced by the interaction between source and level of Met in diets. Similarly, the majority of parameters, except for daily feed consumption and immune response (influenced by level) and egg specific gravity and shell thickness (influenced by source), were not affected by either source or level of Met in the diets. Feed consumption was significantly lower in the birds fed a diet containing 0.42% Met compared to those fed lower levels of Met. The cutaneous basophilic hypersensitivity response to PHA-P and antibody titre (32 and 40 wk) to inoculation of sheep red blood cells increased significantly by increasing the concentration of Met in the diet from 0.30 to 0.36%. Thus, the Met requirement for immune competence was higher than for optimum production. The source of Met significantly influenced the egg specific gravity and shell thickness. The specific gravity and shell thickness of eggs increased significantly when MHA-FA was used as the source of Met in the diet compared to DLM. From the study it is concluded that Met requirement for immune competence (360 mg/b/d) is higher than for optimum production (300 mg/b/d). MHA-FA was comparable with DLM as a source of Met for production performance and immunity, when the bioavailability of MHA-FA was considered as 88% of DLM. Further, MHA-FA improved egg shell quality compared to DLM. (**Key Words :** Performance, Immune Response, DL-methionine, DL-methionine Hydroxy Analogue Free Acid, White Leghorn Layer)

INTRODUCTION

Methionine (Met) is the first limiting amino acid in practical chicken diet. Therefore, it is usually supplemented in practical layer diet for optimal performance (Liu et al., 2004a). Supplementation with limiting amino acid allows the level of protein rich feedstuffs to be reduced, while maintaining the performance, and thus reduce nitrogen excretion (Mandal et al., 2004). In practice Met is commonly supplemented as dry DL methionine (DLM) or liquid DLM hydroxy analogue free acid (MHA-FA). Although both compounds provide Met precursor to the bird, there are some difference between them with respect to chemistry, absorption, transport in the body and metabolism by the tissues (Knight and Dibner, 1984; Dibner, 2003). The information on the relative bioavailability of

MHA-FA compared to DLM in layer chickens is scanty (Harms and Russell, 1994; Liu et al., 2004b; Liu et al., 2005).

Inconsistent conclusions were drawn in the previous studies regarding the bioavailability of MHA-FA relative to DLM, which was attributed to bird age (Liu et al., 2004a), length of trial (Danner and Bessei, 2002), wide variation in bird performance (Harms and Russell, 1994), lack of information about the dietary content of cystine and other amino acids (Vazquez-Anon et al., 2006) and statistical methods (Liu et al., 2004b). Therefore, there is an ongoing controversy about the bioavailability of MHA-FA compared to DLM. Met is important for proper functioning of the immune system, thymus cell proliferation and thus needed a constant supply to ensure that the chicken's body is well defended (Lohakare et al., 2005). MHA-FA is a highly effective source of supplemental Met (Richards et al., 2005) and because of its organic acid nature, could lower the pH of the gastrointestinal tract, may suppress acid sensitive

Table 1. Ingredient and nutrient composition of basal diet (as such basis)

Ingredient	%
Yellow maize	60.27
Soybean meal	19.64
Sunflower meal	8.63
Oyster shell grit	9.51
Dicalcium phosphate	1.30
Salt	0.45
Trace mineral and vitamin premix[1]	0.20
Nutrient composition	
Metabolizable energy (kcal/kg)	2,600
Crude protein	15.78
Lysine	0.76
Methionine	0.28
Calcium	3.64
Available phosphorus	0.34

[1] Supplied (per kg diet): Vitamin A, 16,500 IU; D_3, 3,200 ICU; E, 12 mg; K, 2 mg; thiamin, 1.2 mg; riboflavin, 10 mg; pyridoxine, 2.4 mg; cyanocobalamin, 12 mcg; niacin, 18 mg; pantothenicacid, 12 mg; Zn, 80 mg; Mn, 50 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.

pathogenic bacteria, stimulate absorption of specific nutrients and enhance immune response. However, all the earlier studies compared MHA-FA with DLM considering production parameters as criteria but no single report included immune response parameters. Hence, the present study was undertaken to compare the efficacy of liquid MHA-FA relative to DLM on production parameters as well as immune response in White Leghorn layers.

MATERIALS AND METHODS

Diets, stocks and husbandry

All major feed ingredients were analyzed for protein (AOAC, 1990) and amino acids (NIRS, Adisseo Asia Pacific Pvt. Ltd, Singapore). A basal diet (Table 1) was formulated with maize-soybean-sunflower meal without supplemental Met (0.27% Met). The basal diet was supplemented with DLM and MHA-FA independently to arrive 0.30, 0.36 and 0.42% Met activity in the diet. The Met activity of DLM and MHA-FA considered was 99.7% and 88%, respectively.

Commercial WL layers (24 wk) were randomly distributed into 150 individual California type cages and housed in open side housing system. Considering one bird (individual feeding) as a replicate, 25 such replicates were randomly allotted to one of the three Met levels from both the sources. A regime of 16hr light was provided in a day and all the layers were kept under uniform management throughout the experimental period (24-40 wk).

Response criterion

Body weight, egg production, egg weight and shell quality : Individual body weight of the bird was recorded at the beginning and end of the experiment. Daily, egg production was recorded on individual basis and percent hen day egg production was calculated. Measured quantity of feed was offered daily and the residue left at every 28 d interval was recorded and the feed intake was calculated as g/b/d. Feed efficiency was calculated as the quantity of egg mass-produced per unit feed consumed. All the eggs laid during the last three consecutive days of every 28 days period were collected to measure the egg weight. Twelve eggs were randomly chosen in each treatment group to determine the specific gravity (DensitoMeter, Mettler-Toledo, ISO-14001, Switzerland), shell breaking strength (Universal Testing Machine, EZ test, 120891-04, Japan) shell weight, shell thickness and Haugh unit (HU) (Egg Multi testers EMT-5200, Japan). The cleaned egg-shells were dried for twenty-four hours, weighed and expressed as % of whole egg. The shell thickness was measured at three different locations (middle, broad and narrow ends) using a micrometer gauge (Mitutoyo Code, 7027, Japan).

Immune response : At 32 and 40 weeks, 8 birds in each dietary group were inoculated with 0.1 ml of a 1.0% suspension of sheep red blood cells (SRBC) to measure humoral immune response. After 5 days of post inoculation, the SRBC inoculated birds were bled through the brachial vein to measure antibody titre (log_2) by the microtitre haemmagglutination procedure of Wegmann and Smithies (1966). The reciprocal of the highest dilution where there was complete agglutination was taken as titre. The cellular immune response was assessed by cutaneous basophilic hypersensitivity (CBH) test *in vivo* by using phytohemaglutinin-P (PHA-P). At 36 weeks, 6 birds in each dietary group were selected and the thickness of both left and right wattles were measured by micrometer. Immediately after measurements, 100 μg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS) and 0.1 ml of PBS was injected into right and left wattle (acted as control), respectively. The swelling of both the wattles were measured at 24 h post injection. The response was determined as: CBH response = (Post injection thickness of right wattle-Pre injection thickness of right wattle)-(Post injection thickness of left wattle-Pre injection thickness of left wattle) (Corrier and DeLoach, 1990).

Statistical analysis

Considering source and levels of Met as factors, the data **were** subjected to factorial analysis. When the interaction between the factors was not significant, the effect of main factors was compared using one-way analysis of variance (Snedecor and Cochran, 1989). The differences among the means were compared with Duncan multiple range test (Duncan, 1955). Significance was considered at p≤0.05.

RESULTS

The analyzed amino acid concentrations of feed

Table 2. Amino acid concentration (%) in feed ingredients

Amino acids	Maize	Soybean meal	Sunflower meal
Protein	8.09	43.63	26.72
Lysine	0.22	2.67	1.05
Methionine	0.16	0.60	0.70
Methionine+cystine	0.35	1.25	1.71
Threonine	0.27	1.71	1.06
Tryptophan	0.06	0.62	0.46
Valine	0.40	2.28	1.48
Isoleucine	0.28	2.14	1.27
Leucine	0.96	3.54	1.95
Phenylalanine	0.39	2.33	1.33
Histidine	0.23	1.14	0.78
Arginine	0.38	3.32	2.35

ingredients used for formulation of basal diet are presented in Table 2. The methionine contents in maize, soybean meal and sunflower meal was found to be 0.16, 0.60 and 0.70%, respectively. The interaction between source and levels of Met did not influence body weight gain, egg production, egg weight, daily feed consumed and feed efficiency (Table 3). Similarly, neither source nor levels of Met influenced the above parameters except daily feed consumed by the bird which was significantly influenced by the levels of Met in diets. The feed consumption was significantly lower in the bird fed diet containing 0.42% Met compared to those of 0.30 and 0.36% Met. Though the feed consumption was lower in the bird fed 0.42% Met, egg mass produced per unit feed consumed was not affected.

Neither the interaction between source and levels of Met nor the Met levels influenced egg quality parameters like specific gravity, shell breaking strength, shell weight, shell thickness and Haugh unit (Table 4). However, the source of

Met significantly influenced the egg specific gravity and shell thickness. The specific gravity and shell thickness of eggs increased significantly when MHA-FA was used as the source of Met in diet compared to DLM. No influence of source of Met was observed on other egg quality parameters viz. shell breaking strength, shell weight and Haugh unit.

Both the interaction between source and levels of Met and the source did not influence immune response parameters like CBH response to PHA-P or humoral immune response as measured by antibody titre in response to SRBC (Table 5). However, the levels of Met in diets influenced both the above parameters. The CBH response and antibody titre (32 and 40 wk) increased significantly by increasing the concentration of Met in the diet from 0.30 to 0.36%. Further increasing the concentration of Met beyond 0.36% in diet did not have additional advantage on immune response parameters studied.

DISCUSSION

The analyzed Met content in maize and soybean meal was similar to those reported in literature (NRC, 1994). Met content in sunflower meal was slightly higher than the value reported by NRC (1994) but similar to that of Rama Rao et al. (2006). The concentrations of other amino acids reported were in close agreement with those reported by Rama Rao et al. (2006).

In the present study none of the parameters studied were influenced by the interaction between source and levels of Met in diets. Similarly majority of the parameters except daily feed consumption and immune response (influenced by levels), and egg specific gravity and shell thickness

Table 3. Performance of White Leghorn layers (24-40 wks) fed different levels of DLM and MHA-FA

		Body weight gain (g)	Egg production (%)	Egg weight (g)	Feed consumption (g/bird/day)	Egg mass:feed
Source						
DLM		130.2	87.09	48.69	99.30	0.425
MHA-FA		139.5	88.74	48.86	99.26	0.432
SEM		15.07	1.29	0.36	0.25	0.006
Levels						
0.30		139.1	87.09	49.09	99.63[a]	0.428
0.36		135.5	88.74	48.46	99.59[a]	0.431
0.42		128.8	86.26	48.76	98.62[b]	0.425
SEM		19.06	1.57	0.43	0.31	0.008
Source×level						
DLM	0.30	110.2	89.06	48.43	99.96	0.430
	0.36	141.1	86.50	48.27	99.30	0.419
	0.42	139.3	85.11	49.38	98.62	0.425
MHA-FA	0.30	167.2	85.11	49.76	99.29	0.426
	0.36	131.2	90.97	48.66	99.86	0.443
	0.42	119.4	87.42	48.15	98.62	0.426
SEM		26.5	2.25	0.61	0.41	0.011

[a, b] Means bearing different superscripts in a column differ significantly (p<0.05).
DLM = DL-methionine. MHA-FA = Methionine hydroxy analogue free acid. SEM = Standard error of mean.

Table 4. Egg shell quality of White Leghorn layers fed different levels of DLM and MHA-FA

		Specific gravity	Shell breaking strength (Newton)	Shell weight (%)	Shell thickness (mm)	Haugh unit
Source						
DLM		1.076[b]	33.27	9.37	0.346[b]	61.65
MHA-FA		1.081[a]	34.69	9.23	0.365[a]	59.35
SEM		0.001	0.86	0.10	0.003	1.41
Levels						
0.30		1.078	34.20	9.23	0.365	60.30
0.36		1.078	34.72	9.45	0.361	61.01
0.42		1.079	33.02	9.22	0.345	59.71
SEM		0.001	1.06	0.12	0.004	1.74
Source×level						
DLM	0.30	1.077	34.33	9.38	0.365	62.20
	0.36	1.077	34.26	9.60	0.346	61.19
	0.42	1.074	31.22	9.15	0.337	61.69
MHA-FA	0.30	1.081	34.06	9.09	0.366	58.52
	0.36	1.080	35.18	9.30	0.367	60.83
	0.42	1.081	34.82	9.29	0.364	58.72
SEM		0.001	1.49	0.18	0.005	2.46

[a, b] Means bearing different superscripts in a column differ significantly (p<0.05).
DLM = DL-methionine. MHA-FA = Methionine hydroxy analogue free acid. SEM = Standard error of mean.

Table 5. Immune response of WL layers fed different levels of DLM and MHA-FA

		CBH response	SRBC titre ($\log_2$ value)	
			32 wk	40 wk
Source				
DLM		1.91	7.50	8.04
MHA-FA		1.98	7.54	8.08
SEM		0.06	0.36	0.34
Levels				
0.30		1.51[b]	6.88[b]	6.63[b]
0.36		2.15[a]	7.75[a]	8.75[a]
0.42		2.17[a]	7.93[a]	8.81[a]
SEM		0.08	0.13	0.41
Source×level				
DLM	0.30	1.43	6.75	6.63
	0.36	2.09	8.00	8.88
	0.42	2.20	7.75	8.63
MHA-FA	0.30	1.58	7.00	6.63
	0.36	2.20	7.50	8.87
	0.42	2.14	8.12	8.76
SEM		0.11	0.63	0.58

[a, b] Means bearing different superscripts in a column differ significantly (p<0.05).
DLM = DL-methionine.
MHA-FA = Methionine hydroxy analogue free acid.
SEM = Standard error of mean.
CBH = Cutaneous basophilic hypersensitivity response.
SRBC = Sheep red blood cells.

(influenced by source) were not affected by either source of levels of Met in the diets. The minimum level of Met employed in the present study was 0.30% in the diet. Lack of improvement in any of the production parameters such as egg production, egg weight and feed efficiency by increasing the concentration of Met beyond 0.30% in diet indicated that 0.30% Met in diet appears to be optimum for production. In the present study, birds consumed about 99.3 g feed daily. Thus considering 100g daily feed intake, 300 mg Met per bird per day is required for optimum production. This finding is similar to many of the earlier reports (Harm and Russell, 1994; Panda et al., 2005). NRC (1994) also suggested 300 mg Met per bird per day for laying hens.

The immune response increased significantly by increasing the concentration of Met from 0.3 to 0.36% in the diet. These results indicated Met requirement for immune competence is higher than for optimum production. This finding further supports the results of our earlier study that Met requirement for immune competence was higher than for optimum production (Rama Rao et al., 2003; Panda et al., 2005). Antibodies are proteins; therefore any deficiency of essential amino acids results in poor immune competence. Unlike most of the other essential amino acids whose function is protein accretion, Met has several other biochemical functions and required for maintaining optimum performance (Scott et al., 1982) and immune competence (Rama Rao et al., 2003). Probably higher concentration of Met might have stimulated the cells responsible for immune functions thereby leading to a higher response in the present study (Latshaw, 1991). Many research reports also demonstrated improvements in antibody production in response to SRBC, a T dependant antigen, further ascertain Met involvement in T cell responses (Tsiagbe et al., 1987; Kidd, 2004). Swain and Johri (2000) also suggested higher Met levels for cell mediated immune response than required for growth in broilers. In our study, 0.36% Met in diet appeared to optimum for eliciting higher immune response (both cell mediated and humoral). Thus, assuming a daily intake of 100 g feed, 360 mg Met/b/d is required for optimum immunity.

MHA-FA compared to DLM as source of Met was equally effective in supporting production performance and immune competence in the present study. Thus, the hypothesis taken for MHA-FA bioavailability as 88% compared to DLM was found to be true. Liu et al. (2004b) also reported that bioavailability of MHA-FA relative to DLM was 88% in laying hens. In fact, higher specific gravity and better shell thickness of egg was observed when MHA-FA was used as a source of Met as compared to DLM. This could be attributed to the acid nature of MHA-FA (Richards et al., 2005), which might have lowered the pH of the gastrointestinal tract thereby increased mineral absorption required for shell calcification resulting in higher shell thickness. The exact metabolic processes that result in the bioavailability difference between DLM and MHA-FA are not clear. However, many studies suggest that absorption of Met from different sources though occurs by different mechanisms but at a similar rate (Knight and Dibner, 1986; Han et al., 1990; Richards et al., 2005).

From the study it is concluded Met requirement for immune competence (360 mg/b/d) is higher than for optimum production (300 mg/b/d). MHA-FA could be compared with DLM as source of Met for production performance and immunity, when the bioavailability of MHA-FA was considered as 88% of DLM. Further, MHA-FA improved egg shell quality compared to DLM.

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