The Effects of DL-methionine and DL-methionine Hydroxy Analogue on Growth Performance, Contents of Serum Amino Acids and Activities of Digestive Proteases in Broilers

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ABSTRACT : This study was conducted to determine the effect of DL-methionine (Met) and DL-methionine hydroxy analogue (MHA) supplementation on the growth performance, contents of serum amino acids and activities of digestive protease in broiler chicks from 0 to 3 weeks old . There were three treatments in this study: (1) control (basal diet), (2) 0.24% Met supplementation and (3) 0.368% MHA supplementation. The results showed that Met and MHA supplementation did not significantly ($p>0.05$) improve feed efficiency and body weight gain for broilers from 0-1, 0-2 and 0-3 weeks of age. The serum levels of homocystine, methionine and taurine were significantly ($p<0.05$) higher with supplementation of Met or MHA than with control. The pepsin activity of proventriculus was increased with ($p<0.05$) Met supplementation at 21 days of age and with MHA supplementation at 7 and 14 days of age. The trypsin activity was also increased ($p<0.05$) with MHA supplementation at 7 days of age. The chymotrypsin activity in pancreas and the dipeptidase activity in small intestinal mucosa and content were not affected ($p>0.05$) by Met or MHA supplementation. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 714-718)***

Key Words : Methionine, Broiler, Growth Performance, Serum Amino Acids Pattern, Digestive Proteases

INTRODUCTION

Methionine (Met) is the first-limiting essential amino acid for poultry nutrition (NRC, 1994). When corn and soybean meals are used as basic ingredients, DL-methionine supplementation becomes very common in poultry diet to meet the nutritional requirement (Lien and Jan, 1999). Practically, Met could be replaced by DL-methionine hydroxy analogue (MHA) in part because of the lower cost (Waldroup et al. 1981). Balnave and Oliva (1990) evaluated the biological efficacies of Met and MHA in the diet of growing chicks on an equimolar basis and showed that the biological efficacies of MHA relative to Met was 75-83% for weight gain and 67-75% for feed conversion. In a balance study (Rostagno and Barbosa, 1995), the net absorption of MHA (90.8%) was significantly lower than that of Met (97.2%).

Secretion of pancreatic enzymes could be stimulated by dietary composition (Grossman et al., 1943). Both trypsinogen content in rat (Poort and Poort, 1981) and activities of pancreatic trypsin and chymotrypsin in mule ducks (Lu, 1999) were greatly increased by feeding a protein-rich diet. Furthermore, the composition of dietary amino acids might stimulate the pancreatic secretion. Meyer and Grossman (1972) perfused L-phenylalanine mixtures into dog duodenum and found a stimulation of the pancreatic secretion. Yang et al. (1989a, b, c) showed that the administration of threonine and phenylalanine to chicks could also greatly increase the secretion. However, there is not enough information regarding the effects of dietary supplementation of Met and MHA in broiler chicks. The purpose of this study, therefore, is to examine the effects of dietary supplementation of Met and MHA on the growth performance, composition of serum amino acids and activities of digestive proteases in broiler chicks.

MATERIALS AND METHODS

Day-old Arbor Acres broiler chicks were obtained from a local commercial breeder farm and randomly allotted to three treatments. Treatment 1 was the control diet fed chicks; Treatment 2 was the basal diet supplemented with 0.24% Met (DL-methionine, 99%, Degussa Co., Ltd., Germany); Treatment 3 was the basal diet; supplemented with 0.368% MHA (DL-methionine hydroxy analog calcium, 88%, Degussa Co., Ltd., Germany). The methionine equivalent level in treatments 2 and 3 was supplied on an equimolar basis. The composition of experimental diets was based on the recommendations of the National Research Council (1994) given in Table 1. The difference in assay value of methionine between the Met treatment and MHA treatment was due to the consideration of the equivalent bioefficacy of MHA (74% of Met). Therefore, the assay value of MHA treatment was a little higher than that of Met treatment.

There were 216 chicks randomly assigned to three treatments, each treatment had three replicates with 24 chicks (equal number in male and female), used for determination the growth performance. Chicks were housed

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Table 1. The composition of experimental diets

Ingredients	Basal diet	Basal diet+Met	Basal diet+MHA
Yellow corn, ground	50.0	50.0	50.0
Soybean meal	35.5	35.5	35.5
Soybean oil	6.0	6.0	6.0
Fish meal	5.0	5.0	5.0
Dicalcium phosphate	1.1	1.1	1.1
Limestone, pulverized	1.2	1.2	1.2
Salt, iodized	0.3	0.3	0.3
Vitamin premix [a]	0.1	0.1	0.1
Mineral premix [b]	0.1	0.1	0.1
Choline chloride, 50%	0.15	0.15	0.15
Cellulose	0.55	0.31	-
DL-Methionine	-	0.24	-
DL-Methionine hydroxy analogue Calcium	-	-	0.368
Analyzed value			
Crude protein, %	22.62	22.73	22.85
Methionine, %	0.418	0.682	0.795
Calculated value			
Crude protein, %	22.71	22.89	22.89
Metabolizable energy, kcal/kg	3,150	3,150	3,150
Methionine, % equivalent	0.393	0.631	0.631
Sulfur amino acid	0.753	0.99	0.99

[a] Vitamin premix supplied the followings per kilogram of diet: Vit. A. 12,600 IU; Vit. D_3, 3,000 IU; Vit. E. 18 IU; Vit. K_3, 3.6 mg; riboflavin. 6.6 mg; niacin. 40.8 mg; pantothenic acid. 14.4 mg; Vit. B_{12}, 0.012 mg; folic acid, 0.6 mg; pyridoxine. 1.2 mg.

[b].Mineral premix supplied the followings per kilogram of diet; I, 1mg; Fe, 50mg; Cu, 3 mg; Zn, 60 mg; Mn, 60 mg; Se, 0.26 mg.

in electrically-heated battery brooders throughout the entire 3 weeks of experimental periods. The diets and water were provided *ad libitum*. Another 144 chicks of the same age were allotted to the three treatment diets. Each treatment had 48 chicks which were killed without fasting at days 7, 14 and 21 by cervical dislocation. The blood samples were collected from external jugular vein for amino acid analysis. Pancreas samples were weighed, immediately frozen in liquid N_2 and then stored at -40°C. Proventriculus and small intestine were cut open on a smooth ice plate to collect the contents and the mucosa, respectively. The samples of small intestinal contents and mucosa were weighed, frozen immediately in liquid N_2 and then stored at -40°C.

The activity of pepsin was measured by the method of Rick and Fritsch (1974). One unit of pepsin was defined as one micromole of tyrosine equivalent formed from hemoglobin (Sigma Co. H-2500) in one minute. The activities of trypsin and chymotrypsin were measured by the method of Geiger and Fritz (1984) and of Geiger (1984), respectively. Before those enzyme activities were measured, the crude extracted enzyme solutions prepared from pancreas were incubated with enterokinase at 5°C for 24 hours to activate the trypsinogen and chymotrypsinogen. One unit of trypsin or chymotrypsin was defined as one micromole of 4-nitroanilide formed in one minute when N-benzoyl-L-arginine-4-nitroanilide or N-succinyl-phenylalanine-4-nitroanilide was used as a substrate, respectively. The activity of dipeptidase was measured by the method of Josefsson and Lindberg (1965). One unit of dipeptidase was defined as one micromole of glycyl-leucine dipeptide hydrolyzed in one minute. Protein concentration was determined by the method of Lowry et al. (1951). The contents of amino acids in feed and serum were analyzed by amino acid analyzer (Beckman 6,300, Beckman Instruments, Inc., California, USA). The contents of moisture and protein of the diets and samples were measured as described by the AOAC (1980).

Table 2. The effects of supplementation with DL-methionine and DL- methionine hydroxyanalogue on the growth performance of broilers of different age (Mean±S.D.)

Treatment[1]	Supplementation g/kg	Feed intake g/bird	Body weight gain g/bird	Feed efficiency (Feed/gain)
0-1 weeks of age				
Control	-	125	104	1.20
Met	2.40	139	114	1.22
MHA	5.60	130	110	1.18
SEM		16	3	0.13
0-2 weeks of age				
Control	-	416	334	1.35
Met	2.40	458	336	1.29
MHA	5.60	436	339	1.29
SEM		19	13	0.07
0-3 weeks of age				
Control	-	940	661	1.43
Met	2.40	908	665	1.39
MHA	5.60	923	662	1.39
SEM		45	46	0.02

Control was basal diet only, Met: basal diet supplemented with DL-methionine, MHA: basal diet supplemented with DL-methionine hydroxy analogue calcium.

Table 3. Effects of supplementation DL-methionine and DL-methionine hydroxy analogue on serum amino acids composition of broilers at three weeks of age (Mean±S.D.)

Amino acid N mole/ml	Basal diet	Basal diet+Met	Basal diet+MHA	SEM
Alanine	298.1	209.4	251.8	69.3
Arginine	135.7[a]	104.8[ab]	92.3[b]	32.8
Glutamic acid	118.5[a]	94.8[b]	104.0[ab]	14.0
Glutamine	67.8	66.5	66.7	16.3
Homocystine	89.8[b]	95.8[b]	137.6[a]	31.3
Isoleucine	46.4[a]	34.4[b]	37.2[b]	7.1
Leucine	78.1[a]	63.7[b]	73.8[a]	6.3
Lysine	211.8[a]	99.8[b]	102.2[b]	59.2
Methionine	22.4.9[b]	27.2[a]	26.5[a]	5.5
Phenylalanine	49.5[a]	41.1.[b]	41.3[b]	5.6
Proline	153.5[a]	84.4[b]	92.4[b]	22.1
Serine	447.3[a]	159.5[b]	150.73[b]	97.1
Taurine	93.4[b]	123.1[a]	121.2[b]	17.9
Threonine	100.5	62.8	46.0	86.7
Valine	95.2[a]	68.3[b]	67.5[b]	11.6

[a, b] Data in the same row with different superscripts differ significantly ($p<0.05$).

The experimental data were statistically analyzed by the GLM procedure to compare the difference between the treatment (SAS. 1985). and Duncan's new-multiple range test was used to compare the difference among the mean values of the treatment (Steel and Torrie. 1980).

RESULTS AND DISCUSSION

The effects of equimolar supplementation of Met and MHA on the performance of broiler chicks are summarized in Table 2. The performance of chicks fed with Met or MHA supplementation was not significantly different from that of chicks fed with control in 3-week experimental period. This meant that Met or MHA supplementation did not affected the feed intake, weight gain and feed efficiency (Table 2). These results were consistent with the studies in chicks (Waldroup et al., 1981) and in Pekin ducks (Lu and Lai. 2001). Yanming et al. (1990) also indicated that the absorption and bioavailability of Met and MHA were similar. Collectively, on an equimolar basis, Met and MHA supplementation in corn-soybean basal diets was similar in biological efficacies.

The composition of blood amino acids (Table 3) showed that the amounts of homocystine, methionine and taurine were significantly increased in the chicks fed with Met or MHA supplementation ($p<0.05$). This result was similar to the findings of Fernandez-Figares et al. (1997). It might be related to the sulfur-containing amino acids in the diet far beyond the requirements of the chicks causing their rapid accumulation in blood (Zimmerman and Scott. 1965). In contrast, the amounts of amino acids other than homocystine, methionine and taurine was higher in control than in Met and MHA supplementation. This implied that Met or MHA supplementation might improve dietary amino acid balance and increase amino acid bioavailability (Thomas et al., 1991).

Table 4. Effects of supplementation with DL-methionine and DL-methionine hydroxy analogue on pepsin activity from proventriculus mucosa of broilers (3 weeks)

Days of age	Pepsin activity, U/g protein			
	Basal diet	Basal diet+Met	Basal diet+MHA	SEM
7	1,575[b]	1,639[a]	2,116[a]	65
14	2,128[b]	2,178[ab]	2,264[a]	51
21	2,152[b]	2,667[a]	2,392[b]	96

[a, b] Data in the same row with different superscripts differ significantly ($p<0.05$).

The results of pepsin activity from proventriculus mucosa (Table 4) showed that Met and MHA supplementation could generally stimulate the pepsin activity. The pepsin activity was highest ($p<0.05$) in chicks fed with MHA supplementation at 7 and 14 days of age and in chicks fed with Met supplementation at 21 days of age ($p<0.05$).

The results of trypsin and chymotrypsin specific activities in pancreas (Table 5) indicated that the trypsin activity in chicks fed with MHA supplementation at 7 days of age was highest ($p<0.05$) but not influenced at 14 and 21 days. On the other hand, the trypsin activity in chicks fed with Met supplementation was not affected when compared with control in entire experimental period. Additionally, the dipeptidase activities in small intestinal mucosa and content (Table 6) were also not affected by Met and MHA supplementation.

Previous studies (Go et al., 1970) reported that essential amino acids but those non-essential could stimulate the pancreatic enzyme secretion in humans. It was also found that the pancreatic secretion in rat could be stimulated with DL-tryptophane and DL-phenylalanine supplementation

Table 5. Effects of supplementation DL-methionine and DL-methionine hydroxy analogue on trypsin and chymotrypsin activities in pancreas of broilers

Days of age	Basal diet	Basal diet+Met	Basal diet+MHA	SEM
Total activity of trypsin (U×pancreas weight, g)				
7	0.52[b]	0.57[b]	0.69[a]	0.26
14	0.85	0.86	0.94	0.29
21	2.22	2.11	1.98	0.65
Total activity of chymotrypsin (U×pancreas weight, g)				
7	0.07	0.12	0.12	0.05
14	0.21	0.18	0.35	0.16
21	0.47	0.47	0.53	0.13

[a, b, c] Data in the same row with different superscripts differ significantly ($p<0.05$).

U: Micromoles of 4-nitroanilide released/min.

Table 6. Effects of supplementation DL-methionine and DL-methionine hydroxy analogue on dipeptidase activity in intestinal mucosa and contents of broilers

Days of age	Basal diet	Basal diet+Met	Basal diet+MHA	SEM
dipeptidase activities, U/mg protein of mucosa				
7	50.1	53.7	46.6	21.7
14	54.7	59.5	58.2	21.5
21	54.7	53.2	54.2	21.6
dipeptidase activities, U/mg protein of content				
7	31.3	27.5	38.6	8.5
14	28.3	33.3	36.6	8.3
21	28.4	27.6	33.1	7.5

a, b, c Data in the same row with different superscripts differ significantly ($p<0.05$).

U: Micromoles of glycyl-leucine dipeptide hydrolyzed/min.

(Schneeman et al., 1977). Meyer and Grossman (1972) further pointed out that L-phenylalanine, instead of D-phenylalanine, increased pancreatic secretion in dog duodenum. Konturek et al. (1979) concluded that L-tryptophane and L-phenylalanine exhibited the strongest stimulation of pancreatic protease secretion in dogs. Yang et al. (1989a, b, c) also suggested that threonine and phenylalanine might have a specific regulatory role in the secretion of pancreatic digestive enzymes in chicks when administered simultaneously. Our results indicated that Met or MHA supplementation could increased the activities of pepsin and trypsin in 7 days old, but not in the case of chymotrypsin and dipeptidase activities in more growing days. Therefore, supplementation of Met and MHA over the requirement might not so consistently stimulate the activities of digestive enzymes.

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