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Effect of Arginine Level and Source and Level of Methionine on Performance of Broilers 0 to 18 Days of Age

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Abstract: There is still a debate about the possible difference in the relative bioefficacy between DL-Met and DL-HMB as a source of methionine activity for broilers. This experiment was conducted to determine if the interaction between Arg and Met is influenced by methionine sources (MetS) and methionine level (MetL) in diets for male broilers. A 3 x 2 x 5 factorial arrangement experimental design included three total Arg levels (1.25, 1.35 and 1.45), two Met sources (DL-Met and DL-HMB) and five equimolar levels of supplemental Met (0, 0.05, 0.10, 0.15 and 0.20%) for a total of 30 treatments. Each treatment was fed to 6 replicate pens of 6 male broilers in electrically heated battery brooders from 1-18 days of age. Chickens fed the 1.25% Arg level showed a significantly higher body weight compared to other treatment groups. The FCR and FE were significantly affected by the different levels of Arg in diets. However, FI was not significantly affected by dietary Arg levels. There was no interaction between Arg-MetS and Arg-MetL or any three-way interactions. There was no significant difference between the two Met sources on the performance of broilers. Body weight and FI were not significantly affected by MetL, but an increase of MetL in basal diet significantly improved FCR and FE. The interaction between MetS and MetL had no significant effect on the performance of broilers. These results indicate that the 1.25% total Arg level was sufficient to provide optimum body weight in broiler starter diets. Both MetS had the same relative effectiveness on the performance of broilers when supplemented with equimolar amounts in diets.

Key words: Arginine, methionine, male broiler, performance

INTRODUCTION

It is well known that soy-bean meal is considered the important source of protein in practical poultry diets. Sulfur amino acids are considered the first limiting in chicken diets when soy-bean meal is the primary protein source (Leeson and Summers, 2000) and methionine is the first limiting amino acid in these diets.

For many years, synthetic methionine sources have been commonly supplied as either dry DL-Met or DL-2-hydroxy -4- (methylthio) butanoic acid (DL-HMB) which is added in a liquid free acid form or in a dry form as calcium salt (MHACa). These compounds are absorbed completely through gastrointestinal tract of poultry, converted to L-Met activity and utilized in protein synthesis (Richards *et al*., 2005). The conversion from DL-Met or DL-HMB to L-Met is produced in the same intermediate pathway, 2-keto-4-(methylitho) butanoic acid (Dibner, 2003). However, the conversion from DL-HMB to 2-keto-4-(methylthio) butanoic acid requires the specific enzymes L-2-hydroxy acid oxidase and D-2-hydroxy acid dehydrogenase (Dibner and Knight, 1984). Both enzymes have been found in the liver and kidney of chickens (Gordon and Sizer, 1965) and D-2-hydroxy acid dehydrogenase has been found in the liver, kidney, skeletal muscle, intestine, pancreas, spleen and brain (Dibner and Knight, 1984).

Both methionine sources are considered equivalent sources of L-Met activity for hepatocyte protein synthesis and the differences have only been reported in absorption mechanism and site (Dibner, 2003). Additionally, many previous studies have reported equivalent performance of broilers supplemented with DL-Met and DL-HMB on equimolar basis in diets (Waldroup *et al*., 1981; Elkin and Hester, 1983; Knight and Dibner, 1984; Garlich, 1985; Römer and Abel, 1999). However, there is an ongoing conflict in the literature during the last decade regarding the relative bioefficacy between DL-HMB and DL-Met as a source of L-Met activity in commercial broilers (Lemme *et al*., 2002; Daenner and Bessei, 2003; Dibner, 2003; Drew *et al*., 2003; Meirelles *et al*., 2003; Dibner *et al*., 2004; Motl *et al*., 2005a,b; Liu *et al*., 2006; Vazquez-Anon *et al*., 2006; Liu *et al*., 2007).

Gonzalez-Esquerra and Leeson (2006a) demonstrated an interaction between Arg and Met through the polyamine synthesis and indicated that both Arg and Met participate as precursors to produce the polyamines putresine, spermidine and spermine. The polyamine synthesis plays a principal role in protein synthesis and tissue growth (Seiler, 1992).

A few researchers confirmed the interaction between Arg and Met in broiler chicks. Keshavarz and Fuller (1971a,b) reported that increased levels of Arg in a diet deficient in Met depressed the growth rate. They alleviated the adverse growth depression of high arginine levels by supplementing small amounts of methionine.

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Additionally, they demonstrated that the adverse growth effect of excess arginine is due to increasing demand for methyl groups for the formation of creatine from methionine. Furthermore, Chamruspollert *et al*. (2002) reported that increased Arg level from (15.2-35.2 g/kg) in a Met-deficient (3.5 g/kg) broiler diet decreased the growth rate and feed intake. However, added DL-Met (1.0-2.0g/kg) could have alleviated adverse effect of high levels of Arg.

Thus, it can be hypothesized that the effect of the interaction between Arg and Met on the performance of broilers may be affected by source and level of methionine if there were differences in relative bioefficacy between DL-Met and DL-HMB as a source L-Met activity on the performance of broilers. Therefore, the objective of the present experiment was to study the effect of Arg level and source and level of Met on the performance of broiler chicks.

MATERIALS AND METHODS

A diet was formulated using ground yellow corn, dehulled solvent extracted soybean meal and corn gluten meal as intact sources of protein. The diet was formulated to contain 20% crude protein with 1.25% total Arg and 1.25% total Lys. No minimum specification for Met or TSAA was specified. All other essential amino acids were formulated to meet or exceed minimum amino acid standards suggested by NRC (1994). A dietary electrolyte balance ((Na+K)-Cl) of 250 meq/kg were specified for all the diets and was obtained by varying levels of feed grade sodium chloride and sodium bicarbonate. The crude protein and moisture content of the primary ingredients were determined prior to formulating the diets, with amino acid content and digestibility coefficients suggested by a major amino acid producer (Ajinomoto Heartland, Chicago IL) used in the formulation. Diets were supplemented with complete vitamin and trace mineral mixes. Composition and calculated nutrient content of the basal diet is shown in Table 1.

Experimental diets were prepared containing 1.25, 1.35 and 1.45% total Arg by addition of arginine free base to the basal diet. Within each Arg level, methionine was added from either DL-Met (98% activity) or MHA-Ca (84% activity) to provide 0, 0.05, 0.10, 0.15 and 0.20% supplemental Met. This resulted in a total of 30 experimental treatments.

Male chicks of a commercial broiler strain (Cobb 500; Cobb-Vantress, Siloam Springs, AR) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Six chicks were placed in each of the 180 compartments in electrically heated battery brooders with raised wire floors. Continuous 24 h fluorescent lighting was used. Test diets fed in mash form and tap water were provided for ad libitum consumption. Six replicate pens, stratified across tiers of the battery were assigned to each of the 30 dietary treatments.

Mean body weights by pen were obtained at 1 and 18 d of age. Feed consumption during the test period was determined. Birds were checked twice daily for mortality; any bird that died was weighed to adjust feed conversion.

Data were subjected to analysis of variance as a complete factorial arrangement with Arg level and

Table 1: Composition and calculated nutrient analysis of basal diets

Ingredients	g/kg
Yellow corn	650.60
Soybean meal 47.5%	250.70
Corn gluten meal 60%	41.92
Poultry oil	9.66
Defluorinated phosphate	18.38
Ground limestone	8.84
Vitamin premix[1]	5.00
Feed grade salt	0.88
Sodium bicarbonate	4.50
Mintrex P_Se[2]	1.00
L-Lysine HCl	3.33
L-Threonine	0.19
Variable[3]	5.00
Total	1000.00
Crude protein %	20.00
DEB meq/kg	250.00
ME kcal/kg	3085.65
Met	0.39
Lys	1.25
Trp	0.22
Thr	0.80
Ile	0.83
His	0.54
Val	0.94
Leu	2.04
Arg	1.25
TSAA	0.77
Gly+Ser	1.59
Phe+Tyr	1.71
Sodium	0.26
Chloride	0.15
Dig Met	0.36
Dig Lys	1.14
Dig Trp	0.19
Dig Thr	0.70
Dig Arg	1.16
Dig TSAA	0.68

[1]Provides per kg of diet: vitamin A 7715 IU; cholecalciferol 5511 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [2]Provides per kg of diet: Mn (as manganese methionine 6ydroxyl analogue complex) 40 mg; Zn (as zinc methionine 6ydroxyl analogue complex) 40 mg; Cu (as copper methionine 6ydroxyl analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg. [3]Variable levels of supplemental amino acids

source and level of Met as main effects and all two-way and three-way interactions. Analysis used the general linear models procedure of SAS (SAS Institute, 1991). Mortality data were transformed to square root of n+1 prior to analysis; data are presented as natural numbers. All statements of probability are based on P<0.05.

RESULTS AND DISCUSSION

The effects of arginine level and interactions between arginine level and methionine source and between arginine level and methionine level are shown in Table 2. Birds fed 1.25% total Arg had significantly higher body weight compared to chickens fed 1.35 and 1.45% total Arg. The FCR and FE were significantly affected by the different levels of Arg in diet. There were no significant differences in FCR and FE between chickens fed 1.25 and 1.45 Arg or 1.35 and 1.45 Arg in diets. The FI was not significantly affected by dietary Arg levels. There were no significant two-way interactions between arginine and methionine source and arginine and methionine levels for any performance factor in the present study. There were no significant three-way interactions among Arg level, methionine source and methionine level for any performance factor (Table 3). In addition, there were no significant interactions between methionine source and methionine level for any factor (Table 4).

These results are in close agreement with NRC (1994) which recommended an Arg requirement of 1.25 to optimize performance during 0-3 weeks of age. A similar result was obtained by Labadan *et al*. (2001) who found that the requirement of Arg for maximum weight gain and feed efficiency was 1.24 and 1.28% from 0-2 weeks old. Furthermore, Cuca and Jensen (1990) found that the Arg requirement was 1.10 to 1.28% for growth from 0-3 weeks of age. In addition, increased Arg level up to 1.45% in bird diets improved FCR (Waldroup *et al*., 2006).

Table 2: Effect of Arginine Level (ArgL) and two-way interactions between arginine level and Methionine Source (MetS) and arginine and methionine level (MetL) on performance of broilers 0-18 d

Parameters		Awt18	FCR	FE	FI	Mort
Arginine Level (%)						
1.25		0.580[a]	1.367[b]	0.732[a]	0.793	1.667
1.35		0.562[b]	1.399[a]	0.715[b]	0.787	1.111
1.45		0.566[b]	1.384[ab]	0.724[ab]	0.783	1.389
P-value		0.003	0.009	0.008	0.490	0.809
SEM		0.003	0.003	0.003	0.005	0.604
Arginine Level * Methionine Source						
ArgL	MetS					
1.25	DLM	0.578	1.365	0.733	0.789	1.111
1.25	HMB	0.582	1.370	0.731	0.797	2.222
1.35	DLM	0.560	1.405	0.713	0.786	0.555
1.35	HMB	0.565	1.394	0.718	0.787	1.667
1.45	DLM	0.564	1.398	0.717	0.788	0.555
1.45	HMB	0.568	1.371	0.730	0.778	2.222
P-value		0.987	0.315	0.339	0.525	0.932
SEM		0.005	0.010	0.005	0.008	0.854
Arginine Level * Methionine Level						
ArgL	MetL					
1.25	0	0.587	1.393	0.719	0.817	0.000
	0.05	0.587	1.367	0.732	0.803	0.000
	0.10	0.583	1.355	0.738	0.789	2.778
	0.15	0.586	1.355	0.738	0.794	4.167
	0.20	0.557	1.366	0.733	0.760	1.389
1.35	0	0.555	1.414	0.708	0.786	0.000
	0.05	0.565	1.412	0.709	0.798	1.389
	0.10	0.575	1.383	0.724	0.795	0.000
	0.15	0.565	1.375	0.728	0.776	2.778
	0.20	0.552	1.414	0.708	0.779	1.389
1.45	0	0.556	1.429	0.702	0.793	0.000
	0.05	0.564	1.392	0.720	0.784	1.389
	0.10	0.566	1.367	0.733	0.774	1.389
	0.15	0.572	1.361	0.735	0.778	1.389
	0.20	0.573	1.374	0.729	0.787	2.778
P-value		0.2165	0.8840	0.9102	0.4792	0.733
SEM		0.0084	0.0164	0.0084	0.0130	1.351

[a,b]Different superscripts within a column represent significantly different means (p<0.05).
[1]Awt18= Average weight at 18 day; FCR = Feed Conversion Ratio; FE= Feed Efficiency; FI = Feed Intake; Mort = Mortality Rate.
[2]ArgL= Arginine Level; MetL= Methionine Level.
[3]MetS= methionine sources; DLM= DL-Met.; HMB= 2-hydroxy-4-(Methylthio) butanoic acid

Table 3: Effect of interaction among arginine level and source and level of methionine on performance of broilers 0-18 d

Parameters			Awt18	FCR	FE	FI	Mort
ArgL	MetS	MetL					
1.25	DLM	0	0.587	1.393	0.719	0.817	0.000
		0.05	0.588	1.357	0.737	0.798	0.000
		0.10	0.582	1.360	0.736	0.791	2.778
		0.15	0.583	1.369	0.731	0.797	2.778
		0.20	0.550	1.345	0.744	0.741	0.000
1.25	HMB	0	0.587	1.393	0.719	0.817	0.000
		0.05	0.587	1.378	0.727	0.808	0.000
		0.10	0.584	1.350	0.741	0.788	2.778
		0.15	0.589	1.343	0.745	0.791	5.555
		0.20	0.563	1.386	0.723	0.780	2.778
1.35	DLM	0	0.555	1.414	0.708	0.786	0.000
		0.05	0.559	1.419	0.705	0.793	0.000
		0.10	0.581	1.376	0.727	0.799	0.000
		0.15	0.561	1.386	0.722	0.777	2.778
		0.20	0.543	1.428	0.701	0.775	0.000
1.35	HMB	0	0.555	1.414	0.708	0.786	0.000
		0.05	0.571	1.405	0.712	0.802	2.778
		0.10	0.569	1.389	0.720	0.791	0.000
		0.15	0.569	1.363	0.734	0.775	2.778
		0.20	0.560	1.399	0.716	0.784	2.778
1.45	DLM	0	0.556	1.429	0.702	0.793	0.000
		0.05	0.572	1.405	0.714	0.802	0.000
		0.10	0.560	1.374	0.730	0.769	0.000
		0.15	0.583	1.380	0.725	0.804	0.000
		0.20	0.552	1.401	0.715	0.772	0.000
1.45	HMB	0	0.556	1.429	0.702	0.793	0.000
		0.05	0.556	1.379	0.726	0.766	2.778
		0.10	0.572	1.360	0.736	0.778	0.000
		0.15	0.561	1.342	0.746	0.752	2.778
		0.20	0.595	1.346	0.743	0.801	5.555
P-value			0.618	0.872	0.870	0.861	0.951
SEM			0.011	0.023	0.011	0.018	1.911

[1]Awt18 = Average weight at 18 day; FCR = Feed Conversion Ratio; FE = Feed Efficiency; FI = Feed Intake; Mort = Mortality Rate;
[2]ArgL = Arginine Level, MetS = Methionine Sources; DLM = DL-Met; HMB = 2-hydroxy-4-(Methylthio) butanoic acid, MetL + + added methionine activity

On the other hand, there are few studies that have considered the interaction between Arg and Met source and level. The addition of a high Arg level in broiler basal diets which were Met-deficient (3.60 g/kg) caused a depression in growth which could be alleviated by methionine supplementation (Keshavarz and Fuller, 1971a,b). Therefore, they suggested that the depression in growth was due to an imbalance between arginine and and methionine in the basal diet. Moreover, supplementation of Met in basal diet could alleviate the imbalance. Therefore, they demonstrated that the adverse growth effect of excess Arg is due to the increase demand for methyl groups for the formation of creatine from methionine. The same results were obtained by Chamruspollert *et al*. (2002) who reported that increased Arg level from 1.52-2.52% with Met-deficient (3.50 g/kg) in basal bird diets caused reduction in body weight gain, but not when diets contained 5.5g/kg Met.

Differences between the previous studies and the present study are in the difference of Arg and Met-deficient levels in basal diets which showed insignificant effect on the performance of criteria affected by two-way and three-way interaction. Thus, the preliminary conclusion would be as follows: Chicks fed 1.25% Arg level were significantly higher in body weight compared to those fed the higher levels and both Met level and sources (DL-Met and DL-HMA) have equivalent impact on both routes of Arg metabolism and an equivalent impact on body protein synthesis. This fact was confirmed by Römer and Abel (1999) who stated that both N-balance and N-retention were not affected by adding the two methionine sources in broiler chickens and pigs. Moreover, these results were recently supported by Gonzalez-Esquerra and Leeson (2006b) who reported that birds fed HMB or DL-Met with different Arg:Lys ratios showed the same degree of protein utilization under acute or chronic heat-stress.

There were no significant effect of Met source on the performance parameters (Table 4). Body weight, FI and mortality rate were not significantly affected by Met level. On the other hand, increased Met supplementation to the basal diet showed a significant improvement in FCR and FE. There was no interaction between methionine source and level on performance factors (Table 4).

These results were in agreement with Motl *et al*. (2005b) who found that Met source or an interaction between Met source and level had no significant effect on BW, feed

Table 4: Effect of methionine source and level and interaction between Mets and MetL on performance of broilers 0-18 d

Parameters		Awt18	FCR	FE	FI	Mort
Methionine source						
DLM		0.567	1.389	0.721	0.788	0.741
HMB		0.572	1.379	0.726	0.788	2.037
P-value		0.3298	0.2109	0.2165	0.9849	0.0652
SEM		0.0031	0.006	0.003	0.004	0.493
Methionine level						
0		0.566	1.413[a]	0.709[c]	0.799	0.000
0.05		0.572	1.391[ab]	0.720[bc]	0.795	0.926
0.10		0.575	1.368[bc]	0.732[ab]	0.786	1.389
0.15		0.574	1.364[c]	0.734[a]	0.783	2.778
0.20		0.561	1.384[bc]	0.724[ab]	0.775	1.852
P-value		0.1977	0.0032	0.0037	0.1882	0.1398
SEM		0.005	0.0096	0.0049	0.0076	0.780
Methionine Source * Methionine Level						
DLM	0	0.566	1.412	0.709	0.799	0.000
	0.05	0.573	1.394	0.719	0.798	0.000
	0.10	0.574	1.370	0.731	0.786	1.851
	0.15	0.576	1.378	0.726	0.793	1.851
	0.20	0.548	1.391	0.720	0.763	0.000
HMB	0	0.566	1.412	0.709	0.799	0.000
	0.05	0.571	1.387	0.722	0.792	1.851
	0.10	0.575	1.367	0.732	0.786	0.926
	0.15	0.573	1.349	0.742	0.773	3.704
	0.20	0.573	1.377	0.727	0.788	3.704
P-value		0.241	0.831	0.785	0.296	0.258
SEM		0.007	0.013	0.007	0.010	1.103

[1]Awt18 = Average weight at 18 day; FCR = Feed Conversion Ratio; FE = Feed Efficiency; FI = Feed Intake; Mort = Mortality Rate.
[2]Methionine sources; DLM = DL-Met.; HMB = 2-hydroxy-4-(Methylthio) butanoic acid

conversion and mortality rate when birds were provided equimolar amounts of Met activity in diets; however an increased Met level up to 0.41% showed the best FCR. The supplementation of broiler diets with equimolar amounts of dry DL-Met or Liquid HMB showed insignificant effect on BW, feed conversion and mortality (Motl *et al*., 2005a). Furthermore, Dibnir *et al.* (2003) and (2004) showed that no significant difference between sources of methionine and level on the performance of broilers. The same results were obtained by Liu *et al*. (2007) who reported that no significant effect of Met sources and level and interaction between methionine source and level on average daily gain, average daily feed intake and feed conversion. In addition, both methionine sources showed the same equivalent bioefficacy to improved growth performance and carcass quality.

The results were also previously confirmed by several studies (Waldroup *et al*., 1981; Elkin and Hester, 1983; Knight and Dibner, 1984; Garlich, 1985; Römer and Abel, 1999). These studies reported equivalent performance of broilers fed diets supplemented with DL-Met and DL-HMA on an equimolar basis. In addition, the results of the present study showed significant improvement in FCR and FE related to increased Met level irrespective of Met source. These results might be a consequence of a nonsignificant improvement in body weight and reduction in feed intake attributed to increased methionine levels in diets. However, body weight, feed intake and mortality rate were not significantly affected by Met level because the amount of methionine (0.39%) in the control diet was apparently sufficient and might have been near to the optimum growth rate and could not have been caused a significant response with increase graded methionine level in diets. This was supported by Carew *et al*. (2003) who indicated that chicks fed dietary of Met level 0.40% had no significant effect on body weight and that this level was sufficient for optimum growth during 8-21 days period.

Conclusion: These results indicate that 1.25% total Arg was sufficient for optimum body weight in agreement with NRC (1994). No difference between Met source and level was observed in alteration of Arg metabolism through performance of broilers. Furthermore, both Met sources had the same relative effectiveness on the performance of broilers. As a result, both Met sources have equivalent impact on the performance of broilers when supplemented with equimolar amounts in diets.

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