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Evaluation of Herbal Methionine Source in Broiler Diets†

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Abstract: A study was conducted to evaluate an herbal methionine replacement product in diets for young broiler chicks. A corn-soybean meal diet that was complete in all respects but methionine was prepared and divided into three aliquots. One was supplemented with 0.30% DL methionine and another was supplemented with the herbal methionine replacement product. Each of these was then blended with the unsupplemented basal diet to provide diets with 0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30% of either DL methionine or the herbal methionine replacement product. Each diet was fed to twelve pens of five male broiler type chicks each in electrically heated battery brooders. The diets and tap water were provided for *ad libitum* feeding from day of hatch to 18 d of age. Analysis of the diets indicated that the methionine activity of the diets with DL methionine was close to expected values but no apparent increase in analyzed methionine activity in diets supplemented with the herbal methionine replacement product. Body weight gain and feed conversion of birds fed the diets with DL methionine were significantly superior to that of birds fed the diets with the herbal methionine replacement product. No significant differences were observed between birds fed the two products for weight of digestive or immune organs when expressed as a percentage of body weight. These data suggest that the herbal methionine replacement product is not suitable for use as a methionine source in diets for young broiler chicks.

Key words: Broilers, methionine, herbal replacement, organic feeding, amino acids

INTRODUCTION

Methionine and cystine are considered as the first limiting amino acids in broiler diets based on corn and soybean meal. Methionine is typically supplemented in broiler diets in the form of chemically produced supplements of DL-methionine or methionine hydroxy analogue. Producers of organic diets are presently allowed to use chemical methionine supplements but regulators are seeking to eliminate chemical sources from such diets. Few natural feedstuffs are considered as rich sources of methionine. However, an herbal product has recently been reported to be a satisfactory replacement for DL-methionine in broiler feeds (Kalbande *et al.*, 2009). It is a mixture of various herbs including *Mucuna pruriens*, *Triticum aevesticum*, *Azadirachia indica*, *Trigonella foenumgraecum*, *Allium cepa*, *Allium sativum*, *Boerhavia diffusa* and *Eclipta alba*. Natural additives could Influence humoral Immunity of broilers (Rahmani and Speer, 2005). The objective of this study was to evaluate this herbal product compared to a standard DL methionine source in diets for young broiler chicks.

MATERIALS AND METHODS

A diet was formulated based on corn and soybean meal to meet NRC (1994) suggested levels for total amino acids except for Met and TSAA. The total lysine requirement was set at 1.20%. Lysine HCl, L-Threonine and L-Valine were used to reduce the overall crude protein level and make the diet more Met-deficient (Table 1). Diets were fortified with complete vitamin and trace mineral mixes from commercial sources. The trace mineral mix was composed of inorganic sources with no amino acid complexes.

A large batch of the basal diet was prepared and divided into three aliquots. One of these was supplemented with 0.30% corn starch (NEG CONTROL). The second was supplemented with 0.30% DL-Methionine (DL MET) and the third was supplemented with 0.30% of the herbal methionine substitute product (Methiorep, M/S Ayurvet Ltd., Baddi, India), (HERB MET). Experimental diets were prepared by blending the NEG CONTROL diet with various MET diets in proportions to obtain supplemental levels of 0.05. 0.10, 0.15, 0.20, 0.25 and 0.30% methionine activity from DL MET or HERB MET, respectively. Along with the negative control diet this resulted in a total of thirteen experimental diets. Each of these was fed in mash form to twelve replicate pens of five male broilers housed in electrically heated battery brooders with raised wire floors. All diets were analyzed in duplicate for crude protein and amino acid content by a commercial laboratory specializing in amino acid analysis.

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Male chicks of a commercial broiler strain (Cobb 500) 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. Five chicks were assigned to each of 156 compartments in electrically heated battery brooders with wire floors. Fluorescent lights provided 24 hr of light daily. Care and management of the birds followed recommended guidelines (FASS, 2010). All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Chicks were placed on the diets for ad libitum consumption at day of hatch and grown to 18 d. Body weights by pen were obtained at the start and end of the period and feed consumption was determined. Birds were checked twice daily; any bird that died was weighed and the weight used to adjust feed conversion. At 7 d, chicks were individually examined for incidence of diarrhea. At 18 d, one bird from each pen was randomly selected and euthanized by CO_2 inhalation. The digestive organs of liver, heart, duodenum, jejunum and ileum, cecae and content and immune organs of bursa, spleen and the upper two lobes of the thymus on the left side of the neck were collected and weighed.

Statistical evaluation included weight gain, feed intake, feed conversion, mortality, rate of diarrhea and relative organ weight. Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA using the General Linear Models procedure of the SAS Institute (1991). The main effects of level and source of methionine activity were compared along with their interaction. When significant differences among treatments were found, means were separated using repeated t-tests using the LSMEANS option of the GLM procedure. Mortality, rate of diarrhea and relative organ weight data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers. Following the ANOVA, nonlinear regression analysis was conducted to estimate the supplemental methionine requirement for optimum body weight and feed conversion of chicks fed the diets supplemented with DL methionine. The PROC LIN procedure of SAS (SAS Institute, 1991) was used incorporating the SAS macro of Robbins (1986). The requirement was established as the inflection point of the one-slope regression model (Robbins *et al*., 1979; Yu and Morris, 1999; Waldroup *et al*., 2000).

RESULTS AND DISCUSSION

Diet analysis: Analysis of the diets for crude protein and amino acids indicated good agreement with calculated values (Table 1). Analysis for methionine content indicated that when the level of DL methionine increased in the diet, there was reasonable agreement between the calculated analysis and the actual analyzed amino acid content (Table 2). However, there was little difference in analyzed methionine content between the basal diet and the diets supplemented with the herbal methionine product and no indication that increasing the level of herbal methionine product increased the analyzed level of methionine. Thus, any response to the herbal methionine product would be due to some characteristic of this product other than methionine per se.

Table 1: Composition and calculated nutrient content of test diet. Numbers in parentheses represent analyzed values

Ingredient	g/kg
Yellow corn	628.55
Poultry oil	38.34
Soybean meal	294.55
Ground limestone	6.05
Defluorinated phosphate	18.11
Sodium chloride	2.38
L-Threonine	0.69
L-Lysine HCl	2.26
L-Valine	0.07
Vitamin premix[1]	5.00
Trace mineral mix[2]	1.00
Variable[3]	3.00
Total	1000.00
Crude protein %	20.07 (19.86)
Lysine %	1.20 (1.16)
Threonine %	0.80 (0.79)
Valine %	0.90 (0.92)
Methionine %	0.29 (0.27)
Cysteine %	0.31 (0.30)
Calcium %	1.00
Nonphytate P %	0.45
ME kcal/kg	3195.79

[1]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [2]Provides per kg of diet: Mn (from $MnSO_4{\bullet}H_2O$) 100 mg; Zn (from $ZnSO_4{\bullet}7H_2O$) 100 mg; Fe (from $FeSO_4{\bullet}7H_2O$) 50 mg; Cu (from $CuSO_4{\bullet}5H_2O$) 10 mg; I from $Ca(IO_3)_2{\bullet}H_2O$), 1.0 mg.
[3]Variable amounts of herbal methionine source, DL-Methionine, or corn starch

Table 2: Analyzed methionine content of test diets with DL methionine or herbal methionine replacement product

Source	% Analyzed	Difference*
Basal diet	0.274	-
+0.05 DL	0.332	0.058
+0.10 DL	0.389	0.115
+0.15 DL	0.423	0.149
+0.20 DL	0.455	0.181
+0.25 DL	0.504	0.230
+0.30 DL	0.544	0.270
+0.05 Herbal	0.290	0.016
+0.10 Herbal	0.296	0.022
+0.15 Herbal	0.303	0.029
+0.20 Herbal	0.294	0.020
+0.25 Herbal	0.304	0.030
+0.30 Herbal	0.301	0.027

*Difference between test diet and basal diet in analyzed methionine content

Table 3: Effect of various levels and sources of methionine on performance (means of 12 pens of five male broiler chicks)

% Added	18 d BW (kg) DL	Herbal	Mean	0-18 d FCR DL	Herbal	Mean	0-18 d Feed:Bird (kg) DL	Herbal	Mean	0-18 d Mortality (%) DL	Herbal	Mean
0	------- 0.636 -------		0.636	------ 1.589 -------		1.589	------ 0.956 -------		0.956	------ 5.00 -------		5.00[ab]
0.05	0.668	0.666	0.667	1.502	1.540	1.521	0.938	0.970	0.954	1.67	5.00	3.33[b]
0.10	0.671	0.643	0.657	1.459	1.576	1.518	0.932	0.918	0.925	3.33	1.67	2.50[bc]
0.15	0.692	0.650	0.670	1.421	1.540	1.480	0.933	0.933	0.933	3.33	1.67	2.50[bc]
0.20	0.680	0.625	0.653	1.418	1.627	1.523	0.909	0.930	0.919	0.00	0.00	0.00[c]
0.25	0.706	0.645	0.675	1.423	1.549	1.487	0.936	0.905	0.920	1.67	1.67	1.67[bc]
0.30	0.698	0.643	0.670	1.463	1.564	1.514	0.978	0.946	0.962	6.66	8.33	7.50[a]
Mean	0.679[a]	0.643[b]		1.468[b]	1.567[a]		0.940	0.937		3.10	3.33	
p-value												
Met source	<0.0001			<0.0001			0.769			0.83		
Met Level	0.0425			0.004			0.136			0.02		
S x L	0.0791			<.0001			0.608			0.92		
CV	6.60			4.74			7.29			3.65*		

[ab]Means in rows with common superscripts do not differ significantly ($p \leq 0.05$)

Table 4: Effect of various levels and sources of methionine on digestive organs of male chicks (means of one chick from each of 12 pens)

Met Added (%)	Liver % of BW DL	Herbal	Mean	Heart % of BW DL	Herbal	Mean	Duodenum % of BW DL	Herbal	Mean	Jejunum+ Ileum % of BW DL	Herbal	Mean	Pancreas % of BW DL	Herbal	Mean
0	------- 3.01 -------		3.01	------- 0.70 -------		0.70	------- 0.93 -------		0.93	------- 2.85 -------		2.85	------- 0.34 ------		0.34
0.05	2.69	3.00	2.84	0.65	0.68	0.66	0.93	0.91	0.92	2.75	2.92	2.84	0.36	0.35	0.36
0.10	2.91	3.38	3.15	0.65	0.72	0.69	0.94	0.95	0.95	2.71	2.88	2.80	0.32	0.37	0.35
0.15	2.90	2.89	2.89	0.63	0.68	0.66	0.87	0.91	0.89	2.68	2.73	2.71	0.36	0.33	0.34
0.20	3.12	3.20	3.16	0.73	0.72	0.73	0.95	1.06	1.01	2.79	2.99	2.89	0.38	0.35	0.36
0.25	2.90	3.02	2.96	0.65	0.68	0.66	0.98	0.94	0.96	2.78	2.77	2.77	0.35	0.35	0.35
0.30	2.87	3.21	3.07	0.60	0.76	0.68	0.92	0.90	0.91	2.54	2.90	2.75	0.33	0.38	0.35
Mean	2.92	3.10		0.66	0.71		0.93	0.94		2.73	2.86		0.35	0.35	
p-value															
Met source	0.764			0.854			0.093			0.679			0.089		
Met Level	0.682			0.469			0.748			0.312			0.152		
S x L	0.398			0.635			0.352			0.270			0.635		
SEM	0.442			0.106			0.129			0.361			0.059		

Response to levels and sources of methionine: The effects of source and level of methionine activity on performance of male broilers are shown in Table 3. Chicks fed DL methionine had significantly higher body weights and better feed conversion than did those fed the herbal methionine replacement product. No significant differences in feed intake or mortality were observed between chicks fed the two products. As expected, there was an increase in body weight and improvement in feed conversion as the level of DL methionine was increased in the diets. For body weight the requirement for supplemental DL methionine as determined by the one-slope regression analysis was estimated at 0.245±0.06% and for optimum feed conversion as 0.163±0.018%. When combined with the analyzed methionine content of the basal diet (0.27%) this would result in a total methionine requirement of 0.515% for body weight and 0.433% for feed conversion. The estimate for body weight is in good agreement with the 0.50% suggested by NRC (1994).

The effects of the different sources and levels of supplemental methionine on the digestive organs are shown in Table 4. There was no significant effect of source or level of methionine on liver, heart, duodenum, jejunum plus ileum, or pancreas when expressed as a percentage of body weight of the chick. No significant interactions were noted between source and level of methionine for these measurements. However, there was a numerical trend in which the measurements for chicks fed the DL methionine to have a lower organ weight as percentage of body weight compared to chicks fed the herbal supplement.

The effects of the different sources and levels of supplemental methionine on the immune organs are shown in Table 5. No significant effects of source or level of methionine on thymus, spleen, bursa, or cecae when expressed as a percentage of body weight of the chick. No significant interactions were noted between source and level of methionine for these measurements. However, as in the case of the digestive organs, there was a numerical trend in which the measurements for chicks fed the DL methionine to have a lower organ weight as percentage of body weight compared to chicks fed the herbal supplement.

The effects of the different sources and levels of supplemental methionine on the incidence of diarrhea at seven days of age are shown in Table 6. There was no significant effect of source or level of supplemental methionine on incidence of diarrhea; however, chicks fed the DL methionine had a numerically lower incidence of diarrhea than those fed the herbal supplement.

Table 5: Effect of various levels and sources of methionine on immune organs of male chicks (means of one chick from each of 12 pens)

Met	Thymus % of BW			Spleen % of BW			Bursa % of BW			Cecae % of BW		
Added (%)	DL	Herbal	Mean	DL	Herbal	Mean	DL	Herbal	Mean	DL	Herbal	Mean
0	------- 0.097 -------		0.097	------- 0.126 -------		0.126	-------- 0.18 --------		0.18	------- 1.03 -------		1.03
0.05	0.093	0.103	0.098	0.10	0.10	0.10	0.15	0.16	0.155	1.01	1.09	1.05
0.10	0.096	0.100	0.098	0.10	0.10	0.10	0.18	0.20	0.187	1.08	1.01	1.04
0.15	0.076	0.086	0.081	0.10	0.10	0.10	0.16	0.18	0.167	0.95	0.99	0.97
0.20	0.085	0.086	0.085	0.12	0.10	0.11	0.15	0.18	0.161	0.96	1.09	1.02
0.25	0.072	0.091	0.081	0.11	0.11	0.11	0.15	0.15	0.149	1.04	1.12	1.08
0.30	0.083	0.075	0.079	0.09	0.12	0.11	0.14	0.17	0.154	0.96	1.04	1.00
Mean	0.086	0.091		0.107	0.108		0.157	0.174		1.00	1.05	
Met source	0.180			0.061			0.735			0.209		
Met Level	0.678			0.551			0.851			0.121		
S x L	0.183			0.252			0.139			0.121		
SEM	0.030			0.034			0.044			0.308		

Table 6: Incidence of diarrhea at seven days

% Met	Diarrhea rate %		
added	DL	Herbal	Mean
0	------------- 10.45 -------------		10.45
0.05	6.67	15.00	10.83
0.10	15.00	13.33	14.17
0.15	5.00	20.00	12.50
0.20	10.00	20.00	15.00
0.25	15.00	6.67	10.83
0.30	11.67	15.00	13.33
Mean	10.48	14.29	
p-value			
Met source	0.186		
Met Level	0.132		
S x L	0.537		
SEM	1.340		

The only previous report in the literature regarding the herbal methionine product is that by Kalbande *et al.* (2009). In this study, a control diet was divided into three aliquots with one supplemented with 0.1% of the herbal product Methiorep, one supplemented with 0.1% DL methionine and one remaining unsupplemented as control. It is difficult to ascertain from the report whether the control diet was methionine deficient, as it did not appear that chicks responded to the supplementation with DL methionine and the calculated methionine content of the basal diet met or exceeded the NRC (1994) recommendations for Methionine (0.5% reported in both starter and grower diets). Chicks fed the Methiorep product in the study by Kalbande *et al.* (2009) had significantly greater body weight and better feed conversion than those fed the control diet or those with DL methionine. Therefore, it would appear there is some considerable difference in results between the present study and that of Kalbande *et al.* (2009) in regard to the methionine replacement value for the herbal methionine product. More studies are suggested to determine causes for this discrepancy in results.

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