Bioavailability comparison between herbal methionine and DL-methionine on growth performance and immunocompetence basis in broiler chickens

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Abstract:

BACKGROUND: Herbal methionine can be compared relative to Dl-methionine with evaluation of bioavailability of this source of methionine. **OBJECTIVES:** An experiment was carried out to determine the relative bioefficacy of herbal methionine (H-Met)® relative to DL-methionine (DL-Met) on performance criteria and immunocompetence of Met sources in male broilers. A total of 160 male broilers were fed a Met-deficient basal diet or the basal diet supplemented with three or four concentrations of each Met sources. **METHODS:** Multiexponential and multilinear regressions were used to determined bioavailability of herbal methionine (H-Met)® relative to DL-Met on performance and immunocompetence of broilers. **RESULTS:** Body weight gain and feed intake of the broilers fed H-Met or DL Met improved in the experiment, regardless of Met sources, relative to those broilers that were fed the basal diet. Immunocompetence of broilers were not significant at 28 day of age ($p>0.05$), whereas the broilers were significantly affected by the additional levels of Met sources at 42 day of age. **CONCLUSIONS:** The bioefficacy estimates for H-Met® relative to DL-Met on a product basis were 55% for weight gain, 71% for feed intake, 78% for feed conversion ratio, 70% for dilution 1-choloro 2-3-dinitrobenzene (DNCB), 67% for sheep red blood cell (SRBC), and 68% for phytohemagglutinine (PHA-P). The relative effectiveness of H-Met® compared to that of DL-Met is 68% on average across performance criteria and all immune criteria tested. H-Met® can be supplemented as a new and natural source of Met for the poultry industry.

Introduction

Amino acids are referred to the building blocks of proteins. Synthetic Methionine (Met) -first limiting amino acid in broilers- can be added to many practical diets. Nowadays, the common source of Met used in poultry diets is DL-Met. Currently, DL-Met is produced by chemical synthesis from acrolein, methyl mercaptan, and hydrogen cyanide. Moreover, some consumers of poultry meat prefer to have products from natural sources. Thus, producers consider this tendency of consumers in order to maintain consumer satisfaction and promote poultry products. Recently, herbal Met sources (H-Met®) are available in commercial poultry market. Therefore, it is necessary to compare this new source of Met with DL-Met in poultry nutrition. Also, there are many studies comparing the bioavailability of methionine hydroxy analog-free acid (MHA-FA) with DL-Met (Hoehler et al., 2005; Payne et al., 2006). However,

there are few reports on comparing the bioavailability of H-Met® with DL-Met in broilers. Moreover, it has been shown that Met and Met-metabolites, such as homocysteine, taurine and glutathione, produced in the Met metabolism cycle, significantly influence the immune system and immunocompetence (Ditscheid et al., 2005; Grimble, 2006). Therefore, the objectives of this study were: 1) to evaluate the bioavailability of H-Met® relative to DL-Met and 2) to evaluate the effects of H-Met® on growth performance and immunocompetence of broilers compared to DL-Met.

Materials and Methods

A total of 160 4-day (d)-old male Ross 308 broilers were subject to 8 dietary treatments for 42 d in battery cages. Each treatment was replicated 4 times with 5 birds per replicate. Treatments composed of a basal corn-soybean meal (Table 1) and 3 and 4 series of graded levels of DL-Met (98% purity), and H-Met® (Met: 12.6 purity and Met+Cyc: 16.9%) in all the experiments, respectively (Table 2). Constituent herbs of H-Met® supplemented formulation namely *Andrographis paniculata*, *Ocimum sanctum*, *Asparagus racemosus*, and *Zea mays*. The amount of Met of H-Met was analyzed according to the AOAC (2003) method 982.30. In each treatment, starter, grower, and finisher periods were fed from d 4 to 10, d 11 to 24 and d 25 to 42, respectively. A basal diet was formulated to be adequate for energy and all nutrients except for Met + Cys which were 0.77, 0.68, and 0.61% in the starter, grower and finisher periods, respectively. Chickens were initially maintained at 31°C; the temperature was gradually lowered by 2°C/week (wk) to reach 21°C by the end of wk 5, and this temperature was maintained for the duration of the experiment. The lighting program used was 23 hours of artificial light during the entire experiment period, and feed and water were provided ad libitum from d 1. The experiment was conducted in accordance with local animal-care guidelines.

Measurements (Growth performance): Body weights and feed intakes (FI) were recorded at d 4, 10, 24, and 42, and the body weight gains (BWG) were calculated for the entire period. In addition, feed conversion ratio (FCR) was calculated.

Immune system response: Three tests were carried out in order to estimate immunocompetence of broilers as follows:

Antibody response to sheep red blood cell (Humoral immunity): Sheep red blood cells (SRBC) were used as a test antigen to quantify specific antibody responses. As Niu et al. (2009) described, for SRBC test, a sheep was bled with a syringe containing 3.8% sodium citrate (Anti-coagulant). Sheep red blood was washed 3 times with phosphate buffered salin (PBS), and then at the 21 and 35 days of age, 0.2 mL/kg body weight of 1% SRBC was injected into pectoral muscle of 8 birds in each treatment. On d 7 post-injection, all birds were bled by brachial venipuncture, and 3 mL of blood was collected for primary antibody response, including IgG and IgM. The blood samples were left at room temperature for 2 hours to clot, then blended with a wooden applicator stick and placed in a 4°C refrigerator overnight for maximum sera yield. The antigenic challenge was repeated on d14 after the first challenge, and blood samples were collected on day 3 after the second injection to determine secondary antibody response. Antibody Assay: Serum samples were tested for total antibody response, then specifically for IgM and IgG using the 2-mercaptoethanol (ME) technique as described (Lepage et al., 1996). The serum was pipetted into microcentrifuge tubes and inactivated by heat in a 56°C waterbath for 30 minutes. To assess total antibodies, 50 µL of PBS was placed in the first row of wells in a 96-well V-bottom microtitration plate. To the same wells, 50 µL of serum was added, and plates were sealed and incubated at 37°C for 30 minutes. Plates were removed from an incubator, and 50 µL of PBS was added to the 11 remaining wells in each row. A 2-fold serial dilution of the samples was made on successive rows; 50 µL of a 1% SRBC suspension was added to each well, and plates were again sealed and incubated for 30 min. The IgM (ME-sensitive) and IgG (ME-resistant) antibody titers were assessed using the same procedure as for total titers except that 50 µL of 2-ME was added to the first row of wells. Titers were read by holding plates over a lighted mirror to observe wells showing agglutination. All antibody titers were reported as log 2 of the reciprocal of the last dilution in which agglutination was observed.

Contact hypersensitivity response to dinitro-

chlorobenzene: At 28 and 42 days of ages, 8 birds per treatment were sensitized (Verma et al., 2004) by a single percutaneous application of 1-chloro-2, 4-dinitrobenzene (DNCB-Merck). A total of 250 ml of DNCB (10 mg/mL of acetone and olive oil 4: 1) were applied on a featherless area of the right side, while a similar area on the left side received the solvent without DNCB as a control. Changes in mean skin thickness 24 and 48 h postchallenge were assessed using digital calipers (Mitutoyo, Japan). The average of 3 measurements of skin section was considered as a mean of each replicate.

Contact hypersensitivity response to phyto-hemagglutinin: When birds were 28 and 42 days old, phytohemagglutinin (PHA-P) was injected to a bird/replicate at a dose of 100µg/bird. The cell reaction caused by the PHA-P injection was evaluated as cutaneous basophil hipersensitivity (CBH) according to the methodology described by Corrier and Deloach (1990) and Silva et al. (2010). 15mg of the lyophilized powder were diluted in 15mL of phosphate buffered saline solution (PBS) in order to obtain a dose of 100µg/0.1mL per bird. The inoculation was made in the interdigital space between the third and fourth toes of the right foot of one bird/replicate by intradermal injection. In the same interdigital space of the left foot, 0.1mL PBS was injected as control. The thickness of interdigital spaces was measured before the injection and 24 and 48 hours afterwards, using a digital caliper (Eletronic Digital Caliper CE, with 0.01mm precision). The results were used to calculate the following: 1. response = post-PHA-P injection thickness of the right foot - pre-PHA-P injection thickness of the right foot (mm) and 2. PBS control response = post-PBS injection thickness of the right foot - pre-PBS injection thickness of the left foot (mm). Therefore, cell reaction at each evaluation time was calculated as: CBH = (1) - (2)

Statistical analysis: The data were analyzed as completely randomized designs using analysis of variance procedures by the GLM procedure of statistical analysis software (SAS). Differences between treatment means were tested using Duncan multiple comparison test, and statistical significance was declared at a probability of $p<0.05$. The pen mean was considered the experimental unit for all statistical analyses. A nonlinear exponential model was used to estimate the efficacy of H-Met® relative to DL-Met on a weight basis. As Hoehler et al. (2005) demonstrated that simultaneous nonlinear multi-exponential regression analysis is a valid statistical means for determination of relative bioefficacy of Met sources based on BWG, BWG data and FCR data were analyzed by none-linear multi-exponential regression as suggested by Littell et al. (1997), according to the following equation:

$$y= a+ b \times (_1- e(c_1\times x_1+ c_2\times x_2))$$

Where y= performance criterion, a= intercept (bird performance with basal diet), b= asymptotic response, a+b= common asymptote (maximum performance level), c_1= steepness coefficient for pure DL-Met, c_2= steepness coefficient for H-Met®, and x_1, x_2 = dietary level of DL-Met and H-Met®, respectively.

According to Littell et al. (1997), bioefficacy value for H-Met® relative to DL-Met are given by the ratios of regression coefficients; c_2/c_1.

FI and immune responses data (SRBC, DNCB and PHA-P) were analyzed by multilinear regression as suggested by Littell et al. (1997) using the following equation:

$$Y= a+ (b_1x_1+b_2x_2)$$

Where y= performance criterion; a= intercept, with basal diet; b_1= the slope of DL-Met line; b_2= the slope of H-Met® line; and x_1, x_2 = dietary level of DL-Met and H-Met® respectively.

Results

Performance: Total mortalities over the 42-d periods were very low (0.5%) with no differences among treatments (Data not shown). BWG and FI were improved ($p<0.05$) significantly by the addition of either Met sources relative to the broilers fed the basal diet (Table 3); thus, proving that basal diet was deficient in Met + Cys. With respect to BWG, the maximum performance of DL-Met was achieved at the level of 0.11%, whereas 0.17% was needed for the treatments with H-Met®. These results indicated that the level of 0.17% of H-Met® has the equivalent efficacy of 0.11% of DL-Met based on BWG.

Immunocompetence: The results of our study showed that by supplementing the diet with Met sources, primary immune response (on 28 day) to immune tests was not significant ($p>0.05$, Table 4).

Table 1. The Composition of the starter, grower and finisher basal diets. [(a)] Vitamin premix provided the following per kilogram of diet: Vitamin A: 5,600 IU from all trans-retinyl acetate; Cholecalciferol: 2000 IU; Vitamin E: 20 IU from all-rac-α-tocopherol acetate; Nboflavin: 3.2 mg; Capantothenate: 8 mg; Nicotonic acid: 28mg; Choline Cl: 720 mg; Vitamin B12: 6.4 µg; Vitamin B6: 1.6 mg; Menadione: 1.6 mg (as menadione sodium bisulfate); Folic acid: 0.08 mg; D-biotin: 0.06 mg; Thiamine: 1.2 mg (as thiamine mononitrate); Ethoxyquin: 125 mg. [(b)] Trace mineral premix provided the following in milligrams per kilogram of diet: Mn, 40; Zn, 32; Fe, 32; Cu, 3.2; I, 1.2; Se, 0.06.

Ingredients (%)	Starter	Grower	Finisher
Corn	49.86	62.30	68.50
Soybean meal (44% cp)	31.51	22.08	16.53
Canola meal	10.00	10.00	10.00
Soybean oil	3.71	1.37	0.99
Dicalcium phosphate	1.94	1.62	1.49
Oyster shell	1.52	1.23	1.20
Salt	0.43	0.42	0.37
Vitamin premix a	0.30	0.30	0.30
Mineral premix b	0.30	0.30	0.30
L-Lysine Hcl	0.29	0.27	0.24
Thr %	0.14	0.11	0.08
Calculated Composition:			
ME,kcal/kg	2950	2950	3000
CP %	20.94	17.95	16.08
Calcium %	1.02	0.84	0.80
Available Phosphorus %	0.49	0.42	0.39
Na %	0.19	0.18	0.16
Met %	0.31	0.28	0.26
Met+ Cys %	0.77	0.68	0.61
Lys %	1.24	1.03	0.88
Thr %	0.81	0.68	0.61

However, the data showed the significant effect ($p<0.05$) on secondary response (on 42 day, Table 5). There were differences in immune responses between the broilers fed DL-Met or H-Met® at each inclusion level. The results of our study in the second response showed that by supplementing Met with both sources, antibody levels against SRBC, DNCB and PHA-P in broiler chickens increased, and the maximum immunocompetence according to SRBC was achieved by adding 0.11% DL-Met and 0.17% H-Met® in the diet. With regard to DNCB responses, the maximum immunocompetence was achieved by adding 0.11 and 0.17% DL-Met and 0.17 and 0.22% H-Met® in the diet. Also, PHA-P responses showed that the maximum immunocompetence was achieved by adding 0.11 and 0.17% DL-Met and 0.17 and 0.22% H-Met® in the diet.

Bioefficacy of H-Met® relative to DL-Met: Broilers fed DL-Met and H-Met® performed well; however, according to the regression analysis, the broilers fed DL-Met were able to utilize DL-Met more effectively than those fed H-Met® in all of the response variables measured (Figures 1 to 4). The bioefficacy of H-Met® relative to DL-Met was 55%, 71%, and 78% based on BWG, FI, and FCR, respectively (Figure 1). The overall average of these bioefficacy values is 67% (Table 6), and the bioefficacy of H-Met® relative to DL-Met were 67% based on SRBC test, 70% for DNCB test and 68% for PHA-P test. The overall average of all criteria tested was 68% (Table 6). In this experiment, the addition of each Met sources was made on a weight basis. The design of the trial, either equimolar or weight-to-weight comparison of the two Met sources, did not affect the estimated relative effectiveness (Hoehler et al., 2005).

Discussion

Performance: As Met supplementation levels increased regardless of the sources, FI level significantly increased, and feed conversion ratio (FCR) also increased due to the higher FI in higher Met supplemented diets. The result of growth performance is not in agreement with the result of Halder and Roy (2007) who reported that there are no significant differences between the utilization of H-Met® in comparison with DL-Met at the same level. The result of our study showed that by increasing the level of the Met sources up to 0.11% for DL-Met and 0.17% for H-Met®, BWG and FI increased. However, in the treatments 4 (DL-Met at 0.17%) and 8 (H-Met® at 0.22%) fed broilers consumed more feed but less BWG than those of treatment 3 (DL-Met at 0.11%) and 7 (H-Met® at 0.17%), resulting in increased FCR. The present result is in agreement with Xie et al. (2006) who reported that BWG increased and then decreased as dietary Met increased. Therefore, this result suggests that increasing the levels of Met sources above the broiler Met requirement level results in decreasing BWG and increasing FI, consequently increase in FCR.

Immunocompetence: The results of the present study are in agreement with Takahashi et al. (1993, 1994), and Swain and Johri (2000) who demonstrated neither the excess nor the deficiency of Met in diets influenced the production of primary antibodies in chickens.

Supplementing Met increased secondary anti-

Table 2. Treatments and the levels of supplemented DL-Met and H-Met® of the experimental diets (4-42 d). (*)Required Met according to Ross's (308) catalog is 0.46, 0.39 and 0.36 % for starter, grower and finisher periods respectively.

Treatment	Met source	Addition of Met source (% product)				Difference between amounts of provided Met and required amounts of Ross's (308) catalog(*)
		Starter	Grower	Finisher	Total	
1	BasalDiet	0.31	0.28	0.26	-	-0.15, -0.11, -0.10
2	DL-Met	0.07	0.06	0.05	0.06	-0.08, -0.05, -0.05
3	DL-Met	0.15	0.11	0.10	0.11	0.00, 0.00, 0.00
4	DL-Met	0.22	0.17	0.14	0.17	+0.07, +0.06, +0.04
5	H-Met	0.07	0.06	0.05	0.06	-0.08, -0.05, -0.05
6	H-Met	0.15	0.11	0.10	0.11	0.00, 0.00, 0.00
7	H-Met	0.22	0.17	0.14	0.17	+0.07, +0.06, +0.04
8	H-Met	0.29	0.23	0.19	0.22	+0.14, +0.12, +0.09

Table 3. Performance of broiler chickens fed graded levels of DL-Met and H-Met® from 4 to 42 d of age. (a- d)Means ± SD in a column with no common superscript differ significantly ($p<0.05$). (1)BWG=body weight gain, (2)FI= Feed Intake.

Treatment	Met source	Addition of product (%)	BWG(1) (g) (Mean ± SD)	FI(2) (g) (Mean ± SD)	FCR (Mean ± SD)
1	-	-	2132.67 ± 29^{d}	3720.11 ± 57^{d}	1.74 ± 0.04^{b}
2	DL-Met	0.06	2356.93 ± 12^{c}	4131.88 ± 34^{c}	1.75 ± 0.02^{b}
3	DL-Met	0.11	2490.75 ± 11^{a}	4394.76 ± 35^{b}	1.76 ± 0.02^{b}
4	DL-Met	0.17	2465.62 ± 16^{b}	4643.48 ± 36^{a}	1.88 ± 0.01^{a}
5	H-Met	0.06	2245.49 ± 4^{d}	3736.91 ± 29^{d}	1.66 ± 0.01^{c}
6	H-Met	0.11	2352.47 ± 13^{c}	4146.54 ± 84^{c}	1.76 ± 0.04^{b}
7	H-Met	0.17	2476.45 ± 13^{a}	4407.25 ± 31^{b}	1.78 ± 0.02^{b}
8	H-Met	0.22	2463.87 ± 20^{b}	4686.31 ± 30^{a}	1.90 ± 0.01^{a}

Table 4. Effect of graded levels of DL-Met and H-Met® on haemagglutinin titres against SRBC (HA titre), cell mediated immunity as assessed by contact sensitivity to DNCB and PHA-P injection at 28 days of age. (1)SRBC= Sheep Red Blood Cell, DNCB= dilution 1-choloro 2-3-dinitrobenzene, and PHA-P= phytohemagglutinine. (2)24 and 48 hours after injection.

Treatment	Met source	Addition of product (%)	HA titre		Increase in skin thickness (%)			
			SRBC(1) (Log 2)		DNCB		PHA-P	
			IgG	IgM	24h(2)	48h(2)	24h	48h
1	-	-	1.85 ± 0.019	2.46 ± 0.013	0.83±0.014	0.08 ± 0.010	0.12 ± 0.001	0.07 ± 0.001
2	DL-Met	0.06	1.87 ± 0.010	2.54 ± 0.012	0.83± 0.008	0.08± 0.005	0.12 ± 0.016	0.07 ± 0.006
3	DL-Met	0.11	1.98 ± 0.011	2.54 ± 0.012	0.84± 0.015	0.09 ± 0.016	0.13 ± 0.014	0.08 ± 0.003
4	DL-Met	0.17	1.94 ± 0.009	2.47 ± 0.008	0.84± 0.012	0.08 ± 0.010	0.12 ± 0.005	0.07 ± 0.008
5	H-Met	0.06	1.86 ± 0.014	2.48 ± 0.012	0.83± 0.008	0.08 ± 0.006	0.12 ± 0.013	0.07 ± 0.002
6	H-Met	0.11	1.90 ± 0.013	2.51 ± 0.013	0.83± 0.008	0.08 ± 0.006	0.12 ± 0.004	0.07 ± 0.009
7	H-Met	0.17	1.97 ± 0.012	2.55 ± 0.012	0.84± 0.014	0.09 ± 0.005	0.13 ± 0.011	0.08 ± 0.005
8	H-Met	0.22	1.91 ± 0.011	2.54 ± 0.008	0.84 ± 0.008	0.09 ± 0.016	0.12 ± 0.004	0.07 ± 0.006

body response to sheep red blood cell (SRBC) measured by total IgM and IgG levels (Table 5). The results of humoral immune response are in agreement with other studies (Tsiagbe et al., 1987; Rama Rao et al., 2003), demonstrating that Met is required for some components of the antibody response and Met supplementation increases the anti-SRBC antibody titers. Moreover, the results of cell-mediated immunity are in accordance with the results of the study conducted by Tsiagbe et al. (1987), reporting enhanced mitogen stimulation by PHA-P in chicks fed diets supplemented with Met.

Because antibodies are proteins, any deficiency of essential amino acids results in poor immuno-competence. Met has several biochemical functions such as its roles for protein accretion, optimum performance (Bunchasak, 2009) and immuno-competence (Rama Rao et al., 2003). Increases in antibody titers related to supplemental Met were reported in a study conducted by Tsiagbe et al. (1987).

Effects of total sulfur amino acids (TSAA) can be divided into two routes: 1. a sufficient metabolic

Table 5. Effect of graded levels of DL-Met and H-Met® on haemagglutinin titres against SRBC (HA titre), cell mediated immunity as assessed by contact sensitivity to DNCB and PHA-P injection at 42 days of age. [(a-d)]Means within a column with different superscripts differ ($p<0.05$). [(1)]SRBC= Sheep Red Blood Cell, DNCB= dilution 1-choloro 2-3-dinitrobenzene, and PHA-P= phytohemagglutinine. [(2)]24 and 48 hours after injection.

Treatment	Met source	Addition of product (%)	HA titre		Increase in skin thickness (%)			
			SRBC [(1)](Log 2)		DNCB		PHA-P	
			IgG	IgM	24h[(2)]	48h[(2)]	24h	48h
1	-	-	3.94 ± 0.101^{e}	2.34 ± 0.161^{d}	0.98 ± 0.008^{b}	0.16 ± 0.004^{b}	0.57 ± 0.196^{d}	0.19 ± 0.037^{b}
2	DL-Met	0.06	4.03 ± 0.118^{d}	2.43 ± 0.106^{c}	0.99 ± 0.011^{b}	0.17 ± 0.005^{b}	0.62 ± 0.255^{b}	0.20 ± 0.020^{b}
3	DL-Met	0.11	4.15 ± 0.229^{a}	2.54 ± 0.153^{a}	1.23 ± 0.005^{a}	0.28 ± 0.012^{a}	0.73 ± 0.225^{a}	0.23 ± 0.070^{a}
4	DL-Met	0.17	4.13 ± 0.188^{bc}	2.53 ± 0.107^{ab}	1.23 ± 0.005^{a}	0.27 ± 0.008^{a}	0.72 ± 0.160^{a}	0.23 ± 0.037^{a}
5	H-Met	0.06	3.94 ± 0.100^{e}	2.34 ± 0.098^{d}	0.99 ± 0.005^{b}	0.16 ± 0.005^{b}	0.59 ± 0.040^{cd}	0.19 ± 0.067^{b}
6	H-Met	0.11	4.02 ± 0.125^{d}	2.43 ± 0.111^{c}	0.99 ± 0.010^{b}	0.17 ± 0.010^{b}	0.61 ± 0.201^{bc}	0.20 ± 0.048^{b}
7	H-Met	0.17	4.14 ± 0.134^{ab}	2.54 ± 0.124^{a}	1.23 ± 0.008^{a}	0.28 ± 0.013^{a}	0.73 ± 0.188^{a}	0.23 ± 0.026^{a}
8	H-Met	0.22	4.12 ± 0.142^{c}	2.52 ± 0.097^{b}	1.23 ± 0.008^{a}	0.27 ± 0.004^{a}	0.71 ± 0.165^{a}	0.23 ± 0.071^{a}

Table 6. Estimated effectiveness of H-Met® relative to DL-Met based on BWG (body weight gain), FI (feed intake), feed conversion ratio (FCR) and immune response (SRBC, DNCB, PHA-P) of broiler chickens. Relative effectiveness of H-Met® was significantly lower than that of DL-Met (see Figures 2 to 5 for details). [(1)]BWG= body weight gain and FI= Feed Intake. [(2)]SRBC= Sheep Red Blood Cell, DNCB= dilution 1-choloro 2-3-dinitrobenzene, and PHA-P= phytohemagglutinine.

Variable (%)	Performance			Immune response					
	BWG	FI	FCR	SRBC		DNCB		PHA-P	
				IgG	IgM	24h	48h	24h	48h
Bioefficacy	55	71	78	67	67	69	70	68	68
Mean		67		67		70		68	
Total Mean				68					

supply of TSAA from the diet and tissue protein break down that supports the synthesis of many protein and peptides involved in normal functioning of the immune system and 2. producing glutathione, homocysteine, and taurine that influence inflammatory aspects of the immune response (Grimble, 2006; Bunchasak, 2009). Ditscheid et al. (2005) explained the metabolism of Met as follows: this metabolism includes activation to S-adenosylmethionine (SAM), the most important donor of methyl group. After demethylation to S-adenosylhomocysteine (SAH), homocysteine is formed. Several mechanisms have been discussed if homocysteine precursor SAH accumulated: the binding of the endothelium-derived relaxing factor nitric oxide and the production and the inhibition of transmethylation reactions (Perna, 2003). To avoid an overload of homocysteine and its pathophysiological consequences, homocysteine has to be metabolized rapidly. There are two metabolic pathways: remethylation and transsulphuration. 5-methyltetrahydrofolate is a methyl group donor for remethylation pathway, which is essential for the conversion of homocysteine to Met (Ditscheid et al., 2005). In the second pathway, cystathionine can be converted to cysteine. Cysteine plays an important role as a precursor of glutathione and taurine (Ditscheid et al., 2005). Sulfate and taurine are the major endproducts of TSAA metabolism (Grimble, 2006). Perhaps improvement in immunocompetence is related to the positive effects of Met and its metabolism production. Met supplementation improves leukocyte migration inhibition, cellular immune response and humoral immune response (Swain and Johri, 2000; Attia, 2005). The present results indicate that immunocompetence is influenced by the levels of Met sources ($p<0.05$), which are related to the control of TSAA metabolism and metabolic changes in response to changes in Met levels.

In regard to cellular immunity, the cutaneous hypersensitivity response of toe-webs to T cell mitogens, such as PHA-P, is often used to assess T cell-mediated immunity in vivo in chickens (Corrier and Deloach, 1990), and the cutaneous PHA-P response is characterized by an infiltration of lymphocytes and other inflammatory cells including basophils and macrophages at injection sites (Stadecker, 1977). However, the mechanisms by which dietary Met modulates immune responses are

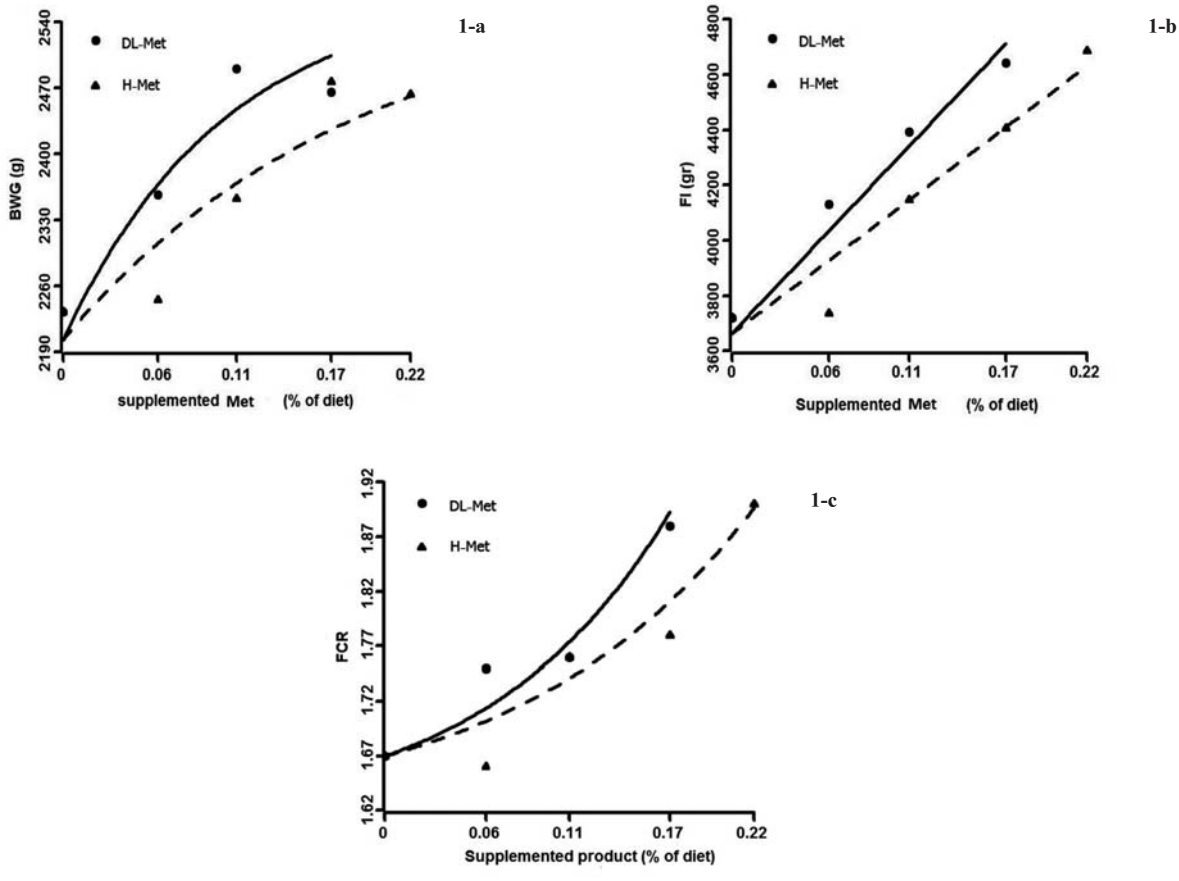

Figure 1. Bioefficacy of H-Met® relative to DL-Met using body weight gain (BWG) (a), feed intake (FI) (b) and feed conversion ratio (FCR) (c) in male Ross 308 broilers (4-42 days of age). Zero level indicates control. Values in parentheses indicate the 95% confidence interval. (*)Values are significantly less than 88%; $p< 0.05$. (1-a) $Y= 2202.7+ 374.8 (1-e^{-(9.64x1+5.33x2)})$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 55%* (40- 71). R^2= 84%. (1-b) $Y= 3663.01+ (6172.71x_1+ 4400.72x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 71%* (63- 79). R^2= 93%. (1-c) $Y= 1.67- 0.06 (1-e^{(9.13x1+7.11 x2)})$. Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 78%* (70- 86). R^2=85%.

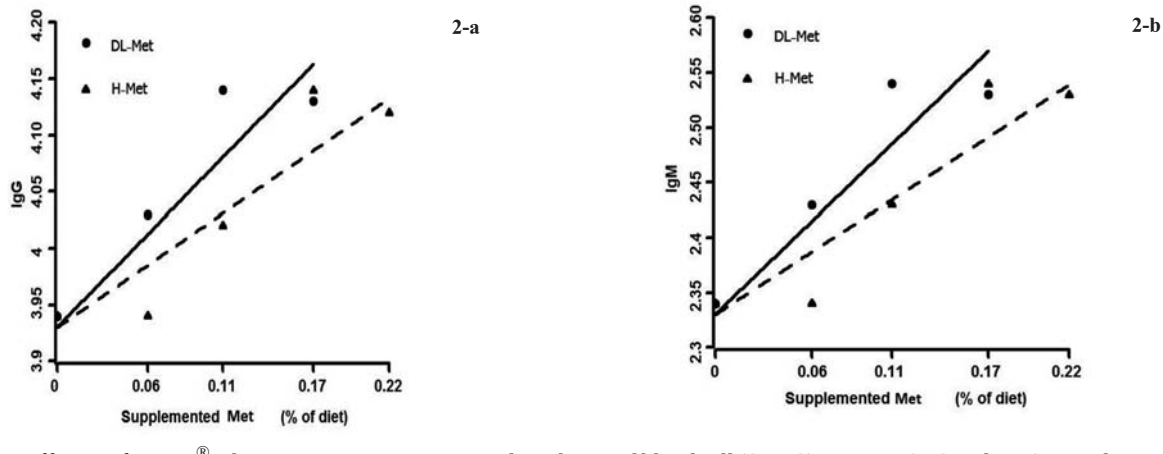

Figure 2. Bioefficacy of H-Met® relative to DL-Met using secondary sheep red blood cell (SRBC) response (IgG and IgM), in male Ross 308 broilers. Zero level indicates control. Values in parentheses indicate the 95% confidence interval. (*)Values are significantly less than 88%; $p< 0.05$. (2-a) $Y= 3.93+ (1.37x_1+ 0.92x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 67%* (60- 74). R^2= 80%. (2-b) $Y= 2.33+ (1.41x_1+ 0.95x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 67%* (60- 74). R^2= 81%.

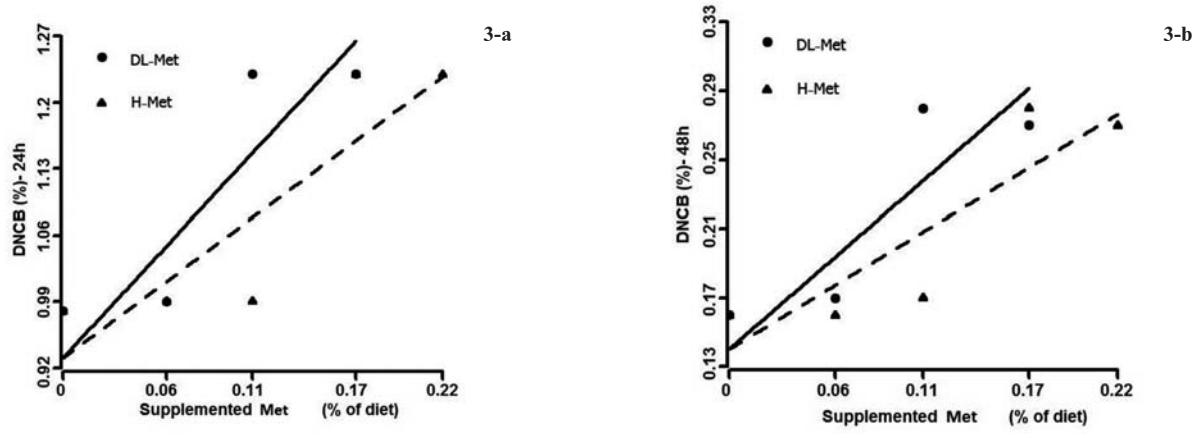

Figure 3. Bioefficacy of H-Met® relative to DL-Met using secondary 1-choloro 2-3-dinitrobenzene (DNCB) response, 24 and 48 hours after injection in male Ross 308 broilers. Zero level indicates control. Values in parentheses indicate the 95% confidence interval. (*)Values are significantly less than 88%; $p< 0.05$. (3-a) $Y= 0.93+ (1.97x_1+ 1.35x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 69%* (57- 81). R^2= 78%. (3-b) $Y= 0.14+ (0.89x_1+ 0.62x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 70%* (57- 83). R^2= 77%.

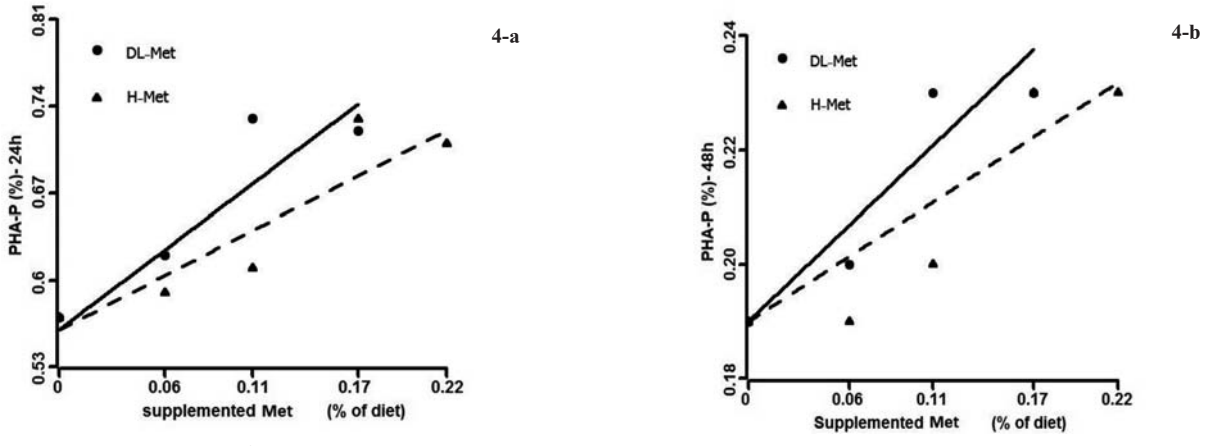

Figure 4. Bioefficacy of H-Met® relative to DL-Met using secondary phytohemagglutinine (PHA-P) response, 24 and 48 hours after injection in male Ross 308 broilers. Zero level indicates control. Values in parentheses indicate the 95% confidence interval. (*)Values are significantly less than 88%; $p< 0.05$. (4-a) $Y= 0.56+ (1.07x_1+ 0.73x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 68%* (57- 79). R^2=78%. (4-b) $Y= 0.19+ (0.28x_1+ 0.19x_2)$, Relative effectiveness: DL-Met (x_1) = 100%. H-Met (x_2) = 68%* (55- 81). R^2= 77%.

not well understood. One possibility is that Met can regulate certain immunomodulators, such as cytokines (e.g. interleukin-1) (Klasing and Barnes, 1988) or hormones (e.g. insulin-like growth factor-I, triiodothyronine and thyroxine) (Rosebrough et al., 1998; Rosebrough et al., 1996).

Bioefficacy of H-Met® Relative to DL-Met: In the present study, the addition of each Met sources was made on a weight basis. Hoehler et al. (2005) demonstrated that the design of the trial could be done either based on equimolar or weight-to-weight comparison of the two Met sources, although the results are not exactly the same.

There are several hypotheses as to why H-Met® has a lower bioefficacy relative to DL-Met. Obviously, there are some physical and chemical differences between DL-Met and H-Met®, and these differences could play a role in bioefficacy differences. In addition, there are some possibilities for lower bioefficacy of H-Met® relative to DL-Met as Hoehler et al. (2005) and Payne et al. (2006) explained for comparing DL-Met and MHA-FA. The poor utilization of the polymeric forms of H-Met® relative to DL-Met polymeric form may be one of the main reasons for its lower bioefficacy. Another potential reason is that DL-Met can absorb faster

because it has transporters with higher affinity and greater velocity than H-Met® transporters.

Conclusions and applications: 1. The relative effectiveness of H-Met® was significantly lower than that of DL-Met in broiler chickens. The average bioefficacy was 68% for H-Met® on all criteria tested.

2. H-Met® can be administered as a new and a natural source of Met in poultry industry.

Acknowledgements

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مجله طب دامی ایران، ۱۳۹۳، دوره ۸، شماره ۳، ۱۷۸-۱۶۹

مقایسه زیست فراهمی متیونین گیاهی و متیونین سنتتیک بر پایه عملکرد رشد و پاسخ ایمنی در جوجه‌های گوشتی

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چکیده

زمینه مطالعه: متیونین گیاهی می‌تواند نسبت به متیونین سنتتیک با تعیین زیست فراهمی این منبع متیونین مقایسه شود. **هدف:** کارایی زیستی نسبی متیونین گیاهی در مقایسه با DL- متیونین بر پایه عملکرد و پاسخ ایمنی منابع متیونین در این آزمایش روی جوجه‌های گوشتی نر بررسی شد. ۱۶۰ جوجه گوشتی نر با جیره پایه فاقد متیونین و یا جیره پایه‌ای که ۳ و ۴ سطح دو منبع متیونین به‌آن اضافه‌شده بودند تغذیه شدند. **روش کار:** رگرسیون نمایی چند گانه نمایی و چند گانه خطی برای تخمین زیست فراهمی متیونین گیاهی نسبت به متیونین سنتتیک بر پایه عملکرد و پاسخ ایمنی جوجه‌های گوشتی به‌کار گرفته‌شد. **نتایج:** افزایش وزن و خوراک مصرفی جوجه‌های گوشتی، بدون در نظر گرفتن نوع منبع متیونین، نسبت به جوجه‌های تغذیه شده با جیره پایه به طور معنی‌داری افزایش یافت ($p<0/05$). در سن ۲۸ روزگی، پاسخ ایمنی جوجه‌های گوشتی معنی‌دار نبود ($p>0/05$) در حالیکه در سن ۴۲ روزگی، جوجه‌های گوشتی به طور معنی‌داری با افزایش سطوح متیونین تحت تأثیر قرار گرفتند. **نتیجه‌گیری نهایی:** کارایی زیستی متیونین گیاهی نسبت به متیونین سنتتیک بر پایه محصول ۵۵٪ (برای افزایش وزن)، ۷۱٪ (برای خوراک مصرفی)، ۷۸٪ (برای ضریب تبدیل غذایی) و ۷۰٪ (برای محلول ۱- کلرو ۲ و ۳ دی نیترو بنزن)، ۶۷٪ (برای سلول خونی قرمز گوسفند) و ۶۸٪ (برای فیتوهماگلوتنین) می‌باشد. کارایی زیستی نسبی متیونین گیاهی در مقایسه با متیونین سنتتیک ۶۸٪ به طور میانگین بر پایه عملکرد رشد و کلیه پاسخ‌های ایمنی می‌باشد. متیونین گیاهی می‌تواند به عنوان یک منبع جدید و طبیعی از متیونین در صنعت طیور به‌کار گرفته شود.

واژه‌های کلیدی: کارایی زیستی، جوجه‌های گوشتی، پاسخ ایمنی، منابع متیونین، رگرسیون چندگانه

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