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Relative biological efficacy of methionine hydroxy analogue-free acid compared to DL-methionine in the broiler chickens

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Abstract

Background: In the broiler's diets based on corn–soya bean meal, methionine (Met) and cystine (Cys), known as sulphur amino acids (SAAs), are the first limiting indispensable amino acids because of their limited presence, which are supplemented with different synthetic sources. Evaluation of the biological effectiveness of these sources can be important in their correct replacement, especially in the starter and growth diets.

Objectives: The current study was done to assess the relative biological efficacy (RBE) of liquid Met hydroxy analogue-free acid (MHA-FA) in comparison with DL-Met (DL-Met) based on broiler performance traits at different levels of digestible SAA in the 1–11 (starter) and 11–25 (grower) days of age periods.

Methods: Two experiments were developed with treatments consisting of a basal diet without Met addition that met the nutrient and energy requirements of broilers with the exception of SAAs (Met + Cys) and five increasing Met doses for both sources (DL-Met and/or MHA-FA), resulting in digestible SAA concentrations from 0.62% to 1.02% of diet in the starter period (Trial 1) and 0.59% to 0.94% of diet in the grower period (Trial 2). The multi-linear regression model and slope ratio method were employed to calculate the RBE of MHA-FA compared with DL-Met for measured variables.

Results: In both experiments, the results obtained during the starter and grower periods with the different Met supplementations show significant growth responses to digestible SAAs levels. By increasing dietary DL-Met and/or MHA-FA levels, the growth performance traits and immune responses were improved (quadratic; $p < 0.05$). The RBE of MHA-FA compared to DL-Met on an equimolar basis was estimated 66%–89% (59%–79% on a weight-to-weight basis).

Conclusions: It is concluded that the RBE of MHA-FA in comparison with DL-Met depends on broiler chicken age and what attribute is being evaluated.

KEYWORDS
broiler chickens, growth performance, immunity, relative biological efficacy

1 | INTRODUCTION

Methionine (Met) and cystine (Cys), known as sulphur-containing amino acids (SAA), are the first limiting amino acids in practical poultry diets because of their limited presence in protein sources of plant origin (Ghavi et al., 2021; Pontin et al., 2018). In animal tissue, four main pathways are responsible for Met metabolism: protein synthesis, trans-methylation to form S-adenosyl Met, deamination to form oxaloacetate and trans-sulphuration to form L-cysteine (Wan et al., 2017). In the scientific literature, several roles for Met are summarized, including that it plays a vital role in many metabolic reactions and energy production (Rehman et al., 2019), boosts livability (Reda et al., 2020), is an initiator amino acid in the protein synthesis (Pontin et al., 2018), is an antioxidant that increases productivity by improving the activity of the antioxidant system (Elnesr et al., 2019) and modulates the immune response.

Broiler diet is commonly supplemented as dry DL-Met (DL-Met) containing about 99% of active substance or as DL-2-hydroxy-4-[methyl] butanoic acid, known as liquid Met hydroxy analogue-free acid (MHA-FA) containing 12% water and 88% active substance (Lemme et al., 2002; Sauer et al., 2008). MHA-FA lacks amino group within its structure and features a hydroxyl group at the asymmetric carbon atom, whereas DL-Met possesses an amino group (Kluge et al., 2016). This chemical difference results in substantial differences between them regarding chemistry, absorption, transport within the body and metabolism by different tissues (Dibner, 2003; Martin-Venegas et al., 2006).

Information about the relative biological efficacy (RBE) of MHA-FA compared with DL-Met is a relevant factor for cost-effective purchasing, feed formulation and animal production (Kim et al., 2019; Sauer et al., 2008). Through the years, there's been considerable research into the potency and usage of these various Met sources, and it has been reported that both Met sources allow for accurate balancing of the dietary SAAs in poultry nutrition. MHA-FA has a value of 88% Met based on its normal chemical structure. The availability of this 88% value has then been shown to vary from 60% to 100% (Leeson & Summers, 2005). In the literature, there is a controversial discussion on the biological effectiveness of MHA-FA compared with DL-Met; the average RBE of MHA-FA products compared to DL-Met is reported to be in the range of 75%–80% on an equimolar basis (Esteve-Garcia & Llaurado, 1997; Huyghebaert, 1993; Lemme et al., 2002; Liu et al., 2006; Rostagno & Barbosa, 1995; Schutte & Jong, 1996; Zou et al., 2015). Even though two meta-analyses of the biological effectiveness of those two compounds have now been performed, the outcomes of both of these analyses are very different (Sauer et al., 2008; Vazquez-Anon et al., 2006). One concludes that DL-Met features a higher activity as a supply of Met in broilers than MHA-FA on an equimolar basis (Sauer et al., 2008), whereas the other one revealed that both compounds have the same activity (Vazquez-Anon et al., 2006).

Given this background, considering that the question of relative potency of products such as MHA-FA often arises in the selection of Met source, the current study was done to determine the biological effectiveness of MHA-FA relative to DL-Met in broiler chickens during starter (1–11 days of age) and grower (11–24 days of age) periods. For this purpose, two dose–response experiments were performed, and responses (growth performance indices, breast weight and immunity against avian influenzas [AI] virus inoculation) were considered to calculate the RBE of MHA-FA in compassion to DL-Met by multiple linear regression and the slope ratio method.

2 | MATERIALS AND METHODS

2.1 | Housing and management

The house temperature during the first 3-day-old was set at the range of 32–34°C, and with increasing age, it gradually decreased (0.5°C/day) to reach at the range of 20–22°C and was kept constant thereafter. During the whole rearing period, the relative humidity was 50%–60%. The birds received continuous lighting during the first 24 h, 23L:1D on days 2 and 3, then maintained an 18L:6D schedule throughout the experimental period. All birds were reared in floor pens with wood shavings as litter and had free access to mash feed and water throughout the experiment period.

2.2 | Birds and experimental design

In Trail 1, newly hatched feather-sexed male Ross-308 chicks (n 792, Ross 308, mean weight 50 ± 1.60 g/birds) were randomly allotted into one of 66 pens (12 chicks each; 0.1 m^2/bird) and arranged in a completely randomized design with 11 treatments and 6 replicates per treatment. A batch of basal starter diet for 1–11 days of age period was formulated to meet requirements as recommended by the strain Management Guide (Aviagen, 2022) except SAAs (Met + Cys), then divided into 11 equal portions, 1 portion non-supplement, graded levels of DL-Met at the rate of 0.8, 1.6, 2.4, 3.2 and 4.0 g/kg were added to five portions, and other 5 portions were supplemented with MHA-FA at the rate of 0.9, 1.8, 2.7, 3.6 and 4.5 g/kg in expense of corn starch and mixed to provide 11 experimental diets (Tables 1 & 2). The experiment lasted for 11 days (1–11 days of age).

Similarly, in Trail 2, newly hatched feather-sexed male chicks were obtained and fed a commercial starter diet (22% CP and 3000 kcal ME/kg) until 10 days of age. At 11 days of age, with deleted heavier and lighter birds, a total of 660 chicks (253 ± 8.14 g/birds) were assigned to 11 treatments, with 6 replicates per treatment and 10 birds each. A batch of basal grower diet was formulated for 11–24 days of age to meet requirements as recommended by the strain Management Guide (Aviagen, 2022) except SAA (Met + Cys), then divided into 11 equal portions, 1 portion non-supplement, 5 portions were supplemented with DL-Met at the rate of 0.7, 1.4, 2.1, 2.8 and 3.5 g/kg and other 5 portions were supplemented with MHA-FA at the rate of 0.8, 1.6, 2.4, 3.2 and 4.0 g/kg in expense of corn starch and mixed to provide 11

TABLE 1 Ingredients and nutrient composition of the basal diets.

Items	Trial 1 (days 1–11)[a]	Trial 2 (days 11–24)[b]
Ingredient, g/kg as-fed basis		
Corn (ME = 3498 kcal/kg, CP = 7.8%)	519.4	551.9
Soya bean meal (ME = 2800, CP = 45.22%)	413.3	376.5
Soya bean oil (ME = 8820 kcal/kg)	20.1	29.0
Limestone	11.6	10.6
Dicalcium phosphate	18.7	16.6
Sodium chloride	4.3	4.4
Vitamin premix[c]	2.5	2.5
Mineral premix[d]	2.5	2.5
L-Lysine-HCL	1.3	1.2
L-Threonine	1.8	0.8
Filler (corn starch)	4.5	4.0
Determined nutrient composition[e], as-fed basis		
Metabolizable energy, kcal/kg	3000	3100
Crude protein, %	23.0	21.5
Calcium, %	0.96	0.87
Available phosphorus, %	0.48	0.43
Sodium, %	0.20	0.20
Digestible lysine, %	1.28	1.15
Digestible methionine, %	0.32	0.30
Digestible sulphur amino acids, %	0.62	0.59
Digestible threonine, %	0.86	0.77

Abbreviations: CP, crude protein; ME, metabolizable energy.

[a] The experimental diets were provided in a such way that a batch of basal diet (without methionine supplementation) was made which met the nutrients and energy requirements of broiler chickens during the starter period with the exception of sulphur amino acids (SAAs) and then divided into 11 equal portions, 1 portion non-supplement, 5 portions were supplemented with DL-methionine (DL-Met 99%, Evonik Degussa GmbH) at the rate of 0.8, 1.6, 2.4, 3.2 and 4.0 g/kg and other 5 portions were supplemented with liquid methionine hydroxy analogue-free acid (MHA-FA 88%, Adisseo) at the rate of 0, 0.9, 1.8, 2.7, 3.6 and 4.5 g/kg in expense of filler (corn starch) and mixed to provide 11 experimental diets.

[b] The experimental diets were provided in a such way that a batch of basal diet (without methionine supplementation) was made which met the nutrients and energy requirements of broiler chickens during the grower period with the exception of SAAs and then divided into 11 equal portions, 1 portion non-supplement, 5 portions were supplemented with DL-methionine (DL-Met 99%, Evonik Degussa GmbH) at the rate of 0.7, 1.4, 2.1, 2.8 and 3.5 g/kg and other 5 portions were supplemented with liquid methionine hydroxy analogue-free acid (MHA-FA 88%, Adisseo) at the rate of 0.8, 1.6, 2.4, 3.2 and 4.0 g/kg in expense of filler (corn starch) and mixed to provide 11 experimental diets.

[c] Vitamin premix supplied the followings per kilogram of diet: vitamin A (all-trans-retinol), 12,000 IU; vitamin D3 (cholecalciferol), 5000 IU; vitamin E (α-tocopherol), 18 IU; vitamin K3 (menadione), 2.65 mg; vitamin B1 (thiamin), 2.97 mg; vitamin B2 (riboflavin), 8.0 mg; vitamin B3 (niacin), 57.42 mg; vitamin B5 (pantothenic acid), 17.86 mg; vitamin B6 (pyridoxine), 4.45 mg; vitamin B9 (folic acid), 1.9 mg; vitamin B12 (cyanocobalamin), 0.02 mg; vitamin H2 (biotin), 0.18 mg; choline chloride, 487.5 mg, and antioxidant 1.0 mg.

[d] Mineral premix supplied the followings per kilogram of diet: Zn (zinc sulfate), 110 mg; Mn (manganese sulfate), 120.6; Fe (iron sulfate), 40.5; Cu (copper sulfate), 16.1; I (calcium iodate), 1.26; Se (Sodium Selenite), 0.31; choline chloride, 474.0.

[e] The determined ingredient analysis was used to calculate nutrient composition (crude protein, calcium and sodium were measured by the AOAC (2002) methods; metabolizable energy, digestible amino acids and available phosphorus were measured by using the near infra-red analysis.

experimental diets (Tables 1 & 2). The experiment lasted for 14 days (11–24 days of age).

In Trail 2 on day 5, all birds were injected with the inactivated bivalent vaccine of Newcastle disease (ND) and AI by subcutaneous route in the back of the neck (H9N2/ND 0.3 mL/bird, Razi Vaccine & Serum Research Institute, Iran). Moreover, on day 11, all birds were inoculated via the oral route with a commercial live-ND vaccine (Avinew NeO, Boehringer Ingelheim).

2.3 | Data collection and sampling

In both experiments, the chicks of each pen were weighed in groups at the beginning and end of each experiment. In order to minimize the error resulting from the digestive tract contents weight, the birds were starved for 4 h before weighing. The feed consumption of each pen was calculated by subtracting the amount of feed remaining at the end of each experiment from the total feed given during the experimental

TABLE 2 Experimental design.

Trial 1 (days 1–11)				Trial 2 (days 11–24)			
DL-Met inclusion, g/kg	MHA-FA inclusion, g/kg	Dietary dig. Met level[a], %	Dietary dig. SAAs level[a], %	DL-Met inclusion, g/kg	MHA-FA inclusion, g/kg	Dietary dig. Met level[a], %	Dietary dig. SAAs level[a], %
Non-supplement		0.32	0.62	Non-supplement		0.30	0.59
–	0.9	0.40	0.70	–	0.8	0.37	0.66
–	1.8	0.48	0.78	–	1.6	0.44	0.73
–	2.7	0.56	0.86	–	2.4	0.51	0.80
–	3.6	0.64	0.94	–	3.2	0.58	0.87
–	4.5	0.72	1.02	–	4.0	0.65	0.94
0.8	–	0.40	0.70	0.7	–	0.37	0.66
1.6	–	0.48	0.78	1.4	–	0.44	0.73
2.4	–	0.56	0.86	2.1	–	0.51	0.80
3.2	–	0.64	0.94	2.8	–	0.58	0.87
4.0	–	0.72	1.02	3.5	–	0.65	0.94

Abbreviations: DL-Met, DL-methionine; MHA-FA, methionine hydroxy analogue-free acid; dig. Met, digestible methionine; dig. SAAs, digestible sulphur amino acids.

[a]The ingredient analysis was determined by using the near infra-red and methionine equimolar content of 88% in MHA-FA and 99% in DL-Met commercial products were used to calculate experimental diet digestible Met and digestible sulphur amino acid composition.

period and adjusted for mortality. The growth performance traits as final live body weight (LBW), weight gain (WG), feed intake (FI) and feed efficiency (FE) were calculated.

At the end of both experiments (11 day old in Trail 1, and 24 days old in Trail 2), one bird from each repetition related to each treatment (six birds/treatment) was randomly selected after 4 h of feed withdrawal but had free access to drinking water and was weighed and slaughtered. After slaughtering, the carcass (skinless), breast muscle (boneless and skinless) and dressing (skin + feathers) were weighed and then calculated as a percentage of LBW (g/100 g LBW).

At 24 days of age, one bird per replicate (six birds/treatment) was randomly selected, and 2 mL blood sample was collected from the brachial vein into a non-heparinized tube for antibody assay against ND virus (NDV) and AI virus (AIV) inoculations. After allowing for the completion of clotting, blood samples were centrifuged at 1900 *g* for 10 min at 4°C to extract serum. Subsequently, serum samples were frozen at −20°C for later analysis.

An immunological evaluation was carried out using HI kits (IDEXX, Labs Inc.) for antibody testing against NDV inoculation. Moreover, antibody titre against AIV inoculation was performed using commercially available ELISA kits (IDEXX, Labs Inc.). ELISA absorbance was measured at 650 nm using an ELISA reader (Bio-Tek Instruments Inc. ELX 800) according to standard procedures.

2.4 | Statistical analysis

The pen mean was considered the experimental unit for all statistical analyses. All data were resulted from this study were tested for normality by using the univariate plot normal procedure, and after removing the outlier data were analysed using the General Linear Model procedure of SAS 9.1 software (SAS, 2014). The differences between treatment groups were compared using the Tukey test with the adjustment for multiple comparisons ($p < 0.05$). The linear and quadratic responses to dietary DL-Met and/or MHA-FA supplementation levels were calculated by using polynomial orthogonal contrasts.

In both trials, a multiple linear regression model was applied to estimate the RBE value of MHA-FA compared to DL-Met (Agostini et al., 2016) by using SAS 9.1 NLIN Proc (Littell et al., 1997):

$$Y = a + \beta_1 X_1 + b\beta_2 X_2 + \varepsilon, \quad \text{RBE} = \frac{\beta_2}{\beta_1}$$

where *Y* is the dependent variable, *a* is the intercept (parameters with the basal diet), β_1 is the slope ratio for DL-Met, β_2 is the slope ratio for MHA-FA, X_1 is the independent variable (dietary supplemented levels of DL-Met), X_2 is the independent variable (dietary supplemented levels of MHA-FA), ε is the random error and RBE is the relative biological efficacy of MHA-FA compared to DL-Met.

3 | RESULTS

The results for measured variables in Trail 1 are presented in Table 3. Analysis of variance showed, in response to increasing dietary digestible Met level by DL-Met and/or MHA-FA supplementation during the starter (1–11 days of age) phase, the main effect of Met supplemental source was significant on variables including LBW at 11 days of age, FI, WG ($p < 0.05$) and FE ($p < 0.001$). The main effect

TABLE 3 Growth performance and carcass traits of broilers fed graded levels of DL-methionine (DL-Met) or liquid methionine hydroxy analogue-free acid (MHA-FA) during the 1–11 days of age (Trial 1)[a].

Inclusion, g/kg DL-Met	Inclusion, g/kg MHA-FA	Digestible Met (SAAs) level[b], %	Final live body weight, g/bird	Feed intake, g/bird	Weight gain, g/bird	Feed efficiency, g/1000 g FI	Carcass relative weight[c], g/100 LBW	Breast relative weight[d], g/100 g LBW	Dressing relative weight[e], g/100 g LBW
Non- supplement		0.32 (0.62)	221^{b}	306^{c}	170^{b}	556^{b}	49.36	13.99^{b}	5.98
–	0.9	0.40 (0.70)	259^{a}	359^{ab}	208^{a}	579^{ab}	49.73	14.76^{ab}	6.68
–	1.8	0.48 (0.78)	265^{a}	367^{a}	213^{a}	579^{ab}	50.58	15.28^{ab}	6.20
–	2.7	0.56 (0.86)	261^{a}	350^{ab}	210^{a}	600^{ab}	51.04	17.39^{a}	6.46
–	3.6	0.64 (0.94)	260^{a}	336^{b}	210^{a}	627^{a}	50.81	17.20^{a}	6.42
–	4.5	0.72 (1.02)	256^{a}	347^{ab}	205^{a}	591^{ab}	49.28	15.87^{ab}	5.94
0.8	–	0.40 (0.70)	256^{a}	339^{ab}	204^{a}	601^{ab}	50.86	15.99^{ab}	6.28
1.6	–	0.48 (0.78)	261^{a}	338^{b}	211^{a}	623^{a}	50.48	15.80^{ab}	6.55
2.4	–	0.56 (0.86)	267^{a}	343^{ab}	215^{a}	628^{a}	51.28	17.52^{a}	6.33
3.2	–	0.64 (0.94)	274^{a}	354^{ab}	224^{a}	633^{a}	51.12	17.82^{a}	5.68
4.0	–	0.72 (1.02)	271^{a}	346^{ab}	221^{a}	637^{a}	50.49	16.19^{ab}	6.21
SEM			5.54	6.26	5.57	14.74	1.04	0.67	0.32
Digestible Met (SAAs) level, %									
0.40 (0.70)			257	349	206	590^{b}	50.29	15.37^{b}	6.48
0.48 (0.78)			263	353	212	601^{ab}	50.53	15.54^{b}	6.37
0.56 (0.86)			264	347	213	614^{ab}	51.16	17.46^{a}	6.39
0.64 (0.94)			267	345	217	630^{a}	50.97	17.51^{a}	6.05
0.72 (1.02)			264	346	213	614^{ab}	49.88	16.03^{ab}	6.08
SEM			3.48	3.72	3.51	9.25	0.736	0.46	0.23
Supplemental source									
MHA-FA			260^{b}	352^{a}	209^{b}	595^{b}	50.29	16.10	6.34
DL-Met			266^{a}	344^{b}	215^{a}	624^{a}	50.84	16.66	6.21
SEM			2.19	2.35	2.22	5.85	0.46	0.28	0.14
p-Value									
level			0.391	0.629	0.273	0.043	0.744	0.002	0.565
Source			0.048	0.021	0.041	0.001	0.403	0.173	0.523
Level × source			0.173	0.001	0.188	0.550	0.960	0.932	0.415
MHA-FA dose response									
Linear			0.001	0.001	0.001	0.148	0.136	0.021	0.175
Quadratic			0.001	0.001	0.001	0.231	0.145	0.046	0.163
DL-Met dose response									
Linear			0.001	0.016	0.001	0.016	0.243	0.007	0.417
Quadratic			0.003	0.041	0.004	0.047	0.281	0.015	0.394

Note: In each column for each effect, values with different superscripts (a–c) are significantly different ($p < 0.05$).

Abbreviations: FI, feed intake; LBW, live body weight.

[a] Every value is the means of 30, 12 and 6 replicates for supplemental source, digestible Met (Met + Cys) level and interaction effects, respectively.

[b] Based on the MHA-FA and DL-Met content of 88% and 99% of Met equivalent in the commercial product, respectively.

[c] Skinless.

[d] Boneless and skinless.

[e] Dressing (skin + feathers).

of dietary digestible SAA (Met + Cys) level was significant on FE ($p < 0.05$) and breast relative weight (BRW) ($p < 0.01$). The main effect of Met supplemental source and dietary digestible SAA level on carcass relative weight (CRW) and dressing relative weight (DRW) was not significant ($p > 0.05$). The interaction effects between Met source and SAA level were not significant ($p > 0.05$) on all of traits, with the exception of FI. Corresponding to inclusion graded levels of DL-Met (+0.8 g/kg of diet) and or MHA-FA (0.9 g/kg of diet) to diet, the analysis of regression results showed 11-day LBW, WG, FE and BRW improved with quadratic polynomial trend ($p < 0.05$), which peaked at 3.2 g/kg of diet for DL-Met and/or 3.6 g/kg of diet for MHA-FA supplementation level. The birds fed an un-supplemented diet (containing 0.32% digestible Met and 0.62% digestible SAAs) showed the lowest LBW, WG, FE and BRW. The addition of DL-Met at the level of 3.2 g/kg of diet and/or MHA-FA at the level of 3.6 g/kg of diet (0.32% Met equivalents) led to significantly higher variable responses. In compassion with the birds fed basal diet (un-supplemented), the birds fed diet supplemented with 3.2 g/kg DL-Met and/or 3.6 g/kg MHA-FA showed 17%, 21%, 21% and 27% and/or 11%, 14%, 20% and 23% higher in 11-day LBW, WG, FE and BRW, respectively.

The results for measured variables in Trail 2 are shown in Table 4. In response to increasing dietary digestible SAA levels by DL-Met and/or MHA-FA supplementals during grower (11–24 days of age) period, the main effect of Met source was significant on WG, FE, BRW and immune response against AIV inoculation ($p < 0.05$). The main effect of digestible SAA level was significant on all traits ($p < 0.05$), exception FI and immune response against AIV inoculation. The interaction effects between Met source and digestible level were not significant ($p > 0.05$) on all traits. Corresponding to graded inclusion levels of DL-Met (+0.7 g/kg) and/or MHA-FA (0.8 g/kg of diet), the LBW at 24 days of age, WG, FE, CRW, BRW and immune response against AIV inoculation were improved with a quadratic trend ($p < 0.05$). The birds fed un-supplemented diet (containing 0.30% digestible Met and 0.59% digestible SAA) showed the lowest LBW, WG, FE, BRW, RDW and immune responses against NDV and AIV inoculation. The addition of DL-Met at the level of 1.4 g/kg and/or MHA-FA at the level of 1.6 g/kg of grower diet led to 22% and 28% and/or 16% and 19% increases in 24-day LBW and WG, respectively. The highest improvement on FE and BRW was shown by the birds fed grower diet supplemented with DL-Met at the level of 1.4 g/kg and/or MHA-FA at the level of 2.4 g/kg, which were 16% and 31% and/or 6% and 18% higher than those fed non-supplemented diet, respectively.

The results obtained from multi-linear regression model and slope ratio method for measuring the RBE of MHA-FA compared with DL-Met are reported in Table 5 and Figures 1–3. During the starter (1–11 days of age) period, the RBE of MHA-FA was estimated 87.57%, 68.54% and 87% as efficacious as DL-Met at a equimolar basis for WG (Figure 1A), FE (Figure 1B) and BRW (Figure 1C), respectively. Similarly, in the grower (11–24 days of age) period, the RBE of MHA-FA was estimated 84.15%, 66.15%, 79.35%, 89.20% and 83.90% as efficacious as DL-Met at a equimolar basis for WG (Figure 2A), FE (Figure 2B), CRW (Figure 2C), BRW (Figure 2D) and immunity against AIV inoculation (Figure 3), respectively.

4 | DISCUSSION

In the current experiment, agreement with many other authors (Daenner & Bessei, 2003; Liu et al., 2006; Mandal et al., 2004; Schutte & Pack, 1995), starter and grower diet supplementations with DL-Met and/or MHA-FA led a clear improvement in growth performance, carcass and breast yield and immune responses; this achievement indicates that corn–soya bean meal basal diet formulated for broiler chickens has limitations in SAA (Met + Cys). These findings support the hypothesis that dietary amino acid fortification can improve live performance (Liu et al., 2006) and increase muscle yield (Nukreaw et al., 2011; Rehman et al., 2019) of modern broilers.

The birds were performed responses well to fed graded either Met source. However, the broilers fed DL-Met than those fed MHA-FA more effectively performed (Figures 1–3). This result was consistent with some previous publications indicating lower efficacy of MHA-FA in promoting growth performance and muscle deposition of the birds when compared to DL-Met (Esteve-Garcia & Llaurado, 1997; Lemme et al., 2002; Mandal et al., 2004). However, our result was not consistent with some other publications (Liu et al., 2006). The discrepancy might be attributive to different diet types, experimental design and application of statistical methodologies for interpretation of data.

The main objective of the current study was to compare the RBE of two Met sources (MHA-FA and DL-Met on an equimolar basis) in the broiler chickens fed corn–soya bean meal diet in the starter and grower phases. The result showed the addition of graded levels of DL-Met and/or MHA-FA caused a numerical increase in growth performance, breast weight, dressing weight and immunity against ND and AIV inoculation responses. But there were significant differences in measured variables among the broilers fed DL-Met or MHA-FA at each equivalent inclusion Met level; the growth performance of broilers fed diet containing equal SAA formulated by MHA-FA supplementation was lower than that of birds fed diet formulated by DL-Met supplementation (Tables 3 and 4). This finding confirmed the reports of earlier researchers (Esteve-Garcia & Llaurado, 1997; Huyghebaert, 1993; Jansman et al., 2003; Rostagno & Barbosa, 1995; Sauer et al., 2008; Schutte & Jong, 1996). The current study estimated that the RBE of MHA-FA (Table 5 and Figures 1–3) is very variable, corresponds to broiler age and what traits are considered for comparison and is lower (at the range of 66%–89% or 59%–79% relative to DL-Met on equimolar or weight-to-weight basis, respectively) than the poultry feed manufacturer used (88% equivalence of MHA-FA relative to DL-Met). This achievement is supported by those of Sauer et al. (2008), who performed a meta-analysis to compare the biological efficacy of MHA-FA with DL-Met in broiler chickens and estimated relative biological effectiveness of MHA-FA in a comparison to DL-Met at the range of 79%–81% on an equimolar basis in broiler chickens. Moreover, Jansman et al. (2003) obtained in broilers only a marginal difference of 77% and 76% in biological efficacy for the WG and feed conversion ratio, respectively. Moreover, a low efficacy of MHA-FA (73% on equimolar basis) relative to DL-Met was reported (Hoehler et al., 2005).

TABLE 4 Growth performance, carcass traits and immune response of broilers fed graded levels of DL-methionine (DL-Met) or liquid methionine hydroxy analogue-free acid (MHA-FA) during the 11–24 days of age (Trial 2)[a].

Inclusion, g/kg											
DL-Met	MHA-FA	Digestible Met (SAAs) level[b], %	Final live body weight, g/bird	Feed intake, g/bird	Weight gain, g/bird	Feed efficiency, g/1000 g FI	Carcass relative weight[c], g/100 LBW	Breast relative weight[d], g/100 g LBW	Dressing relative weight[e], g/100 g LBW	NDV immune responses, $\log_2$	AIV immune responses, $\log_2$
Non- supplement		0.30 (0.59)	809^{b}	1037	566^{b}	545^{b}	58.57^{b}	19.52^{d}	8.04^{a}	5.68	3.25^{c}
–	0.8	0.37 (0.66)	836^{ab}	1045	592^{ab}	564^{b}	59.25^{ab}	21.43^{bcd}	8.31^{a}	6.04	4.00^{abc}
–	1.6	0.44 (0.73)	934^{ab}	1114	675^{ab}	609^{a}	60.77^{ab}	21.66^{bcd}	7.02^{ab}	6.26	3.25^{c}
–	2.4	0.51 (0.80)	883^{ab}	1037	620^{ab}	600^{ab}	60.92^{ab}	22.90^{b}	7.59^{ab}	5.79	3.00^{c}
–	3.2	0.58 (0.87)	886^{ab}	1071	625^{ab}	582^{ab}	60.10^{ab}	22.96^{b}	8.04^{a}	5.79	4.00^{abc}
–	4.0	0.65 (0.94)	891^{ab}	1071	638^{ab}	594^{ab}	59.80^{ab}	22.83^{b}	7.51^{ab}	4.26	4.50^{ab}
0.7	–	0.37 (0.66)	875^{ab}	1027	631^{ab}	613^{a}	59.18^{ab}	20.17^{cd}	6.58^{b}	6.12	3.42^{bc}
1.4	–	0.44 (0.73)	985^{a}	1135	726^{a}	638^{a}	61.75^{a}	23.69^{ab}	6.93^{b}	6.74	4.25^{abc}
2.1	–	0.51 (0.80)	892^{ab}	1036	638^{ab}	615^{a}	61.06^{ab}	23.26^{ab}	8.46^{a}	6.64	5.25^{a}
2.8	–	0.58 (0.87)	903^{ab}	1104	652^{ab}	598^{ab}	61.49^{a}	25.52^{a}	7.62^{ab}	5.10	3.75^{bc}
3.5	–	0.65 (0.94)	929^{ab}	1063	677^{ab}	637^{a}	60.11^{ab}	22.27^{bc}	7.75^{ab}	4.83	3.50^{bc}
SEM			33.27	45.14	31.49	13.99	0.64	0.53	0.31	0.60	0.29
Digestible Met (SAAs) level, %											
0.37 (0.66)			856^{b}	1036	611^{b}	588^{b}	59.22^{b}	20.80^{c}	7.45^{ab}	6.08	3.71
0.44 (0.73)			960^{a}	1125	700^{a}	623^{a}	61.26^{a}	22.67^{b}	6.97^{b}	6.50	3.75
0.51 (0.80)			888^{ab}	1036	629^{ab}	607^{ab}	60.99^{a}	23.08^{ab}	8.02^{a}	6.22	4.13
0.58 (0.87)			895^{ab}	1087	638^{ab}	590^{ab}	60.80^{ab}	24.24^{a}	7.83^{ab}	5.44	3.88
0.65 (0.94)			910^{ab}	1067	657^{ab}	616^{ab}	59.95^{ab}	22.55^{b}	7.63^{ab}	4.55	4.00
SEM			23.54	31.82	22.14	9.84	0.44	0.37	0.23	0.41	0.16
Supplemental sources											
MHA-FA			886	1068	630^{b}	590^{b}	60.17	22.35^{b}	7.69	5.63	3.75^{b}
DL-Met			917	1073	665^{a}	620^{a}	60.72	22.98^{a}	7.47	5.88	4.03^{a}
SEM			14.89	20.12	14.00	6.23	0.28	0.23	0.15	0.26	0.10
p-Value											
level			0.046	0.256	0.044	0.048	0.010	0.001	0.024	0.119	0.278
Source			0.153	0.851	0.042	0.001	0.162	0.044	0.279	0.422	0.041
Level × source			0.966	0.979	0.988	0.641	0.739	0.102	0.104	0.613	0.121
MHA-FA dose response											
Linear			0.058	0.661	0.045	0.019	0.021	0.004	0.156	0.153	0.078
Quadratic			0.121	0.692	0.023	0.033	0.030	0.016	0.199	0.134	0.042
DL-Met dose response											
Linear			0.034	0.367	0.031	0.022	0.001	0.001	0.495	0.161	0.002
Quadratic			0.047	0.408	0.041	0.043	0.002	0.001	0.437	0.143	0.002

Note: In each column for each effect, values with different superscripts (a–c) are significantly different ($p < 0.05$).

Abbreviations: AIV, avian influenza vaccination; Dig SAAs, digestible sulphur amino acids; FI, feed intake; LBW, live body weight; NDV, Newcastle disease vaccination.

[a] Every value is the means of 30, 12 and 6 replicates for supplemental source, digestible Met (Met + Cys) level and interaction effects, respectively.

[b] Based on the MHA-FA and DL-Met content of 88% and 99% of Met equivalent in the commercial product, respectively.

[c] Skinless.

[d] Boneless and skinless.

[e] Dressing (skin + feathers).

TABLE 5 Parameters of linear models describe the relationship between response criteria with supplemental levels and relative biological efficacy values of methionine sources[a].

						Relative bio-efficacy[b], %		
		Estimated β coefficient				MHA-FA		
Items	Y-intercept	DL-Met	MHA-FA	p-Value	R^2	Equimolar basis	Weight to weight basis	DL-Met
Trail 1; fed during the starter period (days 1–11)								
Final live body weight	207.53	0.0291	0.0254	0.0001	0.43	87.35	77.65	100
Daily weight gain	156.61	0.0291	0.0254	0.0001	0.43	87.57	77.84	100
Feed efficiency	532.37	0.0458	0.0314	0.0001	0.27	68.54	60.92	100
Breast relative weight	12.66	0.0020	0.0017	0.0001	0.24	87.00	77.33	100
Trail 2; fed during the grower period (days 11–24)								
Final live body weight	709.64	0.0370	0.0325	0.0001	0.32	87.86	78.10	100
Daily weight gain	476.66	0.0336	0.0282	0.0001	0.29	84.15	74.80	100
Feed efficiency	533.97	0.0144	0.0095	0.0001	0.23	66.15	58.81	100
Carcass relative weight	57.85	0.0005	0.0004	0.0042	0.15	79.35	70.54	100
Breast muscle weight	17.24	0.0010	0.0009	0.0001	0.43	89.20	79.29	100
Immune responses against AIV	2.78	0.0002	0.0002	0.0398	0.1	83.90	74.58	100

Abbreviations: DL-Met, DL-methionine; Dig SAAs, digestible sulphur amino acids; MHA-FA, methionine hydroxy analogue-free acid.

[a] The traits were analysed by multi-linear regression model; $Y = a + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$..

[b] The bio-efficacy values were calculated as slope ratio method: $\frac{\beta_2}{\beta_1}$.

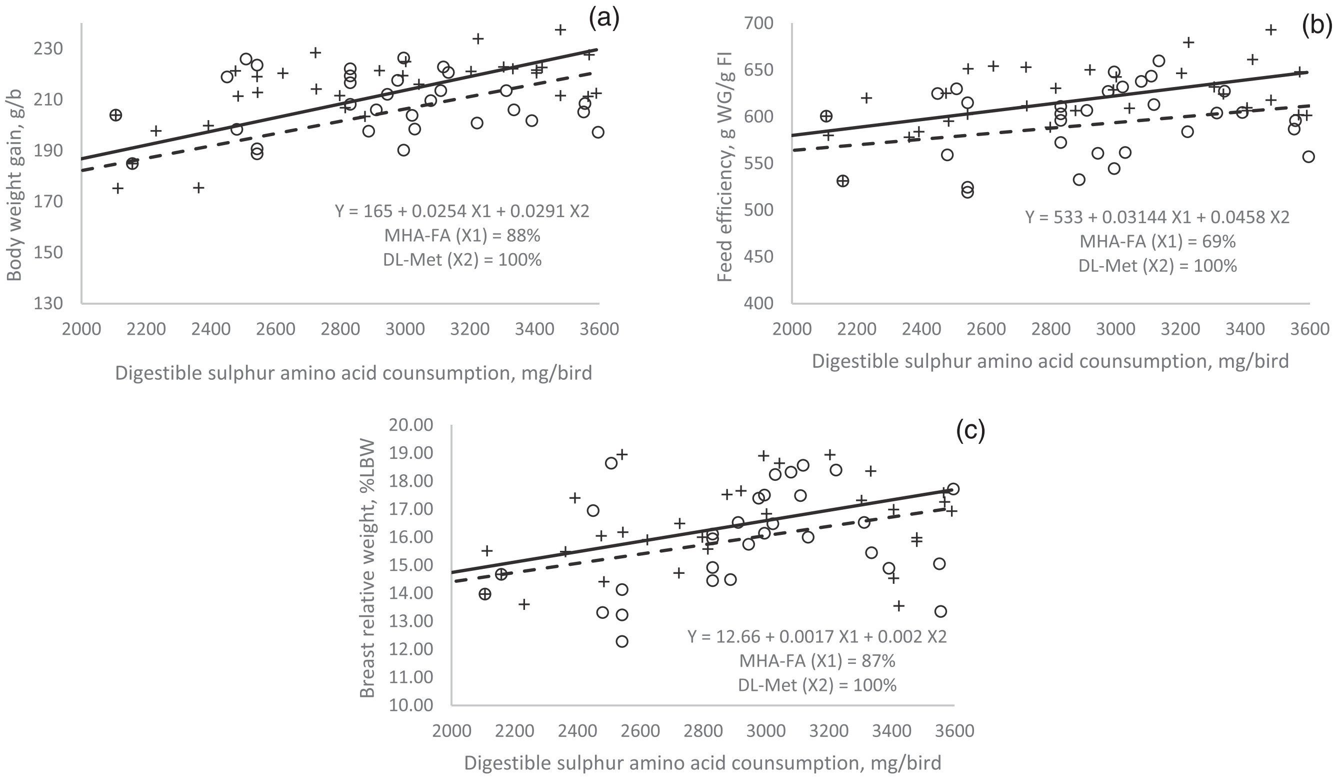

FIGURE 1 Plot of (A) live body weight gain (WG) (g/bird), (B) feed efficiency (g WG/1000 g feed intake [FI]) and (C) breast muscle relative weight (bone and skinless, % of live body weight) of broiler chickens during starter period (1–11 days of age) as a function of digestible sulphur amino acid consumption (mg/bird) of diets were supplemented by methionine hydroxy analogue-free acid (MHA-FA) (○) and or DL-methionine (DL-Met) (+). Predicted line (––) show the relative biological effectiveness of MHA-FA as a compression of DL-Met (——) by using a multi-linear regression model and slope ratio method (Trail 1).

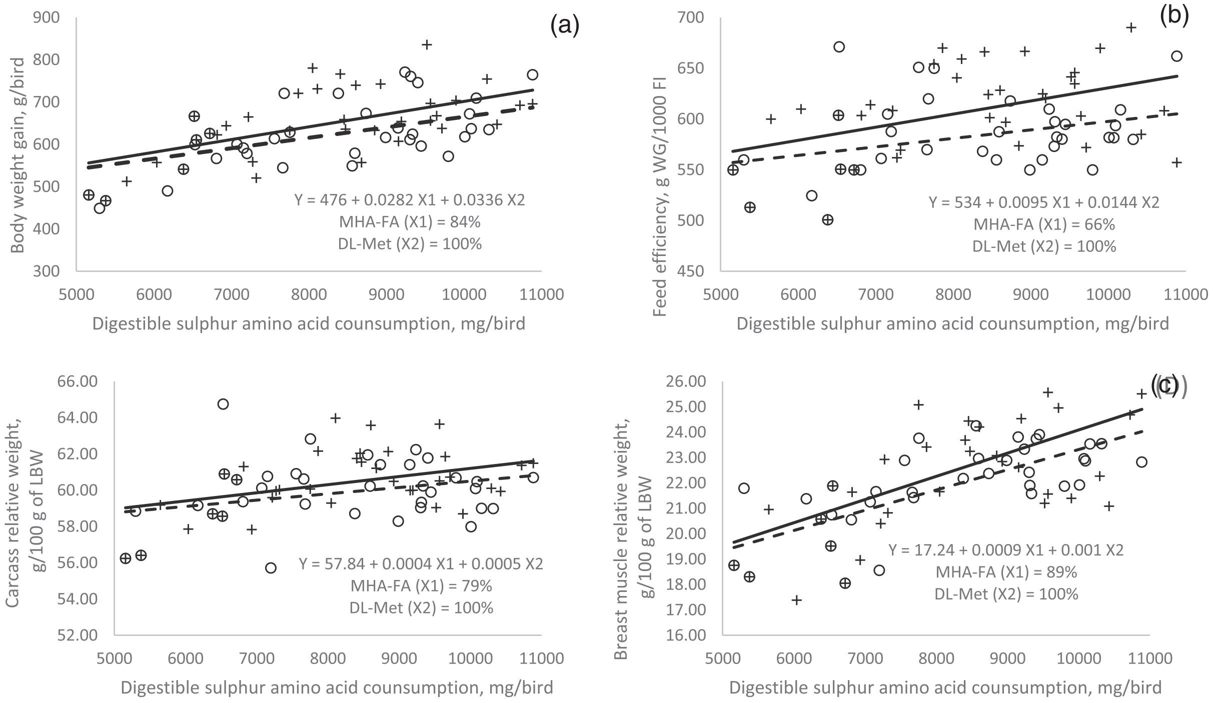

FIGURE 2 Plot of (A) body weight gain (WG) (g/bird), (B) feed efficiency (g WG/1000 g feed intake [FI]), (C) carcass relative weight (skinless, % of live weight) and (D) breast yield relative weight (bone and skinless, % of live weight) of broiler chickens during grower period (11–24 days of age) as a function of digestible sulphur amino acid consumption (mg/bird) of diets were supplemented by (○) methionine hydroxy analogue-free acid (MHA-FA) and or (+) DL-methionine (DL-Met). Predicted line (−−) show the relative biological effectiveness of MHA-FA as a compression of DL-Met (——) by using a multi-linear regression model and slope ratio method (Trail 2).

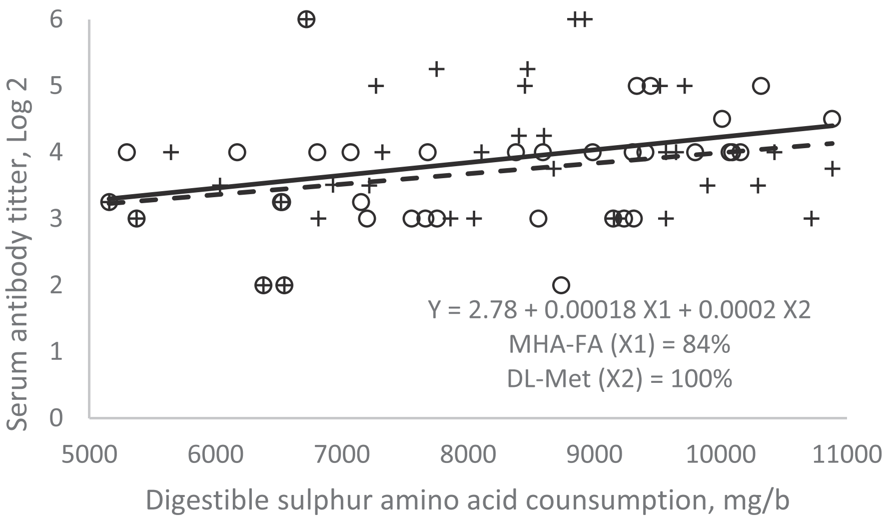

FIGURE 3 Plot of immune response against avian influenza vaccination of broiler chickens as a function of digestible sulphur amino acid consumption (mg/bird) of grower diet supplemented by methionine hydroxy analogue free acid (MHA-FA) (○) and or DL-methionine (DL-Met) (+). Predicted line (−−) show the relative biological effectiveness of MHA-FA as a compression of DL-Met (——) by using a multi-linear regression model and slope ratio method (Trail 2).

Several mechanisms that might explain the lower biological efficacy of MHA-FA, whether functioning alone or in combination, include the following:

I. The poor utilization of MHA-FA is one of the significant reasons for its lower biological efficacy relative to DL-Met (Payne et al., 2006).

II. DL-Met actively absorbed by Na^+-dependent system (transported against a concentration gradient), but MHA-FA is absorbed by the H^+-dependent system which is slower than the Na^+ system (Maenz & Engele-Schaan, 1996b).

III. The Met transporter features a higher affinity and greater velocity of transport for DL-Met than MHA-FA. These declare that the body seems to make use of DL-Met quicker and more proficiently than its MHA-FA counterpart (Maenz & Engele-Schaan, 1996b).

IV. During passage through the gastrointestinal tract, MHA-FA may convert into non-absorbable by-product (Maenz & Engele-Schaan, 1996a; Payne et al., 2006). A substantial portion of MHA is lost due to microbial degradation in the small intestine (Drew et al., 2003).

V. The incorporation of Met into the tissues is significantly higher when administered as DL-Met relative to MHA-FA (Lingens & Molnar, 1996).

VI. Higher potency of MHA relates to variable degradation in body tissues and/or degree of elimination by the kidney than DL-Met (Leeson & Summers, 2005).

It was suggested that this reduced efficacy was due to the inefficiency of the conversion of D- and L-isomers of the analogues, possibly due to some missing factor such as a peptide in this type of diet. However, there is virtually no difference in utilization of hydroxy isomers when diets contain intact product proteins, and under such feeding conditions, the equimolar concentration of DL-Met and products such as MHA-FA seem comparable (Leeson & Summers, 2001).

In contrast, equal bio-efficacy of MHA-FA (Conde-Aguilera et al., 2016; Elkin & Hester, 1983; Hoehler et al., 2005; Jansman et al., 2003; Lemme et al., 2002; Liu et al., 2006; Swennen et al., 2011; Vazquez-Anon et al., 2006; Xi et al., 2007) compared with DL-Met has also been reported in broiler chicks. The outcomes in the different experiments have been inconclusive or appeared to be inconsistent because of differences in bird age, length of trial and SAA levels, and mainly because of the insufficient sensitivity of the respective bioassays (Huyghebaert, 1993; Lemme et al., 2002; Littell et al., 1997).

5 | CONCLUSION

On the basis of the current study results, dietary supplementation of DL-Met and/or MHA-FA as a source of Met for broilers fed corn–soya bean meal diets in the starter (1–11 days of age) and grower (11–24 days of age) periods effectively improved growth performance, breast weight and immunity. The relative biological effectiveness of MHA-FA in comparison with DL-Met on an equimolar basis in broilers was obtained 66%–89% (59%–79% on a weight-to-weight basis). Overall, the estimated biological efficacy of MHA-FA compared to DL-Met varied depending on what productive parameter was taken for optimization and broiler chicken age.

AUTHOR CONTRIBUTIONS

Heydar Zarghi designed the experimental trail. Saeed Ghavi carried out the experimental trail and lab analysis. Heydar Zarghi performed the statistics, tabulated the data and wrote the draft and reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

No potential conflicts of interest were reported by the authors.

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DECLARATIONS

The authors declare that all of the authors listed in the manuscript are employed at an academic or research institution where research or education is the primary function of the entity. Moreover, this manuscript was independently submitted by the authors.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted in the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW

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