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Effects of DL- and L-methionine supplementation on growth performance, carcass quality and relative bioavailability of methionine in broilers fed maize-soybean-based diets

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ABSTRACT. This experiment was conducted to compare the effects and bioavailability of DL-methionine (DL-Met) and L-methionine (L-Met) supplementation in maize-soybean meal-based diets on performance and carcass traits of broiler chickens. A total of 728-day-old male broilers were divided into 7 groups with 9 replicates (7 replicates of 12, and 2 replicates of 10 chicks) using a 2 × 3 + 1 factorial arrangement in a completely randomised design. Dietary treatments consisted of basal diets (BD) (including 0.619, 0.555 and 0.523% digestible methionine + cysteine (Met + Cys) for starter, grower and finisher periods, respectively) supplemented with three levels (0.155, 0.310 and 0.455%) of either DL-Met or L-Met: BD, BD + 0.155% DL-Met, BD + 0.310% DL-Met, BD + 0.455% DL-Met, BD + 0.155% L-Met, BD + 0.310% L-Met and BD + 0.455% L-Met. The interaction between sources and levels of Met supplementation did not influence overall growth performance, yield of carcass and parts, as well as relative internal organ and feather weight. Source of methionine had no significant effect on overall growth performance, yield of carcass and parts, relative internal organ and feather weight, while methionine addition significantly improved growth performance and carcass and cut yields. The slope-ratio assay showed that the relative bioavailability (RBV) of L-methionine to DL-methionine for BW, FCR and BMY of broilers were 123, 91.5 and 88.0%, respectively, i.e. the differences were not significant between the two Met sources. In conclusion, our data indicated that when DL-Met and L-Met were included in feeds at practical levels, they were equally effective as a source of methionine for broilers.

Introduction

Methionine (Met), as an essential amino acid, plays many crucial roles in chick metabolism, including protein synthesis and feather development (Bunchasak, 2009). Met serves as a methyl group and sulphur donor in methylation and trans-sulphuration reactions, respectively, as well as a precursor of some important intermediates (cysteine (Cys), carnitine, S-adenosylmethionine, glutathione, and taurine etc.) in metabolic pathways (Bunchasak, 2009). Met is also involved in the synthesis of certain polyamines and immune system proteins (Bunchasak, 2009; Fang et al., 2010). Researchers demonstrated that methionine deficiency impaired growth performance and breast meat yield (% live weight), while increasing abdominal fat (% live weight) in broiler chickens (Liu et al., 2006; 2007). Additionally, deterioration in immunity (Zhang and Guo, 2008) and meat quality (Liu et al., 2007) were also observed.

Met is the first limiting amino acid in a typical maize-soybean meal-based broiler diets (Dilger and Baker, 2007), and feed grade Met sources, such as DL-Met (equal racemic mixture of D- and L-isomers

of Met), hydroxy analogue of methionine calcium salt (HMTBa-Ca) and hydroxy analogue of methionine (HMTBa or MHA) are all commonly added to broiler diets to balance dietary sulphur amino acid levels according to bird requirements (Kim et al., 2019). Until recently, a commercial product of L-methionine (L-Met) was not available because its chemical production was more expensive and complex than that of DL-Met, and there was no efficient production process based on fermentation. Consequently, studies investigating the effects of different dietary methionine sources on broiler chickens have for decades mainly focused on comparison of DL-Met and MHA. Both sources must be converted to L-Met in order to be used in protein synthesis and other metabolic pathways via an enzymatic conversion of D-methionine to L-methionine in the liver and kidneys (Baker, 2006; Shen et al., 2015). This conversion has been reported to occur at 90% efficiency, mainly in the liver and kidneys of chickens (Ribeiro et al., 2005). However, the expression of D-amino acid oxidase was shown to be significantly low in young animals (D'Aniello et al., 1993). L-isomer is a biologically functional form of Met, as it can be readily used in intestinal cells and directly incorporated into proteins during their synthesis (Fang et al., 2010). L-isomer was demonstrated to be absorbed two times faster compared to D-isomer (Tipton et al., 1966).

It can therefore be assumed that dietary L-Met may be more beneficial than D-Met, which has been supported by previous studies involving broilers (Ribeiro et al., 2005; Shen et al., 2015) and turkey poults (Park et al., 2018). The results of the aforementioned studies showed slight advantages of L-Met compared to DL-Met, but with some inconsistencies which could arise from differences in species, age, and response variables examined. In contrast to these results, some other studies revealed that DL-Met was as effective as L-Met in improving growth performance and carcass characteristics in broilers, but there were differences in their effects on several biochemical pathways in the broiler body (Dilger and Baker, 2007; Zhang et al., 2018). In addition, the data on the biological efficacy of L-Met compared to DL-Met are limited and the results are not fully consistent. Reviewing the literature on the bioavailability of different Met sources, Garlich (1985) and Baker (2006) found a nearly 100% efficiency of both DL- and L-Met. In contrast, Wang et al. (2019) reported that the relative bioavailability of L-Met to DL-Met in terms of body weight gain (BWG) and feed conversion ratio (FCR) was 141.5 and 189.1%, respectively, in 3 weeks old broilers. Thus, the published scientific studies comparing the biological effectiveness of L-Met and DL-Met are limited and their results are largely inconsistent. Recently, Esteve-Garcia and Khan (2018) questioned the efficiency of the D- to L-Met conversion process and emphasized the presence of inconclusive results regarding the bioavailability of different Met isomers and sources in broilers. Moreover, a significantly lower level of Met + Cys (0.69, 0.66, and 0.62% for birds in the starter, grower, and finisher phase, respectively) than recommended, obtained by Millecam et al. (2021) with L-Met supplementation, have demonstrated that further research comparing DL- and L-Met in broiler chickens is still required.

Recent advances in fermentation techniques and the use of genetically modified organisms have facilitated conventional L-Met production (Willke, 2014). This development makes feed-grade L-Met a significant alternative supplementary source of methionine and raises the question whether its substitution for DL-Met would be favourable in broiler nutrition. Knowledge of biological effectiveness of various Met sources is required in order to effectively utilize them in poultry feed production. Therefore, the aims of this study were to compare the effects of two supplemental Met sources (DL- or L-methionine) on growth performance, carcass yield and viscera ratio, and to determine the relative bioavailability (RBV) of L-Met to DL-Met by using the slope-ratio assay in broilers fed maize-soybean meal-based diets.

Material and methods

All procedures were approved by Ankara University Animal Experiments Local Ethics Committee (2015-9-122).

Birds and housing

A total of 728 one-day-old male Ross 308 broilers were used in this experiment. Chicks were reared in floor pens equipped with nipple drinkers and a hanging feeder. Wood shavings were used as litter material in the pens. Feed in the form of mash and water were supplied *ad libitum* during the experiment. All birds were raised in an environmentally controlled poultry house according to the methodology of Aviagen (2014) throughout the study. The lighting programme started with 23 h of light from day 1 to 7, followed by 22 h of light to day 14, and 20 h of light to day 21. Temperature was set at 33 °C for the first 3 days, then gradually decreased

to 23 °C by day 21 and subsequently maintained at this level until the end of the experiment. All other management practices followed the guidelines of the Ross broiler management handbook (Aviagen, 2014).

Experimental design and diets

One-day-old chicks were weighed and then randomly allotted to 7 treatments with 9 replicates (7 replicates of 12, and 2 replicates of 10 chicks) using a 2 × 3 + 1 factorial arrangement in a randomised complete block design with a common basal diet. Maize-soybean meal-based basal diets were formulated to be deficient in Met + Cys without any crystalline Met addition, but all other essential nutrients were provided in an adequate amount to meet broiler requirements (Aviagen 2014), and contained 0.619, 0.555 and 0.523% digestible Met + Cys, for starter (0–11 days), grower (12–25 days) and finisher (26–39 days) periods, respectively (Table 1). Treatments consisted of basal diets (BD) with the addition of three increasing levels of feed grade (99% purity powder) either of DL- or L-Met (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) at the expense of maize as follows: BD, BD + 0.155% DL-Met, BD + 0.310% DL-Met, BD + 0.455% DL-Met, BD + 0.155% L-Met, BD + 0.310% L-Met and BD + 0.455% L-Met. Proximate and amino acid analyses in feed ingredients were performed using near-infrared reflectance spectroscopy (NIRS). Protein and amino acid analyses in the experimental

Table 1. Ingredient and nutrient composition of basal diets, as-fed basis

Ingredients, %	Starter, 0–11 days	Grower, 12–25 days	Finisher, 26–39 days
Maize (7.2% CP)	50.1	58.1	61.8
Soybean meal (47% CP)	40.4	33.1	29.5
Sunflower oil	5.19	4.98	5.14
Limestone (37.7% Ca)	1.02	0.81	0.79
Dicalcium phosphate (22.5% Ca and 18% P)	2.22	2.00	1.86
Sodium chloride	0.30	0.31	0.30
Sodium bicarbonate	0.030	0.020	0.040
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.20	0.20	0.20
Choline chloride (96%)	0.040	0.040	0.040
L-lysine sulphate (54.6%)	0.22	0.17	0.13
L-threonine	0.080	0.060	0.050
L-valine	0.080	0.040	0.020
Calculated values, %			
metabolisable energy, kcal/kg	3025	3100	3150
CP	22.9 (22.6)	20.0 (20.4)	18.5 (18.8)
calcium	1.05	0.90	0.85
available phosphorus	0.50	0.45	0.42
sodium	0.16	0.16	0.16
chloride	0.23	0.24	0.23
choline, mg/kg	1700	1600	1500
ether extract	7.35 (7.58)	7.28 (6.21)	7.50 (6.90)
crude fibre	2.77 (3.58)	2.60 (3.37)	2.50 (2.54)
DEB, mEq/kg	253	220	208
lysine	1.42 (1.40)	1.20 (1.17)	1.08 (1.09)
SID Lys	1.29	1.09	0.98
methionine	0.332 (0.353)	0.297 (0.304)	0.280 (0.301)
SID Met	0.304	0.273	0.257
cystine	0.375 (0.371)	0.336 (0.319)	0.316 (0.315)
Met + Cys	0.712 (0.725)	0.637 (0.623)	0.599 (0.616)
SID Met+Cys	0.619	0.555	0.523
threonine	0.95 (0.98)	0.81 (0.81)	0.74 (0.76)
SID Thr	0.82	0.70	0.64
valine	1.14 (1.14)	0.97 (0.97)	0.89 (0.93)
SID Val	1.02	0.87	0.79

CP – crude protein, DEB – dietary electrolyte balance, SID – standardized ileal digestible; [1] supplied per kg diet: IU: vit. A 10 000, vit. D_3 4 500; mg: vit. E 65, vit. B_1 2.8, vit. B_2 6.5, vit. B_6 3.2, vit. B_{12} 0.017, vit. K_3 3.5, pantothenic acid 18, niacin 55, biotin 0.18, folic acid 1.9; [2] supplied per kg diet: mg: Fe 20, Cu 16, Zn 110, Mn 120, I 1.25, Co 0.9, Se 0.3; the analysed values are presented in parenthesis

Table 2. Analysed amino acid composition of diets, %, as-fed basis

Nutrients	Basal diet	DL-Met			L-Met		
		0.155%	0.310%	0.455%	0.155%	0.310%	0.455%
Starter, 0–11 days							
crude protein	22.6	22.6	23.2	23.3	23.0	23.0	23.1
methionine	0.35	0.51	0.67	0.79	0.51	0.65	0.85
cystine	0.37	0.37	0.37	0.37	0.38	0.37	0.37
Met + Cys	0.72	0.87	1.05	1.17	0.89	1.02	1.22
lysine	1.40	1.40	1.39	1.39	1.44	1.37	1.38
Grower, 12–25 days							
crude protein	20.4	20.0	20.6	20.7	20.2	20.0	20.1
methionine	0.30	0.44	0.57	0.74	0.46	0.57	0.71
cystine	0.32	0.32	0.31	0.32	0.33	0.32	0.32
Met + Cys	0.62	0.76	0.87	1.05	0.79	0.89	1.04
lysine	1.17	1.17	1.16	1.18	1.18	1.15	1.18
Finisher, 26–39 days							
crude protein	18.8	19.0	18.3	19.2	18.1	18.5	19.0
methionine	0.30	0.43	0.56	0.73	0.44	0.57	0.74
cystine	0.31	0.31	0.30	0.31	0.31	0.30	0.30
Met + Cys	0.61	0.75	0.86	1.04	0.75	0.88	1.04
lysine	1.08	1.06	1.03	1.06	1.05	1.03	1.05

diets were conducted using wet chemistry (Llames and Fontaine, 1994; AOAC International, 2000), while proximate analysis (except protein) was performed by NIRS (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) (Table 1 and 2).

Growth performance

Chicks and feed were weighed on day 0, 11, 25 and 39; BWG, feed intake (FI) and FCR (using BWG and FI) were calculated per pen for each growth phase (starter, days 0 to 11; grower, days 12 to 25; finisher, days 26 to 39) and the whole experimental period. Daily mortality was also recorded for each replicate. Dead chicks were removed and weighed daily to calculate mortality and adjust growth performance data.

Carcass and carcass part yields and digestive organ weight

At the end of the experiment, the feed was removed 6 h before processing, then 2 birds per pen, with an average pen weight, were selected and leg-banded. Selected birds were exsanguinated by cutting the jugular vein, allowed to bleed for approximately 1.5 min, scalded at 55 °C for 30 s and then defeathered in a rotary picker. Feather weight was calculated from the difference in body weight before and after the rotary picker step. Viscera and abdominal fat, which consisted of adipose tissue from the proventriculus surrounding the gizzard down to the cloaca, were excised. Afterwards, the weight of pancreas, liver and abdominal fat was determined. Carcass (without head, feet and giblets), thighs, drumsticks and breast (bone-in and skin-on) were obtained and subsequently weighed. The yield of carcass, thighs, drumsticks (bone-in and skin-on), breast meat (bone-in and skin-on), feather, pancreas, liver and abdominal fat were calculated as a percentage of live body weight (BW).

Statistical analysis

Data for all response variables related to different phases of the trial were analysed as a completely randomised block design, with a factorial arrangement of [(2 × 4) − 1] for Met sources and its graded level, respectively, using the general ANOVA procedure implemented in SAS release 9.2 (SAS Institute, 2008). Polynomial contrasts were also applied to determine the linear effects of DL-Met and L-Met levels on response criteria along with the effects of Met sources. Mortality data were subjected to the chi-square test. Pens were treated as experimental units. When significant differences ($P < 0.05$) were found between the groups, means were separated using the Tukey HSD test. BW, FCR, and breast meat yield (BMY) data were analysed by non-linear multi-exponential or multi-linear regression, as suggested by Littell et al. (1997), and according to the following equation for calculating the RBV of L-Met compared to DL-Met: y = a + b * (1 − exp (c1 * x1 + c2 * x2)), where: y – performance criterion, a – performance achieved with the basal diet (y-intercept), b – asymptotic response (difference between a and asymptote), a + b – common asymptote (maximum performance level), c1 – slope coefficient of the L-Met curve, c2 – slope

coefficient of the DL-Met curve, x1 – dietary level of L-Met, x2 – dietary level of DL-Met. The RBV values for DL-Met relative to L-Met were expressed as the ratios of regression coefficients, c_2/c_1 – RBV, according to Littell et al. (1997).

Results

Growth performance

The effects of sources and additional methionine levels on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality are shown in Table 3. Feed intake was not significantly affected by any of the factors in any phase, except for the finisher where methionine supplementation above 0.155% significantly increased (linear, $P = 0.002$) FI compared to the basal diet. FI increased (linear, $P = 0.016$) with incremental Met concentrations, while no effects of Met source were observed in days 0–39.

In the starter period, the interaction between Met source and its level on BWG were significant ($P = 0.008$). Unlike L-Met, the highest additional level of DL-Met (above 0.310%) significantly |reduced BWG, leading to a similar BWG compared to the basal diet. However, no significant effects were found of supplementation level × source or methionine source on BWG for other phases, and thus the whole treatment period. Even the lowest additional level of methionine significantly increased BWG compared to the basal diet in grower and finisher phases, and overall a further linear improvements were also observed (linear, $P < 0.001$) with incremental doses.

No significant interaction effects were observed of the main factors and source on FCR in the starter and whole treatment period. In the starter period, methionine supplementation at 0.310% significantly reduced (linear, $P = 0.040$; quadratic, $P = 0.021$) FCR compared to the basal diet, and additional methionine at 0.155% was sufficient to obtain a significant reduction in FCR after the starter period, and the entire period, in which linear and quadratic improvements ($P < 0.001$) were observed with increasing Met doses. A significant interaction was observed between the source and level of Met on FCR during grower ($P < 0.001$) and finisher ($P = 0.043$) periods. The highest supplemental Met level (above 0.310%) significantly reduced FCR when DL-Met was added to the diet, but the opposite

Table 3. Effect of sources and additional level of methionine on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality in male broiler chickens

Methionine source	Additional level, %	Starter, 0–11 days			Grower, 12–25 days			Finisher, 26–39 days			Overall, 0–39 days			Mortality, %
		FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR	
Basal diet	0.000	371	258^{c}	1.44	1237	739	1.68^{a}	1961	955	2.05^{a}	3568	1953	1.83	1.92
DL-Met	0.155	396	284^{ab}	1.40	1242	794	1.57^{bcd}	1964	1061	1.86^{b}	3600	2138	1.69	0.96
DL-Met	0.310	372	286^{a}	1.30	1244	788	1.58^{bc}	2037	1118	1.82^{bc}	3652	2192	1.67	0.96
DL-Met	0.455	366	270^{bc}	1.36	1247	826	1.51^{d}	2054	1119	1.84^{bc}	3676	2214	1.66	2.88
L-Met	0.155	394	280^{ab}	1.41	1255	799	1.57^{bc}	1999	1079	1.85^{b}	3647	2158	1.69	2.88
L-Met	0.310	393	289^{a}	1.36	1227	805	1.53^{cd}	2068	1103	1.84^{bc}	3658	2197	1.67	4.81
L-Met	0.455	394	289^{a}	1.36	1276	804	1.59^{b}	2035	1144	1.78^{c}	3702	2237	1.66	1.92
SEM[1]		6.1	1.9	0.021	6.8	6.6	0.011	12.2	11.6	0.014	18.8	17.6	0.012	0.587
Main effects														
Source														
DL-Met		379	280	1.35	1244	803	1.55	2018	1099	1.84	3643	2181	1.67	1.60
L-Met		394	286	1.38	1253	803	1.56	2033	1109	1.82	3669	2197	1.67	3.21
SEM[2]		7.0	1.8	0.022	7.9	7.0	0.010	14.4	10.9	0.010	22.0	15.7	0.009	0.621
Level														
0.000		371	258^{b}	1.44^{a}	1237	739^{b}	1.68^{a}	1961^{b}	955^{c}	2.05^{a}	3568^{b}	1953^{c}	1.83^{a}	1.92
0.155		395	282^{a}	1.40^{ab}	1248	796^{a}	1.57^{b}	1981^{ab}	1070^{b}	1.86^{b}	3623^{ab}	2148^{b}	1.69^{b}	1.92
0.310		382	287^{a}	1.33^{b}	1236	797^{a}	1.56^{b}	2052^{a}	1111^{ab}	1.83^{bc}	3655^{ab}	2194^{ab}	1.67^{bc}	2.88
0.455		381	279^{a}	1.36^{ab}	1261	815^{a}	1.55^{b}	2045^{a}	1132^{a}	1.81^{c}	3689^{a}	2226^{a}	1.66^{c}	2.40
P-values														
source		0.146	0.058	0.602	0.622	0.986	0.395	0.542	0.620	0.261	0.564	0.534	0.978	0.916
level, linear		0.742	<0.001	0.040	0.342	<0.001	<0.001	0.002	<0.001	<0.001	0.016	<0.001	<0.001	0.159
level, quadratic		0.299	<0.001	0.021	0.601	0.101	0.009	0.567	0.006	<0.001	0.760	0.001	<0.001	0.542
source × level interaction		0.433	0.008	0.846	0.620	0.532	<0.001	0.753	0.749	0.043	0.960	0.966	0.962	0.916

SEM [1] – pooled standard error of the mean including basal control, n = 63; SEM [2] – pooled standard error of the mean, n = 54; [a–d] – means within the same column with different superscripts are significantly different at $P < 0.05$

effect was recorded when L-Met was applied in the grower period. The highest additional L-Met dose significantly improved FCR compared to the 0.155% level, whereas no significant difference was observed between supplemental DL-Met levels in the finisher period. Mortality was not significantly affected by any of the factors studied.

Carcass and carcass part yields and digestive organ weight

Only the level of Met supplementation had a significant effect on the yield of carcass and cuts, with the exception of the weight of drumsticks and feathers, as well as the relative weight of the pancreas, liver and abdominal fat (Table 4). Carcass and BMY were already increased (linear and quadratic, $P < 0.001$) with the addition of Met at 0.155% compared to the basal diet, and further significant improvement in carcass yield was observed at the 0.310% supplementation level. However, the highest additional dose of Met significantly reduced (linear, $P = 0.004$) the relative thigh weight compared to the basal diet. Broilers fed the diets with 0.310 and 0.455% supplemental Met had a significantly lower (linear, $P = 0.009$) relative weight of abdominal fat compared to chicks fed the Met-deficient basal diet. Quadratic decreases ($P < 0.001$) in relative weights of the pancreas and liver with progressive Met addition were reversed at the highest level of supplementation.

Relative bioavailability of methionine sources

The data of the present study fitted well with the asymptotic nonlinear model, which was considered to be adequate to compare the two sources. The regression model estimated the RBV of L-Met to DL-Met for final BW, FCR (0–39 days) and BMY at 123%, 91.5% and 88.0%, respectively (Figure 1, 2 and 3). The R^2 of the RBV for all three parameters was above 0.97, which indicated the fitting of the model. Confidence intervals for the RBV of BW, FCR and BMY were estimated between 70.4–175.6, 69.7–113.3 and 71–183%, respectively, showing no significant difference in the bioavailability of both Met sources for these parameters.

Table 4. Effect of sources and additional level of methionine on relative weights (weight/BW, %) of carcass and cuts, abdominal fat, liver and pancreas in male broiler chickens (39 days)

Methionine source	Additional level, %	Carcass	Breast	Drumstick	Thigh	Abdominal fat	Liver	Pancreas	Feather
Basal diet	0.000	66.1	20.8	10.8	19.4	1.91	2.25	0.22	5.38
DL-Met	0.155	68.5	24.0	10.8	18.6	1.64	2.15	0.20	5.08
DL-Met	0.310	69.8	24.5	10.7	18.9	1.74	1.91	0.18	5.16
DL-Met	0.455	69.9	24.8	10.5	18.7	1.66	2.14	0.20	5.28
L-Met	0.155	68.2	23.7	10.7	19.0	1.73	2.03	0.18	5.11
L-Met	0.310	70.6	25.0	10.6	18.8	1.36	1.93	0.18	4.84
L-Met	0.455	68.9	24.4	10.7	18.3	1.52	2.15	0.20	5.26
SEM[1]		0.29	0.26	0.08	0.14	0.061	0.032	0.001	0.063
Main effects									
Source									
DL-Met		69.4	24.4	10.7	18.7	1.68	2.07	0.19	5.17
L-Met		69.2	24.3	10.7	18.7	1.54	2.04	0.19	5.08
SEM[2]		0.19	0.16	0.07	0.11	0.054	0.029	0.003	0.061
Level									
0.000		66.1^{c}	20.8^{b}	10.8	19.4^{a}	1.91^{a}	2.25^{a}	0.22^{a}	5.38
0.155		68.3^{b}	23.9^{a}	10.8	18.8^{ab}	1.69^{ab}	2.09^{b}	0.19^{bc}	5.09
0.310		70.2^{a}	24.7^{a}	10.6	18.9^{ab}	1.56^{b}	1.92^{c}	0.18^{c}	5.00
0.455		69.4^{a}	24.6^{a}	10.6	18.5^{b}	1.58^{b}	2.15^{ab}	0.20^{b}	5.27
P-values									
source		0.658	0.864	0.886	0.972	0.201	0.550	0.506	0.435
level, linear		<0.001	<0.001	0.124	0.004	0.009	0.029	0.009	0.454
level, quadratic		<0.001	<0.001	0.847	0.546	0.185	<0.001	<0.001	0.076
source × level interaction	0.210	0.686	0.927	0.598	0.252	0.685	0.632	0.672	

SEM [1] – pooled standard error of the mean including basal control, n = 126; SEM [2] – pooled standard error of the mean, n = 108; [abc] – means within the same column with different superscripts are significantly different at $P < 0.05$

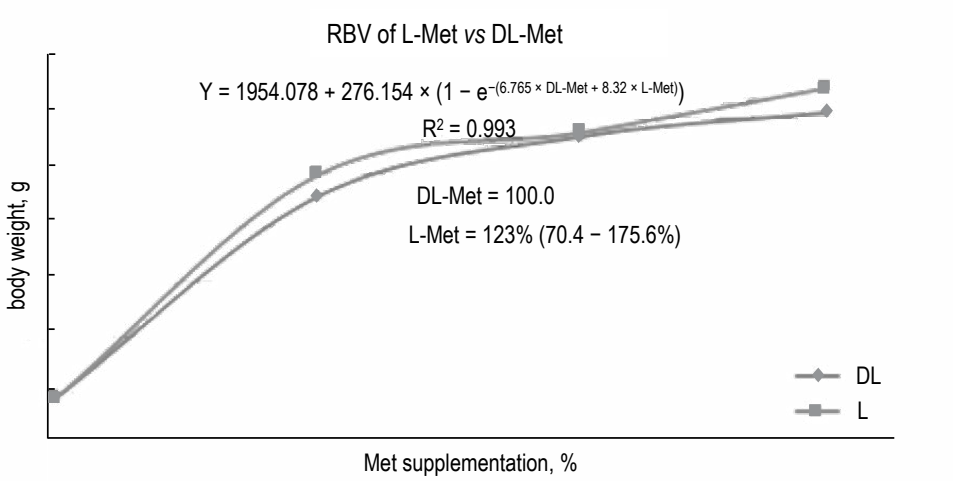

Figure 1. Slope-ratio assay of the relative bioavailability of L-Met to DL-Met based on final body weight as the response criteria of broiler chickens on d 39. Values in brackets indicate 95% confidence intervals. RBV:Relative bioavailability value. Confidence intervals for each parameter include all RBV of L-Met which means no significant (P > 0.05) RBV for L-Met

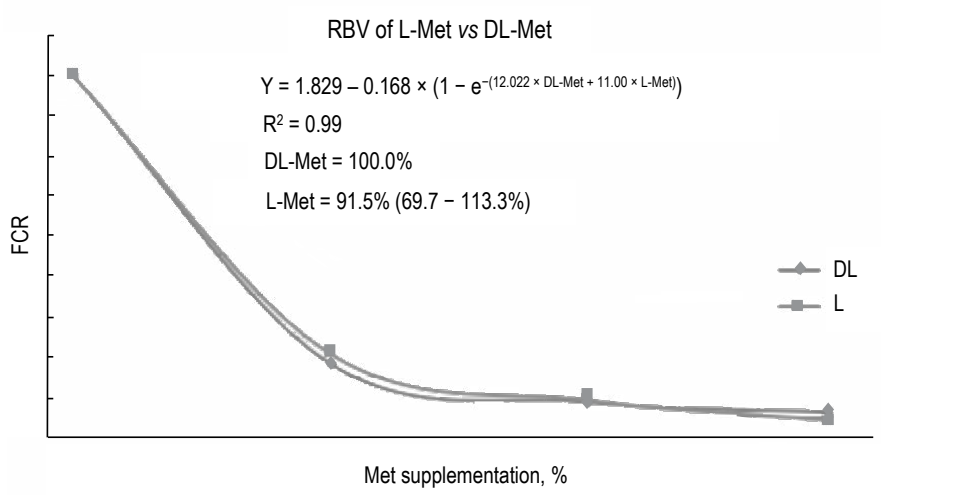

Figure 2. Slope-ratio assay of the relative bioavailability of D-Met to DL-Met based on feed conversion as the response criteria of broiler chickens through d 0 to 39. Values in brackets indicate 95% confidence intervals. RBV: Relative bioavailability value. Confidence intervals for each parameter include all RBV of L-Met which means no significant (P > 0.05) RBV for L-Met

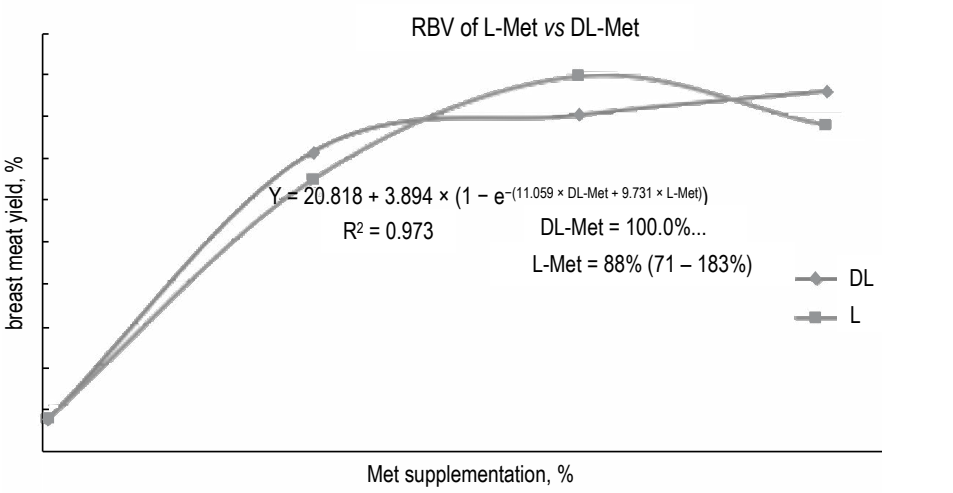

Figure 3. Slope-ratio assay of the relative bioavailability of L-Met to DL-Met based on breast meat yield as the response criteria of broiler chickens on d 39. Values in brackets indicate 95% confidence intervals. RBV: Relative bioavailability value. Confidence intervals for each parameter include all RBV of L-Met which means no significant (P > 0.05) RBV for L-Met

Discussion

As indicated in Tables 1 and 2, the results of crude nutrient and amino acid analyses for each diet confirmed the calculated values regarding incremental supplementation of both DL-and L-Met, as well as the concentration of other essential amino acids in each feeding phase, which demonstrated that the dietary objective was achieved.

Essential amino acid requirements and their appropriate proportions (ideal amino acid concept) must be adequately met to ensure proper growth of broiler chickens. Methionine is considered the first limiting amino acid in broiler diets, and its deficiency may cause reduced growth performance, as well as metabolic and immune system disorders (Bunchasak, 2009). In this respect, Kubińska et al. (2016) reported that DL-Met supplementation increased plasma immunoglobulin concentrations and antioxidant status in turkeys fed Met deficient diets, thereby demonstrating physiological effects of Met supplementation. Furthermore, Met deficiency and disrupted ideal Met:Lys ratio were reported to prevent broilers from properly utilising dietary amino acids, resulting in reduced BWG but increased FCR (Zhan et al., 2006). Similarly, in our study, methionine-deficient basal diets, with a lower digestible Met:Lys ratio than recommended (0.24% vs 0.40% for starter, 0.25% vs 0.41% for grower and 0.26% vs 0.42% for finisher) by Aviagen (2014), adversely affected growth performance of broiler chickens. Methionine supplementation linearly improved BWG and FCR of broilers during the entire treatment period. Our study was in line with previous research (Liu et al., 2006), which indicated that supplemental methionine, regardless of source, increased carcass and breast meat yield, but decreased abdominal fat content. The reduced thigh yield could be related to increased breast meat yield, as observed by some researchers (Liu et al., 2007). Similar to Zhang et al. (2018), an increased relative weight of the liver and pancreas was observed, which could be associated with elevated amino acid metabolism induced by an imbalance in the Met:Lys ratio. The supposition that vegetable diets could lead to feathering, cannibalism or feather pecking problems, due to lower cystine (Cys) levels in feed, was not confirmed in this study. In contrast to Elkin and Hester (1983), who reported that birds fed a diet with 0.66% Met + Cys ate feathers from the floor under field rearing conditions, we did not observed this phenomenon even for the Met + Cys-deficient basal diet. Pacheco et al. (2018) reported that the lower Cys content in diets, the higher the amount of Met converted into Cys and the lower Met conversion into Met cycle intermediates. The latter phenomenon may reduce body protein accretion, and thus reduce growth performance, as observed in the present study. In the current experiment, a significant interaction of Met source and its level was observed for BWG at the starter period. When broilers received the highest dose of DL-Met (0.455%), BWG was significantly reduced, while the addition of L-Met at the same dose did not cause this effect. Shen et al. (2014) showed that the utilisation of Met isomers in animals was likely a function of age, which was also observed by Wang et al. (2019). Therefore, the aforementioned BWG result could presumably be associated with adverse effects of D-Met accumulation on metabolism due to D-amino acid oxidase deficiency in young chicks (D'Aniello et al., 1993). Physiologically, animal cells can only utilise L-isomers of amino acids, and each D-isomer must be converted to the corresponding L-isomer before being used in protein synthesis. D-methionine has been reported to be utilised by chickens with 90% efficacy relative to L-methionine (Baker, 2006). Furthermore, relative utilisation of DL-Met a racemic mixture of D- and L-isomers was reported to be 95% of that recorded for L-Met (Baker, 2006). Thus, it can be assumed that the addition of pure L-Met to the feed should lead to more efficient absorption and protein synthesis, and thus better growth and performance. Jankowski et al. (2017) found a significant interaction effect of Met dosage and its source (DL-Met, L-Met) on concentrations of some redox parameters in turkeys. This physiological dynamics may be the underlying factor causing this interaction effects also in our study. However, contrary to previous studies, Met sources tested in the current work did not affect overall broiler performance, and the effect of the source × level interaction was not significant for any final performance and carcass quality parameters. This was consistent with some previous reports in broilers (Dilger and Baker, 2007; Pacheco et al., 2018; Ullrich et al., 2019; Sahebi-Ala et al., 2021), indicating that DL- and L-Met exerted a similar effect on growth performance and carcass characteristics of broilers. Similar results were obtained by Murawska et al. (2018), who found no significant differences between DL- and L-Met supplementation in terms of turkey growth performance, which was also consistent with our results.

The RBV of Met sources is still a subject of debate that requires careful consideration by poultry nutritionists in order to effectively utilise each

source for cost-effective production. The calculation of the RBV in the present study was based on the non-linear multi-exponential model, which is a common accepted design for this kinds of comparisons to define the RBV and compare different products, since ANOVA appears to be insufficient to estimate the difference between the closely matching treatments (Esteve-Garcia and Khan, 2018). The data of the present study appeared to fit well with the non-linear model, which was considered suitable to compare the two sources. The RBV of L-Met compared to DL-Met estimated in the present study for BW, FCR and breast meat yield of broilers was 123, 91.5 and 88.0%, respectively, .i.e. the obtained values were similar to each other. This was consistent with our previous results, demonstrating that the addition of L-Met provided no significant advantages over DL-Met. Some earlier studies also found that these two sources had similar bioavailability in broilers, with the exception of FCR (Wang et al., 2019), turkeys (Kuzmicky et al., 1977) and pigs (Zeitz et al., 2019), which confirmed our results. Yet other evidence published in the literature indicated that L-Met had a higher RBV than DL-Met, and L-Met supplemented-diets showed greater performance than those fed DL-Met in turkeys (Noll et al., 1984), pigs (Shen et al., 2014) and broilers (Shen et al., 2015). Esteve-Garcia and Khan (2018) reported that the RBV of L-Met for BW and FCR was 112 and 129.8%, respectively, in a 37-day study , while Wang et al. (2019) estimated the RBV of L-Met compared to DL-Met for BWG, FCR and breast meat yield to be 141.5, 189.1 and 116.8%, respectively, in a 21-day study on broiler chickens.

Although it is commonly assumed that the level of enzymes responsible for converting isomers to L-Met in the body is insufficient, it should be noted that D-amino acid oxidase and transaminase capacity to convert D-Met to L-Met in the tissues may not be limiting, as demonstrated in early studies (Brachet and Pugserver, 1992). Transaminase has also been found to be ubiquitous, and thus it is not a limiting step in the transformation process of broilers (Zhang et al., 2018). Therefore, the overall performance and RBV obtained in the present study allow to speculate that the efficiency of DL isomer to L-Met conversion may not be a limiting factor when DL-Met is supplemented at standard levels without exceeding the Met + Cys requirement, and when birds are not exposed to specific stress conditions, as in the present trial with better environmental conditions.

Conclusions

In this study, the addition of methionine to basal diets deficient in this amino acid was demonstrated to improve growth performance and yield of carcass and cuts of broilers. However, different methionine sources were shown to exert no significant effect on the overall performance and relative bioavailability of the calculated parameters. Considering all the values for live performance and carcass parameters of 39-day-old chickens obtained in this study, the average RBV of L-Met in comparison to DL-Met was 100.84%. Therefore, it can be concluded that no significant differences in growth performance and carcass quality parameters should be expected when supplementing broiler diets either with DL-Met or L-Met.

Conflict of interest

The Authors declare that there is no conflict of interest.

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