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RESEARCH ARTICLE



An investigation of the assumed efficacy of methionine hydroxy analogue compared to DL-methionine by measuring growth performance, carcass traits, and GHR and IGF-I expression in broilers

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ABSTRACT

This experiment was conducted to investigate the assumed efficacy of 65% on the product basis of methionine hydroxy analog (OH-Met, 88% aqueous solution of DL-2-hydroxy-4-(methylthio) butanoic acid) relative to DL-methionine (DL-Met) in broiler chickens. A total of 792-day-old male chickens were randomly allotted to 7 dietary treatments consisting of a methionine deficient-basal diet based on corn-soybean meal and 6 different diets obtained by supplying the basal diet with methionine at 3 levels (25, 100 and 125%; levels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements) from either DL-Met or OH-Met at a ratio of 65:100 (DL-Met to OH-Met on a product basis) in the corresponding treatments.

Regardless of the source, methionine supplementation enhanced ($p < .05$) growth performance, carcass and breast meat yield. Supplemental level and the source of Met had no significant effect on the proximate chemical composition of breast meat ($p > .05$). Comparison of effects of OH-Met and DL-Met supplemented at 100–65% weight ratio revealed no differences at any Met + Cys level and for performance criterion confirming the applied concept. Expression of growth hormone receptor (GHR) mRNA, which showed a positive correlation with body weight gain, carcass yield and breast percentage, in broiler liver significantly increased with 100% and 125% relative addition of DL-Met, while only 100% of OH-Met addition did ($p < .05$) when compared to the basal diet. The expression of the insulin-like growth factor-I (IGF-I) gene was not significantly affected by either Met source or supplementation level.

In conclusion, our data indicated that DL-Met can be substituted with a 1.54 times higher amount of OH-Met in corn-soybean meal based broiler diets.

HIGHLIGHTS

- Methionine supplementation enhanced growth performance, carcass and breast meat yield of broiler chickens fed corn-soybean meal based diets.
- DL-Met and OH-Met showed similar growth performance, carcass and cuts yields and breast meat traits when a 1.54 times higher amount of OH-Met, on a product basis, was added.
- DL-Met can be substituted with a 1.54 times higher amount of OH-Met in corn-soybean meal based broiler diets.

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Introduction

Methionine (Met) is an essential amino acid that plays a crucial role in protein synthesis and is considered to be the first limiting amino acid in corn-soybean-based broiler diets. Supplemental Met sources, including crystalline DL-methionine (DL-Met; 99% of active substance) and methionine hydroxy analog (OH-Met; 88% of active substance), are thus used to meet the sulphur amino acid requirements of broiler chickens.

Both sources must be converted to L-Met after being absorbed in the small intestine to be used for protein synthesis and other metabolic pathways (Dibner and Knight 1984). Compared to DL-Met, OH-Met has a hydroxy group instead of an amino group and thus undergoes a different enzymatic process during conversion to L-Met. Furthermore, while some studies have reported comparable or even higher absorption of OH-Met compared to DL-Met (Dibner et al. 1992;

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Richards et al. 2005), others have indicated poorer absorption (Maenz and Engele-Schaan 1996; Drew et al. 2003; Lemme and Mitchell 2008), raising questions about the bioefficacy of OH-Met relative to DL-Met. Hence, there has been ongoing research on the efficacy of OH-Met compared to DL-Met in broiler chickens. Even though a large number of studies have been conducted to compare the efficiency of OH-Met relative to DL-Met, there is still a great deal of controversy in this subject. Some researchers have found no significant differences in broiler performance and carcass traits when comparing both Met sources on an equimolar basis (Liu et al. 2006; Ullrich et al. 2019) while the others showed that OH-Met was markedly inferior to DL-Met (Kim et al. 2019). Likewise, the estimations of relative bioavailability (RBV) of OH-Met compared to DL-Met are varying but an average RBV of 65% on a product basis was indicated by some researchers (Lemme et al. 2002; Hoehler et al. 2005; Lemme et al. 2020). In this respect, there are some studies comparing OH-Met with DL-Met based on the assumed RBV of 65% by diluting DL-Met (Lemme et al. 2002; Hoehler et al. 2005; Lemme et al. 2020), however, in order to validate or disprove the above recommended replacement ratio of the products, comparison of these sources by adding OH-Met at a level approximately 1.5 times higher than that of undiluted DL-Met remains to be investigated. Moreover, a different dosing approach, unlike testing only at one or two supplementation levels, which are slightly at or above the requirement, may benefit the knowledge of the replacement ratio of these products in the feed, which is a critical factor for optimisation of broiler production. Protein deposition in breast meat is being considered as an attribute of the utilisation of Met sources, yet it has rarely been investigated in broiler studies comparing DL-Met and OH-MET (Liu et al. 2006; Ullrich et al. 2019). Indeed, in none of these studies, the addition of Met sources was set according to RBV of 65%. Met addition positively affects protein synthesis *via* several pathways, including regulating genes related to growth (Del Vesco et al. 2013). However, little information is available about how different Met sources affect gene expression that are involved in the growth of broiler chickens, which might be beneficial in a thorough evaluation of the efficacy of OH-Met compared to DL-Met. The lack of research on correlations of gene expression with performance and carcass yield also needs to be addressed in this respect.

Therefore, the aim of this study was to investigate the assumed efficacy of 65% on the product basis of

OH-Met compared to DL-Met by measuring growth performance, carcass traits, and GHR and IGF-I expression in broilers fed corn-soybean meal based diets.

Material and methods

Birds and housing

A total of 792 one-day-old male Ross 308 broilers were reared in 48 floor pens with 24 pens (1.4×0.9 m each) containing 16 chicks and 24 pens (1.5×0.9 m each) containing 17 chicks, each littered with wood shavings and equipped with nipple drinkers and a hanging feeder. Mash feed and water were provided *ad libitum* during the trial. The temperature was set at 33 °C for the first 3 d, then gradually decreased to approximately 23 °C at the end of the third week. The birds received 23 h, 22 h, and 20 h of fluorescent (white, 30 lux for the first 7 day then decreased to 15 lux) illumination by the end of d 7, 14, 40, respectively.

Experimental design and diets

One-day-old birds were weighed and then randomly allotted to 7 treatments with 7 replicates (except the common basal diet which had 6 replicates) using a $2 \times 3 + 1$ factorial arrangement, two sources of Met (DL-Met and OH-Met) and three levels of supplementation (25%, 100% and 125%; levels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements) with a common basal diet in a randomised complete block design. Corn-soybean meal-based basal diets were formulated to be deficient in Met + Cys without any Met addition, but all other essential nutrients were provided in an adequate amount for starter (0–10 d), grower (11–24 d), and finisher (25–40 d) periods according to nutrient recommendations for corn based feed and 2.5–3 kg target live weight of Ross 308 (Aviagen 2019) (Table 1). Dietary treatments consisted of basal diets with the addition of feed grade either DL-Met (99% purity powder) or 88% aqueous solution of DL-2-hydroxy-4-(methylthio) butanoic acid, known as liquid methionine hydroxy (OH-Met), at three different levels which are equal to 25, 100 and 125% of required Met addition to meet SID Met + Cys requirements either from DL-Met or OH-Met. In the respective treatments, OH-Met was added 1.54 times higher than the amount of DL-Met due to the assumed relative bioavailability of 65% on a product basis as validated by Lemme et al. (2020) who demonstrated that RBV of DL-Met diluted to 65% purity was close to the expected value of 65% which was similar to that of OH-Met (Table 2).

Table 1. Composition of basal diets, as-fed basis (%).

Ingredients	Starter (0–10 d)	Grower (11–24 d)	Finisher (25–40 d)
Corn	55.60	59.40	62.40
Soybean meal	36.60	32.20	28.40
Sunflower oil	3.41	4.34	5.40
Dicalcium phosphate, 19%	2.19	2.00	1.79
Limestone	0.83	0.73	0.71
Lysine sulphate, 54.6%	0.37	0.33	0.28
Vitamin-mineral premix ^a	0.30	0.30	0.30
Sodium chloride	0.29	0.29	0.29
Sodium bicarbonate	0.21	0.21	0.22
L-threonine	0.16	0.13	0.10
Choline chloride, 75%	0.08	0.08	0.08
L-valine	0.04	0.03	0.01
Calculated values %			
AMEn, kcal/kg	3000	3100	3200
Crude fibre	2.13	2.02	1.93
Ether extract	5.79	6.76	7.85
Ash	6.73	6.20	5.78
Calcium	1.00	0.90	0.83
Available phosphorus	0.48	0.44	0.40
Sodium	0.19	0.19	0.19
Chloride	0.22	0.22	0.22
Crude protein	22.20(22.30)	20.22(20.56)	18.46(18.21)
Lys	1.40(1.43)	1.26(1.29)	1.13(1.12)
SID ^b Lys	1.28	1.15	1.03
Met	0.32(0.31)	0.29(0.29)	0.27(0.26)
SID Met	0.29	0.27	0.25
Cys	0.35(0.35)	0.32(0.32)	0.30(0.29)
SID Cys	0.29	0.27	0.25
Met + Cys	0.67(0.66)	0.62(0.61)	0.58(0.55)
SID Met + Cys	0.58	0.54	0.51
Thr	0.98(0.98)	0.88(0.90)	0.79(0.77)
SID Thr	0.86	0.77	0.69
Val	1.07(1.09)	0.97(0.98)	0.87(0.87)
SID Val	0.96	0.87	0.78

^aVitamin-mineral premix: Supplied per kg diet: 10,000 U vitamin A, 4500 U vitamin D3, 65 mg vitamin E, 2.8 mg vitamin B1, 6.5 mg vitamin B2, 3.2 mg vitamin B6, 0.017 mg vitamin B12, 3.5 mg vitamin K3, 18 mg pantothenic acid, 55 mg niacin, 0.18 mg biotin, 1.9 mg folic acid. 20 mg Fe, 16 mg Cu, 110 mg Zn, 120 mg Mn, 1.25 mg I, 0.9 mg Co, 0.3 mg Se.

^bSID: standardised ileal digestible; the analysed values are presented in parentheses.

AMEn: apparent metabolizable energy, N-corrected.

Table 2. Experimental design and supplementation levels of DL-methionine (DL-Met) and methionine hydroxy analog (OH-Met).

Diets	Met sources	Additional Met ^a (%)	The addition of Met sources ^b (%)		
			0–10 d	11–24 d	25–40 d
Basal diet	No addition	0	0	0	0
DLM25	DL-Met	25	0.093	0.084	0.075
DLM100	DL-Met	100	0.370	0.331	0.296
DLM125	DL-Met	125	0.464	0.418	0.374
OH-Met25	OH-Met	25	0.141	0.127	0.114
OH-Met100	OH-Met	100	0.565	0.504	0.452
OH-Met125	OH-Met	125	0.707	0.637	0.571

^aLevels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements.

^bAddition on the product basis.

The main ingredients and experimental feeds were analysed for proximate (AOAC 2005), amino acid (Llames and Fontaine 1994), and AMEn content by near infra-red reflectance spectroscopy (NIRS; Evonik Nutrition & Care GmbH, Hanau, Germany) (Valdes and Leeson 1992; Black et al. 2009) (Table 1 and 3). Supplemented OH-Met was analysed according to the method proposed by VDLUFA (1997) (Table 3).

Growth performance

Birds were weighed by digital weighing scales (for the first 10 d with a 0.1 g precision, afterwards with a 5 g precision) for measuring body weight (BW) at the beginning of the experiment, on day 10, 24 and 40 for each replicate. Feed intake (FI) was recorded for each growing period: 0–10, 11–24, 25–40 and 0–40 d on a per pen basis. The feed conversion ratio (FCR) was

Table 3. Analysed amino acid composition of the diets, as-fed basis (%).

Nutrients	Basal diet	DLM25	DLM100	Diets DLM125	OH-Met25	OH-Met100	OH-Met125
Starter (0–10 d)							
Crude protein	22.30	22.40	22.90	23.10	22.30	22.50	22.50
Met	0.31	0.41	0.69	0.80	0.32	0.31	0.31
Cys	0.35	0.34	0.35	0.34	0.34	0.34	0.34
Met + Cys	0.66	0.76	1.04	1.12	0.66	0.66	0.66
OH-Met	–	–	–	–	0.13	0.53	0.68
Lys	1.43	1.42	1.44	1.44	1.42	1.44	1.44
Grower (11–24 d)							
Crude protein	20.60	20.40	20.30	20.10	20.70	20.50	20.40
Met	0.29	0.37	0.62	0.69	0.29	0.30	0.29
Cys	0.32	0.32	0.31	0.32	0.31	0.32	0.31
Met + Cys	0.61	0.70	0.93	1.01	0.60	0.61	0.60
OH-Met	–	–	–	–	0.10	0.48	0.61
Lys	1.29	1.27	1.29	1.29	1.28	1.31	1.30
Finisher (25–40 d)							
Crude protein	18.20	18.90	18.40	18.80	18.70	18.80	18.30
Met	0.26	0.35	0.52	0.62	0.27	0.27	0.27
Cys	0.29	0.30	0.30	0.30	0.30	0.30	0.30
Met + Cys	0.55	0.65	0.81	0.92	0.56	0.56	0.56
OH-Met	–	–	–	–	0.11	0.41	0.53
Lys	1.12	1.13	1.09	1.13	1.14	1.14	1.14

DLM25: DL-Methionine 25%; DLM100: DL-Methionine 100%; DLM125: DL-Methionine 125%; OH-Met25: Methionine hydroxy analog 25%; OH-Met100: Methionine hydroxy analog 100%; OH-Met125: Methionine hydroxy analog 125%.

calculated for 0–10, 11–24, 25–40 and 0–40 d using FI and body weight gain (BWG). Mortality was recorded daily for each pen, and the weight of dead birds was used to adjust FCR.

Carcase and cuts yield, digestive organ weight, and breast meat traits

At the end of the experiment, 2 chicks per pen close to the average broiler weight of the respective pen were selected for processing the following feed withdrawal 6 h before. Each bird was weighed and its legs banded for identification. Each bird was exsanguinated by cutting the jugular vein, bled for approximately 1.5 min, scalded at 55 °C for 30 s, and lastly, defeathered in a rotary picker. Viscera and abdominal fat were removed. Afterwards, weights of the liver (without gallbladder), pancreas and abdominal fat were obtained. Carcase, thighs, drumsticks, and breast meat with bone in and skin on were weighed. Relative weights of the carcase, abdominal fat, thighs, drumsticks, and breast meat were calculated as a percentage of live body weights.

The right *Pectoralis major* muscle was excised and placed in a polyethylene bag, and then stored at –20 °C until analysis. The samples for proximate analysis were obtained from frozen right *Pectoralis major* muscle without thawing by dissecting the muscle area (approximately 6 × 3.5 × 2.5 cm muscle tissue only) manually minced, then the concentrations of moisture, crude protein, crude fat and ash analysis were determined according to the procedures of the AOAC

(2005). The ether extract content of the breast meat was calculated by subtracting crude protein from the organic matter content.

Gene expression in the liver

The liver samples were collected from each replicate ($n = 7$ for each treatment) at the end of the study and preserved in RNeasy Lysis Solution (Thermo Fisher Scientific, USA) at –20 °C for total RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR) analysis of IGF-I and GHR. For RNA extraction, 1 mL/100 mg tissue PureZol™ RNA Isolation Reagent was used (Biorad, USA) according to the manufacturer protocol. Tissue samples were homogenised using a bead mill homogeniser and then centrifuged for 15 min at a temperature of 14,000 g at 40 °C, and fluid isopropanol was collected and transferred in each tube to a clean tube of 500 µL. The excesses were disposed of and the precipitate was treated with 1 mL 75% ethanol. The solution was centrifuged for 5 min at 14,000 g and the supernatant was discarded. The pellet was dried for 15 min, and the content was resuspended in ultrapure water free of RNase. The total RNA concentration was measured using a nano spectrophotometer. The integrity of RNA on 1% agarose gel stained with 10% bromide ethidium was evaluated under UV light. DNase I (Biorad, USA) treatment was performed on the RNA samples to eliminate residues of DNA as suggested by the manufacturer. iScript™ cDNA Synthesis Kit (Biorad, USA) was used to synthesise cDNA according to manufacturer

recommendations. Real-time polymerase chain reaction (RT-PCR) was performed using Fluorescent dye SYBR Green (SsoAdvanced Universal SYBR Green Supermix (Biorad, USA)). RT-PCR products were analysed on CFX96 Real-Time PCR Detection System (Biorad, USA). Previously published (Del Vesco et al. 2013) primers were used to amplify GHR and IGF-I genes. 145 base pair long amplicon of GHR gene amplified using AACACAGATACCCAACAGCC and AGA-AGTCAGTGTTCGTCAGGG; and 140 base pair long amplicon of IGF-I gene amplified using CATTTCTTC TACCTTGGC and TCATCCACTATTCCCTTG primers as forward and reverse, respectively. β -actin used as a housekeeping gene and 136 base pairs long amplicon amplified using TGCTGTGTTCCCATCTATCG and TTGGT-GACAATACCGTGTTC primers as forward and reverse, respectively. All analyses were performed at 60 °C as the annealing temperature in a volume of 25 μ L and in duplicates. Relative expression levels were calculated according to the $2^{-\Delta CT}$ method (Livak and Schmittgen 2001). The reaction efficiency value was considered as 1 (one).

Statistical analysis

Data for all response variables related to the different phases of the trial were analysed as a completely randomised block design, with a factorial arrangement of $2 \times 3 + 1$ for Met sources and that of graded level, respectively by using the general ANOVA procedure of SAS (version 9.2; 2008; SAS Institute, Cary, NC). The pen was considered an experimental unit for performance criteria. When significant differences ($p < .05$) were found among groups, means were separated using the Tukey HSD test. Mortality results were assessed by the chi-square test. T tests were applied to $2^{-\Delta CT}$ data ($p \leq .05$). Spearman correlations have been calculated between gene expression values and BWG, FCR, relative weights of the carcass, breast meat, pancreas, and liver as a percentage of BW.

Results

Growth performance

Effects of levels and sources of supplemental Met on feed intake, body weight gain, FCR, and mortality are given in Table 4. There were interactions between the level and source of supplemental Met for feed intake in finisher and overall ($p < .05$). In the starter and grower period, incremental Met supplementation gradually increased feed intake ($p < .05$) as no significant effect of Met source was observed ($p > .05$). Met

addition to the basal diet improved feed intake, BWG, and FCR in all growth periods ($p < .05$). Met source however had no significant effect on the respective parameters ($p > .05$). Mortality was not significantly affected by any factors studied as well ($p > .05$).

Carcass and cuts yield, digestive organ weight, and breast meat traits

Effects of addition levels and sources of Met on relative weights (weight/BW, %) of the carcass, breast meat, drumstick, thigh, abdominal fat, pancreas, and liver are shown in Table 5. Met supplementation significantly increased carcass, breast meat, and thigh yield while reduced drumstick yield ($p < .05$) and tended to decrease the relative weight of abdominal fat (Linear, $p = .059$). Decreased relative weights of the pancreas and liver were also observed due to Met addition to the basal diet ($p < .05$). The source of supplemental Met had no significant effect on any of these parameters ($p > 0.05$). The breast meat content of dry matter, ash, crude protein, and crude fat was not significantly affected by the sources and supplemental level of Met ($p > .05$) (Table 6).

Gene expression in the liver

The relative fold expressions of GHR and IGF-I mRNA in broiler liver are given in Figure 1. A significant interaction between the level and source of supplemental Met was observed for GHR expression ($p < .05$), while any of the factors studied had no effect on IGF-I expression ($p > .05$).

Expression of GHR showed a positive correlation with BWG (+0.386), relative weights of carcass and breast meat (+0.451 and +0.548), and negative correlation with FCR and relative weight of pancreas (−0.376 and −0.351) as shown in Table 7 ($p < .05$).

Discussion

Tables 1 and 3 show that the analysed amino acid levels of the basal and experimental diets were in close agreement with the calculated values, which demonstrated that the dietary objective was largely achieved.

Growth performance

Compared to the basal diet, the addition of either Met source increased feed intake in the current study. Broilers fed DLM100 had higher feed intake than those fed DLM25, whereas similar feed intake was observed

Table 4. Effects of levels and sources of methionine on feed intake (FI, g), body weight gain (BWG, g), feed conversion ratio (FCR), and Mortality (%) in male broiler chickens.

Methionine source	The level of addition ¹ (%)	Starter (0–10 d)			Grower (11–24 d)			Finisher (25–40 d)			Overall (0–40 d)		
		FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR	FI	BWG	Mortality
Basal diet	0	221	174	1.267	756	439	1.731	1634c	798	2.063	2609c	1411	1.857
DL-Met	25	285	237	1.202	1076	748	1.439	2298b	1270	1.812	3657b	2255	1.623
DL-Met	100	294	242	1.218	1144	874	1.309	2656a	1623	1.637	4083a	2739	1.491
DL-Met	125	297	245	1.216	1140	879	1.298	2658a	1636	1.627	4085a	2759	1.480
OH-Met	25	286	229	1.253	1106	814	1.362	2473ab	1325	1.875	3853ab	2367	1.629
OH-Met	100	294	246	1.198	1132	866	1.309	2532ab	1544	1.641	3953a	2656	1.489
OH-Met	125	296	233	1.273	1098	848	1.295	2587a	1619	1.600	3981a	2701	1.475
SEM		3.989	3.609	0.009	18.965	21.159	0.021	50.763	42.650	0.024	70.725	64.807	0.019
Main effects													
Source													
DL-Met		292	241	1.212	1120	834	1.349	2537	1510	1.692	3942	2584	1.531
OH-Met		292	236	1.242	1112	843	1.321	2530	1496	1.705	3929	2575	1.531
Level													
0		221c	174c	1.267a	756c	439c	1.731a	1634c	798c	2.063a	2609c	1411c	1.857a
25		286b	233b	1.228bc	1091b	781b	1.399b	2385b	1297b	1.844b	3755b	2311b	1.626b
100		294ab	244a	1.208c	1138a	870a	1.309c	2594a	1583a	1.639c	4018a	2697a	1.490c
125		297a	239ab	1.245b	1119ab	864a	1.296c	2622a	1627a	1.614c	4033a	2730a	1.478c
p values													
Source		0.985	0.170	0.134	0.672	0.598	0.144	0.894	0.723	0.622	0.835	0.834	0.332
Level, linear		0.000	0.000	0.686	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.577
Level, quadratics		0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.144
Source × Level interaction		0.983	0.233	0.207	0.322	0.060	0.121	0.041	0.413	0.434	0.050	0.191	0.988

¹Levels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements.^{ab}means within the same column with different superscripts are significantly different at $p < .05$.

DL-Met: DL-Methionine; OH-Met: Methionine hydroxy analog.

Table 5. Effects of addition levels and sources of methionine on relative weights (weight/BW, %) of carcass and Cuts, abdominal fat, pancreas and liver in male broiler chickens (40 d).

Methionine source	The level of addition ¹ (%)	Carcass	Breast	Drumstick	Thigh	Abdominal fat	Pancreas	Liver
Basal diet	0	69.96	20.69	11.10	19.68	1.54	0.30	2.06
DL-Met	25	74.29	25.36	10.24	20.61	1.81	0.23	1.77
DL-Met	100	77.41	28.46	10.04	20.27	1.45	0.18	1.64
DL-Met	125	77.17	28.19	10.19	20.37	1.52	0.18	1.73
OH-Met	25	74.59	24.97	10.72	20.57	1.63	0.22	1.74
OH-Met	100	76.64	27.98	10.42	20.33	1.32	0.20	1.72
OH-Met	125	77.09	28.31	10.31	20.28	1.29	0.17	1.77
SEM		0.30	0.32	0.06	0.11	0.04	0.00	0.01
Main effects								
Source								
DL-Met		76.29	27.34	10.15	20.42	1.59	0.20	1.71
OH-Met		76.11	27.09	10.48	20.39	1.41	0.20	1.74
Level								
0		69.96c	20.69c	11.10a	19.68b	1.54ab	0.30a	2.06a
25		74.44b	25.17b	10.48b	20.59a	1.72a	0.23b	1.76b
100		77.02a	28.22a	10.23b	20.30ab	1.38b	0.19c	1.68c
125		77.13a	28.25a	10.25b	20.32a	1.40b	0.18c	1.75bc
p values								
Source		0.59	0.52	0.21	0.92	0.10	0.75	0.43
Level, linear		0.00	0.00	0.00	0.11	0.05	0.00	0.00
Level, quadratic		0.00	0.00	0.00	0.05	0.35	0.00	0.00
Source × Level interaction		0.50	0.85	0.31	0.99	0.77	0.31	0.54

¹levels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements.^{abc}means within the same column with different superscripts are significantly different at $p < .05$.

DL-Met: DL-Methionine; OH-Met: Methionine hydroxy analog.

Table 6. Effects of levels and sources of methionine on proximate composition (%) of breast meat in male broiler chickens (40 d).

Methionine source	The level of addition ^a (%)	Dry matter	Ash	Crude Protein	Crude Fat
Basal diet	0	23.63	1.30	21.06	1.28
DL-Met	25	23.95	1.22	21.36	1.37
DL-Met	100	23.92	1.19	21.25	1.47
DL-Met	125	23.27	1.18	20.42	1.66
OH-Met	25	23.62	1.22	21.13	1.27
OH-Met	100	24.14	1.20	21.48	1.46
OH-Met	125	23.90	1.19	21.41	1.31
SEM		0.12	0.02	0.13	0.11
Main effects					
Source					
DL-Met		23.71	1.20	21.01	1.50
OH-Met		23.91	1.20	21.35	1.36
Level					
0		23.63	1.30	21.06	1.28
25		23.90	1.21	21.31	1.38
100		23.95	1.20	21.32	1.42
125		23.59	1.18	20.92	1.50
p values					
Source		0.61	0.99	0.39	0.71
Level, linear		0.71	0.33	0.69	0.11
Level, quadratic		0.60	0.44	0.55	0.78
Source × Level interaction		0.69	1.00	0.37	0.93

^alevels of addition relative to the required amount of additional Met to meet SID Met + Cys requirements.

DL-Met: DL-Methionine; OH-Met: Methionine hydroxy analog.

among different levels of OH-Met in the finisher and the whole treatment period. Despite the lack of certainty regarding the precise mechanism underlying this interaction, it is plausible that the distinctive odour and relatively low pH of OH-Met (EFSA 2012) hindered a further increase in feed intake beyond the lowest level of supplementation (25%), particularly during the finisher phase when a greater amount of consumption occurred. Regardless of the source, Met

supplementation to the basal diet improved feed intake, BWG, and FCR in all growth periods in this study. These results demonstrated that a basal diet was successfully formulated for Met-deficient broilers and that the growth performance of broilers fed Met-deficient diets improved with Met supplementation. Given that no further significant improvement was observed on feed intake, BWG, and FCR in any phase with the highest Met supplementation (125%) over 100%

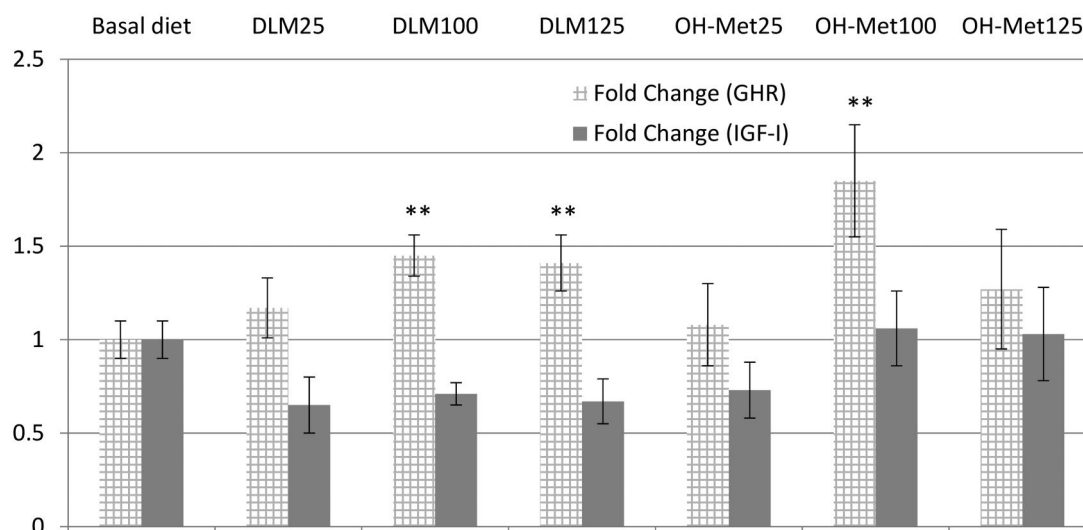


Figure 1. Relative fold expression of GHR and IGF-I mRNA in broiler liver. Changes in GHR and IGF-I gene expression in the liver are normalised to β -actin reference genes and expressed relative to the basal diet group as the mean fold difference ($2^{-\Delta\Delta CT}$). Values are means of 7 biological replicates and 3 technical replicates. ** $p < .01$ Means differed from the basal diet group. DLM25, DLM100, DLM125 and OH-Met25, OH-Met100, OH-Met125 mean that respective levels of addition of either DL-Met or OH-Met relative to the required amount of additional Met to meet SID Met + Cys requirements. DLM25: DL-Methionine 25%; DLM100: DL-Methionine 100%; DLM125: DL-Methionine 125%; OH-Met25: Methionine hydroxy analog 25%; OH-Met100: Methionine hydroxy analog 100%; OH-Met125: Methionine hydroxy analog 125%.

Table 7. Correlation between gene expression and BWG (0–40 d), FCR (0–40 d), relative weights (weight/BW, %) of carcass, breast meat, pancreas, and liver.

Gene expressions		BWG	FCR	Carcass	Breast	Pancreas	Liver
GHR	<i>r</i>	0.386	−0.376	0.451	0.548	−0.351	−0.177
	<i>p</i> values	.012	.014	.002	<.001	.021	.256
IGF-I	<i>r</i>	−0.247	0.155	0.089	0.065	−0.111	0.215
	<i>p</i> values	.115	.328	.572	.678	.480	.166

BWG: body weight gain.

demonstrated that 100% diets allowed for maximising performance in the current study as well. Compared to breeder expectations, a slightly lower (about 6%) final BW with DLM100 noted, however, this could be expected due to providing feed in mash form in this study. Met addition surpassing the requirements caused an impaired ratio of Met + Cys:Lys, which may prevent the utilisation of Met for growth, leading to no further significant improvements observed here. Our trial set-up has the ambition to validate a recommendation proposing that OH-Met can be substituted for DL-Met at a 100:65 ratio without compromising performance. Overall, our results validate this recommendation by obtaining similar growth performance from either Met source at low (25%), moderate (100%) and high (125%) supplemental level. Similarly, Li et al. (2023) found no difference in the growth performance of broilers for either supplementation level of OH-Met and DL-Met (added at 65% of OH-Met, w/w) to reach 75% or 100%

of Met + Cys requirements. Payne et al. (2006) observed no notable difference between DL-Met and OH-Met in BWG and FCR of male Ross 308 broilers (1–47 d of age) in their study where graded levels of DL-Met were made at a constant ratio of 65:100 relative to the OH-Met level on product basis. However, supplementation levels and dietary Met + Cys levels (close or above the requirements established) chosen for assessing the efficiency of OH-Met and DL-Met may lead different results from previously mentioned studies as observed in the literature (Agostini et al. 2016; Uddin et al. 2022; Batonon-Alavo et al. 2023). Lemme et al. (2020) revealed that substantial performance differences between OH-Met and DL-Met at low supplementation diminished with increasing addition levels. Indeed, diluted DL-Met with a purity of 65%, which was used as an internal standard, provided similar performance compared with pure DL-Met when supplemented high enough (Lemme et al. 2020).

Carcase and cuts yield, digestive organ weight, and breast meat traits

In line with our growth performance data, Met supplementation to a Met-deficient basal diet mainly improved carcass and parts yield as no further improvements were observed in carcass and breast meat yield above 100% of Met supplementation. These results are underlying the fact that broilers fed corn-soybean-based diets require additional Met and this requirement needs to be met with an appropriate level of supplementation in order to ensure proper carcass yield as well as growth performance. The source of supplemental Met, however, had no significant effect on carcass and parts yield, which agrees with previous studies (Lemme et al. 2002, 2020) where OH-Met and DL-Met were demonstrated to provide similar improvements when supplementation was made at a constant ratio of 100:65 (100 parts OH-Met, 65 parts DL-Met on product basis). Decreased drumstick yield with Met supplementation was observed in the current study as reported by Pontin et al. (2018) and Sangali et al. (2014), which may be mainly related to increased breast meat and thigh yield. The liver is the main site of Met metabolism and Met deficiency may cause hypertrophy as observed in the current study, which is likely due to increased workload of the liver *via* upregulation of some genes related to amino acid oxidation (Zhang et al. 2018) and remethylation (Aggrey et al. 2018). Likewise, Met deficiency resulted in increased relative weight of the pancreas likely because of elevated amino acid metabolism in the current study, as indicated by Çenesiz et al. (2022). The decreased relative weights of the liver and pancreas observed with the addition of either DL-Met or OH-Met to the basal diet suggests that the adverse effects of Met deficiency on these organs could be alleviated with Met supplementation regardless of source. Met may act as a lipotropic agent through its role as an amino acid in balancing protein synthesis or through its role as a methyl donor and involvement in choline, betaine, folic acid, and vitamin B metabolism (Young et al. 1955). Therefore, Met supplementation to the basal diet tended to reduce abdominal fat, and this lipotropic effect of Met was observed much markedly between the lowest and the rest of the supplemental level in the current study. However, no significant difference was observed between DL-Met and OH-Met on the relative weight of abdominal fat as also reported by some previous studies (Liu et al. 2006, 2007; Conde-Aguilera et al. 2016; Zeitz et al. 2018; Kim et al. 2019). However, the same researchers have obtained varying results related to the effects of Met addition on the relative weight of abdominal fat. Liu et al. (2006, 2007) and Zeitz et al.

(2018) found a decrease in the relative weight of abdominal fat with the addition of Met to the basal diet, as Conde-Aguilera et al. (2016) and Kim et al. (2019) observed no difference.

Met in the poultry diet is essential, besides structuring proteins, for the initialisation of translation (Brosnan et al. 2007), and therefore, different sources and amounts of dietary Met may influence the meat quality of broiler chickens. In the current study, however, the breast meat content of dry matter, ash, crude protein, and crude fat was not significantly affected by the sources and supplemental level of Met ($p > .05$) (Table 6). Some previous studies also reported that different sources and supplemental levels of Met had no remarkable effect on the nutrient content of breast meat in broiler chickens (Liu et al. 2006; Ullrich et al. 2019). However, some previous research (Esteve-Garcia and Llaurodo 1997; Wallis 1999; Lemme et al. 2002; Johri et al. 2004) indicating lower efficacy of OH-Met in promoting muscle deposition of the birds when compared to DL-Met was inconsistent with our results. These discrepancies might be attributed to different diet types, the extent of Met deficiency and level of supplementation, and the application of statistical methodologies for the interpretation of data. In the current study, the addition level of Met was shown to have a greater effect on yield than the nutrient content of the breast meat as no notable source effect.

Gene expression in the liver

Met addition positively affects protein synthesis *via* several pathways including regulating genes related to growth such as GH. GH mainly affects growth *via* IGF-I, of which synthesis and release requires GH-GHR binding thus increased GHR expression is expected with Met supplementation (Del Vesco et al. 2013), as observed in the current study (Figure 1). Likewise, the correlation coefficients and probability values shown in Table 7 confirm a significant relationship between GHR expression and both growth performance and carcass yield. Expression of GHR showed a positive correlation with BWG (+0.386), relative weights of carcass and breast meat (+0.451 and +0.548), and a negative correlation with FCR (−0.376) in the current study. Expression of GHR mRNA in broiler liver significantly increased at the 100% (1.45 vs 1.00) and 125% (1.41 vs 1.00) relative additions of DL-Met, while only 100% of OH-Met additions increased (1.85 vs 1.00) ($p < .05$) when compared to the basal diet (Figure 1). These data show that the effects of supplemental Met levels on GHR expression vary depending on the

source, most likely due to differences in bioavailability as mentioned previously. Unlike GHR, the level or source of supplemental Met had no significant effect on IGF-I expression in the current study, which agrees with Del Vesco et al. (2015) who obtained similar findings on 42 d of age in broilers. Furthermore, Del Vesco et al. (2015) reported that methionine supplementation decreased the expression of atrogen-1 involved in protein breakdown. Zeitz et al. (2019) reported that suboptimum dietary Met concentrations increased the expression of the autophagy-related genes ATG5 and BECN1 on day 35. Likewise, Wen et al. (2014) observed that Met supplementation resulted in reduction of eukaryotic initiation factor 4E binding protein 1 (eIF4EBP1), forkhead box O4 (FOXO4) and atrogen-1 gene expression in chicks. These results indicate that the effects of supplemental Met are not restricted to IGF-I activity and may affect growth by reducing protein degradation through downregulation some respective genes, which may also be the case in the current study.

Conclusions

The supplementation of Met to a corn-soybean meal-based diet improved growth performance and carcase and cuts yields without any significant difference in the proximate chemical composition of breast meat in the current study. DL-Met and OH-Met showed similar BWG, FCR, carcase yield and proximate chemical composition of breast meat when a 1.54 times higher amount of OH-Met was added due to assumed 65% of bioefficacy compared to DL-Met on a product basis. The effects of the supplemental level of Met on GHR expression, which showed a positive correlation with growth performance and carcase yield, vary depending on the source as no difference was observed in IGF-I expression. Overall, these results from the current study demonstrate that a 1.54 times higher amount of OH-Met could replace DL-Met in corn-soybean meal based broiler diets without compromise in growth performance, carcase and cuts yields and breast meat traits.

Ethical approval

All experimental procedures in the current study were approved by Ankara University Animal Experiments Local Ethics Committee (2020-10-82).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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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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