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Original Research Article

Dietary supplementation with 2-hydroxy-4-methyl(thio) butanoic acid and DL-methionine improves productive performance, egg quality and redox status of commercial laying ducks

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ABSTRACT

This experiment aimed to study the effects of supplemental methionine sources, 2-hydroxy-4 methyl(thio) butanoic acid (HMTBa) and DL-Methionine (DL-Met), on productive performance, egg quality, and redox status of laying ducks. A total of 792 healthy 25-wk-old Longyan laying ducks with similar body weights were randomly allotted to 11 treatment groups. Each treatment group had 6 replicates of 12 ducks. The trial lasted for 16 wk. Ducks were fed a basal deficient diet (Met: 0.24%; Met + Cys: 0.51%) or supplemented with DL-Met or HMTBa at 0.05%, 0.12%, 0.19%, 0.26%, and 0.33% of diet, respectively. Compared with the basal diet, supplementation with either DL-Met or HMTBa increased the average egg weight, egg mass, and decreased feed to egg ratio during the whole trial period ($P < 0.05$). Albumen weight and its ratio to total egg weight were increased, but yolk and shell ratio, albumen height, Haugh unit and shell breaking strength were decreased ($P < 0.05$). Dietary DL-Met or HMTBa supplementation increased taurine, methionine, leucine, tryptophan and arginine content, and decreased serine and lysine content in plasma ($P < 0.05$). The redox status of laying ducks was improved by enhancing the glutathione peroxidase and catalase activities, glutathione content and its ratio relative to glutathione (oxidized) content and decreasing malondialdehyde content and increasing mRNA expression of superoxide dismutase-1, glutathione peroxidase-1, hemeoxygenase-1 and nuclear factor-like 2 in liver and ileum with the supplementation of DL-Met or HMTBa ($P < 0.05$). Liver health status measured by average area proportion lipid droplet was improved with supplementation of DL-Met or HMTBa ($P < 0.05$). Villus height and villus height to crypt depth ratio in the ileum and the ileal gene expression of tight junction protein and occludin were increased with DL-Met or HMTBa supplementation ($P < 0.05$). Taken together, these results suggested that the efficacy of dietary supplementation of HMTBa was similar to DL-Met, and it ranged from 98% to 100% for productive performance and egg albumen ratio in laying ducks (25 to 41 wk).

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1. Introduction

Methionine (Met) is the first limiting amino acid in corn-soybean meal-based poultry diets and its dietary provision is indispensable for maintenance, growth, and development (Zeng et al., 2015; Castro et al., 2019; Lemme et al., 2020). The dietary supplemental Met sources in poultry mainly include the synthetic

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forms i.e., DL-methionine (DL-Met, 99% purity) and 2-hydroxy-4-methyl(thio) butanoic acid (HMTBa, 88% purity). The sources of these Met differ in cellular absorption, transport, and site of bioconversion pathways (Zhang et al., 2015). HMTBa is absorbed mainly by monocarboxylate transporter 1, coupled with the activity of the Na^+/H^+ exchanger, while uptake of D-Met and L -Met takes place via multiple carrier-mediated systems. HMTBa is absorbed along the entire gastrointestinal tract, especially the upper gastrointestinal tract. Intestine, liver and kidney all can remove D-Met and HMTBa from circulation and metabolize them to L-Met through oxidation and transamination (Zhang et al., 2015). Both DL-Met and HMTBa are efficient precursors of L-Met (Vázquez-Añón et al., 2006; Zou et al., 2015). However, literature depicts conflicting reports about the relative efficacy from different Met sources in broilers (Lemme et al., 2022, 2020; Sauer et al., 2008; Agostini et al., 2016; Uddin et al., 2022) and pigs (Shoveller et al., 2010; Krutthai et al., 2015). In growing ducks, Kluge et al. (2016) reported that HMTBa and DL-Met had similar efficacy during the first 3 wk of life. Supplementation of DL-Met in diets might ameliorate the oxidative status of the liver, while HMTBa would partially improve the intestinal oxidative status of Peking ducks (Guo et al., 2018). These reports indicate that there is an inconsistency pertaining to the comparison of HMTBa to DL-Met in different species, age, and response variables.

A few studies have investigated the putative effects of supplemental levels or source(s) of Met in laying ducks (He et al., 2003; Fouad et al., 2016) and duck breeders (Ruan et al., 2018). Essentially, DL-Met was the main Met source in these studies. It demonstrates a general paucity of information about the potential role of HMTBa in laying ducks. Therefore, the present study was planned to investigate the bio-efficacy of HMTBa compared with DL-Met and their comparative effects on productive performance, egg quality, intestinal morphology, and their antioxidant potential in commercial laying ducks.

2. Materials and methods

2.1. Animal ethics statement

This work was conducted in accordance with the Guidelines of the Animal Care and Use Committee of the Guangdong Academy of Agricultural Sciences (2020002).

2.2. Experimental design and diets

A total of 792 Longyan laying ducks at 25 wk old with similar body weights and in good health condition were randomly divided into 11 groups. Six replicates with 12 ducks allotted to each group. The total duration of the trial was 16 wk. A basal deficient diet (BD; Met: 0.24%; Met + Cys: 0.51%) was provided for ducks. Both DL-Met (99%; Adisseo, France) and HMTBa (88%; Adisseo, France) were supplemented at 0.05%, 0.12%, 0.19%, 0.26%, and 0.33% on iso-molar basis to reach Met + Cys of 0.56%, 0.63%, 0.70%, 0.77%, and 0.84%, respectively. The cage (45 cm × 30 cm × 50 cm) provided with a feeder and nipple drinker (Guangzhou Huanan Poultry Equipment, Guangzhou, China) was selected to house duck. Diets and water were supplied ad libitum. The basal diet composition and nutritional ingredient are listed in Table 1. The crude protein, Met and Cys levels in corn, soybean meal, peanut meal and wheat bran were analyzed before diet formulation. The Met and Cys levels in the diets were analyzed in a fully automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan), and the crude protein was analyzed by a Kjeltec 8400 Analyzer Unit (FOSS Analytical AB, Hoganas, Sweden); both according to the Chinese National Standard method (GB/T 18246-2019).

2.3. Sample collection

The egg quality was determined with 3 eggs of average weight from each replicate at the end of 4, 8, 12 and 16 wk, and all detections were finished on the day of collection.

On day of the trial ending, the ducks with healthy and normal egg production were selected from each replicate, after the ducks fasted for 12 h, blood was obtained from the wing vein into the evacuated tubes, containing heparin sodium. The plasma was harvested after centrifuged at 3,000 × *g* for 10 min and then kept at −20 °C for further analysis.

Thereafter, the sampled ducks were weighed before slaughter. Tissue samples from the liver and ileum were taken to measure various antioxidant and immune indices, intestinal morphology, and hepatic steatosis. In addition, the abundance of antioxidant-related genes in the liver and ileum, and the intestinal barrier-related genes abundance in the ileum were tested.

2.4. Productive performance and egg quality

The number and weight of eggs and the feed consumption were recorded every day. After egg weight and shell weight were measured for shell ratio calculation, the breaking strength was detected by an egg force reader (Israel Orka Food Technology Ltd.) and the eggshell thickness was determined by an eggshell thickness gauge (Israel Orka Food Technology Ltd., Ramat Hasharon, Israel). Then, the egg analyzer (Israel Orka Food Technology Ltd.) was used to measure yolk color, egg albumen height and Haugh unit.

Table 1
Dietary composition and nutrient levels of the diets (DM basis).

Item	Content
Ingredients, g/kg	
Corn[1]	400
Mazie starch	110
Wheat bran[1]	100
Soybean meal[1]	236
Peanut meal[1]	20
L-Lysine sulfate	1.0
Limestone	85
$CaHPO_4$	13
NaCl	3
Premix[2]	10
Rice hull powder	22
Total	1000
Nutrient levels, %	
AME, MJ/kg	10.06
Crude protein	16.5
Calcium	3.65
Available phosphorus	0.35
Lysine	0.95
Methionine[3]	0.24
Methionine + cysteine[3]	0.51

AME = apparent metabolizable energy.
[1] The analyzed values of crude protein, Met, and Cys in corn, wheat bran, soybean meal, and peanut meal were 8.04%, 16.48%, 46.97%, 52.8% of CP; 0.17%, 0.23%, 0.64%, 0.55% of Met; 0.18%, 0.38%, 0.69%, 0.69% of Cys, respectively.
[2] Provided per kilogram of diet: vitamin A 12,500 IU; vitamin D_3 4,125 IU; vitamin E 15 IU; vitamin K 2 mg; thiamine 1 mg; riboflavin 8.5 mg; calcium pantothenate 50 mg; niacin 32.5 mg; pyridoxine 8 mg; biotin 2 mg; folic acid 5 mg; vitamin B_{12} 5 mg; Mn 100 mg; I 0.5 mg; Fe 60 mg; Cu 8 mg; Se 0.2 mg; Co 0.26 mg.
[3] The number are analyzed values.

2.5. Plasma amino acids

After hydrolysis, the content of essential amino acids, alanine (Ala), arginine (Arg), aspartic acid (Asp), cystine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), ornithine (Orn), proline (Pro), serine (Ser), and taurine (Tau) in plasma were analyzed by the analyzer of amino acid (HITACHI L-8900, Hitachi, Ltd., Tokyo, Japan).

2.6. Antioxidant indices in plasma, liver, and ileum

The content of glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD) and catalase (CAT) activity, malondialdehyde (MDA), oxidized glutathione (GSSG), glutathione (GSH) and protein carbonyl (PC) in plasma, liver, and ileum were detected by commercial kits with code of A005-1, A001-1, A007-1, A003-2, A061-1, A006-2 and A087-1, respectively (Nanjing Jiancheng Biological Product Co. Ltd, Nanjing, China).

2.7. Immune indices in plasma and ileum

The interleukin-1 (IL-1), IL-8, and IL-10 content in plasma, liver, and ileum were determinate by ELISA kits of code ml043001-2, ml036912-2 and ml061196-2, respectively (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China).

2.8. Gene expression in liver and ileum

The Trizol reagent (Invitrogen, Carlsbad, CA) was used to reverse RNA samples to prepare cDNA. Twenty microliters PCR reaction mixture (primer concentration: 0.3 μM) containing the iTaq Universal SYBER Green Supermix (TaKaRa, Tokyo, Japan) was used to detect the target gene abundances with the CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA). The relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and normalized to avian β-actin. The primers and the gene information are shown in Table S1.

2.9. Intestinal histology

The sample of the ileum were embedded in Tissue-Tek to prepare cryosections (5 μm). Then the sections were stained with hematoxylin-eosin (H&E) and observed by scanning electron microscopy (Opelco, Washington, DC). The tip of the villi to the villous–crypt junction (villus height) and from this junction to the base of the crypt (crypt depth) were measured to detect the ratio of villus height to crypt depth.

2.10. Hepatic steatosis

The segments of the liver were cryosectioned and fixed on the slides coated with polyline. Slides were stained with Oil Red and hematoxylin and eosin, and examined under an electron microscopy (Opelco, Washington, DC). The image pro plus 6.0 was used to calculate the area and quantity of red grease in the view. The lipid droplets area and numbers, and the average lipid droplets area proportion were determined.

2.11. Statistical analysis

Each replicate was taken as an experimental unit. The Mixed Model (PROC MIXED) procedure of SAS, version 8 (SAS Inst. Inc., Cary, NC) was used to analysis the effect of dietary supplementation for each response variable. Met supplemental levels in diets were assessed using regression analysis with linear and quadratic effects. The effects of Met sources (BD vs. DL-Met, BD vs. HMTBa, and DL-Met vs. HMTBa) were evaluated by preplanned contrasts with the average of 4 supplemental levels (Zhang et al., 2019). Nonlinear exponential regression analysis was applied to evaluate the relative bioavailability of HMTBa to DL-Met due to the nonlinear responses for productive performance and egg component traits (Littell et al., 1997).

The nonlinear equation was $y = a + b \times [1 - \text{Exp}(c_1x_1 + c_2x_2)]$, specifically y = variable (egg weight, egg mass, FCR, etc.), a = intercept (value for the basal diet), b = asymptotic response, $a + b$ = common asymptote (maximum level), c_1 = slope ratio for DL-Met, c_2 = slope ratio for HMTBa, and x_1 and x_2 = dietary daily intake of Met of DL-Met and HMTBa treatments, respectively. The relative bioavailability values of HMTBa to DL-Met were given by the ratio of their c values = c_2/c_1. Data are expressed as means and pooled SEM. A P-value < 0.05 was considered significant.

3. Results

3.1. Productive performance

Compared with ducks fed the BD, both DL-Met or HMTBa supplementation increased average egg weight, egg mass, and average daily feed intake (ADFI), and decreased feed to egg ratio during the whole trial period ($P < 0.05$, Table 2), without differences between DL-Met and HMTBa ($P > 0.05$). With the increasing supplemental levels, the FCR was quadratically decreased with both DL-Met and HMTBa ($P < 0.05$). The egg mass was quadratically increased only with dietary increments of HMTBa ($P < 0.05$). For the productive performance, no significant differences between HMTBa and DL-Met on egg mass (98.7%; CI: [95.0 to 102.4]), average egg weight (100.2%; CI: [98.8 to 101.5]), and FCR (98.1%; CI: [93.2 to 103.0])

Table 2
Productive performance of ducks fed graded levels of either DL-Met or HMTBa compared to the basal diet.[1]

Item	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[2]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL-Met	BD vs. HMTBa	DL-Met vs. HMTBa
Egg production, %	85.9	84.9	85.2	84.6	84.3	83.9	83.5	84.8	84.5	85.3	84.6	0.24	L**Q*	NS	0.149	0.136	0.931
Average egg weight, g	63.3	66.7	67.8	67.5	67.6	66.9	67.0	67.5	67.6	67.4	67.1	0.18	NS	NS	<0.001	<0.001	0.889
Egg mass, g/d	54.3	56.6	57.8	57.0	57.0	56.0	55.9	57.3	57.1	57.6	56.7	0.20	NS	Q*	<0.001	<0.001	0.951
ADFI, g/d	163	165	165	165	164	165	165	165	164	165	164	0.2	NS	NS	0.020	0.045	0.550
FCR, g:g	3.01	2.92	2.86	2.89	2.89	2.94	2.95	2.88	2.88	2.86	2.90	0.009	Q*	Q**	<0.001	<0.001	0.771

BD = basal diet; DL -Met = DL -methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid; FCR = feed to egg ratio.
[1] Mean of 6 replicates (12 ducks per replicate) per treatment.
[2] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$.

(Fig. 1) were observed. The details on productive performance parameters for different periods up to 16 wk are available in Table S2.

3.2. Egg quality

For the egg quality, both DL-Met and HMTBa supplementations increased egg weight, length, and width compared with ducks fed the BD during the trial period ($P < 0.05$, Table 3). A quadratic increase with DL-Met was observed for these parameters ($P < 0.05$). The egg shape index tended to decrease with DL-Met or HMTBa supplementation. This decrease appeared linear with HMTBa treatments and quadratic with DL-Met treatment ($P < 0.05$). The albumen height and Haugh unit were decreased with DL-Met or HMTBa supplementation ($P < 0.01$), and there was a linear pattern for albumen height and quadratic pattern for the Haugh unit only for DL-Met supplementation ($P < 0.05$). Compared with the BD, shell thickness was not affected ($P > 0.05$), but breaking strength was decreased with the supplementation of DL-Met ($P < 0.05$) while only a tendency was found for HMTBa ($P = 0.062$). Dietary DL-Met or HMTBa supplementation increased shell weight but decreased shell ratio compared with BD treatment ($P < 0.05$). A significant linear and quadratic effect between shell ratio and DL-Met supplementation ($P < 0.05$) was observed. The albumen weight and its ratio were increased, but the yolk ratio was decreased with the supplementation of DL-Met or HMTBa compared with BD treatment ($P < 0.001$). Besides, albumen weight was quadratically increased with DL-Met supplementation, and the yolk ratio was linearly decreased with HMTBa supplementation ($P < 0.05$). The efficacy of HMTBa relative to DL-Met for egg albumen ratio was not significantly different (98.5%; CI: [94.9 to 102.2] (Fig. 1). The egg quality data at the end of wk 4, 8,12, and 16 during the trial are shown in Table S3.

3.3. Plasma amino acids

Dietary DL-Met and HMTBa supplementations increased plasma Tau, Met, Leu, Trp, and Arg content, and decreased Ser and Lys content compared with ducks fed the BD. Higher contents of Tau, Leu, and Trp were observed with HMTBa supplementation compared with DL-Met group while lower Ser and higher Arg content were observed with DL-Met supplementation compared with the HMTBa group. No differences in plasma Met and Lys content were detected between DL-Met and HMTBa groups ($P < 0.05$, Table 4). In addition, supplemental DL-Met linearly or quadratically increased plasma Tau (linear, quadratic; $P < 0.001$), Met (linear, quadratic; $P < 0.05$) and Arg content (linear, $P < 0.05$), and decreased Ser content (linear, $P < 0.05$) with increasing levels, whereas supplemental HMTBa linearly or quadratically increased

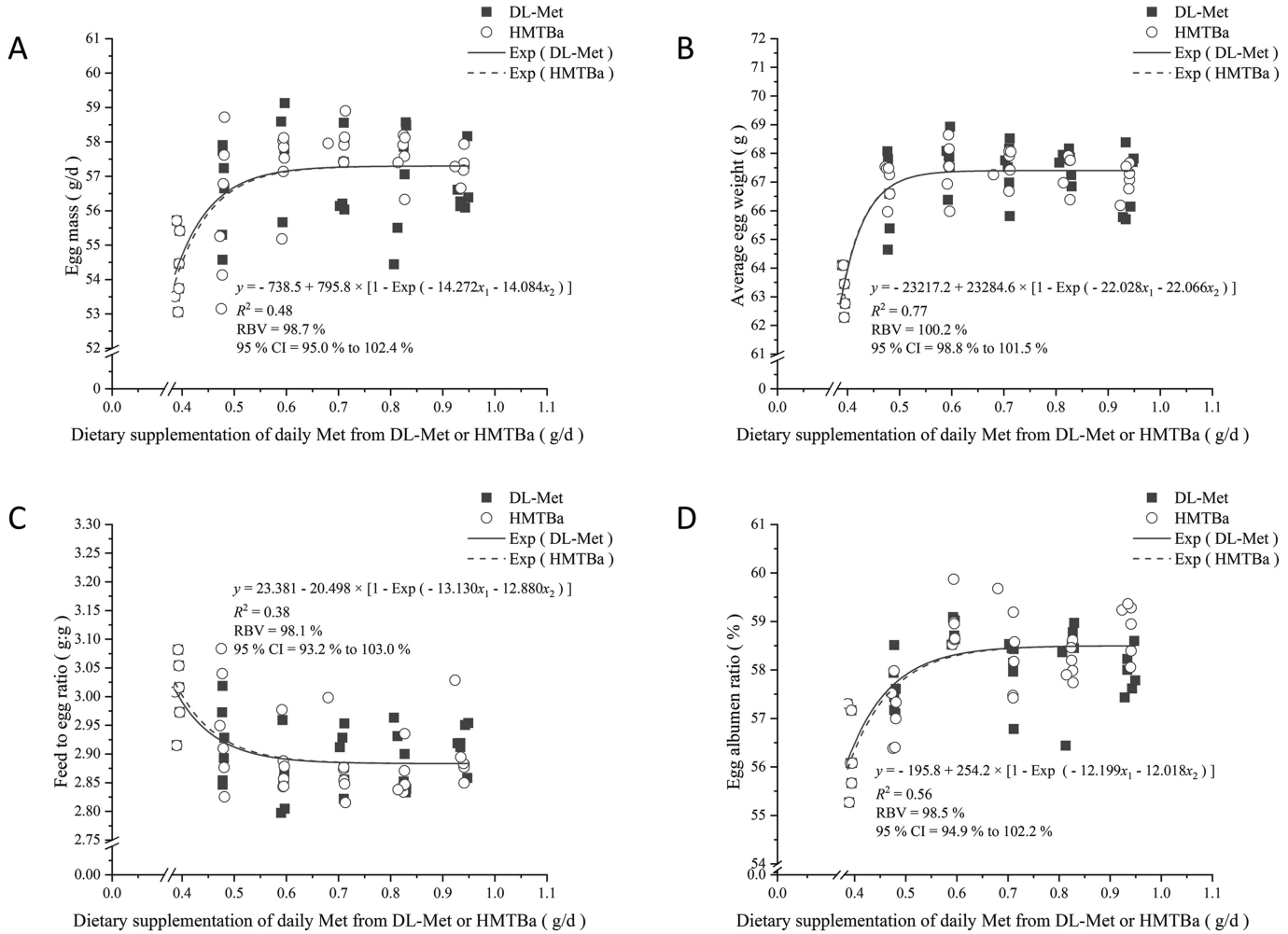

Fig. 1. Egg mass (A), average egg weight (B), feed to egg ratio (C), and egg albumen ratio (D) with increasing dietary supplementation of daily intake of Met from either DL-Met or HMTBa in laying ducks (25 to 41 wk). RBV = relative bioavailability; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.

Table 3
Egg quality of ducks fed graded levels of either DL-Met or HMTBa compared to the basal diet.[1]

Item	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[2]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL-Met	BD vs. HMTBa	DL-Met vs. HMTBa
Egg weight, g	64.1	67.0	68.2	68.5	68.5	67.7	67.9	68.3	68.1	67.7	67.9	0.19	Q*	NS	<0.001	<0.001	0.979
Egg length, mm	58.5	58.9	59.3	59.1	59.1	58.9	59.2	59.4	59.1	58.8	59.1	0.07	Q*	NS	0.028	0.016	0.681
Egg width, mm	43.9	44.7	45.0	45.1	45.2	44.9	45.0	44.9	45.0	45.1	44.9	0.05	Q*	NS	<0.001	<0.001	0.800
Shape index	1.33	1.32	1.32	1.31	1.31	1.32	1.32	1.32	1.32	1.31	1.31	0.002	Q*	L*	0.076	0.073	0.975
Albumen height, mm	7.89	7.71	7.64	7.39	7.55	7.46	7.50	7.63	7.36	7.54	7.74	0.036	L*	NS	0.007	0.008	0.938
Haugh unit	87.2	85.7	84.7	83.2	84.6	83.8	84.2	84.6	83.1	84.4	85.0	0.22	Q*	NS	<0.001	<0.001	0.618
Shell thickness, mm	0.340	0.338	0.339	0.342	0.340	0.342	0.338	0.336	0.342	0.339	0.340	0.001	NS	NS	0.767	0.804	0.347
Shell strength, N	43.2	42.7	39.9	41.3	41.6	41.8	41.2	40.7	40.4	42.5	43.1	0.25	NS	NS	0.045	0.062	0.803
Shell weight, g	6.22	6.44	6.39	6.43	6.30	6.35	6.36	6.34	6.44	6.39	6.40	0.019	NS	NS	0.016	0.013	0.892
Shell ratio, %	9.69	9.58	9.42	9.38	9.31	9.40	9.39	9.31	9.45	9.42	9.48	0.022	L*Q**	NS	<0.001	<0.001	0.849
Yolk weight, g	21.9	21.9	21.7	22.3	22.3	22.1	22.9	21.7	21.9	22.0	21.5	0.07	NS	NS	0.402	0.705	0.424
Yolk ratio, %	34.2	32.8	32.0	32.6	32.6	32.7	33.7	32.0	32.1	32.4	31.8	0.11	NS	L*	<0.001	<0.001	0.369
Albumen weight, g	36.0	38.6	40.1	39.8	39.9	39.2	38.7	40.2	39.8	39.4	40.0	0.17	Q*	NS	<0.001	<0.001	0.672
Albumen ratio, %	56.3	57.6	58.8	58.0	58.3	57.9	57.1	58.9	58.4	58.1	58.9	0.12	NS	NS	<0.001	<0.001	0.335

BD = basal diet; DL -Met = DL -methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (3 eggs per replicate) per treatment.
[2] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$.

Table 4
Plasma amino acids of either DL-Met or HMTBa supplementation in laying ducks (ng/mL).[1]

Item[2]	BD	DL -Met, %					HMTBa, %					SEM	Regression analysis[3]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL -Met	BD vs. HMTBa	DL-Met vs. HMTBa
Tau	20.8	23.4	26.8	35.0	46.1	53.8	29.0	43.6	45.7	41.7	45.1	1.51	L***Q***	L*Q*	<0.001	<0.001	0.028
Asp	7.36	7.88	6.81	6.52	6.35	6.93	7.28	6.62	6.11	5.36	7.10	0.195	NS	NS	0.517	0.225	0.323
Thr	30.3	33.7	35.4	30.8	25.9	31.2	38.7	38.3	33.3	31.3	32.0	0.85	NS	NS	0.710	0.131	0.050
Ser	67.6	66.7	62.8	43.2	43.1	48.9	64.7	67.0	52.8	47.0	56.9	1.42	L*	NS	<0.001	0.003	0.015
Glu	140	184	180	157	172	152	160	142	147	158	150	2.7	NS	NS	<0.001	0.177	<0.001
Gly	29.8	27.3	34.1	26.9	26.1	29.9	31.8	34.4	30.8	30.9	32.2	0.57	NS	NS	0.611	0.238	0.005
Ala	69.2	77.6	81.7	69.0	71.0	65.4	66.5	67.4	66.7	68.0	62.5	1.45	NS	NS	0.465	0.565	0.027
Val	24.4	27.6	27.6	26.9	25.9	27.5	29.2	33.3	27.7	27.3	28.2	0.55	NS	NS	0.160	0.016	0.070
Cys	10.3	10.5	10.7	11.0	10.3	13.6	9.3	10.8	9.72	10.7	11.1	0.23	NS	NS	0.210	0.954	0.041
Met	7.85	11.9	13.7	16.5	15.1	19.7	12.4	17.7	15.8	17.0	16.4	0.54	L**Q*	Q*	<0.001	<0.001	0.560
Ile	14.9	14.7	16.0	14.7	13.2	16.0	15.5	16.1	16.4	16.1	14.6	0.26	NS	Q*	0.946	0.356	0.141
Leu	20.0	22.0	25.6	23.3	20.9	23.7	24.2	25.2	25.5	24.3	24.3	0.41	NS	NS	0.030	0.001	0.048
Trp	36.5	40.3	39.4	47.1	34.6	40.1	43.2	44.5	41.8	41.9	45.6	0.63	NS	NS	0.036	<0.001	0.004
Phe	21.4	22.3	22.6	25.2	21.0	23.4	22.5	24.0	21.8	23.5	23.6	0.43	NS	NS	0.351	0.289	0.821
Or	2.39	2.92	2.74	2.33	2.64	3.65	2.99	3.46	3.23	2.63	2.65	0.094	NS	NS	0.142	0.059	0.454
Lys	57.7	51.6	60.7	51.2	41.4	54.3	56.9	55.2	50.8	46.3	46.6	0.93	NS	L**Q*	0.022	0.011	0.652
His	5.33	5.28	7.22	5.03	4.23	3.44	6.40	4.41	3.81	4.51	3.36	0.174	NS	NS	0.477	0.041	0.020
Arg	35.1	37.9	38.9	47.9	42.0	45.9	36.1	38.0	42.0	39.3	45.1	0.71	L*	L*	<0.001	0.016	0.043
Pro	19.0	20.4	21.4	20.8	20.3	20.3	23.3	24.6	23.5	22.6	22.9	0.39	NS	NS	0.210	0.001	<0.001

BD = basal diet; DL -Met = DL -methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (2 ducks per replicate) per treatment.
[2] Tau = taurine; Asp = aspartic acid; Thr = threonine; Ser = serine; Glu = glutamic acid; Gly = glycine; Ala = alanine; Val = valine; Cys = cystine; Met = methionine; Ile = isoleucine; Leu = leucine; Trp = tryptophan; Phe = phenylalanine; Orn = ornithine; Lys = lysine; His = histidine; Arg = arginine; Pro = proline.
[3] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

plasma Tau (linear, quadratic; $P < 0.05$), Met (quadratic, $P < 0.05$), Ile (quadratic, $P < 0.05$), and Arg content (linear, $P < 0.05$), and decreased Lys content (linear, quadratic; $P < 0.05$) with increasing levels.

3.4. Antioxidant indices in plasma, liver, and ileum

In plasma, both DL-Met and HMTBa supplementations in the diet decreased protein carbonyl (PC) content and increased GSSG content. The GSH content tended to increase with both DL-Met ($P = 0.06$) and HMTBa ($P = 0.075$) without reaching statistical significance, and resulted in a lack of difference in the ratio of GSH to GSSG compared with ducks fed the BD ($P > 0.05$, Table 5). Dietary DL-Met rather than HMTBa supplementation increased plasma CAT activity in contrast to ducks fed the BD, and higher CAT activity was observed with DL-Met supplementation relative to the HMTBa group ($P < 0.05$). In addition, increasing DL-Met supplemental levels decreased PC content and increased GSH-Px activity (linear, quadratic; $P < 0.05$). Both GSH-Px activity and GSH content in plasma were increased with increasing HMTBa supplemental levels (linear, quadratic; $P < 0.05$).

With respect to the liver antioxidant indices, both dietary DL-Met and HMTBa supplementations increased GSH content and its ratio to GSSG, GSH-Px and CAT activities, and decreased MDA content in contrast to ducks fed the BD ($P < 0.05$), without a difference between 2 Met sources. Dietary DL-Met supplemental levels increased GSH-Px (quadratic, $P < 0.001$) and CAT (linear, quadratic; $P < 0.01$) activities, GSH (linear, quadratic, $P < 0.05$) content and its ratio relative to GSSG content (linear, quadratic; $P < 0.05$), and decreased MDA (quadratic, $P < 0.05$), PC (linear, $P < 0.01$), and GSSG (linear, quadratic; $P < 0.05$) content. Dietary HMTBa supplemental levels increased GSH-Px (linear, quadratic;

Table 5
Antioxidant indices in plasma, liver and ileum of either DL-Met or HMTBa supplementation in laying ducks.[1]

Item[2]	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[3]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL -Met	HMTBa	BD vs. DL-Met	BD vs. HMTBa	DL-Met vs. HMTBa
Plasma																	
MDA, nmol/mL	7.82	7.50	8.00	8.08	7.97	7.34	7.45	7.38	8.07	8.57	8.16	0.186	NS	NS	0.950	0.885	0.719
PC, nmol/mL	0.226	0.196	0.199	0.157	0.167	0.139	0.222	0.169	0.189	0.146	0.203	0.0057	L**Q*	NS	0.003	0.028	0.168
GSH-Px, U/mL	102	103	108	110	108	109	103	107	106	109	111	0.9	L*Q*	L**Q*	0.098	0.119	0.866
GSH, μmol/L	33.8	37.0	35.0	37.9	37.7	35.6	33.1	32.9	34.3	39.1	43.1	0.52	NS	L*Q**	0.060	0.075	0.849
GSSG, μmol/L	4.09	4.42	5.12	4.22	4.83	4.92	5.32	4.75	4.33	4.93	4.74	0.084	NS	NS	0.030	0.011	0.488
GSH to GSSG ratio	7.92	8.50	6.84	9.29	7.99	7.28	6.22	7.06	7.97	8.12	9.14	0.186	NS	NS	0.920	0.710	0.415
T-SOD, U/mL	131	140	162	129	150	165	159	122	135	108	136	4.6	NS	NS	0.254	0.961	0.061
CAT, U/mL	1.56	2.67	3.70	1.87	1.59	2.32	1.62	2.05	1.73	1.78	1.51	0.112	NS	NS	0.009	0.588	<0.001
Liver																	
MDA, nmol/mgprot	3.81	3.45	3.28	3.30	3.36	3.41	3.12	3.23	3.17	2.90	3.16	0.061	Q*	NS	0.040	0.002	0.055
PC, nmol/mgprot	1.30	1.25	1.23	1.23	1.22	1.17	1.28	1.22	1.19	1.15	1.14	0.018	L**	L***Q**	0.254	0.142	0.560
GSH-Px, U/mgprot	581	602	621	630	626	613	600	613	633	622	627	4.7	Q***	L*Q*	0.033	0.031	0.963
GSH, μmol/gprot	53.0	57.5	58.6	61.8	60.1	60.2	55.4	58.6	60.5	59.1	60.7	0.65	Q*	L*Q*	0.004	0.012	0.531
GSSG, μmol/mgprot	117	111	109	106	107	105	113	113	109	110	108	1.7	L*Q*	L**Q*	0.181	0.377	0.426
GSH to GSSG ratio	0.455	0.522	0.559	0.587	0.574	0.578	0.493	0.545	0.557	0.537	0.566	0.0107	L*Q**	L*Q*	0.006	0.029	0.271
T-SOD, U/mgprot	305	317	313	306	298	294	315	316	308	309	301	2.0	NS	NS	0.988	0.555	0.321
CAT, U/mgprot	968	1007	1164	1222	1308	1339	1012	1086	1201	1356	1307	22.2	L***Q**	L**Q*	<0.001	<0.001	0.641
Ileum																	
MDA, nmol/mgprot	6.25	4.91	3.98	3.56	4.30	4.10	5.02	3.79	3.13	3.55	3.95	0.199	Q*	Q**	0.003	<0.001	0.470
PC, nmol/mgprot	0.893	0.635	0.704	0.579	0.601	0.478	0.736	0.767	0.691	0.668	0.610	0.0189	L*	L*	<0.001	<0.001	0.004
GSH-Px, U/mgprot	253	284	295	297	290	280	289	302	313	298	305	3.7	Q*	Q*	0.005	0.000	0.106
GSH, μmol/gprot	9.11	10.0	10.7	10.8	10.6	10.6	9.76	10.3	10.5	10.8	11.1	0.14	Q*	L**Q**	0.003	0.004	0.850
GSSG, μmol/mgprot	90.0	80.6	81.2	83.3	80.0	79.6	85.7	85.5	86.5	87.2	81.8	1.15	NS	NS	0.037	0.272	0.080
GSH to GSSG ratio	0.102	0.124	0.133	0.130	0.133	0.135	0.116	0.124	0.123	0.125	0.137	0.0024	NS	L*	0.001	0.006	0.201
T-SOD, U/mgprot	335	361	354	344	326	333	349	362	368	363	357	4.3	NS	Q***	0.597	0.125	0.081
CAT, U/mgprot	102	134	121	158	148	133	142	154	158	166	143	4.1	NS	Q*	<0.001	<0.001	0.084

BD = basal diet; DL-Met = DL-methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (2 ducks per replicate) per treatment.
[2] MDA = malondialdehyde; PC = protein carbonyl; GSH = glutathione; GSH-Px = glutathione peroxidase; T-SOD = total superoxide dismutase; CAT = catalase; GSSG = glutathione (oxidized).
[3] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

$P < 0.05$) and CAT (linear, quadratic; $P < 0.05$) activities, GSH (linear, quadratic; $P < 0.05$) content and its ratio relative to GSSG content (linear, quadratic; $P < 0.05$), and decreased PC (linear, quadratic; $P < 0.01$), and GSSG (linear, quadratic; $P < 0.05$) content.

In the ileum, supplementation of DL-Met and HMTBa increased GSH-Px and CAT activities, GSH content and its ratio to GSSG, and decreased MDA and PC content in contrast to ducks fed BD ($P < 0.05$). The PC content was lower in DL-Met supplementation group than in HMTBa supplementation group ($P < 0.05$). In addition, the supplementation of DL-Met rather than HMTBa decreased GSSG content compared with BD ($P < 0.05$). There was no statistical difference between sources ($P = 0.080$). Quadratic effects on MDA, GSH-Px and GSH and a linear effect of PC were observed with increasing doses of DL-Met and HMTBa ($P < 0.05$). Moreover, increased levels of HMTBa led to increase the ratio of GSH to GSSG (linear, $P < 0.05$), T-SOD (quadratic, $P < 0.001$) and CAT (quadratic, $P < 0.05$) activities.

3.5. Immune indices in plasma and ileum

Dietary DL-Met and HMTBa supplementations decreased IL-1, IL-8, and IL-10 content in plasma, and DL-Met supplementation decreased IL-1 content relative to HMTBa treatment ($P < 0.05$, Table 6). In addition, dietary HMTBa supplemental levels decreased IL-8 (linear, quadratic; $P < 0.05$) and IL-10 (quadratic; $P < 0.05$) content in the plasma.

In the ileum, lower contents of IL-1 and IL-8 were observed with DL-Met and HMTBa supplementations in contrast to ducks fed with the BD ($P < 0.05$), and significantly lower IL-8 content was observed with HMTBa supplementation relative to DL-Met supplementation ($P < 0.05$). In addition, the ileal IL-1 and IL-8 contents decreased with increasing DL-Met and HMTBa supplemental levels (linear, quadratic; $P < 0.05$), and there was a quadratic effect between ileal IL-10 content and HMTBa supplementation ($P < 0.01$).

3.6. Gene expression in liver and ileum

In the liver, both DL-Met and HMTBa supplementations increased gene expression of *SOD1*, *HO-1* and *Nrf2*. The gene expression of *GPX1* was significantly increased with supplementation of DL-Met relatively to BD ($P < 0.05$) but not with HMTBa (Table 7); however, no differences in these genes were observed between the 2 sources. In the ileum, ducks supplemented with either DL-Met or HMTBa had increased mRNA values of *SOD1*, *GPX1*, *HO-1*, and *Nrf2* relatively to ducks fed BD. The ileal *Nrf2* mRNA value was increased more with DL-Met supplementation compared with HMTBa supplementation ($P < 0.05$). The gene expression of *CAT* was increased with the supplementation of HMTBa but not with DL-Met in contrast to ducks fed BD ($P < 0.05$). Besides, hepatic *SOD1* (linear, $P < 0.05$), and ileal *GPX1* (quadratic, $P < 0.05$) and *HO-1* (linear, quadratic; $P < 0.05$) mRNA values were increased with dietary supplemental levels of DL-Met. Dietary HMTBa supplemental levels increased gene expression of *GPX1* (linear, quadratic; $P < 0.05$), *CAT* (linear, $P < 0.05$), *HO-1* (linear, quadratic; $P < 0.05$) both in liver and ileum, and *Nrf2* (quadratic, $P < 0.05$) in the ileum.

Besides, the supplementation of DL-Met and HMTBa in the diet increased the gene expression of *ZO-1* and occludin in the ileum ($P < 0.05$), occludin mRNA level was quadratically increased accompanied by the increasing of HMTBa supplemental levels ($P < 0.05$).

Table 6
Immune indices in plasma and ileum of either DL-Met or HMTBa supplementation in laying ducks.[1]

Item[2]	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[3]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL-Met	BD vs. HMTBa	DL-Met vs. HMTBa
Plasma																	
IL-1, pg/mL	38.4	29.2	30.7	28.6	31.0	29.7	32.7	33.7	33.5	33.6	33.4	0.37	NS	NS	<0.001	<0.001	<0.001
IL-8, pg/mL	29.6	22.8	25.1	22.8	23.3	21.9	25.0	24.8	22.3	22.3	22.3	0.36	NS	L*Q**	<0.001	<0.001	0.751
IL-10, pg/mL	24.7	18.1	19.0	16.5	18.2	16.5	18.9	18.5	18.9	17.6	18.7	0.33	NS	Q*	<0.001	<0.001	0.067
Ileum																	
IL-1, pg/mgprot	5.35	5.20	5.12	4.62	3.97	3.96	4.85	4.81	4.67	3.96	3.86	0.093	L**Q*	L**Q*	0.004	<0.001	0.338
IL-8, pg/mgprot	6.45	6.19	5.48	4.22	3.45	3.11	3.89	3.86	3.67	3.18	3.02	0.154	L***Q**	L*Q*	<0.001	<0.001	<0.001
IL-10, pg/mgprot	2.74	2.75	2.46	2.50	2.60	2.45	2.78	2.85	2.79	2.49	2.13	0.044	NS	Q**	0.175	0.341	0.477

BD = basal diet; DL-Met = DL-methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (2 ducks per replicate) per treatment.
[2] IL-1 = interleukin-1; IL-8 = interleukin-8; IL-10 = interleukin-10.
[3] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 7
Gene expression in liver and ileum of either DL-Met or HMTBa supplementation in laying ducks.[1]

Item[2]	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[3]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL-Met	BD vs. HMTBa	DL-Met vs. HMTBa
Liver																	
SOD1	0.93	1.14	1.01	1.11	1.45	1.38	1.13	1.19	1.01	1.35	1.26	0.033	L*	NS	0.006	0.015	0.586
GPX1	1.07	1.16	1.26	1.34	1.37	1.13	1.08	1.11	1.21	1.27	1.24	0.024	NS	L**Q*	0.030	0.168	0.152
CAT	1.04	1.03	1.19	1.13	1.64	1.26	0.95	1.17	1.29	1.21	1.52	0.038	NS	L*	0.072	0.110	0.716
HO-1	0.94	1.34	1.30	1.31	1.38	1.20	1.14	1.18	1.19	1.51	1.60	0.034	NS	L**Q*	<0.001	<0.001	0.779
Nrf2	1.04	1.32	1.26	1.23	1.41	1.16	1.14	1.29	1.23	1.31	1.18	0.026	NS	NS	0.014	0.046	0.391
Ileum																	
SOD1	1.04	1.38	1.38	1.11	1.26	1.13	1.25	1.27	1.28	1.46	1.26	0.029	NS	NS	0.036	0.010	0.390
GPX1	1.05	1.43	1.71	1.53	1.43	1.13	1.15	1.34	1.45	1.51	1.42	0.036	Q*	L*Q**	<0.001	0.004	0.256
CAT	1.00	1.15	1.06	1.28	1.34	1.22	0.98	1.18	1.36	1.18	1.52	0.032	NS	L*	0.053	0.024	0.559
HO-1	1.04	1.04	1.01	1.16	1.60	1.61	0.98	1.32	1.42	1.58	1.46	0.037	L*Q*	L* Q*	0.006	<0.001	0.192
Nrf2	1.01	1.32	1.68	1.47	1.57	1.62	1.15	1.37	1.52	1.35	1.23	0.036	NS	Q*	<0.001	0.004	<0.001
ZO-1	1.15	1.29	1.44	1.36	1.70	1.44	1.27	1.36	1.72	1.84	1.30	0.036	NS	NS	0.003	<0.001	0.328
Claudin	1.54	1.62	1.84	2.18	1.50	1.69	1.62	2.03	1.59	2.00	1.81	0.048	NS	NS	0.156	0.094	0.645
Occludin	1.06	1.36	1.47	1.14	1.53	1.22	1.18	1.38	1.42	1.32	1.28	0.032	NS	Q*	0.011	0.021	0.648

BD = basal diet; DL-Met = DL-methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (2 ducks per replicate) per treatment.
[2] *SOD1* = superoxide dismutase-1; *GPX1* = glutathione peroxidase-1; *CAT* = catalase; *HO*-1 = hemeoxygenase-1; *Nrf2* = nuclear factor (erythroid-derived 2)-like 2; *ZO-1* = tight junction protein.
[3] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$, **$P < 0.01$.

3.7. Intestinal morphology and hepatic steatosis

Ducks supplemented with either DL-Met or HMTBa had increased villus height and villus height to crypt depth ratio in the ileum than ducks fed BD. These indices were linearly increased with the supplemental levels of DL-Met ($P < 0.05$, Table 8). The average area proportion of lipid droplets was decreased with DL-Met and HMTBa supplementations compared with BD ($P < 0.05$), and the number of lipid droplets was lower in ducks fed with HMTBa compared with DL-Met ($P < 0.05$).

4. Discussion

The current study indicates that the dietary supplementation of DL-Met or HMTBa improves productive performance in laying ducks, including increasing egg weight and mass, and decreasing FCR. Past studies in laying hens (Castro et al., 2019) and laying ducks (He et al., 2003; Fouad et al., 2016) have also shown similar findings with Met supplementation. We also observed that supplementation of Met from 2 sources improved the immune and antioxidant status of ducks, as indicated by the decreased content of IL-1 and IL-8 in plasma and ileum, and the increased content of GSH, GSH-Px, and CAT. These results are supported by the previous studies in egg-laying ducks (Fouad et al., 2016) and duck breeders (Ruan et al., 2018) wherein dietary supplementation of Met supplied as DL-Met, improved the glutathione redox system and the productive performance. The current study also found that the Met metabolism and intestinal development were promoted by Met supplementation, which could be associated with better productive performance, as reported in broiler breeder hens that the hepatic sulfur amino acid metabolism was affected by dietary Met sources and levels (Wan et al., 2017). Similarly, Liu et al. (2022) showed that dietary supplemented with appropriate Met levels would improve the amino acid metabolism and intestinal development, thereby improving the laying performance of layer chicks.

The present study showed that egg and albumen weight were increased while yolk component was decreased with Met supplementation. These results are consistent with Ruan et al. (2018) who also elucidated that the albumen weight and proportion were improved with dietary Met levels (DL-Met) in duck breeders. The positive effect on egg and albumen weight was partly due to its improvement of ovalbumin expression in the oviduct magnum (Ruan et al., 2018). Met is used to synthesize proteins and other amino acids, and altering the Met intake will affect egg weight, albumen, and yolk component of eggs in laying hens (Liu et al., 2017). In this case, the changes of albumen weight with DL-Met

Table 8
Intestinal morphology and hepatic steatosis of either DL-Met or HMTBa supplementation in laying ducks.[1]

Item[2]	BD	DL-Met, %					HMTBa, %					SEM	Regression analysis[3]		Contrast		
		0.05	0.12	0.19	0.26	0.33	0.05	0.12	0.19	0.26	0.33		DL-Met	HMTBa	BD vs. DL -Met	BD vs. HMTBa	DL-Met vs. HMTBa
Ileum																	
Villus height, μm	643	757	815	862	806	868	843	824	815	826	883	12.1	L*	NS	<0.001	<0.001	0.445
Crypt depth, μm	142	133	120	139	123	133	132	139	123	129	143	2.5	NS	NS	0.187	0.342	0.517
Villus height to crypt depth ratio	4.55	5.77	6.80	6.27	6.81	6.98	6.41	5.96	6.69	6.48	6.19	0.137	L*	NS	<0.001	0.001	0.471
Liver																	
Area proportion of lipid droplet, %	3.79	2.80	2.63	1.67	5.83	0.37	0.22	3.11	1.85	0.28	0.28	0.428	NS	NS	0.447	0.078	0.080
Number of lipid droplet	380	1656	1451	287	2169	238	166	1064	459	176	180	138.6	NS	NS	0.077	0.947	0.004
Average area proportion of lipid droplet, $\times 10^3$	7.85	1.26	1.77	3.25	2.02	0.51	0.22	1.09	2.98	0.84	0.50	0.438	NS	NS	<0.001	<0.001	0.434

BD = basal diet; DL-Met = DL-methionine; HMTBa = 2-hydroxy-4 methyl(thio) butanoic acid.
[1] Mean of 6 replicates (2 ducks per replicate) per treatment.
[2] NS = not significant, L = linear effect, Q = quadratic effect, *$P < 0.05$.

and HMTBa supplementations are possibly due to the change of its protein, such as ovalbumin. In addition, the Haugh unit and albumen height decreased to some extent with dietary supplementations of DL-Met and HMTBa. It was reported that ovomucin (approximately 3.5%) is responsible for the thick gel characteristics of liquid egg whites, and its content of the egg affected the Haugh unit and albumen height (Omana et al., 2010). This probably indicated that the ovomucin content of the egg probably changed with DL-Met and HMTBa addition and led to the changes in Haugh unit and albumen height. Besides, the increase in egg weight with DL-Met or HMTBa supplementation could most likely be due to the increased egg albumen weight in the present study. Moreover, supplementation of DL-Met and HMTBa in diets of laying ducks increased the shell weight of eggs, but decreased egg albumen height and Haugh unit, shell ratio, and breaking strength. Haugh unit is negatively related to the egg weight, thus increased egg weight and decreased albumen height with DL-Met and HMTBa supplementations led to a decreased Haugh unit. In addition, the egg size including length and width was increased but the shape index was decreased which implied that the eggs were big and round with the supplementation of Met from 2 sources. This change of egg shape is possibly one of the important factors that result in the change of eggshell breaking strength. In addition, Bunchasak et al. (2012) reported that the amount of calcium deposited in the eggshell did not change in laying hens. This could be a probable reason for the decrease in shell breaking strength. The decrease in shell ratio with Met supplementation was mainly due to the increased egg weight regardless of an increase in shell weight. Consistent with the present finding, Fouad et al. (2016) also reported that the supplementation of Met decreased shell thickness and breaking strength in egg-laying ducks.

D-Met and HMTBa in the diet need to be converted into L-Met before metabolism (Dibner, 2003). In the present study, the supplemental DL-Met or HMTBa increased Tau, Met, Ile, Trp, and Arg, but decreased the Ser and Lys in plasma. This indicates that the process of Met metabolism including transmethylation, remethylation, and transsulfuration was enhanced, thereby affecting the amino acids and protein synthesis in laying ducks. L-Met first enters the transmethylation step and then is hydrolyzed to homocysteine, which can either go through to the remethylation or the transsulfuration pathway. The remethylation process ultimately gives rise to the de novo synthesis of L-Met, through the supplementation of a methyl group to homocysteine (Martin-Venegas et al., 2006). In this respect, dietary DL-Met or HMTBa supplementation increased plasma Met content by activating the transmethylation and remethylation process. The transsulfuration pathway involves the conversion of homocysteine and Ser to Cys (Stipanuk, 2004). It is an irreversible pathway and leads to Met catabolism. The Cys can be further incorporated into protein or used in the synthesis of Tau or GSH (Grimble et al., 1992). This could explain the increased Tau and decreased Ser content in plasma with DL-Met or HMTBa supplementation. Moreover, increased GSH in the ileum and increased Tau level in plasma with increasing Met are in consistent with plasmatic Ser disappearance. Together, these changes suggest that the transsulfuration pathway and downstream sulphur compounds are facilitated by dietary Met. Furthermore, compared with DL-Met, lower Cys and higher Tau content in plasma were observed with HMTBa supplementation. This indicates that more Cys was oxidized to synthesize Tau with the supplementation of HMTBa compared with DL-Met. Besides, Leu and Trp contents in plasma were increased with dietary DL-Met and HMTBa supplementation, which probably results from the synergistic effect of neutral amino acids (Kanai et al., 2013). Moreover, higher Leu and Trp contents in plasma was observed with HMTBa in contrast to DL-Met supplementation. Furthermore, there was an increase in Arg in plasma, and the change of Arg was consistent with the increases of Glu and Pro content in the current study. It was reported that the Glu and Pro could be utilized as a precursor for citrulline synthesis, which could be converted into Arg in the kidney (Marini et al., 2017). However, Lys content in plasma was decreased with the supplementation of DL-Met and HMTBa, this effect might be related to absorption antagonism reported between Lys and Arg.

The antioxidant status in the plasma, liver, and ileum of laying duck improved with the supplementation of DL-Met and HMTBa, in line with previous studies in laying hens and broilers (Wang et al., 2019). Methionine can be catabolized to synthesize Tau and GSH (Martín-Venegas et al., 2006)(), which play vital antioxidant functions in the liver and intestine (Surai et al., 2020). Moreover, the antioxidant capacity was affected by the glutathione redox cycle. The GSH is oxidized by ROS to glutathione disulfide (GSSG), and GSSG can, in turn, be reduced back to GSH by glutathione reductase, or be eliminated from the cell (Wu et al., 2004). This study found the Tau content in plasma increased, and GSSG content decreased in the ileum, and GSH content and the ratio of GSH to GSSG increased in the liver and ileum with the supplemental DL-Met or HMTBa in laying ducks. This suggested that the liver and ileum GSH system was affected by the addition of DL-Met and HMTBa in the diet, which was consistent with the results of Wang et al. (2019). In addition, dietary Met supplementation affected the activity and expression of the crucial molecules in the antioxidant system in laying ducks: increased GSH-Px and CAT capacities in liver and

ileum, decreased MDA and protein carbonyl content in plasma and ileum, and increased mRNA values of *GPX1* and *Nrf2* in the ileum. Moreover, the Tau content in plasma was much higher with the supplementation of HMTBa than DL-Met, and the ileal *CAT* and hemeoxygenase-1 (*HO-1*) expression levels were increased in ducks fed with HMTBa rather than DL-Met. In this case, HMTBa had a more effective ability in improving the antioxidant status than DL-Met in laying ducks. The underlying reason might be that the HMTBa was more effective in generating S-adenosylmethionine and participating in oxidation resistance, as reported in broilers (Wang et al., 2019). Consistent with the study in meat duck by Zhao et al. (2018), who also reported that compared with DL-Met, supplementation of HMTBa dramatically improved the total antioxidant capacity, the activities of GSH-Px, and the concentration of GSH in the pectoralis major muscle. HMTBa has been reported to be more efficient in alleviating oxidative damage induced by heat stress in broiler chickens compared with DL-Met (Willemsen et al., 2011).

The lipid droplet, as the main energy store of adipose tissue, plays an important role in lipid homeostasis. The average area proportion of lipid droplets in the liver of laying ducks was decreased by adding DL-Met or HMTBa to the diet. This result indicated that lipid peroxidation in the liver was alleviated with dietary DL-Met or HMTBa supplementation. Similarly, Peng et al. (2018) have reported that the lipid homeostasis of broilers was modulated by dietary Met levels. Dietary Met deficiency decreased the hepatic lipid export, whereas, high dietary Met addition activated the hepatic lipid catabolism, and then increased or decreased the hepatic lipid accumulation, respectively (Peng et al., 2018). In addition, an enhancement of lipid metabolism in adipose tissue in young growing pigs fed a Met-deficient diet was reported (Castellano et al., 2015). This effect of Met deficiency was explained by the increase in the expression levels of genes participating in glucose uptake, lipogenesis but also lipolysis, and activities of NADPH enzyme suppliers in subcutaneous and perirenal adipose tissues of Met-deficient pigs (Castellano et al., 2015). Importantly, the function of the liver was improved in laying ducks with the supplementation of DL -Met and HMTBa in the diet.

Dietary supplementation with DL-Met or HMTBa increased the immune status of laying ducks in the present study as shown by the decreasing IL-1, IL-8 and IL-10 content in plasma and decreasing IL-1 and IL-8 content in the ileum. Furthermore, higher villus height and greater villus height to crypt depth ratio were observed in the ileum, together with higher mRNA values of ileal *ZO-1* and occludin in laying ducks with supplementation of DL-Met and HMTBa. The results mentioned above suggest that dietary Met supply modulated the intestinal mucosal barrier and improved the development of the ileum. This positive effect on the development of the gastrointestinal tract might be attributed to its broad antioxidative function, as it was observed that the GSH content and its ratio to GSSG increased, GSH-Px and CAT capacities increased, and MDA, GSSG, and protein carbonyl content decreased in the ileum with DL-Met and HMTBa supplementations in the current study. Similarly, Shen et al. (2015) reported that supplementation of either L-Met or DL-Met had beneficial impacts on villus development in association with increased GSH production and levels of total antioxidant capacity, and decreased protein oxidation in the duodenum in young chickens. The improvement of intestinal morphology with the DL-and L-Met was also observed in meat-type ducks (Zhang et al., 2019).

Though different mechanisms for cellular absorption, transport, and metabolism exist for these 2 Met sources (Fang et al., 2010; Martin-Venegas et al., 2006; Wang et al., 2019), no significant difference was observed in bio-efficiency for productive performance and egg component traits (ranged from 98.1% to 100.2%) in laying ducks in the current study. This concurs with Kluge et al. (2016), who also confirmed that HMTBa and DL-Met had similar efficacy in growing ducks. Similarly, there was no difference in body weight gain of broiler chickens between the 2 sources when fed at or below Met requirements for the growth phases (Uddin et al., 2022). In addition, HMTBa improved antioxidant and immune status, Met metabolism, and ileum morphology of laying ducks, the same as the effects of DL-Met supplementation.

5. Conclusions

In summary, dietary supplementation with DL-Met or HMTBa improved the productive performance, egg albumen component, antioxidant, and immune status, Met metabolism, and ileum morphology of laying ducks. In our study, the relative efficacy of supplemental HMTBa and DL-Met in diets was determined as non-statistically different for productive performance and egg albumen ratio of laying ducks (25 to 41 wk).

Author contributions

Yanan Zhang and **Zhiwei Zhuang:** performed experiments and analyzed data. **Yanan Zhang**, **Tahir Mahmood**, and **Yves Mercier:** designed research. **Yongyan Jin**, **Xuebing Huang**, and **Kaichao Li** analyzed parameters. **Shuang Wang**, **Weiguang Xia**, **Shenglin Wang**, and **Miao Yu:** performed data analysis. **Yanan Zhang:** wrote the manuscript. **Tahir Mahmood** and **Yves Mercier:** edited the manuscript. **Wei Chen** and **Chuntian Zheng:** read, edited and accepted the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.04.006.

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