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Non Ruminant Nutrition

NON RUMINANT NUTRITION

Supplemental methionine exerted chemical form-dependent effects on antioxidant status, inflammation-related gene expression, and fatty acid profiles of broiler chicks raised at high ambient temperature[1]

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Abstract

This study was to explore metabolic effects of two forms and concentrations of supplemental methionine in grower and finisher diets for broiler chickens raised at high temperature. Male Cornish cockerel chicks (total = 360, day-old) were divided into four groups (10 pens/treatment, 9 chicks/pen) and fed with 100% or 130% required methionine in the diets as DL-methionine (**DL-MET**) or 2-hydroxy-4-(methylthio)butanoate (**HMTBA**). The room was maintained at 4 to 13 °C above the suggested thermoneutral temperature. The higher concentration of both DL-MET and HMTBA enhanced ($P < 0.05$) hepatic GSH concentrations of the growers and plasma ferric reducing ability of the finishers. The DL-MET-fed growers had greater ($P < 0.05\%$) muscle GSH and hepatic unsaturated fatty acid concentrations than those fed HMTBA. Expression of inflammation-related genes in the liver of finishers was affected ($P < 0.05$) by interaction effects of the methionine form and concentration. In conclusion, effects of the extra methionine supplementation on the high ambient temperature-related metabolic responses of broilers varied with their age and(or) tissue and the methionine form.

Key words: antioxidant, high temperature, methionine, oxidative stress, poultry

Introduction

Approximately 95.5 million tons of chicken was produced worldwide in 2018, where United States, China, and Brazil were the top three producers (USDA, 2019a). In the United States, the major broiler production is in the southern states with high ambient temperature during the summer (USDA, 2019b). Thus, fast-growing broilers raised in those states are likely to experience heat stress and suffer from impaired growth performance, compromised immune responses, and poor health status (Quinteiro-Filho et al., 2010; Lara and Rostagno, 2013). All these consequences may be associated with the induced oxidative stress (Altan et al., 2003; Lin et al., 2006a). Antioxidants, such as vitamin E, carotenoids, and sulfur-containing amino acids (methionine and cysteine) are known for scavenging reactive oxygen species (**ROS**) generated from oxidative stress (Holst and Williamson, 2008; Oroian and Escriche, 2015).

Methionine is an essential sulfur-containing amino acid required for tissue growth and protein synthesis, and is the first-limiting amino acid to broilers fed commercial corn and soybean meal diets. Synthetic DL-methionine (**DL-MET**) and 2-hydroxy-4-(methylthio)butanoate (**HMTBA**) are two commonly used supplements in animal diets. Chemically, HMTBA is the hydroxyl analog of methionine (the amino group in methionine is replaced with a hydroxyl group). To be utilized in the body, HMTBA needs to be converted into L-methionine in a two-step process that takes place mainly in the liver and to some extent in other tissues including small intestine and kidney (Gordon and Sizer, 1965; Martín-Venegas et al., 2006). It has been a long-time debate if MTBA is less bioavailable than DL-MET due to differences in absorption, conversion, and utilization (van Weerden et al., 1982; Elwert et al., 2008; EFSA, 2018). Notably, methionine has been shown to protect the brain (Butterfield and Lauderback, 2002; Butterfield et al., 2010), liver (Singal et al., 2011; Zhu et al., 2012), and muscle (Wang et al., 2009; Willemsen et al., 2011) from oxidative damages. This is because methionine exposed on the surface of proteins is readily oxidized into methionine sulfoxide, which protects the integrity and function of other critical residues in the proteins from oxidation (Levine et al., 1996, 2000; Atmaca, 2004). Moreover, methionine can be converted into homocysteine in the one-carbon cycle in the liver (Miller, 2003). Subsequently, homocysteine is converted into cysteine through transsulfuration (Miller, 2003). Cysteine is not only a potent antioxidant itself, but also a precursor for the synthesis of glutathione (**GSH**) (Elias et al., 2005; Stipanuk et al., 2006; Uthus and Brown-Borg, 2006), a potent scavenger of ROS (Ross, 1988; Valko et al., 2007). Dietary supplemental methionine has been shown to improve antioxidant status, anti-inflammatory response, growth performance, and wellbeing of broilers (Swain and Johri, 2000; Rama Rao et al., 2003; Elwert et al., 2008; Chen et al., 2013). However, few studies have determined if those benefits could be enhanced by elevating the methionine supplementation and(or) vary with its chemical form in broilers exposed to a high ambient temperature.

Therefore, this study was conducted to test a working hypothesis that elevating supplemental DL-MET and HMTBA from the 100% to the 130% of the required digestible methionine concentrations into corn-soybean meal-based grower and finisher diets for broilers would help the animals cope with the high ambient temperature-induced metabolic stress. Our objectives were to compare if the two forms and concentrations of supplemental methionine exerted similar or different effects on: 1) growth performance, meat quality, feather coverage, and bone strength of broilers; and 2) antioxidant status, health indicators, inflammation-related gene expression, and lipid and fatty acid profiles in several tissues of broilers.

Materials and Methods

The experiment was conducted at the Large Animal Research and Teaching Unit, Cornell University, Ithaca, NY. The protocol was approved by the Cornell University Institutional Animal Care and Use Committee (Protocol number: 2010-0106).

Animals, Diets, and Management

A total of 360 (day-old) Cornish Cross cockerels were purchased from Moyer's Chicks (Quakertown, PA). The birds were reared in 1 m^2 floor-pens in environmentally controlled rooms with 2:22 h dark-light cycles during the entire experiment. The temperature in the room for the starter period was set at optimal according to the industrial guide (Cobb-Vantress, 2018). Thereafter, the room temperature was kept at 31 °C (in comparison with the suggested steady decreases from 27 to 18 °C over the age of days 14–42) to impose heat stress on the birds. The birds were randomly allotted into four groups (10 pens/treatment, 9 birds/pen) based on the initial body weights. All birds were fed the same corn and soybean meal based starter diet (days 0–10). During the grower (days 11–22) and finisher (days 23–42) periods, different experimental diets were fed to the birds. Supplemental DL-MET was >99% pure (MetAMINO, Evonik Industries, Essen, Germany). A liquid methionine hydroxyl analogue product containing 88% DL-HMTBA was diluted at 2:1 with Sipernat silica (a food grade ingredient consists 99% of silicon dioxide provided by Evonik SIPERNAT) and created a dry product for feed mixing with a final product concentration of 58% HMTBA. To meet the recommended (100%) methionine + cysteine requirement by broilers based on AMINOChick 2.0 (Evonik Nutrition & Care GmbH, Germany), 3.09 and 2.77g of DL-MET/kg diet was added to the grower and finisher diets, respectively. For the HMTBA treatment groups, the concentration of HMTBA in the product (58%) and an equimolar bioefficacy of 75% for HMTBA compared with DL-MET (EFSA, 2018) were considered so that 7.19 and 6.44 g of HMTBA product/kg diet was added to the grower and finisher diets, respectively. To prepare diets with additional supplemental methionine (130% of the recommendation), 4.02 g MET/kg or 9.34 g HMTBA/kg was added into the grower diets and 3.60 g MET/kg or 8.37 g HMTBA/kg was added into the finisher diets. Actual concentrations of all nutrients except for Ca in all experimental diets were determined by analysis (Evonik Nutrition & Care GmbH) and the analyzed values are given in Table 1. Animals were given free access to water and feed throughout the experiment.

Sample Collection

Body weights and feed intakes of each pen were recorded weekly and at the end of each phase. At the end of grower and finisher periods, two chickens from each pen were euthanized by carbon dioxide followed by cervical dislocation. Blood was collected from the heart by heparinized needles. Plasma was then separated by centrifugation at 12,000 × *g* for 15 min at 4 °C (Beckman GS-6R centrifuge, Brea, CA). After the liver, breast muscle, adipose, and thigh muscle were collected, a portion of the tissues was frozen in liquid nitrogen and then stored at –80 °C for gene expression measurement. The remaining tissues were kept on dry ice and stored at –20 °C for meat quality test and biochemical analyses. After removing the muscle, tendon, and ligament, the left tibia from each bird was collected and stored at –20 °C for strength test.

Plasma Health Indicators

Plasma activities of alanine aminotransferase (**ALT**), alkaline phosphatase (**AKP**), and tartrate-resistant acid phosphatase (**TRAP**) and concentrations of plasma inorganic phosphorus (**PIP**), glucose, total cholesterol (**TC**), triglyceride (**TG**), nonesterified fatty acid (NEFA), and uric acid were analyzed following methods described in previous studies (Magnuson et al., 2018; Sun et al., 2018).

Tissue Lipid and Fatty Acid Profiles

The lipid profiles (TC, TG, and NEFA) and fatty acid profiles were measured in the liver, adipose tissue, breast, and thigh following methods in previous studies (Magnuson et al., 2018; Sun et al., 2018). The gas chromatography-mass spectrometry (model HP

Table 1. Composition (g/kg) of experimental diets for broiler chicks

	Starter	Grower				Finisher			
	DL-MET	DL-MET		HMTBA		DL-MET		HMTBA	
Ingredients, g/kg	100%	100%	130%	100%	130%	100%	130%	100%	130%
Corn[1]	557.6	627	626	623	621	665	664	661	659
Soybean meal	357	297	297	297	297	261	261	261	261
Soybean oil	30.6	30.0	30.0	30.0	30.0	35.5	35.5	35.5	35.5
Dicalcium phosphate	20.7	18.5	18.5	18.5	18.5	14.9	14.9	14.9	14.9
Limestone	15.4	10.5	10.5	10.5	10.5	9.0	9.0	9.0	9.0
Vit./min. premix[2]	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90
L-Lysine	4.25	3.42	3.42	3.42	3.42	3.00	3.00	3.00	3.00
DL-Met, >99%[3]	3.84	3.09	4.02	0.00	0.00	2.77	3.60	0.00	0.00
DL-HMTBA, 58%	0.00	0.00	0.00	7.19	9.34	0.00	0.00	6.44	8.37
Salt	3.11	3.10	3.10	3.10	3.10	3.48	3.48	3.48	3.48
L-Valine	1.07	0.66	0.66	0.66	0.66	0.35	0.35	0.35	0.35
L-Threonine	1.26	0.95	0.95	0.95	0.95	0.84	0.84	0.84	0.84
Choline chloride 60%	1.07	1.11	1.11	1.11	1.11	1.27	1.27	1.27	1.27
Sodium bicarbonate	0.82	0.89	0.89	0.89	0.89	0.00	0.00	0.00	0.00
L-Isoleucine	0.46	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.26
Analytical values									
ME, kcal/kg	2,947	2,943	2,950	2,950	2,935	3,047	3,024	3,011	3,005
Crude protein %	21.0	20.0	20.0	20.0	20.0	19.0	18.3	18.2	18.8
Methionine %	0.66	0.57	0.64	0.36	0.32	0.53	0.60	0.31	0.30
HMTBA %	0.00	0.00	0.00	0.41	0.58	0.00	0.00	0.39	0.54
Cysteine %	0.32	0.31	0.31	0.31	0.31	0.30	0.29	0.29	0.29
Methionine + cysteine %	0.98	0.88	0.95	0.67	0.63	0.83	0.89	0.60	0.59
Lysine %	1.35	1.27	1.22	1.22	1.26	1.20	1.10	1.13	1.15
Phosphorus %	0.62	0.61	0.62	0.61	0.57	0.57	0.56	0.55	0.55
Calcium[4] %	1.05	0.90	0.90	0.90	0.90	0.76	0.76	0.76	0.76

[1]Analytical nutrient values of corn: ME, 3,320 kcal/kg; crude protein, 77.5 g/kg; lysine, 2.51 g/kg; methionine, 1.64 g/kg. Analytical nutrient values of soybean mean: ME, 2,370 kcal/kg; crude protein, 474 g/kg; lysine, 29.3 g/kg; methionine, 66.3 g/kg.
[2]Vitamin and mineral mixture provided the following nutrients per kilogram of diets: vitamin A, 4,550 IU; vitamin E, 7.5 IU; vitamin D 3, 450 IU; vitamin K, 0.752 mg; riboflavin, 3.75 mg; pantothenic acid, 3 mg; niacin, 15.2 mg; vitamin B_{12}, 0.006 mg; biotin, 0.152 mg; folic acid, 0.376 mg; thiamine, 1.07 mg; pyridoxine, 3.78 mg; choline, 1,575 mg; Cu, 12 mg; I, 0.053 mg; Mn, 30.2 mg; Se, 0.09 mg; Zn, 53.0 mg; Fe, 67.8 mg.
[3]DL-methionine (MetAMINO Evonik Industries, Essen, Germany) with >99% purity and a liquid methionine hydroxy analogue product containing 88% DL-HMTBA, diluted at 2:1 with sipernat silica to create a dry product for feed mixing and resulting in a final product concentration of 58% HMTBA, were used as the sources of supplemental methionine in the diets.
[4]Calcium levels were calculated.

5890 A with an HP 5970 series mass-selective ion-monitoring detector, Hewlett-Packard, Palo Alto, CA) with the internal standard of tritridecanoin was used to analyze the fatty acid profiles.

Tissue and Plasma Antioxidant Status

Concentrations of total GSH, glutathione disulfide (**GSSG**) in the plasma, liver, breast, and thigh and concentrations of malondialdehyde (**MDA**) in the livers, adipose, breast, and thigh were assayed using methods of previous studies (Magnuson et al., 2018; Sun et al., 2018). The ferric reducing ability of plasma (FRAP) was determined using Benzie and Strain's method (Benzie and Strain, 1996). Concentrations of protein carbonyl in the liver were determined using a method developed by Levine et al. (1990). An ELISA kit (Cayman Chemical, Ann Arbor, MI) was used to determine concentrations of corticosterone in plasma. Activities of glutathione peroxidase (**GPx**), glutathione transferase (**GST**), glutathione reductase (**GR**), and superoxide dismutase (**SOD**) were measured in the breast, thigh, liver, and adipose tissue using methods adapted from previous studies (Massey and Williams, 1965; McCord and Fridovich, 1969; Mannervik and Guthenberg, 1981; Flohe and Gunzler, 1984).

Quantitative Real-Time PCR

Abundances of interlukin-6 (IL-6), interlukin-10 (IL-10), tumor necrosis factor alpha (**TNFα**), heat shock protein 70 (**HSP70**), heat shock protein 90 (**HSP90**), protein kinase B (**AKT**), P38 mitogen-activated protein kinases (**P38MAPK**), and c-Jun N-terminal kinase (**JNK**) mRNA in the liver were determined. Primers used for these tested genes are listed in Supplementary Table 1. Total mRNA was isolated and purified from the liver using TRIzol Reagent (Life Technologies, Carlsbad, CA) following the established method (Chomczynski and Sacchi, 1987). The mRNA reverse transcription was done by the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Grand Island, NY). The Real-time qPCR (7900 HT; Applied Biosystems) and the $2^{-\text{delta delta Ct }(\Delta\Delta Ct)}$ equation (Livak and Schmittgen, 2001) were then used to quantify the mRNA expression levels.

Meat Quality, Bone Strength, Breast Muscle Myopathy, and Feather Coverage

The meat quality of breast and thigh muscle including pH, water holding capacity, and texture profile was assessed following previously established methods (Sun et al., 2018). The bone strength was measured by testing the energy at maximum load, extension at maximum load, maximum slope, and maximum

load in a 3-point test using the method described in previous studies (Turner and Burr, 1993; Gatrell et al., 2017). Before collecting the breast tissues, severities of woody breast and while stripling of the breast were scored by five individuals independently on a scale of 1–5 with 1 being a normal breast and 5 being a severely diseased breast. Photos of chicks were taken at weeks 5 and 6. Feather coverage was scored based on the photos on a scale of 1–5 with 1 being almost no feathering or less than 25% of the body covered, 2 being 25%–50% feather coverage, 3 being 50%–75% feather coverage, 4 being 75%–90% feather coverage, and 5 being 100% feather coverage.

Statistical Analysis

Software R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) was used for the data analysis. Pen was considered as the experimental unit. Two-way ANOVA was used to evaluate the main effects (the chemical form and concentration of supplemental methionine), and Duncan's multiple range test was used to compare the treatment means. Data were presented as means ± SEM, and the significance level for differences was $P < 0.05$.

RESULTS

Growth Performance and Plasma Health Indicators

Neither the form nor the concentration of supplemental methionine affected the body weight, average daily gain, or feed conversion ratio of birds throughout the study (Table 2). The feed intake of the birds fed DL-MET was 7% higher ($P < 0.05$) than that of the birds fed HMTBA during the finisher phase. The 130% methionine supplementation decreased ($P < 0.05$) plasma uric acid concentration of the finishers and PIP concentrations of both growers and finishers, but elevated ($P < 0.01$) plasma AKP activity of the finishers compared with the 100% methionine supplementation (Table 3). In the finisher phase, the plasma AKP activity and uric acid concentration of the DL-MET-fed groups were 25% and 14% higher ($P < 0.05$), respectively, than those of HMTBA-fed groups. The plasma ALT activity of the finishers was 32% lower ($P < 0.05$) in the DL-MET-fed groups than that in HMTBA-fed groups. In the grower phase, an interaction effect ($P < 0.05$) of the methionine form by concentration was found on the plasma glucose concentration.

Lipid and Fatty Acid Profiles

Neither the form nor the concentration of methionine affected the tissue TC, TG, or NEFA concentrations (Table 4) except for that the TG concentration in the thigh was higher ($P < 0.05$) in the HMTBA-fed groups than that in the DL-MET-fed groups. Compared with the 100% methionine supplementation, the 130% methionine supplementation elevated ($P < 0.01$) concentrations of monounsaturated fatty acid (MUFA) in the liver of the growers, but decreased ($P < 0.05$) concentrations of MUFA, and polyunsaturated fatty acid (PUFA) in the adipose tissue of the finishers (Table 5). Concentrations of total fatty acid, SFA, MUFA, and PUFA in the liver of the DL-MET-fed birds were higher ($P < 0.05$) than those of the HMTBA-fed birds in the grower phase. In the finisher phase, concentrations of all measured fatty acids in the breast and the SFA concentration in the thigh of the DL-MET-fed birds were lower ($P < 0.05$) than those in the breast and thigh of the HMTBA-fed birds. Interaction effects ($P < 0.05$) of the methionine form by concentration were found on concentrations of MUFA and PUFA in the adipose tissue of the grower chicks.

Antioxidant Status

In the growers, the 130% methionine supplementations elevated ($P < 0.05$) GSH concentrations in the liver and GSSG concentrations in the liver and thigh compared with the 100% methionine supplementations (Table 6). In the finishers, the 130% methionine supplementations decreased ($P < 0.01$) concentrations of GSH in the plasma and GSSG in the liver, but elevated ($P < 0.05$) concentrations of GSSG in breast compared with the 100% methionine supplementations. The GSH

Table 2. Effect of different chemical forms and concentrations of methionine supplementations on growth performance of broiler chicks[1]

Form		DL-MET		HMTBA			P-value		
Concentration	Period	100%	130%	100%	130%	SEM	Form	Conc	Interaction
Body weight, g/chick	Starter	324	331	332	329	3.82	0.44	0.47	0.49
	Grower	1,378	1,410	1,421	1,407	21.3	0.35	0.68	0.29
	Finisher	2,612	2,631	2,593	2,559	53.1	0.40	0.89	0.63
Average daily gain, g/chick/day	Starter	26.0	26.7	26.7	26.5	0.35	0.43	0.47	0.19
	Grower	62.0	63.5	64.1	63.4	1.16	0.39	0.76	0.38
	Finisher	82.3	81.4	80.0	76.8	2.40	0.17	0.42	0.65
	Total	59.4	61.5	62.0	62.0	1.12	0.19	0.37	0.37
	Total (G-F)	73.9	74.2	73.0	71.9	1.70	0.37	0.85	0.70
Feed intake, g/chick/day	Starter	39.3	39.9	39.7	40.1	0.49	0.62	0.34	0.86
	Grower	107	107	107	106	1.36	0.90	0.80	0.49
	Finisher	142^{ab}	144^{a}	134^{b}	134^{ab}	2.82	<0.01	0.43	0.97
	Total	102	103	99.1	99.6	1.13	0.02	0.51	0.79
	Total (G-F)	123	124	120	120	1.53	0.02	0.59	0.76
Feed/gain	Starter	1.52	1.48	1.49	1.53	0.02	0.70	0.88	0.15
	Grower	1.72	1.69	1.68	1.67	0.02	0.15	0.49	0.57
	Finisher	1.73	1.78	1.72	1.78	0.05	0.93	0.34	0.98
	Total	1.73	1.74	1.71	1.74	0.03	0.60	0.55	0.69
	Total (G-F)	1.73	1.78	1.72	1.78	0.05	0.62	0.53	0.85

[1]Data are expressed as means ($n = 10$). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

Table 3. Effect of different chemical forms and concentrations of methionine supplementations on plasma indicators of broiler chicks[1]

Form	DL-MET		HMTBA			P-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
ALT, U/L								
Grower	0.97	0.86	1.08	0.97	0.07	0.16	0.14	0.99
Finisher	1.38	1.27	1.74	1.76	0.16	0.03	0.79	0.73
AKP, U/mL								
Grower	726	769	720	775	49.1	0.99	0.34	0.90
Finisher	390^{bc}	654^{a}	343^{c}	493^{b}	59.2	<0.01	<0.01	0.15
PIP, mg/dL								
Grower	65.1	62.9	66.0	61.2	2.11	0.77	0.02	0.38
Finisher	59.9^{a}	54.6^{b}	56.4^{ab}	55.1^{ab}	1.55	0.39	0.05	0.22
Glucose, g/L								
Grower	4.23	4.68	4.72	4.40	0.12	0.43	0.51	0.03
Finisher	3.42	3.30	3.38	3.61	0.13	0.51	0.31	0.05
Uric acid, mmol/L								
Grower	342	339	329	346	13.1	0.88	0.47	0.84
Finisher	565	449	450	435	27.0	0.04	0.02	0.08
TC, mg/dL								
Grower	76.7	72.8	73.3	73.6	2.12	0.55	0.41	0.33
Finisher	96.7	103	103	107	4.56	0.35	0.42	0.96
TG, mg/dL								
Grower	40.8	46.7	43.6	51.8	3.97	0.35	0.08	0.75
Finisher	37.7^{a}	29.0^{b}	33.7^{ab}	33.6^{ab}	2.01	0.65	0.06	0.05
NEFA, μmol/L								
Grower	0.10	0.10	0.11	0.09	<0.01	0.10	0.19	0.59
Finisher	0.11	0.11	0.11	0.12	<0.01	0.32	0.91	0.78

[1]ALT, alanine amino transferase; AKP, alkaline phosphatase; PIP, inorganic phosphorus; TC, total cholesterol; TG, triglycerides; NEFA, nonesterified fatty acids. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly (P < 0.05).

concentration in the breast and GSH and GSSG concentrations in the thigh of the DL-MET-fed growers were higher (P < 0.05) than those of the HMTBA-fed birds. The 130% methionine supplementations enhanced (P < 0.05) FRAP compared with the 100% methionine supplementations in the finishers. Interaction effects (P < 0.05) of the methionine form and concentration were found on the hepatic GSSG concentration of the growers and GSSG concentrations in the breast and thigh of the finishers.

The 130% methionine supplementation decreased (P < 0.05) activities of GPx in the thigh and GST and SOD in the adipose tissue of the growers compared with the 100% methionine supplementation (Table 7). Similarly, the 130% methionine supplementations decreased (P < 0.05) activities of GPx in the liver and GPx and GST in the adipose tissue of the finisher, compared with the 100% methionine supplementations. Activities of GPx in the breast, GR in the thigh, GR and GST in the adipose tissue, and all assayed antioxidant enzymes in the liver of growers were lower (P < 0.05) in the DL-MET-fed groups than those in the HMTBA-fed groups. But the GPx activity in the thigh of growers fed DL-MET was higher (P < 0.05) than that of the HMTBA-fed birds. Interaction effects (P < 0.05) of the methionine form by concentration were found on activities of SOD in the breast, GST in the liver, and GR in the thigh and adipose tissue of the growers and GPx in the breast of the finishers.

Inflammation-Related Gene Expressions

The concentration by form of methionine supplementations exerted an interaction effect (P < 0.05) on the mRNA levels of all tested genes in the liver except for TNFα and JNK (Table 8). The 130% DL-MET supplementation increased mRNA levels of IL-6, AKT and P38MAPK compared with the 100% DL-MET supplementation, but the concentrations of HMTBA supplementation did not affect their mRNA levels. Conversely, mRNA levels of IL-10 and HSP70 were lowered in the 130% HMTBA supplemented birds than those of the 100% HMTBA supplemented birds, but their levels were unaffected by the concentrations of DL-MET supplementation. The mRNA level of HSP90 was higher in the DL-MET-fed birds than that of the HMTBA-fed ones at the 130% supplementation, while no difference on its mRNA level between the chemical forms was observed at the 100% supplementation. The mRNA levels of JNK and HSP90 were lower in DL-MET-fed birds than those of the HMTBA-fed birds.

Meat Quality, Breast Muscle Myopathy, Bone Strength, and Feather Coverage

The dressing percentage, meat to bone ratio, pH, and WHC of both breast and thigh muscles were unaffected by either the form or concentration of supplemental methionine (Supplementary Table 2). The 130% methionine supplementations enhanced (P < 0.05) the chewiness of the breast compared with the 100% methionine supplementations. Neither the form nor the concentration of supplemental methionine affected the breast muscle myopathy scores (Supplementary Table 3). The 130% methionine supplementations decreased (P < 0.05) the energy at maximum load and the extension at maximum load of the tibia of the finishers compared with the 100% methionine supplementations (Supplementary Table 4). The energy at maximum load of tibia of the grower chicks was higher (P < 0.01) in the DL-MET-fed groups than that in HMTBA-fed groups. The

Table 4. Effect of different chemical forms and concentrations of methionine supplementations on tissue lipid profiles of finisher broiler chicks[1]

Form	DL-MET		HMTBA			P-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
Breast								
TC, mg/g protein	4.13	3.79	3.70	4.20	0.26	0.95	0.73	0.13
TG, mg/g protein	19.8	22.0	24.5	23.4	2.54	0.24	0.83	0.51
NEFA, μmol/g protein	7.83	7.24	7.02	7.19	0.61	0.54	0.76	0.58
Thigh								
TC, mg/g protein	2.28	2.06	2.43	2.21	0.15	0.31	0.15	0.99
TG, mg/g protein	17.1[b]	23.0[ab]	28.4[ab]	31.1[a]	4.16	0.02	0.31	0.70
NEFA, μmol/g protein	3.15	4.15	4.85	3.78	0.55	0.28	0.90	0.08
Liver								
TC, mg/g protein	9.92	9.32	9.15	8.33	0.57	0.13	0.22	0.85
TG, mg/g protein	21.5	19.2	20.0	17.6	1.74	0.40	0.21	0.95
NEFA, μmol/g protein	48.0	46.9	44.8	47.4	2.18	0.54	0.73	0.39
Adipose tissue								
TC, mg/g protein	55.1	62.9	69.5	65.8	9.42	0.39	0.86	0.57
TG, mg/g protein	1,125	1,284	1,078	1,175	164	0.64	0.44	0.85
NEFA, μmol/g protein	159	197	209	188	30.2	0.49	0.80	0.34

[1]TC, total cholesterol; TG, triglycerides; NEFA, nonesterified fatty acids. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

feather coverage scores of the broilers at weeks 5 and 6 were not affected by either the form or the concentration of supplemental methionine (Supplementary Table 5).

Discussion

The present study was performed to address the missing information in the literature on the comparative effects of two major supplemental methionine forms: DL-MET and HMTBA, at two concentrations in the grower and finisher diets of broilers on their metabolic responses to high ambient temperatures. To our best knowledge, this was the first of such attempts despite many past studies on the two forms of methionine supplements. One of the most interesting findings from our study was that supplementing 30% extra methionine in either form improved antioxidant status of broilers under such environmental condition. The improvements were manifested with enhanced GSH concentrations in the liver of the growers and elevated FRAP of the finishers. Although similar effects of supplemental methionine on tissue GSH concentrations were found by other groups in broilers (Zeitz et al., 2018; Zhang et al., 2018) and quails (Del Vesco et al., 2014), those animals were raised under normal temperatures. The observed benefits may be explained by the potential that methionine could be converted into cysteine for the synthesis of GSH (Stipanuk et al., 2006; Uthus and Brown-Borg, 2006). Likewise, FRAP was improved by supplementing methionine and other antioxidant nutrients such as vitamins C and E to broilers (Dušinská et al., 2003; Jena et al., 2013). Notably, the DL-MET supplementation produced higher GSH concentrations in the breast and thigh than the HMTBA supplementation in several instances.

In contrast, the extra methionine supplementation decreased SOD activity in the adipose tissue and GPx activity in the thigh of the growers, and also decreased the GPx activity in the liver and GPx and GST activities in the adipose tissue in the finishers. Comparatively, the DL-MET-fed growers showed lower activities of various antioxidant enzymes in the assayed tissues than the HMTBA-fed birds. Seemingly, there was a methionine form-dependent effect on the tissue antioxidant defense responses to the high ambient temperature. Our previous study also indicated that supplementing diets with potent antioxidant astaxanthin for both broilers and laying hens under high ambient temperatures decreased tissue activities of GPx, GR, and GST (Magnuson et al., 2018; Sun et al., 2018). Supplementing broiler diets with methionine also decreased GPx activity in the liver and intestines (Zeitz et al., 2018). Thus, there seemed to be a coordinated function or adaptive response between the intrinsic antioxidant enzyme production and the extrinsic antioxidant nutrient enrichment. Future research should be performed to assess this type of coordination in comparing the relative efficacy of different forms of methionine supplements in improving tissue antioxidant status of broilers. Furthermore, it is intriguing that the effects of supplemental methionine on the responses of GSH, FRAP, and antioxidant enzyme activities were not consistent across the assayed tissues, the two ages of birds, or the two forms and concentrations of methionine. While revealing underlying mechanisms for these variations or discrepancies may take a long time, we should currently develop a practical method to assess if those sporadic enhancements of tissue GSH or antioxidant enzyme activities indeed contribute to the resistance of broilers to the high ambient temperature or other stressors.

Another interesting finding from our study was that the extra DL-MET supplementation elevated the hepatic gene expression of IL-6, AKT, and P38MAPK, while the extra HMTBA supplementation decreased the gene expression of IL-10 and HSP 70. Meanwhile, the hepatic mRNA levels of HSP 90 and JNK were higher in the DL-MET-fed groups than those in the HMTBA-fed groups. Proinflammatory cytokine IL-6 was previously found to be elevated under inflammatory status (Scheller et al., 2011; Hunter and Jones, 2015). The elevated IL-6, along with ROS generated from oxidative stress, might up-regulate mitogen-activated protein kinases (MAPK) such as P38MAPK and JNK and subsequently induces AKT to protect cells from oxidative injury or death (Wang et al., 2000; Cantley, 2002; Zhang et al., 2016). On the other hand, IL-10 and heat shock proteins (HSP70, HSP90) were reported to be up-regulated under oxidative stress to exert their anti-inflammatory effects (Parsell and Lindquist, 1993;

Table 5. Effect of different chemical forms and concentrations of methionine supplementations on fatty acid concentrations of broiler chicks[1]

Form	DL-MET		HMTBA			P-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
Fatty acids, mg/g tissue								
Liver								
Total								
Grower	16.6^{ab}	17.7^{a}	13.1^{b}	14.3^{ab}	1.56	0.05	0.49	0.97
Finisher	14.9	15.8	15.1	15.1	1.57	0.92	0.80	0.80
SFA								
Grower	8.62	8.72	6.66	7.41	0.77	0.05	0.58	0.67
Finisher	7.26	8.06	7.70	7.76	0.61	0.90	0.55	0.58
MUFA								
Grower	3.86^{b}	6.48^{a}	3.78^{b}	4.52^{ab}	0.59	0.02	<0.01	0.02
Finisher	4.21	4.12	3.30	4.17	0.67	0.57	0.61	0.53
PUFA								
Grower	3.33^{a}	3.22^{a}	2.36^{b}	2.55^{ab}	0.18	<0.01	0.95	0.57
Finisher	3.04	3.13	3.15	3.18	0.17	0.69	0.77	0.91
Breast								
Total								
Grower	7.71	8.16	7.24	6.84	0.62	0.18	0.97	0.51
Finisher	5.72^{b}	5.71^{b}	7.93^{a}	8.01^{a}	0.40	<0.01	0.93	0.91
SFA								
Grower	2.30	2.66	2.35	2.42	0.16	0.55	0.23	0.39
Finisher	2.02^{b}	2.00^{b}	2.61^{a}	2.62^{a}	0.13	<0.01	0.94	0.90
MUFA								
Grower	2.62	3.03	3.09	2.63	0.23	0.95	0.78	0.08
Finisher	1.86^{b}	1.83^{b}	2.63^{a}	2.61^{a}	0.13	<0.01	0.81	0.71
PUFA								
Grower	2.32	2.56	2.29	2.05	0.19	0.17	0.98	0.23
Finisher	1.79^{c}	1.88^{bc}	2.37^{a}	2.27^{ab}	0.13	<0.01	0.97	0.49
Thigh								
Total								
Grower	7.80	7.79	7.98	8.23	0.42	0.47	0.76	0.75
Finisher	8.63	7.90	8.85	8.32	0.45	0.49	0.19	0.83
SFA								
Grower	2.32	2.50	2.56	2.56	0.14	0.35	0.55	0.57
Finisher	2.79^{ab}	2.46^{b}	3.08^{a}	2.85^{ab}	0.14	0.02	0.04	0.71
MUFA								
Grower	2.87	2.75	3.24	3.01	0.14	0.08	0.31	0.73
Finisher	3.06	2.71	3.38	2.96	0.15	0.10	0.06	0.83
PUFA								
Grower	2.45	2.37	2.55	2.70	0.13	0.15	0.77	0.42
Finisher	2.95	2.70	3.06	2.79	0.16	0.55	0.13	0.95
Adipose tissue								
Total								
Grower	64.9	78.6	73.1	66.3	7.31	0.57	0.99	0.39
Finisher	86.3	79.0	88.5	83.1	4.52	0.49	0.19	0.83
SFA								
Grower	16.5	21.9	18.7	17.2	2.00	0.35	0.80	0.28
Finisher	27.9	26.3	29.6	28.5	1.52	0.21	0.39	0.86
MUFA								
Grower	23.5	29.8	30.3	22.7	2.59	0.93	0.67	0.01
Finisher	25.7	21.5	25.8	23.6	1.51	0.43	0.03	0.45
PUFA								
Grower	18.9	22.8	22.4	17.7	1.88	0.63	0.62	0.05
Finisher	22.1	18.7	22.5	20.7	1.32	0.32	0.04	0.53

[1]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

Dokka et al., 2001; Yenari et al., 2005; Kalmar and Greensmith, 2009; Iyer and Cheng, 2012; Latorre et al., 2014). Supplementing human subjects with antioxidant mixtures enhanced synthesis of heat shock proteins (Peng et al., 2000; Howard et al., 2002), which was somewhat similar to the elevated gene expression of HSP90 in the DL-MET-fed groups. In comparison, supplementing rats and human subjects with antioxidants decreased IL-6 but elevated IL-10 in the blood. (Alleva et al., 2005; Lowes et al., 2013;

Table 6. Effect of different chemical forms and concentrations of methionine supplementations on antioxidant status of broiler chicks[1]

Form	DL-MET		HMTBA			*P*-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
Grower								
Plasma, nmol/ml								
GSH	3.72	4.45	3.52	3.67	1.69	0.82	0.71	0.64
GSSG	0.31	0.42	0.35	0.37	0.02	0.94	0.32	0.52
Liver, µmol/g								
GSH	9.05^{ab}	11.2^{a}	7.08^{b}	10.2^{ab}	1.23	0.21	0.03	0.67
GSSG	3.17^{ab}	4.31^{a}	3.08^{b}	2.08^{b}	0.38	0.71	<0.01	<0.01
Breast, µmol/g								
GSH	0.26^{ab}	0.38^{a}	0.22^{b}	0.21^{b}	0.04	0.01	0.09	0.10
GSSG	0.016^{ab}	0.019^{a}	0.010^{b}	0.017^{ab}	0.002	0.15	0.06	0.52
Thigh, µmol/g								
GSH	0.47^{ab}	0.53^{a}	0.34^{b}	0.34^{b}	0.05	<0.01	0.60	0.65
GSSG	0.053^{b}	0.097^{a}	0.039^{b}	0.062^{ab}	0.010	0.02	<0.01	0.29
Finisher								
Plasma, nmol/g								
GSH	2.88^{a}	0.86^{b}	2.37 ^{a}	1.90^{ab}	0.41	0.67	0.01	0.09
GSSG	0.25^{ab}	0.30^{a}	0.32^{a}	0.24^{b}	0.05	0.67	0.05	0.25
Liver, µmol/g								
GSH	2.37^{b}	2.86^{ab}	3.24^{ab}	3.57^{a}	0.37	0.24	0.36	0.91
GSSG	0.11^{a}	0.083^{ab}	0.069^{b}	0.051^{b}	0.007	0.05	<0.01	0.61
Breast, µmol/g								
GSH	0.195	0.23	0.20	0.24	0.04	0.99	0.28	0.76
GSSG	0.032^{b}	0.078^{a}	0.047^{ab}	0.053^{ab}	0.010	0.86	0.02	0.04
Thigh, µmol/g								
GSH	0.25	0.36	0.21	0.26	0.04	0.06	0.07	0.43
GSSG	0.059^{a}	0.036^{b}	0.028^{b}	0.047^{ab}	0.005	0.16	0.67	<0.01
MDA, µmol TEP equivalent/mg protein								
Liver	29.1	26.6	27.3	29.2	2.54	0.30	0.87	0.11
Breast	23.5	23.1	25.4	22.6	2.58	0.91	0.92	0.43
Thigh	66.7	74.4	80.2	71.2	5.02	0.82	0.57	0.64
Adipose tissue	1,202	1,400	1,364	1,107	152	0.67	0.85	0.14
FRAP, mmol Trolox equivalent/L plasma								
Grower	16.7	17.6	16.5	15.6	1.07	0.31	0.99	0.42
Finisher	31.9^{b}	41.0^{a}	31.4^{b}	36.0^{ab}	2.83	0.31	0.02	0.42
Serum corticosterone, ng/mL								
Finisher	179	198	219	258	47.7	0.32	0.56	0.85
Liver protein carbonyl, nmol/ mg								
Finisher	1.67	1.81	1.84	1.49	0.36	0.88	0.82	0.48

[1]GSH, glutathione; GSSG, glutathione disulfide; MDA, malondialdehyde; FRAP, ferric reducing ability of plasma, TEP, 1,1,3,3-tetraethoxypropane. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

de Oliveira et al., 2014; Hou et al., 2015). Treating macrophage like cells (RAW 264.7) with natural anti-inflammatory products inhibited the activation of MAPKs (de Oliveira et al., 2014; Hou et al., 2015). These results were in disagreement with our above-described findings. Although the chemical form and concentration of supplemental methionine in the diets for broilers indeed affected the hepatic inflammation-related gene expression, there is no simple interpretation to explain the observed mixed effects in the present study.

The third finding from the current study was that the extra methionine supplementation enhanced the concentrations of MUFA in the liver of growers. Because MUFA and PUFA are susceptible to oxidation and peroxidation, and supplemental antioxidants can prevent those destructions (Wood et al., 2004; Niu et al., 2018), our finding provides evidence from a different angle for the antioxidant potential of supplemental methionine. The greater MUFA and PUFA concentrations in the liver of birds fed DL-MET than those fed HMTBA might also imply a better antioxidant efficacy of the DL-MET. However, as the high ambient temperature stress became more intensified from the grower to the finisher period, we observed opposite effects of the extra methionine supplementation and(or) DL-MET on fatty acid profiles in the breast and adipose tissue. It is relevant to note that lipid synthesis and activation of fatty acid synthase are related to proinflammatory status (Carroll et al., 2018), while breakdown of fatty acids might be related to anti-inflammatory phenotypes (Carroll et al., 2018). Previous chicken and pig studies have shown elevated lipid synthesis and storage under heat stress conditions (Ain Baziz et al., 1996; Geraert et al., 1996; Lin et al., 2006b; Jahanian and Rasouli, 2015; Qu and Ajuwon, 2018). Intriguingly, the decreased MUFA and PUFA concentrations in the liver of the finishers fed the extra methionine or DL-MET

Table 7. Effect of different chemical forms and concentrations of methionine supplementations on antioxidant enzymes activities of broiler chicks[1]

Form	DL-MET		HMTBA			P-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
Grower								
Breast, U/mg protein								
GR	4.07	4.34	3.46	3.69	0.31	0.34	0.70	0.97
GPx	24.5	31.5	34.2	35.5	1.31	<0.01	0.08	0.23
GST	45.3	43.5	44.0	40.7	2.07	0.64	0.56	0.87
SOD	1.46^{a}	0.99^{ab}	0.96^{b}	1.14^{ab}	0.06	0.30	0.40	0.05
Thigh, U/mg protein								
GR	89.8^{b}	141^{a}	146^{a}	140^{a}	6.26	0.01	0.05	0.01
GPx	12.1^{a}	9.90^{b}	8.17^{bc}	6.50^{c}	0.46	<0.01	<0.01	0.70
GST	80.9	83.8	80.7	73.5	1.94	0.18	0.57	0.20
SOD	0.21	0.19	0.32	0.22	0.03	0.29	0.20	0.28
Liver, U/mg protein								
GR	11.5^{ab}	9.91^{b}	13.9^{a}	13.0^{ab}	0.58	0.02	0.26	0.76
GPx	8.57^{ab}	7.39^{b}	10.4^{a}	9.70^{ab}	0.44	0.02	0.26	0.76
GST	136^{c}	123^{c}	177^{b}	219^{a}	8.73	<0.01	0.29	0.04
SOD	0.043^{b}	0.038^{b}	0.067^{ab}	0.091^{a}	0.013	0.02	0.52	0.34
Adipose tissue, U/mg protein								
GR	10.8^{b}	4.62^{b}	12.0^{b}	31.4^{a}	1.92	<0.01	0.05	<0.01
GPx	41.7	35.2	41.4	49.7	3.63	0.35	0.91	0.32
GST	57.2^{b}	69.0^{b}	64.9^{b}	92.4^{a}	3.89	0.03	<0.01	0.25
SOD	7.21^{ab}	6.29^{b}	9.46^{a}	5.78^{b}	0.50	0.35	0.02	0.14
Finisher								
Breast, U/mg protein								
GR	5.69	9.14	7.32	6.84	0.64	0.79	0.25	0.13
GPx	27.1	42.6	42.4	31.9	3.13	0.71	0.68	0.04
GST	79.3	106	92.2	97.7	5.48	0.83	0.16	0.35
SOD	1.05	1.50	1.04	1.37	0.14	0.81	0.18	0.83
Thigh, U/mg protein								
GR	14.0	12.1	11.9	11.8	0.46	0.31	0.22	0.33
GPx	15.5	16.3	14.6	14.4	0.48	0.14	0.77	0.60
GST	79.8	73.9	81.7	77.0	1.75	0.48	0.14	0.87
SOD	0.33	0.21	0.31	0.39	0.03	0.22	0.73	0.16
Liver, U/mg protein								
GR	14.9	15.8	16.2	15.6	0.37	0.47	0.82	0.34
GPx	26.0^{a}	22.0^{b}	25.8^{ab}	23.7^{ab}	0.72	0.59	0.04	0.49
GST	241	220	237	229	5.85	0.82	0.24	0.61
SOD	0.19	0.18	0.10	0.20	0.02	0.33	0.21	0.12
Adipose tissue, U/mg protein								
GR	21.4^{ab}	19.5^{b}	23.0^{ab}	29.3^{a}	1.01	0.52	0.53	0.12
GPx	85.7^{a}	49.4^{b}	61.9^{ab}	55.7^{b}	4.85	0.34	0.03	0.11
GST	110^{ab}	103^{ab}	125^{a}	92.0^{b}	3.95	0.75	<0.01	0.07
SOD	34.0^{b}	35.3^{ab}	47.2^{a}	44.3^{ab}	2.27	0.02	0.86	0.62

[1]GR, glutathione reductase; GPx, glutathione peroxidase; GST, glutathione transferase; SOD, superoxide dismutase. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

might be associated with a relatively long term-improved antioxidant and anti-inflammatory status.

Compared with the commercial standards, the overall growth performances of birds during the starter and grower phases were similar, but impaired during the finisher phase by the high room temperature. Specifically, the body weight, average daily gain, and feed intake were 14%, 13%, and 22% lower than the commercial targets (Cobb-Vantress, 2018). The impairment was consistent with results reported in previous studies (Rostagno and Barbosa, 1995; McKee et al., 1997; Kirunda et al., 2001; Quinteiro-Filho et al., 2010). As heat stress is known to decrease feed intake, the greater feed intake in the DL-MET-fed groups than that in the HMTBA-fed groups suggested a potential benefit of supplementing the former under high ambient temperature conditions. Likewise, benefits of supplemental methionine into the amino acid-deficient diets on growth performance were shown in broilers raised under thermoneutral conditions (Swain and Johri, 2000; Rama Rao et al., 2003; Chen et al., 2013). In the present study, the extra methionine supplementation-compromised growth performance compared with the commercial goals and the lack of difference from the normal level of supplementation may be related to the chronic high temperature employed, especially toward the end of the study when the room temperature was

Table 8. Effect of different chemical forms and concentrations of methionine supplementations on liver inflammation-related gene expressions of finisher broiler chicks[1]

Form	DL-MET		HMTBA			*P*-value		
Concentration	100%	130%	100%	130%	SEM	Form	Conc	Interaction
IL-6	1.00^{b}	2.52^{a}	2.41^{a}	1.42^{ab}	0.38	0.39	0.76	<0.01
IL-10	1.00^{ab}	1.13^{ab}	1.67^{a}	0.45^{b}	0.19	0.48	<0.01	<0.01
TNFα	1.00	1.17	1.23	1.09	0.13	0.53	0.94	0.23
HSP70	1.00^{ab}	1.78^{a}	1.60^{a}	0.68^{b}	0.23	0.09	0.26	<0.01
HSP90	1.00^{ab}	1.48^{a}	1.10^{ab}	0.79^{b}	0.18	0.02	0.37	0.02
AKT	1.00^{b}	1.77^{a}	1.46^{ab}	1.10^{ab}	0.22	0.32	0.14	0.01
P38MAPK	1.00^{b}	1.32^{a}	1.11^{ab}	0.92^{b}	0.12	0.16	0.51	0.01
JNK	1.00^{b}	1.16^{ab}	1.42^{a}	1.36^{ab}	0.12	0.03	0.70	0.40

[1]IL-6, interleukin 6; IL-10, interleukin 10; TNFα, tumor necrosis factor alpha, HSP70, heat shock protein 70; HSP90, heat shock protein 90; AKT, protein kinase b; P38MAPK, P38 mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase. Data are expressed as means (n = 10). The main effects (the chemical form and concentration of supplemental methionine) were analyzed by two-way ANOVA and Duncan's multiple range test was used to compare the treatment means. Means in the same row without a common letter differ significantly ($P < 0.05$).

12 °C higher than the suggested temperature. That might be too extreme for the extra methionine to compensate for the performance loss of the birds. Although the extra methionine supplementation indeed partially ameliorated adverse effects of the high ambient temperature on health and antioxidant status of the birds, these ameliorations were not sufficient to affect growth performance.

In conclusion, supplementing 30% extra methionine, either as DL-MET or HMTBA, into the corn-soybean meal-based diets for the grower and finisher broilers raised in high ambient temperature affected their antioxidant status, the inflammation-related gene expression in the liver, and the fatty acid profiles in several tissues. However, the effects varied with the tissues and ages of the birds, the selected measures, and the forms and concentrations of the supplemental methionine. Future research should be performed to explore how coordinated adaptations of the intrinsic ROS scavengers and antioxidant enzymes affect functions and efficacy of supplemental DL-MET vs. HMTBA in helping broilers to cope with various environmental stresses.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Conflict of interest statement

Rose Whelan is an employee of Evonik Nutrition & Care GmbH, Hanau, Germany. All other authors declare no conflicts of interest.

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