# Effects of dietary supplementation of methionine and its hydroxy analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperatures

H. Willemsen,*[1] Q. Swennen,*[2] N. Everaert,* P.-A. Geraert,† Y. Mercier,† A. Stinckens,* E. Decuypere,* and J. Buyse*

*Laboratory for Livestock Physiology, Immunology and Genetics, Department of Biosystems, KU Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium; and †Adisseo France SAS, F-92160 Antony, France

**ABSTRACT** Heat stress is known to impair performance and to induce oxidative stress in poultry. The aim of the present study was to compare the effects of dietary supplementation of DL-methionine (DL-M) or the synthetic analog 2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA) on broiler growth performance, plasma hormone levels, and some oxidative stress-related parameters under conditions of chronic exposure to high temperatures (HT). From 2 to 6 wk of age, male broiler chickens were reared under either a constant temperature of 32°C until 6 wk of age or a normal temperature scheme (gradual decrease to 18°C at 5 wk of age). Chicks in both the normal and HT treatments were provided with a commercial grower diet supplemented with either 1.0 or 1.2 g/kg of DL-M or 1.0 or 1.2 g/kg of DL-HMTBA. Because there were no effects of supplement dose, data were pooled over both doses within each temperature treatment. The chronic HT treatment impaired feed intake and BW gain, but these negative effects were less pronounced when the chickens received DL-HMTBA. Exposure to HT was also associated with decreased ($P < 0.001$) plasma thyroid hormones and increased ($P < 0.0001$) plasma corticosterone levels. At 4 wk of age, and irrespective of the supplemental source, chickens subjected to HT were characterized by significantly lower plasma TBA-reactive substance levels. In contrast, at 6 wk of age, plasma TBA-reactive substance levels were significantly increased by HT, but this effect was observed only for the chickens receiving DL-M and not for those receiving DL-HMTBA. High temperatures induced a significant increase in hepatic total glutathione (GSH) and oxidized GSH levels, regardless of the supplemental source. However, the hepatic ratios of reduced GSH to total GSH and reduced GSH to oxidized GSH were highest in chickens supplemented with DL-HMTBA. In conclusion, DL-HMTBA supplementation partially prevented the growth-depressing effects of chronic heat exposure compared with DL-M supplementation. It can be inferred that DL-HMTBA is more efficient in alleviating HT-induced oxidative damage because of a more favorable reduced GSH-to-total GSH ratio.

**Key words:** DL-methionine (analog), oxidative status, heat stress, broiler chicken

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## INTRODUCTION

The effect of supplementing 2 commercially available methionine sources [DL-methionine (**DL-M**) and DL-2-hydroxy-4-methylthiobutanoic acid (**DL-HMTBA**)] on the growth performance of broiler chickens exposed to thermoneutral and high temperatures have been extensively investigated. For many years, scientists have addressed the differential effects of both methionine sources on growth performance, with rather inconclusive data, depending on dietary conditions (Vázquez-Añón et al., 2006), level of DL-M deficiency or supplementation (Elkin and Hester, 1983; Van Weerden et al., 1983; Garlich, 1985; Balnave and Oliva, 1990; Sauer et al., 2008), arginine-to-lysine ratio (Balnave et al., 1999; Ribeiro et al., 2005), or level of cysteine (Thomas et al., 1983; Pillai et al., 2005). Some researchers have observed that under high-temperature (**HT**) conditions, birds fed DL-HMBTA showed better growth, better feed efficiency, and lower mortality compared with chickens supplemented with DL-M (Swick and Pierson, 1988; Swick et al., 1990). Ribeiro et al. (2001) found that birds under thermoneutral conditions performed better


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[1]Corresponding author: hilke.willemsen@biw.kuleuven.be
[2]Present address: Biomedical Research Institute, Hasselt University and Transnational University Limburg, School of Life Sciences, Diepenbeek, Belgium 3590.

when supplemented with DL-HMTBA compared with DL-M. In another study, Ribeiro et al. (2006) found no differences in growth performance resulting from supplementation of either source in broilers exposed to HT. Conversely, Balnave and Oliva (1990) found improved feed efficiency under HT conditions with DL-M supplementation, but not with DL-HMTBA supplementation. In view of these inconsistent results, there is a need for additional research on the effect of DL-M and analogs on broiler performance under normal or heat-stress conditions.

Furthermore, information is lacking concerning the effects of supplementing the DL-M analog on the pro-oxidant or antioxidant status of broiler chickens under conditions known to induce oxidative stress. Oxidative stress is responsible for damage to macromolecules such as lipids, proteins, carbohydrates, and DNA through the generation of reactive oxygen species (**ROS**). Exposure to a temperature of 32°C for 6 h was capable of inducing oxidative stress, as reflected in increased plasma TBA-reactive substance (**TBARS**) levels resulting from lipid peroxidation in 5-wk-old broiler chickens (Lin et al., 2006). Therefore, it can be stated that heat stress is a practical way to induce oxidative stress in broiler chickens (Altan et al., 2003; Maini et al., 2007; Mujahid et al., 2007). Because DL-M and DL-HMTBA are precursors of cysteine and glutathione (**GSH**; Martín-Venegas et al., 2006; Métayer et al., 2008), special attention is paid to the generation of GSH, a measure of the nonenzymatic antioxidant defense mechanism, in the livers of supplemented broilers raised in HT.

The aim of this experiment was to compare the effects of dietary DL-M and DL-HMTBA supplementation on broiler growth performance, plasma hormone levels, and some oxidative stress-related parameters under chronic high ambient temperatures.

## MATERIALS AND METHODS

The experimental setup was approved by the Ethical Commission for Experimental Use of Animals of KU Leuven.

### Experimental Design

Three hundred twenty male Ross broiler chickens were obtained from a local hatchery at 1 d of age (Belgabroed, Merksplas, Belgium). Broilers were placed in deep-litter floor pens and provided with a 23L:1D lighting schedule. During the first 2 wk, a commercial starter diet (22.3% CP and 3,043 kcal of ME/kg; Table 1) was provided on an ad libitum basis. The temperature was set at 34°C at 1 d of age and then gradually decreased to reach 25.5°C at 2 wk of age.

At 2 wk of age, chickens were randomly assigned to 1 of 2 temperature treatments [HT and control temperature (**CT**)], with 160 birds per treatment. Within each treatment room, birds were further randomly assigned to 1 of 8 pens; 4 pens received a commercial grower diet (Adisseo, France, Antony, France) supplemented with 1.0 or 1.2 g/kg of DL-M, and 4 pens received the same diet supplemented with 1.0 or 1.2 g/kg of DL-HMTBA. The supplements provided DL-M in excess of the NRC (1994) requirement (4 g/kg). The HT treatment was 32°C, whereas the CT gradually decreased from 25.5°C to 18°C, as typically occurs in a commercial setting. The 2 temperature treatment rooms were identical in every respect. The duration of the temperature treatments was 4 wk.

### BW, Feed Intake, and Sample Collection

Individual BW and feed intake per pen were recorded on a weekly basis. The treatment rooms were checked daily for dead birds. At 4 and 6 wk of age, blood was collected from the jugular vein in heparinized tubes and kept on ice. After centrifugation (3,024 × *g*, 10 min, 4°C), plasma was collected and stored at −20°C until analysis. Eight broiler chickens per treatment group (4 chickens per pen) were killed by decapitation, and the liver, right breast muscle, abdominal fat pad, and heart (fat was trimmed off) were excised and weighed. Proportional organ weights were calculated as (weight of organ/BW) × 100. At 6 wk of age, a liver sample was taken, immediately frozen in liquid nitrogen, and stored at −80°C until analysis.

### Plasma Metabolites and Hormones

Plasma triiodothyronine (**$T_3$**) and thyroxine (**$T_4$**) concentrations were measured by RIA as described by Darras et al. (1996). The antisera for $T_3$ and $T_4$ were purchased from Byk-Belga (Brussels, Belgium).

**Table 1.** Diet components and calculated contents of the diet

| Item | Amount |
|---|---|
| Ingredient (%) | |
| Corn | 25 |
| Wheat | 36.3 |
| Soybeans | 8 |
| Soybean meal | 21.4 |
| Palm oil | 5.2 |
| Limestone | 1.2 |
| Monocalcium phosphate | 1.6 |
| Salt | 0.3 |
| Premix[1] | 0.42 |
| DL-Methionine | 0.28 |
| L-Lysine | 0.3 |
| Calculated content (%) | |
| ME (kcal/kg) | 3,152 |
| CP | 19.08 |
| Crude fat | 8.79 |
| Crude fiber | 3.37 |
| Lysine | 1.180 |
| Methionine | 0.561 |
| Methionine + cysteine | 0.890 |

[1]Premix supplied the following amount of vitamins and minerals per kilogram of diet: vitamin A, 12,000 IU; vitamin $D_3$, 2,000 IU; vitamin E, 50 IU; vitamin $K_3$, 2.5 mg; vitamin $B_1$, 2.0 mg; vitamin $B_2$, 6 mg; calcium pantothenate, 15 mg; vitamin $B_6$, 3 mg; vitamin $B_{12}$, 0.02 mg; nicotinic acid, 30 mg; vitamin H, 0.1 mg; folic acid, 1 mg; Fe, 80 mg; Cu, 8 mg; Mn, 60 mg; Co, 0.4 mg; Zn, 40.4 mg; I, 0.8 mg; Se, 0.2 mg.

Intraassay CV were 4.5 and 5.4% for $T_3$ and $T_4$, respectively. Plasma corticosterone levels were measured by using a commercially available double-antibody RIA (IDS Ltd., Boldon, UK). The intraassay CV for corticosterone was 3.9%. Plasma insulin-like growth factor-I (**IGF-I**) concentrations were measured by the RIA method of Renaville et al. (1993).

### Redox Balance

Plasma creatine kinase (**CK**; VetTest 9820366) and uric acid (VetTest 9820378) concentrations were determined using a VetTest 8008 analyzer (Idexx Laboratories Inc., Westbrook, ME). The apparatus is based on dry chemical technology and a colorimetric reaction. Sample analysis was carried out on selective testing discs (Idexx Laboratories Inc.) by means of a laser reading the bar codes. Plasma lipid peroxidation was estimated by spectrophotometric determination of TBARS as described by Lin et al. (2004a,b), with some modifications. Thiobarbituric acid-reactive substances are expressed as nanomoles of malondialdehyde per milliliter of plasma. Plasma ferric reducing/antioxidant power (**FRAP**) was determined by using a method described by Benzie and Strain (1996). Plasma superoxide dismutase (**SOD**) activity was assessed using an SOD assay kit (Dojindo Molecular Technologies Inc., Rockville, MD) according to the manufacturer's recommendations, using a microplate reader (Victor$^3$ V Multilabel Counter 1420, PerkinElmer, Waltham, MA).

### Liver GSH Analysis

For the quantification of GSH and oxidized GSH (**GSSG**) concentrations in liver samples, an enzymatic recycling method using GSH reductase was used. The preparation of samples and colorimetric end-point measuring method were executed according to the manufacturer's recommendations (GSH assay kit, no. 703002, Cayman Chemical Company, Ann Arbor, MI). Reduced GSH levels were calculated by twice subtracting the GSSG concentration from the total GSH concentration. Total GSH, GSSG, and reduced GSH were expressed in nanomoles per milligram of total protein. The total protein concentration of samples was determined using a Pierce BCA protein assay (Thermo Scientific, Rockford, IL).

### Statistical Analysis

For each age, data were analyzed separately by 3-way factorial ANOVA (SAS version 9.1; SAS Institute Inc., Cary, NC), with temperature schedule, supplemental source, and supplemental dose as fixed effects. Because preliminary studies showed no evidence of the effect of room, this explanatory variable was excluded from the model. Because no significant effects of supplement dose and no interactions between supplement dose and other classification variables were found, data were pooled per supplemental source for the dose. Data were reanalyzed by 2-way ANOVA, with temperature schedule and supplemental source as the classification variables. The model was

$$Y_{ij} = \mu + \alpha_i + \tau_j + \alpha\tau_{ij} + e_{ij},$$

where $Y_{ij}$ is the dependent variable, $\mu$ is the overall mean, $\alpha_i$ is the main temperature schedule effect (i = CT or HT), $\tau_j$ is the main supplemental source effect (j = DL-M or DL-HMTBA), $\alpha\tau_{ij}$ is the interaction effect between temperature schedule and supplemental source, and $e_{ij}$ is the residual error term. If a significant model was discerned, means were further compared using Tukey's test. Statistical significance was accepted when $P \leq 0.05$.

## RESULTS

### BW, Feed Intake, and Proportional Organ Weights

Figure 1 shows the weekly BW from 2 wk of age onward. From wk 3 for both supplemental sources, chickens reared in the CT had higher BW than those subjected to chronic HT (wk 3, $P = 0.0003$; wk 4, $P < 0.0001$; wk 5, $P < 0.0001$; wk 6, $P < 0.0001$). Supplemental source had an effect on growth performance at 5 and 6 wk of age. Chickens supplemented with DL-HMTBA had higher BW than those supplemented with DL-M (wk 5, $P = 0.0441$; wk 6, $P < 0.0001$). As expected, the chronic HT exposure impaired feed intake (Figure 2). This effect was significant at wk 4 ($P = 0.0054$), wk 5 ($P = 0.0005$), and wk 6 of age ($P = 0.0001$), irrespective of the supplemental source. Mortality rate averaged 3.7, 0, 6.25, and 3.7% for the DL-M- and DL-HMTBA-supplemented thermoneutral chickens and the DL-M- and DL-HMTBA-supplemented HT chickens, respectively.

The effects of supplemental source and exposure to high ambient temperature on BW and proportional weights of the liver, heart, abdominal fat, and breast muscle of 4- and 6-wk-old broilers are presented in Tables 2 and 3, respectively. At 4 wk of age, broilers exposed to the high ambient temperature had smaller proportional heart weights ($P < 0.0001$) compared with those of thermoneutral birds. The proportional abdominal fat pad weight was increased ($P = 0.0051$) by the exposure to HT. The proportional weights of the right breast muscle were influenced by the supplemental source ($P = 0.0099$), with chickens supplemented with DL-M having higher proportional right breast muscle weights. Proportional liver weights were not affected by any of the treatments [temperature treatment (**TT**): $P = 0.0636$; supplemental source (**SS**): $P = 0.1696$; interaction (**Int**), $P = 0.9910$].

At 6 wk of age, no effects of the imposed environmental temperature or supplemental source were found on proportional liver (TT, $P = 0.9071$; SS, $P = 0.8458$; Int,

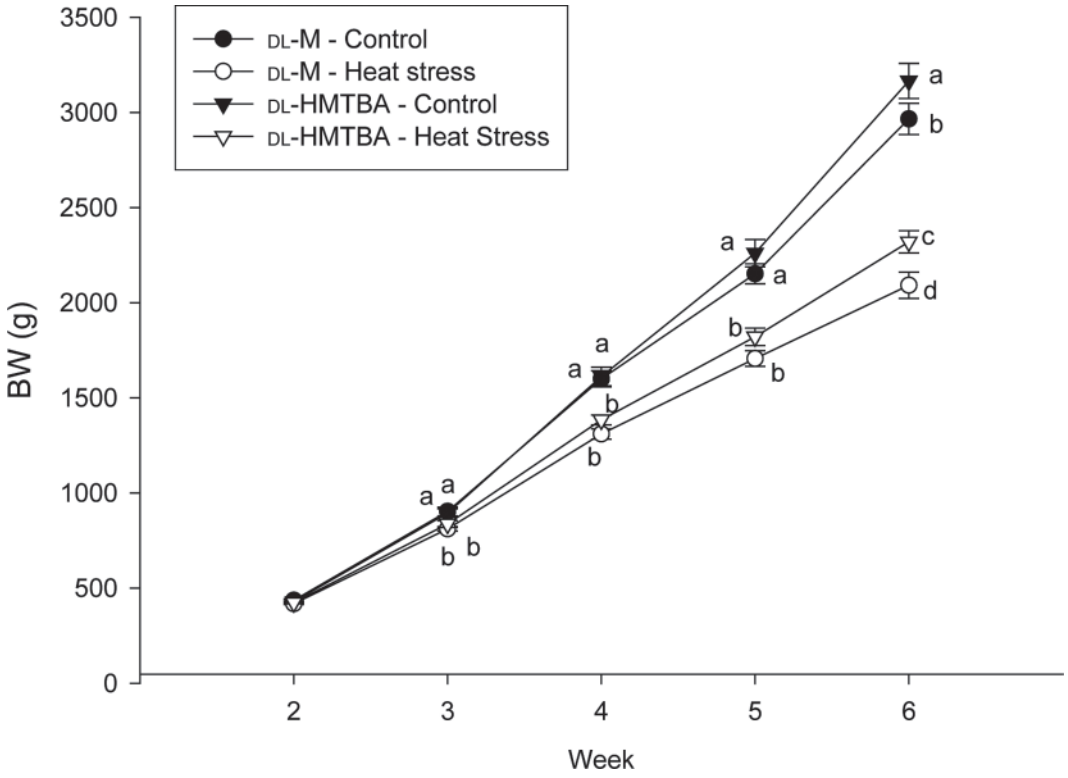


**Figure 1.** Weekly BW from wk 2 onward of broiler chickens reared on a normal temperature schedule or under heat stress (32°C) and supplemented with either DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA; 1 and 1.2 g/kg treatments combined). Values are shown as mean ± SEM (n = 80 chicks per treatment group). Different letters (a–d) denote a significant ($P < 0.05$ or lower) difference between treatments.

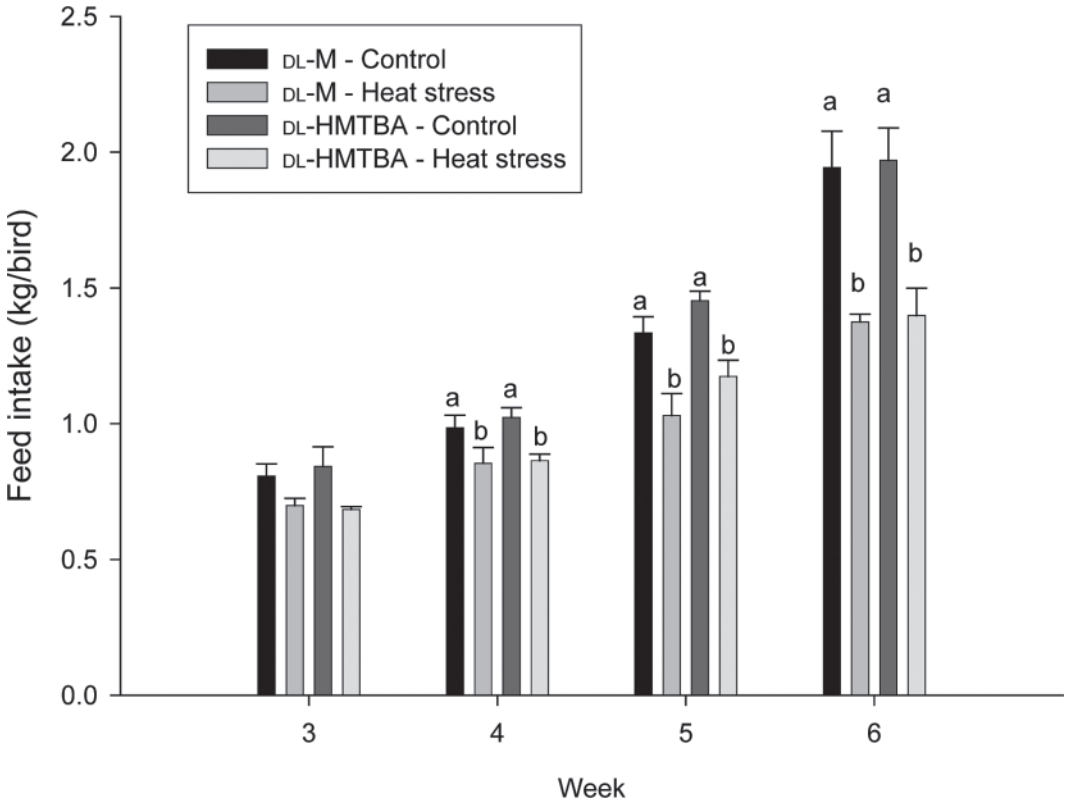


**Figure 2.** Weekly feed intake (kg/chicken) from wk 3 onward of broiler chickens reared on a normal temperature schedule or under heat stress (32°C) and supplemented with either DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA; 1 and 1.2 g/kg treatments combined). Values are shown as mean ± SEM (n = 4 pens per treatment group). Different letters (a, b) denote a significant ($P < 0.05$ or lower) difference between treatments.

**Table 2.** Proportional organ weights of 4-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| | Proportional weight | | | |
|---|---|---|---|---|
| Item | Liver (%) | Heart (%) | Abdominal fat (%) | Breast muscle (%) |
| Main effect | | | | |
| Temperature treatment | | | | |
| CT | 2.23[1] | 0.40[a] | 1.62[b] | 7.24 |
| HT | 2.39 | 0.2[b] | 1.88[a] | 7.11 |
| Methionine source | | | | |
| DL-M | 2.26 | 0.35 | 1.69 | 7.34[a] |
| DL-HMTBA | 2.38 | 0.35 | 1.81 | 7.01[b] |
| Pooled SEM | 0.042 | 0.0082 | 0.047 | 0.064 |
| | *P*-value | | | |
| Temperature treatment | 0.0636 | <0.0001 | 0.0051 | 0.2964 |
| Methionine source | 0.1696 | 0.4818 | 0.1656 | 0.0099 |
| Interaction | 0.9910 | 0.3460 | 0.8458 | 0.6786 |

[a,b]Means within a column with different superscripts differ significantly according to the corresponding probabilities.

[1]The number of chicks per treatment was 32.

$P = 0.5222$), abdominal fat pad (TT, $P = 0.8422$; SS, $P = 0.5198$; Int, $P = 0.1525$), or right breast muscle (TT, $P = 0.1651$; SS, $P = 0.4921$; Int, $P = 0.8426$) weights (Table 3). However, the proportional heart weights of the thermoneutral chickens were higher than those of the chickens exposed to HT ($P < 0.0001$).

## Redox Balance

At 4 wk of age, neither the HT nor the supplemental source had an effect on plasma FRAP capacity (TT, $P = 0.3461$; SS, $P = 0.2271$; Int, $P = 0.4590$), the activity of SOD (TT, $P = 0.5699$; SS, $P = 0.6825$; Int, $P = 0.2008$), or CK (TT, $P = 0.3319$; SS, $P = 0.8201$; Int, $P = 0.3988$) in the plasma (Table 4). However, a temperature effect ($P < 0.0001$) was found for plasma TBARS levels, with a lower level for the chickens subjected to HT, irrespective of the supplemental source. In addition, chickens reared in HT conditions were characterized by higher plasma uric acid levels ($P = 0.0269$).

At 6 wk of age, no statistically significant effects of the imposed temperature regimen or supplemental source were observed for plasma FRAP capacity (TT, $P = 0.3741$; SS, $P = 0.1942$; Int, $P = 0.4716$), SOD activity (TT, $P = 0.6160$; SS, $P = 0.9156$; Int, $P = 0.9302$), or uric acid concentration (TT, $P = 0.7710$; SS, $P = 0.9927$; Int, $P = 0.1232$; Table 5). However, a temperature effect was found for both the plasma

**Table 3.** Proportional organ weights of 6-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| | Proportional weight | | | |
|---|---|---|---|---|
| Item | Liver (%) | Heart (%) | Abdominal fat (%) | Breast muscle (%) |
| Main effect | | | | |
| Temperature treatment | | | | |
| CT | 2.11[1] | 0.34[a] | 2.02 | 8.75 |
| HT | 2.13 | 0.26[b] | 2.07 | 7.98 |
| Methionine source | | | | |
| DL-M | 2.14 | 0.30 | 1.99 | 8.17 |
| DL-HMTBA | 2.10 | 0.30 | 2.11 | 8.57 |
| Pooled SEM | 0.073 | 0.0089 | 0.0089 | 0.27 |
| | *P*-value | | | |
| Temperature treatment | 0.9071 | <0.0001 | 0.8422 | 0.1651 |
| Methionine source | 0.8458 | 0.8438 | 0.5198 | 0.4921 |
| Interaction | 0.5222 | 0.7370 | 0.1525 | 0.8426 |

[a,b]Means within a column with different superscripts differ significantly according to corresponding probabilities.

[1]The number of chickens per treatment was 32.

**Table 4.** Plasma TBA-reactive substances (TBARS), ferric reducing/antioxidant power (FRAP), plasma superoxide dismutase (SOD), uric acid levels, and creatine kinase (CK) activity of 4-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| Item | TBARS (nmol/L) | FRAP (μmol of $Fe^{2+}$/L) | SOD (U/mL) | Uric acid (mg/dL) | CK (U/mL) |
|---|---|---|---|---|---|
| Main effect | | | | | |
| Temperature treatment | | | | | |
| CT | 1.88[a,1] | 1,001 | 4.18 | 327[b] | 4,978 |
| HT | 1.39[b] | 948 | 4.40 | 384[a] | 5,682 |
| Methionine source | | | | | |
| DL-M | 1.58 | 939 | 4.22 | 354 | 5,401 |
| DL-HMTBA | 1.71 | 1,008 | 4.35 | 359 | 5,258 |
| Pooled SEM | 0.060 | 28 | 0.24 | 13 | 358 |
| | *P*-value | | | | |
| Temperature treatment | <0.0001 | 0.3461 | 0.5699 | 0.0269 | 0.3319 |
| Methionine source | 0.2139 | 0.2271 | 0.6825 | 0.8489 | 0.8201 |
| Interaction | 0.5556 | 0.4590 | 0.2008 | 0.5064 | 0.3988 |

[a,b]Means within a column with different superscripts differ significantly according to corresponding probabilities.

[1]The number of chicks per treatment was 32.

TBARS levels and CK activity. Plasma TBARS levels were increased by exposure to the HT ($P = 0.0085$) only for chickens supplemented with DL-M, and not DL-HMTBA, as reflected in the temperature × supplemental source interaction ($P = 0.0227$). The plasma CK activity was lower for chickens exposed to HT ($P = 0.0025$), and this was true for both supplemental sources.

Liver samples of 6-wk-old broiler chickens exposed to the ambient HT contained higher concentrations of total GSH ($P = 0.0056$) compared with those of birds reared under a normal temperature (Table 6). Irrespective of the supplemental source, GSSG concentrations were higher ($P < 0.0001$) in the chickens subjected to the HT. Exposure to the ambient HT was associated with decreases in the reduced GSH-to-total GSH ratio ($P < 0.0001$) and in the reduced GSH-to-GSSG ratio ($P < 0.0001$). Furthermore, chickens supplemented with DL-HMTBA were characterized by higher hepatic ratios of reduced GSH to total GSH ($P < 0.0057$) and reduced GSH to GSSG ($P = 0.0423$) compared with chickens supplemented with DL-M.

**Table 5.** Plasma TBA-reactive substances (TBARS), ferric reducing/antioxidant power (FRAP), plasma superoxide dismutase (SOD), uric acid levels, and creatine kinase (CK) activity of 6-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| Item | TBARS (nmol/L) | FRAP (μmol $Fe^{2+}$/L) | SOD (U/mL) | Uric acid (mg/dL) | CK (U/mL) |
|---|---|---|---|---|---|
| Temperature treatment and methionine source | | | | | |
| CT | | | | | |
| DL-M | 1.15[b,1] | 830 | 4.19 | 321 | 15,629 |
| DL-HMTBA | 1.35[ab] | 742 | 4.20 | 291 | 14,253 |
| HT | | | | | |
| DL-M | 1.55[a] | 837 | 3.85 | 297 | 10,034 |
| DL-HMTBA | 1.38[ab] | 812 | 3.96 | 325 | 8,221 |
| Pooled SEM | 0.04 | 22 | 0.27 | 9 | 969 |
| Main effect | | | | | |
| Temperature treatment | | | | | |
| CT | 1.26[2] | 783 | 4.19 | 305 | 14,922[a] |
| HT | 1.47 | 824 | 3.91 | 313 | 9,181[b] |
| Methionine source | | | | | |
| DL-M | 1.36 | 833 | 4.01 | 309 | 12,832 |
| DL-HMTBA | 1.37 | 778 | 4.08 | 309 | 11,495 |
| | *P*-value | | | | |
| Temperature treatment | 0.0085 | 0.3741 | 0.6160 | 0.7710 | 0.0025 |
| Methionine source | 0.7975 | 0.1942 | 0.9156 | 0.9927 | 0.3911 |
| Interaction | 0.0227 | 0.4716 | 0.9302 | 0.1232 | 0.9062 |

[a,b]Means within a column with different superscripts differ significantly according to the corresponding probabilities.

[1]The number of chickens per treatment was 16.

[2]The number of chicks per treatment was 32.

**Table 6.** Hepatic total glutathione (GSH), oxidized GSH (GSSG), reduced GSH, ratio of reduced and total GSH and ratio of reduced GSH and GSSG of 6-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| Item | Total GSH (nmol/mg of protein) | GSSG (nmol/mg of protein) | Reduced GSH (nmol/mg of protein) | Reduced GSH: total GSH | Reduced GSH: GSSG |
|---|---|---|---|---|---|
| Main effect | | | | | |
| Temperature treatment | | | | | |
| CT | 77.6[b,1] | 6.7[b] | 63.4 | 82.4[a] | 9.7[a] |
| HT | 94.3[a] | 11.9[a] | 71.8 | 74.8[b] | 6.0[b] |
| Methionine source | | | | | |
| DL-M | 83.5 | 9.7 | 64.8 | 77.2[b] | 7.3[b] |
| DL-HMTBA | 88.3 | 9.2 | 70.01 | 80.0[a] | 8.1[a] |
| Pooled SEM | 3.1 | 0.6 | 2.5 | 0.9 | 0.4 |
| | *P*-value | | | | |
| Temperature treatment | 0.0056 | <0.0001 | 0.1024 | <0.0001 | <0.0001 |
| Methionine source | 0.3958 | 0.6817 | 0.3028 | 0.0057 | 0.0423 |
| Interaction | 0.7830 | 0.0909 | 0.7806 | 0.1753 | 0.3110 |

[a,b]Means within a column with different superscripts differ significantly according to the corresponding probabilities.

[1]The number of chicks per treatment was 32.

### Plasma Hormones

The effects of supplemental source and exposure to the ambient HT on plasma $T_3$, $T_4$, corticosterone, and IGF-I concentrations are presented in Table 7. Four-week-old broilers exposed to the ambient HT exhibited lower circulating $T_3$ ($P < 0.0001$) and $T_4$ ($P = 0.0007$) concentrations and higher corticosterone ($P < 0.0001$) concentrations in plasma than did birds exposed to thermoneutral temperatures. Plasma IGF-I concentrations were affected by both the temperature treatment and the supplemental source. Chickens exposed to the ambient HT were characterized by lower plasma IGF-I ($P = 0.0002$) concentrations compared with those of birds reared under the CT. Plasma IGF-I levels of chickens supplemented with DL-HMTBA were higher ($P = 0.0011$) than those of chickens supplemented with DL-M.

At 6 wk of age, chickens reared under the ambient HT showed lower plasma $T_3$ ($P < 0.0001$) and $T_4$ ($P < 0.0001$) levels than those reared under CT conditions, irrespective of the supplemental source (Table 8). In contrast, no temperature or supplementation effect was found on plasma corticosterone concentrations of 6-wk-old chickens (TT, $P = 0.2077$; SS, $P = 0.0899$; Int, $P = 0.4266$). The chronic HT induced a reduction ($P < 0.0001$) in circulating IGF-I levels. However, this effect was more pronounced for the chickens supplemented with DL-M ($P = 0.0007$). This was observed in lower plasma IGF-I levels for chickens supplemented with DL-M, compared with DL-HMTBA, and exposed to the ambient HT.

## DISCUSSION

### Growth Performance and Plasma Indicators

It is well known that chronic heat stress imposes a metabolic burden on animals, including humans (Williamson et al., 1985). In an attempt to try to cope with

**Table 7.** Plasma triiodothyronine ($T_3$), thyroxine ($T_4$), corticosterone, and insulin-like growth factor I (IGF-I) levels of 4-wk-old broiler chickens subjected to a control temperature (CT) schedule or a chronic high temperature (HT; 32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| Item | $T_3$ (ng/mL) | $T_4$ (ng/mL) | Corticosterone (ng/mL) | IGF-I (ng/mL) |
|---|---|---|---|---|
| Main effect | | | | |
| Temperature treatment | | | | |
| CT | 1.34[a,1] | 4.34[a] | 11.1[b] | 30.2[a] |
| HT | 0.87[b] | 2.96[b] | 35.4[a] | 24.6[b] |
| Methionine source | | | | |
| DL-M | 1.05 | 3.34 | 19.1 | 24.6[b] |
| DL-HMTBA | 1.16 | 3.95 | 27.9 | 29.7[a] |
| Pooled SEM | 0.06 | 0.21 | 2.7 | 0.8 |
| | *P*-value | | | |
| Temperature treatment | <0.0001 | 0.0007 | <0.0001 | 0.0002 |
| Methionine source | 0.2758 | 0.1106 | 0.0528 | 0.0011 |
| Interaction | 0.9577 | 0.6212 | 0.4266 | 0.2695 |

[a,b]Means within a column with different superscripts differ significantly according to the corresponding probabilities.

[1]The number of chicks per treatment was 32.

**Table 8.** Plasma triiodothyronine ($T_3$), thyroxine ($T_4$), corticosterone, and insulin-like growth factor I (IGF-I) levels of 6-wk-old broiler chickens subjected to a control temperature schedule or chronic heat stress (32°C, from 2 wk of age onward) and receiving a dietary supplemental source of DL-methionine (DL-M) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA)

| Item | $T_3$ (ng/mL) | $T_4$ (ng/mL) | Corticosterone (ng/mL) | IGF-I (ng/mL) |
|---|---|---|---|---|
| Temperature treatment and methionine source | | | | |
| CT | | | | |
| DL-M | 1.05[1] | 5.38 | 11.28 | 36.5[a] |
| DL-HMTBA | 1.04 | 4.68 | 9.61 | 31.4[a] |
| HT | | | | |
| DL-M | 0.68 | 2.27 | 15.04 | 19.2[c] |
| DL-HMTBA | 0.65 | 1.75 | 10.46 | 25.0[b] |
| Pooled SEM | 0.040 | 0.27 | 0.92 | 1.06 |
| Main effect | | | | |
| Temperature treatment | | | | |
| CT | 1.04[a,2] | 5.01[a] | 10.42 | 33.8[a] |
| HT | 0.66[b] | 2.02[b] | 12.69 | 22.1[b] |
| Methionine source | | | | |
| DL-M | 0.84 | 3.74 | 13.21 | 27.4 |
| DL-HMTBA | 0.85 | 3.25 | 10.04 | 28.3 |
| | | | *P*-value | |
| Temperature treatment | <0.0001 | <0.0001 | 0.2077 | <0.0001 |
| Methionine source | 0.8228 | 0.1464 | 0.0899 | 0.8230 |
| Interaction | 0.8492 | 0.8278 | 0.4266 | 0.0007 |

[a–c]Means within a column with different superscripts differ significantly according to the corresponding probabilities.

[1]The number of chickens per treatment was 16.

[2]The number of chicks per treatment was 32.

heat stress, birds decrease their feed intake to reduce their metabolic rate.

Compared with supplementing the diets with DL-M, supplementing them with DL-HMTBA alleviated some of the negative effects of exposure to the ambient HT. This result is in agreement with those of Swick and Pierson (1988) and Swick et al. (1990), who observed better growth performance and lower mortality rates in broilers exposed to HT conditions and receiving DL-HMTBA, in comparison with DL-M. However, Ribeiro et al. (2001, 2006) found no differences in broiler growth performance between DL-M and DL-HMTBA supplementation under HT conditions, although Balnave and Oliva (1990) observed better performance in chickens subjected to HT conditions and supplemented with DL-M. In the current study, the higher BW of chickens supplemented with DL-HMTBA under HT conditions was accompanied by higher levels of plasma IGF-I, which might explain the better growth performance of chickens supplemented with DL-HMTBA. Insulin-like growth factor-I is a potent anabolic hormone in chickens (as reviewed by Buyse and Decuypere, 1999).

High environmental temperatures are known to reduce the metabolic rate in domestic fowl (Williamson et al., 1985), which is clearly reflected in the present study in the consistent reduction in plasma $T_3$ and $T_4$ concentrations after 2 and 4 wk of continuous exposure to the HT. The relationship between $T_3$ and $T_4$ concentrations in the plasma of chickens subjected to HT conditions probably results from a decreased peripheral deiodination and thyroidal $T_4$ output (Darras et al., 1996).

Notably, proportional heart weights were the only proportional organ weights that were significantly affected by the temperature treatments. Chickens subjected to HT conditions had smaller proportional heart weights, irrespective of the supplemental source. This result is consistent with the studies of Yahav and Plavnik (1999), who observed decreased proportional heart weights with increasing environmental temperatures in turkeys. Acclimation to HT conditions coincides with plasma expansion to provide enough fluid to peripheral organs involved in heat dissipation (Marder and Arad, 1989; Yahav et al., 1997). Consequently, hematocrit levels will drop. A linear positive relationship exists between hematocrit and proportional heart weight (Yahav et al., 1997). This might explain the decrease in proportional heart weights in chickens subjected to high temperatures observed in the current study.

Determination of plasma CK is used as an indicator for muscle cell membrane integrity (Sandercock et al., 2001). At 4 wk of age, neither the ambient HT nor the supplemental source had an effect on plasma CK activity. However, between 4 and 6 wk of age, plasma CK activity increased by 192% (DL-M) and 212% (DL-HMTBA) in broilers reared at the CT. This finding is in agreement with previous observations that the plasma CK activity of broilers increases exponentially during this stage of the rearing period (Sandercock et al., 2001; Malheiros et al., 2003; Buyse et al., 2009). In comparison with the changes in plasma CK of the broilers subjected to HT conditions, the sharp increase in plasma CK activity in thermoneutral birds could be

related to their faster growth, and hence higher metabolic rates.

### Redox Balance

In the present study, exposure to the HT was used to induce oxidative stress to examine the role that the dietary DL-M analog might play in alleviating the possible damage to the cells caused by increased generation of ROS. Irrespective of the supplemental source, 2 wk of chronic HT exposure significantly elevated plasma corticosterone levels, indicating that the chickens were stressed. Lin et al. (2004a,b) demonstrated that chronic dietary administration of corticosterone for 2 wk induced cellular oxidative injury, as indicated by increased plasma and liver TBARS. It was unexpected in the present study that the plasma TBARS concentrations would be significantly lower in chickens after 2 wk of chronic HT exposure, regardless of the supplemental source. Several mechanisms might explain these findings: 1) The nonenzymatic defense system, as indicated by significantly increased plasma uric acid levels in chickens subjected to the HT, was activated in these first 2 wk of HT exposure, and the successful alleviation of oxidative injury caused by HT exposure may be via the elevated plasma corticosterone levels. Uric acid is considered a potent scavenger of free radicals in mammals (Hellsten et al., 1997) and particularly in birds (Simoyi et al., 2002). Indeed, Lin et al. (2004a) concluded that in parallel with the significant induction of oxidative stress by corticosterone, the nonenzymatic antioxidant capacity (as indicated by the elevated plasma uric acid levels) during stress was already being activated after 3 d of dietary corticosterone administration. There was, however, no change in the FRAP capacity, notwithstanding the elevated plasma uric acid levels. 2) Plasma SOD activity, as an indicator of the enzymatic defense system, was not changed by the HT, but it could be that the activities of this and other scavenging enzymes in the liver or in red blood cells were activated. 3) Another explanation for the lower TBARS levels in chickens subjected to the HT would be the effect of the methionine itself because both supplemental sources induced a similar decrease in plasma TBARS levels in chickens exposed to the HT. It has already been proved that these sulfur-containing compounds are the precursors of very powerful antioxidants such as taurine and glutathione (Métayer et al., 2008) and therefore could be a possible reason for the lower lipid peroxidation damage in chickens subjected to the HT.

However, at 6 wk of age, plasma TBARS levels were higher in chickens supplemented with DL-M and exposed to the HT compared with those reared under the CT, but this was not the case in birds supplemented with DL-HMTBA. Hence, we can infer that DL-HMTBA might be more beneficial than DL-M in protecting cells from ROS. Martín-Venegas et al. (2006) reported that DL-HMTBA is converted to cysteine (the precursor of glutathione) more efficiently than is DL-M. This might strengthen the assumption that DL-HMTBA might be more beneficial than DL-M in cellular protection against ROS (Swennen et al., 2011). The hepatic ratios of reduced GSH to total GSH and reduced GSH to GSSG were highest in chickens supplemented DL-HMTBA and confronted with the HT. Hence, these chickens have developed a better nonenzymatic antioxidant defense mechanism, and this phenomenon is believed to be a causative mechanism for the alleviated growth depression attributable to HT in chickens supplemented with DL-HMTBA compared with their counterparts supplemented with DL-M.

In summary, the objective of this study was to investigate the potential antioxidant power of 2 commercially available methionine sources (DL-M and DL-HMTBA). It was clear that DL-HMTBA supplementation partially prevented the growth-depressing effect of chronic exposure to HT compared with DL-M supplementation. In addition, both DL-M and DL-HMTBA appeared to alleviate oxidative damage (as indicated by lower lipid peroxidation) caused by chronic HT exposure, but DL-HMTBA was more effective. On the basis of this study, it can be inferred that the more efficient effect of DL-HMTBA was due to a more favorable reduced GSH-to-total GSH ratio.

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