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Acute and Chronic Stress Response in Japanese Quail (*Coturnix Coturnix Japonica*) Supplemented with Dietary Methionine and DL-2-Hydroxy-4-(Methylthio)-Butanoic Acid on the Performance and Blood Chemistry

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Abstract:

To measure the extent of acute and chronic stress response in Japanese quails on the performance and blood chemistry. An experiment was conducted, quails were fed with dietary treatment containing either DL-methionine (DLM) or 2-hydroxy-4-methylthiobutanoic acid (HMTBA) at adequate (starter 0.92%, grower 0.77%) or higher (starter 1.33%, grower 1.08%) concentrations from three to six weeks of age under both normal (24°C) and elevated (33°C) environmental temperatures. Blood samples were collected at 7 hours and 21 days post heat treatment to measure the effect of acute and chronic stress response on the blood parameters. The results obtained shown that heat stress produces reactive oxygen species (ROS) effect in the quails in response significantly impaired the quails performance.

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No significant effects were obtained for either the adequate (DLM & HMTBA) or higher (DLM & HMTBA) concentrations on the performance of the quails. All blood chemistry were altered during acute heat stress but reverted to normal during the period of chronic heat challenge. However, quails were able to adapt in response to heat stress via blood chemistry from acute to chronic stress.

Key words: Acute, Chronic, blood chemistry, DL-methionine, Japanese quails, 2-hydroxy-4-methylthiobutanoic-acid, performance, stress response.

INTRODUCTION

The major effects of hot climates or temperature change on the poultry species are declined feed intake, body weight gain, and increase the gain feed ratio and physiological changes in birds' metabolism (Attia *et al.*, 2006). High environmental temperatures impair performance, productivity, and welfare of Japanese quails (Kul & Seker, 2004; Vercese *et al.*, 2012; Caurez and Olo, 2013).

Heat stress produces the effect of reactive oxygen species (ROS) within the body. These molecules are highly reactive cause damage to various biological tissues and impair quail performance (Bolek *et al.*, 2013; Diarra, 2007). Moreover, to their potential to cause tissue damage, ROS is an active regulator of cell signaling and immune responses.

Synthetic methionine (DLM & HMTBA) supplementation in the diet of birds has been shown as a technique of ameliorating the influence of ROS linked with high environmental temperature (Swick *et al.*, 1990; Knight *et al.*, 1994; Chen *et al.*, 2003, 2005; Gonzalez-Esquerra & Leesson, 2006). Methionine is a precursor of cysteine and taurine, an important component of the body's antioxidant defense system which is very vital in the detoxification of ROS (Bolek *et al.*,

2013). Taurine has several central biological roles, such as osmoregulation, antioxidation, membrane stabilization, conjugation of bile acids, and modulation of calcium signaling.

Despite several studies on methionine and its sources in the diets of the birds, there is still limited information on the stress responses in quails to DLM and HMTBA, and how this amino acid influence the quail performance and welfare under elevated temperature. Thus, the objective of this study was to measure the acute and chronic stress response in Japanese quail supplemented with dietary methionine and DL-2-hydroxy-4-methylthio-butanoic acid on the performance and blood chemistry.

MATERIALS AND METHODS

Ethical consent

The research was conducted in accordance with all applicable laws and rules set forth by the Ministry of Animal and Fishery, Yobe State on Animal use and handling for the research purpose.

Birds and husbandry

One hundred and ninety-six (196) one week old male Japanese quails were used in this study. Four dietary treatments were formulated with each treatment having seven replicates (seven quails per replicate). The dietary treatment provided containing either 2-hydroxy-4-methylthiobutanoic acid (HMTBA) or DL-methionine (DLM) at adequate (starter 0.92%, grower 0.77%) or higher concentration (starter 1.33%, grower 1.08%) concentrations. At three weeks of age, birds were leg-tag and kept in cages within the environmentally controlled houses and were assigned to either elevated (33°C, 12 hours per day) or normal temperature (24°C) combined with the pair-feeding. The

quails were kept under these conditions for another three weeks.

Blood samples were collected by heart puncture 7 hours after heat treatment to measure the acute heat stress effects on the blood parameters. Samples were also collected after 3 weeks following the heat treatment to measure the chronic heat stress on the same parameters. Data were analyzed using a 3-way ANOVA of the SAS software (SAS, 9.3).

RESULTS AND DISCUSSION

Quails supplemented with HMTBA have a little higher body weight gain, feed intake and lower FCR as compared with those fed DLM (Table 1). A similar trend was noted for the higher concentrations of HMTBA and DLM fed. However, following heat stress treatment, body weight gain (BWG) and feed intake were significantly reduced while the FCR increased (Table 1). Though quails performance of those kept at normal temperature pair-fed (NT-PF) was found not significantly differed from those of normal temperature (NT). There was a decline in the BWG approximately 15% in the pair-fed group when compared with those kept at NT. It might have been due to the effect of raised in environmental temperature. Reduction in BWG of other groups could be associated with decreased feed intake.

Table 1. The main effect mean following heat challenge on the performance of Japanese quails supplemented with DLM and HMTBA for 21 days

Parameters	Body weight gain (g/bird)	Feed intake (kg/pen)	FCR (g/g)
Adequate concentration			
DLM	120	1.32	1.30
HMTBA	128	1.39	1.26
SEM	6.27	0.12	0.06

Higher concentration			
DLM	120	1.51	1.31
HMTBA	130	1.48	1.27
SEM	4.78	0.1	0.03
Temperature treatments			
NT	139	1.48	1.28
NT-PF	124	1.35	1.30
HT	118	1.20	1.42
SEM	9.12	0.3	0.05
Probability			
ANOVA	<0.01	<0.01	<0.01
Heat*Diets	<0.01	<0.01	<0.01

Adeq: Adequate methionine, NT: normal temperature, DLM: DL-methionine, HMTBA: 2-hydroxy-4-methylthiobutanoic acid, NT-PF: normal temperature-pair fed, HT: high temperature.

The response of the quails to acute heat challenge decreased the blood carbon dioxide (pCO_2), hemoglobin (Hgb), and hematocrit (HCT or PCV) concentrations though increased the blood pH levels when compared to normal environmental treatments (Table 2). There was no significant different between the DLM and HMTBA treated groups for both 7 hours and 21 days blood parameters.

Table 2. The main effect mean following heat challenge on the blood parameters of Japanese quails supplemented with DLM and HMTBA for 7 hours

Parameters	pH	pCO_2	HCT (%)	Hgb (g/dL)
Adequate concentration				
DLM	7.13	49.02	19.51	7.01
HMTBA	7.19	45.75	22.08	6.84
SEM	0.05	1.33	0.41	0.06
Higher concentration				
DLM	7.11	54.81	19.31	7.01
HMTBA	7.09	57.73	19.04	6.86
SEM	0.02	0.01	0.31	0.11
Temperature treatments				
NT	7.05	55.33	20.98	7.19
NT-PF	7.09	58.91	19.23	6.67
HT	7.13	53.52	19.75	7.00

SEM	0.03	3.10	0.23	0.09
Probability				
ANOVA	<0.01	<0.01	0.02	0.01
Met source	0.27	0.04	0.18	0.06
Met conc.	0.18	0.03	0.37	0.30
Temperature	<0.01	<0.01	<0.01	<0.01

Adeq.: Adequate methionine, NT: normal temperature, DLM: DL-methionine, HMTBA: 2-hydroxy-4-methylthiobutanoic acid, NT-PF: normal temperature-pair fed, HT: High temperature.

The blood parameters were not significantly affected by chronic heat stress, indicating that quails were able to adapt to the elevated temperatures and return to same blood chemistry within the 21 days (Table 3) similar to those of 7 hours (Table 2).

Table 3. The main effect mean following heat challenge on the blood parameters of Japanese quails supplemented with DLM and HMTBA for 21 days

Parameters	**pH**	**pCO_2**	**HCT (%)**	**Hgb (g/dL)**
Adequate concentration				
DLM	7.16	50.00	19.73	6.91
HMTBA	7.11	48.15	19.88	7.04
SEM	0.03	1.53	0.61	0.12
Higher concentration				
DLM	7.00	58.45	19.73	7.07
HMTBA	7.10	60.13	19.32	6.99
SEM	0.07	0.02	0.21	0.20
Temperature treatments				
NT	7.01	56.17	19.82	7.29
NT-PF	7.19	59.04	19.45	6.71
HT	7.16	55.22	19.80	7.20
SEM	0.09	2.55	0.83	0.41
Probability				
ANOVA	0.08	0.39	0.22	0.31
Met source	0.47	0.48	0.18	0.21
Met conc.	0.78	0.69	0.37	0.33
Temperature	0.03	0.19	0.15	0.20

Adeq.: Adequate methionine, NT: normal temperature, DLM: DL-methionine, HMTBA: 2-hydroxy-4-methylthiobutanoic acid, NT-PF: normal temperature-pair fed, HT: high temperature.

Despite the different patterns of utilization and absorption of DLM and HMTBA, the present data reveal that both DLM and HMTBA are similar in efficiency as methionine sources. Also, the performance of the quails was found similar with regard these sources. The current data suggest that supplementing quail diet with higher concentrations of methionine sources beyond bird requirements has little protective value during the short period of continuous heat stress.

Studies have revealed that high temperatures negatively affect the performance of birds supplemented with DL-methionine to a greater degree than those fed with DL-2-hydroxy-4-methylthio-butanoic acid (Swick and Pierson, 1988; Swick *et al.*, 1990; Knight *et al.*, 1994; Gonzalez-Esquerra and Leesson, 2006). These studies were in agreement with the present findings. However, Ribeiro *et al.* (2008), found that under heat stress condition, birds supplemented with DL-2-hydroxy-4-methylthio-butanoic acid (HMTBA) as compared with DL-methionine (DLM) achieve better weight gain and feed conversion. The current findings were in line with the results found by Ribeiro *et al.* (2008). Moreover, Willemsen *et al.* (2011) established that HMTBA inclusion to some degree might have growth depressing effects of chronic heat stress when compared with DLM inclusion. These authors further explained that HMTBA is more effective in ameliorating oxidative tissue damage induced by high ambient temperatures that produce the effect of reactive oxygen species (ROS) due to its ability to better the production of glutathione via trans-sulfuration (Martin-Venegas *et al.*, 2006).

In conclusion, the result indicated that supplementing diets with either DL-methionine or 2-hydroxy-4-methylthio-butanoic acid did not improve the performance of quails under the effect of reactive oxygen species (ROS) resulted from heat stress. However, supplementing the diet with higher concentrations of DLM and HMTBA beyond quail's

requirements has mild protective value during the short period of continuous heat stress, but better the sustainability during the period of chronic heat stress. The future additional study is required to elucidate the mechanism responsible for the performance of quails supplemented with DLM and HMTBA.

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