Ergothioneine Modulates Biomarkers of Oxidative Stress, 
Biochemical Profiles and Rectal Temperatures of Arabian Stallions 
Following Exercise in A Hot-Humid Environment

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ABSTRACT

These experiments were performed to determine the effect of ergothioneine (EGT) and 
environmental parameters on some physiological profiles of Arabian stallions 
following an 1800 m race in a hot-humid environment. Twelve stallions having a mean 
weight of 401 ± 7.33 kg and aged 5.28 ± 1.14 years were used as subjects. They were 
divided into two groups of treated stallions (n = 6) and untreated stallions (n = 6). 
Group I which was the experimental group was administered with EGT (0.5 mg/kg 
orally) every week for two months while group II which served as controls was not 
treated. The temperature and the relative humidity of the experimental site were 
determined for six days and on the day of the experiment. The temperature-humidity 
index (THI) was also calculated. Vital parameters and some biochemical parameters of 
all stallions were determined before the commencement, immediately after, and one 
hour after the exercise. Some biomarkers of oxidative stress and serum biochemical 
parameters (aspartate aminotransferase, creatinine and lactate dehydrogenase) of the 
stallions were also determined. The biochemical parameters were higher (P < 0.05) in 
the untreated group than in the treated group. The results obtained showed that EGT 
lowered the rectal temperature and modulated biomarkers of oxidative stress and 
biochemical profiles.

Keywords: biochemical parameters; ergothioneine; heart rate; rectal temperature; 
respiratory rate
INTRODUCTION

Horses are unique animals when compared with other animals; their respiratory, circulatory systems and thermoregulatory are among the most important systems involved in exercise (Mcmiken et al., 1983; Cottin et al., 2006; Adah et al., 2020). Physiological and biochemical parameters are used to evaluate the responses of horses to exercise following interventions. These interventions include training, periods of rest and dietary supplementation (Mader et al., 2006; Schimdt et al., 2010). Exercise is necessary for health improvement and fitness (Courouce et al., 2000; Padilla et al., 2006) and training status is used to understand how exercise influences physiological and biochemical responses (Marques et al., 2002; Gerard et al., 2014). Biochemical and physiological responses are closely connected to changes in the autonomic nervous system; therefore, they are useful to determine changes under different supplements interventions (Aubert et al., 2003; Hamlin and Hopkins, 2003). The work output of the muscles of horses during exercise depends on physiological capacities to deliver large volumes of blood to the tissue and the splenic reserve supply (Evans and Rose 1988; Mader et al., 2006; Bashir and Rasedee, 2009; Kerley et al., 2018).

Ambient temperatures and relative humidity that are higher than the thermoneutral zone of horses induce higher metabolic rates associated with decreased evaporative heat losses, impaired thermoregulation, hyperthermia, electrolyte imbalance, and alteration in biochemical parameters (Hodgson et al., 1994; Rensis and Scaramuzzi, 2003; Hartmann et al., 2015). A high ambient temperature and relative humidity impair the performance of a horse due to cardiorespiratory deficiencies, effects on core body temperatures, and changes in biochemical profiles (Schrama et al., 1996; Nenaber et al., 1999).

Ergothioneine (EGT) is a unique supplement because it does not auto-oxidize at physiological pH and does not promote the generation of hydroxyl radicals from H2O2 and Fe2+ ions (Fenton reaction) (Cheah et al., 2017; Yoshida et al., 2017). Important physiologic features of EGT include its prompt clearance from peripheral circulation into tissues with little metabolism and high stability and its long half-life (approximately 30 days). It has a reduced tendency to auto-oxidize or produces free radicals from peroxide and iron at normal body pH (Kenneth et al., 2004; Cheah et al., 2016). EGT requires the specific carrier protein called organic cation transporter novel type-1 (OCTN-1) to be transported across the cell membrane (Morris et al., 1991; Grigat et al., 2007). OCTN-1 is found mainly in the kidneys, trachea, erythrocytes, lungs, heart, and the bone marrow (Richard et al., 2009; Marone et al., 2016). Contrary to other
antioxidants such as glutathione or N-acetyl-L-cysteine, EGT metabolises slowly and is resistant to disulfide formation (Tamai et al., 1997; Nakamura et al., 2008). The formation of ergothioneine disulfide occurs only at low body pH in the presence of Cu²⁺ or H₂O₂. Ergothioneine acts as a cation chelator (Cheah et al., 2018, Halliwell et al., 2018), has a bioenergetics effect (Tamai et al., 1997), and acts as an immune regulator (Paul and Snyder, 2009). It is also an antioxidant (Cheah et al., 2016; Tang et al., 2018) and is widely distributed within organs and body tissues prone to oxidative stress (Kerley et al., 2018).

The aim of this study is to evaluate the effect of EGT on selected physiological and biochemical profiles following exercise in Arabian stallions in a hot-humid environment.

MATERIAL AND METHODS

Experimental animals

Twelve healthy stallions of the Arabian breed with a mean weight of 401 ± 7.33 kg served as experimental animals. The stallions which were used for pleasure riding were obtained from a standard stable in Ilorin (8° 30′ N, 4° 33′ E), situated in the Southern Guinea Savannah vegetation zone of Nigeria. The experiment was performed during the hot-humid season characterized by high ambient temperatures and relative humidity. The stallions were housed in a concrete-walled stable covered with corrugated iron roofing sheet. They were fed with hay and their feed was supplemented with concentrate (groundnut bran). Also, clean and cold water was also provided ad libitum. The stallions were screened for gastrointestinal parasites, haemoparasites, and ectoparasites, and only healthy animals were used as subjects. The ethical review committee of the Ahmadu Bello University, Zaria approved and endorsed the research study (ABUCAUC/2017/VET.MED/APP/019).

Determination of thermal environmental parameters

The ambient temperatures were taken at 06.00 h, 12.00 h and 18.00 h for six consecutive days and on the day of the experiment using dry- and wet-bulb thermometers (Brannan, UK). From the data obtained, relative humidity (RH) and temperature-humidity index (THI) at each hour of measurement were determined. The THI was determined using the equation of Hartmann et al. 2015, as follows:

\[
\text{THI} = (\text{DBT} \times 0.8) + \{(\text{RH}/100 \times (\text{DBT}-14.4) + 46.4) \}
\]

Where, DBT = Dry Bulb Temperature and RH = Relative Humidity

Experimental design

The stallions were divided into two groups of six stallions each: Group I stallions were administered with EGT at a dose of 0.5 mg/kg by oral administration every week for two
months before subjecting them to exercise while stallions in Group II serve as control were not administered with EGT before exercise.

Before the commencement of the experiment, the heart rate (HR), respiratory rate (RR) and rectal temperature (RT) of each stallion were determined in all the stallions. The HR of each horse was measured using the Equine Polar Heart Rate monitor (Polar Electro Oy, Kempele, Finland), while RR was determined by counting the number of respiratory flank movements per minute as described by Williams (2016). The RT was determined using a digital clinical thermometer (The Hartman’s Company, England). The thermometer was inserted about 5 cm into the rectum of each animal, and the RT value was read after a beep sound was heard, indicating the end of the reading.

Exercise protocol

The stallions were mounted by riders of an average weight of 70.43 ± 3.78 Kg and subjected to a race of 1800 m on a race track. Rectal temperature, heart, and respiratory rates were determined in the stallions pre-exercise, 5 min post-exercise, and 1-hour post-exercise. Immediately after exercise, stallions rested under a tree shade.

Collection of blood samples

A blood sample of 10 ml was collected by jugular venipuncture from each of the stallions pre-exercise and post-exercise. The samples were collected into plain containers without anticoagulant so as to harvest serum. The blood samples were transported to a physiology research laboratory in a Coleman box for the analyses of biomarkers of oxidative stress and some biochemical parameters.

Determination of biochemical parameters

The serum activities of the enzymes lactate dehydrogenase, aspartate aminotransferase and concentration of creatinine were determined using a clinical chemistry analyzer Kone Pro (Konelab, Thermo Clinical Lab systems Oy, Finland) while the activities of superoxide dismutase, catalase, glutathione peroxidase and concentrations of malondialdehyde were determined using spectrophotometric methods.

Analyses of data

Data generated from the study were expressed as mean ± SEM and analysed using the Student t-test to compare the two groups. Data generated from thermal environmental parameters were analysed using the one-way analysis of variance. Data analyses were done using GraphPad Prism (Version 5.3).

RESULTS

Table 1 shows the thermal environmental parameters of the study site during the hot-dry season. The dry-bulb temperature (DBT) increased
significantly (P < 0.05) from 22.6 ± 1.23°C at 06.00 h (6 am) to 38.6 ± 6.53°C at 12.00 h (12 pm). There was a significant (P < 0.05) increase in relative humidity from 64.4 ± 2.34% at 06.00 h (6 am) to 74.3 ± 6.73% at 12.00 h (12 pm). The temperature-humidity index (THI) increased significantly (P < 0.05) from 76.41 ± 0.56 at 06.00 h (6 am) to 83.36 ± 4.53 at 12.00 h (12 pm) when it attained its peak.

Table 1. Thermal environmental parameters of the experimental site during the hot-humid season

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Dry-Bulb Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Temperature-Humidity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>06.00</td>
<td>22.6 ± 1.23a (22 - 24)</td>
<td>64.4 ± 2.34a (63 - 68)</td>
<td>76.41 ± 0.56a (68.71 - 83.65)</td>
</tr>
<tr>
<td>12.00</td>
<td>38.6 ± 6.53b (37-39)</td>
<td>74.3 ± 6.73b (72 - 78)</td>
<td>83.36 ± 4.53b (81.32 - 89.01)</td>
</tr>
<tr>
<td>18.00</td>
<td>36.5 ± 0.17 (36-37)</td>
<td>78.8 ± 5.98 (76 - 81)</td>
<td>83.24 ± 3.49 (83.21 - 84.95)</td>
</tr>
<tr>
<td>Overall Mean ± SEM</td>
<td>37.22 ± 4.17 (36.11 - 37.33)</td>
<td>75.19 ± 5.98 (73.65 - 81.11)</td>
<td>81.45 ± 5.18 (78.11 - 84.76)</td>
</tr>
</tbody>
</table>

a,bMeans for the same column having different superscript letters are significantly (P < 0.05) different. Values in Parentheses are Minimum – Maximum

Table 2 shows the biochemical response of the stallions. All biochemical parameters measured were significantly lower (P < 0.05) in the treated group than in the untreated group.

Figure 1 shows heart rate responses of stallions before exercise, immediately after exercise, and 1 hour after exercise. There was a significant difference (P < 0.05) between the treated group and the untreated group immediately after exercise with the treated group having a higher heart rate.
### Table 2. Biochemical parameters of stallions subjected to exercise in the hot-humid season following administration of ergothioneine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treated (n = 6)</th>
<th>Not-treated (n = 6)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>124.60 ± 10.92</td>
<td>137.43 ± 10.16</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>183.4 ± 9.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>230.14 ± 17.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>316.0 ± 18.25</td>
<td>314.23 ± 21.97</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>365.9 ± 10.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>457.13 ± 21.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Creatinine µmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>40.57 ± 5.37</td>
<td>47.64 ± 6.86</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>43.27 ± 1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.07 ± 7.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase u/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>0.82 ± 0.21</td>
<td>1.77 ± 0.29&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>1.69 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.89 ± 2.63&lt;sup&gt;b2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalase u/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>4.97 ± 0.23</td>
<td>6.93 ± 0.76&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>5.24 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.54 ± 5.14&lt;sup&gt;b2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>259.90 ± 6.32</td>
<td>262.7 ± 7.02&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>288.0 ± 8.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>393.0 ± 17.82&lt;sup&gt;b2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde µmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>5.61 ± 0.34</td>
<td>5.23 ± 0.14&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>7.26 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.53 ± 5.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1,2</sup>Means for the same column having different superscript numbers are significantly (P < 0.05) different.

<sup>ab</sup>Means for the same row having different superscript letters are significantly (P < 0.05) different.
The respiratory rates of the stallions as shown in Figure 2 show that the respiratory rate of the treated group was significantly higher (P < 0.05) than the untreated group immediately after exercise. The rectal temperature of the untreated group was higher (P < 0.05) than the treated group (Figure 3).
DISCUSSION

The thermal environmental parameters recorded during the study period were characterised by high THI, AT, and RH, typical of the hot-humid season of the Southern Guinea Savannah zone of Nigeria. The mean AT values recorded during the study period was higher than the established thermoneutral zones of 5–25 °C for stallions (Kohn et al. 1999; Wallsten et al., 2012). The mean 74.3 ± 0.73 and 78.8 ± 0.77 % relative humidity recorded at 12.00 h and 18.00 h are clearly above the 70 % recommended for stallions (Schrama et al., 1996; Wallsten et al., 2012). High ambient temperature and high relative humidity are the most important thermal environmental indices, causing heat stress in stallions (Nienaber et al. 1999). The heat generated during exercise which is a by-product of metabolic production of energy may accumulate when ambient temperatures and relative humidity are high. The hot-humid environment in this study imposed additional heat stress on stallions during exercise resulting in the inability of the horses to dissipate heat effectively (Hogson et al., 1993; Hodgson et al., 1994).

The high THI (76.41 ± 0.24 to 83.24 ± 0.49) recorded in this present study is higher than the accepted value of 68 recommended for horses (CIGR 1984; Noordhuizen. and Noordhuizen2017) indicating that meteorological conditions prevailing in the study area were unfavourable for horse performance. Therefore, measures and supplements aimed at alleviating the effects of high THI are necessary to reduce the risks of heat stress and enhance performance.

Figure 3. Rectal temperature of stallions subjected to exercise during the hot-humid season
Rectal temperature is the most accurate method of evaluating the core body temperature and is considered an important index of the body’s heat load (Geor and Mccutcheon, 1998). Lower rectal temperature post-exercise in the ergothioneine treated group indicates a better heat dissipation and less heat load, compared to the untreated group after exercise. Supplementation with ergothioneine before exercise suggests that all organs and tissues involved in heat dissipation were protected from the negative effects of exercise stress, thereby reducing the risk of exercise-induced hyperthermia, rhabdomyolysis, and heat stroke (Hodgson et al., 1994).

The finding that the heart rate of the stallions administered with EGT was higher although not statistically significant immediately after exercise than in the control group may indicate the modulatory effects of EGT. Ergothioneine has been reported to be a potent antioxidant in vivo with cytoprotective effects on organs and tissues prone to oxidative damage and inflammation (Tamai et al., 1997; Lawan et al., 2010). Exercise induces increased oxygen consumption which affects the oxidant/antioxidant status. Increased oxidants cause an imbalanced equilibrium between oxidants and antioxidants, resulting in oxidative stress, bradycardia, and reduced heart rate (Williams 2016; Martin et al., 2000). This study has established that EGT may be a potent antioxidant in vivo. Halliwell et al. (2018) reported an avid uptake of EGT via a specific carrier solute carrier 22 family member 4 (SLC22A4) by organs challenged by oxidative stress. The higher mean respiratory rates in the treated group indicate a more effective function by the respiratory system of the treated group. Unlike many species, horses have a high metabolic rate but a small surface area for dissipation of heat (Franklin et al., 2010). The respiratory system plays a very important role in exercise and the dissipation of heat and must function effectively. The evaporative heat loss from the respiratory system is considered one of the important mechanisms for maintaining heat balance (Rensisand Scaramuzzi, 2003; Franklin et al., 2010). The increased respiratory response recorded in this study may be due to the stimulation of peripheral receptors that transmit impulses to the thermal centre in the hypothalamus by ergothioneine which has been reported to cross the blood-brain barrier. This may result in activation of the respiratory centre to send impulses to the diaphragm and intercostal muscles resulting in increased respiratory rates (Evans and Rose, 1988). Ergothioneine has also been reported to accumulate in the mitochondria and lungs which are sites that are very prone to oxidative imbalance (Repine and Elkins, 2012). Horses are obligate nasal breathers, so the integrity of their respiratory system is paramount for their well-being during and post-exercise. The higher respiratory rate recorded after exercise
in the treated group suggests a modulatory role by EGT. Our findings agree with the work of Repine and Elkins (2012) who reported that EGT is a potent antioxidant and anti-inflammatory agent in the respiratory system.

The activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LH) were lower in the treated group than in the untreated group after exercise. AST and LH have very high activities in the skeletal muscles and the elevation of their activities in the serum suggests damage to the muscles (Harris et al., 1998; Verdegaal and Franklin, 2015). The lower activities of these enzymes recorded in the treated group suggest lesser damage to the skeletal muscle due to the stress of exercise and the functional protective effects of ergothioneine. This result agrees with the report of Cheah et al. (2017) who demonstrated that the administration of ergothioneine in human subjects of good health status reduced oxidative and inflammatory damage to the skeletal muscle.

Creatinine, a byproduct of muscle metabolism, was lower in the treated group suggesting protection of muscles from damage by ergothioneine. Following intense exercise and muscle damage, creatinine was released from the muscles, increasing serum creatinine levels (Harris et al., 1991; Lai et al., 2016). This finding agrees with the work of Joseph et al. (2012), who reported an increased creatinine level following exercise in a hot environment in individuals not administered with any supplement.

The serum activities of catalase, glutathione peroxidase, and superoxide dismutase which are defense enzymes against oxidative stress were higher in the untreated group than in the treated group (Hanzawa et al., 2002; Younus, 2018). The results demonstrated lower oxidative stress in the treated group than in the untreated group suggesting effective protection by ergothioneine against ROS.

The serum concentration of malondialdehyde, a by-product of lipid peroxidation was higher in the untreated group than in the treated group post-exercise. Lipid peroxidation occurs when ROS overwhelms and reacts with lipids containing double carbon bonds, particularly polyunsaturated fatty acids (Ayala et al., 2014). Under normal peroxidation rates, the cells stimulate their maintenance through highly organized antioxidant defense systems that up-regulate antioxidants, resulting in an adaptive stress response (Khoubsabjafare et al., 2015). However, when lipid peroxidation rates are high, the degree of oxidative insults overwhelms antioxidant ability, and the cells undergo apoptosis or necrosis programmed-cell death (Hodgson et al., 2014). It was, therefore, concluded that EGT exerted modulatory roles on biochemical parameters and lowered rectal temperatures of Arabian stallions.
following exercise in a hot-humid environment and may be useful in preventing hyperthermia in stallions following exercises in a hot-humid environment.

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