



Modulatory roles of ergothioneine on heat shock protein-70, tumour necrosis factor-alpha, and rectal temperatures of Arabian stallions following race of 2000 m in a hot-dry environment

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ABSTRACT

Experiments were performed to determine the effect of ergothioneine on rectal temperature and the serum concentrations of heat shock protein-70 (HSP-70) and tumor necrosis factor- α (TNF- α) in stallions following a race of 2000 m in a hot-dry environment. Eighteen stallions weighing approximately 400 kg each were used for the experiment. They were divided into three groups of six stallions each. Group I (EEX) was the experimental group that was administered ergothioneine (0.5 mg/kg per os), while group II (EEC) did not receive ergothioneine before exercise. The third group (EEN) was neither administered ergothioneine nor exercised. The dry-bulb temperature and the relative humidity of the experiment were determined for six days and on the day of the experiment. The temperature-humidity index was also calculated. Rectal temperature, serum HSP-70, and TNF- α concentrations of all horses were measured before commencement, immediately after, and 2 h after the exercise. The dry-bulb temperature and relative humidity which showed diurnal fluctuations increased significantly ($p < 0.05$) between 06.00 h and 12.00 h (22.6 ± 1.23 and 38.6 ± 6.5 , respectively). Serum TNF- α and HSP-70 levels of the stallions in the EEX group were higher than the values obtained in the EEC and EEN groups ($p < 0.05$). The values of rectal temperature obtained were lower ($p < 0.05$) in the EEX group than in the other groups. Therefore, it could be concluded that ergothioneine modulated rectal temperature, as well as TNF- α and HSP-70 concentrations in the stallions, and might be beneficial to horses during exercise.

Keywords

Ergothioneine, hot-dry season, tumor necrosis factor- α , heat shock protein-70, rectal temperature

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Abbreviations

HSP-70: Heat shock protein-70

TNF- α : Tumor necrosis factor- α

EEX: Group administered ergothioneine before exercise

EEC: Group was not administered ergothioneine but exercised

EEN: Group not administered ergothioneine and not exercised

THI: Temperature-humidity index

RH: Relative humidity

DBT: Dry-bulb temperature

OCTN-1: Organic cation transporter novel type-1

ELISA: Enzyme-linked immunosorbent assay

Introduction

Horses are elite athletes compared to other animals; their respiratory, musculoskeletal, and cardiovascular systems are the most important systems during exercise [1, 2, 3]. Exercise testing is used to evaluate fitness and training status in these animals [4], and it is crucial to understand how exercise influences physiological responses so that the tests can be applied correctly [5, 6].

Determination of 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 [7, 8]. In comparison with other animals, the horse appears to have the disadvantage of having a fast metabolic rate and a small surface area for heat dissipation, especially when sweat evaporation is the primary mechanism of heat dissipation. Sweating dissipates at least two-thirds of the metabolic heat load in most workout situations [9].

Rectal temperature is closely linked to heat load following exercise; therefore, it is useful to examine health status under different physiological conditions. It is of value in evaluating the effects of training and also to examine the core body temperature capacity which is an important index of thermoregulation in horses [10, 11]. The work output of muscles and heat dissipation via the skin of horses during exercise depends on the heart size and capacity to deliver large volumes of blood to the tissues and the splenic reserve supply [12, 13]. It also depends on the capacity of the respiratory system to supply sufficient oxygen for cellular respiration [14, 15, 16].

Exposure of body tissues and cells to environmental stressors during exercise can cause serious disruptions in cellular homeostasis, which is primarily caused by harmful protein modifications, such as unfolding, misfolding, or aggregation. Cells produce a set of proteins known as HSPs or stress proteins in response to stress proteins, such as myosin heavy chain. In horses, exercise is a physiological stressor that has been established to affect the levels of HSPs. The HSPs are upregulated in response to cellular stress and are necessary to maintain cellular functions and prevent cellular death [17].

In horses, decreased skeletal muscle mass and strength have been associated with a range of chronic illnesses and higher mortality [18]. Consequently, understanding the underlying adaptability of skeletal muscle is crucial for maximizing health and longevity. Skeletal muscles have an exceptional ability to change phenotype in response to mechanical/metabolic stimuli, with muscle fiber size increasing (i.e., hypertrophy) or decreasing (i.e., atrophy) [19].

When secreted from muscle, TNF- α plays a cru-

cial role in a muscle state change. TNF- α is a pro-inflammatory cytokine that has been linked to muscle tissue loss, particularly in disease processes, such as cachexia [20] and wasting syndrome [21]. However, high levels of circulating TNF- α after exercise, for example, have been linked to higher muscle mass and strength in healthy adult horses due to the anti-inflammatory functions under certain conditions [20].

Ergothioneine is a relatively stable antioxidant because it does not auto-oxidize at physiological pH and does not enhance the production of hydroxyl radicals from H₂O₂ and Fe²⁺ ions as a Fenton reaction [22]. The fast clearance from the circulation into retained tissues with little metabolism and high stability, as well as its long half-life (approximately 30 days) and reduced tendency to auto-oxidize or produce free radicals from peroxide and iron at physiologic pH, are all beneficial physiologic properties of ergothioneine [23]. Ergothioneine requires the specific carrier protein OCTN-1 to cross the cell membrane [24, 25]. OCTN-1 is mainly found in the kidneys, trachea, erythrocytes, lungs, heart, and bone marrow [23]. Unlike other antioxidants, such as glutathione or N-acetyl-L-cysteine, EGT acts as a cation chelator [26], bioenergetics factor [27], the immune regulator [28], and antioxidant [29], and is widely distributed within the body tissues [30].

The aim of this study was to evaluate the effect of ergothioneine on rectal temperature and HSP-70 and TNF- α concentrations in response to exercise in horses in a hot-dry environment.

Results

The thermal environmental parameters obtained during the period of the study are presented in Table 1. The mean DBT increased significantly from 22.6°C \pm 0.24°C at 06.00 h to 38.6°C \pm 0.53°C at 12.00 h ($p < 0.05$) (Table 1). The mean THI also rose significantly from 76.41°C \pm 0.24°C to 83.36°C \pm 0.53°C at 06.00 h and 12.00 h, respectively ($p < 0.05$). The rectal temperature responses are shown in Table 2. The rectal temperature obtained in the EEX group (37.82°C \pm 1.91°C) was lower ($p < 0.05$) than the value obtained in the EEC group (38.87°C \pm 4.61°C) 15 min post-exercise.

Figure 1 shows serum HSP-70 concentrations of the stallions. The HSP-70 concentration of the EEX group was significantly ($p < 0.05$) higher than the values obtained in the EEC and EEN groups.

Figure 2 shows the concentration of TNF- α in the stallions. The TNF- α concentration of the EEX group was significantly ($p < 0.05$) higher than the values obtained in the EEC and EEN groups.

Table 3 shows serum HSP-70 concentrations of the stallions. HSP-70 concentration of the EEX group

was significantly ($p < 0.05$) higher than the values obtained in EEC and EEN groups.

Table 4 shows the concentration of TNF- α in the stallions. The TNF- α concentration of the EEX group was significantly ($p < 0.05$) higher than the values obtained in EEC and EEN groups.

Discussion

The thermal environmental data recorded during the study period were characterized by high temperat-

ue humidity index, ambient temperature, and relative humidity, typical of the hot dry season in the Southern Guinea Savannah zone of Nigeria. The mean AT values recorded during the research period were higher than the established thermoneutral zones of 5°C-25°C for horses [32, 33]. Moreover, the mean RH of 74.3% \pm 0.73% and 78.8% \pm 0.77% recorded at 12.00 h and 18.00 h, respectively, are clearly above the 70% recommended for horses [34].

The results indicated that the natural meteorological conditions were stressful to the horses and may impair their respiratory and cardiovascular responses and also impair their performance [10]. High ambient temperature and high RH are the most important meteorological indices, that cause heat stress in horses [7]. The heat generated during exercise, which is a byproduct of the inefficient metabolic production of energy, can accumulate when ambient temperature and RH are high because a hot, humid environment as observed in this study imposes added thermal stress on horses during exercise resulting in the inability of horses to dissipate heat effectively [15, 35].

The high THI of 76.41 \pm 0.24 to 83.24 \pm 0.49 recorded in the present study is higher than the accepted level recommended for horses [34] indicating that meteorological conditions prevailing in the study area were unfavorable 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. The rectal temperature of the EEX group was lower than the EEC group after exercise suggesting a modulatory role of ergothioneine on the thermoregulatory system of the stallions after exercise. Supplementation with ergothioneine before exercise suggests that the agent helps in a more efficient heat dissipation mechanism by a process that requires further

Table 1.

Thermal environmental parameters of the experimental site in the hot-dry season

Time of the Day	Dry-Bulb Temperature (°C)	Relative Humidity (%)	Temperature-Humidity Index
06.00	22.6 \pm 1.23 ^a	64.4 \pm 2.34 ^a	76.41 \pm 0.56 ^a
	(22-24)	(63-68)	(68.71-71.65)
12.00	38.6 \pm 6.53 ^b	74.3 \pm 6.73 ^b	83.36 \pm 4.53 ^b
	(37-39)	(72-78)	(81.32-89.01)
18.00	36.5 \pm 0.17 ¹	78.8 \pm 5.98 ²	83.24 \pm 3.49 ²
	(36-37)	(76-81)	(83.21-84.95)
Overall Mean \pm SEM	37.22 \pm 4.17	75.19 \pm 5.98	78.19 \pm 5.18

^{a,b} Means in the same column with different superscript letters are significantly ($p < 0.05$) different

^{1,2} Means in the same row with different superscript numbers are significantly ($p < 0.05$) different

Values in parentheses are minimum to maximum

Table 2.

Rectal temperatures of stallions

	EEN (°C)	EEC (°C)	EEX (°C)
Pre-exercise	37.76 \pm 3.61	37.69 \pm 2.97	37.58 \pm 2.77
15 min post-exercise	37.44 \pm 2.91 ^a	38.87 \pm 4.61 ^b	37.82 \pm 1.91 ^a
2 h post-exercise	37.43 \pm 3.01	37.87 \pm 3.13	37.53 \pm 2.74

^{a,b} Means in the same row with different superscript letters are significantly ($p < 0.05$) different

EEN: Not treated, not exercised

EEC: Not treated but exercised

EEX: Treated with ergothioneine before exercise

Table 3.

Heat shock protein-70 concentration in stallions subjected to exercise (values are in ng/ml)

	EEN (Mean \pm SEM)	EEC (Mean \pm SEM)	EEX (Mean \pm SEM)	P values
Pre-exercise	112.32 \pm 13.33	114.34 \pm 12.76	114.54 \pm 13.67	0.257
15 min post-exercise	117.54 \pm 13.65 ^a	118.54 \pm 14.55 ^a	137.34 \pm 26.55 ^b	0.019
2 h post-exercise	109.3 \pm 10.76 ^a	117.44 \pm 11.55 ^a	143.52 \pm 28.77 ^b	0.023

^{a,b} Means in the same row with different superscript letters are significantly ($p < 0.05$) different

EEN: Not treated, not exercised

EEC: Not treated but exercised

EEX: Treated with ergothioneine before exercise

study, thereby reducing the risk of exercise-induced hyperthermia, rhabdomyolysis, and heat stroke [36].

The concentration of HSP-70 was higher in the EEX group compared to the EEC and EEN groups indicating a modulatory role by ergothioneine. Ergothioneine may enhance the synthesis of HSP-70 by a mechanism that requires further study. HSP-70 has been demonstrated to decrease inflammatory processes by suppressing oxidative stress, reducing apoptosis and hyperplasia, as well as inhibiting the expression of adhesion molecules that lead to leukocyte extravasation and inflammatory cytokine production. HSP-70 interacts with several signal transduction pathways that affect cell homeostasis, proliferation, differentiation, and cell death, and is related to less atherosclerotic intima thickening and a lower risk of coronary artery disease [37]. The concentration of TNF- α was also higher in the EEX group than in the other groups. Ergothioneine might have activated TNFR2 receptors by a mechanism not yet understood. TNF-TNFR2 interaction activates the reciprocal PI3K/Akt pathway [38]. The TNFR2-Etk-vascular endothelial growth factor receptor 2 (VEGFR 2) complex, which is important in cell adhesion, migration, survival, and proliferation, is formed by

this pathway. The TNF-TNFR2 interaction also activates the PI3K/Akt pathway in the opposite direction. The TNFR2-Etk-VEGFR2 complex is implicated in cell adhesion, migration, survival, and proliferation after exercise [39]. Therefore, it can be inferred that supplementing racing horses with ergothioneine may

Table 4.

Tumour necrosis factor- α concentration in stallions subjected to exercise (values are in pg/ml)

	EEN (Mean \pm SEM)	EEC (Mean \pm SEM)	EEX (Mean \pm SEM)	P values
Pre-exercise	142.32 \pm 16.13	144.34 \pm 12.76	146.54 \pm 12.67	0.224
15 min post-exercise	144.54 \pm 16.25 ^a	161.84 \pm 14.55 ^a	187.54 \pm 26.55 ^b	0.027
2 h post-exercise	147.36 \pm 15.86 ^a	167.74 \pm 11.55 ^a	183.52 \pm 28.77 ^b	0.031

^{a,b} Means in the same row with different superscript letters are significantly ($p < 0.05$) different

EEN : Not treated, not exercised

EEC : Not treated but exercised

EEX : Treated with ergothioneine before exercise

Materials and Methods

Experimental Animals

The experiment involved 18 healthy Arabian stallions with a mean body weight of 401 \pm 32.11 kg (395-404 kg) and an age of 5.7 \pm 0.54 years (5-6 years). The stallions were from a standard stable in Ilorin, Nigeria (8 30 N, 4 33 E) that was exclusively used for pleasure riding. They were kept in a concrete-walled stable with a corrugated iron roof. They were fed hay with concentrate as a supplement. A constant supply of clean, cool water was also made available. Only apparently healthy animals were included in the research after the stallions were screened for gastrointestinal para-

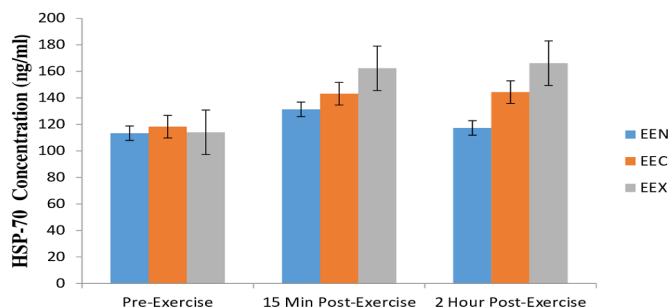


Figure 1. Heat shock protein-70 (HSP-70) concentration of the stallions
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX: Treated with ergothioneine before exercise

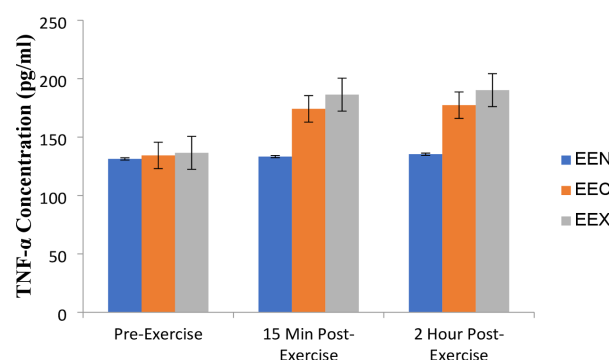


Figure 2. Tumor necrosis factor- α (TNF- α) concentration of the stallions
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX: Treated with ergothioneine before exercise

sites, haemoparasites, and ectoparasites.

Experimental Design

The stallions were divided into three groups of six horses each: Group I (EEX) horses were treated with ergothioneine at a dose of 0.02 mg/kg daily for two months before being subjected to exercise, Group II (EEC) were not treated before exercise, and the third group (EEN) neither were administered ergothioneine nor exercised. Prior to the commencement of the experiment, RT was taken using a digital clinical thermometer (Hartman's Company, England). The thermometer was inserted into the rectum of each animal, and the RT value was recorded after a beep sound indicating the end of the reading.

Exercise Protocol

Each of the stallions in the EEX group was saddled by properly dressed and trained riders weighing 70.56 ± 4.23 kg on a conventional horse race track and raced for 2000 m at maximum speed. Horses were kept in the shade immediately after exercising.

Blood Sampling

Blood samples (10 ml) were taken from each stallion using 18-gauge needles before the experiment, as well as 15 min and 2 h after exercise. The collection site was disinfected before sampling with a cotton swab bathed in methylated spirit. The samples were taken from the jugular vein and were placed in plain vacuum containers.

Determination of Thermal Environmental Parameters

The DBT and RH were determined using the wet and dry-bulb thermometer (Mark, England). The THI was calculated using the formula of Hartmann et al. [31]:

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

Assessment of Heat Shock protein-70

MyBioSource horse HSP-70 ELISA detection kit (San Diego, California, USA) was used to measure the serum HSP-70 levels. It is an ELISA kit for detecting HSP-70 in microwell, strip plate format. The ELISA analytical biochemical technique used HSP-70 antibody-antigen interactions (immunosorbency) and a colorimetric detection device to detect HSP-70 antigen in serum.

Measurement of Serum Tumor Necrosis Factor- α

The concentration of TNF- α in the serum was evaluated using the TNF- α ELISA kit (Mybiosource.com, San Diego, California, United States). Sandwich enzyme immunoassay is the method used in the kit. The package included a microtiter plate that was pre-coated with an anti-TNF- α antibody. A biotin-conjugated antibody specific for tumor necrosis factor- α was added to the appropriate microtiter plate wells with serum. Afterwards, each microplate well was treated with Avidin coupled with horseradish peroxidase. Only the color of the wells containing TNF- α , biotin-conjugated antibody, and enzyme-conjugated Avidin changed once the TMB substrate solution was added. The color shift was detected spectrophotometrically at a wavelength of 450 nm 10 min after the enzyme-substrate reaction was stopped by adding sulphuric acid solution. By comparing the optical density of the samples to the standard curve, the concentration of TNF- α in the samples was measured.

Data Analysis

The results of this experiment are presented as mean \pm SEM and were tested for normality using the Shapiro-Wilk test. All the data were found to be normally distributed. The one-way analysis of variance was used to analyze all the data, followed by the Tukey post-hoc test. The analyses were carried out using the software Graph Pad Prism (version 5.3).

Authors' Contributions

ASA and JOA conceptualized and designed the study. ASA, JOA, PIR, and TA wrote and edited the manuscript. DAA and ASA performed the experiments and analyzed the data.

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Conflicts of Interest

The authors state that they have no competing interests.

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