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# Evaluation of Trace Elements, Oxidant, and Antioxidant Status in Polo and Dressage Horses with or without Lameness

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**Abstract**

HORSES are used for sports such as polo or dressage are prone to exercise-induced oxidative stress, due to increased energy demands during exercise. There are no reports of levels of Reactive oxygen species (ROS ) formation, antioxidant response, and trace minerals' profile variations between polo and dressage horses, particularly in conjunction with the occurrence of lameness. This study, therefore, compared the serum concentrations of trace elements (TE) and oxidants/antioxidants between polo and dressage horses. Forty healthy horses, comprising polo (33) and dressage (7) were sampled. The polo horses were made up of 21 females and 12 males, while the dressage horses were made up of 2 females and 5 males. Blood (5mls) was collected from the jugular vein into plain bottles for the determination of serum concentrations of selected oxidants and antioxidants, ascorbic acid, Manganese, Copper, Zinc and Magnesium using spectrophotometric and colorimetric techniques. Means of the oxidants and antioxidants were compared between groups using an independent T-test. Correlation between the oxidants, antioxidants and trace elements was determined using Pearson correlation coefficients. Results were considered significant at $P \leq 0.05$. Serum malondialdehyde (MDA) concentration was higher (($P < 0.05$)) in polo (2.21 ± 1.05 U/L) than dressage horses (1.71 ± 0.48 U/L). Similarly, serum MDA and $GP_X$ concentrations were higher ($P < 0.05$) in males (2.58 ± 1.08 U/L; 25.30 ± 6.52 U/L) than in female horses (1.93 ± 0.90 U/L; 16.2 ± 4.80 U/L). However, concentrations of MDA were significantly ($P < 0.05$) lower in shod (1.63 ± 0.74 U/L) than in unshod horses (1.63 ± 0.74 U/L). Also, serum magnesium was significantly ($P < 0.05$) lower in polo (2.67 ± 0.88 mg/dl) than in dressage horses (3.717 ± 1.97 mg/dl). It can be concluded that the use, sex, and shoeing status of horses affect concentrations of MDA and antioxidants. This could have implications for the precipitation of degenerative or metabolic diseases related to oxidative stress in predisposed horses.

**Keywords**: Horses, Polo, Dressage, Trace minerals, Oxidants, Antioxidants

## Introduction

Lipid peroxidation, mediated by free radicals is a major cause of cellular damage. Reactive oxygen species are produced both during physiological and pathological processes in tissues [1]. The failure of the body to curtail lipid peroxidation (due to a deficient antioxidant system), results in oxidative stress (OS) induced formation of lipid peroxides and reactive oxygen species (ROS) leading to the accumulation of their final products; malondialdehyde (MDA) in different body tissues [2]. Age-related decline in mitochondrial population and activities plays important role in protection free radical induced oxidative damage. Reactive oxygen

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species up-regulates pro-inflammatory cytokines and matrix metalloproteinase (MMP) enzymes leading to cartilage degradation [3].

Dressage is a horse riding sport in which the rider and the horse performed series of predetermined movements from memory [5]. The purpose is to develop in a horse natural athletic ability through standardized progressive training methods, thus maximizing the horse riding potential. Polo on the other hand is an equine sport played on horseback in which two teams play against each with the aim of scoring using a long wooden mallet [2].

Equine athletes, such as racing, polo, or dressage horses have been documented to suffer exercise related immune or inflammatory disorders [6]. Intense exercise induce subclinical tissue damage with resultant activation of the immune system. Exercise-induced activation of the immune systems have been demonstrated in horses to be comparable to acute phase responses [7]. Horses exposed to increased levels of physical stress experience changes in oxidative stress biomarkers following its onset. Acute exercise increases ROS production in horses, while chronic endurance training has been shown to enhance antioxidant-scavenging capacity in horses. Several reports have shown that horse races induce oxidative stress and skeletal muscle damage, which is often negatively associated with athletic performance [6, 7]. Thus, it is logical to expect that the serum composition will be affected by the activity level of a horse. Although there are several reports on the influence of horse activities on the serum concentrations of oxidant and antioxidant compounds, most of the studies have focused on racing horses, while none has compared the oxidant/antioxidant status between polo and dressage horses.

Trace elements (TE) are dietary minerals required in minute amount for the proper development. Deficiencies in essential TEs such as copper (Cu), zinc (Zn) and manganese (Mn) have been reported to induce developmental orthopaedic diseases in horses [8]. Zinc as an essential TE associated with regulatory process of keratinization and maintenance of the structural integrity and health of the hoof [9]. Selenium and Vitamin E function as antioxidants to protect body tissues from free radical damage and have been associated with [10] white-line diseases of the hoof in the horse [11]. Deficiency of manganese results in epiphyseal plate narrowing, and decrease in blood vessel migration into growth plate. Recent studies have shown that the serum concentration of TEs varies with the diet of the horse, sex, and living condition [12, 13]. From the aforementioned, TEs could probably play a crucial role in the pathophysiology of equine lameness and their dynamics under different use (polo or dressage), sex, and shoeing status of a horse may provide useful insights for improved management practices for the enhancement of the overall health and well-being of sport horses. However, there have been no reports on the differences in serum concentration of TEs in equestrian sports horses under different conditions of use, shoeing, lameness, and sex. Therefore, the objective of this study was to determine and compare the serum concentrations of oxidant (MDA) and antioxidants (SOD, GSH, $GP_{X,}$ and ascorbic acid) between polo and dressage horses and further examine the differences in serum concentration of TEs (Mn, Cu, Zn, and Mg) between polo and dressage horses with or without lameness, shoes, and different sexes.

## Material and Methods

### *Study Animals*

The horses used for this study were Polo horses of the Ibadan Polo club (Eleyele, Ibadan, Oyo State, Nigeria) and dressage horses at the Nigeria Police Command (Eleyele), Ibadan. History of the diet of the horses showed that all the horses were fed grass, wheat bran, and finished feed. Approval for this study was obtained from Research and Ethics Committee, Federal University of Agriculture (FUNAAB/COLVET/CREC/2018/07/02), and informed consent was obtained from the appropriate authorities before the commencement of the study. Each horse was individually examined physically and the findings were documented. The history of each horse was obtained using a structured questionnaire. Thereafter, each horse was walked and trotted by the handler to assess the gait subjectively according to the criteria dictated by the American Association of Equine Practitioners. The shoeing status of each horse as well as the condition of the shoes was noted.

### *Blood Collection*

Before blood collection, each horse was walked and moderately trotted by the handlers. Blood (5mls) was obtained through jugular vein of each horse into universal bottle for harvesting of serum and determination of serum concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase ($GP_x$), ascorbic acid, Manganese (Mn), Copper (Cu), Zinc (Zn) and Magnesium (Mg). The blood samples were allowed to clot and serum separate, to allow the serum to be aspirated into Eppendyorf tubes. The harvested serum was stored at -20℃ until ready for assay.

### *Determination of Serum Concentrations of MDA, Antioxidants, and Trace Elements*

### *Malondialdehyde*

The serum concentration of MDA was determined spectrophotometrically as earlier described [14]. Approximately 0.05mL of horse serum was mixed with 1.0mL of Thiobarbituric acid

(TBA) reagent, incubated for 15 minutes in hot water bath. The tube was placed under a running, centrifuged for 10 minutes at 1000 *rpm*. The supernatant absorbance was read against a blank at 535nm. Concentration of MDA was determined using the formula:

$$\text{Concentration (M)} = \frac{\text{Absorbance}}{\varepsilon}$$

* Where 1.56 x $10^5 M^{-1}cm^{-1}$ is the extinction coefficient (ε) of the MDA-TBA complex at 535 nm.

*Superoxide dismutase (SOD)*

The serum concentration of SOD was determined spectrophotometrically [14]. Test tubes were marked blank (B) and test (T). 100µL of the equine serum sample was pipetted into test tube T. 100µL buffered potassium phosphate and 830µL of distilled water were pipetted into tubes B and T. The tubes were incubated at 25°C for 10 minutes and 20µL of pyrogallol was added. The tubes were mixed by inversion and the absorbance recorded with a spectrophotometer for three minutes at 420nm. Difference in absorbance and the average absorbance difference was calculated (Δ420/min).

SOD concentration was calculated using the formula:

$$\%\ \text{Inhibition} = \frac{(\Delta A420\text{nm/min Blank} - \Delta A\ 420\text{nm/min of the sample}) \times 100}{\Delta A\ 420\text{nm/min Blank}}$$

*Glutathione (GSH)*

The serum GSH concentration was measured spectrophotometrically [14]. 0.5mL of the equine serum (5µL of serum + 495µL of $dH_2O$) was added to 0.5mL Trichloroacetic acid (TCA) and centrifuged for 15 minutes at 3000rpm. 0.4mL of supernatant and 0.8mL of 0.4M Tris buffer pH 8.9 was mixed with 20µL of freshly prepared 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Absorbance was read with a spectrophotometer at 412nm against a blank containing water instead of the equine serum, 5 minutes after the addition of DTNB. Serum GSH concentration was measured as:

$$\text{mM GSH} = \frac{\text{Absorbance x 2.44 x dilution factor}}{14.15}$$

* Where $14.15 Mm^{-1}cm^{-1}$ is the molar extinction coefficient of NTB and 2.44 is the dilution factor of the sample in a 1.22mL assay mixture

**Glutathione peroxidase (GPX)**

The serum concentration of $GP_X$ was determined spectrophotometrically [14]. Two test tubes were labelled blank (B) and test (T). 200µL of 0.4M buffered potassium, 100µL of sodium azide, 200µL of 10mM GSH, 100µL of 0.2mM hydrogen peroxide and 200µL of serum were placed into tube T, while 400µL of 0.4M buffered potassium phosphate buffer, 100µL of sodium azide, 200µL of 10mM GSH and 100µL of 0.2mM hydrogen peroxide were placed in tube B. The tubes were incubated for 10 minutes, followed by addition of 10% trichloroacetic acid (TCA) to both tubes. The mixtures were then centrifuged for 15 minutes at 3,000rpm. 400 µL of the supernatant was added to 800µL of 0.4M Tris buffer and 20µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Absorbance was determined following addition of DTNB using spectrophotometer at 412nm. Activity of $GP_X$ was determined in terms of concentration of GSH utilized as mM GSH = Absorbance / 14.15

* Where 14.15 is the molar extinction coefficient of NTB

$GP_X$ activity was calculated as:

$$\text{U/min} = \frac{\text{initial GSH concentration} - \text{Final GSH concentration}}{\text{Time}}$$

*Ascorbate*

Serum ascorbic acid concentration was measured colorimetrically [15] using Ascorbate Assay Kit™ (Sigma-Aldrich, USA). The assay catalyst was diluted 10-fold with water and mixed well. Thereafter 30 mL of diluted catalyst was added to each standard and well. 50 mL of the Master Reaction Mix was then added to the wells. The wells were mixed and incubated for 3-5 minutes at room temperature. The absorbance of the color change was then determined at 570 nm. Concentration of Ascorbic Acid (C) was calculated as Sa/Sv = C; where

Sa = Ascorbic Acid concentration in unknown sample from a standard curve

Sv = volume of serum added to the wells

*Copper*

The serum concentration of Cu was measured colorimetrically using copper colorimetric assay kit™ (Abnova, Taiwan). Test tubes were labeled as Sample Test (ST), Sample Blank (SB), and Reagent Blank (RB). Distilled water (100 µL) was pippeted into RB. Standard (20 µL) and distilled water (80 µL) was pippeted into SB, while equine serum (100 µL) was pippeted into ST. Reagent A (35 µL) was added to each tube and mixed by vortexing. 100 µL of ST, SB, and RB was placed in separate wells of a clear flat-bottom 96-well plate. Working Reagent (150 µL) was added to each well and mixed. Resultant mixture was incubated at room temperature and absorbance was determined at 359 nm. Corrected absorbance (ST-{SB + RB}) was used to calculate Cu concentration using the extinction co-efficient derived from the calibration curve.

*Manganese*

The serum concentration of Mn was determined colorimetrically [15] using Mn Assay Kit™ (Sigma Aldrich CO, UK). 10 µL of the equine serum was pipetted into duplicate wells. Assay Buffer (10 µL) was added into duplicate wells. Substrate Preparation (50 µL) was added to each well. Xanthine Oxidase Preparation (25 µL) was then added to each well. The mixture was incubated at room temperature for 20 minutes. Optical density was read at 450 nm and a standard curve was created. A standard equation was then derived to calculate the Mn concentration in the equine samples.

*Magnesium*

Serum concentration of Mg was measured using the spectrophotometry method. Three tubes were labeled as Sample Test (ST), Sample Blank (SB), and Reagent Blank (RB). Equine serum (20 µL) was added to ST and SB, while Tris solution (20 µL) to RB. 80µl of Calmagite was added to the tubes, vortex mixed, and incubated at for 10 min. 60µl of ethylene glycol tetra-acetic acid was added to ST and RB, while dietylamine (200µl) was added to SB. Absorbance was recorded at 480 nm after 5 min. Corrected absorbance of the sample (ST-{SB + RB}) was used to calculate Mg concentration using calibration curve derived extinction co-efficient

*Zinc*

The serum Zn concentration was measured colorimetrically using Zinc assay Kit™ (Sigma-Aldrich, USA). Zn Reagent Mix (200 mL) was pippeted into each well. The wells were mixed and then incubated for 10 minutes at room temperature. The absorbance of the colour change was then recorded at 570 nm (A570).

The concentration of Zn (C) was calculated as Sa/Sv = C; where

Sa = Amount of zinc from standard curve

Sv = Volume of serum sample added to well

*Data Analysis*

Serum concentrations of oxidants, antioxidants and TEs were presented as means and standard deviations. Correlations between TEs and oxidants/antioxidants in horses with lameness were done using Pearson's correlation coefficient test. Differences in concentrations of TEs, MDA, and antioxidants between dressage and polo horses, between horses with lameness and those without lameness were determined using an unpaired student t-test. Differences were significant at $P \leq 0.05$. [14].

## Results

A total of forty (40) horses comprising 33 polo horses and 7 dressage horses were sampled. The polo horses were made up of 21 females and 12 males, while the dressage horses were made up of 2 females and 5 males. The mean height of the polo horses was 156.5 ± 12.4 cm (height range = 146 – 172 cm), while the mean height of the dressage horses was 147.0 ± 11.6 cm (height range = 139 – 157 cm). Also, the mean weight of the polo horses was 431.1 ± 137.8kg (weight range ≡ 297 – 525kg), while the mean weight of the dressage horses was 313.8 ± 128.7kg (weight range ≡ 236 – 382kg). Both the heights and body weights were significantly ($P < 0.05$) higher in the polo horses than in the dressage horses. Eight (8/33) of the polo horses were shod, while none of the dressage horses (0/7) were shod. History of the diet of the horses showed that all the horses were fed grass, wheat bran, and finished feed. None of the horses was fed on any special supplement. The health history of the polo horses showed that (22/33) had previous episodes of colic, while (6/7) of the dressage horses had a previous history of colic. In addition, 19/33 of the polo horses were evaluated to be lame, while 3/7 of the dressage horses were evaluated to be lame.

The variation of serum oxidants and antioxidants with sex in the horses is shown in Table 1. The concentration of MDA was significantly ($P < 0.05$) higher in male horses than females. Similarly, $GP_X$ concentration was higher ($P < 0.05$) in male horses than in the female horses. However, concentrations of SOD, GSH, and ascorbic acid did not differ significantly between the sexes. The concentrations of Zn and Mg tended to be higher in females than in male horses (Table 1). However, the values were not statistically significant. Conversely, concentrations of Cu and Mn tended to be higher in the male than female horses. The values were also not statistically significant ($P > 0.05$).

The effect of shoeing on concentrations of oxidants and antioxidants in horses is shown in Table 2. The MDA was significantly ($P < 0.05$) lower in shod than unshod horses. Similarly, concentration of GSH was significantly ($P < 0.05$) lower in shod horses compared to unshod horses. However, SOD and ascorbic acid were higher ($P < 0.05$) in shod horses compared to unshod horses. Equally, there was no significant difference in the serum concentration of $GP_X$ between shod and unshod horses. The effect of shoeing on the concentrations of TEs in horses is shown in Table 2. Zinc, Mg, and Mn tended to be lower in shod horses than in unshod horses. However, the values were not statistically significant. Moreover, Cu serum did not differ significantly between shod and unshod horses.

The effect of lameness on serum concentrations of oxidants and antioxidants in horses is shown in Table 3. The SOD and GPX tended to be lower in lame horses than in normal horses. However, the values were not statistically significant. Similarly, there were no significant differences in the concentrations of MDA, GSH, and ascorbic acid

between lame and normal horses, although values tended to be lower in lame than normal horses. The effect of lameness on TEs in horses is shown in Table 3. Serum concentrations of Zn, Cu, Mg, and Mn tended to be lower in lame horses compared with normal horses. However, all the measured TEs did not vary significantly between lame and normal horses.

Concentrations of MDA was significantly ($P < 0.05$) higher in polo horses than in dressage horses (Table 4). Similarly, SOD was higher in polo than in dressage horses, although the differences were not significant. On the other hand, the GSH, $GP_X$, and ascorbic acid did not differ between polo and dressage horses, although the values tended to be lower in polo than in dressage horses. The values of Mg were significantly ($P < 0.05$) lower in polo than in dressage horses (Table 4). Also, the concentration of Zn was significantly lower in polo horses than in dressage horses, although the values were not significant. Copper and Mg levels did not differ significantly between polo and dressage, although the values tended to be higher in polo.

The Pearson correlations between oxidants, antioxidants, and TEs in lame, polo, and dressage horses are shown in Tables 5 – 7. Copper and ascorbic acid were positively correlated with SOD, while Mg and Mn were positively correlated with MDA in lame horses (Table 5). In polo horses, serum Zn, Mg, and ascorbic acid were positively correlated with SOD, while Mn was positively correlated with MDA and Cu (Table 6). Furthermore, in dressage horses, Cu was positively correlated with SOD and $GP_X$, and Mn was positively correlated with MDA (Table 7).

## Discussion

The demographic records of the studied horses showed that the majority of the polo horses were females, while a majority of the dressage, were male. It has been reported that sex preferences exist, regarding the use of horses in sports [16]. Mares were reported to be more favored for polo and racing, while stallions are preferred for dressage. This might explain the higher proportion of females among the polo horses, and males among the dressage horses, observed in the present study. Although stakeholders within the equestrian sports industry have sex preference for horses, Current studies revealed a low inter-study agreement on the effects of sex on trainability, responsiveness, excitability anxiety display [17].

In this study, the finding of higher serum concentrations of MDA in males than in female horses, and concentrations of antioxidants being higher in the males (although not significant) except for SOD, may suggest that there is a sexual dimorphism twist to the occurrence of lipid peroxidation in horses. For example, from human studies, it has been recognized that mitochondria from females generated only half the amount of superoxide radicals generated by males [18]. Correspondingly, a higher total antioxidant status was reported in female neonates, than in their male counterparts [19]. However, previous studies in horses did not report sex differences in the plasma concentrations of oxidants and antioxidants [20, 21]. In the present study, it appears that male horses have an overall higher stimulation of the OS pathways (high MDA) which may consequently have induced the production of more antioxidant enzymes (as required) by males. It is not immediately clear if there is any advantage in male horses, of being able to handle OS better than their female counterparts.

The imbalance in the number of females versus male horses used in this study could be a confounding factor in the significance levels obtained when comparing the variations of oxidants and antioxidants. This limitation of the unequal number of subjects per sex, justifies further studies, using balanced models, to ascertain the role of sex in the levels of, and responses to, OS in sport horses.

Previous studies have shown that the effect of sex on serum TEs is not consistent and are species dependent [11, 22]. In mice, only serum Zn differed significantly between sexes [22]. In donkeys, TE differences were only noticed for serum Mn, Cu, and Zn between male and female animals [11]. In horses, significant differences have only been reported in selenium and chromium [23] between the sexes. This present study showed no significant differences in serum TEs between males and females. This may be because the TEs determined in this study were different from those reported to be affected by sex in horses, from previous studies [23]. This assertion, and the actual role of sex in the profile of equine serum TEs, needs further future validation, especially also, as it relates to sports horses.

Horseshoes help to protect the hoof of the horse from excessive wear and tear. It also prevents the hoof wall from splitting. In general, it keeps the horse working in comfort and enables a balanced foot with a normal axis and is particularly important in athletic horses [24]. This study indicates a lower level of OS (MDA) and higher antioxidant (SOD) status in shod than in unshod horses. This finding supports the theory that shoeing ameliorates OS on the horses by generally keeping the horse in a more comfortable body condition. Thus, the shoeing of horses, especially, sports horses should be encouraged to improve equine welfare.

The quality of the keratinized structures of the hoof is dependent on minerals such as calcium (Ca), Zn, or Cu [25]. Thus, it could be speculated that serum concentrations of TEs such as Zn, Cu, Mg, and Mn will be higher in shod horses than in their unshod counterparts, due to continuous uptake of

these TEs for continuous hoof development as a result of the regular wear and tear. Interestingly, there were no significant differences in the concentrations of the TEs between shod and unshod horses in this present study. This finding may be attributable to the smaller number of shod horses compared with the unshod horses or may be an indication that the serum concentrations of TEs in horses are affected by multifactorial variables, for example, the dietary content of these essential elements or other physiological parameters. To gain a better understanding of all other potential factors that can influence the dynamics of serum TE in relation to shoeing status, further comprehensive studies are warranted.

Pathology of lameness in dairy cows has been associated with oxidative stress through induction of hoof tissues dyskeratosis, chondrocyte apoptosis, and cartilage degeneration [26]. In a recent study, also in dairy cows, increased MDA and a low SOD were reported in lame cows [27]. However, there is a dearth of information on the role of OS on hoof lameness in horses. In this study, we found no significant differences in the oxidant, MDA, between lame and walking horses, and also in the serum SOD and $GP_{X,}$ but the antioxidant enzymes tended to be lower in the lame horses than the normal horses. The lower concentration of antioxidants in lame hoses compared with healthy horses is in agreement with similar observations in lame cows and may be associated with the depletion of antioxidants secondary to OS. In addition, this study also showed that Zn, Cu, Mn, and Mg tended to be lower in lame horses compared with healthy horses. Comparably, decreases in serum Zn, Cu, and Mn have been reported in lame dairy cows [28]. These observations may be associated with the role of some of these TEs e.g. Cu, Zn, as critical components of the antioxidant system, scavenging free radicals during OS.

Physical activity of varying intensities has been demonstrated to induce OS not only in human athletes but also in show jumpers and dressage horses, pentathlon horses, and racehorses [29]. However, there has been no comparison made, of the severity of OS between different equine activities. In this study, concentrations of MDA were significantly higher in polo compared to dressage horses. This may suggest that the intensity of energy metabolism (which is directly associated with free radical generation) during polo is greater than in dressage or show jump horses. However, more controlled studies will be required to confirm if this observation is accurate. In the same vein, Mg was significantly lower in polo than in dressage horses. This may be due to increased consumption of water and general metabolism, leading to higher urinary excretion rates in polo horses because of increased exercise intensity. Results here mirror those of human marathon runners whose serum Mg was significantly lowered, without a corresponding change in concentrations of Ca, Cu, or Zn following a marathon race [30].

Result of SOD being positively correlated with Cu and ascorbic acid, while MDA being positively correlated with Mn and Mg in lame horses, is probably due to the role of Cu as a constituent of SOD. Superoxide dismutase is an enzyme that catalyze the conversion of superoxide free radicals to water [31]. The SOD exists in two isoforms, namely Cu and Zn isoforms. In humans, a positive correlation was reported between SOD and ascorbic acid following a single bout of exercise in women [32]. The findings on antioxidants in this study are like that, reported for humans following exercise and suggest the possible role of ascorbic acid in ameliorating signs of lameness by decreasing tissue damage originating from OS. Ascorbic acid also facilitates the regeneration of new tissue, thereby promoting healing. Therefore, it can be advocated that ascorbic acid be supplemented in lame polo horses to promote tissue regeneration and healing.

Further, in humans, a negative correlation has been recently reported between MDA and Mg [33]. This is contrary to the findings of this study, where a positive correlation was found between MDA and Mg in lame horses. The research findings suggest that lameness may be associated with increased release of Mg from body tissues into the plasma, secondary to tissue damage. This however requires a more comprehensive study, to confirm the actual relationship between systemic oxidant preponderance and Mg metabolism in horses.

Finally, a few limitations by way of the number and distribution of horses, in interest groups, sampled in this study existed and appeared to skew the data. For instance, only 8 out of the 40 horses sampled were shod. Also, only 7 out of the 40 horses were dressage horses, while the rest were used for polo. This unequal distribution of the study animals between groups could be attributable to the socioeconomic realities, consisting, of levels of demand and supply, the scale of awareness, deployed management practices, and the general stride of the equine industry in Nigeria. Another possibly confounding situation experienced during this study was the period of time during sample collection. For the polo horses, the samples were collected outside of a polo tournament indicating that the horses were sampled outside of the peak activity period. This may have influenced the values of oxidants, antioxidant enzymes, and TEs obtained in this study. However, it is important to note that both the polo and dressage horses were trotted moderately before the samples were obtained. Nevertheless, the data obtained from this study represent a solid baseline and repository for understanding the relationship between oxidative stress, sex, shoeing, and lameness in polo and dressage horses in Nigeria.

## Conclusion

It can be said that the biological parameter (sex), management and usage practices (shoeing and type of sport), and the pathological condition of lameness, affect the serum concentrations of MDA and antioxidants significantly, but not the concentration of TEs. This information is useful for harnessing management practice upgrades for the prevention and control of metabolic and degenerative diseases in sports horses. Data from this study could also contribute to guiding informed decisions on selection criteria that would favor optimum delivery in these high-performance equines.

*Data availability statement:* The data supporting the results presented in the paper is available on request.

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*Conflicts of interest*

The authors report there are no competing interests to declare.

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**TABLE 1. Effect of sex on serum concentration of oxidants, antioxidants, and trace elements in horses**

| Oxidant/Antioxidant Concentration | Male (N ≡ 17) | Female (N ≡ 23) |
|---|---|---|
| Malondialdehyde (U/L) | 2.58 ± 1.08 | 1.93 ± 0.90* |
| Superoxide dismutase (U/L × $10^9$) | 6.92 ± 2.39 | 7.32 ± 4.32 |
| Glutathione (U/L) | 237.30 ± 6.32 | 234.96 ± 4.80 |
| Glutathione peroxidase (U/L) | 25.30 ± 6.52 | 16.2 ± 4.80* |
| Ascorbic acid (mg/L) | 4.08 ± 0.90 | 4.04 ± 1.17 |
| Zinc (mg/L) | 1.17 ± 0.39 | 1.42 ± 0.50. |
| Copper (µg/dl) | 57.75 ± 7.28 | 54.10 ± 5.54 |
| Magnesium (mg/dl) | 2.83 ± 1.40 | 2.86 ± 1.11 |
| Manganese (ng/ml) | 4.67 ± 1.61 | 4.07 ± 1.30 |

* $P < 0.05$ is significant

**TABLE 2. Effect of shoeing on serum concentration of oxidants and antioxidants in horses**

| Oxidant/Antioxidant Concentration | Shod (N ≡ 8) | Unshod (N ≡ 32) |
|---|---|---|
| Malondialdehyde (U/L) | 1.63 ± 0.74 | 2.25 ± 1.01* |
| Superoxide dismutase (U/L × $10^9$) | 9.38 ± 1.74 | 6.66 ± 1.82* |
| Glutathione (U/L) | 233.00 ± 3.52 | 236.31 ± 5.57* |
| Glutathione peroxidase (U/L) | 15.50 ± 5.23 | 19.78 ± 11.70 |
| Ascorbic acid (mg/L) | 4.63 ± 1.06 | 3.91 ± 1.06* |
| Zinc (mg/L) | 1.25 ± 0.46 | 1.37 ± 0.49. |
| Copper (µg/dl) | 55.50 ± 4.99 | 55.13 ± 10.67 |
| Magnesium (mg/dl) | 2.63 ± 1.0 | 2.91 ± 1.23 |
| Manganese (ng/ml) | 3.88 ± 1.55 | 4.34 ± 1.30 |

* $P < 0.05$ is significant

**TABLE 3. Effect of lameness on serum concentration of oxidants and antioxidants in horses**

| Oxidant/Antioxidant Concentration | Lame (N ≡ 22) | Normal (N ≡ 18) |
|---|---|---|
| Malondialdehyde (U/L) | 2.00 ± 0.81 | 2.28 ± 1.17 |
| Superoxide dismutase (U/L × $10^9$) | 6.59 ± 2.06 | 7.94 ± 5.21 |
| Glutathione (U/L) | 235.45 ± 5.18 | 235.89 ± 5.63 |
| Glutathione peroxidase (U/L) | 18.50 ± 11.94 | 19.44 ± 9.53 |
| Ascorbic acid (mg/L) | 4.05 ± 1.17 | 4.06 ± 1.00 |
| Zinc (mg/L) | 1.27 ± 0.45 | 1.44 ± 0.51 |
| Copper (µg/dl) | 53.36 ± 5.41 | 57.44 ± 8.84 |
| Magnesium (mg/dl) | 2.64 ± 1.21 | 3.11 ± 1.13 |
| Manganese (ng/ml) | 4.18 ± 1.46 | 4.33 ± 1.37 |

* $P < 0.05$ is significant

**TABLE 4. Comparison of serum concentration of oxidants and antioxidants in polo and dressage horses**

| Oxidant/Antioxidant Concentration | Polo (N ≡ 33) | Dressage (N ≡ 7) |
|---|---|---|
| Malondialdehyde (U/L) | 2.21 ± 1.05 | 1.71 ± 0.48* |
| Superoxide dismutase (U/L × $10^9$) | 7.36 ± 4.09 | 6.43 ± 2.22 |
| Glutathione (U/L) | 235.45 ± 5.04 | 236.57 ± 6.90 |
| Glutathione peroxidase (U/L) | 17.72 ± 8.9 | 24.57 ± 6.91 |
| Ascorbic acid (mg/L) | 4.03 ± 1.13 | 4.14 ± 0.90 |
| Zinc (mg/L) | 1.30 ± 0.46 | 1.57 ± 0.53 |
| Copper (µg/dl) | 56.78 ± 10.88 | 47.71 ± 8.04 |
| Magnesium (mg/dl) | 2.67 ± 0.88 | 3.71 ± 1.97* |
| Manganese (ng/ml) | 4.36 ± 1.47 | 3.71 ± 0.95 |

* $P < 0.05$ is significant

**TABLE 5. Correlation between serum concentration of oxidant, antioxidant, and trace elements in lame horses**

| Correlations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **SOD** | **MDA** | **GSH** | **GPX** | **ZN** | **CU** | **MG** | **MN** | **Vit. C** |
| SOD | Pearson Correlation | 1 | -0.057 | -0.155 | 0.2 | 0.074 | .570** | -0.157 | -0.006 | .559** |
| | Sig. (2-tailed) | | 0.803 | 0.49 | 0.372 | 0.745 | 0.006 | 0.485 | 0.98 | 0.007 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| MDA | Pearson Correlation | -0.057 | 1 | 0.292 | -0.015 | 0.128 | -0.042 | 0.144 | .715** | -0.298 |
| | Sig. (2-tailed) | 0.803 | | 0.187 | 0.948 | 0.57 | 0.854 | 0.523 | 0 | 0.178 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| GSH | Pearson Correlation | -0.155 | 0.292 | 1 | 0.167 | 0.146 | -0.242 | -0.04 | 0.126 | -0.152 |
| | Sig. (2-tailed) | 0.49 | 0.187 | | 0.456 | 0.516 | 0.279 | 0.858 | 0.576 | 0.499 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| GPX | Pearson Correlation | 0.2 | -0.015 | 0.167 | 1 | -0.087 | -0.013 | 0.108 | -0.141 | 0.049 |
| | Sig. (2-tailed) | 0.372 | 0.948 | 0.456 | | 0.699 | 0.954 | 0.632 | 0.531 | 0.828 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| ZN | Pearson Correlation | 0.074 | 0.128 | 0.146 | -0.087 | 1 | 0.006 | 0.101 | 0.278 | 0.243 |
| | Sig. (2-tailed) | 0.745 | 0.57 | 0.516 | 0.699 | | 0.98 | 0.653 | 0.21 | 0.277 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| CU | Pearson Correlation | .570** | -0.042 | -0.242 | -0.013 | 0.006 | 1 | -0.247 | 0.068 | 0.32 |
| | Sig. (2-tailed) | 0.006 | 0.854 | 0.279 | 0.954 | 0.98 | | 0.269 | 0.762 | 0.147 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| MG | Pearson Correlation | -0.157 | 0.144 | -0.04 | 0.108 | 0.101 | -0.247 | 1 | 0.279 | -0.055 |
| | Sig. (2-tailed) | 0.485 | 0.523 | 0.858 | 0.632 | 0.653 | 0.269 | | 0.209 | 0.81 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| MN | Pearson Correlation | -0.006 | .715** | 0.126 | -0.141 | 0.278 | 0.068 | 0.279 | 1 | -0.088 |
| | Sig. (2-tailed) | 0.98 | 0 | 0.576 | 0.531 | 0.21 | 0.762 | 0.209 | | 0.697 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| Vit. C | Pearson Correlation | .559** | -0.298 | -0.152 | 0.049 | 0.243 | 0.32 | -0.055 | -0.088 | 1 |
| | Sig. (2-tailed) | 0.007 | 0.178 | 0.499 | 0.828 | 0.277 | 0.147 | 0.81 | 0.697 | |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |

** Correlation is significant at the 0.01 level (2-tailed).

**TABLE 6. Correlation between serum concentration of oxidant, antioxidant, and trace elements in polo horses**

| Correlations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **SOD** | **MDA** | **GSH** | **GPX** | **ZN** | **CU** | **MG** | **MN** | **Vit. C** |
| SOD | Pearson Correlation | 1 | -0.2 | -0.178 | 0.085 | .366* | -0.145 | .369* | -0.173 | .382* |
| | Sig. (2-tailed) | | 0.265 | 0.322 | 0.639 | 0.036 | 0.421 | 0.035 | 0.336 | 0.028 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| MDA | Pearson Correlation | -0.2 | 1 | 0.199 | 0.129 | -0.008 | 0.133 | 0.044 | .693** | -0.11 |
| | Sig. (2-tailed) | 0.265 | | 0.267 | 0.475 | 0.966 | 0.461 | 0.806 | 0 | 0.541 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| GSH | Pearson Correlation | -0.178 | 0.199 | 1 | -0.025 | -0.246 | 0.312 | 0.07 | 0.309 | -0.167 |
| | Sig. (2-tailed) | 0.322 | 0.267 | | 0.891 | 0.167 | 0.077 | 0.7 | 0.08 | 0.354 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| GPX | Pearson Correlation | 0.085 | 0.129 | -0.025 | 1 | -0.054 | -0.019 | -0.078 | 0.022 | -0.018 |
| | Sig. (2-tailed) | 0.639 | 0.475 | 0.891 | | 0.764 | 0.918 | 0.664 | 0.904 | 0.922 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| ZN | Pearson Correlation | .366* | -0.008 | -0.246 | -0.054 | 1 | -0.17 | 0.326 | 0.062 | 0.337 |
| | Sig. (2-tailed) | 0.036 | 0.966 | 0.167 | 0.764 | | 0.345 | 0.064 | 0.732 | 0.055 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| CU | Pearson Correlation | -0.145 | 0.133 | 0.312 | -0.019 | -0.17 | 1 | -0.147 | .356* | 0.17 |
| | Sig. (2-tailed) | 0.421 | 0.461 | 0.077 | 0.918 | 0.345 | | 0.415 | 0.042 | 0.345 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| MG | Pearson Correlation | .369* | 0.044 | 0.07 | -0.078 | 0.326 | -0.147 | 1 | 0.167 | -0.021 |
| | Sig. (2-tailed) | 0.035 | 0.806 | 0.7 | 0.664 | 0.064 | 0.415 | | 0.354 | 0.909 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| MN | Pearson Correlation | -0.173 | .693** | 0.309 | 0.022 | 0.062 | .356* | 0.167 | 1 | 0.031 |
| | Sig. (2-tailed) | 0.336 | 0 | 0.08 | 0.904 | 0.732 | 0.042 | 0.354 | | 0.866 |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| Vit. C | Pearson Correlation | .382* | -0.11 | -0.167 | -0.018 | 0.337 | 0.17 | -0.021 | 0.031 | 1 |
| | Sig. (2-tailed) | 0.028 | 0.541 | 0.354 | 0.922 | 0.055 | 0.345 | 0.909 | 0.866 | |
| | N | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

**TABLE 7. Correlation between serum concentration of oxidant, antioxidant, and trace elements in dressage horses**

| Correlations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **SOD** | **MDA** | **GSH** | **GPX** | **ZN** | **CU** | **MG** | **MN** | **Vit. C** |
| SOD | Pearson Correlation | 1 | -0.482 | -0.507 | 0.449 | 0.18 | .761* | -0.195 | -0.326 | 0.381 |
| | Sig. (2-tailed) | | 0.273 | 0.246 | 0.313 | 0.699 | 0.047 | 0.675 | 0.475 | 0.4 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| MDA | Pearson Correlation | -0.482 | 1 | -0.092 | -0.583 | 0.091 | -0.321 | 0.593 | .872* | 0.108 |
| | Sig. (2-tailed) | 0.273 | | 0.845 | 0.17 | 0.846 | 0.483 | 0.161 | 0.01 | 0.817 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| GSH | Pearson Correlation | -0.507 | -0.092 | 1 | 0.187 | 0.032 | -0.131 | -0.438 | -0.225 | -0.337 |
| | Sig. (2-tailed) | 0.246 | 0.845 | | 0.689 | 0.945 | 0.779 | 0.325 | 0.628 | 0.459 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| GPX | Pearson Correlation | 0.449 | -0.583 | 0.187 | 1 | -0.577 | .777* | -0.154 | -0.713 | -0.072 |
| | Sig. (2-tailed) | 0.313 | 0.17 | 0.689 | | 0.175 | 0.04 | 0.742 | 0.072 | 0.878 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| ZN | Pearson Correlation | 0.18 | 0.091 | 0.032 | -0.577 | 1 | -0.188 | -0.451 | 0.375 | 0.149 |
| | Sig. (2-tailed) | 0.699 | 0.846 | 0.945 | 0.175 | | 0.686 | 0.31 | 0.408 | 0.751 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| CU | Pearson Correlation | .761* | -0.321 | -0.131 | .777* | -0.188 | 1 | -0.194 | -0.317 | 0.122 |
| | Sig. (2-tailed) | 0.047 | 0.483 | 0.779 | 0.04 | 0.686 | | 0.676 | 0.489 | 0.795 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| MG | Pearson Correlation | -0.195 | 0.593 | -0.438 | -0.154 | -0.451 | -0.194 | 1 | 0.304 | 0.402 |
| | Sig. (2-tailed) | 0.675 | 0.161 | 0.325 | 0.742 | 0.31 | 0.676 | | 0.507 | 0.372 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| MN | Pearson Correlation | -0.326 | .872* | -0.225 | -0.713 | 0.375 | -0.317 | 0.304 | 1 | -0.139 |
| | Sig. (2-tailed) | 0.475 | 0.01 | 0.628 | 0.072 | 0.408 | 0.489 | 0.507 | | 0.766 |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Vit. C | Pearson Correlation | 0.381 | 0.108 | -0.337 | -0.072 | 0.149 | 0.122 | 0.402 | -0.139 | 1 |
| | Sig. (2-tailed) | 0.4 | 0.817 | 0.459 | 0.878 | 0.751 | 0.795 | 0.372 | 0.766 | |
| | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |

* Correlation is significant at the 0.05 level (2-tailed).

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