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Minerals and Trace Elements Profile of Apparently Healthy Horses in Ibadan Polo Club, Oyo State, Nigeria

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ABSTRACT

Roles of trace elements and minerals in the overall performance, health and wellbeing of horses cannot be underestimated *vis-a -vis* deleterious effects on normal functioning of living organisms posed by prolonged exposure to toxic metals. We evaluated the levels of some minerals and trace elements and compared these parameters among different breed, age and gender. Forty-six apparently healthy horses in Ibadan Polo Club, Ibadan, Nigeria were used for the study. Minerals and trace elements analyses were performed using the inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro®, Varian Medical Systems, Inc., California, USA) and inductively coupled plasma mass spectrometry (ICP-MS) (Varian 820 MS®, Varian Medical Systems, Inc., California, USA). Student's T-test was applied to establish differences in the values of minerals and trace elements in relation to breed, gender and age. The results revealed significant differences in the levels of Magnesium (Mg), Iron (Fe), Iodine (I) and in the level of Silicon (Si), Chromium (Cr), and Iridium (Ir) in minerals and trace elements respectively with significantly higher values in Sudanese compared to Cross-bred horses. Mg, Fe, I, Cr, and Ir values were significantly higher in Mares compared to Stallion. Values of Fe, I, Si, Mo and Cr were significantly higher in young horses compared to adult horses. Conclusively, significant variations were observed in the minerals and trace elements values regarding breed, sex and age of Horses in Ibadan Polo Club, Nigeria. Provision of regular supplement of minerals lick and trace elements advised especially for Cross-bred, Stallion and adult horses in Ibadan Polo Club.

Keywords: Minerals; Polo horses; Trace element; Ibadan; Nigeria

INTRODUCTION

Trace elements and minerals play a significant role in equine health and performance, (Coenen, 2013). Kalashnikov *et al.* (2018) had previously demonstrated that trace element levels in hair are importantly correlated with the speed in trotter horses. Therefore, monitoring of horses' trace element status is considered an important tool for the evaluation of equine nutrition and level of exposure. The inclusion of Fe, K, and Zn chelated with glycine in horse's feed resulted in exceptionally high levels of metal in horse hair (Armelin *et al.*, 2003). Equines are food and milk-producing animals in many countries, therefore determining the status of toxicants in equine biological components is relevant to human health, and therefore should not be underestimated (Kozak *et al.*, 2002). McGorum *et al.* (2003) also discovered variations in systemic levels of Cu, Mg, and selenium (Se) between horses with Equine grass sickness and healthy horses. But no reason was given for this. Trace elements have also been implicated especially magnesium in a worldwide endocrinopathy disease of horses called equine metabolic syndrome (EMS). Determination of serum and blood nutritional parameters is an essential component of assessing the nutritional health of animals. The role of minerals as body structural components and in hormonal and enzymatic body activities cannot be underestimated. The process of regulation of cells differentiation and replication requires minerals as constituents of body fluid necessary for normal body metabolism (Balamurugan *et al*., 2017). Available literature reveals that horse age, housing system, breed, and season have a tremendous effect on the trace element level in horse hair (Topczewska, 2012; Topczewska and Krupa, 2013). Despite the nutritional and toxicological importance of establishing levels of trace elements in horses. To the best of our knowledge, there is no available information on the levels of minerals *vis-a-vis* trace elements profile of Nigerian horses and the variation in these parameters with regards to breed, gender and age, especially the Polo horses. Even in countries where they have data on horse's trace element parameters, there are conflicting reports between outcomes of published studies, and still, the physiological processes involved in mineral poisoning are also poorly understood. The determination of the trace elements profile is also important in order to obtain the necessary information regarding the animal's health and also the establishment of either the increase or decrease in these parameters will be useful in the diagnosis and institution of appropriate treatment regimen for the management of equine metabolic and toxicological related conditions (Al-Bulushi *et al*., 2017). The establishment of these reference values for the Nigerian breed of horses and the comparison based on gender, age, and breed will be of utmost benefit to Nigeria's equine industry. We therefore highlight and elaborate on the findings of the present study, which serves as baseline data that will be of help to equine clinician and horse's owners in the aspect of diagnosis and management of equine metabolic, nutritional deficiencies, and toxicological-related diseases in Nigerian horses.

MATERIALS AND METHODS

Ethical Statement

The appropriate international standards according to the guidelines for the ethical use of animals in research were duly followed during the course of this study (Sherwin *et al.,* 2003).

Description of the Study Site

The study was conducted at Ibadan Polo Club, situated near Eleyele in Ibadan North West Local government area of Oyo state, Nigeria. Ibadan Polo Club is located at Latitude: 7.40417452811. Longitude: 3.87146768914. The polo field is covering an area of about 300 yards in length and 160 yards in breadth. The climatic condition of the area is tropically wet and dry with a lengthy wet season and relatively stable temperatures year-round. The mean temperature is 26.46^{o} c, and the minimum is 21.42°c, while the relative humidity is about 74.55mmHg (Adebola *et al*., 2015)

Sample Collection

Blood samples were aseptically collected from apparently healthy horses that are of different breed, age, and gender. The samples were collected from 46 horses at rest during the morning period during the rainy season of April, May and June 2020. Samples were immediately transported to the laboratory for analyses within minutes of collection. The locations represent areas where a large number of horses were present and used for polo games. Breeds were morphologically classified (Olaogun *et al*., 2022) as Crosses and Sudanese breeds, Age was estimated using the birth record of the animals and were classified as young and adult (Adedokun *et al*., 2023).

The criteria for enlistment in the sampling were horses assessed for illness or diseases and certified healthy before sampling and their owners consented to the sampling of their horses. All animals sampled were subjected to a similar feeding protocol in which they were fed on pastures and good quality hay at 2kg/100kg body weight and grains such as barley and/or concentrate at 1kg/100kg body weight without any mineral supplementations. Horses were being provided with water ad-libitum. Blood samples were collected by jugular venipuncture, and separated sera were stored at - 20°c prior to testing as previously described by (Olaogun and Jeremiah, 2018; Olaogun and Onwuzuruike, 2018; Olaogun and Oyetoyinbo, 2020).

Minerals and Trace Elements Analyses

The concentration of minerals and trace elements in serum was determined by atomic emission and mass spectrometry in the testing laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo state Nigeria. A total of 46 serum samples were analyzed (apparently healthy ones); 3 samples could not be processed due to hemolysis. The samples were processed adopting inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro®, Varian Medical Systems, Inc., California, USA) and inductively coupled plasma mass spectrometry (ICP-MS) (Varian 820 MS®, Varian Medical Systems, Inc., California, USA) as earlier described by (Schweinzer *et al.,* 2017). Both techniques were used to measure the concentration of minerals and trace elements. ICP-MS was employed in assessing selenium (Se), cobalt (Co), iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), manganese (Mn), and nickel (Ni). While magnesium (Mg), iodine (I), mercury (HG), silicon (Si), molybdenum (Mo), fluoride (Fl), cadmium (Cd), and iridium (Ir) were estimated using ICP-MS.

Statistical Analyses

Data were entered into a Microsoft Excel® 2016 and Statistica 13 (Microsoft Corporation, USA) spreadsheet and then analysed. The data were processed using generally accepted descriptive statistics and expressed in the form of mean ± standard error of the mean (Mean± SEM). Student's T-test was used to compare the mean and the differences were considered statistically significant at $p \leq 0.05$

RESULTS

The serum analyses of minerals among the breed of horses only showed significant differences in the levels of Magnesium (Mg), Iron (Fe), and Iodine (I) with significantly higher values in Sudanese breeds compared to Cross-bred horses (Table 1).

Table 1: Comparison of levels of minerals in Cross-bred and Sudanese breed of horses in Ibadan, Nigeria

Minerals	Cross	Sudanese	P-values
Magnesium (Mg/l)	32 ± 0.76^{a}	36 ± 0.74^{b}	0.002
Iron (Mg/l)	3.3 ± 0.1^{a}	3.9 ± 0.06^{b}	0.0004
Manganese (Mg/l)	0.17 ± 0.2^{a}	0.08 ± 0.006^{a}	0.390
Copper (Mg/l)	0.20 ± 0.003^{a}	0.21 ± 0.007^{a}	0.342
Zinc (Mg/l)	0.92 ± 0.01^{a}	0.8 ± 0.01^{a}	0.158
Iodine (Mg/l)	0.15 ± 0.003^{a}	0.19 ± 0.006^{b}	0.001
Selenium (Mg/l)	0.13 ± 0.004^{a}	0.13 ± 0.006^{a}	0.786

Mean ± SEM values with different superscripts within columns $^{a, b}$ is significantly different ($P<0.05$).

Comparison of values of mean ± standard error of the mean (Mean ± SEM) of trace elements among breed of horses showed statistically significant differences in the level of Silicon (Si), Chromium (Cr), and Iridium (Ir) with significantly higher values in Sudanese breeds compared to Cross-bred horses (Table 2).

Comparison regarding gender reveals significant differences in the Mg, Fe, and I levels. Female horses (Mares) possessed significantly higher values of Mg, Fe, and I when compared with the values in males (Stallions) respectively (Table 3).

Table 2: Comparison of trace element concentration in Cross-bred and Sudanese breed of horses in Ibadan, Nigeria

Trace element	Cross	Sudanese	P-values
Mercury (Mg/l)	44 ± 4.0 [a]	41 ± 4.8 [a]	0.648
Silicon (Mg/l)	0.081 ± 0.004 [a]	0.092 ± 0.003 [b]	0.001
Molybdenum (Mg/l)	0.095 ± 0.005 [a]	0.103 ± 0.005 [a]	0.568
Cobalt (Mg/l)	0.09 ± 0.005 [a]	0.09 ± 0.006 [a]	0.760
Chromium (Mg/l)	0.09 ± 0.005 [a]	0.11 ± 0.004 [b]	0.004
Fluorine (Mg/l)	0.065 ± 0.005 [a]	0.063 ± 0.005 [a]	0.460
Cadmium (Mg/l)	0.043 ± 0.005 [a]	0.060 ± 0.008 [a]	0.153
Iridium (Mg/l)	0.020 ± 0.003 [a]	0.039 ± 0.007 [b]	0.060
Nickel (Mg/l)	0.071 ± 0.002 [a]	0.064 ± 0.004 [a]	0.7600

Mean ± SEM values with different superscripts within columns [a, b] is significantly different ($P<0.05$).

Table 3: Minerals level in Male (Stallion) and Female (Mare) horses in Ibadan, Nigeria

Minerals	Stallions (male)	Mare (female)	P-values
Magnesium (Mg/l)	33 ± 0.78 [a]	37 ± 0.76 [b]	0.003
Iron (Mg/l)	3.4 ± 0.1 [a]	4.0 ± 0.07 [b]	0.0005
Manganese (Mg/l)	0.19 ± 0.1 [a]	0.09 ± 0.005 [a]	0.380
Copper (Mg/l)	0.22 ± 0.005 [a]	0.23 ± 0.009 [a]	0.357
Zinc (Mg/l)	0.94 ± 0.03 [a]	1.0 ± 0.03 [a]	0.163
Iodine (Mg/l)	0.17 ± 0.005 [a]	0.21 ± 0.008 [b]	0.0011
Selenium (Mg/l)	0.11 ± 0.005 [a]	0.11 ± 0.005 [a]	0.972

Mean ± SEM values with different superscripts within columns [a, b] is significantly different ($P<0.05$).

Gender comparison of horses' trace elements indicated significant differences in the values of Cr and Ir with relatively higher significant differences in their values in female compared to male horses (Table 4).

Comparison of values of different minerals analysed in relation to age group classification shows statistically significant differences in Iron (Fe), Manganese (Mn) and Iodine (I) parameters respectively. The values of Fe and I were significantly higher in young horses compare to adult horses. Mn value was significantly higher in adult group of horses compare to horses in young age category (Table 5).

Table 6 indicated comparison of the trace elements values in relation to age group classification. There were higher significant values observed in the values of Silicon, Molybdenum and Chromium in young horses compare to adult category of horses.

Table 4: Trace element concentration in Male (Stallion) and Female (Mare) horses in Ibadan, Nigeria

Trace element	Stallions (male)	Mare (female)	P-values
Mercury (Mg/l)	42 ± 6.0 [a]	39 ± 5.8 [a]	0.703
Silicon (Mg/l)	0.077 ± 0.004 [a]	0.088 ± 0.007 [a]	0.105
Molybdenum (Mg/l)	0.091 ± 0.005 [a]	0.098 ± 0.007 [a]	0.434
Cobalt (Mg/l)	0.05 ± 0.003 [a]	0.05 ± 0.004 [a]	0.854
Chromium (Mg/l)	0.05 ± 0.003 [a]	0.07 ± 0.006 [b]	0.005
Fluorine (Mg/l)	0.061 ± 0.003 [a]	0.058 ± 0.003 [a]	0.455
Cadmium (Mg/l)	0.043 ± 0.005 [a]	0.055 ± 0.006 [a]	0.158
Iridium (Mg/l)	0.013 ± 0.003 [a]	0.032 ± 0.007 [b]	0.030
Nickel (Mg/l)	0.063 ± 0.003 [a]	0.058 ± 0.006 [a]	0.4900

Mean ± SEM values with different superscripts within columns [a, b] is significantly different ($P<0.05$).

Table 5: Comparison of levels of minerals in Young and Adult horses in Ibadan, Nigeria

Minerals	Adult	Young	P-values
Magnesium (Mg/l)	35 ± 0.58 [a]	36 ± 0.90 [a]	0.274
Iron (Mg/l)	3.5 ± 0.2 [a]	4.1 ± 0.08 [b]	0.0002
Manganese (Mg/l)	0.14 ± 0.5 [a]	0.05 ± 0.009 [b]	0.003
Copper (Mg/l)	0.40 ± 0.005 [a]	0.41 ± 0.009 [a]	0.564
Zinc (Mg/l)	0.96 ± 0.01 [a]	0.12 ± 0.01 [a]	0.206
Iodine (Mg/l)	0.17 ± 0.005 [a]	0.21 ± 0.008 [b]	0.002
Selenium (Mg/l)	0.15 ± 0.003 [a]	0.16 ± 0.005 [a]	0.924

Mean ± SEM values with different superscripts within columns [a, b] is significantly different ($P<0.05$).

Table 6: Trace element concentration in Young and Adult horses in Ibadan, Nigeria

Trace element	Adult	Young	P-values
Mercury (Mg/l)	43 ± 2.0 [a]	40 ± 2.8 [a]	0.722
Silicon (Mg/l)	0.086 ± 0.007 [a]	0.097 ± 0.005 [b]	0.002
Molybdenum (Mg/l)	0.095 ± 0.005 [a]	0.098 ± 0.005 [b]	0.004
Cobalt (Mg/l)	0.07 ± 0.007 [a]	0.08 ± 0.008 [a]	0.560
Chromium (Mg/l)	0.11 ± 0.003 [a]	0.13 ± 0.002 [b]	0.003
Fluorine (Mg/l)	0.075 ± 0.008 [a]	0.073 ± 0.008 [a]	0.390
Cadmium (Mg/l)	0.054 ± 0.007 [a]	0.071 ± 0.010 [a]	0.333
Iridium (Mg/l)	0.022 ± 0.005 [a]	0.044 ± 0.009 [a]	0.060
Nickel (Mg/l)	0.068 ± 0.004 [a]	0.061 ± 0.006 [a]	0.808

Mean ± SEM values with different superscripts within columns [a, b] is significantly different ($P<0.05$).

DISCUSSION

The function of minerals in the maintenance of body structural components and in the enzymes and hormones mechanisms cannot be overemphasised. They also play essential roles in the formation of body fluids, and tissue and regulate the processes of cell proliferation, replication**,** and differentiation (Balamurugan *et al*., 2017). World Health Organization (WHO) categorized trace elements as important and or probably elements with toxicity potential (Mehri, 2020). This metabolic derangement and its toxicity potential are generally known in humans, but the biological roles of these important elements are not yet fully understood in both humans and animals (Mehri, 2020; Boer *et al*., 2014). The determination of the trace elements and minerals status of any domestic animal is essential for the general well-being and welfare of such animal or stable. The performance of the stable or a particular horse is a function of its optimal levels of minerals and trace elements. Considering the value of the Polo game to the owners of Polo horses, minerals and trace elements assessment is necessary to prevent deficiencies and or oversupplies of important nutrients in animal feed (Humann-Ziehank *et al.,* 2013). In the present study, we determined the concentration of minerals and trace elements in Nigerian Polo horses, and these values were compared between breed, gender, and age of horses. The findings of the present study may be taken as essential baseline data or indicative of the level of exposure to some specified elements through the feeds or the environment. We employed the use of serum for the evaluation of trace elements and minerals in the present study. This is in conformity with the previous description of blood sampling procedure as being less invasive (Herdt and Hoff, 2011). Stanek *et al*. (2016) and Forrer *et al*. (2001), used serum to establish the concentration of micro and macro minerals in horses as these represent the quantity of these minerals in the entire body of the horses.

Use of serum for analyses of trace elements and minerals may have advantages over the use of horses' hair as most horse owners will not want the hair of their animals to be trimmed due to aesthetic reasons. The significantly higher values of Mg, Fe, and I in Sudanese breed compared to Cross-bred horses in the present study may be related to the better adaptability of Sudanese horses to the environment and diets compared to Cross-bred horses. This finding disagrees with the report of (Weigert *et al*., 1981) who reported that nutritional differences in different breeds of horses do not affect the trace elements concentration. This observation is in tandem with the findings of (Or *et al*., 2004) who discovered variations in the levels of trace elements among different breeds of horses. Significantly higher values of Mg, Fe, and I were observed in female horses (Mares) compared with the values in male horses (Stallions) observed in the present study. This is not in agreement with (Kalashnikov *et al*., 2019) who reported no significant differences in the concentration of Cu, I, and Se in the gender pattern of essential minerals among English thorough bred horses. This observation also contradicts the findings of Cygan-(Szczegielniak and Stasiak, 2022) who reported no significant differences in the values of trace elements among male and female horses. These differences may be associated with the organ that the trace elements were assessed from, because our own assessment was on horses' serum, while the earlier reports were assessed on the hair of horses. The gender pattern observed in this study with significant higher values of Chromium and Iridium in mares compared to stallions, and four other elements having relatively higher concentration in Mares compared to Stallions out of total of nine elements analysed. These observations also conform with the earlier findings of (Kalashnikov *et al*., 2019) who reported a similar higher value in the concentration of horses' hair trace elements in Mares compared to Stallions. Gender has previously been demonstrated to have significant influence on trace elements metabolism. For instance (Cywinska *et al*., 2011) had previously reported significantly higher serum haptoglobin concentrations in mares compared to stallions. These variations in gender pattern of trace elements, in particular, heavy metals may be associated with metallothionein metabolism, that is been controlled by sex hormones (Jeffery *et al*., 1989). Though there are no sufficient data to support this assertion. The relatively higher values of minerals and in most of the trace elements seen in young horses compared to adult horses observed in this study is similar to the earlier observation of (Fleming *et al*., 2006) who has described persistent increase in serum Fe and Zn in young horses till maturity. This variation may be due to hormonal influence especially activity of growth hormones in young animals, immunogenicity potential, diets, feeding habit, care and management that varies between young and old animals. Most often, the horse owners usually provide improved diet, care, and management in general for young horses compare to adult horses. This also conform with the earlier report of (Stowe and Herdt, 1992) who also discovered persistent increased in the level of selenium in young horses until maturity was attained.

Conclusion

We therefore conclude that there were significant variations in the levels of minerals and trace elements regarding breed, sex and age of Horses in Ibadan Polo Club, Nigeria. The levels of minerals and trace elements appeared better in Sudanese breed, female horses and young horses compared to relatively low values observed in Cross-bred, male and adult horses. Provision of regular supplement of minerals lick and trace elements were advised especially for Cross-bred, Stallion and Adult horses. Needs for periodic training of owners of horses on nutritional management of Polo horses is also advocated.

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Conflict of Interest

The authors declare that there is no conflict of interest

Author's Contributions

Conceptualisation, OSC; methodology, OSC.; ARAM, EOO; data analysis, OSC; writing original draft, OSC; writing, review and editing, OSC, ARAM, EOO; All authors have read and agreed to the publication of the final manuscript.

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