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Age, Sex, Breed and Activity-Related Effects on Some Haematological Parameters of Horses in South Western Nigeria

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ABSTRACT

Haematological parameters are good indicators of the physiological status used by equine practitioners for health assessment. The aim of this study was to evaluate the influences of sex, age, breed and activity on haematological parameters of apparently healthy horses from three South-western states in Nigeria. Blood samples from 100 apparently healthy horses with age ranges from 1 to 18 years old, both sexes, mixed breeds, and engaged in different activities were obtained for hematological analysis and erythrocyte osmotic fragility test. The results showed that the haematological values are comparable with normal reference values for horses. The age, sex, breed and activities had exerted some effect on the hematological values with significantly ($p<0.05$) higher hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cells count (WBC) in the stallions than mares. Older horses had significantly ($p<0.05$) lower Hb and MCH values than younger ones. The Nigerian Local horse (indigenous) and the cross breed had significantly ($p<0.05$) higher Hb and MCHC. Horse used for recreation activities had significantly ($p<0.05$) higher values when compared with other horse activities. Similarly, horse activities influenced the erythrocyte osmotic fragility. In conclusion, this study showed that the haemogram values for horses from South-western Nigeria are within the acceptable reference values for horses. Some effect of sex, age, breed and activities were observed on the haematological parameters of the horse with stallions, the indigenous, their cross breed and those engaged in recreational activities having significantly higher Hb, MCH, MCHC and WBC values.

Keywords: Hematology; Erythrocyte indices; Horse: South-Western Nigeria

INTRODUCTION

Traditionally, horses in Nigeria are used for transportation, riding, farm activities like ploughing, sugar mills run, and ceremonial purposes such as durbar, carnival and wedding caravans (RIM, 1992; Garba, 2006). In recent years, there has been an increase in the population of horses due to the evolvement of different equestrian events in various part of the country with more individuals now keeping and rearing horses. Among the purposes of horse keeping pleasure riding, polo, racing, transportation, breeding, durbar, carnival and wedding caravans, agricultural activities and security operations and parades by mounted police and Army are predominant (Mayaki *et al*., 2018). Furthermore, there has been a shift from keeping predominantly stallion to mix sex to encourage breeding as well as importation of different breeds in other to meet the demands of the growing equestrian industries. This increasing population trend has made collection of information about physiological and pathological conditions of horses in Nigeria indispensable in the clinical evaluation of their health.

Haematological parameters are good indicators of the physiological status used by equine practitioners to monitor health and classify disease severity and progression, treatment response, as well as an aid in prognostic assessment (Buendia *et al*., 2021). They are also used to provide information about the metabolic situations and fitness level of horses (Adamu *et al*., 2013). The normal reference intervals for haematology parameters for horses are widely reported. These reference intervals may not be fully applicable under all conditions because they are known to be influenced by several factors like age, sex, breed, exercise or type of activities, biological rhythms, management, season and geographic location (Witkowska-Piłaszewicz *et al*., 2020; Udeh *et al*., 2021; Sawesi *et al*., 2023; Silva Rua *et al*., 2024; Akinniyi *et al*., 2025).

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Studies in Nigeria from the North (Ilorin, Maiduguri), and South (Enugu, Ibadan, Port Harcourt) parts have reported varying references values for haematological parameters for horses (Ebge-Nwiyi *et al*., 2012; Ihedioha and Agina, 2014; Ememe *et al*., 2015; Udeh *et al*., 2021; Basiru *et al*., 2022; Adedokun *et al*., 2023). There are concerns on values reported by these previous studies because the studies either used a particular breed and/or sex (Stallion) or smaller sample sizes. More recently, the only study in south western Nigeria from Oyo state used 20 horses, predominantly mares and only two breeds (Sudanese and Cross breed) were considered (Adedokun *et al*., 2023). The Nigerian local breed (Arewa) and common horse activities were not included. It has been established that the correct interpretation of haematological tests for horses requires specific reference values from studied populations taking into consideration all peculiar factors because of the important variations observed in the local environment. Therefore, equine veterinarians must interpret available reference intervals with caution. Hence, the need to compliment the paucity of information on these parameters for the mixed breeds horse population and the influence that horse activities have on their haematological parameters in South-West Nigeria. The three states in the southwestern Nigeria where equestrian activities are predominant were considered for this study. Thus, aim of this study was to evaluate the influences of sex, age, breed and activity on haematological parameters of apparently healthy horses from Oyo, Ogun and Lagos states.

MATERIALS AND METHODS

Study Area

The study was carried out in three southwestern states of Nigeria: Lagos (6.5˚N, 3.6˚E), Ogun (6.9˚N, 3.6˚E) and Oyo (8.16˚N, 3.16˚E). The three states were selected was based on the availability and accessibility to horses. The study was consented to by horse owners' and approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/033-0423/18).

Study animal and Blood sample collection

A total of 100 horses comprising of 60 from Oyo state, 15 from Ogun state and 25 from Lagos states were used for this study. The horses comprised of both sexes, different breeds that were used for security (police mounted troop), sports (polo and racing), recreation, teaching and research. Their ages were determined using either reliable records or dentition. All horses were examined and sampled in the morning between the hours of 8:00 and 10:00am in their normal habitat while being handled by their keepers before their routine activities to minimalize stress. Approximately 5mls of blood was collected from the jugular vein of each horse using 18-gauge needle and transferred into labelled heparinized bottles. The blood samples were transported on an ice pack to the Physiology laboratory, Faculty of Veterinary Medicine, University of Ibadan.

Analysis of Haematology analysis

The Packed cell volume (PCV) was determined by microhaematocrit method, red blood cells count (RBC) and white blood cells count (WBC) by using the improved Neubauer slide, and Hb value was determined using cyanmethaemoglobin method (Coles, 1986). The MCV, MCH and MCHC were calculated from the values of PCV, RBC and Hb, using the standard methods (Coles, 1986). Erythrocyte osmotic fragility (EOF) was determined according to the method described by Azeez *et al*. (2009). Briefly, 0.02 ml of blood was added to tubes containing increasing concentration of phosphate-buffered saline (PBS) solution at pH 7.4 (0, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, and 0.9% NaCl). The contents of each tube were gently mixed and incubated at room temperature (29 ℃) for 30 minutes. The content of each tube was then centrifuged at 959g for 10 minutes and the supernatant was decanted. The optical density of the supernatant was determined spectrophotometrically at 540 nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100 % using the formula:

% haemolysis = (Absorbance od sample / Absolute of 0% NaCl solution) x 100.

Data Analysis

Data are presented as mean ± standard deviation and analyzed using student t-test and One-way ANOVA followed by Tukey Kramer post hoc test. All analysis were done using GraphPad Prism version 9, (GraphPad Software, San Diego, California USA) and the probability (p) value of < 0.05 was considered significant.

RESULTS

The horses studied consisted of 46.0% stallion and 54.0% mare. Their ages ranges from 3 to 20 years (median age 8 years) and were of different breeds: Nigerian Local horse (33.0%), Sudanese (47.0%), Argentine (11.0%), South African (4.0%) and Cross-breed (5.0%).

The results of haematological parameters of the horses in relation to sex showed that Hb, MCH, MCHC and WBC were significantly lower ($p<0.05$) in the female than in the male horses (Table 1). The haematological parameters based on age indicated that horses under 5 years old had significantly higher ($p < 0.05$) Hb and MCH values compared to horses aged 11–15 years and those older than 15 years (Table 2).

There were significant differences in the Hb, MCH, MCHC and WBC values among all the five breeds studied (Table 3). The Hb, MCHC and WBC were significantly ($p<0.05$) higher in the Nigerian Local horse (indigenous) and the cross breed, when compared to other breeds. The cross breed also had significantly ($p<0.05$) higher MCH when compared with the Sudanese and South African breeds. On the basis of assigned horse activity (Table 4), there were significant ($p<0.05$) differences in most haematological parameters except for RBC and MCV. Horses used for recreation were observed to have significantly ($p<0.05$) higher values when compared with horses used for other activities.

The erythrocyte osmotic fragility (EOF) of the horses based on sex shows that erythrocyte osmotic resistance is similar for both sexes, except at 0.5% NaCl concentration where the females had slightly higher red cell fragility than the males, although the variation was not statistically ($p>0.05$) significant (Figure 1). Similar trend was

observed with the EOF when compared according to the different age categories of the horses sampled (Figure 2). All the breeds also had similar EOF with no significant ($p>0.05$) difference except for the South African breed with significantly ($p<0.05$) higher fragility than the Sudanese breed at 0.5% NaCl (Figure 3). However, the EOF of horses based on assigned activity shows relative variations between the groups (Figure 4). EOF was significantly lower ($P<0.05$) in horses used for teaching and research than those used for security and recreation at 0.0% NaCl. At 0.5 % NaCl concentration, the EOF in horses used for security was significantly higher than those used for recreation.

Table 1: Effect of sex on haematological parameters in apparently healthy horses from three Southwestern states, Nigeria.

		Sex	
Parameter	**Total (n=100)**	**Stallion (n = 46)**	**Mare (n = 54)**
PCV (%)	35.38±6.10	35.57±6.72	35.32±5.68
Hb (g/dL)	15.57±6.57	17.08±1.66	14.52±4.30*
RBC (x10^6/uL)	7.59±1.66	7.49±1.66	7.70±1.70
MCV (fl)	47.55±11.54	48.35±10.89	46.73±12.11
MCH (pg)	22.06±12.67	24.62±16.53	20.10±8.05*
MCHC (g/dL)	45.25±22.62	49.80±29.53	41.81±14.50*
WBC (x10^3)	9.25±4.00	10.22±4.89	8.61±3.08*

Key: PCV= Packed Cell Volume, Hb = Haemoglobin, RBC = Red blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cells. *Means within row are significantly different at $p<0.05$. n = number of horses

Table 2: Effect of age on haematological parameters in apparently healthy horses from three Southwestern states, Nigeria

	Age (years)			
Variables	**< 5 (n = 15)**	**6 – 10 (n = 57)**	**11 - 15 (n = 20)**	**> 15 (n = 8)**
PCV (%)	33.67±5.20	35.67±5.93	36.75±7.16	32.62±4.93
Hb (g/dL)	16.98±6.12^{ab}	16.70±7.34	13.45±4.27^{b}	12.39±4.08^{a}
RBC (x10^6/µL)	7.49±1.44	7.62±1.56	7.82±2.16	7.36±1.61
MCV (fL)	46.85±8.45	47.72±12.72	46.82±9.98	45.56±9.97
MCH (pg)	23.66±9.02^{ab}	23.98±14.85	18.27±8.94^{b}	17.21±6.16^{a}
MCHC (g/dL)	48.48±22.65	48.73±24.99	37.68±14.38	39.27±16.80
WBC (x10^3/µL)	9.07±3.91	9.42±4.33	9.53±4.08	7.98±2.63

Key: PCV = Packed Cell Volume, Hb = Haemoglobin, RBC = Red blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cells. Values with different superscript along the same row are significantly different at $P<0.05$. n = number of horses.

Table 3: Effect of breeds on haematological parameters in apparently healthy horses from three Southwestern states, Nigeria

	Breeds				
Variables	**Local breed (n = 33)**	**Cross breed (n = 5)**	**Sudanese (n = 47)**	**Argentine (n =11)**	**S/African (n = 4)**
PCV (%)	35.12±6.77	35.00±3.87	34.95±6.04	37.36±6.22	36.25±1.50
Hb (g/dL)	18.49±9.55^{bdf}	18.44±2.87^{eg}	15.00±4.02^{abc}	11.42±1.05^{ade}	10.85±1.40^{cfg}
RBC (x10^6/µL)	7.47±1.85	7.42±1.22	7.64±1.58	8.09±1.94	7.51±0.90
MCV (fL)	47.61±11.74	47.75±5.50	46.34±10.72	48.99±16.09	48.80±6.41
MCH (pg)	27.25±19.27	25.70±7.59^{a}	20.61±6.53^{b}	15.71±5.77	14.62±3.04^{ab}
MCHC (g/dL)	55.38±33.54^{abc}	53.00±9.44	42.75±13.05^{a}	32.64±3.95^{b}	29.92±3.29^{c}
WBC (x10^3/µL)	11.51±5.40^{b}	11.60±3.96^{c}	7.36±1.95^{abc}	9.91±3.42^{a}	9.15±1.23

Key: PCV= Packed Cell Volume, Hb= Haemoglobin, RBC= Red blood cells, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, WBC= White blood cells. Values with different superscript along the same column are significantly different at $p<0.05$. n = number of horses

Table 4. Effect of horse activity on haematological parameters in apparently healthy horses from three Southwestern states, Nigeria

	Horse activity			
Variables	**Sport (n = 67)**	**Security (n = 12)**	**Recreation (n = 15)**	**T/Research (n = 6)**
PCV (%)	35.82±6.13	35.83±5.49^{a}	32.43±5.62^{a}	34.50±3.67
Hb (g/dl)	13.62±3.90^{a}	11.62±2.07^{abc}	28.35±1.45^{bd}	17.93±2.85^{cd}
RBC (x10^6/µL)	7.66±1.56	8.23±2.43	6.87±1.45	7.37±1.10
MCV (fl)	47.86±12.25	42.30±8.88	48.97±11.70	47.25±5.07
MCH (pg)	18.80±6.61^{a}	14.94±5.85^{b}	44.26±18.71^{abc}	25.00±7.00^{c}
MCHC (g/dl)	38.63±12.79^{a}	32.92±7.10^{bd}	88.84±25.63^{abc}	52.19±8.70^{cd}
WBC (x10^3/µL)	7.76±2.21^{a}	9.20±3.28^{b}	15.15±5.79^{abc}	10.95±3.57^{c}

Key: PCV = Packed Cell Volume, Hb = Haemoglobin concentration, RBC = Red blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cells. values with different superscript along the same row are significantly different at $p<0.05$. n = number of horses

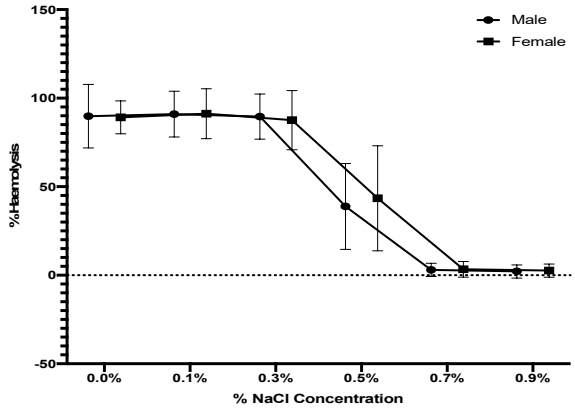

Figure 1. Sex related effect on erythrocyte osmotic fragility in horses from three Southwestern states, Nigeria.

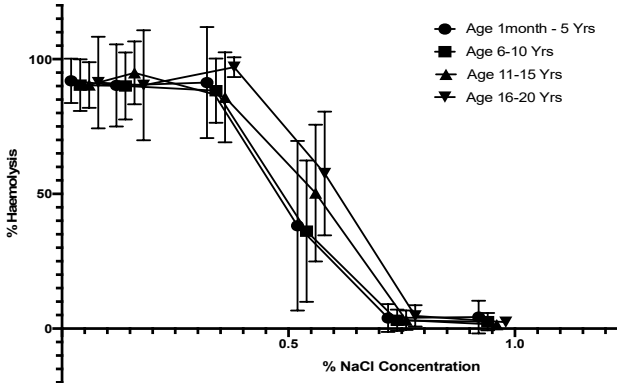

Figure 2: Age related effect on erythrocyte osmotic fragility in horses from three Southwestern states, Nigeria.

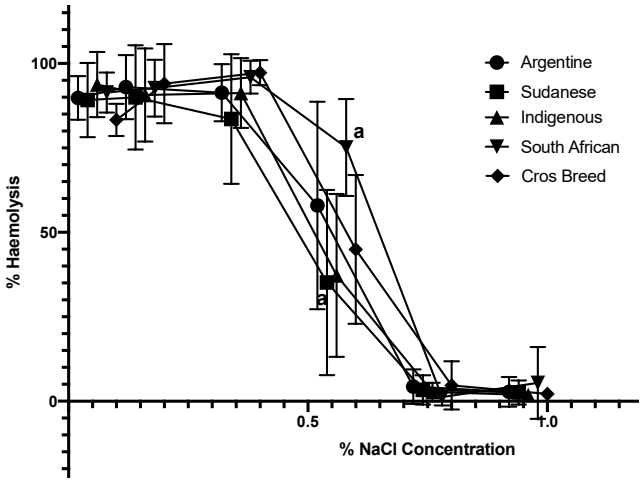

Figure 3: Breeds related effect on erythrocyte osmotic fragility in horses from three Southwestern states, Nigeria. Values with different superscript at the same concentration are significantly different at P<0.05.

DISCUSSION

Blood is an accessible tissue obtained with minimal invasiveness, but an essential diagnostic sample that provides specific and accurate indications of events within the animal body system. Therefore, profiling of hematological parameters is necessary to establish reference values in normal healthy animals for diagnostic purposes within their local environment. This present study profiled the influences of sex, age, breed and activity on haematological parameters of apparently healthy horses in South West, Nigeria.

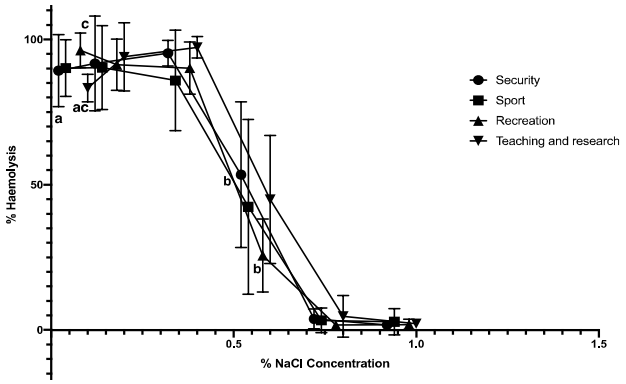

Figure 4: Horse activity effect on erythrocyte osmotic fragility in horses from three Southwestern states, Nigeria. Values with the same superscript alphabet at the same concentration are significantly different @ P<0.05.

The haematological values recorded in this study agreed with accepted reference values reported for apparently health horses by previous local and international researchers worldwide (Schalm *et al*., 1975; Hassan and Hassan, 2003). With the exception of Basiru *et al*. (2022) who reported lower mean values for RBC, PCV and Hb concentration, the mean erythrocytic and leukocytic values for horse in Ilorin in this study were comparable with most of the earlier reports from Borno (Ebge-Nwiyi *et al.,* 2012), Enugu (Ihedioha and Agina, 2014), Port Harcourt (Ememe *et al.,* 2015; Udeh *et al.,* 2021) and Oyo states (Adedokun *et al.,* 2023) in Nigeria as well as those reported for warm-blooded and hot-blooded horses (Buendia *et al*., 2020; Satue *et al*., 2020; Inoue *et al*., 2022).

No effect of age and sex were observed on the PCV, RBC, and MCV among the horses studied as was similarly reported (Ihedioha and Agina, 2014; Udeh *et al*., 2021; Inoue *et al.,* 2022). Although, the male horses had higher mean values of Hb, MCH and WBC than the females. These higher mean values seen in stallion could be the result of androgenic hormone-testosterone on erythropoietin production through increase iron absorption and utilization and higher metabolic demand in males (Bachman *et al.,* 2014). The mechanism responsible for the increase erythropoiesis in male was documented in human where hepcidin inhibition by testosterone caused increase iron-dependent erythropoiesis due to increasing iron availability for RBC production (Bachman *et al*., 2014).

The comparative haematology based on the equine age showed that PCV and MCV values increased with age from horses less than 5 years old to 11 – 15 years old horses while horses older than 15 years had the lowest values. The Hb concentration on the other hand decreased with age. These observations invariably showed like other previous studies that hematological values decrease as horses get older irrespective of age or sex with the lowest mean values seen among horses greater than 15years old (Takasu *et al*., 2013, Ono *et al*., 2019). Satue *et al*. (2020)

and Basiru *et al.* (2022) also reported a reduction in RBC, with compensatory increases in MCV and MCH as horses age. This is probably due to reduced erythropoiesis in the bone marrow as a result low regenerative capacity or myelophthisis (Satue *et al.,* 2009; Mazaheri *et al.,* 2010; Munoz *et al.,* 2012).

This study also shows for the first time to the best of our knowledge, variation in hematological parameters among Nigeria local horse and their cross breed when compared with foreign breeds like Sudanese, South African and Argentine horses kept in South-West Nigeria and it highlights the need for establishing reference values locally for the evaluation of the different breeds under clinical situations. The mean PCV and RBC were lower in the local and cross breed while the Hb and MCHC were significantly higher. However, mean leucocyte values were also significantly higher in the local and cross breeds. This observation seen in the–Local and Cross breed may be attributed to genetic factor as well as climatic adaptation to tropical environment as they are known to be descendant of Arabian and Barb horses which are the main foundation horses for most developed breeds overseas (Dent and Hendricks, 2001). Breed variations in haematological parameters are common in domestic animals. For example, Kiso horses are known to have lower haematological parameters than the Noma horses, despite living in the same environment (Takasu *et al*., 2013, Ono *et al.*, 2019), and genetic factors are more likely to be responsible for the variation.

The comparative haematology of the horses based on horse activities showed that the type of assigned activity of horses has significant influence on most of the animal's haematological parameters analyzed. Similar variations were observed by previous workers reported (Larsson *et al.,* 2013; Burlikowska *et al*., 2015; Massányi *et al.,* 2022; Isović *et al*., 2023). In this study, horses used for recreational activities had significantly higher values particularly for Hb, MCH, MCHC and WBC when compared with other activities, followed by animals used for teaching and research. Horses used for security by mounted Police and Army had the least values, although the haematological values recorded were within the normal reference range for apparently horses. The efficiency of horse metabolic processes under different physical activity depends on the blood constituents particularly the haemogram level (Masko *et al*., 2021; Massányi *et al*., 2022), and the level reflects the horse's compensatory physiological response or adaptations to the intensity of work load. Furthermore, the energy requirements in horses engaged in low intensity exercises like leisure riding and recreation is lower than those engage in high-intensity exercise like flat racing, endurance racing and polo (Witkowska-Piłaszewicz *et al*., 2020). Hence, sport horses are expected to have higher haematological parameters because of the increased tissue oxygen utilization which stimulates compensatory marrow erythropoiesis and increase in the number of circulating erythrocytes (Neuberg-Zuchowicz and Geringer de Oedenberg, 2011) to forestall hypoxaemia.

This variation has clinical implication in the evaluation of these animals during disease conditions and prognostic assessment and advisory following clinical intervention. For example, sport horses with similar levels of anaemia as mounted security horses would be less exercise tolerant and could succumb to hypoxaemia when subjected to its routine activities.

The EOF of horses in South-West Nigeria in this study showed minimal variations with age, sex, and breeds. It is marginally higher in the female than male horses, and in older horses than the younger horses. This shows that the erythrocytes in female and older horses (>15 years old) are more prone to lysis following stress than the other categories and would suggest that females and older animals would more likely develop higher levels of haemolytic anaemia than males and younger animals under similar haemolytic disease condition. Erythrocyte osmotic fragility has been previously reported to increase with the age of the animals (Oyewale *et al*., 1992, Azeez *et al.*, 2011) and age of the erythrocytes, principally due to increased generation of free radicals and reactive oxygen species (ROS) and their damaging effects on membrane protein and lipids (Azeez *et al*., 2009, Ramkumar *et al.,* 2014) as well as the depression of endogenous antioxidants such as glutathione in the body as the animal ages. Brzezińska-Ślebodzińska (2001), explained that oxidative stress results from an imbalance between the effect of pro-oxidants and antioxidant defense mechanisms of the body, and mammalian RBC are particularly susceptible to oxidative damage because they are exposed uninterruptedly to high oxygen tension, with no capacity to repair their damaged components, and higher susceptibility of their membrane components to lipid peroxidation (Galleano and Puntarulo, 1995). This oxidative modification by lipid peroxidation of the membrane thereafter increases the fragility of the RBC to osmotic lysis. Under normal conditions, RBC is rich in antioxidant enzyme defense system that can hydrolyze oxidatively-modified proteins and prevent most of the adverse effects of oxidative stress, but succumb to oxidative damage as the levels of these endogenous antioxidants decreases in the body.

The effect of breed on EOF showed that the South African breed had ($p<0.05$) higher erythrocyte fragility than the Sudanese breed at 0.5% NaCl. indicating a superior South African breed's red cell resistance to lysis at low osmotic gradient. The EOF of horses in relation to activity shows some variations among the groups. At 0.5 % NaCl concentration, the EOF in horses used for security was significantly higher than those used for recreation. Stress factors such as transportation (Adenkola and Ayo, 2009; Yaqub *et al*., 2014), exercise (Hanzawa and Watanabe, 2000), pregnancy (Yaqub *et al.*, 2021) as well as ageing (Azeez *et al.,* 2011) have been reported to increase osmotic lysis. This finding further substantiates the higher haemogram values observed in horses used recreational activities due to less fragility of circulating young or immature erythrocytes. Young erythrocytes and reticulocytes are more resistant to osmotic lysis because of the presence of mitochondria that help to pump out absorbed hypotonic fluid and stabilize the erythrocyte membrane (Clark *et al.,* 2021).

In conclusion, this study shows that the haemogram values for horses from South-western Nigeria is comparable with warm- and hot-blooded horses with

some degree of effects of sex, age, breed and activities on the haematological parameters that can be remarkable in the clinical evaluation and prognostic assessment of these animals during disease conditions. The haematological values reported can therefore serve as additional information for local comparative clinical evaluation of these horses.

Acknowledgement

The authors are grateful to the horse owners for consent to use their horses

Conflict of Interest

The authors have no conflict of interest to declare.

Authors Contribution

The research was conceived and designed by OOG and OTO. The field work, sample collection and processing were by AO, AOO, ATF, AOI, OAA and ODO while data analyzed were by MAM, LYI, AOO, AOI, and AOA. The manuscript was written by MAM, LYI, ODO, and AOA, and revised and approved by AO, AOO, OAA, AOI, ATF, OOG, and OTO.

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