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Clinical, Enzymatic and Hormonal Profile of Apparently Healthy Polo Horses in Ibadan Nigeria

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ABSTRACT

Background and Objective: Clinical examination and other diagnostic procedures, including enzymatic and hormonal assays, are important in equine medicine for disease diagnosis, assessing the extent of damage and monitoring the response to therapy. The study sought to establish the baseline data for some clinical, enzymatic and hormonal parameters and compare them between different ages, breeds and sexes. **Materials and Methods:** A total of 40 apparently healthy polo horses were sampled in Ibadan, Oyo State. Clinical parameters were recorded, blood was drawn through the jugular vein and serum was analysed for enzyme and hormone profiles. The student's t-test was used to compare these parameters and significance was taken at p = 0.05. **Results:** The results revealed more crossbred, female and adult horses in Ibadan, Nigeria. Significantly higher respiratory rate in crossbred horses compared to Sudanese breeds with (31.20±5.93) and (27.20±2.70) was observed, respectively. The respiratory rate was also significantly higher in young horses (34.00±8.49) compared to adult horse sand the adult (27.56±3.03). The values of prolactin, progesterone and oestrogen were significantly higher in female horses compared to males. **Conclusion:** There was a higher population of crossbred, female and adult horses in Ibadan. There were variations in the clinical, enzyme and hormonal values among different ages, sexes and breeds of horses, especially the enzymatic parameters, which seem to be proportionally increasing with the increase in the age of horses in Ibadan, Nigeria.

KEYWORDS

Clinical parameters, enzymatic profile, hormonal profile, polo horses, Sudanese breed

INTRODUCTION

The horse (*Equus caballus*) is a hoofed herbivorous mammal belonging to the Equidae family. It is associated with royalty (kings and noblemen)[1]. The commonest breed of horses in Nigeria includes, a mix of Arewa and their crosses with Dongola, Arabian, Sudanese and Barb breeds that are predominantly raised in Northern Nigeria and used across the country for sports, ceremonial purposes, transportation, draught, research and food[2]. The changes in enzyme levels particularly at the level of the blood are greatly linked with various types of liver pathologies be they infectious or non-infectious such as inflammatory, toxic and metabolic disorders[3]. The consequences of acute and chronic exercise on endocrine homeostasis have been exhaustively reported in the horse and it has been documented that the physical performance

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Data demonstrated by horses in various ways indicates a disturbance to homeostasis that requires an integrative response from the whole system of the horses in question[4,5].

Despite the importance of clinical indices, interpreting them can be difficult due to certain factors. These factors include environmental conditions (lab or field), diet, physical activity, gender, breed, training, age, laboratory procedure, subclinical diseases and host health status[6]. Accurate assessment of laboratory tests relies on the use of reference ranges suited for horses in that region. Despite the growing emphasis on evidence-based veterinary practise[7], there is relatively little substantial scientific evidence for fundamental clinical parameters. There is a dearth of information on various clinical, enzymatic and hormonal parameters in Nigerian horses. The use of reference ranges from different countries and regions might lead to an incorrect interpretation of results and improper treatment or management. When interpreting clinical, enzymatic and hormonal profiles in veterinary medicine, age, breed and gender are important factors to consider.

Therefore, the present study sought to establish the baseline data for some clinical, enzymatic and hormonal parameters of apparently healthy polo horses and determine the likely influence of age, breed and sex on their clinical, enzymatic and hormonal parameters in Ibadan, Nigeria.

MATERIALS AND METHODS

Study area and animals: This study was done between June, 2022 and September, 2022. The study was carried out in Ibadan polo Club, Oyo State. Ibadan polo Club is located at latitude 7.40494° or 7°24'18" North and longitude 3.87204° or 3°52'19" East and the polo field covers an area of about 300 yards in length and 160 yards in width. A total of 40 apparently healthy polo horses were sampled. Information regarding breed and sex was recorded. Age was determined by using dentition as described by Khazaeel *et al.*[8]. Horses under five years old were considered young, while horses over five years old were considered adults.

Clinical evaluation: The clinical parameters evaluated include estimated life weight (kg), respiratory rate (breaths min^{-1}), rectal temperature (°C), blood pressure (mmHg), heart rate (beats min^{-1}) and pulse rate (beats min^{-1}) as previously adopted by Olaogun *et al.*[9,10]. Weight was estimated through measurement of girth circumference and length with appropriate formula[11]. A clinical thermometer manufactured by Eiffel Medical Suppliers Co. Ltd., Shenzhen, Guangdong, China was used to evaluate rectal temperature at a depth of 5 cm. An automatic blood pressure machine manufactured by Shangai Brother Medical Manufacturer Co. Ltd., Shangai, China was used to evaluate the blood pressure, heart rate and pulse rate accordingly.

Blood collection: Five millilitres of blood were collected from each of the horses by jugular venepuncture as previously adopted by Adedokun *et al.*[12]. The blood was dispensed into a plain glass test tube, allowed to clot at room temperature and transported with ice to the General Laboratory, Department of Veterinary Medicine, University of Ibadan. Five millilitres of blood collected were centrifuged at 3000 rpm for 15 min with a clinical table centrifuge and sera were harvested within 1 hr of the collection as previously described by Olaogun *et al.*[13]. The serum was subsequently taken to the Medical Laboratory Department, Adeoyo State Hospital for analyses.

Enzymatic and hormonal analysis: Concentrations of enzymatic parameters, including Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were established by colorimetric method using a reagent kit. The γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP), were measured by adopting the phenolphthaleinmonophosphate method using a reagent kit as earlier adopted by Onyiche *et al.*[14] and Olaogun and Onwuzuruike[15]. Randox laboratory kits manufactured by Fortress

Diagnostics Limited, based in the United Kingdom were the reagents used. Regarding hormonal analysis, the hormonal parameters (luteinizing hormone [LH], follicle-stimulating hormone [FSH], prolactin, progesterone, oestrogen, testosterone and cortisol) were determined by Enzyme-Linked Immunosorbent Assay (ELISA) as previously used by Ajadi *et al.*[16]. The procedure used was as described by the manufacturer of the kit (Randox Laboratories Limited, United Kingdom).

Statistical Analysis: Data generated from the study were subjected to appropriate statistics using the Statistical Package for Social Sciences (SPSS®, version 26). The Shapiro-Wilk test was used to decide whether the clinical, enzymatic and hormonal parameters fit a normal distribution. The student's-test was used to compare the clinical, enzymatic and hormonal parameters of the sexes, breeds and ages. Values of $p \leq 0.05$ were considered significant.

Ethical consideration: The Research Ethics Committee (ACUREC) guidelines of the University of Ibadan were duly followed during the course of this study.

RESULTS

Demography of Horses: Regarding the sexes, 36 (90%) were female while 4 (10%) were male. The two breeds sampled were crossbreed (30, 75%) and Sudanese (10, 25%). In terms of age, 4 (10%) were ≤5 years of age (Young), while 36 (90%) were >5 years of age (Adult) (Table 1).

Clinical, enzymatic and hormonal profiles of apparently healthy polo horses in Ibadan: In terms of the enzymatic parameters, the overall means for AST (IU L^{-1}), ALT (IU L^{-1}), GGT (IU L^{-1}) and ALP (IU L^{-1}) were 85.75±12.14, 9.30±2.43, 6.55±2.06 and 47.80±8.12, respectively. Concerning hormonal parameters, overall means for LH (ng mL^{-1}), FSH (ng mL^{-1}), prolactin (ng mL^{-1}), progesterone (ng mL^{-1}), oestrogen (ng mL^{-1}) and testosterone (ng mL^{-1}) were 11.80±3.07, 8.40±3.02, 10.90±4.54, 3.53±1.87, 26.85±11.01 and 1.28±1.85, respectively (Table 2).

Clinical, enzymatic and hormonal profiles of the Sudanese and crossbreed polo horses in Ibadan: Regarding the enzymatic parameters, there were no significant differences ($p>0.05$) attributable to breed in all the enzymatic parameters: ALT (IU L^{-1}), GGT (IU L^{-1}) and ALP (IU L^{-1}). In terms of hormonal parameters, there were no significant differences ($p>0.05$) attributable to breed in all the hormonal parameters: LH (ng mL^{-1}), FSH (ng mL^{-1}), prolactin (ng mL^{-1}), progesterone (ng mL^{-1}), oestrogen (ng mL^{-1}) and testosterone (ng mL^{-1}) (Table 3).

Clinical, enzymatic and hormonal profiles of the female and male polo horses in Ibadan: There were four significant differences in the hormonal parameters related to sex. The mean prolactin (ng mL^{-1}) of the female polo horses (11.67±4.09) was significantly ($p<0.05$) higher than the male (4.00±1.41).

Table 1: Demographic structure (regarding breed, sex and age) of horses sampled in Ibadan polo Club

Demography	Frequency	Percentage
Breeds		
Sudanese	10	25
Crossbreed	30	75
Sex		
Female	36	90
Male	4	10
Age		
Young	4	10
Adult	36	90
Total	40	100

Table 2: Clinical, enzymatic and hormonal profile of apparently healthy polo horses in Ibadan (n = 40)

Parameters	Mean±SD	Range
Clinical		
Weight	420.15±41.45	328.00-482.00
Temperature (°C)	37.53±0.52	36.40-39.10
Heart rate (beats min^{-1})	42.65±12.05	28.00-72.00
Pulse rate (beats min^{-1})	41.30±8.84	31.00-68.00
Systolic pressure (mm Hg)	92.05±10.75	71.00-114.00
Diastolic pressure (mm Hg)	55.65±9.81	37.00-75.00
Respiratory rate (breaths min^{-1})	28.20±4.00	24.00-40.00
Enzymes		
Aspartate aminotransferase (IU L^{-1})	85.75±12.14	74.00-115.00
Alanine transaminase (IU L^{-1})	9.30±2.43	6.00-13.00
γ-glutamyl transferase (IU L^{-1})	6.55±2.06	3.00-10.00
Alkaline phosphatase (IU L^{-1})	47.80±8.12	34.00-64.00
Hormones		
Luteinizing hormone (ng mL^{-1})	11.80±3.07	6.00-16.00
Follicle stimulating hormone (ng mL^{-1})	8.40±3.02	4.00-13.00
Prolactin (ng mL^{-1})	10.90±4.54	3.00-21.00
Progesterone (ng mL^{-1})	3.53±1.87	0.20-7.00
Oestrogen (ng mL^{-1})	26.85±11.01	0.80-41.00
Testosterone (ng mL^{-1})	1.28±1.85	0.10-8.00

Table 3: Clinical, enzymatic and hormonal profiles of the Sudanese and crossbred polo horses in Ibadan

	Breed	
	Sudanese (n = 30)	Crossbred (n = 10)
Parameters	Mean±SD	Mean±SD
Clinical		
Weight	427.47±38.34	398.20±47.02
Temperature (°C)	37.53±0.58	37.54±0.33
Heart rate (beats min^{-1})	44.06±12.27	38.40±11.52
Pulse rate (beats min^{-1})	43.20±9.01	35.60±5.73
Systolic pressure (mm Hg)	92.66±11.79	90.20±7.50
Diastolic pressure (mm Hg)	55.67±10.04	55.60±10.21
Respiratory rate (breaths min^{-1})*	27.20± 2.70	31.20± 5.93
Enzymes		
Aspartate aminotransferase (IU L^{-1})	87.70±12.65	80.20±9.47
Alanine transaminase (IU L^{-1})	9.07±2.43	10.00±2.55
γ-glutamyl transferase (IU L^{-1})	6.47±2.00	6.80±2.49
Alkaline phosphatase (IU L^{-1})	47.87±8.18	47.60±8.91
Hormones		
Luteinizing hormone (ng mL^{-1})	12.00±3.05	11.20±3.42
Follicle-stimulating hormone (ng mL^{-1})	8.60±3.18	7.80±2.68
Prolactin (ng mL^{-1})	11.07±5.12	10.40±2.4
Progesterone (ng mL^{-1})	3.57±2.05	3.40±1.34
Oestrogen (ng mL^{-1})	26.60±12.32	27.60±6.66
Testosterone (ng mL^{-1})	1.00±1.07	2.12±3.33

Asterisk superscript letter(s) on any indices show significant difference ($p<0.05$)

The mean progesterone (ng mL^{-1}) of the female polo horses (3.88±1.61) was significantly ($p<0.05$) higher than the male (0.35±0.21). The mean oestrogen (ng mL^{-1}) of the female horses (29.72±6.94) was significantly ($p<0.05$) higher than the male (1.00±0.28) (Table 4).

Clinical, enzymatic and hormonal profiles of the young and old polo horses in Ibadan: The mean respiratory rate (breaths min^{-1}) of the young (34.00±8.49) was, however, significantly ($p<0.05$) higher than the adult (27.56±3.03). Regarding the enzymatic parameters, there were no significant differences

Table 4: Clinical, enzymatic and hormonal profiles of the female and male polo horses in Ibadan

	Sex	
Parameters	Female (n = 36) Mean±SD	Male (n = 4) Mean±SD
Clinical		
Weight	424.17±41.71	384.17±12.73
Temperature (°C)	37.52±0.53	37.79±0.42
Heart rate (beats min^{-1})	43.39±12.44	36.00±5.66
Pulse rate (beats min^{-1})	41.89±9.04	36.00±5.66
Systolic pressure (mm Hg)	91.67±11.14	95.50±7.78
Diastolic pressure (mm Hg)	55.56±9.84	56.50±13.44
Respiratory rate (breaths min^{-1})	28.44±4.09	26.00±2.83
Enzymes		
Aspartate aminotransferase (IU L^{-1})	85.67±12.32	86.50±14.85
Alanine transaminase (IU L^{-1})	9.44±2.43	8.00±2.83
γ-glutamyl transferase (IU L^{-1})	6.61±2.15	6.00±1.41
Alkaline phosphatase (IU L^{-1})	48.28±8.23	43.50±7.78
Hormones		
Luteinizing hormone (ng mL^{-1})	12.11±3.07	9.00±1.41
Follicle stimulating hormone (ng mL^{-1})	8.72±3.01	5.50±0.71
Prolactin (ng mL^{-1})*	11.67±4.09	4.00±1.41
Progesterone (ng mL^{-1})*	3.88±1.61	0.35±0.21
Oestrogen (ng mL^{-1})*	29.72±6.94	1.00±0.28
Testosterone (ng mL^{-1})	1.05±1.81	3.35±0.35*

Asterisk superscript letter(s) on any indices show significant difference ($p<0.05$)

Table 5: Clinical, enzymatic and hormonal profiles of the young and adult polo horses in Ibadan

	Age	
Parameters	Young (n = 4) Mean±SD	Adult (n = 36) Mean±SD
Clinical		
Weight	419.94±42.96	422.00±36.36
Temperature (°C)	37.30±0.14	37.56±0.54
Heart rate (beats min^{-1})	33.50±7.78	43.67±12.16
Pulse rate (beats min^{-1})	41.50±14.85	41.23±8.62
Systolic pressure (mm Hg)	87.00±7.07	92.61±11.08
Diastolic pressure (mm Hg)	53.00±1.41	55.94±10.32
Respiratory rate (breaths min^{-1})*	34.00±8.49	27.56±3.03
Enzymes		
Aspartate aminotransferase (IU L^{-1})	84.50±12.84	85.89±12.32
Alanine transaminase (IU L^{-1})	6.50±0.71	9.61±2.35
γ-glutamyl transferase (IU L^{-1})	5.50±0.71	6.67±2.14
Alkaline phosphatase (IU L^{-1})	44.5±14.85	48.12±7.70
Hormones		
Luteinizing hormone (ng mL^{-1})	14.5±0.71	11.50±3.09
Follicle stimulating hormone (ng mL^{-1})	10.00±00	8.22±3.14
Prolactin (ng mL^{-1})	9.50±0.71	11.06±4.77
Progesterone (ng mL^{-1})	4.05±0.07	3.47±1.97
Oestrogen (ng mL^{-1})	35.5±6.36	25.89±11.11
Testosterone (ng mL^{-1})	0.55±0.35	1.36±1.94*

Asterisk superscript letter(s) on any indices show significant difference ($p<0.05$)

($p>0.05$) attributable to breed (young and adult) in all the enzymatic parameters: ALT (IU L^{-1}), GGT (IU L^{-1}) and ALP (IU L^{-1}). In terms of hormonal parameters, there were no significant differences ($p>0.05$) attributable to breed in all the hormonal parameters: LH (ng mL^{-1}), FSH (ng mL^{-1}), prolactin (ng mL^{-1}), progesterone (ng mL^{-1}), oestrogen (ng mL^{-1}) and testosterone (ng mL^{-1}) (Table 5).

DISCUSSION

The present study revealed more crossbred horses compared to Sudanese breed, more female compared to male and more adult compared to young horses. This may be due to natural mating system being adopted by most of the horses' owners in Nigeria. It also appears that female horses were generally more in population in relation to male which maybe basically for procreation potential of female animals as one good male horse is enough for ten female horses' reproductive wise. The values of all indices analysed in this study were within the normal reference range of values, this implies that horses sampled were well taken care of and apparently healthy as assessed during the period of sampling. The demography of horses in the study location as revealed was similar to the trends observed by Alaba *et al.*[17], who also discovered higher number of exotic breed, female and adult horses compared to local breed, male and young horses, respectively. Greater number of crossbred horses compared to local might be due to improved growth, productivity and performance associated with crossbred horses. More mares compared to stallion may be as a result of reproductive potential of female horses regarding birth of foals. Adult horses are generally preferred for polo game rather than young horses.

The normal rectal temperature mean value for the horses in the present study was 37.53°C, when measured with a clinical digital thermometer. This was similar to several reports[18-20]. Giannetto *et al.*[21] and Piccione *et al.*[22], on the other hand, reported a higher temperature. The disparity could be accounted for by the fact that rectal thermometers were inserted 15 cm deep in these studies. Due to the lack of deep rectal probes among horse owners, a reference range set at 5 cm depth is preferable.

The present pulse rate, heart rate and respiratory rate are higher than the generally known reference values[20], which could be due to fright or excitement in the horses. The current study's serum AST and ALP values were lower than those reported in the Western world[3], but higher than those previously reported in Nigeria[1,23]. The decreased AST activity found in Nigerian horses is assumed to be owing to their reduced muscle mass in comparison with their Western world counterparts, as serum AST primary cellular sources are majorly in the skeletal myocytes. The hormonal profiles in the present study are strongly affected by the sex and reproductive phase of the female horses[24], hence, their overall mean values might not be conclusive as a reference value.

The present study shows that prolactin (ng mL^{-1}), progesterone (ng mL^{-1}) and oestrogen (ng mL^{-1}) of the female polo horses were significantly ($p<0.05$) higher than the male. This is predictable because these hormones reveal the characteristics that set females apart from males. In general, females and males produce similar hormones, such as progesterone, oestrogen and testosterone, but their production sites, blood concentrations and interactions with organs and systems differ[25].

In the current study, the respiratory rate of the young polo horses was substantially greater than that of the adults. Young ones have much higher metabolic rates than adults, resulting in a higher oxygen demand, which leads to increased respiratory rates.

According to the findings of this study, the respiratory rate in Ibadan polo horses was affected by age and sex, whereas prolactin, progesterone and oestrogen were affected by sex. Apart from the respiratory rate mean value that was significantly higher in crossbred horses compared to Sudanese, every other parameter showed no significant variation in their values. This variation in respiratory rate may be due to differences in genetic composition and size. This indicates no breed variations in enzymes, hormones and most of the clinical parameters. According to the results of this present study, there was no significant influence of breed on the clinical parameters, enzymatical and hormonal levels amongst horses which was similar to the observation of Shawaf *et al.*[25], who also reported no variation in these parameters among

different breeds of horses. This may be due to the relatively similar genetic composition exists in breeds, similar feeding regimens, housing and management systems that both breeds are being exposed to. This present study also revealed a lack of any significant influence of sex on clinical and enzymatic parameters of horses, this was contrary to the observation of Cruz *et al*.[26], who reported significant variation in the level of some enzymes and clinical parameters among female and male horses. The finding from this study regarding age influence on the parameters revealed no significant differences in all the tested parameters. Except for the significantly higher respiratory rate in young horses compared to adults, this disagreed with the observation of Raymond *et al*.[27], who reported a significant influence of age on clinical and most of the biochemical analyses in horses. This corroborated the findings of Czech *et al*.,[28] who reported no significant influence of age on values of liver enzymes among horses of the Malopolski breed on a farm in South-Eastern Poland. This study has been able to provide baseline data for clinical, enzymatic and hormonal indices among polo horses in Ibadan. It has also been able to reveal slight or no variations when comparisons were made regarding breed, gender and age of horses. The outcome of this study provides relevant information that may be useful in the designing of appropriate equine health care, in the determination of equine health status, assessment of the wellbeing and welfare of equines and in proper management and prevention of equine diseases in general. The low sample size due to financial constraints, inability of some horses' owners to allow their animals to be sampled, bias in the results output due to low sample size and high cost of analysis were part of the limitations of this study. It is therefore recommended that further research with a larger sample size, prolonged sample period and probably a whole year in order to determine the influence of season, feeding and climatic conditions on these parameters. Horse owners should be educated on the reason and importance of research and the need for them to always consent their animals for sampling. Researchers are also encouraged to give feedback to owners of horses about the outcome of their research.

CONCLUSION

It is concluded that crossbred, female and adult horses were more at the Ibadan polo club. There are not many significant variations in clinical, enzymatic and hormonal values regarding breed, gender and age of polo horses in Nigeria. Though clinical, enzymatic ally indices of adult horses were generally higher than young horses and the values of hormones were generally higher in young horses compared to adults. crossbred and adult horses possessed higher resilience potential compared to Sudanese breed and young horses respectively.

SIGNIFICANCE STATEMENT

This study reveals relatively higher resilience of Sudanese breed compared to crossbred horses and the hormonal profile was the first report of such among polo horses in Ibadan Nigeria. There are not many significant variations in clinical, enzymatic and hormonal values regarding breed, gender and age of polo horses in Nigeria. These findings will be most relevant for equine clinicians as baseline values in Nigerian horses, they may also be considered when dealing with horses in health and disease states.

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