

Effect of season, age and sex on haematology and serum biochemistry of sport horses

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ABSTRACT

Blood is an important medium for assessing the health status of individual animals: variations in blood parameters of animals are due to several factors such as altitude, feeding level, age, sex, breed, disease, seasonal variations, temperature and physiological status. This study was designed to evaluate the serum biochemical profile and haematology of sport horses of mixed breeds to determine the effect of season, age and sex on these parameters. A total of 40 horses were assayed thrice in the year of study (2020): early rainy season (March); peak of rainy season (July) and dry season (December). Blood was collected by jugular venipuncture and all the parameters were determined using standard procedures. Serum aspartate transaminase (AST), alkaline phosphatase (ALP), globulin and creatinine were significantly ($P < 0.05$) different across all the seasons assayed, with the rainy season having the highest levels. Total protein and albumin were significantly ($P < 0.05$) higher in the rainy season while total bilirubin and urea were significantly ($P < 0.05$) higher in the dry season. Packed cell volume (PCV), erythrocyte and total leukocyte counts were significantly ($P < 0.05$) higher in the dry season than rainy season. Serum AST and total protein were significantly ($P < 0.05$) higher in the adult horse, while AP was higher ($P < 0.05$) in the young. PCV, RBC & TWBC were higher in the young horses. Season and age affect blood profile and serum biochemistry of mixed breed sport horses and should be considered when using these parameters for clinical diagnosis.

Keywords: Age, blood profile, rainforest, Season, serum biochemistry, sport horses

INTRODUCTION

Horse (*Equus caballus*) is a domesticated mammal of the family Equidae and order Perissodactyla. It is used for various purposes that include draft, transport, sports, ceremonial exhibitions and research purposes. Other uses of horse are warfare, crowd control, as well as food (meat) and source of variety of products (Ihedioha & Agina, 2013). In horses, haematological and serum biochemistry assessments are used as an aid to the clinical diagnosis of variety of diseases, in monitoring recovery during treatment and to assess the health status of a single animal or an entire herd (Ricketts, 1987). Effects of physiological factors such as age, sex, breed, exercise and environment/geographical location on blood profile of horses have been extensively studied and reported in many parts of the world (Lumsden *et al.*, 1980; Krumrych, 2006; Lacerda *et al.*, 2006; Altinsaat, 2008; Ihedioha & Agina, 2013). Changes caused by the disease

conditions, other forms of injury, deprivation and/or stress reflect changes in the blood and serum biochemistry (Coles, 1986, Stockham and Scott, 2008. Ihedioha & Agina, 2013). In horses, haematological and serum biochemistry assessments are used as an aid to the clinical diagnosis of variety of diseases, in monitoring recovery during treatment and to assess the health status of a single animal or an entire herd (Ricketts, 1987). Haematological values and serum biochemistry of horses vary in different locations and seasons, therefore the need to establish reference values for the horse population in these environments (Coles, 1986; Ricketts, 1987; Stockham & Scott, 2008, Ihedioha & Agina, 2013). Many laboratories, clinics and research institutes in the West have reference values for serum biochemistry and haematology in animals, including horses (Lumsden *et al.*, 1980; Krumrych, 2006; Lacerda *et al.*, 2006; Altinsaat, 2008; Ihedioha & Agina, 2013). However, there is paucity of data

on the effects of season, age and sex on haematological profile and serum biochemistry of Nigerian sport horses. Ihedioha & Agina (2013) reported the effect of season, age and sex on only the serum biochemistry of horses in Nsukka, a dry and humid environment. The present study therefore evaluated the haematological profile and serum biochemistry of apparently healthy exotic sport horses in the rain forest zone of Nigeria and determined the influence of age, sex and seasons on these parameters.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND HOUSING

The study was conducted on mixed breed horses in Port Harcourt polo club Rivers State, Southern Nigeria. Port Harcourt has coordinates of 4.8156° N and 7.0498° E. Forty horses were sampled, consisting of 26 females and 14 males of which nine were young and thirty-one were adult horses, including Argentine and Sudan breeds. They were aged using tooth eruption and wear. The horses were in standard stables, and fed on wheat bran, sorghum, hay and fresh pasture. Water was given *ad libitum*. Only apparently healthy adult horses were included in the study.

COLLECTION OF SAMPLES

Five millilitres of blood was collected from each of the horses by jugular venipuncture. Blood samples for haematological examination were dispensed into sample bottles containing anticoagulant (EDTA) while samples for serum biochemical analyses were collected into plain sample bottles, allowed to clot and the serum was separated by centrifugation at 3000rpm for 10minutes. Samples were stored at 4 °C until analysed.

ANALYTICAL PROCEDURES

Packed cell volume (PCV) was determined by the microhaematocrit method. Red Blood Cell and White Blood Cell counts were done using haemocytometer method (Thrall & Weiser, 2002) while PCV was determined using microhaematocrit method (Bull & Hay, 2001). Haemoglobin levels were determined using cyanomethaemoglobin method and Red blood cell indices were calculated using standard formulae. Serum creatinine levels were assayed following the modified Jaffe method (Blass *et al.*, 1974; Lamb & Price, 2008), using Quimica Clinica Aplicada (QCA) Creatinine test kit (QCA, Spain). The serum urea levels were determined by the modified Berthelot-Searcy method (Lamb and Price 2008), using the Dialab Urea (urease/colorimetric) test kit (Dialab, Neudorf, Austria). Total bilirubin was determined by colourimetric method as described by Jendrassik and Grof

(1938). Total protein was determined by the Biuret method (Lubran, 1978). Serum albumin was determined using the bromocresol green method (Doumas *et al.*, 1973) and globulin was determined by subtracting the albumin value from total protein (Johnson, 2008). Alkaline phosphatase was determined using the phenolphthalein monophosphate method (Babson *et al.*, 1966) and aspartate transaminase level was assayed using the Reitman-Frankel method (Reitman & Frankel, 1957).

STATISTICAL ANALYSIS

Results are presented as Means \pm Standard error mean. Data were analysed by one way Analysis of Variance (ANOVA). Means were separated using least square deviation (LSD) and significance was accepted at $P < 0.05$.

RESULTS

EFFECT OF SEASON ON HAEMATOLOGY OF SPORT HORSES

The PCV, RBC and Total white blood cell were 32.18 ± 0.63 %, $7.69 \pm 0.15 \times 10^6/\mu\text{l}$ and $8.76 \pm 0.63 \times 10^3/\mu\text{l}$ for the rainy season respectively and were significantly ($P < 0.05$) lower than the values for the dry season which were 38.12 ± 1.62 %, $9.06 \pm 0.36 \times 10^6/\mu\text{l}$ and $13.45 \pm 0.61 \times 10^3/\mu\text{l}$ respectively (Table I). Haemoglobin concentration and Mean corpuscular volume values for the rainy season were 16.92 ± 0.50 g/dl & 43.70 ± 0.05 fl respectively while similar values for dry season were 16.55 ± 0.49 g/dl and 43.80 ± 0.34 fl respectively. However, there was no significant difference ($P > 0.05$) between the two seasonal values.

Table I. Effect of season on haematology of sport horses

PCV- packed cell volume; RBC- red blood cell count; TWBC- total white blood cell count; MCH- means corpuscular haemoglobin; MCV- mean corpuscular volume; MCHC- mean corpuscular haemoglobin concentration.

Parameters	Early rainy season	Rainy season	Dry season
Haemoglobin(g/dl)	17.52 ± 0.37^a	16.92 ± 0.05^a	16.55 ± 0.49^a
PCV (%)	30.75 ± 1.14^a	32.18 ± 0.63^a	38.12 ± 1.62^b
RBC ($\times 10^6$)	7.22 ± 0.26^a	7.69 ± 0.15^a	9.06 ± 0.36^b
TWBC ($\times 10^3/\mu\text{l}$)	9.96 ± 0.57^a	8.76 ± 0.63^a	13.45 ± 0.61^b
MCH (pg)	4.49 ± 0.58^a	22.10 ± 0.74^b	18.37 ± 0.20^c
MCV(fl)	43.45 ± 0.41^a	43.70 ± 0.05^a	43.80 ± 0.34^a
MCHC (g/dl)	58.36 ± 1.47^a	52.65 ± 1.79^a	43.70 ± 0.57^a

^{abc}mean in the same row with different superscripts are significantly different at $P < 0.05$

EFFECT OF SEASON ON SERUM BIOCHEMISTRY OF SPORT HORSES

The serum AST and ALP activities of the horses were significantly ($P < 0.05$) higher during the rainy season

(95.00±0.48 and 67.25±0.35) compared to that of the dry seasons (104.18±0.34 IU/L and 91.25±0.62 IU/L) respectively (Table II). Total protein, albumin and globulin levels were significantly higher during the rainy season compared to that of the dry season. Total proteins, albumin and globulin were 7.24±0.01 g/dl, 3.66±0.01 g/dl and 3.58±0.01g/dl respectively for the rainy season and was significantly higher ($P<0.05$) than the values for the dry season which were 6.62±0.01, 3.37±0.01 and 3.24±0.02 respectively. Total bilirubin, urea and creatinine levels were significantly ($P<0.05$) higher during the dry season (3.55±0.02, 33.67±0.29 and 1.37±0.01) than in the rainy season (3.42±0.03 mg/dl, 32.57±0.1 mg/dl and 1.30±0.01mg/dl).

EFFECT OF AGE ON HAEMATOLOGY OF SPORT HORSES

Effect of age on haematology of sport horses is presented in

Table II: Effect of season on serum biochemistry of Sport Horses

AST-aspartate aminotransferase; ALP-alkaline phosphatase

[Parameters	Early rainy season	Rainy season	Dry season
AST (IU/L)	95.16±0.42 ^a	104.18±0.34 ^b	95.00±0.48 ^a
ALP (IU/L)	71.68±0.42 ^a	91.25±0.62 ^b	67.25±0.35 ^c
Total protein (g/dl)	6.69±0.01 ^a	7.24±0.01 ^b	6.62±0.01 ^a
Globulin(g/dl)	3.53±0.21 ^a	3.66±0.01 ^b	3.37±0.01 ^c
Albumin (g/dl)	3.15±0.02 ^a	3.58±0.01 ^b	3.24±0.02 ^a
Total bilirubin (mg/dl)	3.31±0.03 ^a	3.42±0.03 ^a	3.55±0.02 ^b
Urea (mg/dl)	31.26±0.25 ^a	32.57±0.1 ^a	33.67±0.29 ^b
Creatinine (mg/dl)	1.28±0.01 ^a	1.30±0.01 ^b	1.37±0.01 ^c

^{abc}:means in the same row with different superscripts are significantly different ($P<0.05$).

Table III. The PCV, RBC, MCH and Total white blood cell values were significantly ($P<0.05$) higher in young horses (33.11±2.85 %, 7.86±0.66 × 10⁶/μl, 43.84±0.10 fl, and 11.09±1.38 × 10³/μl respectively) compared to adults (32.94±0.71 %, 7.82±0.16 × 10⁶/μl , 43.39±0.20 fl and 9.91±0.45 × 10³/μl respectively). However, adult horses had significantly ($P<0.05$) higher haemoglobin levels, MCV and MCHC than the young ones.

EFFECT OF AGE ON SERUM BIOCHEMISTRY OF SPORT HORSES

Adult horses had significantly ($P<0.05$) higher serum AST and total protein (99.09±0.88 IU/L, 6.92±0.05 IU/L

respectively) than the young (97.77±1.41 IU/L and 6.78±0.08 IU/L) and lower ALP (79.93±2.01 IU//L) than in the young horses (73.33±2.79 IU/L) (Table IV). The values for serum albumin, globulin, urea, creatinine and total bilirubin were 3.30±0.06 g/dl, 3.44±0.44 g/dl, 32.84±0.26 mg/dl, 1.34±0.02 mg/dl and 3.49±0.49 mg/dl respectively for the young and 3.36±0.37, 3.56±0.22, 32.34±0.26, 1.30±0.2 and 3.39±0.26 respectively for adults. There were however no significant ($P>0.05$) differences in these parameters for the young and adult horses.

EFFECT OF SEX ON HAEMATOLOGY OF SPORT HORSES.

Male horses had higher levels of haemoglobin, PCV, RBC, TWBC, MCH, MCV and MCHC than female horses (Table V). These differences were however, not statistically significant ($P>0.05$).

EFFECT OF SEX ON SERUM BIOCHEMISTRY OF SPORT HORSES.

Female horses had higher levels of AST, ALP and total protein than the males, while the young horses had higher levels of globulin, total bilirubin, creatinine and urea (Table VI). However, these differences were not significant ($P>0.05$).

DISCUSSION

The result of this study demonstrated that the mean values of the various parameters did not differ from the normal value of healthy exotic horses as reported by Schalm *et al.* (1975).The horses assayed in this study were of mixed breed and so had values comparable to foreign breeds. Serum AST, ALP, total protein, total bilirubin, creatinine and urea values in this study during the rainy and dry seasons were comparable to but slightly higher than that reported by Ihedioha *et al.*, 2013 for local Nigerian horses. This study recorded a low AST limit of 95.00 IU, this is higher than the lowest value of 74.38 reported by Ihedioha & Agina (2013). This high AST recorded in the horses could be due to their use as sport horses, because AST is known to be associated with increase in muscular activity and the skeletal myocytes are a major source of AST (Stockham & Scott, 2008). Higher levels of AST and ALP were recorded in the rainy season in comparison to the dry season. This was also reported by Ihedioha & Agina (2013) for horses in Enugu and in other animals including sheep, goat, cattle, and rabbits (Ihedioha &

Table III: Effect of age on haematology of Sport Horses

PCV- packed cell volume; RBC- red blood cell count; TWBC- total white blood cell count; MCH- means corpuscular haemoglobin; MCV- mean corpuscular volume; MCHC- mean corpuscular haemoglobin concentration.

Parameters	Young (n=9) (<2yrs)	Adult (n=31) (>2yrs)
HB(g/dl)	16.42±0.63	17.20±0.30
PCV (%)	33.11±2.85 ^a	32.94±0.71 ^b
RBC (×10 ⁶ /μl)	7.86±0.66 ^a	7.82±0.16 ^b
TWBC (×10 ³ /μl)	11.09±1.38 ^a	9.91±0.45 ^b
MCH (pg)	43.84±0.10 ^a	43.39±0.20 ^b
MCV(fl)	21.78±1.61 ^a	22.22±5.2 ^b
MCHC (g/dl)	51.82±3.94 ^a	53.02±1.2 ^a

^{ab}: means in the same row with different superscripts are significantly different at $P<0.05$.

Table IV: Effect of age on serum biochemistry of Sport Horses

AST-aspartate aminotransferase; ALP-alkaline phosphatase

Parameters	Young (n=9) (<2yrs)	Adult (n=31) (>2yrs)
AST (IU/L)	97.77±1.41 ^a	99.09±0.88 ^b
ALP (IU/L)	79.93±2.01 ^a	73.33±2.79 ^b
Total protein (g/dl)	6.78±0.08 ^a	6.92±0.05 ^b
Globulin (g/dl)	3.44±0.44 ^a	3.56±0.22 ^a
Albumin (g/dl)	3.30±0.06 ^a	3.36±0.37 ^a
Total bilirubin (mg/dl)	3.49±0.49 ^a	3.39±0.26 ^a
Urea (mg/dl)	32.84±0.26 ^a	32.34±0.26 ^a
Creatinine (mg/dl)	1.34±0.02 ^a	1.30±0.20 ^a

^{ab}: means in the same row with different superscripts are significantly different ($P<0.05$).

Agina, 2013). Urea and creatinine levels increased during the dry season in comparison to rainy season. This is in agreement with Ihedioha and Agina, (2013), despite the difference study locations and this increase may be attributed to dehydration and greater loss of water through sweat during sporting activities.

There were no seasonal variations in the PCV, MCV, MCHC and MCH values in this study. This is in agreement with previous studies by Ihedioha *et al.* (2013) for horses in Enugu, Zakari *et al.* (2015) for donkeys, Habibu *et al.*, (2016) and Olayemi *et al.* (2015) for Red Sokoto goats. However, Hb, RBC and TWBC levels were high during the

Table V: Effect of sex on haematology of sport horses

PCV- packed cell volume; RBC- red blood cell count; TWBC- total white blood cell count; MCH- means corpuscular haemoglobin; MCV- mean corpuscular volume; MCHC- mean corpuscular haemoglobin concentration.

Parameters	Female (n=26)	Male (n=14)
Haemoglobin(g/dl)	16.37±0.33 ^a	17.57±0.46 ^a
PCV (%)	32.26±0.84 ^a	33.85±1.75 ^a
RBC (×10 ⁶ /μl)	7.69±0.19 ^a	8.09±0.40 ^a
TWBC (×10 ³ /μl)	9.65±0.58 ^a	11.14±7.36 ^a
MCH (pg)	43.42±0.24 ^a	43.64±0.11 ^a
MCV(fl)	22.06±0.65 ^a	22.23±0.97 ^a
MCHC (g/dl)	52.65±1.58 ^a	52.94±2.33 ^a

dry season than in the rainy season. This is in agreement with earlier reports on donkey (Zakari *et al.*, 2015) and red sokoto goats (Habibu *et al.*, 2016). These increases may be attributed to dehydration during the dry season resulting to haemoconcentration. Male horses had higher values of Hb, PCV and RBC. Although these were not statistically different, it corroborates previous reports by Satue *et al.* (2009) who reported that male horses have slight RBC, Hb & PCV than the females. This insignificance in the haematology of both sexes may be because the male and female athletic horses are used for the same purposes from time to time throughout the year. Adult horses had significantly higher AST and total protein than the young and lower AST than the young. This agrees with the report by Ihedioha & Agina, (2013) who recorded higher ALP in the young horses. Increased ALP levels reflects could be due to bone changes due to osteoclastic activity in young horses (Ihedioha & Agina, 2013) while higher AST levels in adults reflects more muscular activities than in the young animals.

The PCV, RBC, MCH and MCV values were significantly higher in young horses compared to adults, but Hb levels were lower than that of the adults. This corresponded to previous reports by Altinsaat (2008) and Mikniene *et al.* (2014). Also Muñoz *et al.* (2012) reported higher PCV, RBC and Hb in Spanish foals than those in adult Spanish horses. Grondin & Dewitt (2010) reported that new-born foals have higher PCV, RBC, and HB because erythrocytes are of foetal origin, thus the involvement of the liver and spleen in erythropoiesis (Zakari *et al.*, 2015). However, the lower haemoglobin concentrations in the young horses may result from the fact that the life span of erythrocytes is shorter in

young animals due to the destruction of erythrocytes by the spleen.

CONCLUSION

We conclude that serum biochemistry and haematological parameters of mixed breed sport horses were influenced by season and age and not by sex. Therefore, these factors should be considered when using these parameters for clinical diagnosis in these horses.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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