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Influence of Age on Haematology, Serum Biochemistry and Lipid Profile of Stallions in Ilorin, Nigeria

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ABSTRACT

This study determined the influence of age on haematological and serum biochemical parameters and lipid profiles of stallions in Ilorin, Nigeria. Blood samples from 50 adult stallions were collected from different stables within Ilorin metropolis, and haematological and serum biochemical parameters were determined according to standard procedures. The stallions were grouped into three: <5 years (n=12), 6-11 years (n=33) and >11 years (n=5). The values obtained were subjected to a one-way ANOVA ($p<0.05$) using Tukey's multiple comparison test as post test to determine the significant differences in the haematological and serum biochemical parameters in relation to age differences. Stallions with less than 5 years of age showed significant ($p< 0.001$) increase in PCV, red blood cell and haemoglobin counts when compared with stallions aged 6-11 years and above 11 years. Serum albumin and triglycerides for the <5 years of age stallion significantly reduced and increased respectively when compared to the stallions aged 6-11 years and above 11 years. There was no significant difference in cholesterol, white blood cell, neutrophils and lymphocytes counts. Age influences haematological and serum biochemical parameters in stallions. This is more noticeable in stallions aged five years and below. The findings from this study will guide clinicians in interpretations of haematological and serum biochemical values in different age group of stallions.

Keywords: Haematology; Ilorin; Serum biochemistry; Stallion

INTRODUCTION

In Nigeria, horses are used for cultural ceremony, draft, transportation, sports and research purposes (Garba *et al.*, 2015; Agina and Ihedioha, 2017). Nigerian horses are also known as Arewa, Dongola, Bornu, Djerma, Hausa or West African Dongola. The horses are characterised by a convex profile facial bone with about 152-157cm in height. Their colour include bay, black, chestnut, gray while white or white patterns are less frequent (Valerie *et al*., 2016).

Measurement of haematological and serum biochemical parameters of animals is one of the tools that provides valuable data to evaluate health condition of animals, to confirm tentative diagnoses, as well as to evaluate treatments and outcomes in both, a single animal or herds (Cruz *et al.*, 2017).

Haematological examination is essential in clinical diagnosis of disease whether infectious or non-infectious disease in equine practice (Aiello *et al*., 2016). Its indications are extended to the routine management of horses such as routine foal examinations, horse performance monitoring, pre-anaesthetic checks and pre-procurement examinations. Serum biochemical parameters analysis is also an essential tests use in diagnosis since their analytical value notify a clinician about serum biochemical alterations which can be use in the assessment of pathological changes in visceral organs of the body such as liver, kidney, pancreas, heart and muscles (Ihedioha and Agina, 2013).

Haematological and serum biochemical parameters of horses can be affected by various factors which include the age, sex, breed, exercise, nutritional status and geographical location (Egbe-Nwiyi *et al*., 2012; Ihedioha and Agina, 2013; Mikniene *et al.*, 2014; Grden *et al.*, 2019).There is no doubt that the awareness of these factors is a prerequisite to distinguish between physiological and pathological blood pictures (Rubinoa, *et al*., 2006).

There is dearth of information on haematological and serum biochemical parameters of stallions in relation to age. Therefore, this study evaluated the influence of age on haematological parameters and some selected serum biochemical parameter in a population of stallion in Ilorin, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Ilorin, Kwara State, Nigeria. Ilorin is one of the largest city in the north-central part of

Nigeria (National Bureau of Statistics, 2017). Ilorin is located on latitude 8.4799°N, longitude 4.5418°E in north-central, Nigeria, and situated 320 meters above the sea level (Abiodun and Gbenga, 2016).

Experimental Stallion

Fifty apparently healthy male horses were considered for this study aged between 3 to 16 years old. They were from different stables owned by the Emir; the royal chieftaincies, Nigeria Army Sobi Barracks and the Nigeria Police Force (mounted troop) within Ilorin city in the early rain season (March-May). The stallions were grouped by age, Group A (1 year – 5 years) n=12; Group 2 (6 years – 10 years) n=33 and Group C (11 years – 16 years) n=5 for haematological analysis, while 5 horses from each group were used for the serum biochemical analysis. Their ages were evaluated based on tooth eruption and wear (Evans and Jack, 2007).

Blood Collection

Procedure for blood collection was done (under physical restraint) through venepuncture of the jugular vein. 5ml of blood sample was collected aseptically from each of the horses and dispensed into sterile EDTA sample bottle for haematology analysis. Another 5ml of blood sample was collected into sterile plain bottle for serum biochemical analysis. Blood samples collected were transported to the laboratory under a regulated temperature (12^{o}C-20^{o}C) using an insulated cold box.

Haematology and Serology Analyses

The blood samples upon getting to the laboratory were further analysis. The samples collected inside EDTA sample bottles were used for haematology;

Using a sterile grease-free glass slide a thin smear of blood was made from each of sample to determine the blood cells for each of the sample. Furthermore, plain capillary tube was used to withdrawn blood from each of the sample which was spun using haematocrit centrifuge to determine the packed cell volume (PCV) of each of the sample using haematocrit reader (Ihedioha and Agina, 2013).

Furthermore, white blood cells (WBC), platelets, red blood cells, differential cells count, Haemoglobin, and haematological indices such as Mean corpuscular volume (MCV) were analysed and estimated (Jain and Schalm, 1986).

The blood samples collected into the sterile plain bottles were immediately spun using bench centrifuge for extraction of serum. The serum extracted was dispensed into Eppendorf tube and stored inside freezer at -20^{o}C for further serum biochemical analyses which include; protein, albumin, BUN, ALT, creatinine quantification and the lipid profile of the sample (Ihedioha and Agina, 2013).

All the serum biochemical analyses were done according to manufacturer manuals using Fortress Diagnostics® and Pointe Scientific® kits. Total protein from the sera samples was determined using test principle by quantified the protein through a reaction whereby the copper ions in alkaline solution react with protein peptide bonds to give a purple-coloured biuret complex. Furthermore, creatinine from the sera samples was determined using Jaffe method. Albumin in the sera samples were determined quantitatively through bromocresol green (BCG) dye-binding method.

Also, Quantification of cholesterol from the sera samples were done through a principle in which the amount of cholesterol present in serum as cholesterol esters were hydrolysed by cholesterol esterase. Total serum cholesterol is made of three basic component which are; triglycerides, high-density lipoproteins and low-density lipoproteins. High-density lipoproteins (HDL) and low-density lipoproteins (LDL) was determined through a test mechanism whereby LDL are precipitated by adding phosphotungstic acid in the presence of magnesium ions to the sample. The HDL fraction remains in the supernatant was then valued through cholesterol assay**.** Triglycerides from the sera samples were also determined through similar method. Finally, LDL was determined using the formula;

LDL= $Total\ Cholesterol - \frac{Triglycrides}{5.0} - HDL\ Cholesterol$ (in mg/d)

Statistical Analysis

All data collected was summarized, tabulated and the descriptive statistics i.e., mean and standard deviation of each sample was determined using Microsoft® Excel 2019 (Redmond, VA, USA). Data analysis was done using GraphPad Prism® version 5.0.3 for Windows (GraphPad Software, San Diego, California USA), the values obtained were subjected to a one-way ANOVA ($p<0.05$) followed by Tukey's multiple comparison test to determine the significant differences in the haematological and serum biochemical parameters in relation to age differences.

Ethical Statement

The ethical review Committee of the Faculty of Veterinary Medicine, University of Ilorin, approved and endorsed the research study with approval reference number: UERC/FVM/2021/011.

RESULTS AND DISCUSSION

The variations in the haematological values and selected serum biochemical values of the stallions, grouped by age into three main groups (group A, B and C) are shown below in Table 1 and 2 respectively. For the haematological parameters (Table 1), statistically significant differences were found for PCV, RBC, haemoglobin, MCV and MCH. Stallions age <5 years showed significant higher ($p<0.001$) PCV, haemoglobin and RBC values when compared to stallions age between 6 to 11 years and above 11 years. For MCV, there was significant increase in group A than B ($p< 0.05$), increase in group A than C ($p<0.001$) while increase in group B than C had ($p<0.01$). For MCH, there were the same significant increase across the groups; group A value greater than B, group A greater than C and group B greater than C ($p< 0.001$). The lymphocytes had significant increase in group B than group C ($p<0.05$). Haematological parameters (PCV, haemoglobin, leucocytes and platelets) of the stallions analysed were within the normal reference intervals when compared with reference values reported for all breeds (Aiello *et al*., 2016).More so, our findings are similar to that of Egbe-Nwiyi *et al*., (2012) among Nigerian local horses in Maiduguri, Nigeria. Erythrogram values for each group

reported for this study have similar value with that of thoroughbred PCV and haemoglobin mean value reported by Santos *et al*. (2014) while RBC value for this study was low compared to Santos *et al*., (2014) and MCV value was high for this study when compared with Santos *et al*. (2014). However, MCHC, leucocytes and thrombocytes values were similar when compared with Santos et al. (2014). The erythrocyte indices and leucocytes values of male Arabian breed horse categorized as adult (Altinsaat, 2008) have similar value across the groups in this study with exception of RBC value which was low and this could be as result of difference in nutritional status or underlining infections. While the MCV and MCH values were high for this study. A similar comparison was also recorded between this study and the study done on Žemaitukai horses (Mikniene *et al.*, 2014). In addition to the exceptions seen in (Altinsaat, 2008) study, the haemoglobin value for this study was low compared to Žemaitukai horses (Mikniene *et al.*, 2014). This difference may be attributed to the altitude of the location and the management practices such as diet.

The RBC values tend to decline with increment in age of the horse (Osec and Estnik, 2002; Altinsaat, 2008; Ribeiro *et al*., 2008; Mikniene *et al.*, 2014; Aiello, Moses and Allen, 2016), this was also recorded in this study with significant difference ($p<0.001$) in RBC across the all groups. MCH and MCHC values tend to increase with age of horse (Osec and Estnik, 2002; Altinsaat, 2008; Mikniene *et al.*, 2014), this was also recorded for this with significant differences ($p<0.001$) across group for MCH value. Values for PCV and haemoglobin declined with age in this study which was in agreement with the study done on Žemaitukai horses (Mikniene *et al.*, 2014). However, PCV and haemoglobin values tend to increase with age in both Arabian and Lipizzan horses as reported by (Osec and Estnik, 2002; Altinsaat, 2008) respectively. From this study, it showed that there were significant differences ($p<0.001$) in all the erythrogram parameters when comparing; group A (1-5 years) with both group B (6-10) and group C (11-16) with exception of MCHC values where no significant difference ($p>0.05$) was recorded across groups. The leucogram and thrombrogram values of this study are in tandem with report from previous studies (Altinsaat, 2008; Mikniene *et al.*, 2014; Grden *et al.*, 2019). There was significant difference ($p<0.05$) between group B and group C for lymphocytes value, showing increment in lymphocytes value with increase in age. This however contradict report from previous work (Altinsaat,2008; Ribeiro *et al*., 2008; Mikniene *et al.*, 2014). The differences between lymphocytes value seen in this study and that of previous studies could be as a result varied physical activities, level of stress in these stallions and previous exposure to parasites and disease agents (Cruz *et al.*, 2017). Stress and physical exercise stimulate the production of steroids which in turns stimulate erythropoiesis.

The results of lipid profile and serum biochemical parameters is shown in Table 3 & 4. Statistically significant differences were found for cholesterol, HDL, LDL, triglycerides, albumin, globulin, albumin-globulin ratio and ALT among different age groups of stallions.

The serum biochemical parameters (Albumin and creatinine) of the stallions analysed were within the normal reference intervals when compared with reference values reported for most breeds (Mikniene *et al.*, 2014; Grden *et al.*, 2019). The lipid profile varied arbitrarily and low when compared with study done by (Egbe-Nwiyi *et al*., 2012; Ihedioha and Agina, 2013) on Nigerian horses (the breeds were not specified). This could be as a result differences in breeds, physical activity, nutritional status and sample analysis of the sampled horses used for the various study (Egbe-Nwiyi, Kalu and Naphtali, 2012; Ihedioha and Agina, 2013; Aiello, Moses and Allen, 2016). From this study, there was significant decrease ($p<0.001$) in value of group A to C, significance decrease ($p<0.01$) from group B to C for triglycerides value showing reduction in triglycerides value with increment in horse age. Although, there was no significant increase between creatinine values across the groups, group C creatinine value was significantly high compare to the other groups, this maybe as a result of muscular development and activity seen more prominently in older horses (Stockham and Scott, 2008;). The ALT values across groups were slightly lower when compared with values obtained by Ihedioha and Agina (2013) for Nigerian horses.

Conclusion

It was concluded that haematological parameters (PCV, haemoglobin, thrombrogram, and leucogram) of the Stallions were similar to previous reports in other breeds. Furthermore, the RBC and PCV decreased with age. It was also concluded that the serum biochemical parameters (Protein, albumin and creatinine) of the Stallions were similar to values reported in previous studies. However, the lipid profile, BUN, and ALT were lower in this study.

Table 1: Haemogram of Stallions in Different Age Groups

Parameters (Mean±SD)	Group A <5 years (n=12)	Group B 6-11 years (n=33)	Group C >11 years (n=5)
PCV (%)	37.75±2.90[a]	31.67±3.13[ab]	29.00±3.39[b]
RBC (x10^{12}/L)	5.48±0.49[a]	4.47±0.52	3.61±0.59
Haemoglobin (g/dl)	12.46±0.98[a]	10.43±1.04[b]	8.68±1.18[c]
MCV (fl)	66.58±0.90[a]	70.21±1.62[b]	73.00±2.65[b]
MCH (pg)	22.75±0.24[a]	23.29±0.35[b]	24.12±0.82[b]
MCHC (g/dl)	32.98±0.12	33.15±0.12	33.56±1.76

[a, b, c] superscripts with different letters within rows are statistically significant at $p<0.05$

Table 2: Leukogram of Stallions in Different Age Groups

Parameters (Mean±SD)	Group A <5 years (n=12)	Group B 6-11 years (n=33)	Group C >11 years (n=5)
WBC ($x10^9$/L)	9. 19±2.17	8.28±2.62	8.29±1.69
Neutrophil ($x10^9$/L)	5.15±1.14	5.05±2.10	6.12±1.75
Band cells ($x10^9$/L)	0.04±0.08	0.04±0.07	0.02±0.04
Lymphocyte($x10^9$/L)	3.78±1.37	3.06±0.88	4.47±0.96
Monocyte ($x10^9$/L)	0.18±0.08	0.15±0.09	0.21±0.07
Eosinophil ($x10^9$/L)	0.04±0.06	0.03±0.04	0.06±0.05
Basophil ($x10^9$/L)	0.01±0.02	0.00±0.00	0.00±0.00
Platelets ($x10^9$/L)	231.40±89.69	244.10±93.66	208.00±79.00

Table 3: Variations in Lipid Profiles of Stallions in Different Age Groups

Parameters (Mean±SD)	Group A <5 years (n=12)	Group B 6-11 years (n=33)	Group C >11 years (n=5)
Cholesterol (mg/dl)	$46.30±4.12^a$	$63.00±1.92^b$	$92.3±7.95^c$
HDL (mg/dl)	$25.70±2.65^a$	$35.10±1.92^b$	$58.60±4.95^c$
LDL (mg/dl)	$25.60±2.41^a$	$33.90±1.96^b$	$54.21±4.02^c$
Triglyceride(mg/dl)	$39.03±1.88^a$	$27.81±1.72^b$	$4.57±1.11^c$

$^{a, b, c}$ superscripts with different letters within rows are statistically significant at $p<0.05$

Table 4: Variations in Serum Biochemistry of Stallions in Different Age Groups

Parameters (Mean±SD)	Group A <5 years (n=12)	Group B 6-11 years (n=33)	Group C >11 years (n=5)
Total protein (g/dl)	7.43±0.20	7.60±0.33	7.38±0.98
Albumin (g/dl)	$2.78±0.22^a$	$3.22±0.28^b$	$3.79±0.43^b$
Globulin (g/dl)	4.65±0.07	4.38±0.51	3.62±0.76
Albumin: Globulin	$0.59±0.05^a$	$0.74±0.14^a$	1.07±0.23
Creatinine (umol/l)	$53.40±1.82^a$	$70.80±3.3^b$	$185±1.3^c$
ALT (g/dl)	$130.00±8.56^a$	$109.04±5.41^b$	$60.28±7.78^c$
BUN (mg/dl)	19.44±2.82	18.82±1.88	19.24±1.77

$^{a, b, c}$ superscripts with different letters within rows are statistically significant at $p<0.05$

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors Contributions

BA designed the study, reviewed literature on the topic and edited the final version of the manuscript, IAO did the sample collection and processing, literature search and draft the manuscript. OFH did data analysis and manuscript editing while SF was part of sample collection and analysis group. All authors contributed to the final versionof the manuscript.

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