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# Physical Assessment, Hematological, and Serum Amyloid A Levels Pre- and Post-Galloping Exercise in Arabian Horses in Maiduguri and Jere, Borno State, Nigeria

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## Abstract

Long-duration activities, such as endurance races, cause significant changes in hematological and biochemical components as a result of central and peripheral exhaustion. The aim of the present study was to assess the physical health status as well as hematological and serum amyloid A (SAA) levels pre- and post-galloping exercise in Arabian horses in Maiduguri and Jere, Borno State, Nigeria. Before and after the galloping exercise, the health status of 50 healthy Arabian horses was physically assessed and blood samples were collected for hematological and serum biochemistry analyses. Packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb) concentrations, white blood cell (WBC), and differential leucocytes such as neutrophils, eosinophils, basophils, monocytes, and lymphocytes were determined using ballotment methods and haematocrit cyanmethaemoglobin. SAA levels pre- and post-galloping were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. The physical parameters such as temperature, heart, and respiratory rates values were significantly increased at $p < 0.001$, 0.001, 0.050, and 0.001, respectively, after the galloping exercise as compared with the pre-galloping values. Haematological parameters such as PCV, RBC, and Hb were increased significantly at $p < 0.001$, 0.001, and 0.015, respectively, as well as eosinophils and monocytes at $p < 0.040$ and 0.001, respectively, after galloping as compared with the pre-galloping values. The mean values of SAA increased significantly ($p < 0.007$) post-galloping. Therefore, it was concluded that the galloping type of exercise affects some physical parameters such as temperature, respiration, and heart rates as well as various hematological parameters and biochemical parameters such as SAA, and an increase in SAA could be due to resistance provided by the sandy terrain, leading to exhaustion in exercising the muscles of horses in the study area.

## Keywords

Exercise; hematology; serum amyloid A; Arabian horses

## 1. Introduction

This is the first documented study conducted under sandy terrain about galloping exercise in Arabian horses in the studied area. Horses are useful in agriculture, transport, policing, games, sports, and others [1–3]. Humans, horses, camels, and greyhounds are the four primary athletic species although horses are the quickest. As a result, horses drew the most interest in the study of athletic science, second only to humans [4]. A horse's competence is the consequence of a complex interplay of numerous factors. The horse's age, gender, breed, genetic potential, stride, nutrition, behaviour, strength, and neuromuscular coordination, as well as

the ability for labor or fitness, are all factors to consider [5,6]. In Nigeria, there are approximately 240,000 horses predominantly of Arabian breed with a large population in Borno State [7]. Exercise is among the stressful events an animal may face during his life, and during elevated exercise, the metabolism of horses undergoes transitory homeostatic alterations [8,9]. Due to severe environmental conditions and central and peripheral exhaustion, protracted activities, such as endurance races, provide a significant stimulus to those components [10]. It has been reported that exercise influences hematological parameters [11]. In relation to its age, sex, breed, degree of fitness, health, and dietary sufficiency, a healthy horse retains a concentration of red and white blood cells, as well as other blood components, within the normal range [12–14]. After activity, deviations from the normal range in one or more blood components, either above or below the range's boundaries, might indicate a response to exhaustion by a working horse. In reaction to a number of stresses, acute phase proteins (APP) such as serum amyloid A (SAA) are released into the bloodstream [15,16]; another generally held belief is that APP is common in horses and is caused by glycogen depletion in exercising muscles, which leads to the release of IL-6 [17]. Pleiotropic effects are promoted by IL-6, which raises the synthesis of SAA in the liver [18]. Hepatocytes produce the majority of it as part of the APP [16]. The most significant metabolic alterations involve a huge family of proteins produced by the liver, known as APPs, being greatly increased or reduced [15,16,19]. The present study was aimed to evaluate the influence of galloping exercise on physical and hematological parameters as well as SAA levels pre- and post- galloping in Arabian horses located in Maiduguri and Jere local Government Areas of Borno State.

## 2. Materials and Methods

### *2.1. Study Area and Sample Population*

This study was conducted in Maiduguri and Jere local Government areas, Borno State, which lies within the semi-arid zone of the Northeastern part of Nigeria, with an area of 69,436 sq km. Maiduguri lies between latitude 11°N and longitude 13°E. It has a mean day temperature of 38°C. The horses used in the present study are stallions of the Arabian breed which are most predominant in Borno State, Nigeria.

### *2.2. Sample Size Determination and Study Design*

Cross-sectional study design and convenient sampling methods were used to select fifty stallion horses in Maiduguri and Jere. The galloping type of exercise was selected for fifty stallion horses of the Arabian breed weighing between 350 kg and 450 kg and grouped into two sessions of a twenty-five kilometer galloping that was covered in one hour, twenty-seven stallions were from Maiduguri and twenty-three from Jere, respectively. Physical conditions such as the body conformation and body condition score of the horses (the horses selected for the study have approximately the same body condition score; hence, this result was not presented in the study) were determined as well as the vital parameters which include the temperature, respiration, and heart rates before and after exercise in the study area. The galloping exercise was conducted on a sandy terrain in the study area.

### *2.3. Collection of Blood Samples*

Blood samples were collected from the left jugular vein of each sampled horses using the procedure described in [20]. The jugular vein area was cleansed with a swab moistened with 70% v/v alcohol, and it was allowed to dry. Using 21 gauge needles, approximately 5 ml of the blood sample were aseptically collected before the exercise and 5 ml after the galloping exercise from each of the horses into heparinized vacutainer tubes for biochemical analyses. SAA levels were determined using ELISA kit (Cusabio), and ethyl diaminotetraacetic acid (EDTA) vacutainer tubes for hematological tests such as PCV, RBC, Hb, WBC, and differential leucocytes were determined using Microhaematocrit, Cyanmethaemoglobin, and Battlement Methods as described by Coles [21]. The EDTA vacutainer tubes were rocked and rolled gently to allow for uniform mixing of the blood with the anticoagulant. The samples were labelled appropriately. Blood was drawn from horses that appeared healthy and at rest, and a thorough clinical examination was performed to determine their health state. The samplings were carried out with caution to ensure that the horses were not compelled by the process, which could have skewed off the horses' hematological and immunologic characteristics. Before the galloping exercise, the blood sample was taken 24 hours before the galloping starts and when the horses were at rest (between 8 and 10:30 a.m.) and tested in the event's on-site laboratory. The galloping exercise began at 4 p.m., and ended at 6:30 p.m. on the same day. The post-galloping blood sample was collected, and the clinical parameters of the horses were checked after a maximum of 20 to 30 minutes of the recovery period of the galloping events [22–26].

### *2.4. Data Analyses*

Nonparametric test: Shapiro Wilk W test was used to compare the pre- and post-galloping values, and the data was examined using a student paired t-test. The changes on hematological and physical parameters during galloping were analyzed with JMP version 11 software (SAS Institute Inc, Cary, NC). Values were considered statistically significant at $p < 0.05$.

## 3. Results

The determined physical parameters revealed that temperature, respiration, and heart rates showed significant differences, as compared to the pre-exercise values as shown in **Table 1**. The revealed mean values of PCV, HB, and RBC pre- and post-galloping were significantly different. The mean values of differential leucocytes count, monocytes, and eosinophils pre- and post-galloping were significantly different as shown in **Table 2**. The mean values of SAA pre- and post-galloping revealed a significant difference as shown in **Table 3**.

**Table 1:** Changes in the physical parameters of Arabian horses in Maiduguri and Jere as a result of galloping events 20 to 30 minutes of the recovery period (n = 50).

| Physical parameters | Pre-galloping | Post-galloping | *P*-values |
|---|---|---|---|
| Body temperature (°C) | $36.52^{b} \pm 0.19$ | $38.82^{a} \pm 0.14$ | $0.001^{*}$ |
| Heart rate (Beat/mins) | $64.46^{b} \pm 0.81$ | $85.46^{b} \pm 0.96$ | $0.050^{*}$ |
| Resp. rate (Breath/mins) | $21.20^{b} \pm 0.36$ | $24.52^{a} \pm 0.39$ | $0.001^{*}$ |

All values are expressed as mean ± SE; values with different superscript $^{a, b,}$ within rows are significantly different at $^{*}p < 0.05$.

**Table 2:** Changes in the hematological parameters of Arabian horses in Maiduguri and Jere as a result of galloping events 20 to 30 minutes of the recovery period (n = 50).

| Blood Parameters | Pre-galloping | Post-galloping | *P*-values |
|---|---|---|---|
| Packed Cell Volume (L/L) | $29.76^{b} \pm 0.69$ | $31.72^{a} \pm 0.71$ | $0.001^{*}$ |
| Haemoglobin (mmol/L) | $11.55^{b} \pm 0.43$ | $12.91^{a} \pm 0.48$ | $0.001^{*}$ |
| RBC (x $10^{12}$/L) | $6.27^{b} \pm 0.30$ | $7.03^{a} \pm 0.34$ | $0.015^{*}$ |
| WBC (x $10^{9}$/L) | $9.25^{a} \pm 0.22$ | $9.49^{a} \pm 0.18$ | 0.312 |
| Neutrophils (x $10^{9}$ /L) | $2.96^{a} \pm 0.09$ | $2.97^{a} \pm 0.10$ | 0.881 |
| Lymphocytes (x $10^{9}$ /L) | $5.21^{a} \pm 0.16$ | $5.27^{a} \pm 0.13$ | 0.603 |
| Monocytes (x $10^{9}$ /L) | $0.37^{b} \pm 0.02$ | $0.42^{a} \pm 0.02$ | $0.040^{*}$ |
| Eosinophils (x $10^{9}$ /L) | $0.66^{b} \pm 0.03$ | $0.81^{a} \pm 0.03$ | $0.001^{*}$ |
| Basophils (x $10^{9}$ /L) | $0.03^{a} \pm 0.01$ | $0.06^{a} \pm 0.02$ | 0.231 |

All values are expressed as mean ± SE; values with different superscript $^{a, b,}$ within rows are significantly different at $^{*}p < 0.05$. RBC = red blood cells; WBC = white blood cells.

**Table 3:** Pre- and post-galloping serum amyloid A (SAA) of Arabian horses in Maiduguri and Jere after 20 to 30 minutes of the recovery period (n = 50).

| Blood Parameters | Pre-galloping | Post-galloping | *P*-values |
|---|---|---|---|
| SAA (mg/mL) | $7.07^{b} \pm 0.64$ | $10.43^{a} \pm 0.95$ | $0.007^{*}$ |

All values are expressed as mean ± SE; values with different superscript $^{a, b,}$ within rows are significantly different at $^{*}p < 0.05$.

## 4. Discussion

This is the first reported galloping exercise in Arabian horses in the studied region, which was conducted on sandy terrain to test the physical health statuses, hematological, and serum amyloid A (SAA) levels in Arabian horses in Maiduguri and Jere, Borno State, Nigeria, before and after the galloping exercise. However, there are some variations with other studies, possibly as a result of the sandy terrain of the studied area. It is possible that the variances are attributable to the sandy terrain's resistance to the horses' galloping efforts. In the present study, only stallions were used for the galloping type of exercise; this is done to avoid fluctuations in the final results which might create bias in the study as the presence of mares during such an event might cause distraction due to the mating behaviour. It was observed that if mares and stallions were allowed to perform together at the same time on the same terrain, they tend to sustain injuries because of the exhibited mating behaviour. The results revealed that the galloping exercise has caused some fluctuations in the physical parameters such as temperature, respiration, and heart rate in the horses. This could be due to the requirement of the muscles for more oxygen during the galloping exercise. The finding in the present study is consistent with the previous research reported in **[11,14]**, that reported similar findings on endurance horses in Malaysia. This increase could be as a result of the conditioning of the horses to the galloping exercise because most of the horses used for this research were also event horses. The considerable rise in PCV, Hb, and RBC in the present study could be attributed to sympathetic stimulation-induced splenic mobilization of erythrocytes, which is intensity-dependent as reported in **[12]**. Ok-Deuk and Yong-Soo (2017) also reported a significant increase in PCV and Hb values after exercise due to the effects of environmental factors such as the shape of the track, topography, the type of the actual exercise, the breed of the horse, and the exercise career **[27]**. These elevations were equivalent in size to those seen in earlier investigations as reported in **[28]**. After the race, however, there were no modifications in the overall leukocyte counts or neutrophils. A shift of both lymphocyte subsets after strenuous exercise could explain such a response. However, the present study revealed an increase in monocytes and eosinophils within the normal range value; this could be as a result of vigorous activity such as galloping in a non-inflammatory environment that can trigger an acute-phase response. This Modification observed in the total blood cell count might be interpreted as normal exercise-induced reactions in the performance of horses such as jumping, endurance, and polo. Bente and Anders (2000) as well as Cuniberti *et al.* (2012) found that neutrophil concentrations rose during and after exercise, while lymphocyte concentrations rose during acute activity and fell below baseline after long-duration exercise **[29,30]**. The amount of lymphocyte number change in exercise in the horse, according to Malinowski *et al.* (2006) is dependent on age and training. Both splenic contraction, which is regulated by catecholamine, and sweating, which causes fluid loss, may be blamed for the rise in the total number of red blood cells, packed cell volume, and hemoglobin concentration **[28,30]**.

In the current study, the results obtained after exercise revealed that SAA concentrations increased 1.5 times as compared to pre-galloping levels. Exercise has a significant impact on acute phase proteins (APPs) as reported in **[31]**. The increase in SAA could be attributable to the beginning of the galloping training. Satué *et al.* also reported a significant increase in hematological parameters as a result of splenic contraction which is densely innervated and can normally deposit up to quarter of the total red blood cells in the body, after exercise or haemorrhage; the spleen can release this deposition; therefore, hematocrit PCV value can show up to a fifty percent (50%) increase **[32]**. The mean PCV and RBC values increased up to 20-30% after exercise. Fan *et al.* (2002)

also reported a significant increase in PCV, RBC, and HB which could be due to its large capacity of sequestering blood cells in the spleen, and WBC in prolonged exercise induced leukocytosis as a result of cortisol release [5]. Our findings confirmed that SAA is perhaps the most responsive APP in horses despite the fact that the event appears to produce a modest reaction when compared to high intensity endurance riding.

## 5. Conclusion

In conclusion, the SAA utilized in the present study could be used as an indicator of post-galloping value in athletic Arabian horses as a result of resistance provided by the sandy terrain which might probably cause glycogen depletion, leading to exhaustion. Individual diversity in response to exercise is evident, with modest increases in SAA levels observed in certain horses. To determine the clinical usefulness of SAA measurement in galloping horses in the study region, more research with repeated post-galloping samplings over a longer period is needed. Our findings back up the idea that galloping exercise in sandy tracts might change SAA, packed cell volume, hemoglobin, and red blood cells. Furthermore, some differential leucocytes count those that have never been reported before such as monocytes and eosinophils to have shown an increase in the current study, but within the normal range values. These early discoveries came about as a result of organized galloping on a controlled number of Arabian horses.

## Authors' Contributions

Lawan Adamu and Muhammed Modu Bukar planned and designed the study. Yakaka Wakil collected the data and carried out the study, while Isa Gulani ran the experiments. Lawan Adamu carried out statistical analysis. All authors read and approved the manuscript.

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## Data Availability Statement

The data supporting the findings of this study are available upon request from the corresponding author.

## Conflicts of Interest

The authors declare that there is no conflicts of interest.

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## Ethical Approval

The study has been approved by the ethical committee of the Faculty of Veterinary Medicine, University of Maiduguri. In addition, the study also complied with the guidelines of the Declaration of Helsinki.

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