**FULL PAPER** *Physiology*

# Hemato-biochemical responses to packing in donkeys administered ascorbic acid during the harmattan season

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(Received 4 February 2012/Accepted 2 November 2012/Published online in J-STAGE 16 November 2012)

ABSTRACT. Experiments were performed to investigate the effect of ascorbic acid (AA) in reducing hemato-biochemical changes in pack donkeys during the cold-dry (harmattan) season. Six experimental donkeys administered orally AA (200 mg/kg) and six control donkeys not administered ascorbic acid were subjected to packing. Blood samples were collected from all donkeys for hematological and biochemical analyses. In the control donkeys, packed cell volume (PCV), erythrocyte count and hemoglobin concentration (Hb) decreased significantly ($P<0.05$) at the end of packing. In the experimental donkeys, there was no significant difference between the pre- and post-packing values of PCV, erythrocyte count and Hb. In the control donkeys, the neutrophil and neutrophil:lymphocyte ratio increased significantly ($P<0.05$) post packing, but in the experimental donkeys, the pre- and post-packing values were not significantly different. The eosinophil count increased significantly ($P<0.05$) in experimental and control donkeys post packing. In conclusion, packing exerted significant adverse effects on the hematological parameters ameliorated by AA administration. AA may modulate neutrophilia and induce a considerable alteration of erythroid markers in donkeys subjected to packing during the harmattan season.
KEY WORDS: ascorbic acid, biochemistry, donkey, hematology, packing

doi: 10.1292/jvms.12-0038; *J. Vet. Med. Sci.* 77(2): 133–138, 2015

Strenuous exercise modifies hematologic parameters [9]. According to Pearson and Vall [22], packing in donkeys is a form of physical exertion that may be stressful. The sympathetic nervous system generally plays an important role in the mediation of responses to exercise, modifying cardiovascular function [7], which alters hematologic parameters. Changes occurring in hematologic parameters, especially in the erythrocytes, induced by physical work in horses [14, 25] and donkeys [21] have been documented. Erythrocytes are naturally prone to stress, because of their constant exposure to high oxygen tension, high content of polyunsaturated fatty acid in their membranes and high amount of hemoglobin-bound iron [34]. Vani *et al.* [34] reported that erythrocytes are an ideal model for studying the effects of stress since they lack a nucleus and have short life span. The susceptibility of erythrocytes to hemolysis increases in stressful conditions [19]. Responses of leukocytes and their subpopulations to physical work are also well established [23]. Exercise-induced leukocytosis has often been compared to an inflammation-like reaction. After strenuous work, the leukocyte count increases by a factor of two, leading to neutrophilic leukocytosis, which is similar to the response to physiological insults by the immune system [5]. The neutrophil:lymphocyte ratio is a good indicator of stress, as the ratio increases in stressed animals [1]. It may serve as a biomarker of stress in donkeys. The biochemical parameters usually evaluated to determine the extent of stress include electrolytes ($Na^+$, $K^+$, $Cl^-$ and $H^+$), urea, glucose and total protein [17]. Acids and bases are usually added continuously to the body fluids as a result of either ingestion of feed or production of energy during cellular metabolism [31]. However, environmental factors, including the thermal microclimate, exert a significant effect on the energy exchange between the animal and its environment. This interaction may alter the normal acid-base balance. All serum biochemical parameters, except total protein, have been reported to be significantly affected by workload in donkeys [17].

The harmattan season has been described as the most thermally stressful of all the seasons, hot-dry (March–April), rainy (May–October) and cold-dry (harmattan) (November–February), in the northern guinea savannah zone of Nigeria [15]. In Nigeria, the season occurs from December to February and coincides with the peak harvest period of the year, when donkeys are used to transport farm produce to market for sale. Stresses due to concomitant effects of packing and meteorological factors during the harmattan season may alter the hemato-biochemical parameters in donkeys.

Ascorbic acid (AA) is a potent antioxidant compound in the mitigation and prevention of adverse effects of stress in livestock [3, 18, 19]. It has been used as dietary supplement even in domestic animals that are capable of AA synthesis, because it is depleted when such animals are stressed [13]. AA as an antioxidant donates free molecules of hydrogen that detoxify harmful reactive oxygen species, especially when the natural antioxidants in the body are exhausted or overwhelmed [32]. There is paucity of information on the hemato-biochemical responses of donkeys administered AA

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Table 1. Thermal environment conditions from the study period (Mean ± SEM)

| Period | Time | Ambient temperature (ºC) | Relative humidity (%) |
|---|---|---|---|
| Pre-packing | 6:00 hr | 13.67 ± 1.30 (11–15) | 20.67 ± 3.33 (14–24) |
| During packing | 10:00–14:00 hr | 28.37 ± 0.99 (22.7–30.3) | 37.67 ± 15.03 (11–63) |
| Post-packing | 14:30 hr | 36.50 ± 5.22 (27–45) | 41.67 ± 16.80 (14–72) |
| | 16:00 hr | 28.83 ± 4.66 (19.8–35.3) | 13.33 ± 3.83 (9–20) |
| | 18:00 hr | 26.50 ± 2.59 (22–31) | 15.00 ± 2.08 (11–18) |
| Overall mean | | 26.38 ± 2.96 | 22.67 ± 5.07 |

The numbers in parenthesis are minimum and maximum values.

and subjected to packing as well as meteorological stress during the harmattan season. Although *Equidae* do not need AA supplementation in their diet, Snow *et al.* [29] showed that when horses are subjected to exercise stress, the body requirement for AA may exceed that synthesized by the body.

The aim of the present study was to investigate the effect of AA in reducing the hematological and biochemical changes in pack donkeys during the harmattan season.

## MATERIALS AND METHODS

*Study area*: The study was approved by the Ethics Committee of Ahmadu Bello University (ABU), Zaria, Nigeria. It was carried out during the harmattan season in an area extending from the Research Pen in the Department of Veterinary Physiology and Pharmacology, ABU, Zaria (11°10'N, 7°38'E), to Panhauya village (11°7'N, 7°37'E) behind ABU in the northern guinea savannah zone of Nigeria. The donkeys covered a distance of about 20 km (to and fro), from the Research Pen to Panhauya village. The terrain of the route, trekked by the donkeys, was narrow and stony. Daily thermal environmental parameters for the locality, covering the experimental period were obtained from the Meteorological Data Processing Unit, Department of Soil Science, Faculty of Agriculture, ABU, Zaria, located about 1 km from the experimental site. The data analyzed for the study period are presented in Table 1. The overall mean ambient temperature (AT) and relative humidity (RH) values recorded during the experiment were 26.38 ± 2.96°C and 22.67 ± 5.07%, respectively. AT values ranged from 11–45°C, while RH values ranged from 9–72%.

*Experimental animals and management*: Twelve, apparently, healthy pack donkeys, between 3 and 4 years old served as experimental subjects. The donkeys comprised six males and six non-pregnant females (sexual ratio in each group was 1:1) with an average weight of 97.90 ± 4.8 kg. They were obtained from a donkey market at Sheme, near Faskari (11°43'N, 7°02'E), Nigeria. The donkeys were divided into experimental and control groups of six donkeys each. The donkeys were preconditioned for two weeks. During this period, they were subjected to clinical examination, screened for parasites and prophylactically treated. The donkeys were kept in the Research Pen during the experimental period, from 21 to 25 February, 2011, and reared under the traditional extensive management system. They were fed guinea corn straw and groundnut hay in the proportion of 4:1 and supplemented with wheat bran. The donkeys were allowed to graze extensively on natural pasture, comprising mainly browse plants and gamba grass (*Andropogon gayanus*). In addition, 1 kg of whole sorghum grain was fed to each donkey per day, and the donkeys were given access to water using drinking troughs. Salt licks were also provided throughout the period of the experiment.

*Administration of ascorbic acid*: All experimental donkeys (n=6) were orally administered ascorbic acid (AA; Sigma Chemical, St. Louis, MO, U.S.A.) dissolved in 20 m*l* of sterile water at a dose of 200 mg/kg body weight [29], while each of the control donkeys (n=6) was orally administered 20 m*l* of sterile water only. The administration was done 30 min before packing the donkeys on each day of the experiment.

*Packing procedure*: All the donkeys were saddled at 07:00 hr with a locally made leather saddle pack frame, filled with chopped dry grasses to provide a cushion effect on the back of the animals. The saddles for each donkey were loose enough to flap on both sides of the body of the donkey. Each donkey was packed with sand at the rate of 50% of the body weight [21] every morning on each experimental day. All loads were balanced evenly with similar weight bulk on either side of the animal, and padding was arranged such that it was thickest along the sides of the backbone [20]. The donkeys were trekked with an average speed of 1.79 ± 0.002 m/sec, during which they covered a distance of 20 km, on every other day for 4 hr (10:00 to 14:00 hr) per day, and for a total of three days as described by Pal *et al.* [21].

*Blood sample collection and analyses*: During packing, before at 06:00 hr and after at 14:00 hr, a blood sample was collected from each animal by jugular venipuncture using a 10-m*l* syringe connected to an 18-gauge needle. The sample was divided into two aliquots (2 m*l* each): one was put in a tube with ethylenediaminetetraacetic acid (EDTA) at the rate of 2 mg/m*l* of blood for hematological analysis, and the second was put in a tube without an anticoagulant. In the whole blood, erythrocyte and leukocyte counts were evaluated using a hemocytometer [27]. The packed cell volume (PCV) was determined by the microhematocrit method, while the hemoglobin (Hb) concentration was evaluated using the cyanmethemoglobin method [4]. Biochemical parameters were determined as described by Dacie and Lewis [8]. The blood collected in a plain vacutainer tube was allowed to clot. It was centrifuged at 1,500 × *g* for 10 min to obtain the serum,

which was evaluated for concentration of electrolytes, $Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$.

*Statistical analysis*: One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-hoc test, was applied to evaluate the differences due to AA administration. $P<0.05$ was considered significant.

## RESULTS

The changes in PCV, Hb and erythrocyte count are shown in Fig. 1a–c, respectively. In the control donkeys, the PCV (32.24 ± 0.67%) significantly declined to 30.48 ± 0.46% in association with packing ($P<0.05$), but in the experimental donkeys, there were no significant differences between the pre-packing (33.16 ± 0.78%) and post-packing (31.79 ± 0.75%) values of PCV (Fig. 1a). In the experimental donkeys, no significant differences were observed between the pre- (11.02 ± 0.26 g/d*l*) and post-packing (10.57 ± 0.25 g/d*l*) Hb concentrations, but in the control donkeys, the Hb concentration significantly decreased (10.71 ± 0.23 to 10.11 ± 0.16 g/d*l*) after the end of packing ($P<0.05$) (Fig. 1b). In the experimental donkeys, no significant differences were observed between the pre- ($5.53 \pm 0.13 \times 10^6/\mu l$) and post-packing ($5.30 \pm 0.13 \times 10^6/\mu l$) erythrocyte counts, but in the control donkeys, the erythrocyte count significantly decreased ($5.43 \pm 0.12 \times 10^6$ to $5.08 \pm 0.08 \times 10^6/\mu l$) after end of packing ($P<0.05$) (Fig. 1c).

There were no significant changes between the pre- and post-packing total leukocyte counts obtained in each group (Fig. 2a). The pre- and post-packing neutrophil counts in the experimental donkeys were not significantly different. In the control donkeys, however, the neutrophil count increased significantly ($P<0.05$) from $3.22 \pm 0.20 \times 10^3$ to $4.54 \pm 0.52 \times 10^3/\mu l$ with packing (Fig. 2a). In both experimental and control donkeys, the pre- and post-packing lymphocyte counts were not significantly different (Fig. 2a). There were no significant changes between the pre- and post-packing monocyte counts obtained in each group (Fig. 2a). The eosinophil count of the experimental group increased significantly ($P<0.05$) from $0.31 \pm 0.05 \times 10^3$ to $0.73 \pm 0.27 \times 10^3/\mu l$ post packing. In the control donkeys, the value also increased significantly from $0.29 \pm 0.06 \times 10^3$ to $0.79 \pm 0.14 \times 10^3/\mu l$ ($P<0.05$) (Fig. 2a) post packing. In the experimental donkeys, the pre-packing neutrophil:lymphocyte ratio of 1.02 ± 0.14 was not different from the ratio of 1.32 ± 0.22 obtained post packing, but the pre-packing value of 0.90 ± 0.11 recorded in the control donkeys increased significantly ($P<0.05$) to 1.52 ± 0.25 at the end of the packing (Fig. 2b).

No significant differences were obtained in the biochemical parameters of $Na^+$, $K^+$, $Cl^-$, $H^+$ and urea throughout the observations (Fig. 3).

## DISCUSSION

The results showed that the AT values of 11–45°C obtained during the study period were predominantly outside the thermoneutral zone of 23–32°C, established for donkeys [26]. The wide AT range of 34°C, characteristic of the sea-

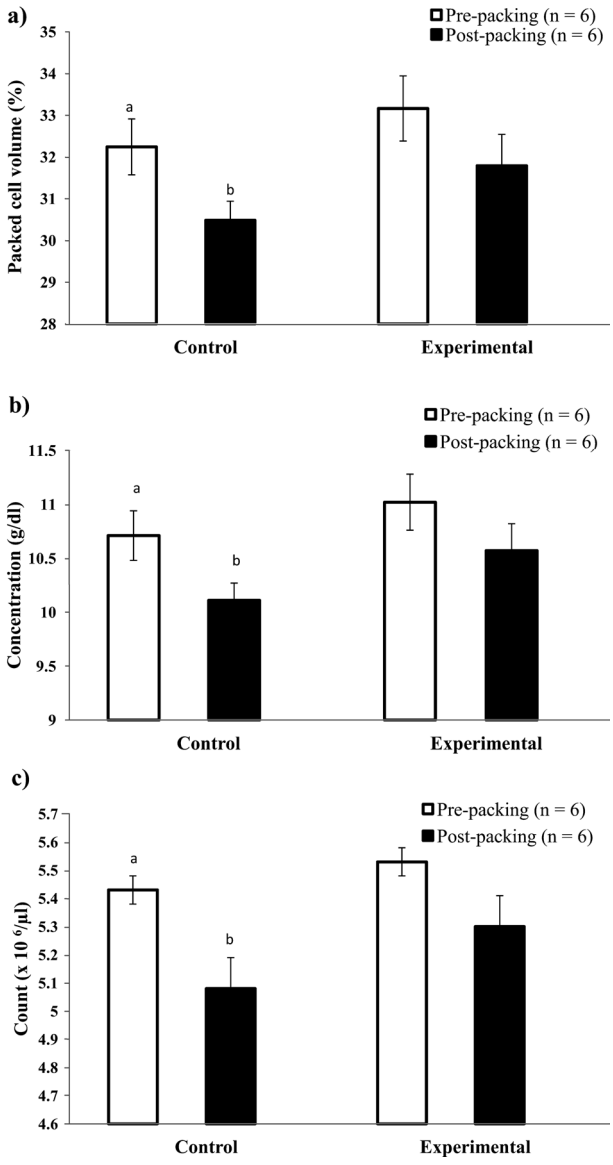


Fig. 1. a) Packed cell volume of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post packing values (n=6). Packed cell volume was measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$. b) Hemoglobin concentration of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post packing values (n=6). Hemoglobin concentration was measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$. c) Erythrocyte count of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post packing values (n=6). Erythrocyte count was measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$.

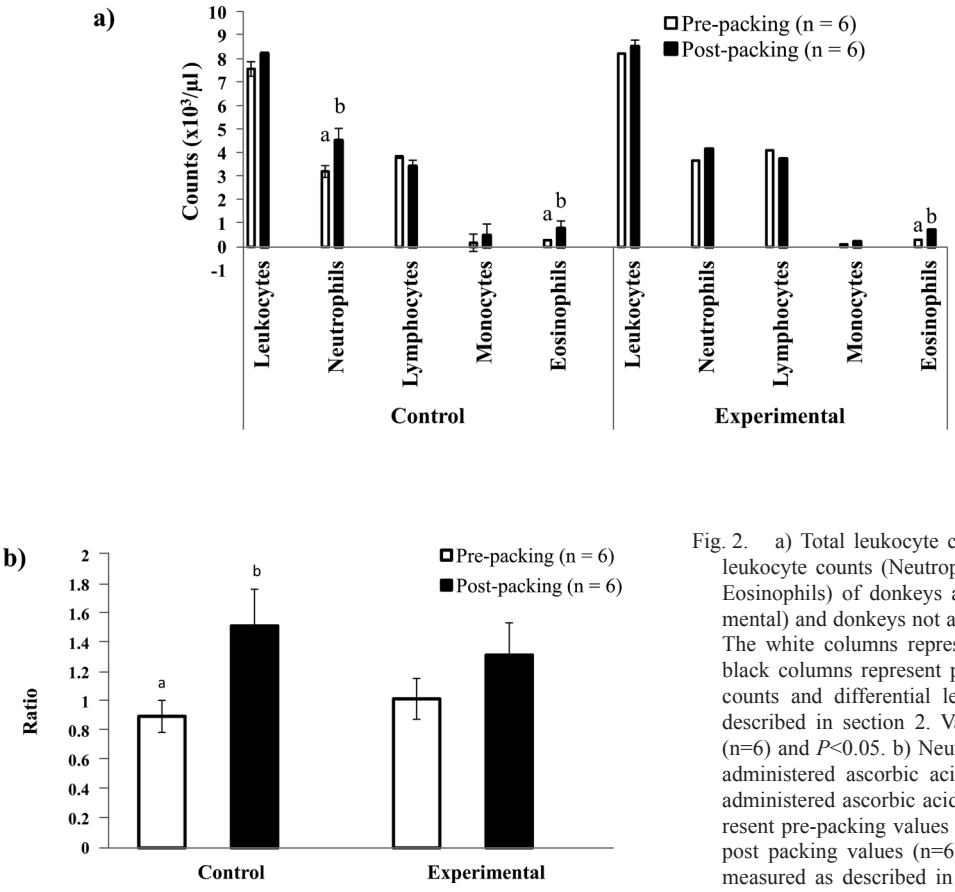


Fig. 2. a) Total leukocyte counts (Leukocytes) and differential leukocyte counts (Neutrophils, Lymphocytes, Monocytes and Eosinophils) of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post packing (n=6). Total leukocyte counts and differential leucocyte counts were measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$. b) Neutrophil:lymphocyte ratio of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post packing values (n=6). Neutrophil:lymphocyte ratio was measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$.

son, confirmed the findings of Igono *et al.* [15] and Ayo *et al.* [2], who showed that the cold-dry (harmattan) season is thermally stressful to livestock. The results also showed that the harmattan season had mostly low humidity, typical of the season. The mean RH value of 22.67 ± 5.07% with the range of 9–72%, obtained during the season showed that the values were predominantly below the thermoneutral zone of 30–70%, established for donkeys [26].

All the hematological values were within the normal range for donkeys [17]. However, the significant decrease in PCV and erythrocyte count in the control donkeys observed in this study did not agree with the findings of Bhatti and Shaikh [5] and Boning *et al.* [6], who obtained an increase in PCV and erythrocyte count following physical exercise in humans. Although it has been shown that the increase in PCV is a function of exercise intensity in dogs [28], the reduction in the values of the parameters recorded in the donkeys may be a result of the exercise (trekking) with packing that the donkeys were subjected to. The intensity of the exercise was apparently not adequate to induce an increase in the PCV and erythrocyte count in the donkeys. Besides, donkeys are known to be hardy animals and very resistant to environmental stress factors [22].

The significant increase in neutrophil count of the control group post packing may have been due to the release of neutrophils from the marginated pool as a result of the exercise (trekking) the donkeys were subjected to. This finding agrees with the result obtained by Pederson and Hoffman-Goetz [23], who reported an increase in leukocyte count following physical exercise in humans. The neutrophilia may be due to a rise in the concentration of hormones (adrenaline, cortisol and growth hormone) during exercise and increased cardiac output, which increase the mobilization of leukocytes from the marginated pool [33]. Physical exercise has been shown to induce inflammation-like reactions [5], which may be a result of oxidative stress [9], a major cause of inflammatory and immune dysfunctions [30]. The fact that the differences in the post-packing neutrophil count was significantly higher compared with the pre-packing value in only the control suggests that AA modulated the stress-induced neutrophilia. Thus, administration of AA ameliorated the stress-induced inflammatory response [11].

The post-packing increase in eosinophil count recorded in the experimental and control donkeys was evidence of an inflammatory reaction [35]. All the donkeys, which were reared under the traditional extensive management system, were exposed to the prolonged effect of dust-laden, harmattan wind and airborne sand dust, which may induce allergic lung eosinophilia. This increase in eosinophil count was in agreement with the findings of He *et al.* [12], who reported that Asian sand dust enhanced lung eosinophilia in mice, and further suggested that the eosinophilia may be due to acti-

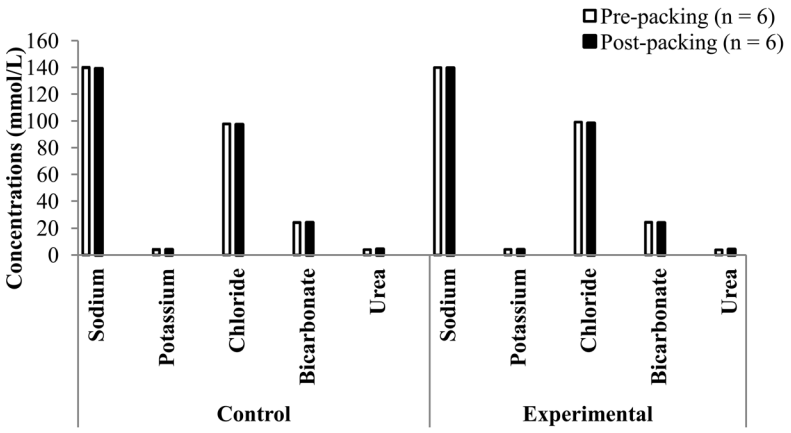


Fig. 3. Biochemical parameters (Sodium, Potassium, Chloride, Bicarbonate and Urea) of donkeys administered ascorbic acid (Experimental) and donkeys not administered ascorbic acid (Control). The white columns represent pre-packing values (n=6). The black columns represent post-packing values (n=6). Biochemical parameters were measured as described in section 2. Values and bars are the mean ± SE (n=6) and $P<0.05$.

vation of a helper T cell 2-associated immune response. In this study, AA may not have had an effect on the eosinophil count. This result was in agreement with the findings of Pederson and Hoffman-Goetz [23], who reported that a single nutrient supplement may not exert physiologically relevant effects on exercise-induced immune modulation. However, the finding was not in agreement with the results obtained by Peters *et al.* [24], who reported that AA ameliorated symptoms of pulmonary disorders. The difference in results may be because their subjects were supplemented with AA for three weeks before the marathon race. In the present study, AA was administered only on each experimental day, 30 min prior to subjecting the donkeys to work.

The significant increase in post-packing neutrophil:lymphocyte ratio compared with the pre-packing ratio in the control donkeys was mainly caused by neutrophilia, and this result supports the previous findings of Kannan *et al.* [16] and Minka and Ayo [18], who observed an increase in neutrophil:lymphocyte ratio in goats subjected to transportation by road for 4 and 8 hr, respectively. This shows that donkeys in the control group, which were not administered with AA, were subjected to a higher level of stress. Conversely, the fact that the pre-packing neutrophil:lymphocyte ratio in the experimental donkeys, which were administered AA, was not significantly different from the ratio obtained post packing showed that the experimental donkeys were less stressed compared with the controls, demonstrating the anti-stress effect of AA.

In the present study, there were no significant differences between the pre-packing and post-packing values of $Na^+$, $K^+$, $Cl^-$, $HCO_3^-$ and urea. This finding was in agreement with that of Ghanem *et al.* [10], who reported an insignificant difference in the concentration of all the electrolytes of rehydrated Awassi sheep deprived of water. However, this finding of the present study was not in agreement with the results obtained by Lemma and Morges [17], who recorded a significant change in $Na^+$, $K^+$, $Cl^-$, $HCO_3^-$ and urea, and emphasized that changes in the parameters were directly dependent on workload. In the present study, the donkeys were apparently not subjected to a heavy enough workload to cause such significant changes. Besides, the differences in the season in which the experiment was performed and breeds of donkeys may be responsible for the variations. Lemma and Morges [17] conducted their experiment using the Abyssinian breed of donkeys during the hot-dry season, while the present study was performed using the Nubian breed of donkeys during the harmattan season. The finding of the present study further confirms a previous report [22] that donkeys are very resistant to stress. This requires further investigation.

In conclusion, packing exerted significant adverse effects on the hematological parameters ameliorated by AA administration. AA may modulate neutrophilia and induce a considerable alteration of erythroid markers in donkeys during the harmattan season.

It is recommended that AA be administered to donkeys before subjecting them to packing in order to reduce the risk of adverse effects of work stress and enhance work output.

ACKNOWLEDGMENT. The authors acknowledge the Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria, for making available the data for thermal environmental parameters used in this study.

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