—Full Paper—

Effects of packing on the diurnal rhythms of respiratory and heart rates in donkeys during the hot-dry season

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This study aimed to evaluate the effects of packing (load carrying) on the diurnal rhythms of respiratory and heart rates in donkeys during the hot-dry season. Twenty 2- to 3-years-old donkeys weighing 93 ± 2.7 kg were divided into two groups to serve as subjects: Group 1 was subjected to packing + trekking, while group 2 was subjected to trekking only. All donkeys trekked 20 km on each experimental day. The procedure was repeated three times at one-day intervals. Thermal environmental parameters were recorded. Respiratory rate (RR) and heart rate (HR) were measured before and immediately (15 min) after the packing and/or trekking period. Continuous recordings of parameters were performed for 27 hr at 3-hr intervals beginning 16 hr after the last packing and/or trekking period. The RR rose significantly (P<0.05) immediately after packing + trekking and trekking only, from 30.15 ± 0.5 and 27.52 ± 0.5 cycles/min before packing + trekking and trekking only, respectively, to 43.78 ± 3.0 and 46.30 ± 1.8 cycles/min after them, respectively. The HR (76.63 ± 4.5 beats/min) in the packing + trekking donkeys was higher (P<0.05) than that of the trekking-only donkeys (62.33 ± 2.7 beats/min). The acrophase and bathyphase of the HR in both groups were recorded during the photophase and scotophase, respectively, with higher values (P<0.05) in the packing + trekking donkeys than in the trekking-only donkeys. The circadian rhythms of RR and HR did not differ between the groups of donkeys. In conclusion, packing + trekking was more stressful to the donkeys, significantly increasing the HR of donkeys subjected to packing + trekking, compared with trekking only.

Key words: *circadian rhythm, donkey, packing, trekking*

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Introduction

Donkeys are suitable for packing (load carrying), especially in difficult terrains because they possess closer limbs and more upright hooves than horses, a straight dorsal top line, low withers, and slow and smooth paces, which make them uniquely suited to load carrying [4]. The major uses of donkeys are packing and transportation of goods and people in many developing countries. Furthermore, donkeys represent an important source of income for many farmers, and therefore there is a need to investigate how to engage them meaningfully without compromising their health and welfare, especially during the hot-dry season, which is known to be thermally stressful [3]. Exhaustive work or exercise, such as racing over several kilometers and packing and trekking over long distances, may cause muscle damage, which may result in inflammatory responses and, consequently, compromise welfare and health [21]. Although the stress responses induced by this may cause disturbances in homeostasis, body regulatory systems are activated to restore the body to a new level of equilibrium [1]. Increasing respiratory and heart rates (RR and HR, respectively) are part of the mechanisms employed by the animal to cope with the body's demand for oxygen. During intense physical activities, there is an increased oxygen demand for working muscles, and the body responds with an increase in the RR to ensure homeostasis [8, 9]. During exercise, oxygen delivery is improved by increasing the volume of inspired air, amount of blood pumped by the heart, oxygen carrying capacity of the blood, and a selective redistribution of the blood flow from the viscera to the working muscles. Evaluation of the RR and HR is important because the key

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parameters most studied are indicators of fitness and workload capacity in equines [1, 15]. Measurements of basic physiological parameters are easy to access and are used as the first indicators of the well-being of working donkeys. The HR of an animal is one of the physiological parameters measured to determine its health status or response to activities with increased energy demands, like packing [2, 25]. In order to ensure more humane handling of the donkey, there is need to obtain information that will maximise their potential use in packing and be beneficial in establishing guidelines for their humane handling for packing purposes in different seasons of the year. The RR and HR of donkeys have been reported to increase after packing exercise [13, 19]. The daily rhythmicity of RR and HR has been reported in donkeys during the hot-dry season [25]. However, the effects of different workloads on the daily rhythmicity of RR and HR during the hot-dry season have not been investigated. The data obtained will improve the welfare and handling of working donkeys by veterinarians and donkey owners.

The aim of the study was to evaluate the effects of packing and trekking on the diurnal rhythms of RR and HR in donkeys during the hot-dry season.

Materials and Methods

Experimental site

The study was carried out during the hot-dry season, from 21 March to 17 April 2021. The hot-dry season is a transitionary period between the cold-dry and rainy seasons that is characterised by high ambient temperature and low relative humidity, and begins in early March and ends in early June [15]. The study area spanned a distance of 20 km in the direction of Sabon Gida from the Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria (11°10′N, 7°38′E), which is 5 km from Panhauya village (11°7′N, 7°37′E) in the Northern Guinea Savannah zone of Nigeria. The terrain of the route was narrow, stony, and undulating, typical of the trekking routes taken by packed donkeys in the zone.

Ethical clearance

Ethical approval for the research was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), and the approval number was ABUCAUC/2021/063.

Study animals and management

Twenty, apparently, healthy pack donkeys (15 uncastrated males and 5 non-pregnant females), that were between 2 and 3 years' old and had an average weight of 93 ± 2.7 kg served as experimental subjects. They were housed in the research pen of the Department of Physiology, Faculty of Veterinary Medicine, ABU, Zaria, and reared under the traditional extensive management system. They were allowed to graze extensively during the day, and supplemented with sorghum straw, groundnut hay and gamba grass (*Andropogon gayanus*) in the morning and evening. In addition, 1 kg of whole sorghum grain was fed to each donkey before the packing and trekking procedures on each experimental day, and the donkeys had access to water in drinking troughs before and after the procedures were completed. Salt licks were also provided throughout the period of the experiment.

Experimental design

The donkeys were pre-conditioned for three weeks. During pre-conditioning, they were exposed to the packing procedures and familiarised with the route used for packing and/or trekking during the experiment. They were divided into two groups of 10 each. Group 1 comprised 8 male and 2 female donkeys that were 2 and 3 years' old, had an average weight of 96.9 ± 4.8 kg, and were subjected to packing and trekking (packing + trekking), while group 2 comprised 7 male and 3 female donkeys that were 2 and 3 years' old, had and average weight of 89.1 ± 2.1 kg, and were subjected to trekking without a load (trekking only). All the donkeys were trekked, a total distance of 20 km in the direction of Sabon Gida from the Faculty of Veterinary Medicine, ABU, Zaria (11°10′N, 7°38′E), which is 5 km from Panhauya village (11°7′N, 7°37′E), and then back to the research pen. The packing and trekking procedures were conducted from 06:00 to 12:00 - 13:00 hr on each experimental day. They were carried out three times one day between them. The continuous recordings of RR and HR were conducted for 27 hr at 3-hr intervals and only commenced at 16 hr (06:00 to 09:00 hr the following day) after the third packing and/or trekking procedure.

Packing procedure

A saddle pack frame, filled with chopped dry grasses, was used to provide a cushion effect on the backs of animals [18]. The saddles for each donkey in the packing + trekking group were loose enough to flap on both sides of the body. They were loaded with sand at a loading rate of 50% of their body weight [11] every morning on each experimental day. All loads were balanced evenly with a similar weight on either side of the animal [13].

Measurement of meteorological conditions

The dry-bulb temperature (DBT) was measured by a wet- and dry-bulb thermometer (Brannan, England) on each day of the experiment at 06:00 hr, every 1-hr thereafter, and then after packing and/or trekking, and relative humidity (RH) was calculated using Osmond's hygrometric table (Narindra

Scientific Industries, Haryana, India). Wind speed was determined using anemometer (AM 816, Littsoyh, Hong Kong, China), and topsoil temperature was recorded using a long-stem soil digital thermometer (Model: TP 300, Jiangsu Xuzhou, China). Temperature-humidity index (THI) was determined using the following formula [12]:

THI = (0.8 × Tdb) + [(RH/100) × (Tdb − 14.4)] + 46.4,

Where, Tdb was the dry-bulb temperature (°C) and RH was the relative humidity.

Measurement of respiratory and heart rates

Measurement of RR and HR were recorded before and after packing and/or trekking on each experimental day, which consisted of three days with one day between them, and also during the long-term experiment period beginning 16 hr after the last packing and/or trekking period, with RR and HR measurements being taken for 27 hr at 3-hr intervals.

RR was measured by observing and counting the number of respiratory flank movements for one min. HR was recorded using a Polar Equine FT1 Healthcheck HR monitor (Polar Electro Oy, Kempele, Finland), which consisted of two main components, a T31 transmitter with a handle bar and an FT1 training computer, and the HR of each donkey was measured in accordance with the instruction manual. The transmitter sent an electrocardiogram-accurate HR signal to the training computer, and the computer displayed the HR.

Statistical analyses

Data were expressed as the mean ± standard error of the mean (mean ± SEM). Student's *t*-test was used to evaluate the statistical differences within the groups before and after packing and/or trekking. The effects of packing were evaluated by comparing the groups using one-way repeated-measures ANOVA, followed by Tukey's *post-hoc* test to compare mean values. Pearson's correlation (*r*) and linear regression analysis were used to determine the relationships between thermal environmental parameters and RR and HR. An online cosinor analysis was used to determine the values of the variables for the circadian rhythm of RR and HR in the donkeys [14]. Four rhythmic parameters were determined: mean level (mesor [M]), amplitude (A), acrophase (Φ; the time at which the peak of rhythm occurred), and bathyphase. Data were analysed using GraphPad Prism, version 8.02 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com.). Values of $P<0.05$ were considered significant [22].

Results

Meteorological parameters

The values of meteorological parameters recorded before and after packing and/or trekking are shown in Table 1. The DBT, RH, and THI before packing and/or trekking were 25.07 ± 0.3°C, 33.33 ± 7.5%, and 70.00 ± 0.1, respectively. After packing and/or trekking, the DBT and THI rose significantly ($P<0.05$) to 35.83 ± 0.2°C and 79.67 ± 0.3, respectively, while the RH decreased to 20.00 ± 0.0%. The wind speed in the afternoon after packing and/or trekking was 1.83 ± 0.9 m/sec. The soil temperature rose from 26.17 ± 0.3°C before packing and/or trekking to 42.97 ± 6.5°C in the afternoon hours after packing and/or trekking.

The overall mean DBT obtained during the 27-hr of continuous recording (30.55 ± 1.3°C) was significantly ($P<0.05$) lower than the value of 35.83 ± 0.2°C recorded immediately after packing and/or trekking, but higher ($P<0.05$) than the 06:00 hr (25.07 ± 0.3°C). Similarly, the overall mean RH recorded during the continuous recording (37.90 ± 5.2%) was higher ($P<0.05$) than the values recorded before and immediately after packing and/or trekking (33.33 ± 7.5% and 20.00 ± 0.0%, respectively). The overall mean

Table 1. Meteorological parameters before and after packing and/or trekking during the hot-dry season

Meteorological parameters	Before packing and/or trekking (06:00 hr)	After packing and/or trekking (12:00–13:00 hr)
Dry-bulb temperature (°C)	25.07 ± 0.3[a] (25.7–24.5)	35.83 ± 0.2[b] (36.0–35.5)
Relative humidity (%)	33.33 ± 7.5[a] (46.0–20.0)	20.00 ± 0.0[a] (0.0–0.0)
Temperature-humidity index	70.00 ± 0.1[a] (72.0–69.0)	79.67 ± 0.3[b] (80.0–79.0)
Wind speed (m/sec)	0.0 ± 0.0 (0.0–0.0)	1.83 ± 0.9 (3.0–2.5)
Soil temperature (°C)	26.17 ± 0.3[a] (26.8–25.7)	42.97 ± 6.5[b] (55.9–35.9)

Values in parentheses represent ranges (maximum − minimum). [a,b] Values with different superscript letters in the same row are significantly ($P<0.05$) different.

Table 2. Meteorological parameters during the 27 hr recordings

Hours	DBT (°C)	RH (%)	THI	WS (m/s)	ST (°C)
06:00	25.0	54.0	72.0	0.0	28.1
09:00	30.0	53.0	79.0	1.2	29.9
12:00	35.0	23.0	78.0	2.1	35.4
15:00	37.0	20.0	81.0	3.0	36.1
18:00	35.5	20.0	79.0	1.4	35.8
21:00	32.0	20.0	87.0	0.0	32.1
00:00	29.0	30.0	74.0	0.0	29.8
03:00	27.5	50.0	75.0	0.0	27.8
06:00	27.0	53.0	75.0	1.8	28.9
09:00	27.5	56.0	76.0	1.8	29.5
Mean ± SEM	30.55 ± 1.3 (37.0–25.0)	37.90 ± 5.2 (56–20)	77.60 ± 1.4 (87–72)	1.26 ± 0.4 (3.0–0.0)	31.34 ± 1.0 (36.1–27.8)

Values in parenthesis represent ranges (maximum − minimum). DBT, dry-bulb temperature; RH, relative humidity; THI, temperature-humidity index; WS, wind speed; ST, soil temperature.

THI value for 27-hr recording (77.60 ± 1.4) was lower (P<0.05) than the value (79.67 ± 0.3) recorded immediately after packing and/or trekking, but higher than the value of 70.00 ± 0.1 obtained before packing and/or trekking. The overall mean soil temperature (31.34 ± 1.0°C) during the continuous recording was lower than that recorded immediately after packing and/or trekking (42.97 ± 6.5°C), but higher than the value of 26.17 ± 0.3°C obtained before packing and/or trekking. The mean wind speed (1.83 ± 0.9 m/sec) recorded immediately after packing and/or trekking did not differ (P>0.05) when compared to the overall mean value obtained during continuous recordings (1.26 ± 0.4 m/sec) (Table 2).

Duration of trekking of donkeys for 20 km during the hot-dry season

During the 3 days of trekking and packing, the group 1 donkeys took a mean duration of 4:15 ± 0.01 hr to cover the 20 km distance,while the group 2 trekking only donkeys took a mean duration of 3:47 ± 0.03 hr, which was significantly (P<0.05) lower compared with the packing + trekking group (Table 3).

Effect of packing on respiratory and heart rates

Table 4 shows the effect of packing and trekking on RR and HR. The RR in the packing + trekking donkeys was significantly (P<0.05) higher after packing and trekking compared with before packing and trekking. Similarly, the RR in the trekking-only donkeys only rose significantly (P<0.05) after trekking compared with before trekking. The HR value (76.63 ± 4.5 beats/min) recorded in packing + trekking donkeys immediately after packing and trekking was significantly (P<0.05) higher than that (62.33 ± 2.7 beats/min) obtained in the trekking-only donkeys. However, at 16 hr after packing and/or trekking, the RR and HR values

Table 3. Duration of trekking (hr) of donkeys for 20 km during the hot-dry season

Days	Packing + trekking	Trekking only
Day 1	4:15	3:50
Day 2	4:12	3:40
Day 3	4:17	3:52
Overall mean (SEM)	4:15 ± 0.01[a]	3:47 ± 0.03[a]

[a] Values with different superscript letters in the same row are significantly (P<0.05) different.

in the packing + trekking donkeys did not differ significantly (P>0.05) from the corresponding values recorded in trekking-only donkeys (Fig. 1). The cosinor parameters of mesor and amplitude in the packing + trekking donkeys did not differ from the corresponding values in the trekking-only donkeys. The acrophase and bathyphase values were significantly (P<0.05) higher in the packing + trekking donkeys compared with those recorded in the trekking-only donkeys (Tables 5).

Relationships between meteorological parameters and respiratory and heart rates

Table 6 shows the correlation coefficients between meteorological parameters and RR and HR. The THI, wind speed, and soil temperature were directly related to the RR and HR, but the relationships were significant (P<0.05) only between wind speed and RR, and between soil temperature and HR or RR. Similarly, RR was directly and significantly (P<0.05) related to HR. Figs. 2 and 3 show the linear regression between THI, RR, and HR. The relationships were positive, with THI explaining 18% and 25% of the variation in RR and 18% and 11% of the variation in HR in the packing + trekking and trekking-only donkeys, respectively, but they were not significant.

Table 4. Effect of packing and trekking on respiratory and heart rates in donkeys during the hot-dry season (mean ± SEM, n=10)

Parameters	Packing + trekking		Trekking only	
	Before	After	Before	After
Respiratory rate (cycles/min)	30.15 ± 0.5[a] (32.0–27.3)	43.78 ± 3.0[b] (58.7–30.0)	27.52 ± 0.5[a] (30.0–26.0)	46.30 ± 1.8[b] (56.7–40.7)
Heart rate (beats/min)	48.81 ± 1.8[a] (55.0–39.7)	76.63 ± 4.5[b] (98.3–59.3)	47.93 ± 2.2[a] (59.7–36.0)	62.33 ± 2.7[c] (77.3–53.0)

Values in parenthesis represent ranges (maximum and minimum). [a,b] Values with different superscript letters in the same row are significantly (P<0.05) different.

Table 5. Mesor, amplitude, acrophase, and bathyphase values for respiratory and heart rates at 16 hr after packing and/or trekking period in donkeys under the natural dark-light cycle during the hot-dry season (mean ± SEM, n=10)

Rhythmicity parameters	Respiratory rate (cycles/min)		Heart rate (beats/min)	
	Packing + trekking	Trekking only	Packing + trekking	Trekking only
Mesor	29.80 ± 2.0 (41.0–21.6)	30.61 ± 0.5 (32.5–27.8)	49.86 ± 1.7 (55.4–42.0)	48.38 ± 1.8 (57.8–41.0)
Amplitude	10.25 ± 2.5 (30.6–4.1)	11.25 ± 1.3 (18.9–2.9)	6.72 ± 1.3 (14.8–1.7)	5.62 ± 0.7 (8.1–1.7)
Acrophase	14:11 ± 0.6 (16:6–11:2)	14:25 ± 0.3 (16:0–12:7)	17:10 ± 0.7[a] (22:1–14:6)	15:37 ± 0.4[b] (17:4–13:2)
Bathyphase	2:11 ± 0.6 (4:6–0:9)	2:25 ± 0.3 (4:0–0:7)	5:09 ± 0.7[a] (10:1–2:5)	3:37 ± 0.4[b] (5:4–1:2)

Values in parenthesis represent ranges (maximum and minimum). [a,b] Values with different superscript letters in the same row are significantly (P<0.05) different.

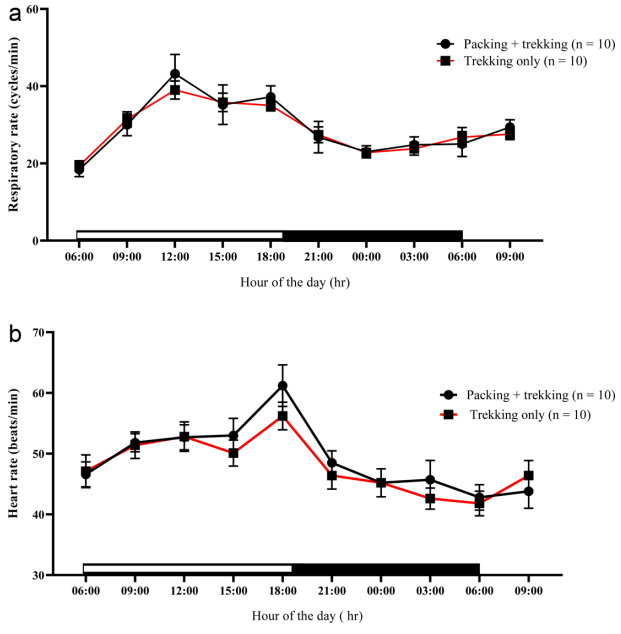

Fig. 1. Diurnal rhythms of respiratory (a) and heart rates (b) in donkeys beginning 16-hr after the packing and/or trekking period during the hot-dry season. The horizontal bar denotes the dark and light phases of the prevailing light-dark cycle. The measurements were made at 3-hr intervals for a period of 27 hr.

Table 6. Relationships among meteorological parameters, respiratory rate and heart rate in donkeys at 16-hr after the packing and/or trekking period during the hot-dry season (n=10)

Correlated parameters	Correlation coefficient (Pearson, r)	
	Packing + trekking	Trekking only
THI and respiratory rate	0.4230[ns]	0.5026[ns]
THI and heart rate	0.4243[ns]	0.3308[ns]
Wind speed and respiratory rate	0.7103**	0.7746***
Wind speed and heart rate	0.3339[ns]	0.4029[ns]
Soil temperature and respiratory rate	0.8615****	0.8761****
Soil temperature and heart rate	0.8116***	0.7759***
Heart rate and respiratory rate	0.7103**	0.7249**

[ns] P>0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Discussion

The values of the meteorological parameters obtained in the study show that the experimental period was characterized by a high DBT and high THI but relatively low RH. The findings were similar to the results obtained by Olaifa *et al*. [15] in donkeys in the same zone, which showed that the hot-dry season was characterized by a high DBT and

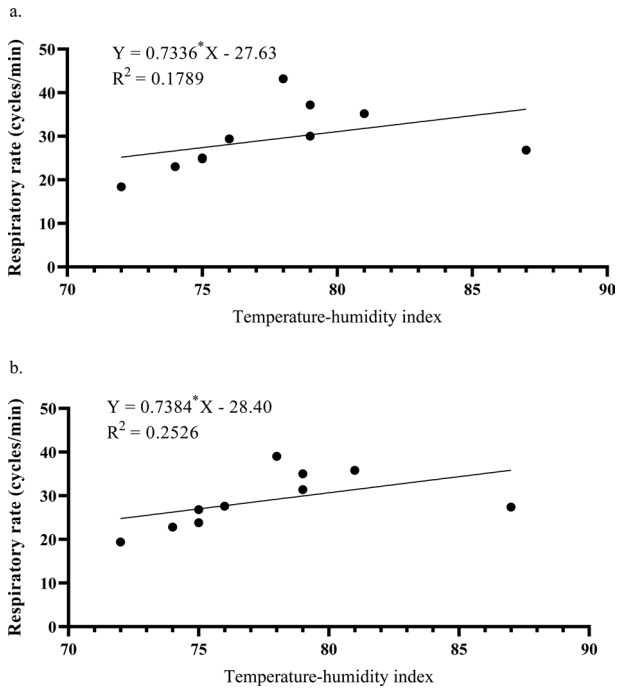

Fig. 2. Regression analysis between temperature-humidity index and respiratory rate in donkeys at 16-hr after packing and/or trekking period during the hot-dry season (n=10). (a)=Packing + trekking (b)=Trekking only.

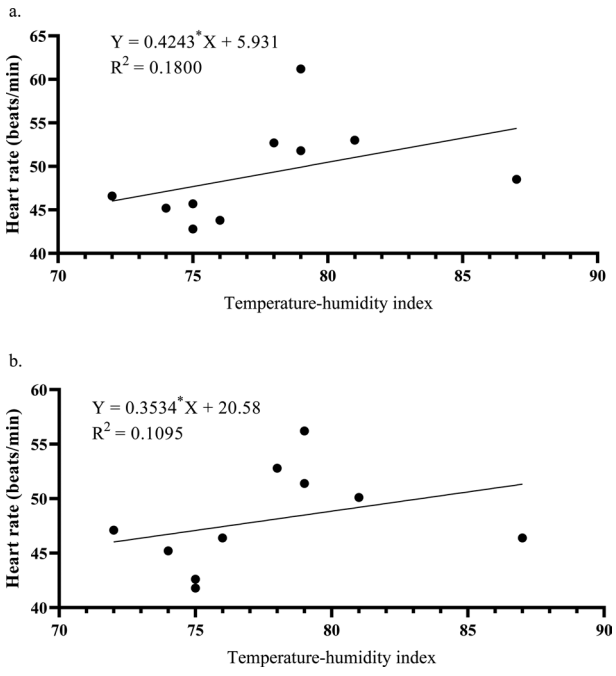

Fig. 3. Regression analysis between temperature-humidity index and heart rate in donkeys at 16-hr after packing and/or trekking period during the hot-dry season (n=10). (a)=Packing + trekking (b)=Trekking only.

THI, adversely affecting the packing performance of the donkeys in the zone. Indeed, the DBT and THI obtained in the current study were outside the thermoneutral zone (23–32°C) established for the donkey in the tropics [7]. Although donkeys have been reported to have adapted to arid conditions prevailing in the Northern Guinea Savannah zone of Nigeria [3, 17, 24], measures adopted to alleviate the impact of meteorological stress on them may be of value in enhancing their work output in the thermally stressful hot-dry season. The findings of the current study show that the work output of the donkeys may be compromised, if they are subjected to packing for a long period during the hot-dry season. The results provide some evidence suggesting that evaluation of the meteorological conditions prevailing in the zone is of value in determining the suitability and duration of working conditions for donkeys in a given period of time. Furthermore, the highest values of the day for the meteorological conditions, particularly DBT, THI, and soil temperature, were recorded in the afternoon (15:00 hr). This result strongly suggests the need to avoid or reduce to the bare minimum the packing activities of donkeys at around 15:00 hr in order to mitigate the risk of adverse effects of environmental heat stress on them.

The results for the duration, required by each group of donkeys to cover the 20 km distance in this study, indicate that load-carrying reduced the speed of trekking in the packing + trekking donkeys compared with the trekking-only donkeys.

The results for the RRs immediately after packing + trekking and trekking only show that packing + trekking and trekking only significantly increased the RRs of the donkeys; due to the adverse effects of the stress of packing and trekking on them. Trekking is a form of exercise in donkeys [16]. Thus, it exerted a significant impact on the donkeys in the hot-dry season by altering their respiration, as evidenced by the increase in their RRs recorded after packing and/or trekking. As the donkeys trekked, energy was generated and expended by the muscle contractile activities to facilitate the movement of the donkeys. The generation of energy involves the breakdown of substrates, which requires oxygen [10], suggesting that packing and trekking of the donkeys were associated with increased consumption of oxygen and, consequently, an increased minute respiratory volume of the lungs. Repeated moderate trekking of donkeys with or without packing may decrease the RR, as the animals become adapted to the stress of trekking and/or packing [26].

Packing + trekking and trekking only did not alter the diurnal rhythm of the RR in the donkeys. Thus, the effect of packing on the donkeys as far as the RR was concerned

was abolished at least 16 hr after the packing and/or trekking period. These findings were based on the fact that the cosinor parameters for the RR in both the packing + trekking and trekking-only donkeys did not differ significantly, although the parameters of both groups demonstrated typical dark-light rhythms. The result shows that the RRs of both groups of donkeys were influenced by the prevailing environmental conditions [25] and that the donkeys used for packing in the zone are relatively adapted not only to the meteorological conditions prevailing in the zone but also to the adverse effects of packing during the hot-dry season [3, 16]. Subjecting donkeys to packing and/or trekking during the hot-dry season for 4 hr exerted a transient effect on the RR. Consequently, donkeys may be exposed to packing and/or trekking after a 16-hr interval without any adverse effect on respiratory activities.

The results also show that HR increased significantly after packing + trekking and trekking only in the donkeys, suggesting that trekking, a form of exercise, enhanced cardiac activities [5, 23]. It has been established that nutrients, including oxygen, glucose, and minerals, are required to ensure the performance of animals subjected to the stress of exercise or trekking and load carrying [6]. Thus, the recorded increase in HR was beneficial because it was an adaptation by the donkeys to the stress due to the totality of unfavorable thermal environmental conditions, trekking, and packing. Further studies are required to evaluate HR variability and its effect on donkeys subjected to packing and trekking under hot-dry conditions.

The acrophase showed that the HR peak was attained at a later hour during the photophase in the packing + trekking donkeys than in the trekking-only donkeys. This finding was due to the combined stressful effects of packing and trekking on the HR as well as their diminishing effects on the packing + trekking donkeys after packing and trekking. The HR acrophase (17:10 hr) of the packing + trekking donkeys in the current study was later than that obtained by Zakari *et al.* [25] in yearling donkeys (15:40 hr) during the hot-dry season. The difference in results may be due to the impact of packing and trekking on the donkeys. The bathyphase was recorded at a later time during the photophase in the packing + trekking donkeys compared with the trekking-only donkeys. This finding may be due to the stress of being subjected to two kinds of physical exertion, with packing and trekking simultaneously having a greater impact on retarding the cardiac activities of these donkeys. The recorded response in the donkeys subjected to both packing and trekking was an adaptive mechanism to sustain the cardiac activities for a longer time and ensure its efficiency [26]. Based on the HR values, the findings of this study strongly suggest that packing + trekking was more stressful on the donkeys than trekking only [16], although the effects of both packing + trekking and trekking only were all abolished at least 16 hr after the packing and/or trekking period. This finding demonstrated that donkeys may be exposed to repeated packing without negative effects, at least at 16-hr intervals.

The direct and significant correlation of the wind speed with the RR in the packing + trekking and trekking-only donkeys shows that wind speed exerted more significant impact on the RR than the HR. Wind blowing against the donkeys during packing for 20-km over 4-hr did not significantly affect the HR but did affect the RR. The soil temperature was significantly and directly related to the HR and particularly the RR, although the THI and HR and RRs were insignificantly correlated. This finding shows that the RR was more subject to the influence of the THI [12]. The results of the current study show that the wind speed and soil temperature had a greater influence on the RR than the HR. Respiration is directly related to the environment, and it influences the performance of the other organs, including the heart. This is because the supply of oxygen to tissues is crucial for the efficiency of the activities of tissues and body organs [20]. Thus, HR and RRs were directly and significantly correlated both in the packing + trekking and trekking-only donkeys.

Conclusion

Packing + trekking significantly increased the HR in donkeys compared with trekking only, suggesting that it was more stressful to the donkeys than trekking only. Repeated packing of donkeys under the hot-dry conditions for 4 hr had no negative effects, and it may be recommended with at least an interval of 16 hr between sessions. Stress mitigation methods should be considered in donkeys before subjecting them to trekking and packing.

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References

1. Arfuso, F., Giannetto, C., Giudice, E., Fazio, F., Panzera, M., and Piccione, G. 2021. Peripheral modulators of the central fatique development and their relationship with athletic performance in jumper horses. *Animals (Basel)* **11**: 743. [Medline] [CrossRef]
2. Arrazola, A., and Merkies, K. 2020. Effect of human attachment style on horse behaviour and physiology during equine-assisted activities-a pilot study. *Animals (Basel)* **10**: 1156. [Medline] [CrossRef]

3. Ayo, J.O., Dzenda, T., Olaifa, F., Ake, S.A., and Sani, I. 2014. Diurnal and seasonal fluctuations in rectal temperature, respiration and heart rate of pack donkeys in a tropical savannah zone. *J. Equine Sci.* **25**: 1–6. [Medline] [CrossRef]
4. Bukhari, S.S.U.H., McElligott, A.G., and Parkes, R.S.V. 2021. Quantifying the impact of mounted load carrying on equids: a review. *Animals (Basel)* **11**: 1333. [Medline] [CrossRef]
5. Contreras-Aguilar, M.D., Cerón, J.J., Muñoz, A., and Ayala, I. 2021. Changes in saliva biomarkers during a standardized increasing intensity field exercise test in endurance horses. *Animal* **15**: 100236. [Medline] [CrossRef]
6. de Melo-Marins, D., Farinha, J.B., Rodrigues-Krause, J., Laitano, O., and Reischak-Oliveira, A. 2021. Redox balance during exercise in the heat in healthy adults: A systematic review. *J. Therm. Biol.* **99**: 102943. [Medline] [CrossRef]
7. Fielding, D., and Krause, P. 1998. Donkeys. p. 119. *In*: The Tropical Agriculturist. Macmillan Education Ltd., London and Basingstoke.
8. Fukushi, I., Yokota, S., and Okada, Y. 2019. The role of the hypothalamus in modulation of respiration. *Respir. Physiol. Neurobiol.* **265**: 172–179. [Medline] [CrossRef]
9. Hooper, H.B., Dos Santos Silva, P., de Oliveira, S.A., Merighe, G.K.F., Titto, C.G., and Negrão, J.A. 2021. Long-term heat stress at final gestation: physiological and heat shock responses of Saanen goats. *Int. J. Biometeorol.* **65**: 2123–2135. [Medline] [CrossRef]
10. Hoyt, D.F., Wickler, S.J., and Garcia, S.F. 2006. Oxygen consumption (VO_2) during trotting on a 10% decline. *Equine Vet. J. Suppl.* **36**: 573–576. [Medline] [CrossRef]
11. Legha, R., Kumar, V., Pal, Y., Dedar, R., Bala, P., Ravi, S., Talluri, T., and Tripathi, B. 2018. Physiological, haematological and biochemical responses in non-descript Indian donkeys working with different pack loads in brick kiln stimulated conditions. *Int. J. Livest. Res.* **8**: 59–66. [CrossRef]
12. Mader, T.L., Davis, M.S., and Brown-Brandl, T. 2006. Environmental factors influencing heat stress in feedlot cattle. *J. Anim. Sci.* **84**: 712–719. [Medline] [CrossRef]
13. Minka, N.S., and Ayo, J.O. 2007. Effects of shade provision on some physiological parameters, behaviour and performance of pack donkeys (*Equinus asinus*) during the hot-dry season. *J. Equine Sci.* **18**: 39–46. [CrossRef]
14. Molcan, L. 2019. Time distributed data analysis by Cosinor. Online application. *bioRxiv*, 805960.
15. Olaifa, F.H., Ayo, J.O., Aluwong, T., and Rekwot, P.I. 2021. The effect of epicatechin − (4β-8) − catechin on some biomarkers of fatigue in packed donkeys (*Equus asinus*) during the dry season in Northern Nigeria. *J. Appl. Anim. Welf. Sci.* **16**: 1–14. [Medline]
16. Olaifa, F.H., Ayo, J.O., Aluwong, T., Rekwot, P.I., and Zakari, F.O. 2019. Ergonomic study of donkeys administered with Pycnogenol® and subjected to packing during the hot-dry season in Northern Nigeria. *Trop. Anim. Health Prod.* **51**: 389–394. [Medline] [CrossRef]
17. Olaifa, F., Ayo, J.O., Ambali, S.F., Rekwot, P.I., and Minka, N.S. 2013. Rectal temperature responses of donkeys administered with ascorbic acid and subjected to load carrying (packing) during the harmattan season in Nigeria. *Trop. Anim. Health Prod.* **45**: 473–477. [Medline] [CrossRef]
18. Oudman, L. (2002). *Donkeys for Traction and Tillage*. p. 25. De Goede Press, Wageningen, Netherlands.
19. Pal, Y., Gupta, A.K., and Kumar, S. 2012. Physical and physiological changes in donkeys during pack load. *Indian J. Anim. Sci.* **82**: 1230–1232.
20. Périard, J.D., Eijsvogels, T.M.H., and Daanen, H.A.M. 2021. Exercise under heat stress: thermoregulation, hydration, performance implications, and mitigation strategies. *Physiol. Rev.* **101**: 1873–1979. [Medline] [CrossRef]
21. Rodrigues Júnior, J.L., Duarte, W., Falqueto, H., Andrade, A.G.P., Morandi, R.F., Albuquerque, M.R., de Assis, M.G., Serpa, T.K.F., and Pimenta, E.M. 2021. Correlation between strength and skin temperature asymmetries in the lower limbs of Brazilian elite soccer players before and after a competitive season. *J. Therm. Biol.* **99**: 102919. [Medline] [CrossRef]
22. Snedecor, G.W., and Cochran, W.G. 1989. Statistical Methods. p. 503. 8th ed., Ames, Iowa State University Press.
23. von Lewinski, M., Biau, S., Erber, R., Ille, N., Aurich, J., Faure, J.M., Möstl, E., and Aurich, C. 2013. Cortisol release, heart rate and heart rate variability in the horse and its rider: different responses to training and performance. *Vet. J.* **197**: 229–232. [Medline] [CrossRef]
24. Zakari, F.O., and Ayo, J.O. 2021. Comparison of body temperature in donkeys using rectal digital, infrared, and mercury-in-glass thermometers during the hot-dry season in a tropical savannah. *Int. J. Biometeorol.* **65**: 1053–1067. [Medline] [CrossRef]
25. Zakari, F.O., Ayo, J.O., Rekwot, P.I., Kawu, M.U., and Minka, N.S. 2018. Diurnal rhythms of heart and respiratory rates in donkeys of different age groups during the cold-dry and hot-dry seasons in a tropical savannah. *Physiol. Rep.* **6**: e13855. [Medline] [CrossRef]
26. Zhang, S., and Zhu, N. 2021. Exercise heat acclimation causes human responses and safety performance improvements. *J. Therm. Biol.* **100**: 103042. [Medline] [CrossRef]