

# Modulatory roles of melatonin on respiratory and heart rates and their circadian rhythmicity in donkeys (*Equus asinus*) subjected to packing during the hot-dry season

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## ABSTRACT

In mammals a central circadian clock is located in the suprachiasmatic nuclei of the hypothalamus, which regulates the innate physiological rhythms to the ambient 24-h light-dark cycle. Melatonin is an essential component of circadian rhythm. The study aimed to evaluate the effects of melatonin administration on the respiratory rate (RR) and heart rate (HR) and their circadian rhythmicity in donkeys subjected to packing (load carrying) during the hot-dry season. Twenty healthy pack donkeys, aged 2–3 years with average weight of  $93 \pm 2.7$  kg were divided into two groups randomly for the experiment. Group 1 donkeys (packing with melatonin) were administered melatonin for seven days during the study and subjected to packing, while group 2 were packed without melatonin administration. Both groups of donkeys were packed three times within the week, one day apart, covering a total distance of 20 km on each experimental day. Meteorological parameters were recorded during the study period. RR and HR were measured pre- and immediately (15 min) post-packing. Continuous measurement of the parameters started 16 h later, after the last packing procedures for a period of 27 h at intervals of 3 h. Temperature-humidity index (THI) pre-packing ( $73.67 \pm 0.7$ ) was lower ( $P < 0.05$ ) than that obtained post-packing ( $80.33 \pm 1.2$ ). The THI recorded during the continuous measurement was at its peak at 15:00 h (86), indicating that the afternoon hours were thermally stressful to the donkeys. The THI was strongly correlated with HR recorded in packing (without melatonin) compared to packing (with melatonin) donkeys. The RR and HR values in both groups of donkeys were significantly ( $P < 0.05$ ) higher immediately after packing. However, the post-packing values of the parameters were not different ( $P > 0.05$ ) between the two groups of donkeys. The mesor (adjusted arithmetic mean) and amplitude of RR and HR in packing (without melatonin) donkeys were significantly ( $P < 0.05$ ) higher than that recorded in packing (with melatonin) donkeys. In conclusion, melatonin reduced negative influence on the circadian rhythmicity (mesor and amplitude) of RR and HR in donkeys by exerting its anti-stress and antioxidant effects. The study has demonstrated the beneficial effect of melatonin and its administration may mitigate excessive respiratory and cardiac activities that may reduce the work output of donkeys during the hot-dry season.

## 1. Introduction

Donkeys are used for packing (load carrying) bricks, sand, firewood, transportation of goods in developing countries of the world (Minka and Ayo, 2007; Bukhari et al., 2021). They are very effective pack animals, especially in areas where the access roads are bad (Minka and Ayo, 2007; Legha et al., 2018). The inability of donkey owners to recognise the onset of fatigue, stress signs has resulted in poor well-being and health status of many working donkeys (Legha et al., 2018; Bukhari et al., 2021). Therefore, studies that will improve the existing data on

the welfare of the donkey are very crucial. The poor performance of donkeys is mostly due to overworking, overloading and exposure to multiple stressors (Ayo et al., 2013). Heat stress, caused by high ambient temperature (AT) and relative humidity (RH), has been reported to lead to reduction in performance in working animals (Pal et al., 2002; Olaifa et al., 2019). Moderate stress has been reported in donkeys covering 9 km distance and carrying loads equivalent to the 50% of their body weight (Pal et al., 2012; Legha et al., 2018). Changes in physiological parameters such as respiratory rate (RR) and heart rate (HR) have been used as indicator of stress in pack donkeys (Ayo et al., 2008, 2014; Legha

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et al., 2018; Frippiat et al., 2021). Increased HR in working donkeys during and after packing indicates physiological responses not only to the load carried, but also the ability of the animals to cope with stresses, generated by trekking over long distances and stressful meteorological parameters (Olaifa et al., 2019). The balance between heat produced plus heat gain from the environment and heat loss in donkeys is enhanced by the adjustment of RR and HR (Ayo et al., 2008, 2014; Lacetera, 2019). During intense physical activities the animal's body, due to its high oxygen demand and increased aerobic metabolism, is exposed to the formation and oxidizing action of reactive oxygen species (ROS) (Czuczajko et al., 2019). Oxidative stress results when the antioxidant capacity of the animal is overwhelmed by increased production of ROS (Powers et al., 2020). The use of antioxidants in animals has been demonstrated to ameliorate the deleterious effects of stress on performance and health (Ocheja et al., 2020; Olaifa et al., 2021). Melatonin is a potent antioxidant and anti-stress agent (Adah et al., 2020; Nazifi et al., 2020; Rastogi and Haldar, 2020), possessing a high safety profile (Zhang et al., 2020). Meteorological conditions such as photoperiod and AT are known to influence circadian rhythms. Circadian rhythms are internally generated; although they could be influenced by external factors, primarily day light (Janott, 2020; Goh et al., 2021). Normal physiological rhythms are controlled by biological clock in the body (Piccione and Caola, 2002). In mammals a central circadian clock is located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Piccione et al., 2005), which regulates the innate circadian physiological rhythms to the ambient 24-h light-dark cycle (Depres-Brummer et al., 1998; Zisapel, 2018; Janott, 2020; Goh et al., 2021). Melatonin acts at the SCN to reduce the wake-promoting signal of the circadian clock, thus promoting sleep. Circadian rhythms of physiological parameters have been studied in human and some species of animals (Mortola, 2004; Piccione et al., 2005). Modulatory effects of melatonin on physiological parameters have been reported in several other studies (Depres-Brummer et al., 1998; Simko et al., 2016; Adah et al., 2020; Niehoff et al., 2021), but not in working donkeys to the best of our knowledge. There is paucity of data on working donkeys. Therefore, studies on the effects of melatonin on working donkeys will contribute to the data available and improve well-being of pack donkeys.

The aim of the study was to evaluate the effects of melatonin administration on the RR and HR and their circadian rhythmicity in donkeys subjected to packing during the hot-dry season.

## 2. Materials and methods

### 2.1. Experimental location

The study was carried out during the hot-dry season between April and May 2021. The study area covered an area from the Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria (11°10'N, 7°38'E) to Sabon Gida, 5 km away from Panhauya village (11°7'N, 7°37'E), Giwa Local Government Area (LGA), Kaduna State, located in the Northern Guinea Savannah zone of Nigeria. The packing covered a total distance of 20 km (to and fro), from the research pen to Sabon Gida on the outskirts of ABU, Zaria. The terrain of the route was narrow, stony, and undulating, typical of the trekking routes taken by packed donkeys in the zone (Minka and Ayo, 2007).

### 2.2. Ethical clearance

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

### 2.3. Experimental animals and management

Twenty, apparently, healthy pack donkeys, between 2 and 3 years' old with average weight of  $93 \pm 2.7$  kg served as experimental subjects.

The donkeys were purchased at a donkey market at Maiadua International Market, Maiadua LGA (13°11'N, 8°12'E), Katsina State, Nigeria. The donkeys 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, 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 packing on each experimental day, and the donkeys had access to water before and after packing using drinking troughs. Salt licks were also provided throughout the period of the experiment.

### 2.4. Experimental design

The donkeys were pre-conditioned for three weeks. During the pre-conditioning period, physical examination was carried out on the donkeys. They were screened for both endo- and ectoparasites, and blood samples were collected for complete haemogram and screened for any infection. The parasitic results indicated presence of *Strongyle* eggs in the samples submitted and all the donkeys were treated with Ivermectin subcutaneously at dosage of 0.2 mg/kg. All other results were within normal range. The donkeys were exposed to packing procedures and familiarised with the route used for packing during the experiment, though they all had previous experience in packing. The donkeys were divided into two groups of 10 each. Melatonin was administered for 7 days to Group 1 donkeys. Both Groups 1 and 2 donkeys were subjected to packing and covered a total distance of 20 km on the same days. This procedure was carried out three times within the week, one day apart.

### 2.5. Administration of melatonin

Group 1 donkeys (n = 10) were orally administered melatonin (Puritan's Pride, Inc. Holbrook, New York, U.S.A.) at a dose of 10 mg per donkey at 20:00 h (Guillaume and Palmer, 1991). The supplement was dissolved in 10 mL of sterile water and aspirated using 20 mL syringe, while Group 2 donkeys (n = 10) were orally administered 10 mL of sterile water only. Melatonin was administered once a day for 7 days during the experiment.

### 2.6. Packing procedure

The donkeys were loaded and saddled at 08:00 h (GMT + 1) after parameters were measured at 06:00 h, with a locally made saddle pack frame, filled with chopped dry grasses to provide a cushion effect on the back of the animals (Oudman, 2002). The saddles for each donkey 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 (Legha et al., 2018) every morning on each experimental day. All loads were balanced evenly with similar weight bulk on either side of the animal. On each day of the experiment, the donkeys were trekked covering a distance of 20 km for a little over 4 h from the research pen of the Department of Physiology, Faculty of Veterinary Medicine, ABU (11°10'N, 7°38'E) to Sabon Gida located at a distance of about 5 km from Panhauya village (11°7'N, 7°37'E), and returned to the starting point between 12:00–13:00 h, as described by Minka and Ayo (2007).

### 2.7. Measurement of thermal environmental conditions

Wind speed was measured using anemometer (AM 816, Littsoyh, Hong Kong, China) and topsoil temperature was evaluated by long-stem soil digital thermometer (Model: TP 300, Jiangsu Xuzhou, China). The dry-bulb temperature (DBT) was recorded on each day of the experiment at 06:00 h and post-packing (12:00–13:00 h), and for continuous measurement beginning at 16 h after the last packing procedures for 27 h at 3 h intervals using a wet- and dry-bulb thermometer (Osmond, Haryana, India), and relative humidity (RH) was calculated using Osmond's

hygrometric table (Narindra Scientific industries, Haryana, India). Temperature-humidity index (THI) was determined using the formula (Mader et al., 2006):

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb - 14.4)] + 46.4$$

Where, Tdb = dry-bulb temperature (°C).

RH = relative humidity

### 2.8. Measurement of respiratory and heart rates

Evaluation of RR and HR were recorded pre- and post-packing three times within the week, with a day interval and at the expiration of 16 h following the third packing procedures for a continuous recording for 27 h at 3-h intervals. The RR was measured by observing and counting the number of respiratory flank movements for 1 min. The HR was recorded using a polar equine FT1 health check (Heart rate monitor, POLAR, polar Electro Oy, Kempele, Finland), consisting of two main components (T31 transmitter with a handle bar and FT1 training computer) used to measure HR of each donkey according to the manual's instruction. The transmitter sent electrocardiogram accurate HR signal to the training computer, while the computer displayed the HR.

### 2.9. Statistical analyses

GraphPad Prism, version 8.02 for Windows (GraphPad Software, San Diego, CA, USA, [www.graphpad.com](http://www.graphpad.com).) was used for the analysis. Data were expressed as mean ± standard error of the mean (Mean ± SEM). The statistical differences within groups pre- and post-packing were evaluated with Student's *t*-test. The effects of packing between the groups were compared 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 evaluate the relationship between thermal environmental parameters and RR and HR. Online cosinor analysis was used to determine the values of the variables of circadian rhythm of RR and HR in the donkeys (Molcan, 2019). Four rhythmic parameters were determined: mesor (adjusted arithmetic mean), amplitude, acrophase (time at which the peak of rhythm occurred) and bathyphase (time at which the lowest point of rhythm occurred). Values of *P* < 0.05 were considered significant (Snedecor and Cochran, 1989).

## 3. Results

### 3.1. Meteorological parameters during the study

Table 1 shows the meteorological parameters pre- and post-packing. During the packing days the DBT (32.67 ± 0.4°C) post-packing recorded was significantly (*P* < 0.05) higher than the value of 24.17 ± 0.4°C obtained pre-packing at 06:00 h, however the RH (79.00 ± 2.6 %) pre-

**Table 1**  
Daily fluctuation in meteorological parameters pre- and post-packing during the hot-dry season.

Packing (Hour of Day)	Meteorological Parameters	Mean ± SEM
Before (06:00 h)	Dry-bulb temperature (°C)	24.17 ± 0.4 (25.0–23.5)
After (12–13:00 h)		32.67 ± 0.4 (33.0–32.0)
Before (06:00 h)	Relative humidity (%)	79.00 ± 2.6 (83.0–74.0)
After (12–13:00 h)		48.67 ± 0.9 (50.0–47.0)
Before (06:00 h)	Temperature-humidity index	73.67 ± 0.7 (75.0–73.0)
After (12–13:00 h)		80.33 ± 1.2 (82.0–78.0)
Before (06:00 h)	Wind speed (m/s)	0.0 ± 0.0 (0.0–0.0)
After (12–13:00 h)		1.03 ± 0.7 (2.3–0.8)
Before (06:00 h)	Soil temperature (°C)	26.03 ± 0.3 (26.3–25.5)
After (12–13:00 h)		32.60 ± 0.5 (33.6–31.9)

Values in parenthesis are maximum – minimum.

packing was higher (*P* < 0.05) than the value recorded post-packing (48.67 ± 0.9 %). The THI (80.33 ± 1.2) post-packing was higher (*P* < 0.05) than pre-packing (73.67 ± 0.7). The post-packing soil temperature value of 32.60 ± 0.5°C was higher (*P* < 0.05) than 26.03 ± 0.3°C obtained before packing.

The DBT was 29.00 ± 1.3°C, fluctuating between 24.0 and 36.0°C, while the RH was 60.60 ± 2.9 % and THI value of 78.20 ± 1.6, fluctuating between 48 and 78 % and 72–86, respectively, during the recordings. The soil temperature was 28.65 ± 1.0°C, fluctuating between 24.7 and 33.7°C. The highest DBT recorded was at 15:00 h, with the value of 36.0°C, while the highest RH occurred at 06:00 h with the value of 78.0 %. The THI was highest at 15:00 h with the value of 86, and the soil temperature was highest also at 15:00 h with the value of 33.7°C (Table 2).

### 3.2. Effect of melatonin on pre- and post-packing values of respiratory and heart rates

The RR in packing (with melatonin) and packing (without melatonin) donkeys were significantly (*P* < 0.05) higher immediately post-packing. The increase in RR immediately after the packing procedure in each group was over 100 %. Similarly, HR values were significantly (*P* < 0.05) higher in both groups of donkeys post-packing compared to pre-packing values. However, the post-packing overall HR mean values in packing (with melatonin) donkeys did not differ (*P* > 0.05) from that of packing (without melatonin) donkeys (Table 3).

Fig. 1 shows the effect of melatonin on circadian rhythm of RR. Following the expiration of 16-h post-packing, the effect of melatonin on the circadian rhythm of RR recorded no significant (*P* > 0.05) difference between the donkeys subjected to packing and treated with melatonin and those that were packed without administration of melatonin, except at the 06:00 h and 18:00 h of photophase and 03:00 h of scotophase. The RR fluctuated from the least value of 18.00 ± 0.9 cycles/min at 06:00 h, to the peak value recorded at 15:00 h (54.33 ± 1.8 cycles/min) in the packing (with melatonin) donkeys. Similarly, in packing (without melatonin) donkeys, the lowest value was recorded at 06:00 h, while the highest value was attained at 15:00 h with the values of 16.00 ± 0.9 and 58.40 ± 2.3 cycles/min, respectively.

After the expiration of 16-h post-packing period, the fluctuations in the HR show that the values were significantly (*P* < 0.05) lower in packing (with melatonin) compared to packing (without melatonin) donkeys; particularly throughout the photophase, except at 09:00 h and 15:00 h. During the scotophase, the values were not different (Fig. 2).

**Table 2**  
Hourly fluctuations in meteorological parameters during therecordings.

Hours	DBT (°C)	RH (%)	THI	WS (m/s)	ST (°C)
06:00	24.0	78.0	73.0	0.0	26.8
09:00	31.0	68.0	81.0	1.4	29.5
12:00	33.5	54.0	84.0	1.2	31.6
15:00	36.0	48.0	86.0	0.0	33.7
18:00	32.0	54.0	82.0	1.3	32.3
21:00	29.0	52.0	77.0	0.0	28.5
00:00	26.0	58.0	74.0	0.0	26.5
03:00	25.5	63.0	74.0	0.0	25.5
06:00	24.0	67.0	72.0	0.0	24.7
09:00	29.0	64.0	79.0	0.0	27.4
Mean	29.0 ± 1.3	60.60 ±	78.20 ±	0.39 ± 0.2	28.65 ± 1.0
±	(36.0–24.0)	2.9	1.6	(1.4–0.0)	(33.7–24.7)
SEM		(78–48)	(86–72)		

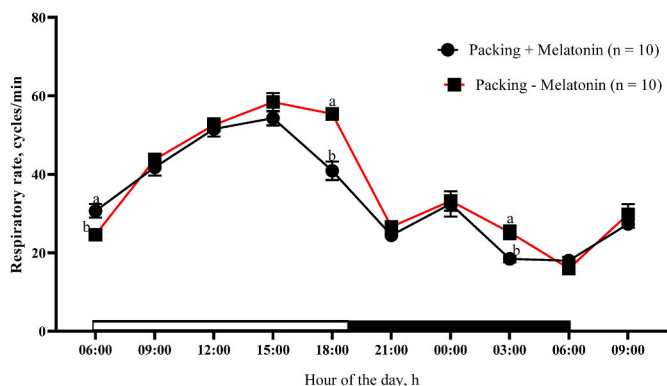
Values in parenthesis are maximum – minimum.

Note: DBT = Dry-bulb temperature, RH = Relative humidity, THI = Temperature-humidity index, WS = Wind speed, ST = Soil temperature.

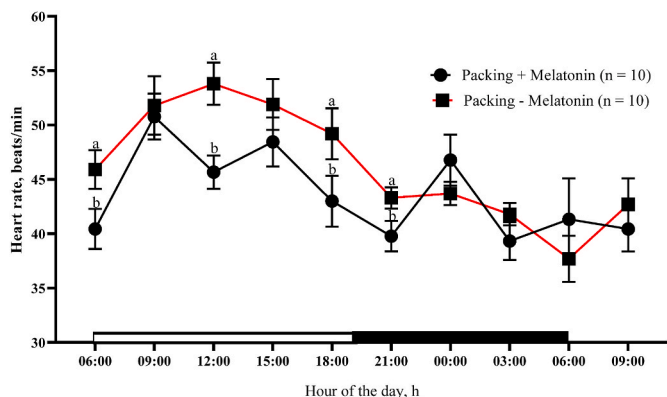
**Table 3**  
Effect of melatonin on respiratory and heart rates in donkeys during the hot-dry season (mean ± SEM, n = 10).

Parameters	Packing + Melatonin		Packing - Melatonin	
	Before	After	Before	After
<b>Respiratory rate (cycles/min)</b>	28.89 ± 0.9 <sup>a</sup> (38.0–20.0)	59.85 ± 1.6 <sup>b</sup> (80.0–40.0)	26.22 ± 1.2 <sup>a</sup> (40.0–18.0)	56.96 ± 2.2 <sup>b</sup> (72.0–26.0)
<b>Heart rate (beats/min)</b>	42.19 ± 1.2 <sup>a</sup> (56.0–30.0)	73.37 ± 1.9 <sup>b</sup> (98.0–55.0)	45.41 ± 1.4 <sup>a</sup> (58.0–36.0)	73.00 ± 2.0 <sup>b</sup> (110.0–58.0)

Values in parenthesis are maximum and minimum. <sup>a,b</sup> = Values with different superscript letters along the same row are significantly (P < 0.05) different.



**Fig. 1.** Effect of melatonin on 16-h post-packing circadian rhythm of respiratory rate in donkeys during the hot-dry season.  
Note: The horizontal bars denote the dark and light phases of the prevailing light-dark cycle. The measurements were made at 3-h intervals for a period of 27 h.  
<sup>a,b</sup> = Values with error bars with different letters are significantly (P < 0.05) different.



**Fig. 2.** Effect of melatonin on 16-h post-packing circadian rhythm of heart rate in donkeys during the hot-dry season.  
Note: The horizontal bars denote the dark and light phases of the prevailing light-dark cycle. The measurements were made at 3-h intervals for a period of 27 h.  
<sup>a,b</sup> = Values with error bars with different letters are significantly (P < 0.05) different.

**3.3. Effect of melatonin on cosinor parameters of respiratory and heart rates**

The mesor and amplitude of RR in packing (with melatonin) donkeys were significantly (P < 0.05) lower than those recorded in packing (without melatonin) donkeys. The acrophase and bathyphase in both

groups did not differ (P > 0.05). The mesor and amplitude of HR were significantly (P < 0.05) higher in the packing (with melatonin) compared to packing (without melatonin) donkeys. The acrophase and bathyphase did not differ (P > 0.05) in the two groups (Table 4).

**3.4. Effect of melatonin on the relationships between meteorological parameters and respiratory and heart rates**

The effect of melatonin on the relationship between meteorological parameters and RR and HR are shown in Table 5. The THI and wind speed were significantly (P < 0.05) correlated with RR, and the relationship was stronger and positive in packing (without melatonin) donkeys. The correlation coefficients between the THI and HR, WS and HR as well as the ST and HR in packing (with melatonin) donkeys show that the values were significantly (P < 0.05) lower, compared to the corresponding values recorded in packing (without melatonin) group. Figs. 3 and 4 show the linear regression analysis results between THI, RR and HR. The relationship was linear as indicated by the regression equations between THI and RR (Y = 2.019\*X - 130.3, R<sup>2</sup> = 0.7518 and Y = 2.523\*X - 168.3, R<sup>2</sup> = 0.8704, packing (with melatonin) and packing (without melatonin), respectively) and THI and HR (Y = 0.4399\*X + 7.794, R<sup>2</sup> = 0.3571 and Y = 0.7877\*X - 17.94, R<sup>2</sup> = 0.6783, packing (with melatonin) and packing (without melatonin), respectively).

**4. Discussion**

The result of the meteorological parameters obtained in the experiment indicates that the thermal environmental conditions were stressful to the donkeys, especially immediately after packing period of 12–13:00 h. The THI value above 78 has been established to be very stressful to animals (Habeb et al., 2018; Lallo et al., 2018; Zakari et al., 2018a). The afternoon THI that fluctuated between 78 and 82, in the current study shows that the afternoon hours were thermally stressful to the donkeys, which agrees with the finding of previous study (Zakari et al., 2020).

The finding of the meteorological parameters during the 27-h continuous measurement were high especially during the photophase. The lower values measured at scotophase, between 03:00 to 06:00 h, indicate that packing of donkeys during the early hours of the day may give a better work output, good welfare and less stressful impact. This is because the environmental conditions during the early hours of the day were relatively favourable and conducive for packing donkeys during the hot-dry season. However the afternoon hours, especially at the 15:00 h, were stressful to the donkeys as they were characterised by high values of thermal environmental conditions. The result of this study has shown that packing of the donkeys during the hot-hours of the day beginning from 12 to 18:00 h should be reduced or completely avoided in order to minimise the negative impact of environmental stress on the

**Table 4**  
Effect of melatonin on 16-h post-packing mesor (adjusted arithmetic mean), amplitude, acrophase and bathyphase of respiratory and heart rates in donkeys under natural dark:light circle during hot-dry season (mean ± SEM, n = 10).

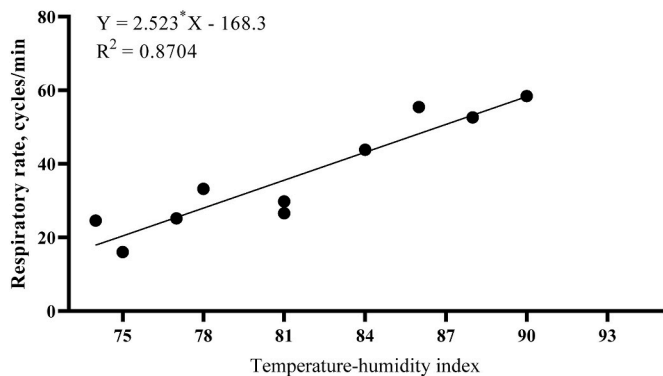
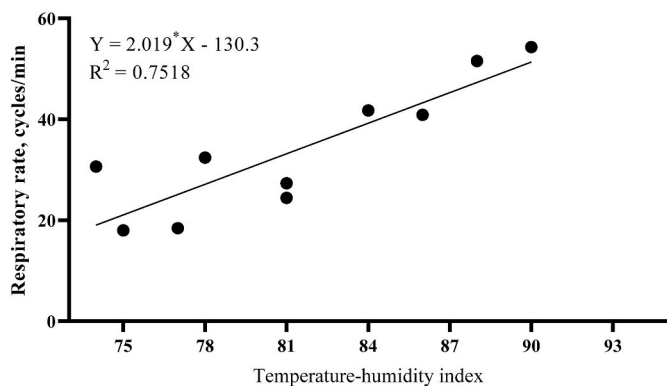
Rhythmicity parameters	Respiratory rate (cycles/min)		Heart rate (beats/min)	
	Packing + Melatonin	Packing - Melatonin	Packing + Melatonin	Packing - Melatonin
<b>Mesor</b>	34.71 ± 0.6 <sup>a</sup> (36.8–31.3)	38.04 ± 0.4 <sup>b</sup> (39.8–35.6)	43.64 ± 1.1 <sup>a</sup> (49.5–41.4)	46.40 ± 1.2 <sup>b</sup> (51.0–40.2)
<b>Amplitude</b>	14.49 ± 0.2 <sup>a</sup> (15.6–13.9)	18.93 ± 0.7 <sup>b</sup> (23.2–15.7)	4.22 ± 0.8 <sup>a</sup> (8.8–1.5)	6.49 ± 0.7 <sup>b</sup> (10.4–3.4)
<b>Acrophase</b>	14.49 ± 0.2 (15.6–13.9)	15.24 ± 0.2 (16.5–14.0)	13.84 ± 1.6 (22.3–3.8)	14.12 ± 0.6 (17.6–10.6)
<b>Bathyphase</b>	2.49 ± 0.2 (3.6–1.9)	3.14 ± 0.2 (4.5–2.0)	2.68 ± 1.1 (10.3–0.0)	2.25 ± 0.5 (5.6–0.0)

Values in parenthesis are maximum and minimum. <sup>a,b</sup> = Values with different superscript letters along the same row are significantly (P < 0.05) different.

**Table 5**  
Effect of melatonin on correlation coefficients of meteorological parameters with respiratory and heart rates in donkeys during the hot-dry season (n = 10).

Correlated parameters	Correlation coefficient (Pearson, r)	
	Packing + Melatonin	Packing – Melatonin
THI and respiratory rate	0.8670***	0.9330****
THI and heart rate	0.5976*	0.8236****
Wind speed and respiratory rate	0.5641*	0.6389*
Wind speed and heart rate	0.5119 <sup>ns</sup>	0.7021**
Soil temperature and respiratory rate	0.9052****	0.9538****
Soil temperature and heart rate	0.5443 <sup>ns</sup>	0.8676****
Heart rate and respiratory rate	0.7545***	0.8979****

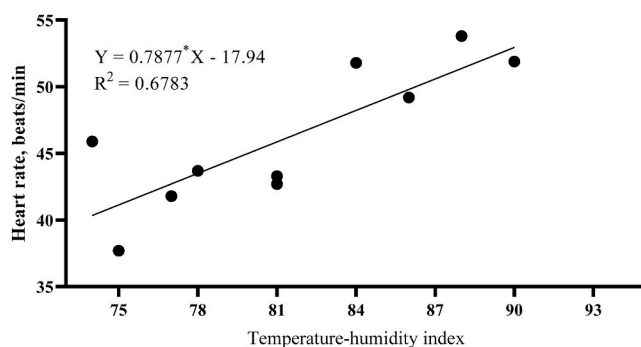
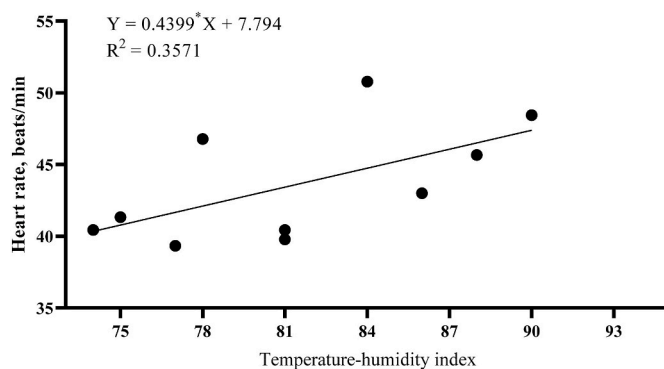
\*\*\*\* = P < 0.0001, \*\*\* = P < 0.001, \*\* = P < 0.01, \* = P < 0.05, <sup>ns</sup> = P > 0.05.  
Note: THI = Temperature-humidity index.



**Fig. 3.** Effect of melatonin on regression analysis between temperature-humidity index and respiratory rate in donkeys post-packing during the hot-dry season (n = 10).  
Note: (a) = Packing + Melatonin (b) = Packing – Melatonin.

donkeys. The finding agrees with the observations of Zakari et al. (2018a), who obtained high values of environmental parameters during the photophase, especially at 15:00 h of the day.

The increase in RR recorded in packing (with melatonin) or packing (without melatonin) donkeys suggests that packing and high environmental heat index significantly impacted the respiratory activities as load carrying requires expenditure of energy, which involves increased consumption of oxygen and increased respiratory rate (Hodgson et al., 1994; De Solis, 2019; Lacetera, 2019). Consequently, packing exerted some load on the respiratory system of the donkeys, both in the treated and those not treated with melatonin. This finding was different from the observation of Foster et al. (1995), who recorded no significant



**Fig. 4.** Effect of melatonin and packing on regression analysis between temperature-humidity index and heart rate in donkeys post-packing during the hot-dry season (n = 10).  
Note: (a) = Packing + Melatonin (b) = Packing – Melatonin.

increase in the RR in donkeys exposed to treadmill exercise. The difference in results may be due to increased workload in the current study. At the expiration of 16-h post-packing, there was no significant difference in the values of RR between the treated and untreated donkeys, except at 18:00 h and 03:00 h of the photophase and scotophase, respectively. The observation shows that melatonin exerted an antioxidant effect by decreasing the RR during that period of photophase and scotophase, when the THI values were high and low, respectively. The finding was an adaptive thermoregulatory response of the donkeys to the increasing heat load during the later hours of the day. The response may result in the increase of the respiratory activity to meet the increasing demand of heat loss through evaporative mechanism (Ayo et al., 2008; Fukushi et al., 2018; Lacetera, 2019).

The mesor of RR in packing (with melatonin) was lower than that of packing (without melatonin) donkeys because of the hypothermic effect of melatonin exerted via anti-stress and antioxidant mechanism; while in packing (without melatonin) donkeys, the hypothermic effect was absent. Body temperature has direct influence on the value of RR (Mortola, 2004) and melatonin has been reported to have capacity to reduce body temperature (Cagnacci, 1996; Depres-Brummer et al., 1998; Atkinson et al., 2005; Simko et al., 2016), thereby indirectly influencing the rhythm of RR. The mesor of RR obtained in the current study was higher compared to the value obtained by Zakari et al. (2018b) in donkeys that were not subjected to packing. The difference in the observations may be due to the impact of packing on the donkeys.

The amplitude of RR was lower in packing (with melatonin) than in packing (without melatonin) donkeys, which shows that melatonin decreased not only the RR but also influenced its fluctuations. This was evidenced by low range values of RR in the packing (with melatonin) donkeys, compared to packing (without melatonin) donkeys. Thus, melatonin enhance the adaptive responses of the donkeys in the packing

(with melatonin) group, compared to the packing (without melatonin) donkeys. The finding has demonstrated the beneficial effect of melatonin and that where it becomes necessary for the donkeys to be packed during the hot-dry season, melatonin administration may mitigate excessive respiratory activity that may impair their work output. [Olaifa et al. \(2013\)](#), [Aluwong et al. \(2013\)](#) and [Sinkalu et al. \(2020\)](#) have demonstrated that antioxidants, such as ascorbic acid, probiotic and melatonin, reduced the range of fluctuations in body physiological parameters. It has been demonstrated by [Sinkalu et al. \(2015\)](#) that wider fluctuations in physiological parameters are an evidence of poor adaptive responses.

There was no significant difference in the acrophase recorded in packing (with melatonin)  $14:49 \pm 0.2$  h and packing (without melatonin) donkeys ( $15:24 \pm 0.2$  h), though, the time at which the RR rhythm reached the peak in donkeys with melatonin was shorter. The finding demonstrates the beneficial role of melatonin in improving performance in individuals subjected to strenuous exercise ([Atkinson et al., 2005](#); [Ochoa et al., 2011](#); [Siuf et al., 2011](#)).

The result in packing (with melatonin) donkeys was similar to the report of [Zakari et al. \(2018b\)](#), who reported that the peak RR occurred at  $14:10 \pm 0.2$  h in yearling donkeys during the hot-dry season, while the acrophase obtained in packing (without melatonin) was significantly higher. This observation may be due to the effect of packing and the antioxidant effect of melatonin in the donkeys that were administered melatonin and subjected to packing.

The relationship between the THI and RR demonstrates that the DBT and RH were responded to by the donkeys by corresponding increase in the respiratory activity. The response was an adaptive mechanism to ensure homeostasis in the donkeys, subjected to packing under the conditions of high DBT and RH ([Ayo et al., 2008, 2014](#); [Lacetera, 2019](#)). The correlation coefficient was, thus, relatively higher in packing (without melatonin) donkeys compared to packing (with melatonin) donkeys. The result thus suggests the ability of melatonin to mitigate the tendency towards exhaustion of the energy required for sustaining normal respiratory activity. Consequently, melatonin administration prolonged the time of attainment of fatigue in the donkeys ([Rastogi and Haldar, 2020](#)). Thus, further studies are required to investigate the effect of THI on other respiratory parameters, such as the tidal volume and, consequently, the minute respiratory volume.

The post-packing values of HR were about two times higher than the normal range of 38–45 beats/minute, established for donkeys in the tropical region ([Fielding and Krause, 1989](#)). The HR responded to the stress of packing and heat stress via increased demand in blood circulation during the hot-dry season, which consequently results in increased post-packing HR values in both the packing (with melatonin) and packing (without melatonin) donkeys ([De Solis, 2019](#)). The finding indicates that the donkeys employed increased blood flow to cope with the demand of packing and trekking ([Hodgson et al., 1994](#)). The increased HR recorded in the study is in agreement with the finding of [Foster et al. \(1995\)](#), who reported significant increase in HR values in donkeys during exercise. In the present study, melatonin did not reduce HR in treated donkeys compared to the group that were not treated. Some studies have reported reduction in HR in humans post-exercise ([Siuf et al., 2011](#); [Simko et al., 2016](#)), while another shows no significant change ([Atkinson et al., 2005](#)). After the expiration of 16-h, the post-packing influence on the circadian rhythm of HR shows that the values obtained in packing (with melatonin) donkeys were significantly lower, compared to those recorded in packing (without melatonin) donkeys, especially during the photophase. The finding indicates that circadian rhythm of HR in the donkeys was significantly influenced by melatonin especially during the photophase, thus the overall mean HR was significantly reduced in packing (with melatonin) donkeys than in packing (without melatonin) donkeys. The finding demonstrates that melatonin decreased the recovery time from the post-packing effects and by implication improved the performance of the donkeys.

The mesor of HR obtained in packing (with melatonin) donkeys was

significantly lower than the value recorded in packing (without melatonin) donkeys. The result demonstrates the involvement of melatonin in homeostasis of the packed donkeys by decreasing the mesor and amplitude of HR, apparently to increase the efficiency and work output in the donkeys. It is possible that melatonin may improve the cardiac activity of the donkeys by enhancing their stroke volume and, consequently, increasing the cardiac output. An increase in the cardiac output via the mechanism of increase in stroke volume may facilitate the efficiency and volume of work output in the pack donkeys, particularly if they are to be subjected to packing for long period during the thermally stressful hot-dry season. The mesor of HR ( $43.64 \pm 1.1$  beats/min) and amplitude ( $4.22 \pm 0.8$ ) recorded in packing (with melatonin) donkeys were lower than mesor ( $50.40 \pm 0.52$  beats/min) and amplitude ( $28.00 \pm 1.80$ ) reported by [Zakari et al. \(2018b\)](#) in yearling donkeys that were not subjected to packing during the hot-dry season. The difference may be due to the antioxidant effect of melatonin. Lower HR has been demonstrated to be beneficial in exercising individuals ([Mlynekova et al., 2016](#); [Reimers et al., 2018](#); [Arrazola and Merckies, 2020](#)), and exercise is a form of stress. Further studies are, however, required to evaluate the HR variability in donkeys, which will give a better assessment of the stressful impact of the thermal environmental conditions.

The lack of difference in acrophase and bathyphase of the HR may be due to the fact that the heart plays a crucial role in pumping blood to the systemic circulation in order to ensure effective blood distribution to critical structures during the packing period. This is particularly true of the skeletal muscles, innervated by the somatic nervous system. However, the vessels are sub-served by the post-ganglionic sympathetic nerve fibres, discharging acetylcholine to ensure vasodilation and, consequently, optimum blood flow to the skeletal muscles during the packing ([Basdanis et al., 2021](#)). Further investigations are required to determine the perfusion rate in the different structures of the donkeys during packing.

The correlation coefficients between meteorological (THI, wind speed and soil temperature) parameters and HR show that the values in the packing (with melatonin) donkeys were lower than those obtained in packing (without melatonin) donkeys. Again, the finding demonstrates the significant role of melatonin in decreasing the HR in packing (with melatonin) donkeys, compared to pack donkeys without administration of melatonin. Wind speed may influence the HR via the respiratory system; thus, the correlation coefficient between the wind speed and HR was significantly lower in packing (with melatonin), compared to packing (without melatonin) donkeys. Thus, the RR was more influenced by the thermal environmental conditions than the HR. The soil temperature was directly correlated with HR, apparently because the donkeys usually lie down after packing ([Minka and Ayo, 2007](#)). Based on thermodynamic mechanism of heat loss via convection and conduction, the high soil temperature may stimulate the HR increase. [Minka and Ayo \(2007\)](#) have shown that HR increases when donkeys are directly exposed to the influence of high soil temperature. The HR value in packing (without melatonin) donkeys was higher than in the donkeys that were packed and treated with melatonin. This finding may be because melatonin decreased body temperature in the donkeys and, consequently, reduced the HR, and the influence of high soil temperature was low in the pack donkeys administered with melatonin.

## 5. Conclusion

Packing under stressful environmental conditions increased RR and HR immediately post-packing. Melatonin reduced the negative influence on the circadian rhythmicity (mesor and amplitude) of RR and HR in donkeys by exerting hypothermic effect via anti-stress and antioxidant mechanisms. It enhanced RR and HR adaptive responses in the packed donkeys, and may increase the efficiency and work output in the donkeys. The finding has demonstrated the beneficial effect of melatonin and that where it becomes necessary for the donkeys to be packed during the hot-dry season, melatonin administration may mitigate excessive

respiratory and cardiac activities that may reduce their work output. Further studies are, however, required to investigate the effect of meteorological parameters and melatonin on other respiratory parameters, such as the tidal volume and, consequently, the minute respiratory volume and HR variability which are better indicators of stress than HR.

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## CRedit authorship contribution statement

**Joseph Olusegun Ayo:** The authors have materially participated in the research, article preparation, The authors have approved the final article, The idea for the paper was conceived, The experiments were designed, The paper was jointly written and edited. **Ayodele Stephen Ake:** The experiment was performed and supervised, trained student volunteers, The data were analysed, The paper was jointly written and edited.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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