Effects of melatonin and transportation on rectal temperature, heterophil/lymphocyte ratio and behaviour of Japanese male quails (*Coturnix japonica*)

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Abstract: The experiment was performed in order to determine the ameliorating effect of melatonin on rectal temperature (RT), heterophil/lymphocyte (H/L) ratio and behaviour of transported quails. On transportation day, the birds were randomly divided into three groups, consisting of 40 birds each. 30 minutes before transportation, quails in group one (melatonin-treated, mel-treated) were administered orally and individually with melatonin at a dose of 0.5 mg/kg body weight dissolved in 1ml of sterile water, while group two (control) and three (sedentary) quails were given equivalent of sterile water. The sedentary quails were not transported. The ambient temperature and relative humidity recorded before transportation and inside the vehicle during transportation were outside the thermoneutral values of 12-24^{0}C and 45%, respectively for the quails. The mean RT value of 42.4 ± 0.7^{0}C recorded in the control quails during the transportation period was higher than the mean RT values of 41.3 ± 0.2^{0}C and 41.4 ± 0.2^{0}C recorded in mel-treated and sedentary quails, respectively. Similarly, the control quails had higher ($P < 0.05$) H/L ratio and decreased ($P < 0.05$) locomotory and vocalization behaviours compared to the corresponding values obtained in mel-treated and sedentary quails, respectively. The result showed that the transportation was stressful to the quails and has induced hyperthermia, lymphopenia, hetetrophilia and a decrease in locomotory and vocalization behaviours. The administration of melatonin has alleviated the effects of transportation stress on the quails.

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1. Introduction

Japanese quail has been used extensively as a laboratory animal as well as a commercial bird for egg and meat production. It low maintenance cost associated with its small body size coupled with its short generation interval, resistance to diseases and high egg production rendered it an excellent laboratory animal (Oguz and Minvielle, 2007; Vali, 2008).

Commercial quail farming is becoming more popular and is being increasingly promoted in a number of Asian and European countries (EFSA, 2004; Vali, 2008, RSPCA, 2011) and of recent in Africa. The advantages of quail farming includes minimum floor space, low investment, comparatively sturdy birds, early market age and sexuality, high rate of egg production and less fed requirement (EFSA, 2004). Besides, Quail meat and egg are tastier than chicken and has less fat contents. It has been shown to promote body and brain development in children and nursing mothers (EFSA, 2004; Sahin *et al*., 2003).

Transportation of animals is known to induce both psychological and physiological stress, and stress- induced behavioural changes may result in accompanying major physiological disturbances such as aberrant behaviours, immune-suppression, and disruption of general homeostasis (Minka and Ayo, 2008; Minka and Ayo, 2010a). It has been established that changes in rectal temperature, neutrophil/lymphocyte ratio and behavioural activities of animals, including birds, are reliable indices of stress, and are often used in the development of management strategies aimed at increasing productivity. They are important for on-the-spot evaluation of the health status and adaptability of animals to various stress factors, including transportation stress (Minka and Ayo, 2008, 2010a). Very often, behavioural changes are the main signs of distress and the first signs of disease in animals (Ayo *et al*. 2002).

In spite of the numerous studies conducted on quails as a laboratory and commercial bird information on the physiological responses of quails to road transportation are limited in the available literature (EFSA, 2004; Gonzalez *et al*., 2007), especially during the hot climatic conditions.

Many environmental stress factors, including high and low ambient temperature, and management factors like transportation, induce oxidative stress and deplete antioxidants and increase oxidative stress in animals (Sahin *et al*., 2003; Tekelioglu *et al*., 2010).

Several studies have demonstrated the ameliorating effect of antioxidant vitamins, electrolytes, probiotics and sedatives on transportation stress in livestock and poultry (Schaefer *et al*., 1997; Ayo *et al*., 2006; Lykkesfeldt and Svendsen, 2007; Minka and Ayo, 2008), but in quail birds such information are grossly inadequate. Of recent, studies have shown that supplementation of vitamin C and melatonin, the hormone of the pineal gland, could be used to reduce the negative effects of heat stress on quails (Sahin *et al.,* 2004a, b). It was shown that melatonin is a powerful antioxidant with a high free radical scavenging activity (Reiter *et al*., 1997; 2004; Reiter *et al*., 2007; Tan *et al*., 2007) and it administration in the daytime leads to a fall in internal temperature (Gilbert *et al*., 1999). These findings suggest that melatonin may play an important role in the thermoregulatory control of body temperature and immune status.

The present study was aimed at examining the effects of road transportation and the administration of melatonin on rectal temperature, immune status and behaviour of male Japanese quails.

2. Material and Methods

2.1 Study area, environmental variables and rectal temperature measurements

The experiment was performed at the Livestock Farm of the College of Agriculture and Animal Science, Ahmadu Bello University, Kaduna (11^{o} 10´N, 07^{o} 38´E), located in the Northern Guinea Savannah zone of Nigeria. The ambient temperatures (AT), relative humidity (RH) and rectal temperature (RT) of the quails were recorded at the experimental site at 07:00, 13:00 and 18:00h daily for seven consecutive days before and after transportation. Values of these parameters were also recorded at 30 min, 1 h and 2 h of the transportation period. The AT and RH were measured using a wet- and dry-bulb thermometer (DTH 1, Clarke Int. Epping, Essex). The measurement of the RT was done using a digital clinical thermometer (Hartman's Company PLC, England), inserted about 2-3 cm into the cloacae through the anus till an alarm sound was heard, indicating the end of the reading.

2.2 Birds and transportation procedures

One hundred and twenty 8-week-old Japanese male quails served as subjects. The quails were raised from day-old in a standard pen to maturity. On transportation day, the birds were randomly divided into three groups, consisting of 40 birds each. Feed and water were withdrawn from all the birds six hours before transportation and during the transportation periods. 30 minutes before loading into a vehicle, quails in group one (melatonin-treated) were administered orally and individually with melatonin at a dose of 0.5 mg/kg body weight dissolved in 1ml of sterile water, while group two (control) quails were given equivalent of sterile water. Thereafter, the birds were loaded in two crates in a modified golf-wagon vehicle and transported for2 h. The third group, sedentary quails were also given sterile water only as in control, but were not transported, rather they were left inside a crate in the pen with no food and water. The handling, loading and transportation of the quails were carried out humanely in accordance with the guidelines governing animal transport welfare by road (EFSA, 2004; RSPCA, 2011). The journey commenced by 11:00 h and was terminated at 13:00 h. The vehicle traveled for 2 h on a typical asphalt single lane road with an average speed of 50-70 km/h from Kaduna (11^{o} 10´N, 07^{o} 38´E) to Birnin Gwari town and from Birnin Gwari back to Kaduna, covering a total distance of about 140 km. The quails were returned to the same pen. Food and water were offered and all conditions were provided for the three groups as it was done before transportation.

2.3 Blood sampling and analysis

Ten birds from each group were colour marked and quickly sacrificed just before the administration of the vitamins and loading to obtain baseline values. Immediately after the journey when the birds were unloaded another set of 10 birds from each group were sacrificed to evaluate the stress due to transportation. Three days post-transportation the rest 20 birds from each group were finally sacrificed.. This arrangement was provided to eliminate the stress of repeated handling and blood sampling of the same birds. From each sample, two blood smears were made on microscopic slides immediately after the blood was collected. The blood smears were dried and stained with Camco Quick Stain II, Buffered Differential Wright Giemsa Stain (Bayer Corp., Diagnostic Division, Elkhart, IN). One hundred leucocytes were counted, and the relative proportions of lymphocytes, heterophils, eosinophils, basophils, monocytes, and the heterophil/lymphocyte (H/L) ratios were determined (Gross and Siegel, 1983).

2.4 Behavioural measurement

Passive behaviour (standing, lying on side and sitting), locomotory behaviour (walking, running and flying) and vocalization behaviour were recorded as earlier described (Altmann, 1974; Buchwalder and Wechsler, 1997; Minka and Ayo, 2008) with a slight modification. Briefly, the behaviours were recorded visually for 3 hours immediately after transportation and unloading of the quails in the pen, with 10 min of observation and 10 min of pause. The measurement of the behaviour was done at the same hour of the day during the pre and post-transportation periods. The number of quails and time spent performing a particular behaviour at the time of observation was recorded. The frequency of vocalization which included crowing, calling, chirping, tweeting and singing were also recorded per group. Vocalization types that lasted over 1 second and were heard by the observer were recorded (Chang *et al*., 2009).

2.5 Statistical analysis

Data were subjected to Student's *t*-test and expressed as mean ± S.E.M. Analysis of variance was used to compare behavioural results between the groups of the quails during the 3 hours post-transportation period. Values of $P < 0.05$ were considered significant.

3. Results

3.1 Environmental variables and rectal temperature

Table 1 showed the environmental variables of the study area and RT before transportation and the data were not different ($p > 0.05$) from those obtained post-transportation period. The RT of the quails fluctuate between the values of 37.5 ± 0.1^0C - 42.0 ± 0.3^0C and had a mean value of 40.4 ± 0.2^0C.

The AT recorded inside the vehicle during transportation rose to a maximum value of 37.4^0C, when the transportation was completed, while the RH fluctuated between the values of 55 - 78%.

Table 1: Environmental data and rectal temperature of quails (n=30) before transportation.

Hour of the day	Ambient temperature (^{0}C)	Relative humidity (%)	Rectal temperature (^{0}C)
06:00	24.0 ± 0.5^0C	50 ± 5.5	37.5 ± 0.1^0C
14:00	38.8 ± 0.2^0C	60 ± 7.5	41.8 ± 0.2^0C
18:00	36.0 ± 0.3^0C	68 ± 8.7	42.0 ± 0.3^0C
Mean± SEM	35.0 ± 0.5^0C	60 ± 5.0	40.4 ± 0.2^0C

The mean RT value of 41.3 ± 0.2^0C recorded in mel-treated quails during transportation was significantly ($P < 0.05$) lower than the mean value of 42.4 ± 0.7^0C recorded in the control quails (Table 2). The mean RT value recorded in sedentary quails was not different ($P > 0.05$) from the mel-treated quail. The mean RT value recorded in the control birds returned to baseline value a day after the transportation and the value was not different from the corresponding values recorded in the sedentary and mel-treated quails.

Table 2: Rectal temperature responses of quails to the effects of transportation and melatonin administration.

	Rectal temperature (^{0}C)			Environment variables	
Transportation Period	Control (n= 30)	Mel-treated (n=30)	Sedentary (n = 30)	Ambient Temperature (^{0}C)	Relative Humidity (%)
30 min	42.3±0.2	41.5±0.2	40.5 ±0.2	29.5	55.6
1 hour	42.4±0.5	40.8±0.5	41.8± 0.5	30.4	70.5
2 hours	42.5±0.2	41.6 ± 0.4	41.9 ± 0.4	37.6	78.0
Mean±SEM	42.4 ± 0.7^a	41.3 ± 0.2^b	41.4 ± 0.2^b	32.5 ± 1.4	66.9 ± 5.7

^{ab}= Mean values with different superscript alphabets are significantly different at $P < 0.05$.

Table 3: Leucocytes counts of quails pre- and post- transportation periods

		Immediately after transportation			3- days post- transportation		
Variables	Baseline (n=30)	Control (30)	Mel-treated (n=30)	Sedentary (n=30)	Control (n=15)	Mel-treated (n=15)	Sedentary (n=15)
Heterophil	22.0 ± 1.2^a	42.0 ± 4.2^b	24.0 ± 5.4^a	20.0 ± 3.6^a	32.0 ± 9.2^b	21.0 ± 3.6^a	21.0 ± 4.5^a
Lymphocyte	60.0 ± 5.1^a	50.0 ± 7.1^b	60.0 ± 7.2^a	54.0 ± 5.5^b	54.0 ± 10.2^b	68.0 ± 8.5^a	58.0 ± 5.5^a
Heterophil/ Lymphocyte ratio	0.37 ± 0.2^a	0.84 ± 0.1^b	0.40 ± 0.02^a	0.38 ± 0.01^a	0.59 ± 0.12^b	0.39 ± 0.02^a	0.36 ± 0.01^a
Eosinophil	3.8 ± 0.4^a	0.8 ± 0.02^b	3.6 ± 1.0^a	2.9 ± 0.9^a	2.0 ± 0.3^b	4.0 ± 1.1^a	3.2 ± 0.5^a
Basophil	0.8 ± 0.2^a	1.0 ± 0.02^a	1.0 ± 0.02^a	0.7 ± 0.01^a	0.7 ± 0.1^a	0.33 ± 0.01^a	1.0 ± 0.03^a
Monocyte	13.4 ± 1.3^a	18.7 ± 1.7^a	12.5 ± 1.5^a	11.5 ± 1.2^a	11.5 ± 1.5^a	11.0 ± 1.3^a	18.0 ± 0.9^a

Mean values with different ($P<0.05$) superscript alphabets along the same row are significantly different at $P<0.05$.

Table 4: Behavioural responses of quails during three hours of post- transportation period

	Number of birds (%)			Time spent (%)			Vocalization frequency/h/group		
Behaviours	Control (n=20)	Mel-treated (n=20)	Sedentary (n=20)	Control (n=20)	Mel-treated (n=20)	Sedentary (n=20)	Control (n=20)	Mel-treated (n=20)	Sedentary (n=20)
Standing	40 ± 6.2^{a}	10 ± 1.2^{b}	15 ± 2.2^{b}	25 ± 4.0^{c}	12 ± 1.5^{b}	15 ± 2.4^{b}			
Lying down	45 ± 5.0^{a}	5 ± 1.5^{b}	10 ± 2.0^{c}	40 ± 2.5^{a}	8.0 ± 5.0^{b}	12 ± 3.5^{b}			
Sitting	20 ± 3.2^{a}	10 ± 1.0^{b}	10 ± 3.2^{b}	30 ± 7.2^{a}	12 ± 4.5^{b}	19 ± 3.2^{b}			
Walking	5 ± 0.7^{a}	60 ± 5.2^{b}	52 ± 10.1^{b}	3.0 ± 0.5^{a}	40 ± 8.2^{b}	35 ± 8.7^{b}			
Running	2 ± 0.2^{a}	30 ± 8.5^{b}	20 ± 6.1^{b}	2.0 ± 0.2^{a}	25 ± 5.5^{b}	22 ± 9.2^{b}			
Flying	0.0^{a}	1.0 ± 0.2^{a}	1.0 ± 0.1^{a}	0.0^{a}	0.5 ± 0.01^{a}	0.1 ± 0.02^{a}			
Vocalization	ND	ND	ND	ND	ND	ND	5.0 ± 2.2^{a}	25 ± 5.5^{b}	20 ± 7.8^{b}

Mean values with different ($P < 0.05$) superscript alphabets along the same row are significantly different at $P < 0.05$. ND = Not done.

3.2 Heterophil/lymphocytes ratio

Table 3 depicts leucocytes counts of the quails before and after transportation. The H/L ratios of the pullets are shown in Figure 1. The pre-transport H/L ratios in all the quails were not different (p>0.05) from one another. The H/L ratios obtained in sedentary and mel-treated quails immediately and three days after transportation were not different (p>0.05) from each other and from the pre-loading values. In control quails the H/L ratio recorded immediately and three days post – transportation periods were significantly (p<0.05) higher than the pre-transportation and corresponding post-transportation H/L ratios recorded in mel-treated and sedentary quails.

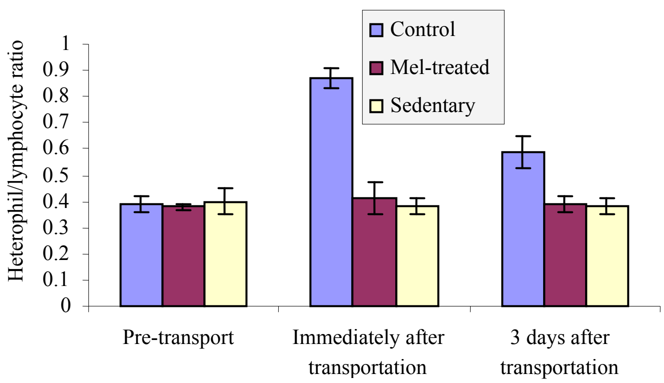

Figure 1: Effects of transportation and melatonin on heterophil/lymphocyte ratio of Japanese quails

3.3 Behavioural activities

The number of quails performing particular behaviour, percentage time spent on the behaviour and the frequency of vocalization per group of birds are shown in Table 4. The result showed that the number of the control quails and the time the quails spent on passive behaviour were significantly higher (P < 0.05) compared to locomotory behaviour and with the corresponding results obtained in mel-treated and sedentary quails. The behaviours of sedentary and mel-treated quails were not different (p < 0.05) from each other.

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4. Discussions

The meteorological variables of the AT and RH recorded before, during and after transportation were outside the established thermoneutral zones of 12-24^{0}C for AT and 64% for RH, respectively for quails (Woodard and Mather, 1964; Rosa *et al*., 2011). The result of meteorological variables suggests that the conditions were unfavourable to the quails, especially during the afternoon hours of the day.

The mean RT value of 40.4 ± 0.2^{0}C recorded before transportation fell within the normal RT range value of 40-41.5^{0}C established for quails (Woodard and Mather, 1964). However, the high RT values above the normal range values recorded in the quails during the afternoon and evening periods were associated with high AT and RH occurring during the day time. Such high AT and RH have been reported to induce heat stress in livestock, including poultry, and the response of the animal to such stress is first by increase in RT as observed in the present study (Sahin *et al.,* 2003, 2004; Gonzalez *et al*., 2007).

The increased in RT values above the normal recommended values recorded in the control quails compared to mel-treated as the journey progressed indicated that the transportation was stressful and has induced hyperthermia in the quails.

Although quails are known to be hardy birds, manual handling, crating and transportation have been identified as potential sources for stress in poultry (Sahin *et al.,* 2003, 2004; Minka and Ayo, 2008, 2010a, 2011). Factors that were responsible for the increase in RT value during the transportation may be the introduction to a novel environment, vehicle noise and vibration, motion, and incremental environmental variables, especially high AT and RH.

The non significant increase in RT values of sedentary quails demonstrated that the few hours of food and water deprivation did not induced any adverse effect on the RT of the quails. Similar observations were made in quails deprived of feed and water for several hours (Underwood *et al*., 1999). The result obtained in sedentary quails confirmed that the increase in RT values recorded in the control quails was mainly due to transportation procedures.

The RT recorded during the journey in mel-treated quails were within the upper critical limit values of 41.5^{0}C established for quails (Woodard and Mather, 1964; Sahin *et al.,* 2004) and lower than the corresponding values obtained in the control birds. The result demonstrated that the administration of melatonin has offered protection against the adverse effects of heat and transportation stress on the RT of the quails. Similar hypothermic effect of melatonin has been reported in human subject Gilbert *et al*. (1999). The result obtained in mel-treated quails showed that the administration of melatonin had reduced the risk of the adverse effects of road transportation and thermal stress on the RT of quails.
The haematological variables recorded in the quails before transportation fell within the recommended normal values reported for male quails (Hassan *et al*., 2003). The result showed that the quails that served as subject of the present study were apparently healthy and ethically fit for the experiment. The significant (P<0.01) decrease in lymphocyte, eosinophil and basophil counts and an increase in heterophil values recorded in control quails immediately after transportation and three days post-transportation showed that the transportation procedures were stressful and has adversely affected the immune system of the birds up to the third day of post-transportation period. The increase in the number of heterophils and a decrease of the lymphocytes resulted in to an increase in H/L ratio in the control quails. Increase in H/L ratio has been reported to reflect the effects of elevated corticosteroids in the circulation induced by stress (Kannan *et al*., 2002). The H/L ratio has been accepted as the most sensitive and reliable index for determining the effect of various stressors in poultry and other livestock (Gross and Siegel, 1983; Minka and Ayo, 2008; Minka and Ayo, 2011). The increase in H/L ratio obtained in the present study agree with the findings of several studies which demonstrated that stress conditions, especially thermal stress decrease both humoral and cellular immune responses (Kannan *et al*., 2002; Minka and Ayo,

2008, 2010b), which resulted to an increase in H/L ratio. Furthermore, a decrease in eosinophil count has been reported to be as a result of physical and emotional stress (Nwe *et al*., 1996; Hassan *et al*., 2003). This indicated that the control quails suffered more from physical and emotional stress induced by road transportation procedures than the mel-treated.

The lower values of H/L ratio recorded in mel-treated quails post-transportation suggested, for the first time, that melatonin reduced the adverse effect of road transportation stress on the immune system of quails. The mechanism of action of melatonin against lymphopenia and neutrophilia may be directly or indirectly through the scavenging of free radicals and other reactive species commonly generated during stress. Melatonin has been reported to be an excellent antioxidants, involved in the prevention and restriction of free radical chain formation and propagation; and consequently, protecting the blood cells from oxidative damage (Reiter *et al*., 1997, 2004). Melatonin has also been shown to be involved in many physiological functions, such as immune response, energy metabolism regulation, production of glutathione and catalase (Karbownik and Reiter, 2001; Sahin *et al*., 2004; Reiter *et al*., 2007). The fact that increased in H/L ratio has been reported to reflect the effects of elevated corticosteroids in the circulation induced by stress (Kannan *et al*., 2002) suggested that melatonin has inhibited the release of cortisol, the fear hormone. Cortisol has been implicated in the destruction of immune cell, especially lymphocytes. Thus, the population of lymphocytes in the control quails was reduced drastically. Furthermore, the reason that melatonin has been reported to inhibit the activity of nitric oxide syntheses, which catalyzes the promotion of nitric oxide (NO), a precursor of cortisol syntheses (Karbownik and Reiter, 2001; Tan *et al*., 2003, 2007) suggested that it administration to quails may reduce the psychological and fear responses of the birds to novelty of transportation procedures. These properties of melatonin is of particular important to the quails because inspite of many years domestication of quails bird's it does not appear to have substantially altered its wild behaviour (RSPCA, 2011). Such wild behaviours have been reported to aggravate stress and increase incidence of injuries and mortality in transported animals (Grandin, 1997; Minka and Ayo, 2010b, 2011). The result of H/L ratio obtained in sedentary quails showed that the heterophil and lymphocyte counts were not affected by the few hours of feed and water deprivation during the experimental period.

The values of the behavoural activities recorded in the quails before transportation were similar to those obtained in domesticated Japanese quails by Ottinger *et al*. (1982), Buchwalder and Wechsler (1997) and Schmid and Wechsler (1997) who showed that quails spent more time on locomotor behaviour than passive behaviour. However, after transportation, a subjective assessment of the quails' behaviours showed that majority of the control quails spent more time on passive behviour (standing, lying by side and sitting) than locomotory behaviour, and the frequency of vocalization was low during the 3 hours of post-transportation period. The present result was different from the results recorded in sedentary and mel-treated quails where the majority of the quails were found to spend more time on locomotory behaviour than passive behaviour during the 3 hours of post-transportation period. This demonstrated that the control quails were excessively stressed and fatigued immediately after the transportation and little energy was left available for locomotory activities. Similar effect of post transportation stress on neuromuscular system has been reported in animals and pullets (Ayo *et al*., 2006; Minka and Ayo, 2008, 2011).

Although several factors have been attributed to vocalization, the reason that less frequency of vocalization was observed in the control quails during the 3 hours of post- transportation period suggested that transportation stress has affected the ability of the quails to vocalize. This may probably be due to fatigue, suppression of steroid hormones like testosterone and neurons like nucleus intercollicularis known to be responsible for vocalization in quails (Panzica *et al*., 1991). The restoration of the behvioural activities a day after transportation in the control quails showed that the effect of transportation stress on the behaviour of the quails was transient.

The non significant difference from pre-transportation values in passive, locomotory and vocalization behaviours observed in mel-treated quails demonstrated that melatonin has reduced the incidence of fatigue and enhanced locomotory activities, probably by it analgesic and anti inflammatory effects (Espositor *et al*., 2010), apart from its role as a powerful antioxidant and inhibitor of stress hormone (Tan *et al*., 2003; Reiter *et al*., 2004; Tan *et al*., 2007).

The result, for the first time, showed that administration of melatonin to quails prior to transportation has enhanced cellular immunity, homeostasis and behavioural imbalance induced by road transportation stress. Thus, it administration has improve the welfare and health status of quails subjected to road transportation.

In conclusion, the result showed that 2 h of road transportation of broiler quails was stressful and resulted in hyperthermia, lymphopenia, neutrophilia and passive behaviours, which were ameliorated by the administration of melatonin.

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