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Full Length Research

Rectal temperature and behaviour of layer hens administered with vitamins C and E and transported during the hot-dry season

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ABSTRACT: A 6 hours experiment involving 90 apparently healthy Shika Brown layer hens of twenty-two weeks age and an average live weight of 1.1 ± 0.2 kg was carried out in a completely randomized design to evaluate the effects of vitamins C and E on rectal temperature (RT) and behaviour of the birds transported by road during the hot-dry season. Two experimental groups consisting of thirty layers each were separately administered orally with vitamins C and E just before transportation, while another 30 layers given sterile water only served as control. At journey time the RT was higher ($p<0.05$) in control compared with treatment groups. After the journey, the maximum RT values of 42.3 ± 0.2°C and 41.9 ± 0.1°C obtained in control and vitamin C groups were significantly ($p<0.001$) and ($p<0.05$) higher than their pre-journey values. There was a non-significant ($p>0.05$) difference in the vitamin E group. When pre- and post-transportation values were compared, the behavioural activities of tonic immobility (TI), regrouping and awareness tests were non-significant ($p>0.05$) in treatment groups, but control showed significant ($p<0.01$) difference for TI, and ($p<0.05$) for regrouping and awareness tests, respectively. In conclusion, vitamins C and E ameliorated the negative psycho-physiological effect of heat stress in transported birds.

Keywords: Heat and transport stress, rectal temperature, behaviour, layer hens, vitamins C and E.

INTRODUCTION

The need to transport birdsstarting from day-old from the hatchery to breeding room, breeding stage and/or grower to laying/fattening (broilers) houses, and finally to the market or slaughterhouse cannot be over-emphasized (FAO, 2013). However, poultry transportation itself is a traumatic exercise as defined by Elrom (2000a), but transporting birds under heat conditions constitute a health risk that elicit undue psycho-physiological response thereby leading to increased morbidity and mortality, poor meat quality and decreased production efficiency (Mitchell and Kettlewell, 2009; Akbarianet al., 2016). Several researchers including ethologists have reported behavioural reactions as the first noticeable response to stress (Jones et al., 1996). The first man to bird interaction during the process of transportation is catching; this activity in itself elicits fear in birds (HSUS, 2014; Bulitta, 2015). Fear responses are seen as escape or panic reactions, which are inappropriate in intensive rearing systems and can cause injury, pain or even death (Jones, 1997). Although fear and stress are not synonymous, fear encompasses adrenergic, dopaminergic, and cholinergic systems, which play a pivotal role in the physiological stress response in poultry as reported previously by Jones (1986). Mitchell and Kettlewell (1998), Hartung (2003) and Bulitta (2015) described factors such as loading and unloading, distance and duration of transport, vibration in the vehicle, sudden climatic changes; increased thermal core within the vehicle, stereotype, nature of road, and speed of the vehicle as major stressors associated with transportation. Tonic immobility (TI) (Zulkifli et al., 2009), regrouping and awareness tests (Sherwin et al., 1993), were reported earlier as good indicators of fear and fatigue in transported birds. Similarly, Sinkalu and Ayo (2008) described the fluctuations in rectal temperature (RT) of birds as a good indicator of heat stress in pullets. The use of vitamins C and E as antioxidants in combating heat stress has been documented in Japanese quails by Ciftci et al. (2005), in pullets by Sinkalu and Ayo (2008) and in broilers by Ismail et al. (2013). Similarly, the beneficial effect of vitamin C supplementation in transported Japanese quails and broiler chickens, respectively have been documented by Jones et al. (1999) and Zulkifli et al. (2001). Furthermore, documents abound on the immunomodulatory effects of the antioxidant systems in poultry biology be it performance or reproduction (Surai, 2016; Haq et al., 2018). However, there is a paucity of information on the physiology of transportation on the hybrid Shika Brown layer (SBL) that is indigenous to the Northern Guinea Savannah zone of Nigeria.

Therefore, the aims of this study were to establish the effects of dietary supplementation of vitamins C and E in this breed of birds transported at high ambient temperatures and to determine their possible ameliorative effects on RT and behavioural activities.

MATERIALS AND METHODS

Experimental site and meteorological data

The study was conducted at the College of Agriculture and Animal Science, Ahmadu Bello University, Mando, Kaduna (11º 10´N, 07º 38´E), in the Northern Guinea Savannah zone of Nigeria. Data of precipitation and wind direction were collated from the Meteorological Unit of the Institute for Agricultural Research, Ahmadu Bello University, Samaru, Zaria, Nigeria. The ambient temperature (AT) and relative humidity (RH) inside and outside the pen were recorded daily and for three days before and after the journey period at 07:00, 14:00 and 18:00 h, respectively. A standard ambient thermometer for AT (Cocet, China and up to 50°C calibration) and wet and dry-bulb hygrometer for RH (Zeal England, Mason's type and up to 320 mm long) were used. The wet and dry bulb values were recorded, and RH calculated using the depression factor as indicated in the manufacturer's manual. Both instruments were obtained from a commercial medical equipment sales outlet in Kaduna.

Management of birds and experimental design

Ninety apparently healthy (22 weeks of age; Shika Brown) layer hens and weighing 1.1 ± 0.2 Kg were randomly divided into three replicate groups (cells) within each of three experimental groups (pens). For the purpose of homogeneity, each pen containing a particular treatment group was partitioned into three equal cells by 5 m plywood, so that each replicate cell contained 10 birds at a stocking rate of 0.373 m^2/bird as defined by Koelkebeck and Cain (1984). All birds were fed a basal layer diet (metabolizable energy (ME), 11.5 MJ/Kg; crude protein (CP), 16.5 g; calcium (Ca), 3.52 g; available phosphorus (P_A)0.25 g) and water was provided for *ad libitum*). In addition, all necessary preventive medications, vaccinations, and other related management practices were strictly adhered to as recommended by the National Veterinary Institute (NVRI), Vom, Plateau State, Nigeria.

One week to the commencement of the experiment, deep-body temperatures of each bird was recorded daily using a clinical digital thermometer (accuracy ± 0.1°C) inserted 5 cm by the wall of the rectum to ascertain their health status. Daily, and for three days before and after the journey, the RT of 10 birds per group was measured at 07:00, 14:00 and 18:00 h, as described previously. The birds were also accustomed to the experimental procedures. On the experimental day and just before loading into the vehicle, the first group were administered orally with vitamin C at a dosage of 200 mg/kg body weight dissolved in 5 ml of sterile water w/v, while the second group were administered orally with vitamin E at a dosage of 200 mg/kg body weight dissolved in 5 ml edible vegetable oil (Chew Hong Edible Oil, Malaysia) w/v and the last group were administered 5 ml sterile water only (control) v/v. Both vitamins were obtained from (VMD, Arendonk, Belgium). The RT of equal numbers of birds per group were recorded inside the vehicle at 1, 2, 3, 4, 5, and finally at 6 h during the journey period. Subsequently, the birds were transported on a tarred road covering a total distance of about 280 km at anaverage speed of 45 km/h. Food was withdrawn from the birds 8 hours before the journey, while water was withdrawn two hours before the journey and throughout the journey period as suggested by Metheringham and Hubrecht (1996). The duration of the journey was 6 h, including stop-over times for measuring the parameters. On completion of the journey, the layers were unloaded at the same spot where they were pre-loaded into the vehicle just before the start of the journey. Food and water were given as done before the commencement of the journey.

The arrangement of birds, vehicle design and journey time

The birds were easily caught by experts and feather tagged in groups by means of adhesive plaster. Thereafter, all groups were uniformly inter-mixed and loaded into nine standard crates (0.8 x 0.6 x 0.3 m) at a stocking density of 0.0350 m^2/bird as defined by Delezie et al. (2007). In order to maintain the homogeneous distribution of environment and "thermal core" load within the vehicle, the guidelines laid out by CATGP (2017) were used and modified by the authors. The crates used for transporting the birds were arranged in groups of three from the front to the rear part of the vehicle. The vehicle used for the journey was a customary 18-seater Toyota Hiace bus that had glass louvres for regulating air movement inside the bus. The top roof was made up of a metal iron from outside and a stock-pile of heat-absorbable foam materials, covered with thick polythene strap from inside. The floor of the vehicle was constructed with metal, and it was covered with 5 cm wood shavings. Meteorological data inside and outside the vehicle were recorded during the journey period using a standard ambient thermometer for AT and wet and dry-bulb hygrometer for RH. All measurements were recorded inside and outside the vehicle at 1, 2, 3, 4, 5, and finally at 6 h during the journey period.

Behavioural activities

Behavioural activities measurements were carried out post-transportation and immediately after unloading.

Tonic immobility (TI) test

A total of 30 birds (10 birds/group) were tested for TI. Each individual was gently caught with both hands, held in an inverted manner, and carried to a separate pen (no visual contact with other birds) for TI measurements. A modification of the procedure described by Benoff and Siegel (1976) was used. Tonic immobility was induced as soon as the bird arrived in the separate room by gently restraining it on its right side and wings for 15 s. The experimenter then retreated approximately 1 m and remained within the sight of the bird but made no unnecessary noise or movement. Direct eye contact between the observer and the chicken was avoided because it may prolong TI duration (Jones, 1986). A stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, the restraining procedure was repeated. If TI was not induced after 3 attempts, the duration of TI was considered 0 s. The maximum duration of TI allowed was 600 s. After the measuring of TI, the bird was returned to its home flock. It was assumed that the catching and returning of birds did not disturb the other members of the flock (Lagadic et al., 1990). The birds were ranked from low (1 to 300 s) to high (>300 s) according to the duration of TI previously described by Beuving et al. (1989), and modified by the experimenter. Those birds showing the shortest TI duration and those scoring the longest durations were classified as not so fearful and fatigued (NFF) or highly fearful and fatigued (HFF), respectively.

Regrouping test

The test was carried out as described by Sherwin et al. (1993). Briefly, birds were removed individually from the transported crates and placed at one end of a 2 m runway constructed with wooden sides, 0.8 m high and 9.4 m apart, on a concrete floor covered with wood shavings. At the other end of the runway, four non-experimental birds were confined with wire mesh so as to be easily visible to the experimental bird. The time taken for the experimental bird to move half-way (1 m) and fully (2 m) along the runway towards the confined birds were recorded. Birds which had not moved either 1 m or 2 m down the runway after 300 s were removed, and their time recorded as 300 s. Latency that it took the birds to leave the starting box (maximum of 180 s) was also recorded. The birds were ranked from fast (<100 s to move across 2 m) to slow (>100 s to move across 2 m). Those birds showing fast movement across 2 m and those showing the slow movement across 2 m were classified as not so fearful and fatigued (NFF) or highly fearful and fatigued (HFF), respectively.

Awareness test

A possible consequence of fatigue caused by transport is reduced awareness of the environment. To test this, the method described by Sherwin et al. (1993) was adopted. Briefly, one sub-group of birds (n=10) in each group was conditioned to become more aware of their surroundings upon hearing a signal, and their reactions upon hearing this signal before and after transport were observed and recorded. Each bird was placed individually on a plastic crate in the centre of an empty room and a variable period (10 to 60 s) was allowed for the bird to settle. The bird was then exposed to a taped auditory conditioned stimulus (15 s duration piano scale of 2 octaves), followed by a mildly aversive unconditioned stimulus (a fine water splash applied from a distance of 3 m). A further variable settling period was allowed to elapse and the procedure repeated. Each bird received two of these training trials on four of the seven days prior to the experiment. At the experimental time, this test was conducted daily for three days before and after transportation as the birds were placed individually on a crate and exposed to the conditioned stimulus. The behaviour of the birds during this 15 s period was recorded from a hidden viewpoint at a distance of 3 m from the birds. The following indicators of awareness were considered: headshaking, feeding, drinking, standing but not feeding, walking, lying, panting, crouching, croaking. The latency to first headshake was taken as a measure of

Table 1. Meteorological data at the experimental site three days before transportation of the (SBL) hens.

Hour	Ambient Temperature (ºC)		Dry-bulb (ºC, Mean ± SEM)		Relative humidity (%, Mean ± SEM)		Rainfall (mm)	Wind Direction
	Maximum	Minimum	Outside Pen	Inside Pen	Outside Pen	Inside Pen		
07:00	26.0	25.0	25.3 ± 1.7	26.5 ± 1.7	97.3 ± 3.3	99.0 ± 3.3	0.0	South - West
14:00	32.0	29.0	30.8 ± 1.9	30.7 ± 1.9	77.0 ± 3.0	81.7 ± 3.0	0.0	South - West
18:00	30.0	27.5	28.8 ± 1.8	31.2 ± 1.9	82.7 ± 3.1	92.0 ± 3.2	0.0	South - West
Mean (SEM)	29.3 ± 1.8	27.2 ± 1.2^{NS}	28.3 ± 1.6	29.4 ± 1.5 ^{NS}	85.7 ± 6.1	91.2 ± 5.3 ^{NS}	0.0 ± 0.0	-

SBL = Shika Brown layer, SEM = Standard error of the mean, NS= Non-significant (p>0.05) difference.

Table 2. Meteorological data of the experimental site for three days post–transportation of the (SBL) hens.

Hour	Ambient Temperature (º C)		Dry-bulb (ºC, Mean ± SEM)		Relative humidity (%, Mean ± SEM)		Rainfall (mm)	Wind Direction
	Maximum	Minimum	Outside Pen	Inside Pen	Outside Pen	Inside Pen		
07:00	26.0	25.0	25.7±0.2	25.5±0.3	94.7±2.7	98.7±1.3	0.0	South - West
14:00	33.0	30.0	31.5±0.8	30.7±0.4	85.7±4.1	91.7±3.8	0.0	South - West
18:00	30.0	28.5	29.7±0.3	29.5±0.5	80.0±5.0	83.7±4.5	0.0	South - West
Mean (SEM)	29.7±2.0	27.8±1.5^{NS}	29.0±1.7	28.6±1.6 ^{NS}	86.8±4.3	91.4±4.3 ^{NS}	0.0±0.0	-

SBL = Shika Brown layer, SEM = Standard error of the mean, ^{NS} = Non-significant (p>0.05) difference.

awareness to the conditioned stimulus as defined previously by Sherwin et al. (1993) and Elrom (2000b). The birds were ranked from fast (<10 s to headshake) to slow (>10 s to headshake). Those birds showing fast headshaking actions and those scoring the slow headshaking were classified as highly aware, and not so fearful and fatigued (HAFF) or mildly aware, and highly fearful and fatigued (MAFF), respectively.

Statistical analysis

StatSoft, Inc., Tulsa, OK.: STATISTICA version 8 package was used and all data were subjected to student's paired t-test. Data were expressed as the mean ± standard error of the mean (mean±SEM). Values of (P<0.05) were considered significant.

RESULTS

The meteorological data before, during and after the journey throughout the study period are shown in (Tables 1, 2 and 3). The AT, dry-bulb temperature (DBT) and RH before and after the journey did not show any significant (p>0.05) difference. However, the maximum and minimum AT showed a consistent pattern of increase from 07:00 h, peaked at 14:00 h, and then decreased towards 18:00 h before and after the journey.

The minimum AT recorded during the study period was 25.0ºC while the maximum for the same period was 33.0ºC. Similarly, the DBT inside and outside the pen throughout the experimental period showed a parabolic increase from 07:00 h to 18:00 h with a peak value at 14:00 h. The pre-transportation values of DBT inside the pen were consistently higher than the values recorded for outside, while reciprocal values were observed post-transportation. The overall mean DBT pre- and post-transportation followed the same pattern.

The RH before and after the journey was higher inside the poultry house than outside and consistently decreased from 07:00 to 18:00 h. The overall pre-transportation percentages RH inside and outside the poultry house were 91.2±5.3 and 85.7±6.1, while the values recorded post-

Table 3. Ambient temperature and relative humidity inside the vehicle during transportation of the SBL hens.

Hour of Journey	Dry-bulb temperature (ºC)	Relative humidity (%)
1	32.0	67.0
2	32.0	67.0
3	34.0	62.0
4	36.0	52.0
5	36.0	48.0
6	32.0	73.0
Mean ± SEM	33.7 ± 0.8	61.5 ± 3.9

SBL = Shika Brown layer; SEM = Standard error of the mean.

Table 4. Maximum rectal temperature (ºC) values of the SBL hens three days before and three days after road transportation (Mean ± SEM; n = 30).

Hour	Before Transportation			After Transportation		
	++Vitamin C	++Vitamin E	++Control	++Vitamin C	++Vitamin E	++Control
07:00	41.7 ± 0.2	41.7 ± 0.2	41.7 ± 0.2	42.0 ± 0.5	41.7 ± 0.1	42.2 ± 0.6
14:00	41.6 ± 0.2	41.6 ± 0.2	41.7 ± 0.1	42.9 ± 0.2	41.9 ± 0.4	42.7 ± 0.9
18:00	41.4 ± 0.4	41.4 ± 0.1	41.4 ± 0.2	41.6 ± 0.1	41.7 ± 0.2	41.9 ± 0.0
Mean ± SEM	41.6 ± 0.1	41.6 ± 0.1	41.6 ± 0.1	41.9 ± 0.1*	41.8 ± 0.1^{NS}	42.3 ± 0.2***

SBL = Shika Brown layer; SEM = Standard error of the mean; ^{NS} = Non-significant (p>0.05) difference; * = p<0.05; *** = p<0.001; ++ n = 10 across row before and after transportation.

Table 5. Fluctuations in mean rectal temperature (ºC) values of the SBL hens three days before and three days after transportation (Mean ± SEM; n = 30).

Hour	Before Transportation			After Transportation		
	++Vitamin C	++Vitamin E	++Control	++Vitamin C	++Vitamin E	++Control
07:00	41.0 ± 0.2	41.0 ± 0.2	41.0 ± 0.2	41.2 ± 0.1	41.3 ± 0.0	41.3 ± 0.4
14:00	41.0 ± 0.2	41.0 ± 0.2	41.0 ± 0.1	41.6 ± 0.3	41.5 ± 0.4	42.0 ± 0.7
18:00	40.8 ± 0.3	40.7 ± 0.2	40.8 ± 0.2	41.2 ± 0.2	41.3 ± 0.2	41.3 ± 0.3
Mean ± SEM	40.9 ± 0.1	40.9 ± 0.1	40.9 ± 0.1	41.3 ± 0.1*	41.4 ± 0.1**	41.5 ± 0.2***

SBL = Shika Brown Layer; SEM = Standard error of the mean; * = p<0.05; ** = p<0.01; *** = p<0.001; ++ n = 10 across row before and after transportation.

transportation showed 91.4±4.3 and 86.8±4.3, respectively. During the journey period, the DBT rose from 32.0ºC at the first hour and peaked at 36.0ºC corresponding to the fourth and fifth hour of the journey and 14:00 to 15:00 h of the day. A clear demonstration; that these were the hottest period of the day in this region of the world. However, the RH showed a rise, fall and another increase at the first, fifth and sixth hours of the journey, respectively. The lowest RH value of 48% was recorded at 15:00 h corresponding to the fifth hour and the hottest period of the journey with a DBT of 36.0ºC. During this period, the birds were observed to be panting heavily. There was no rainfall during the study period, and wind direction was predominantly south-west.

The overall mean maximum RT values (Table 4) before the journey was not significant (p>0.05) within groups. However, the post-transportation values of 42.3±0.2°C in control and 41.9±0.1°C were significantly (p<0.001) and (p<0.05) higher compared to their pre-journey values, respectively. There was no significant (p>0.05) difference in the RT values of vitamin E group before and after the journey. The hourly fluctuations in RT during the journey period (Table 5) in control was significantly (p<0.05) higher compared to the treatment groups.

All behavioural indices (Table 6) after the journey showed fearfulness and fatigue in control birds with significant (p<0.01) difference for TI, and significant (p<0.05) differences for regrouping and awareness tests

Table 6. Behavioural indices of the SBL hens before and after transportation (n = 30) during the study period.

Test	Before Transportation			After Transportation		
	++Vitamin C	++Vitamin E	++Control	++Vitamin C	++Vitamin E	++Control
Tonic immobility (n = 10)						
Number of inductions	1.8 ± 0.3	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
Duration(s)	220.4 ± 16.1	225.8 ± 21.8	234.0 ± 14.7	270.3 ± 17.1^{NS}	250.1 ± 14.0^{NS}	410.9 ± 26.6**
Regrouping (n = 10)						
Latency to leave box (s)	48.8 ± 1.4	48.4 ± 0.9	46.8 ± 1.6	52.2 ± 2.9^{NS}	53.4 ± 3.2^{NS}	46.4 ± 3.9^{NS}
Time to move 1 m (s)	47.0 ± 1.9	50.6 ± 1.1	47.2 ± 1.7	51.4 ± 1.3^{NS}	51.6 ± 3.3^{NS}	42.8 ± 1.9^{NS}
Time to move 2 m (s)	93.2 ± 4.2	92.2 ± 2.8	94.6 ± 1.7	89.4 ± 3.2^{NS}	98.2 ± 3.6^{NS}	107.6 ± 2.3*
Awareness (n = 10)						
Latency to headshake (s)	8.6 ± 0.5	8.2 ± 0.6	8.4 ± 0.5	8.0 ± 0.6^{NS}	8.4 ± 0.5^{NS}	11.6 ± 0.9*

SBL = Shika Brown layer; (s) = Seconds; ^{NS} = Non-significant (P>0.05) difference; * = P<0.05; ** = P<0.01; ++ n = 10 across row before and after transportation.

compared to their pre-journey values, respectively. There were no significant (p>0.05) differences in these indices in all the treatment groups.

DISCUSSION

The results obtained in the present study showed that the transported hens were subjected to the influence of high ambient temperature and high relative humidity, characteristic of the early rainy and late hot-dry seasons in the Northern Guinea Savannah zone of Nigeria. The 27.2±1.2 to 29.3±1.8 and 27.8±1.5 to 29.7± 2.0ºC pre- and post-transportation AT values recorded were predominantly outside the established thermoneutral zone of 12 to 24ºC for birds reared in the temperate region reported by Selyansky (1975) and 18 to 24ºC for the tropical region by Holik (2009).

Similarly, the overall DBT value of 33.7±0.8ºC recorded during the journey time was very stressful for the birds. The combination of high AT, RH and micro-environment “thermal core” load during the journey predisposes the birds to additional heat generation leading to a shift in the mechanism of heat dissipation from sensible to latent form as evidenced by their heavy panting.

Similar findings were reported by Ayo et al. (2006) which confirmed that road transportation induces considerable stress in domestic animals (goats), which could be ameliorated with the administration of vitamin C. In a separate report, Minka and Ayo (2008) observed severe panting after a 6 h transportation period in birds treated with 60 mg/kg body weight of vitamin C, compared to the control group. Furthermore, Minka and Ayo (2011) reported that the deleterious effects of lymphopenia, heterophilia, liveweight loss and mortality induced as a result of handling, loading, and transportation of pullets for 8 h during the hot-dry conditions were alleviated by the oral administration of vitamins C and E.

In a separate experiment, Mitchell and Kettlewell (1998) confirmed that any impairment of airflow through the vehicle ventilation system will result in the accumulation of heat and moisture that in combination will impose heat stress upon the birds. The consequent stimulation of thermal panting will increase evaporative water loss from the animals, adding to the moisture load, further precipitating heat stress and creating a vicious spiralling cycle of hyperthermia. The detrimental effects of such heat loads will be exacerbated by the constraints imposed upon the birds’ behavioural thermoregulatory capacity by the high stocking density within the crates or containers (Grandin, 2014).

The deleterious effects of AT and RH were more pronounced in the control layer chickens. This was evidenced by the consistent fluctuations in RTs obtained as the hour of the day increased. The RT rose from 07:00 h, and peaked at 14:00 h. The decrease recorded at 18:00 h is proportionally related with decreasing AT and showed the attempts of the birds to maintain homeothermy.

The increase in rectal temperature due to heat stress is in agreement with studies of Warriss et al. (1999) and Altan et al. (2000). Similar findings were recorded by Menon et al. (2014), which reported changes in the indices of stress and metabolic homeostasis as physiological response of emus transported by road for 6 h. After the journey, most birds in control groups were observed to be weak and only made strong efforts at responding to an external stimulus or they simply croak (sign of fear) at the human approach, while birds in treatment groups especially vitamin C treated group was observed to be highly alert. Such observations were made by Zulkifli et al. (2009), who reported that high fear (HF) birds were more distressed than their low fear (LF) counterparts after 3 h of heat exposure and crating, suggesting a possible link between thermoregulation and underlying fearfulness.

In a previous study, Campo and Carnicer (1994) showed that heat stress prolonged TI duration in chickens. Hence, it appears that response to thermal stressors is associated with fear-related behaviour. The report in this study on the favourable response of treatment groups to TI index is in agreement with the observation made by Jones et al. (1999), who reported that dietary treatment with vitamin C failed to affect corticosterone concentrations, but did attenuate the TI fear reactions of stressed quails. Similarly, Egbuniwe et al. (2016), reported that TI duration was greatly reduced in broiler chickens treated with ascorbic acid during the hot-dry season. Pre-treatment with drinking water supplemented with vitamin C also reduced the fearfulness of quail. The exact mechanism by which vitamins C and E ameliorate stressful condition is yet to be fully elucidated. Although, Tauler et al. (2003) and Ismail et al. (2013) have pointed to their antioxidants properties as reactive oxygen species (ROS) scavengers per excellence, while Kondratyev (1998) suggested the contributory factors of their other concurrent functions such as; regulation of blood circulation, permeability of blood vessels and their contribution in increasing the phagocytic activities of leukocytes, activation of many vital body enzymes, promotion of immunological processes and general body detoxification.

Conclusion and recommendation

In conclusion, the hens in treatment groups showed greater positive responsiveness to both transport and heat challenge than those of control, particularly as reflected in their responses to all the three behavioural tests which served as stress indicators that they were subjected to. It is therefore recommended that studies should be carried out to investigate the actual mechanism(s) through which these two antioxidants achieve their protective roles against the adverse effects of transportation and high ambient temperatures.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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