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Variation in hematological, biochemical parameters and physiological adaptation of cockerel strains to transportation density in a hot tropical environment

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ABSTRACT: One hundred and eight 8 weeks old Harco (black) and Nera (white) cockerel strains were randomly allotted in 2 x 3 factorial design experiment to investigate the response of cockerel strains transported at different densities in hot humid ecological zone of Nigeria. Birds were transported in two ventilated wooden cages with a motorbike on a rough road at 30 km/hr for 2 hours at three densities [Low density (LD) = 0.86 m^2, Medium density (MD) = 0.64 m^2, and High density (HD) = 0.48 m^2] and each replicated three times. Blood samples were collected pre and post transportation. Physiological indices {respiratory rate (RR), panting rate (PR), skin temperature (ST) and rectal temperature (RT)} were measured post-transportation. There was ($p<0.05$) difference in strain x density interaction on blood parameters except in red blood cell (RBC) and packed cell volume (PCV). The RBC, Heamoglobin (Hb), and PCV values reduced slightly with transportation density for both strains {LD > MD > HD}. Total white blood cell (WBC), alanine amino transferase (ALT), aspartarte amino transferase (AST), total protein (TP), and glucose had ($p<0.05$) strain x density interaction. Variance analysis revealed ($p<0.05$) effect of strain, density, and strain-density interaction on physiological indices post transportation. Values increased with density in this order {HD > MD > LD}. Harco strain had higher values as compared to white strain birds. In conclusion, this study showed that transportation and density affected both strains of birds. White strain birds are relatively resistance to transportation stress as compared to Harco strain.

Keywords: Biochemical, cockerel strains, haematological, physiological, stress.

INTRODUCTION

In livestock production, stresses are usually referred to as reflex response that occurs inevitably when animals are exposed to adverse environmental conditions. It has been described as the cause of many physiological dysfunction of body system, ranging from discomfort traumatic injuries to death (Minka and Ayo, 2007; Oguntunji et al., 2019). Stress increases animal susceptibility to severe infections. The indicators of stress embrace numerous deleterious factors, including pathogenic factor, toxic and biologically active substances and physical factors.

The common sources of stress include the following; climatic stressors (extreme heat and cold, high humidity), environmental stressors (bright light, wet litter, poor ventilation), nutritional stressors (shortages of nutrients, feed intake), physiological stressors (rapid growth, sexual maturity), physical stressors (catching, immobilization, infection, transportation, social stress, overcrowding, poor body weight uniformity), pathological stressors; exposure to infectious agents and immunological stress (Minka and Ayo 2007).

All animals reared directly or indirectly for meat are transported at some point in their lives, which makes transportation an inevitable husbandry practice, which livestock are subjected to (Adenkola and Ayo, 2009; Gupta et al., 2013). However, transportation of food animals, including poultry, is associated with stresses which include

rough handling, loading and unloading, introduction to a new environment, fear, vehicle motion, vibration and postural instability during the journey, long journey hours, deprivation of food and water, extreme climatic conditions, especially during the hot-dry and the harmattan seasons (Ayo and Oladele, 1996). These factors acting concurrently on transported animals impair the normal body functions, leading to increased morbidity and mortality, poor meat quality and decreased productivity (Broom, 2000; Minka and Ayo 2008).

Transportation affects the susceptibility of animals to infection. This is because exposure to stressors during transportation is associated with a reduction in the function of the immune system of animals. This effect is largely mediated through the hypothalamo-adreno-cortical system, the secretion of corticosteroid hormones, suppressing the immune response (Warriss et al., 1995). Sutherland et al. (2009) reported that transportation stress resulted in physiological responses that induced changes in serum metabolites, dehydration, loss of body weight, body/skin temperature, respiratory rate and rectal temperature.

It has been shown that changes in haematological values and serum metabolites are good indicator of stress response in animal (Broom, 2000). Odore et al. (2004) observed a rise in blood hormone levels, lymphocyte, glucocorticoid and adrenergic receptor, cortisol and catecholamine concentrations. The increased values, however, returned to normal at 24 hours and one week after arrival in long-term transportation of calves by road. Adenkola et al. (2009) demonstrated that road transportation induced leucocytosis, neutrophilia, lymphocytosis, eosinophilia in control pigs that were not supplemented with ascorbic acid while total protein, alkaline phosphatase, aspartate amino transferase and neutrophil lymphocyte ratio was found to decrease significantly in pigs administered with ascorbic acid as compared to control group after 4 hours of road transportation during harmattan season in Northern Guinea Savanna zone of Nigeria.

The physiological parameters of rectal temperature (RT), heart rate (HR) and respiratory rate (RR) are known to be the most relevant on-the-spot diagnostic parameters of the state of an animal's health, before any laboratory analysis is carried out, especially in remote rural areas in the tropics, where modern laboratory facilities may be lacking. These parameters are of importance in evaluating the adaptability of domestic animals to various environmental stress factors (Ayo et al., 1998), including transportation stress.

Cockerels are slow growing (male layer) birds usually raised for their meat, which female lines have been selected over many generations for high egg production while the males have been less selected. Fatufe and Matanmi (2008) reported that strains of these birds have different growth rate. However, little or no information is known about physiological response of these strains (Harco -black and Nera- white) to transportation stress with consideration on transportation density in hot humid agro-ecological zones.

Therefore, the present study was designed to investigate influence of transportation density and strain on physiological response of Harco (black) and Nera (white) cockerel birds in hot humid ecological zone, Nigeria.

MATERIALS AND METHODS

Management of experimental birds

One hundred and eight (108) apparently healthy cockerel birds of Harco black and Nera white strain aged eight weeks old, weighing 1.050±0.4 kg served as subjects of the study. The birds were raised in a standard poultry house and fed standard commercial feed from day old till eight weeks old. Water was given *ad libitum*. All necessary medications were administered to the birds. Food and water were withdrawn 12 hours before the journey on the experimental day. Each bird was gently handled in a relatively calm condition and assign to the experimental units for transportation based on density.

Experimental design and loading

Transporting cage was made from a wooden materials and wire mesh. It was partitioned into 3 compartments cells with a wooden plank (low density = 0.86 m^2, medium density = 0.64 m^2, and high density = 0.48m^2) floor space. Each compartment was replicated three times and 6 birds of the same strain were randomly allotted to each cell. Cages were mounted on two motorbikes and travelled at a regular speed of 30 km/h through a rough rural road for 2 hours. Each bird was easily caught in a relatively calm condition during loading and unloading after the journey. The average measured environmental condition during the journey was recorded, temperature (34^{0}C) and relative humidity (43.2 %).

Data collection

Haematological parameters

Blood sample was collected before loading and transportation (pre-transportation) and immediately after the journey (post-transportation). 2.5 ml of blood sample was collected from the wing vein of each bird. All blood samples were analysed for heamatology and serum biochemical according to Oladele et al. (2001) and Schalm et al. (1975). Samples meant for hematology were collected into bottles treated with Ethylene diethyl trichloro acetate (EDTA), an anticoagulant. Hematological parameters measured were haemoglobin concentration

(Hb), packed cell volume (PCV), red blood counts (RBC), and total white blood counts (WBC). Serum metabolites measured were total protein (TP), glucose, and albumin.

Physiological parameters

The physiological parameters were described below as adapted from Oguntunji et al. (2019). Respiratory rate (RR), panting rate (PR), Body temperature (BT) and rectal temperature (RT) were taken in two periods before loading and transportation (pre-transportation) and immediately after the end of the journey (post-transportation).

Respiratory rate (RR): This was determined by counting the number of movements of the abdominal region or vent per minute using stop watch.

Skin temperature (ST ºC): This was taken with an infra-red thermometer in the shaved area under the wing. Body temperature was taken after the sound of alarm of the thermometer.

Rectal temperature (RT ºC): This was taken with the aid of a digital thermometer. The disinfected sensory tip of the thermometer was inserted into the vent and reading was taken after the sound of the alarm of the thermometer.

Panting rate (PR): This was taken as the number of times a bird panted in a minute using a stop watch.

Statistical analysis

Data collected were analysed with the analysis of variance (ANOVA) using a general linear model with the following fixed effect models:

$Y_{ij} = \mu + S_i + D_j + (SD)_{ij} + e_{ij}$

Where: Y_{ij} = Individual observation, μ = Fixed overall mean, Si = Fixed effect of strains (i = 1……2). Dj = Fixed effect of density (j = 1……3), $(SD)_{ij}$ = Interaction effect of strain and density and e_{ij} = experimental error assumed to be independently, identically and normally distributed, with zero mean and constant variance i.e. nd (0, σ^2).

Differences between means were separated with the New Duncan Multiple Range test at 5% probability level. All statistical analyses were performed with the SAS (2002) version 16.

RESULTS

The values of haematological and serum biochemical parameters recorded pre and post transportation for experimental birds were ($p<0.05$) regardless of strains. Measured parameters pre-transportation and loading were lower ($p<0.05$) compared to post-transportation, except for Hb, lymphocytes and eosinophil that had higher values as shown in Figure 1.

Main effects of strain and density on measured blood and physiological parameters

The results of the main effects (strain and density) were presented in Table 1. There was an ($p<0.05$) effect of strain and density on measured blood parameters except for red blood cell (RBC) and packed cell volume (PCV) and the values reported for Nera (white) were lower compared to Harco (black) in all measured blood parameters except Hb. In addition, a unique trend was observed for response of birds to main effect of densities and strains, values obtained for Harco (black) strains were ($p<0.05$) higher than Nera (white). Density effect on measured blood parameters followed a trend (HD > MD > LD) except for haemoglobin (Hb) which followed a reverse order. The results of the main effects of strain and density on physiological parameters are presented in Figures 2 and 3. There was ($p<0.05$) effect of strain on panting rate (PR) and rectal temperature (RT) and the values reported for Nera white were lower compared to Harco black chicken in all the physiological parameters measured. Density had ($p<0.05$) effect on physiological parameters and values followed a trend HD > MD > LD for all parameters measured.

Strain-density interaction effect on measured blood and physiological parameters

As shown in Table 2, there was ($p<0.05$) strain-density interaction effect on measured blood parameters except for red blood cell (RBC), and packed cell volume (PCV). However, values of RBC, Heamoglobin (Hb), and PCV reduced slightly with transportation density in this order for both strains i. e {High density (HD) > Medium density (MD) > Low density (LD)}. The ($p<0.05$) strain x density interaction effect of white blood cell (WBC), alanine amino transferase (ALT), aspartarte amino transferase (AST), total protein (TP), and glucose indicated that regardless of the transportation density (low, medium and high), both strains of cockerel (Harco and Nera) are physiologically affected by the induced transportation stress.

Strain-density interactions on all physiological parameters measured post transportation were ($p<0.05$) (Table 3). The physiological values were ($p<0.05$) lower for LD in both strains of chicken. The density followed a trend for all physiological parameters measured (HD > MD > LD). The Harco black chicken appeared more susceptible to transportation stress during the hot humid weather; having highest values at all the transportation densities as

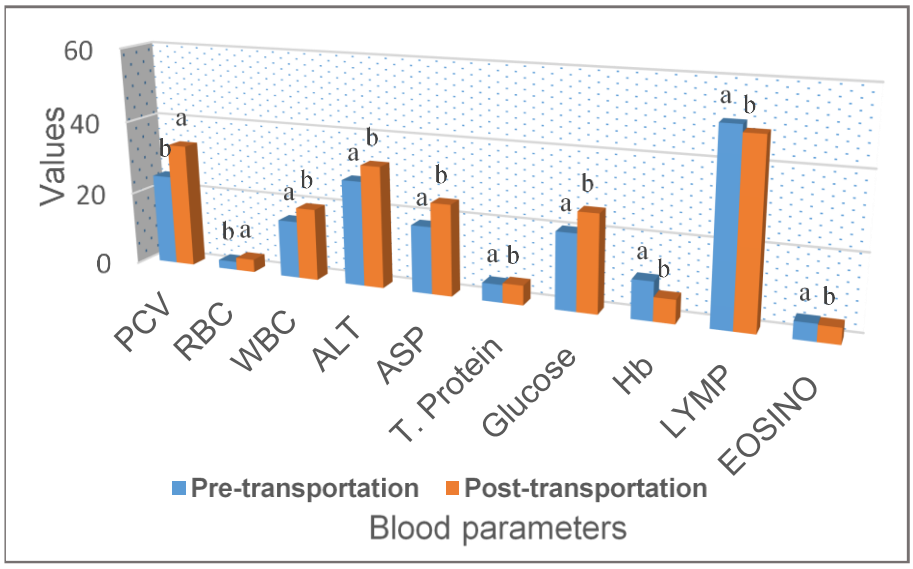

Figure 1. Measured blood hematological values pre and post transportation. [abc] means along the same bar with different superscripts are significantly ($p < 0.05$) different.

Table 1. Effect of strain and transportation density on measured blood parameters.

Parameters	Blood parameters								SEM	P-Value
	RBC ul/l	WBCul /l (10^3)	ALT (IU/L)	AST (IU/L) (10^1)	TP (%) g/100l	Glucose (10^1)	PCV (%)	Hb (%)		
Strain										
HB	3.65^a	20.86^a	32.01^a	24.34^a	5.17^a	21.55^a	31.33^a	9.03^b	0.15	0.002
NW	3.40^a	16.98^b	25.17^b	18.85^b	3.69^b	18.72^b	31.17^a	11.5^a	0.13	0.001
Density										
LD (0.8 $6m^2$)	3.35^a	18.05^{bc}	30.10^c	20.18^{bc}	4.07^{bc}	17.05^{bc}	30.33^a	12.2^c	1.80	0.04
MD (0.64 m^2)	3.45^a	19.07^b	31.01^b	21.08^b	4.87^b	18.85^b	31.03^a	10.0^b	1.10	0.02
HD (0.48 m^2)	3.50^a	21.77^a	33.50^a	24.18^a	5.67^a	21.85^a	32.33^a	8.23^a	1.18	0.003

[abc] means along the same row with different superscripts are significantly ($p < 0.05$) different.

compared to Nera white chicken. The values for respiratory and panting rate increases from pre to post-transportation between the strains and within the strains for the transportation densities.

DISCUSSION

This study presents the first report on effect of transportation stress on physiological adaptation of white and black cockerels to the best knowledge of the authors. Therefore, paucity of related studies for critical comparison of the result. Nevertheless, the results are compared with available reports on poultry and livestock species.

As shown in Figure 1, the significant ($p<0.05$) difference recorded for measured blood parameters pre/post transportation indicated that the induced transportation stress had an effect on the experimental animals. However, all measured blood parameters pre-transportation and loading are within the recommended normal range values established for chicken of the same age and sex group (Byadevlatov et al., 1992). This indicated that the birds were healthy without any form of stress pre-transportation and therefore, fit for the journey.

The ($p<0.05$) strain × density interaction effect on measured blood parameters except ($p>0.05$) for red blood cell (RBC), and packed cell volume, which reduced slightly with transportation density in this order for both strains i. e {High density (HD) > Medium density (MD) > Low density (LD)}. This may be attributed to short transportation distance covered for the experiment. The result corroborated the reports of researchers who stated that food animals on short transportation distance will experience very mild dehydration as compared to long

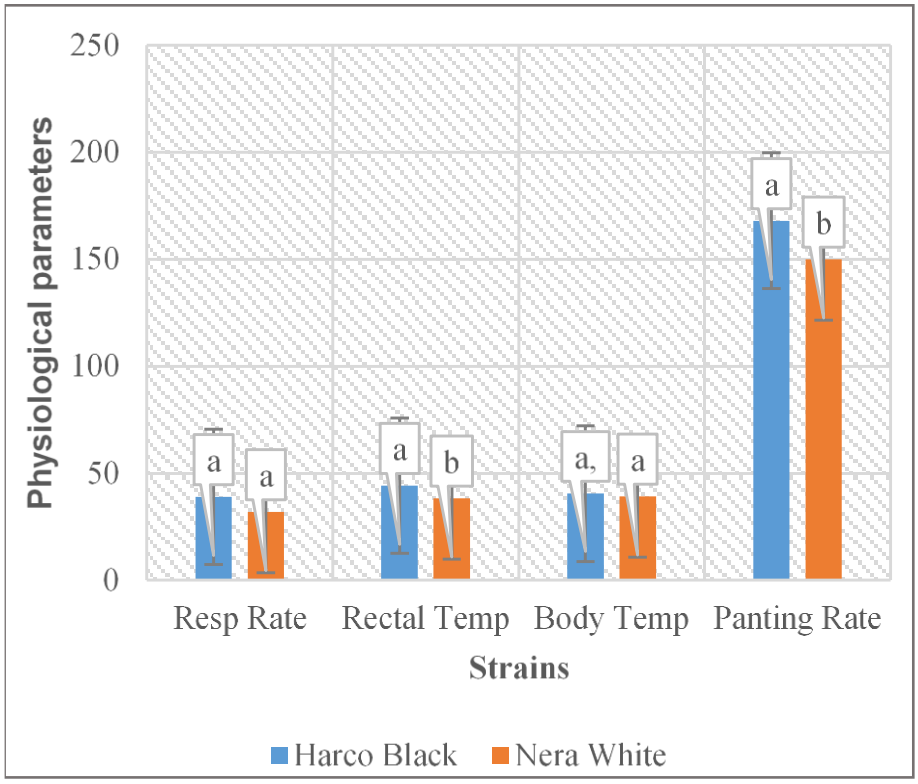

Figure 2. Response of Cockerel strains to physiological parameters post transportation. [abc]means along the same bar with different superscripts are significantly ($p< 0.05$) different.

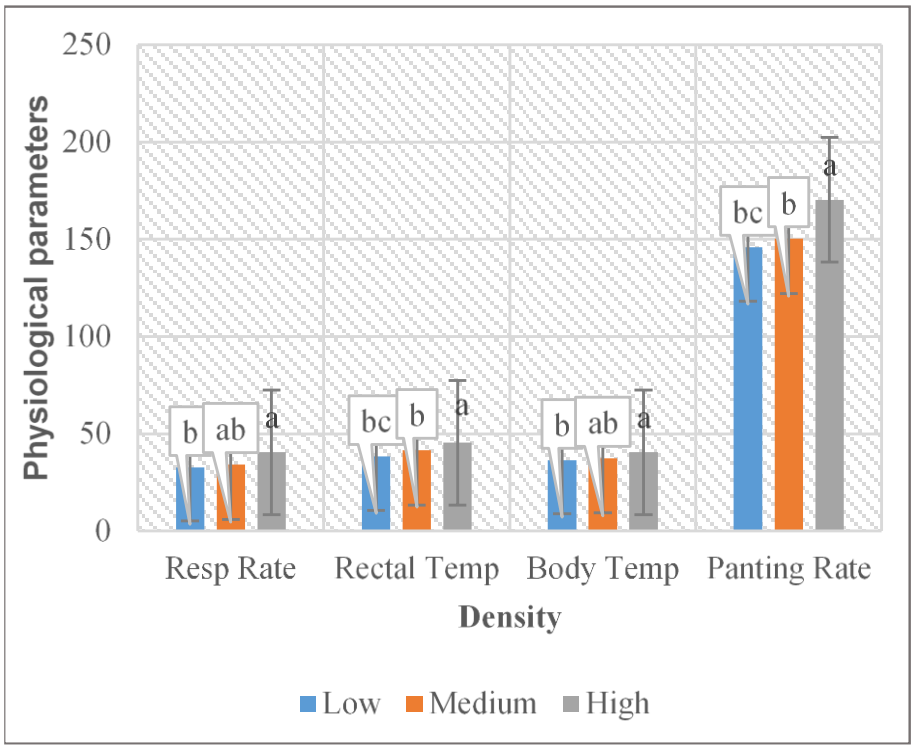

Figure 3. Effect of transportation densities on physiological parameters of birds post transportation. [abc]means along the same bar with different superscripts are significantly ($p< 0.05$) different.

Table 2. Strain x density interaction on measured blood parameters post transportation.

Parameters/density	Harco			White			SEM	P-value
	HD (0.86 m^2)	MD (0.64 m^2)	LD (0.48 m^2)	HD (0.86 m^2)	MD (0.64 m^2)	LD (0.48 m^2)		
RBC ul/l	3.45^{a}	3.50^{a}	3.40^{a}	3.40^{a}	3.48^{a}	3.38^{a}	0.02	0.001
WBC ul/l (10^3)	19.77^{b}	21.06^{ab}	23.96^{a}	16.98^{cd}	18.73^{c}	20.66^{bc}	4.13	0.03
ALT (IU/L)	31.50^{ab}	32.67^{ab}	34.83^{a}	25.17^{c}	27.00^{b}	31.00^{ab}	1.12	0.04
AST (IU/L) (10^1)	21.18^{bc}	22.85^{b}	25.01^{a}	18.85^{d}	20.01^{c}	23.18^{b}	5.10	0.01
TP (%) g/100l	4.67^{bc}	4.83^{ab}	5.20^{a}	3.69^{cd}	4.62^{bc}	5.05^{ab}	0.80	0.002
Glucose (10^1)	19.85^{a}	20.45^{a}	23.65^{ab}	18.72^{a}	19.25^{a}	20.37^{b}	3.18	0.01
PCV (%)	31.33^{a}	31.50^{a}	32.00^{a}	31.17^{a}	31.67^{a}	31.80^{a}	0.37	0.002
Hb (%)	10.23^{ab}	10.63^{ab}	9.92^{a}	11.05^{b}	10.76^{ab}	10.35^{ab}	0.14	0.003

^{abc} means along the same row with different superscripts are significantly ($p< 0.05$) different using Duncan's test as post hoc analysis. Where: HD – High density, MD – Medium density, LD- Low density, TP – Total protein, RBC – Red blood cell, WBC – White blood cell, ALT – Alanine amino transferase, AST – Aspartate amino transferase, PCV – Packed cell volume, Hb – Heamoglobin.

Table 3. Strain x density interaction effect on measured physiological parameters post transportation.

Strain	Density	RR/min	PR/min	BT (^{0}C)	RT (^{0}C)
Black	LD (0.86m^2)	37.87^{b}	158.00^{b}	36.79^{a}	38.48^{a}
Black	MD (0.64m^2)	38.08^{ab}	165.00^{ab}	38.12^{a}	42.72^{ab}
Black	HD (0.48m^2)	40.77^{a}	180.00^{a}	42.85^{a}	46.01^{b}
White	LD (0.86m^2)	34.87^{b}	145.00^{b}	34.79^{ab}	36.48^{b}
White	MD (0.64m^2)	37.08^{a}	150.00^{ab}	35.12^{a}	38.72^{ab}
White	HD (0.48m^2)	39.77^{a}	165.00^{a}	38.85^{a}	40.01^{a}
SEM		1.05	5.64	1.32	2.01
P-value		0.04	0.03	0.01	0.002

^{abc} means along the same column with different superscripts are significantly ($p< 0.05$) different. Where: HD – High density, MD – Medium density, LD – Low density.

transportation distance associated with high stress intensity and very low values of RBC, and PCV (Minka and Ayo, 2008; Sutherland et al., 2009).

The high ($p<0.05$) strain × density interaction effect of white blood cell (WBC), alanine amino transferase (ALT), aspartarte amino transferase (AST), total protein (TP), and glucose indicated that regardless of the transportation density (low, medium and high), both strains of cockerel (Harco and Nera) are physiologically affected by the induced transportation stress. Although, values of measured blood parameters are within the recommended normal range established for birds of same age and sex (Minka and Ayo, 2008). The concentration of AST, and ALT may have increased in the blood after tissue damage, poor muscular tissue reperfusion, decreased heat dissipation, hypoxia and fatigue, due to increase in the permeability of muscle membrane induced by capture, loading and transportation stress (Knowles and Warris 2000; Minka and Ayo, 2008).

Total protein concentration is a marker for protein homeostasis, which increase with dehydration (Elbers et al., 1991). Also, the increase in plasma glucose concentration is mainly due to glycogenolysis associated with the increase in catecholamines and glucocorticoids which were released during the stress of transportation (Sutherland et al., 2009). The oxygen carrying capacity of the blood was reduced as evidenced with significant ($p<0.05$) decrease in Heamoglobin (Hb) concentration with rate of density in both strains (i.e. HD < MD < LD) due to transportation stress and congestion during the journey.

Overall, based on the values of measured blood parameters, it was observed that Nera strain birds are more resistant to the induced transportation stress in hot humid environment as compared to Harco strain birds. This may be due to the plumage colour, as black conserved heat. Further effect of physiological disorder can be attributed to the effect of transportation stress from vehicle noise, bad road network and most importantly increased ambient temperatures (Minka and Ayo, 2008; Oguntunji and Oluwatomini, 2016).

Furthermore, the increase in body temperature (BT) and rectal temperature (RT) post transportation suggest the effect of transportation stress in hot humid tropics on cockerel strains at the varied transportation densities. On the average, Harco black had higher values of 3.0^0C and 4.0^0C for body and rectal temperature respectively as compared to Nera white birds. The transportation density is directly proportional to BT and RT within and between the strains. The result obtained agreed with other authors who explained that road transportation is generally consi-

dered to be stressful to animals, and factors such as crowding (density), temperature, feed/water deprivation, and length of travel must be considered (Dalin et al., 1993; Ritter et al., 2004; Adenkola et al., 2008; Adenkola et al., 2009)

Also, in similar report on pig, Adenkola et al. (2008) reported that rectal temperature recorded during 8 hours and 4 hours journey increased significantly in transported pigs, but the administration of ascorbic acid prior to transportation ameliorates the risk of adverse effects of road transportation. This result emphasized the adverse effect of transportation stress on livestock welfare and corroborate Minka and Ayo (2007) who explained that road transportation of ostrich chicks for 6 hours has a transient stressful effect on the birds and that the physiological parameters of rectal temperature (RT) and respiratory rate (RR) are indicators of welfare of transported birds. However, as observed that Nera white strain birds are more resistant to the induced transportation stress in hot humid environment as compared to Harco black strain birds which could partly be due to the plumage colour, as black conserved heat (Ayoola and Babalola, 2018).

Sweating during heat stress enhances the mitigation of thermal load in the body. However, since birds lack sweat glands, this heat loss mechanism does not practically exist in birds (Ayo et al., 1998). Hence, birds resort to panting and increased respiratory rate. In view of the foregoing and since the principal evaporative heat loss mechanism is panting in hyperthermic animals, the reported ($p<0.05$) respiratory rate (RR) and panting rate (PR) in both strains at HD suggest the induced transportation stress during the stated environmental condition affected the birds physiology. The result is in accordance with Oguntunji et al. (2019), who stated that as body temperature increases above the thermo-neutral range through increased metabolic activities and absorption of heat from environment, non-sensible heat loss mechanisms such as evaporative heat loss through higher respiratory rate and panting takes over. The report of Salama et al. (2013) buttressed this assertion that at high temperature, water loss by evaporation is the most efficient way to dissipate heat, and it occurs by respiratory system (panting) and by sweating.

However, significantly higher values for Harco black suggests that they were more stressed post transportation than Nera white birds and higher PR and RR was necessitated to enhance homeostasis through respiratory evaporation (Fadare et al., 2012). Gupta et al. (2013) stated that an important thermal regulatory reaction to heat stress is increased (RR), which aids in heat dissipation via evaporative cooling as observed in transported birds, the transportation and heat stress acted concurrently to induced physiological stress on birds.

Conclusions

The higher values of measured blood and physiological parameters of Harco black and Nera white birds at LD, MD and HD are indicative of transportation stress. The higher values of blood and physiological parameters reported in Harco black also indicate that they were more sensitive to induced transportation stress as compared to Nera white birds. Although, the two strains were affected by transportation densities, the result of this study suggested that transportation of these bird strains should be done at low transportation density viz-a-viz other mechanisms to alleviate transportation stress in hot humid tropics for efficient bird welfare.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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