



CHANGES IN STRESS MARKERS AND THERMOREGULATORY RESPONSE OF BROILERS, NOILERS, AND NATIVE CHICKENS TO TRANSPORTATION STRESS

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(Received 22 January 2025; Revision Accepted 5 March 2025)

ABSTRACT

The present study evaluated the changes in stress markers and the thermoregulatory response of broilers, noilers, and native chickens to transportation stress. A total of 45 chickens of three breeds (matured native chicken, noiler chicken, and broiler chicken) were used in this study and each breed had 15 chickens respectively and the breeds served as the treatments. The chickens were randomly placed in the transportation crates in a completely randomized design. The birds' rectal temperature (RT) and body temperature (BT) were measured before, during, and after transportation. Five (5) ml of blood was collected from the wing vein of five birds of the three strains, respectively, chosen randomly, into sample bottles/tubes for haematological and changes in the evaluation of stress markers. Data obtained from the study was subjected to a one-way analysis of variance. The result showed that the body temperature of the broiler before, during, and after transportation was significantly ($p < 0.05$) higher than those of noiler and the local chicken. Cortisol concentration in the broilers was significantly ($p \leq 0.05$) higher than cortisol levels in the noilers and Local chickens. Cortisol levels recorded after transportation were significantly ($p \leq 0.05$) higher than before. Most of the haematological parameters of the three breeds of birds significantly ($p \leq 0.05$) increased after transportation. The values of the stress markers were significantly ($p \leq 0.05$) higher in the Broiler and Noiler chickens than in the Local chickens. After transportation, the serum glucose of the noiler significantly ($p \leq 0.05$) increased while the calcium and sodium decreased. It was concluded that broilers were susceptible to transportation stress at a short distance while noilers and local chickens were prone to transportation stress at a longer duration.

KEYWORDS: Noilers, Poultry Transportation, Stress Markers

INTRODUCTION

Stress can be described as responses elicited by an animal in a bid to adapt or protect itself from the adverse effects of stressors (Zhang *et al.*, 2017). Physiological stress in animals can be caused by extreme temperature (heat or cold), overcrowding or high stocking density, starvation (feed and/or water deprivation or withdrawal), and harmful handling such as restraint, noise, and transportation. In Cross River State Nigeria, chickens are the most transported farm animal; they are transported and sold alive because of limited stockpiling space and scattered farm locations in the absence of a central slaughterhouse. This makes transportation a crucial activity along this value chain.

Stress resulting from the transportation of meat-type chicken has a significant impact on welfare, meat yield, and meat quality, which causes major economic losses in poultry enterprises (Barbut, 2015). The transport stressors can be categorized into psychological, physical stressors, and physiological factors; the psychological stressors include social mixing, fear and pain, food, and water deprivation, promoting exhaustion of the natural antioxidant capacity of the bird, exposing the cells to harmful reactive oxygen species (Jayaprakash *et al.*, 2016), while the physical stressors include catching, crating, and the environment during transportation, such as hot or cold temperatures, wind, vehicle vibration, and airflow, which cause physical injuries.

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The antemortem stress on animals increases the production and consumption of epinephrine and glucocorticoids (Arikan *et al.*, 2017). These stressors promote major physiological disturbances such as aberrant behaviours, immune suppression, and disruption of general homeostasis (Minka and Ayo 2008; 2010) thereby reducing the quality and quantity of meat.

Important indices of stress due to road transportation in livestock include rectal temperature (RT) (Anoh, *et al.* 2018; 2017; Minka and Ayo 2007) and live weight (Minka and Ayo 2010). Signs of transportation stress have been reported in different animal species by previous studies; for example, increased heart rate (Anoh, *et al.* 2018a), increased rectal temperature (Anoh, *et al.* 2016; 2018b; Minka and Ayo 2007), increased adrenal-cortical activity (Minka and Ayo 2007), increased morbidity and mortality (Chirase *et al.* 2004). By studying stress markers, such as cortisol levels, heterophils/lymphocyte variability, and other physiological and behavioral indicators, we can gain valuable insights into the impact of transportation on the well-being of the chickens.

The Noiler chicken a cross between the Nigeria indigenous chicken and the Plymouth breed of chicken was developed by Amo farm Sieberer Hatchery Limited for smallholders to address the challenges of food insecurity and financial dependency among the rural populace, especially women (Yakubu and Ari, 2018). It has been emphasized that the introduction of tropically adapted genotypes with high performance, diseases, and high environmental temperature resistance is beneficial to resource-poor poultry farmers (Yakubu and Ari, 2018). The responses to stress of different breeds of chickens to transportation conditions may vary, which is why it's important to identify and understand these markers. By doing so, we can potentially improve transport conditions, minimize stress levels, and ultimately enhance the quality of the meat produced as well as animal welfare and additionally reduce mortality rates, and better meat quality for consumers. The study aimed to evaluate the changes in stress markers and the thermoregulatory response of broilers, noilers, and native chickens transported by road.

MATERIALS AND METHODS

Study Location

The study was carried out from 18th to 21st May, in Calabar and Akamkpa. The location is situated at 11° 10' N, 07° 38' E, located in the swamp savannah zone of Nigeria. The Calabar environment has an altitude of 91 m above sea level, an average rainfall of 1600mm which starts from late April to November. The study location has an average temperature of 36°C and an average relative humidity of 75% (Weather and Climate, 2023).

Pre-conditioning period

Three types of chicken (matured native chicken, noiler chickens, and broiler chicken) were used in this study. The native chickens were purchased from a local chicken vendor in Bogobiri Calabar while the noiler and broiler chicken were obtained from a reputable poultry farm in Calabar. Feed and water were served ad libitum to the birds for three consecutive days and were withdrawn 6 hours before the commencement of the study.

Experimental Design

A total of 45 chickens were used for this study. The chickens (15/breed) were randomly placed in the transportation crates in a completely randomized design.

Data collection

Thermoregulatory evaluation

At 12 h in the afternoon before the transportation, the Rectal Temperature (RT) and Body Temperature (BT) of the chickens were measured. The RT and BT were measured by inserting a digital clinical thermometer (Electron thermometer manufactured in China) for about 1 to 2 mins into the rectum and wings of each bird, respectively until an alarm sound was produced, indicating the end of the readings. The measurement was taken before and after the transportation

Haematological and changes in stress markers evaluation

Five (5) ml of blood was collected from the wing vein of five birds of the broiler, noilers, and native chickens respectively, chosen at random, into sample bottles/tubes with and without anticoagulants before and after transportation for the evaluation of the changes in stress markers in the blood and serum of the chickens. The blood samples in the tubes containing the anticoagulants were used to evaluate for full and differential counts as well as for the heterophil: Lymphocyte ratio. Serum was harvested after centrifuging the blood sample in the plane bottle and stored at -10°C until when analyzed. Serum glucose, protein, sodium, and calcium were evaluated as stress markers with the help of an auto-analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc. San Antonio, Texas, USA, according to the manufacturer's instructions.

Transportation Period

The crates with the birds were loaded in a Hi-jet mini-bus and transported on the road. The birds were loaded in perforated rectangle crates of 1.5m (Length) x 1m (width) and 0.4m (height) from Calabar to Akamkpa and back to Calabar, covering a distance of 80 km at a speed of about 70 km/h. During the journey, RT and BT values were recorded and immediately on arrival in Calabar when the journey was completed.

Statistical Analysis

Data obtained from the study were subjected to a one-way analysis of variance using the general linear model procedure of Statistix software 10.0 version.

Significant differences among treatment means were separated using the least significant difference in the Statistix package.

RESULTS AND DISCUSSION

Thermoregulatory response of chickens transported by road

The thermoregulatory response of the chickens transported by road (Table 1) showed that the body temperature of the broiler before, during, and after transportation was significantly ($p < 0.05$) higher than those of the noiler and the local chicken. During and after transportation, the differences in body and rectal temperature changes in the broilers and noilers were significantly ($p < 0.05$) higher than the body and rectal temperature of the local chickens.

The rectal temperature of the broiler chicken during and after transportation were above the normal range indicating that the birds were stressed. The adult chicken's normal body temperature ranges from 41–41.5°C (Zhou *et al.*, 1997; Luthra, 2017). The observed low temperature recorded in the local chicken and broilers agrees with Scott *et al.* (1998). The authors observed that chickens raised in a free-

range cage showed lower signs of fear and a shorter duration of tonic immobility than chickens raised inside cages and in confinement when transported for over 60 km or for longer than one hour. Fear could lead to nervousness and subsequently stress. The genetic makeup of the local chicken which can tolerate heat stress could have been responsible for the low rectal temperature as well. These traits are also present in the noilers. Also, the levels of deposition of the adipose tissues in the broiler are high compared to the fatty tissues present in the local chicken and noilers. High adipose tissue has been attributed to heat stress (Anoh *et al.*, 2018). Krannen *et al.* (2000) showed that the high growth rate of broiler chickens, in combination with inadequate oxygenation, made them highly sensitive to hypoxia. Furthermore, Watt *et al.* (2011) reported that other stressors, such as high animal density inside crates or fluctuations in ambient temperature during transport, can affect the body heat of broiler chickens. Chepete (2008) reported an increase in the rectal temperature of broilers with a corresponding increase in environmental temperature. The increased rectal temperature at that time of transportation indicated that the thermoregulatory mechanisms were completely failing (Etches *et al.*, 1989).

Table 1: Thermoregulatory response of three strains of chicken transportation by road

| Parameters | Broilers | Noilers | Native Chicken | SEM |
|------------------------------|--------------------|--------------------|--------------------|------|
| Before transportation | | | | |
| Body Temperature (°C) | 37.10 ^a | 34.67 ^b | 34.45 ^b | 0.27 |
| Rectal Temperature (°C) | 39.66 ^a | 37.43 ^b | 37.33 ^b | 0.29 |
| During transportation | | | | |
| Body Temperature (°C) | 38.00 ^a | 36.33 ^b | 35.16 ^b | 0.22 |
| Rectal Temperature (°C) | 44.50 ^a | 40.00 ^b | 38.16 ^c | 0.19 |
| After transportation | | | | |
| Body Temperature (°C) | 39.00 ^a | 36.66 ^b | 35.66 ^b | 0.43 |
| Rectal Temperature (°C) | 46.33 ^a | 40.67 ^b | 38.33 ^c | 0.33 |

^{a, b, c} Means on the same row with different superscripts are significantly different ($p < 0.05$)

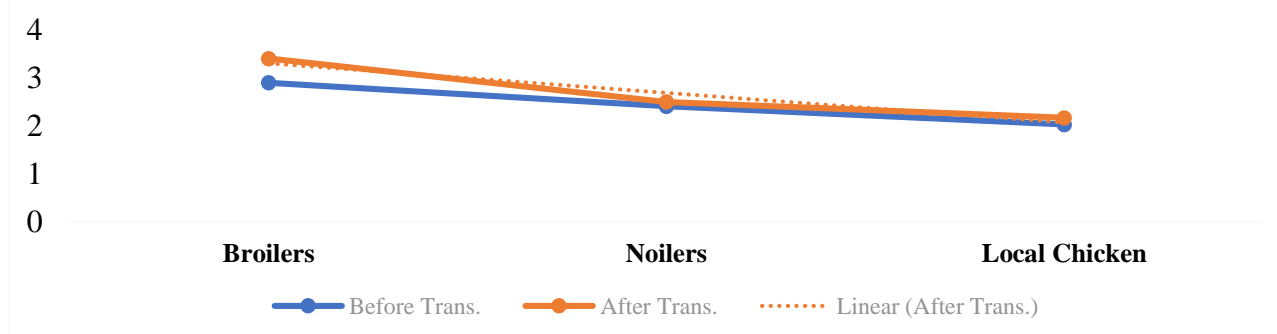
SEM: Standard Error of Mean.

Cortisol Concentration of Three Strains of Chicken Transported by Road

Cortisol concentration in the Broiler was significantly ($p \leq 0.05$) higher than cortisol levels in the Noilers and

Local chicken (Fig. 1). Generally, cortisol levels recorded after transportation in this study were significantly ($p \leq 0.05$) higher than before transportation.

Fig. 1: Cortisol Concentration of Three Strains of Chicken Transported by Road



The cortisol levels recorded after transportation were higher than cortisol levels before transportation agreeing that transportation stress had a significant impact on the chickens (Freeman *et al.* 2013). Transportation induces the release of higher levels of cortisol into the chicken blood. This is an indicator of stress response (Zhang, *et al.*, 2009; Kang and Kuenzel 2014; Huth and Archer, 2015). This also implied that transportation stress affected the broiler chickens more than the noiler and the local chicken. Additionally, the Broilers may have been more susceptible to the transportation stress due to their larger body size, weight, and adipose tissue deposit which can lead to increased stress and discomfort during transport (Maxwell *et al.* 2018). The increased cortisol levels after transportation may be attributed to the physical and psychological stress caused by the transportation process, including noise, vibration, and changes in temperature and humidity (Nazifi *et al.* 2017). Also, the handling and loading of the chickens onto the transport vehicle may have caused additional stress, leading to increased cortisol levels (Al-Masri *et al.*, 2020). Increases in cortisol levels have been

reported in Turkey (Knowles *et al.*, 2010) and rabbits (Marai *et al.*, 2017; Padilla *et al.*, 2018) subjected to transportation and heat stress respectively.

Haematological evaluation of chickens transported by road

The haematological parameters of the three strains of chicken before the transportation of the birds (Table 2), showed that the noilers had a superior haematological status as they recorded a significantly ($p \leq 0.05$) high haemoglobin, packed cell volume, mean capsular haemoglobin, and mean capsular volume. This was followed by the values of the local chicken for these parameters. The broiler chicken, however, recorded a significantly ($p \leq 0.05$) high white blood cells and blood platelets. Most of the haematological parameters of the three breeds of birds significantly ($p \leq 0.05$) increased after transportation. The Noiler birds for instance recorded a significantly high haemoglobin, white blood cells, packed cell volume, mean capsular haemoglobin, and mean capsular volume. The values were similar to those of the Broiler chicken. Apart from the red blood cells and mean capsular haemoglobin concentration, haematological values of the local chicken, were low.

Table 2 Effect of transportation stress on the haematological evaluation of three breeds of chicken

| Parameters | Broiler | Noiler | Native Chicken | SEM |
|---|---------------------|---------------------|---------------------|------|
| Before Transportation | | | | |
| Haemoglobin (g/dL) | 4.67 ^b | 6.23 ^a | 5.13 ^b | 0.13 |
| Red Blood Cells (x10 ¹¹ /L) | 3.50 | 3.83 | 3.83 | 0.13 |
| White Blood Count (x10 ⁹ /L) | 5.30 ^a | 3.10 ^b | 4.20 ^c | 0.19 |
| Packed Cell Volume (%) | 16.00 ^c | 23.00 ^a | 18.66 ^b | 2.19 |
| MCH (pg) | 11.23 ^c | 16.00 ^a | 13.67 ^b | 1.96 |
| MCV (1fL) | 46.00 ^b | 57.00 ^a | 46.33 ^b | 4.95 |
| MCHC (%) | 27.00 | 27.33 | 28.67 | 2.46 |
| PLT (x10 ⁹ /L) | 152.33 ^a | 103.67 ^c | 113.33 ^b | 2.47 |
| After Transportation | | | | |
| Haemoglobin (g/dL) | 5.30 ^b | 6.30 ^a | 4.97 ^b | 0.24 |
| Red Blood Cells (x10 ¹¹ /L) | 4.13 ^a | 3.43 ^b | 4.27 ^a | 0.13 |
| White Blood Count (x10 ⁹ /L) | 4.30 ^a | 4.70 ^a | 3.27 ^b | 0.24 |
| Packed Cell Volume (%) | 21.00 ^a | 23.00 ^a | 19.00 ^c | 0.65 |
| MCH (pg) | 13.33 ^b | 17.67 ^a | 13.33 ^b | 0.74 |
| MCV (1fL) | 52.33 ^b | 65.67 ^a | 45.00 ^c | 3.05 |
| MCHC (%) | 24.00 ^c | 27.00 ^b | 31.00 ^a | 1.05 |
| PLT (x10 ⁹ /L) | 120.67 ^a | 118.00 ^b | 104.00 ^c | 2.59 |

a, b, c Means on the same row with different superscripts are significantly different (p<0.05)

SEM: Standard Error of Mean.

The effect of transportation stress on the differential blood counts of three strains of chicken is shown in Table 3. Before transportation, the Noiler recorded a (p ≤ 0.05) significantly high eosinophils and neutrophils, the values of the eosinophils were similar to those of Broilers. The differential counts of the Local

chicken were generally low. After transportation, eosinophils and neutrophils significantly increased in the Broiler chicken and the local chicken respectively. Eosinophils in the Noiler Chicken was low compared to the eosinophils of the Broilers and Local chicken.

Table 3: Effect of transportation stress on the differential blood counts of three strains of chicken

| Parameters | Broiler | Noiler | Native Chicken | SEM |
|-----------------------------------|--------------------|--------------------|--------------------|------|
| Before Transportation | | | | |
| Basophils (x10 ⁹ /L) | 0.00 | 0.00 | 0.00 | 0.00 |
| Eosinophils (x10 ⁹ /L) | 7.66 ^a | 7.67 ^a | 7.00 ^b | 0.43 |
| Neutrophils (x10 ⁹ /L) | 12.67 ^b | 13.33 ^a | 11.67 ^c | 0.47 |
| After Transportation | | | | |
| Basophils (x10 ⁹ /L) | 0.00 | 0.00 | 0.00 | 0.00 |
| Eosinophils (x10 ⁹ /L) | 9.66 ^a | 4.00 ^c | 5.33 ^b | 0.43 |
| Neutrophils (x10 ⁹ /L) | 21.56 ^a | 17.73 ^b | 16.55 ^b | 1.33 |

a, b, c Means on the same row with different superscripts are significantly different (p<0.05)

SEM: Standard Error of Mean.

The implications of these findings are significant, as high and low values of PCV, RBC, and other parameters can indicate various physiological responses (Kumar *et al.*, 2011). High PCV values indicate increased blood viscosity, which may be beneficial for birds under stress (Sahin *et al.*, 2012), while low values may indicate anaemia or blood loss (Borges *et al.*, 2018). High RBC counts indicate increased oxygen-carrying capacity (Gonzalez *et al.*, 2017), while low values may indicate anaemia or iron deficiency (Kumar *et al.*, 2011). High WBC counts

indicate an immune response to stress or infection (Maxwell *et al.*, 2006), while low values may indicate immunosuppression (Borges *et al.*, 2018).

Lymphocytes and heterophil/lymphocytes ratio of Chickens transported by road

The values of the lymphocytes and heterophil/lymphocytes ratio (Table 4) before transportation showed that the stress markers were significantly (p ≤ 0.05) high in the Broiler and Noiler chickens compared to the Local chickens. The same trend was observed and recorded after transportation.

Unlike in the serum markers, the values of lymphocytes and heterophil/lymphocytes ratio were generally high after transportation.

The results of this study are consistent with previous studies that have reported increased lymphocyte and heterophil/lymphocyte ratio values in response to

stress (Gross *et al.*, 2017). Transportation stress increases the values of lymphocytes and heterophil/lymphocyte ratio, indicating higher levels of stress (Knowles *et al.*, 2010) leading to increased stress and discomfort of the broilers and noilers compared to the local chicken. The heterophil/lymphocyte ratio is a well-established stress marker in chickens, and increased values indicate higher levels of stress (Maxwell *et al.* 2018).

Table 4: Differential blood count stress marker of three strains of Chicken transported by road

| Parameters | Broiler | Noiler | Native Chicken | SEM |
|------------------------------|--------------------|--------------------|--------------------|-------|
| Before Transportation | | | | |
| Lymphocytes (x109/L) | 79.67 ^a | 79.33 ^a | 70.00 ^b | 0.64 |
| Heterophil/Lymphocyte Ratio | 0.60 ^a | 0.58 ^a | 0.54 ^b | 0.03 |
| After Transportation | | | | |
| Lymphocytes (x109/L) | 87.33 ^a | 79.66 ^b | 73.00 ^b | 1.23 |
| Heterophil/Lymphocyte Ratio | 0.8 ^a | 0.6 ^a | 0.5 ^b | 0.003 |

a, b, c Means on the same row with different superscripts are significantly different ($p < 0.05$)

SEM: Standard Error of Mean.

Serum metabolite stress markers of Chickens transported by road

The effect of transportation stress on serum metabolites stress markers is shown in Table 5. Before the commencement of transportation, glucose, total protein, and calcium were significantly ($p \leq 0.05$) high in broiler chicken. The noiler had similar glucose, total protein, and calcium levels that were similar to the broilers. Only the sodium level of the local chicken was similar to the broilers. The serum metabolite stress

markers (glucose, total protein, and calcium) of the local chicken were significantly ($p \leq 0.05$) low. After transportation, the serum glucose of noiler significantly ($p \leq 0.05$) increased while the calcium and sodium decreased. The serum calcium and sodium of broilers were significantly ($p \leq 0.05$) high; the value of calcium was similar to those of the local chicken. Generally, the values of most of the markers decreased after transportation.

Table 5: Serum metabolite stress markers of three strains of Chicken transported by road

| Parameters | Broiler | Noiler | Local Chicken | SEM |
|------------------------------|---------------------|---------------------|---------------------|------|
| Before Transportation | | | | |
| Glucose (mmol/L) | 2.60 ^a | 2.20 ^{ab} | 1.80 ^b | 0.30 |
| Total Protein (G/L) | 3.70 ^a | 3.76 ^a | 1.37 ^b | 0.54 |
| Calcium (Ca) (mmol/L) | 6.47 ^a | 5.40 ^{ab} | 3.77 ^b | 0.28 |
| Sodium (Na) (mmol/L) | 119.67 ^a | 112.00 ^b | 120.00 ^a | 4.93 |
| After Transportation | | | | |
| Glucose (mmol/L) | 1.80 ^b | 2.10 ^a | 1.80 ^b | 0.06 |
| Total Protein (G/L) | 2.60 | 2.80 | 2.70 | 0.06 |
| Calcium (Ca) (mmol/L) | 5.57 ^a | 4.77 ^b | 5.50 ^a | 0.07 |
| Sodium (Na) (mmol/L) | 131.00 ^a | 120.33 ^b | 113.00 ^c | 0.96 |

a, b Means on the same row with different superscripts are significantly different ($p < 0.05$)

SEM: Standard Error of Mean.

After transportation, there were significant changes in serum metabolite stress markers among the three strains of chicken. Noiler chickens recorded a significant increase in serum glucose levels, indicating that transportation stress may have caused a spike in glucose levels. This is consistent with previous studies that reported increased glucose levels in response to stress (Freeman *et al.* 2013). The study found that stress increases glucose levels and alters glucose

metabolism in chickens, affecting energy homeostasis. Calcium and sodium levels decreased in noilers after transportation, suggesting that transportation stress may have disrupted mineral homeostasis. Stress decreases calcium and sodium levels in chickens, disrupting mineral homeostasis and affecting bone health and welfare (Nazifi *et al.*, 2017). Nazifi *et al.* (2017) reported decreased calcium and sodium levels in response to stress.

CONCLUSION AND RECOMMENDATION

It was concluded that broilers were susceptible to transportation stress at a short distance while noilers and local chickens were prone to transportation stress at a longer duration. The findings of this study recommend cluster farming among poultry farmers with a central slaughterhouse for chickens to minimize the transportation of chickens over long distances.

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