EFFECT OF TRANSPORTATION ON WELFARE OF INDIGENOUS CHICKEN IN MACHAKOS COUNTY, KENYA

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DECLARATION

This thesis is my original work and has never been presented for an award in any other University or institution of higher learning.

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DEDICATION

This work is dedicated to my entire family, my loving husband and children. Your love, support, encouragement and sacrifices during this process is highly appreciated.

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ABBREVIATIONS AND ACRONYMS

CK	**:**	Creatine kinase
CORT	**:**	Corticosterone
FAO	**:**	Food and Agriculture Organization
GOK	**:**	Government of Kenya

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ABSTRACT

The aim of this study was to investigate effect of transportation on welfare of indigenous chicken. A sample of 8 hens were randomly selected from the target population. Each treatment had an equal number of mature indigenous chicken hens weighing between 1.25 and 2.4 kg. The first batch of 4 birds were tied together and loaded on to an open vehicle roof top. The second batch of 4 birds was loaded into traditional transport cages and the cage loaded on top of the transport vehicle. Transport conditions (temperature, relative humidity, air speed, vehicle velocity) were measured by use of automatic data loggers. Bird physiological conditions (temperature, pH, weight, hormones, behavior changes) were measured for each treatment separately at the beginning and the end of 2 hours journey on a tarmac road stretch of 109.5 km from Kyua to Athi River in Machakos County. t-tests were run to determine the effect of each treatment and difference between treatments. It was established that in general road transportation of indigenous chicken in Machakos County adversely affected welfare of indigenous chicken. The study showed that transportation whether in traditional cages or on open vehicle roof top led to increased body temperature, increased serum cortisol and reduced body weight. However, comparatively, the change in temperature for birds in the cage was significatly higher than for birds transported on the open vehicle roof top. On the contrary, transportation on the open vehicle roof top had a significantly higher mean loss of body weight and higher levels of serum cortisol compared to transportation in the cages. Finally, increased panting, fatigue and closing of eyes were observed in both treatments. The results are in line with findings from similar studies done on layers and broilers suggesting that transport condition acts as a stress stimuli leading to physiological, biochemical and behavior changes in chicken with a negative impact on their welfare. The data also contributed to a clearer understanding on the difference between transportation of indigenous chicken in traditional cages and on open vehicle roof tops. The study was cross sectional and hence generalizability of the results is limited to its context and time of study. The study recommends that transportation cages should be properly designed, constructed and fitted to meet the thermoregulation and comfort requirements of the birds at all times during transportation. Further, care should be taken to protect the birds from adverse temperatures and direct sunlight as well as wind. In addition, transportation time should be limited to reduce potential adverse effects with long journeys having rest and recovery periods. For government agencies, the study recommends full enforcement of regulations on standard chicken transportation to safeguard on their welfare. For future research, the study recommends morning and late afternoon studies, at various times of the year and comparative studies using cocks. The findings provide empirical evidence that helps in improving transportation conditions, care of the indigenous chicken on transit as well as help in directing policy in the industry.

1.0 INTRODUCTION

1.1 Background of the Study

Indigenous chicken production is the largest and fastest growing poultry sub sector in Kenya. Over the years, indigenous chicken production has progressed to become one of the most important livestock enterprises particularly in rural households where over 70% of the country's population live and derive their livelihood (Wachira et al., 2018). According to the 2018 annual livestock statistics report by the State Department of Livestock, Government of Kenya (GOK), there are approximately 40 million indigenous chicken in Kenya (GoK, 2018). These birds are an important source of food, income and employment and form an integral source of livelihood to over 80% of the rural households, with many social and cultural uses including preferred gifts to friends and family, slaughtered during cultural rites and insurance against drought. The poultry industry also has linkages with other sectors of the economy that include input supplies, feed manufacturing, breeding, transportation and processing (Wachira et al., 2018).

Indigenous chicken rearing in Kenya is mostly carried out on small scale and in form of free-range system across different geographical areas and transported by road over long distances to urban areas for marketing and processing (Wachira et al., 2018). This makes transportation a crucial activity along this value chain. The modes of transportation mostly involve the placement of birds either in transport containers, which are subsequently loaded on to the available means of transport (vehicles, motorbikes, and bicycles), simply hung on open vehicle rooftops, bike handles, and carts, or carried by hand for transportation to their final destination. According to Mitchell and Kettlewell (2009), all procedures and practices involved in poultry transportation and the microenvironments prevailing in containers and means of transportation may impose varying degrees of stress upon the birds, which may result in compromise of their welfare status, health and productive efficiency depending upon the magnitude of the stress imposed.

To address these concerns, globally particular attention has been focused on animals' welfare with regard to animal transport practices (Qi et al., 2017). In Kenya, the Prevention of

Cruelty to Animals Act (CAP 360) is in favour of introduction of regulations to improve the welfare of animals during transportation. The law demands that animals while being transported are supposed to be supplied with adequate food, water and shelter in comfortable carriers (GoK, 2012). However, the standards are not explicitly defined to properly curtail transportation of chicken in substandard conditions.

1.1.1 Transportation Stress

Transportation induced stress in chicken is perceived as adaptive or protective responses by birds on transit to protect against adverse effects of transportation conditions such as feed and water withdrawal, handling, crating density, transport time, ambient temperature, vehicle design, trailer microclimate and lairage time (Qi et al., 2017). Multiple interactive stressors associated with aspects of transportation have been cited to be responsible for adverse effects on the physiological and biochemical status of birds on transit (Jayaprakash et al., 2016). These effects have a direct relationship to the birds' productivity, quality of their products and welfare.

Transport related stresses are mainly triggered by the nature of bird handling. That is, how the birds are held, loaded and offloaded onto the transport vessel. This is further aggravated by adverse transportation microenvironment (Qi et al., 2017). Mitchell and Kettlewell (2009) identified major factors, which may act in isolation or in combination to impose various degrees of stress to the birds during transportation to include thermal demands of the transport microenvironment, acceleration, vibration, motion, impacts, fasting, thirst/dehydration, social disruption and noise. However, according to Nilipour (2002), thermal demands constitute the major threat to animal well-being and productivity as it may lead to imposition of thermal loads upon the birds on transit resulting in moderate to severe thermal stress and consequent reduced welfare, increased mortality due to either heat or cold stress, muscle damage and associated changes in product quality (Mitchell & Kettlewell, 2009).

The degree of these impacts however depends on the mode of transportation, speed, microenvironment, time and distances covered. Stressful conditions stimulate adaptive response such as regulation of body temperature, behavior changes and release of stress hormones from the adrenal gland, which is the main stress response organ (Vuuren, 2011). That is, stress influences parameters of bird physiological responses, like the release of corticosterone (CORT), glucose and creatine kinase (CK) from the adrenal gland (Qi et al., 2017). These hormones are to prepare the birds' response by stimulating glycogenesis for release of energy into the birds' muscles and blood (Tang et al, 2013). Quantities of these indicators in the blood stream thus act as indicators for evaluating stress levels of the birds on transit. This is crucial in improving the management of transport conditions and care to meet the needs of the birds (Mitchell & Kettlewell, 2009).

1.1.2 Animal Welfare

The rationality underpinning the concept of animal welfare is that animals have a wide range of needs that make natural living possible. Thus, animal welfare has been associated with their well-being; happiness; thriving and successful progress in life (Nicol & Davies, 2013). However, due to contextual variability and relative importance put on animal welfare factors in different parts across the globe, welfare of any animal is determined by an individual animal's perception of physical and emotional state (Webster, 2016). In general according to World Organization, for animal health, animal welfare refers to how well an animal is able to cope with the conditions in which it lives (Nicol & Davies, 2013). These conditions encompass biological function, affective state (experiences), feelings, natural behavior and living (Hemsworth et al., 2015; Phillips, 2009).

The major driver for studies evaluating the influence of stress on animal welfare is the hypothesis that stress interferes with the natural adaptation and coping mechanism of an animal, adversely affecting the animals' natural biological function, affective state and natural living (Nicol & Davies, 2013). Globally, the animals' Five Freedoms frameworks have been highly influential for the overall assessment of quality of animal life and welfare. The freedoms as illustrated by Webster (2016) include freedom from thirst, hunger and malnutrition. That is, animals should have ready access to a diet to maintain full health and vigor.

Second is the Freedom from thermal and physical discomfort.Animals should be provided with suitable environment including shelter and a comfortable resting area. Third is the Freedom from pain, injury and disease (prevention or rapid diagnosis and treatment). Fourth is the Freedom from fear and distress (provision of sufficient space, proper facilities and company of the animal's own kind). Lastly, is the Freedom to express normal behavior, ensuring conditions that avoid mental suffering. These five freedoms form the basis of the four guiding principles of animal handling of good feeding, good housing, good health, and appropriate behavior, which form the key pillars that govern animal welfare (Nicol & Davies, 2013).

1.2 Statement of the Problem

The government of Kenya has enacted the prevention of cruelty to animals' act (CAP 360) and other legislations to improve animal welfare during transit. Despite the recognized effects of transportation practices on the birds and the need for enhancing animal welfare during transit, the enforcement of the animal welfare laws and regulations has remained a major challenge in Kenya. In Kenya and in Machakos County in particular, it is common for indigenous chicken to be spotted on open vehicle rooftops or hung on motor bikes enroute to markets and other destinations. These transportation conditions more often than not fall short of meeting sound animal welfare standards by subjecting the birds to uncomfortable transit microenvironment. This has remained a major concern, as it is apparent that these transport conditions do influence the physiological parameters of the birds.

Several studies have been carried out to establish the effect of transportation on chicken welfare and quality of chicken products (Arikan et al., 2017; Bulitta, 2015; Jayaprakash et al., 2016; Lengkey et al., 2013). However, most of these studies have focused on broilers and are based on regions outside Kenya. It is imperative to incorporate existing knowledge to major types of breeds and to assess how these breeds are equipped to respond to "transportation induced stress" and how these characteristics are influenced by different climatic conditions in different localities as well as different transport means.

Machakos County has unique climatic conditions whereby findings from different environmental conditions may not wholly apply.Further, indigenous chickens have unique characteristics different from other chicken breeds.This presents a knowledge gap. The study therefore sought to investigate the effect of transportation on welfare of indigenous chicken in Machakos County. The findings will be important in providing empirical evidence that helps in improving transportation conditions, care of the indigenous chicken on transit as well as help in directing policy.

1.3 Objectives of the Study

The main objective of the study was to investigate the effect of transportation on welfare of indigenous chicken in Machakos County.

Specific objectives

i.   To determine the effect of transportation on body temperature of indigenous chicken in Machakos County.
ii. To investigate the effect of transportation on blood pH of indigenous chicken in Machakos County.
iii. To examine the effect of transportation on body weight of indigenous chicken in Machakos County.
iv. To establish the effect of transportation on stress hormones of indigenous chicken in Machakos County.
v.   To investigate the effect of transportation on behavior of indigenous chicken in Machakos County.

1.4 Research Questions

i.   Does transportation affect body temperature of indigenous chicken in Machakos County?
ii. Does transportation affect blood pH of indigenous chicken in Machakos County?
iii. Does transportation affect body weight of indigenous chicken in Machakos County?

iv.   Does transportation affect level of stress hormones of indigenous chicken in Machakos County?

v.   Does transportation affect behavior of indigenous chicken in Machakos County?

1.5 Justification of the Study

Since welfare concerns have a direct influence on the chicken productivity and quality of their products (Nilipour, 2002), it implies that these concerns have an indirect impact on the economic contribution of the sub sector. Therefore, a study to create an understanding on the effect of transportation on welfare of indigenous chicken is significant not only in improving transportation conditions for the chicken, but also provide information crucial in tapping opportunities of improving the earnings of rural households, whose livelihoods depend on the sub sector. In the long-term contributing towards poverty reduction and wealth creation.

Significant amount of research has been conducted on the effect of transportation on welfare of layers and broiler chickens in Asia, Europe and the Americas (Arikan et al., 2017; Bulitta, 2015; Jayaprakash et al., 2016; Lengkey et al., 2013). Most of these studies have not adequately explored the effects of transportation on indigenous chicken in Africa and particularly in Machakos County. This presents a knowledge gap with regard to this important breed of chicken. It was of importance to clearly demonstrate how indigenous chicken respond to transportation stress within the context of Machakos. The study therefore sought to bridge this knowledge gap by investigating the effect of transportation on welfare of indigenous chicken in Machakos County.

1.6 Limitations

The study context was limited to Machakos County, Kenya. The study was also limited to the effect of transportation on indigenous chicken transported by tarmac road for 2 hours over a distance of 109.5 km from Kyua to Athi River in Machakos County. Further, the study adopted an experimental research design and was cross-sectional and limited to describing the study parameters at the time of the study.

1.7 Scope of the Study

The scope of this study depended on its conceptualization. The study focused on investigating the effect of transportation on welfare of indigenous chicken. The study narrowed on the effect of transportation induced parameters namely ambient temperatures, relative humidity and air speed on stress response parameters namely, body temperature, blood pH, body-weight, stress hormones and behavior changes. The study only looked at transportation induced stress by road transportation using vehicles on a tarmac road. Geographically, the study focused on Machakos County, Kenya. The study explored this effect on birds transported on a tarmac road stretch of 109.5 km from Kyua to Athi River.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The chapter reviews and discusses past literature to identify knowledge gaps and provide a basis for the study. The chapter is organized into five sections namely, status of chicken transportation, transportation and chicken welfare, empirical literature review, summary of literature review and research gaps.

2.2 Status of Chicken Transportation

According to Food and Agriculture Organization (FAO) of the United Nations, globally there are over 23 billion poultry kept and raised in a wide range of production systems to provide mainly meat, eggs and manure (FAO, 2016). Average annual global production of eggs is approximately 73 million tons while that of poultry meat is approximately 100 million tons with majority (92%) of poultry meat production coming from specialized broiler chicken production systems. Layers contribute only 6% of the total while small-scale free-range systems mainly consisting of indigenous chicken contribute 8% of global annual eggs production and 2% of global annul poultry meat production (Mottet & Tempio, 2017). These estimates however widely vary depending on regions.

Small-scale free-range systems make significant contribution to eggs and poultry meat production in Eastern Europe, South Asia, Sub-Saharan Africa and to a lesser extent in East Asia, Latin America and the Caribbean (Mottet & Tempio, 2017). In sub Saharan Africa, more than 80% of the poultry production is of indigenous chicken reared mostly in small-scale and in free-range system (Dunya et al., 2015). Generally, chickens are reared on farms and transported to markets or slaughterhouses. Particularly in sub-Saharan Africa, collation of chicken from small-scale farms is necessary to attain critical masses for marketing or slaughter. This necessitates transportation of the marketed chicken to markets or slaughterhouses, making transportation a crucial activity along the value chain.

Globally, the number of poultry annually transported to markets or slaughterhouses is greater than any other livestock. Estimates by FAO indicated that 87% of annually transported poultry are broiler chicken, 1.1% are layers and 11.9% are other poultry species (Weeks, 2014). Studies on transported broilers records an average mortality of 0.2%, however this may vary greatly (Weeks, 2014). In Kenya, 30% of marketed indigenous chicken are sold directly to other farmers, 50% directly to final consumers within the locality, 15% to rural brokers and 5% to retailers and hotels (Okello et al., 2010). Basically, all marketed indigenous chicken in Kenya undergo some kind of transportation to the final consumer.

Compared to the developed countries of Europe, America and Asia, chicken transportation in the developing world is quite different. For instance, while animal transportation in the developed world provides services geared towards meat quality, transportation of live animals in the developing world especially in Africa does not always consider animal welfare and meat quality issues (Frimpong et al., 2014). Chicken are transported over very long distances on poor roads. Further, treatment of birds on transit especially the indigenous chicken often falls short of humane standards. Quite often, they are chased around at the farm during catching, predisposing them to fear and stress. Moreover, transportation vehicles are not specialized and are mostly unsuitable and birds are often transported in public service vehicles (Frimpong et al., 2014).

Additionally, the chickens are loaded onto public vehicle rooftops with no covers to protect the birds from the vagaries of the weather such as rain, high temperatures and high relative humidity (Frimpong et al., 2014). Besides, the birds are sometimes packed in overcrowded carriers and are exposed to long lairage times (Qi et al., 2017). In most cases, the birds are transported in two phases. First, individual birds or few birds are transported from farms to collection centers in small village markets for aggregation and onward transportation by road to major urban centers. From the farms, the birds are mainly carried in traditional containers, which are subsequently loaded on to either a motorbike, bicycle, human head, back or simply hanged on bikes, carts or carried by hand.

In Kenya, it is a common feature for indigenous chicken to be transported atop public service vehicles, bicycles, motorbikes and by hand. In the Eastern Kenya region, a study by Mutua (2018) indicated that 48% of indigenous chicken are loaded onto open roof top carriers of public vehicles for transportation to and from the markets. The study also showed that 10% are transported by bicycles, 24% by motorbikes and 18% by hand. As illustrated by Nyaga (2008), in most cases the indigenous chickens are not carried in specified containers but are simply tied together and hung onto the transportation vessel. This mode of transportation predisposes the indigenous chicken to stress through thermal variations and discomfort, which raises fundamental welfare concerns.

2.3 Transportation and Chicken Welfare

Having lived in relatively uniform environments, sudden changes induced by transportation practices such as withdrawal of food and water, exposure to vibrations, confinement, different micro-climatic conditions, noise or extreme light predispose chicken to unfavorable conditions (Sossidou et al., 2009). The major sources of transportation induced stress include extreme transportation ambient environment, transportation time, handling methods, state of the road, and starvation. Conditions such as high temperature, humidity, air speed and deprivation of food and water are major causes of thirst, heat stress, pain, hunger and even mortalities (Frimpong et al., 2014). Even though stress has been assumed as an invariable consequence of chicken transportation (Qi et al., 2017), stress during transportation can be minimized by improving transportation facilities and handling methods (Frimpong et al., 2014).

Welfare concerns are measured by physiological response indicators, which require an establishment of basal values of the indicators under various conditions (Sossidou et al., 2009). The extent of welfare effect is thus, defined by evaluating the difference between the basal value and the resultant value as well as the maximum absolute value resulting from the treatment. For instance when using change in body temperature due to transportation, marginal change in body temperature as well as the maximum deviation from the basal temperature at a particular time of the day is critical in estimating the total effect of transportation on the birds (Broom, 2008).

For transportation induced stress to occur in chicken during transit, relay of external stimuli (stress signals) follow two major physiological pathways in order to reach adrenal gland which is the main stress response organ (Vuuren, 2011). First is the Sympatho-adrenal Medullary System, which is an interactive physiological connection between sympathetic nervous systems and the adrenal medulla. When the body receives external sensory information, the sympathetic nervous system sends a signal, which activates the adrenal medulla leading to downstream glucocorticoid production by the adrenal cortex (Vuuren, 2011). This triggers a change in the bird's physiological, biochemical and behavior to respond to the effect of stress stimuli.

The second path involves the hypothalamic-pituitary-adrenal axis (HPA axis) which is an interactive neuroendocrine unit comprising of the hypothalamus, the pituitary gland, and the adrenal glands (Qi et al., 2017). Stimulation of the hypothalamus triggers a chain of hormonal secretions (Corticotrophin-releasing hormone (CRH) followed by Adrenocorticotropic hormone (ACTH)), which in turn circulates to adrenal cortex to stimulate the release of glucocorticoids into the bloodstream (Vuuren, 2011). Regardless of the path, transport stress influences parameters of bird physiological responses, like corticosterone (CORT), glucose and creatine kinase (CK) (Qi et al., 2017). Quantities of these substances in the blood stream thus acts as indicators for evaluating stress levels of the birds on transit (Mitchell & Kettlewell, 2009).

Several empirical studies have been conducted to explore the effects of transportation on chicken welfare, productivity and quality of the chicken products on different breeds under different environmental conditions. Lengkey et al. (2013) investigated the effects of transportation on broiler meat pH and tenderness in Bandung, Indonesia. The study indicated that prolonging transportation distances increases the meat pH but the increased pH values remain within the normal meat pH (5.2-6.6). However, the meat tenderness was decreased from 176.5 mm/g/10 sec when the birds were slaughtered on farm to 124.75 mm/g/10 sec when the birds were transported for 4 hours before slaughter.

A similar study by Bulitta (2015), in Ethiopia investigated the effects of handling as far as animal welfare is concerned during transport and marketing. The study-examined heart rates during loading, driving speed, road conditions and concluded that based on transport conditions, vibration levels, animal behavior, stress hormones and pH values, handling and transport had a negative effect on animal welfare (Bulitta, 2015). Further, a study by Jaya-prakash et al. (2016), explored the transportation in broiler chicken in India. The study identified thermal changes, acceleration, motion, vibration, fasting, withdrawal of water, social disruption, noise and internal vehicle thermal microenvironment as the major sources of stresses leading to loss of body weight and eventual mortalities. The study rec-ommended use of natural antioxidants such as vitamins A, C and E from plant material during transportation to alleviate the stress and improve birds' condition.

Additionally, Arikan et al. (2017), investigated total losses associated with the season, transportation distance, and slaughter age during the transportation of broilers from poultry farms to slaughterhouses in Turkey. The study indicated that losses increased with dis-tances, whereas total transportation losses in spring and winter were found to be relatively lower. The study showed that long distance transportation in the winter considerably in-crease total losses to levels similar to those obtained in the summer.

2.4 Empirical Literature Review

Previous empirical evidence is explored in this section to establish research gaps. The sec-tion is organized in line with the response parameters which define the study objectives. The indicators of the response are measurable in terms of the change in body temperature, blood pH and body weight.

2.4.1 Transportation Microenvironment and Chicken Body Temperature

Extreme temperatures have been identified as a potential cause of adverse effects on phys-iological response of chicken and other farm animals. Exposure to temperatures below freezing points or higher than room temperatures is a common cause of poor welfare in poultry. The relationship between environmental temperature and rectal and deep body temperatures has been previously studied in both heat-stressed and cold-stressed birds with

Broom (2008) recommending that chickens ambient temperature should be regulated to avoid substantial risk of high mortality and poor welfare.

A study by Knezacek et al. (2010) investigated temperature gradients in transport trailers and changes in broiler rectal and core body temperature during winter transportation in Saskatchewan, Canada. The study evaluated the effect of ambient temperature, crate temperature and journey length on change in rectal temperature and core body temperature. At 99% level of confidence, the study established a positive relationship between change in broiler rectal temperature and crate temperature (r^2=0.5737, p<0.0001) as well as journey length (r^2=0.3264, p=0.0002). However, the relationship between change in rectal temperature and ambient temperature was insignificant (r^2=0.2261, p=0.0103).

The study also showed an insignificant relationship between core body temperature and ambient temperature (r^2=0.2615, p<0.2522) as well as crate temperature (r^2=0.3136, p<0.1662). However, the study established a positive relationship between core body temperature and journey length (r^2=0.5634, p<0.0078). The study concluded that body temperature recordings indicated the potential for the development of both hypothermia and hyperthermia, showing that cold stress can occur near air inlets and heat stress in poorly ventilated areas (Knezacek et al., 2010). Despite the findings, the study was limited to broiler transportation under winter conditions in Canada. Further, the study only showed correlation rather than causation.

Dadgar et al. (2010) investigated effect of microclimate temperature during pre-slaughter transportation on broiler chicken meat quality. Assuming a normal average chicken temperature between 40.5 and 42.5 ℃, the study established that the birds transported at temperatures below 0 ℃ exhibited a significant (p< 0.0001) decrease of 0.8 ℃ in their core body temperatures (39.7°C) compared with the birds exposed to temperatures between 10 and 20 ℃ and above 20 ℃, which had similar core body temperatures of 40.5 and 40.7 ℃, respectively. Birds transported at temperatures between 0 and 10 ℃ also showed a significant small decrease of 0.3 ℃ in their core body temperature (40.2 ℃) compared with warmer temperatures tested. The study was however delimited to broilers and Canada.

Nazareno et al. (2016) sought to determine a model to predict mean surface temperature of broiler chicks and live load microclimate conditions during transport by using neural networks in the state of São Paulo, Brazil. The mean surface temperature of chicks was measured with an infrared thermometer in both loading and unloading stages. The transportation vehicle had a controlled thermal environment set to a temperature range between 23-25 °C and relative humidity between 60 and 70%. The study established mean surface temperature for shipment to be at 36.1 ℃, loading at 35.2 ℃ and unloading at 37.1°C). Maximum value of 38.2 ℃ was established for shipment, 37.2 ℃ for loading and 40.0°C for unloading. Minimum value of 32.7 ℃ was established for shipment, 31.8 ℃ for loading and 30.9 ℃ for unloading. The study concluded that mean surface temperature for shipment was within the recommended range (31.6- 36.9 ℃) while for mean surface temperature for load, only the maximum value was out of the optimum range of the average surface temperature. For mean surface temperature for unload, all values were out of the optimum range, indicating that the unloading caused thermal stress in the chicks (Nazareno et al., 2016). Despite being insightful, the study was based on broiler chicks carried in trucks with controlled temperatures and relative humidity.

Aldridge (2017) investigated thermal environment during transport of market age commercial broiler chickens in the South-Central Region of the USA with trips lasting between 60 and 125 minutes. The study found out that for the low ambient temperature trips (-16.4 to 2.8° C) controlled by plastic wraps, the temperature within the trailer for the first half of the trip was higher (p<0.05) than the temperature during the second half of trip. However, temperatures were 11.8 ℃ above ambient but remained below optimal conditions. Secondly, the study illustrated that for moderate ambient temperature (6.22- 23.35 ℃), first half temperatures were higher (<0.05) than that of the second half but the differences was significantly small with a mean difference of 0.17 ℃. Third, for transport within a range of 29.05 to 40.14 ℃, an increase (p<0.05) in temperature was seen from the first to second half of transportation duration. However, temperatures experienced by broilers were an average of 3.99 ℃ below ambient for the first half of the transport duration. Temperatures rose during the second half of transportation to only 1.63 ℃ below ambient. The study

concludes that optimal temperatures, to ensure the wellbeing of broilers, may not be maintained across the trailers depending on humidity, wind velocity, and duration (Aldridge, 2017).

A study by Cockram and Dulal (2018) shows that external temperatures >18°C can cause a steep increase in the broilers body temperature. Heat stress is aggravated by high relative humidity. For instance, relative humidity levels of 70%−80% can result in the onset of severe physiological stress at temperatures ≥25−26 °C (Mitchell & Kettlewell, 1998). Temperature of 38°C, relative humidity of 23% and air velocity of 2.2 ms^{-1} can be fatal to some birds (Chepete, 2008). Cockram and Dulal (2018) explain that under heat stress, the ability of the bird to lose heat from evaporative cooling is dependent on a gradient in temperature and (or) moisture between the bird and the surrounding environment. For example, at 2°C, evaporative cooling only represents 30% of heat loss, at 25 °C and 71% relative humidity, latent heat loss is more important than sensible heat loss, but at 30 °C and 90% relative humidity, latent heat loss is minimal.

In cold conditions, the lower critical temperatures for broilers is 24 ℃, below which the birds must reduce their heat loss and (or) increase heat production to maintain their body temperature and if the environmental temperature exceeds the capacity of the birds to maintain their body temperature, they become hypothermic and they will die when their body temperature decreases to 19 ℃ or 20 ℃ (Cockram & Dulal, 2018). From the literature review, when broilers are transported, the environmental conditions can affect the body temperature of the birds and even cause death on arrival.

2.4.2 Transportation Microenvironment and Chicken Blood pH
Transport induced heat or cold stresses have been shown to have varying degrees of effects on blood and muscle metabolism of chicken in various conditions. Particularly acute heat or cold stress is considered to provoke release of adrenaline in the blood causing significant change in the composition of blood and serum metabolites. For instance, stressful conditions may lead to depletion of muscle glycogen reserves causing higher ultimate pH values in meat and result in low residual levels of glucose (Tang et al., 2013). Several studies have

been conducted to investigate the effect of thermal stress on chicken blood pH. However, most of these studies have focused on effect on muscle pH.

A study by Dadgar et al. (2010) examined muscle pH of broilers transported under different temperature regimes. The study showed that breast meat pH and color values are affected by many pre-slaughter factors, including environmental temperature during transportation. The study established that ultimate pH for breast meat of birds exposed to temperatures below 0 ℃ was significantly higher (p< 0.0001; ultimate pH = 5.98) compared with ultimate pH for breast meat of birds exposed to temperatures between 0 and 20 ℃ (ultimate pH = 5.91). The ultimate pH of breast meat was significantly lower (p < 0.0001) by -0.1 unit for birds exposed to temperatures >20 ℃ with an average ultimate pH of 5.84 compared with the cooler temperatures. The study was however limited to broilers and Canada.

A follow up study by Dadgar et al. (2012) assessed the effect of acute cold exposure on broiler breast muscles and thigh muscles. The experiment was conducted on male birds at ages of 5 and 6 weeks. The birds were exposed to temperature ranges of −9 to −15 ℃ during a 3-hour transit. Control was set at 20 ℃ in a simulated transport chamber. After transportation, the birds were rested for 2 hours before slaughter. The study established 84% incidences of dark, firm, dry quality defect in thigh meat compared to 42% incidences on breast muscles. This showed that thigh muscles were affected more severely than breast muscle by exposure to cold temperatures during transportation. This study however insightful was based only on male birds.

A similar study by Hasan (2012) evaluated the influence of chicken transportation time and lairage before slaughtering on the occurrence of Pale, Soft and Exudative (PSE) meat and the quality of meat and canned meat processed locally under commercial transportation conditions in the Syrian winter. The study used 150 broiler chickens, which were submitted to 13-hour pre-slaughter fasting periods, transported on road in an open truck at 11°C for transport periods of 3-5 hours then subjected to lairage under natural ventilation for 2-4 hours before slaughtering. The study showed that the pH of the samples during 24 hours

postmortem showed that 80% of the samples were considered as normal meat (pH>5.80) and 20% of the samples were considered as PSE meat (pH = 5.8).

Sowiñska et al. (2013) investigated the effect of different variants of pre-slaughter procedures in the winter on body weight loss in broiler chickens, and on the proximate chemical composition and physicochemical properties of meat. The acidity (pH) of breast muscles was measured 15 minutes and pH 24 hours post mortem. A comparison of pH 15 minutes post mortem and pH 24 hours post mortem values in all groups revealed certain differences in the rate of glycolysis. Acidity decreased by 0.69 units in group transported for 300 km, compared with 0.62, 0.60 and 0.62 units in groups not transported, transported for 100 km and transported 200 km respectively. Indicating that transport distance has correlation with pH.

Castellini et al. (2016) evaluated the effect of transport length on *in vivo* oxidative status and breast meat characteristics in two chicken genotypes of naked necked and Rose 308 chickens reared under free-range conditions. The study suggested that transport for 4 hours prior to slaughter resulted in higher muscle pH, higher water retention and decreased antioxidant compounds such as vitamins E, A and xanthophylls. On average the pH of breast muscle 4 hour transported chickens showed higher muscle pH at 0 hours (6.875) compared to 2 hours (6.22) and 24 hours (6.155) post-mortem. However, average pH declines for 4 hour transported chickens after 24 hours' post-mortem was lower (0.72) than for 0 hour transported chickens after 24 hours' post-mortem (0.93). The study also indicated that naked necked birds showed lower pH values (0, 2 and 24 h) than Ross 308 strain mainly when immediately slaughtered. On the contrary, the pH decline was lower (0.74 versus 0.70) for the longer transportation. The study concluded that pH decline and the rigor mortis occur more rapidly in the breast of slow-growing strain (naked necked), mainly because they struggle more (Ross 308). The study was however delimited to Italy and was conducted in a highly controlled rearing condition.

Bonou et al. (2018) investigated the influence of pre-slaughter transportation duration stress on carcass meat quality of indigenous chicken reared under traditional system in

Benin. The study showed that pH of breast muscles was higher in the one and two hours transported birds compared to birds transported for 30 minutes. The same tendency was observed in the thigh muscles. The breast meat pH of the males was observed to be higher than for females. The same tendency was observed in the thigh muscles.

2.4.3 Transportation Microenvironment and Chicken Body Weight

Investigation into economic losses due to live weight shrinkage and mortality during transportation of 42 days old broilers in Turkey by Aral et al. (2014) revealed an average live weight shrinkage in broilers of 5.43% (minimum of 2.13% and maximum of 12.27%) after an average of 349 minutes of transportation. The study also showed that the live weight shrinkages increased (4.33%-6.63%) as the duration of transportation increases from 120 minutes to over 600 minutes respectively.

A study by Sowiñska et al. (2013) investigated effect of different variants of pre-slaughter procedures during winter period on body weight loss in broiler chickens, and on the proximate chemical composition and physicochemical properties of the meat. The results of the study conducted that elongation of transport period significantly influenced the increase of broiler weight loss. Transportation for 100 Km led to 1.41% weight loss while transportation for 200 km led to 2.36% weight loss while transportation for 300 km led to 2.65% weight loss.

A study by Li et al. (2017) examined transport induced stress on chicks. The study established that body weight decreased by 0.614 grams after 2 hours of transportation, 0.008 grams after 4 hours and 0.393 grams after 8 hours. Similarly, Arikan et al. (2017) analyzed the effects of transportation distance, slaughter age, and seasonal factors on total losses in broiler chickens in Brazil. The study reported an average live weight loss per broiler (grams/broiler) of 259.40 g, 307.35 g, and 350.14 g for short (≤50 km), medium (51-150 km) and long distances (≥151 km), respectively. This indicated that losses significantly increased with transportation. Broilers slaughtered at a younger age presented lower total losses than those slaughtered at an older age. The highest total loss was determined in the

summer, which was not statistically different from that for autumn. However, total transportation losses in spring and winter were found relatively lower. The study showed that long distance transportation in the winter considerably increased total losses to levels similar to those obtained in the summer. It must be noted that the study measured the losses inclusive of the excreta. The study also did not explicitly indicate the cause of the losses.

2.4.4 Transportation Microenvironment and Chicken Blood Hormones

Studies on the effect of stressors are predominantly illustrated by changes in plasma concentrations of corticosterone (CORT). However, while circulating concentrations of CORT are undoubtedly very useful in quantifying stress levels, it reliability has been is questioned since different studies report highly variable differences in findings (Scanes, 2016). Further it is questioned whether the techniques employed in detecting CORT have been adequately validated for chicken plasma/serum due to observable variability in the results.

For instance, levels of plasma concentrations of CORT in unstressed chickens vary over a very wide range. Kang and Kuenzel (2014) reported 0.05 ng/l as the basal CORT level in broiler chicken as determined by radioimmunoassay. Huth and Archer (2015) reported basal plasma corticosterone in broilers at 0.612 ± 0.1 ng/ml as determined by ELISA test. Olanrewaju et al. (2014) employed ELISA test and reported that plasma corticosterone of unstressed birds ranges between 1.783 ng/ml to 2.098 ng/ml. Mirfendereski and Jahanian (2015) using ELISA test reported 5.578 ng/ml for unstressed chicken while Zhang et al. (2009) reported basal CORT in broilers at 33.38 ng/ml as determined by ELISA. Similarly, plasma concentrations in stressed chickens also widely vary from as low as 0.23 ng/ml (following acute 1 h immobilization stress) (Kang & Kuenzel, 2014) to 2.022 ± 0.423 ng/ml (Huth & Archer, 2015) to 7.476 ng/ml (for desnsity induced stress-7 hens/cage) to 37.36 ng/ml (45 minutes transport with 45 minutes recovery) (Zhang et al., 2009).

Study by Zhang et al. (2009) investigated the effect of transport stress on blood metabolism, glycolytic potential, and meat quality in day old and 46 days old male broilers. The study established that transport time significantly affected plasma glucose level ($P < 0.05$)

and glycogen level ($P = 0.06$) in breast muscles. The study showed that glucose concentration increased slightly during the first 45 min of transport and then decreased dramatically in the long-term (3 hours) transported broilers ($P< 0.05$) while long-term transportation decreased the concentration of breast glycogen ($P = 0.06$). Meanwhile, long-term recovery after transport contributed to the homeostasis of blood corticosterone (CORT, $P= 0.05$) and low levels of glycogen ($P< 0.05$), lactate ($P< 0.01$), and glycolytic potential ($P< 0.01$) in thigh muscles. These findings however insightful were concluded based on muscle properties of male broilers and ducks.

These contradictions on the effect of transportation on blood serum parameters such as hormonal levels, glucose and pH has been attributed to lack of unified methods, measurement devices, measurement conditions and timings as suggested by (Qi et al., 2017). Therefore, since several studies provide contradictory findings a critical mass of empirical evidence is required for conclusivity.

2.4.5 Transportation Microenvironment and Chicken Behavioral Response

Environmental conditions including temperature and relative humidity of poultry transport vehicles, in general are not effectively controlled. Therefore, birds on transit are subjected to stressful conditions due to varying temperatures and relative humidity. Handling and transportation can cause stress to birds, ranging from mild discomfort, morbidity and aversion to death with the proportion of broiler chickens dead on arrival having been reported to vary from around 0.15% to 0.67% with the mortality rate increasing with the length of the journey (Chikwa et al., 2019).

Transportation environmental change induced stress in general, causes inestimable and negative impacts on the birds' welfare, growth, development, production, reproduction as well as decrease the quality of chicken and meat (Li et al., 2015). In response to changes in the environmental conditions, chicken like other warm-blooded animals utilize multiple ways for maintaining homeostasis when subjected to varying environmental conditions. Such responses include both physiological and behavioral responses (Chikwa et al., 2019).

Previous studies have attempted to characterize the behavioral and physiological responses of poultry on transportation in either field or lab conditions (Aldridge, et al., 2019). For instance, a study by Lara and Rostagno (2013) showed that under high temperature conditions, birds tend to alter their behavior seeking thermoregulation to decrease body temperature. The study identifies reduced feeding, increased drinking, increased panting, elevated wings and limited movements as some of the behavioral responses by birds under heat stress. These changes however vary in intensity and duration depending on individual birds and their breeds.

Similarly, a study by Li et al. (2015) investigated effects of heat stress on the daily behavior of day-old broiler chicks in Hainan, China. The results showed that, compared with the control group (kept at room temperature and relative humidity; density 8.33 birds/m^2), the duration and frequency of drinking and lying-down behaviors of chicks subjected to acute heat stress (Temperature of $40 \pm 0.5 °C$; Relative humidity of $82 \pm 6\%$) increased. Whereas, the duration of feeding and standing significantly decreased ($p < 0.01$). These results showed that heat stress significantly affects behavior of broiler chicks, including feeding, drinking, lying, standing, and walking.

Some of these behaviour responses have however been indicated to elicit an even more negative effect on the birds. For instance (Chikwa et al., 2019) argue that increased panting at high temperature causes accumulation of water and compromise its efficient evaporative heat loss and increases thermal load upon birds and the vicious cycle starts all over again. Similarly, Mitchell and Kettlewell (2009) argued that the thermoregulatory effort expended by the birds through panting causes respiratory overventilation, which produces excessive elimination of carbon dioxide and thus increases blood pH. Thus, at high thermal loads, thermoregulatory and acid-base homeostatic mechanisms may become antagonistic and severe acidbase disturbances may be superimposed upon the direct effects of hyperthermia (Mitchell & Kettlewell, 2009).

2.5 Summary of Literature Review and Research Gap

Empirical literature reviewed shows strong evidence that alteration of the normal microenvironment of chicken caused by transportation has a direct effect on physiological functioning of transit chicken. Transportation induced stress has been shown to influence transit chicken's body temperature (Cockram & Dulal, 2018; Dadgar et al., 2010; Knezacek et al., 2010; Nazareno et al., 2016). Likewise, transportation influences transit chicken's blood pH (Bonou et al., 2018; Castellini et al., 2016; Dadgar et al., 2010; Dadgar et al., 2012; Hasan, 2012; Sowiñska et al., 2013;). Further, transportation may lead to reduced weight loss in transit chicken (Aral et al., 2014; Arikan et al., 2017; Sowiñska et al., 2013).

Despite these findings being informative, majority of the studies on the effect of transportation on chicken have been conducted on intensively reared broilers (Aral et al., 2014; Arikan et al., 2017; Hasan, 2012; Knezacek et al., 2010; Nazareno et al., 2016). There are very few studies conducted on outdoor reared indigenous chicken (Bonou et al., 2018; Castellini et al., 2016).

Secondly, majority of the studies were conducted in temperate regions (Castellini et al., 2016; Dadgar et al., 2010; Dadgar et al., 2012; Knezacek et al., 2010; Sowiñska et al., 2013). The few empirical studies conducted in the tropics also do not focus on sub Saharan Africa (Aral et al., 2014; Arikan et al., 2017; Hasan, 2012) and particularly in Kenya and Eastern region of Kenya (Bonou et al., 2018).

Third, specific studies focusing on the effect of transportation on blood pH have primarily looked at post mortem muscle pH (Bonou et al., 2018; Castellini et al., 2016; Dadgar et al., 2010; Dadgar et al., 2012; Hasan, 2012; Sowiñska et al., 2013). Studies measuring pH change in the blood are far from adequate. In summary, validation of findings from studies done elsewhere is thus required. Hence, the current study sought to investigate the effect of transportation in indigenous chicken in Machakos County, Kenya.

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Study Area

The study was conducted in Machakos County. As shown in Figure 3.1, geographically Machakos County borders Nairobi and Kiambu counties to the West, Embu to the North, Kitui to the East, Makueni to the South, Kajiado to the South West, and Muranga and Kirinyaga counties to the North West. The rationale was to choose a County with the high density of indigenous chicken within the arid and semi-arid counties in Kenya. With indigenous chicken population of 1.4 million, Machakos County has 1.27 indigenous chicken per capita (GoK, 2018).
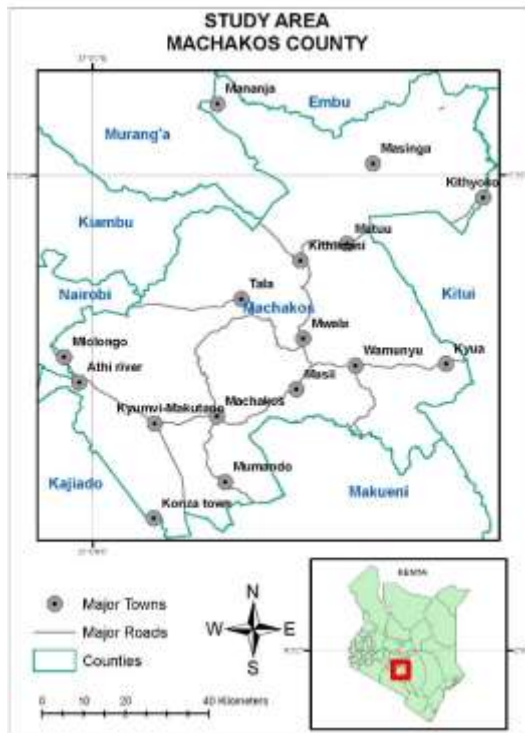
Figure 3.1: Machakos County
Source (GoK, 2017)

The local climate is semi-arid with an altitude ranging from 1,000 to 2,100 meters above sea level. The county has a bimodal rainfall pattern. The major rainy season is between October and December while the other rainy season is between March and May. The coldest month in the county is July while the warmest month is February (GoK, 2018). The average annual maximum temperature is 28 ℃, the average lowest temperature is 15 ℃ and the average mean temperature during most parts of the year is 25 ℃. The county enjoys an average annual relative humidity of 65%. (GoK, 2018).

3.2 Research Design

This study adopted an experimental research design with standardized procedures for data collection, management, analysis and reporting. Standardization ensured high internal validity or experimental control (Ross & Morrison, 2004).

3.3 Study Population

The general study population for this study comprised all indigenous chicken transported from Machakos County whether within or across the County. The 2018 annual livestock statistics report by the State Department of Livestock, Government of Kenya (GOK), indicated that at any given time, there were approximately 1.4 million indigenous chicken in Machakos County. Annually, approximately 435,000 of these birds are transported by road to various market destinations within and across the county (Okello et al., 2010). Hence, the target population comprised of 435,000 indigenous chicken from Machakos County, which are annually transported by road and marketed within and across the County.

In Machakos County, approximately 2.9 million indigenous chicken are marketed within and across the county (GoK, 2017). A study by Okello et al. (2010) established that farmers sell 30% of marketed indigenous chicken in rural Kenya directly to other farmers, 50% are sold directly to final consumers within the locality, 15% to rural brokers and 5% to retailers and hotels. The rural brokers then make 75% of their sales to urban brokers and 25% directly to final consumers.

According to the study, brokers mostly transport the live birds in open carriers of or inside passenger vehicles to their final destinations, (75% to urban brokers) and (25% to other consumers/hotels/retailers) (Okello et al., 2010). The target population therefore, comprised of approximately 15% (435,000) of the marketed indigenous chicken annually collected from Machakos County farmers and transported by vehicles across the County to major markets. The choice of the target population was informed by the assumption that the alternative means of transportation such as motorbikes, bicycles, ox/hand carts and hand are mostly used to cover relatively shorter distances and their impacts may not be as profound. The test birds were sourced from one farm in Katangi Ward, Machakos County.

3.4 Sample Size and Sampling Design

In animal research, determining of how many animals should be used in an experiment is a critical decision since a too small sample size can miss the real effect in an experiment, while a sample size that is larger than necessary will lead to ethical concerns on the animals' welfare (Arifin & Zahiruddin, 2017). Emphasis on power analysis approach to sample determination as the most scientifically favored method is well documented (Charan & Kantharia, 2013). Power-based sample size calculation formula is as follows.

Sample size = $2 \, SD^2 \, (Z^{\alpha/2} + Z^{\beta})^2/d^2$

Where;

SD= Standard deviation observed from previous studies

$Z^{\alpha/2} = Z^{0.05/2} = 1.96$ (from Z table) at Type I error of 5%

$Z^{\beta} = 0.842$ (from Z table) at 80% power (Type II Error)

d=effect size = difference between means

The formula is simplified as below.

Sample size = $2 \, SD^2 \, (1.96 + 0.842)^2/d^2 = 15.7 SD^2/d^2$

However, this formula requires prior knowledge of effect size (d^2) and standard deviation (SD^2). This information was not readily available for this study since most studies have been done in exotic breeds of chicken especially broilers under conditions not similar to the study context. Thus, the study adopted the "resource equation" approach as an alternative approach to calculating the sample size. As a rule of thumb, based on resource equation approach, for an independent t-test, the acceptable range of degree of freedom (df) for error term is between 10 and 20. According to Charan and Kantharia (2013), if df is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results.

However, according to Arifin and Zahiruddin (2017) resource equation for group comparison with repeated measures (one between and one within factors) there are two error degree of freedoms (DFs). The between-subject error degree of freedom and within-subject

error degree of freedom. The between-subject error degree of freedom is calculated as follows.

DF = N - k = kn - k = k (n - 1),

and the within-subject error degree of freedom is calculated as shown.

DF = (N - k) (r - 1) = (kn - k) (r - 1) = k (n - 1) (r - 1)

Hence, the error degree of freedom is the sum of these two dfs.

DF=Between-subject error + DF Within-subject error

DF=k(n−1) + k(n−1) (r−1) = k(n−1) (1+r−1) = kr(n−1)

Where;

N = total number of subjects

k = number of groups

n = number of subjects per group

r = number of repeated measurements

By rearranging the terms, n is obtained as DF/kr + 1

The current study used t-test to compare means between two groups (cage transportation and open roof top transportation) and with two repeat measures (pre-treatment, and post-treatment). Hence the sample sizes per group was calculated as shown below.

Minimum = 10/(2*2) + 1 = 3.5 rounded up to 4 birds/group

Maximum = 20/(2*2) +1 = 6 birds/group

In this study, between 4 and 6 birds were required per group to keep the DF within the acceptable range of 10 to 20. This was considered adequate to limit welfare concerns. Hence, 4 subjects (hen) were used to test differences between treatments for mean body temperature, blood pH, weight, stress hormones and behavioral responses between two groups at an α level, $p = 0.05$. Studies show that transportation causes physiological responses in poultry with varying effects on different gender (Aarif et al., 2013; Khosravinia, 2015). A study by Abioja et al. (2020), under humid tropical conditions indicated that based on heat stress index, female indigenous chickens are more prone to stress than the males due to the different physiological make-up of the female chickens. Thus, the study was based on the most affected sex of the chicken.

In this study, the sampling unit comprised of the female indigenous chicken transported by road using vehicles across Machakos County while the experimental unit comprised of specific female indigenous chicken on which treatments were randomly assigned for data collection. Sampling of the route was purposive while sampling of the indigenous chicken involved in the experiment adopted complete randomized design. Kyua-Athi river tarmac road stretch of 109.5 km was selected. The choice of the route was to control for possible variability in the vibrations between tarmac and non-tarmac roads.

Complete randomized design was adopted in assigning treatments. In completely random design, there is random assignment of subjects to treatments, thereby eliminating any systematic errors (Ross & Morrison, 2004). The total sample was randomly divided into groups and different treatments applied to the groups, one treatment for each group with the rationale that if the treatments differ from each other then, the various treatment groups will have different mean values (Alkutubi et al., 2012). The fundamental assumption in complete randomized design was that each member of the population has a chance of participating in the experiment and each experimental unit has an equal and independent chance of receiving any one of the treatments. Likewise, observed values in any one group represent a random sample of all possible values of all experimental units under that particular treatment (Alkutubi et al., 2012).

3.5 Conceptual Framework

Independent Variable

Dépendent Variable

```
┌─────────────────────────┐                          ┌─────────────────────────────┐
│      Transportation     │                          │      Stress Response        │
│     Microenvironment    │                          │        Parameters           │
│ (Stress Inducing        │        H_{O1}            │  ┌───────────────────────┐  │
│  Parameters)            │ ─────────────────────▶   │  │     Changes in        │  │
│                         │                          │  │   Body Temperature    │  │
│  ┌───────────────────┐  │                          │  └───────────────────────┘  │
│  │                   │  │                          │                             │
│  │     Ambient       │  │        H_{O2}            │  ┌───────────────────────┐  │
│  │   Temperature     │  │ ─────────────────────▶   │  │     Change in         │  │
│  │                   │  │                          │  │     Blood PH          │  │
│  └───────────────────┘  │                          │  └───────────────────────┘  │
│                         │                          │                             │
│  ┌───────────────────┐  │        H_{O3}            │  ┌───────────────────────┐  │
│  │     Ambient       │  │ ─────────────────────▶   │  │     Change in         │  │
│  │ Relative Humidity │  │                          │  │    Body Weight        │  │
│  │                   │  │                          │  └───────────────────────┘  │
│  └───────────────────┘  │                          │                             │
│                         │        H_{O4}            │  ┌───────────────────────┐  │
│                         │ ─────────────────────▶   │  │     Change in         │  │
│  ┌───────────────────┐  │                          │  │  Cortisol & Serum     │  │
│  │     Ambient       │  │                          │  │      glucose          │  │
│  │    Air Speed      │  │                          │  └───────────────────────┘  │
│  │                   │  │        H_{O5}            │  ┌───────────────────────┐  │
│  └───────────────────┘  │ ─────────────────────▶   │  │     Change in         │  │
│                         │                          │  │     Behavior          │  │
│                         │                          │  │ (Panting, plumage,    │  │
│                         │                          │  │ agitation, posture,   │  │
│                         │                          │  │   movement)           │  │
└─────────────────────────┘                          │  └───────────────────────┘  │
                                                      └─────────────────────────────┘
```

The arrows are labelled H_{O1}, H_{O2}, H_{O3}, H_{O4}, and H_{O5}.

Figure 3.2: Conceptual Model

3.6 Research Procedure

3.6.1 Pilot Study

Before the actual study, a pilot study was conducted. A pilot study was a small-scale version of the study to test the proposed research protocols, data collection instruments, sample recruitment strategies, and other research techniques in preparation for the actual study (Hassan et al., 2006). A pilot study was critical testing the reliability of the data collection tools. Reliability defines the extent to which a measurement instrument provides stable and consistent result when repeated under constant conditions (Taherdoost, 2016). Test-retest reliability was measured by administering the test instruments twice on the same experimental units under the same conditions to test whether they return the same results. The pilot test further served to assess the inclusion and exclusion criteria of the experimental units, preparation of the reagents, instruments and treatments as well as training of researchers and research assistants (Junyong, 2017). The pilot study involved a full administration of the entire research protocol to help identify challenges in the research design and procedure for improvement.

According to Sorzanoa, Tabas-Madrid et al. (2017) pilot size somewhere between 5 and 20 animals is only appropriate to the extent that it generates adequate accuracy of mean estimate for constructing a confidence interval containing the true distributional parameter. Based on Arifin and Zahiruddin (2017) resource equation calculation for group comparison with repeated measure, pilot sample size of 3 test subjects was used to meet the minum threshold. The birds acted as their controls to create an understanding on the physiological changes post treatment. Hence, the comparison of pretreatment and post treatment.

3.6.2 Data Collection Procedure

Data collection involved measurements, observations and data analysis. Data was collected through measurement of the test variables. The study model was such that transport conditions such as the ambient temperature, relative humidity, air speed and transport time induce stress response parameters such as changes in body temperature, blood pH, body weight, stress hormones and behavior changes. Data collection was as described in Table 3.1.

Table 3.1: Treatment Design

Treatment 1	Treatment 2	Control
4 birds tied together and loaded on an open roof top of the transport vehicle	**4** birds loaded into transport crate and the crate loaded on an open roof top of the transport vehicle	The birds acted as their own control by comparing reading before and after
Assumptions: Birds transported with and without transport crate experience different microenvironments		

N=Sample size per group

Equal number (4) of mature female indigenous chicken weighing between 1.75 and 2.4 kg were used. Completely Randomized Design, with two levels (at the beginning, T_0 and the end of the journey, T_1) was used to assign the treatments. Measurements of the ambient conditions for the treatments were as shown in Table 3.2. Similarly, ambient conditions were measured.

Table 3.2: Measurement of ambient conditions

Parameter	Point of Measurement	Frequency	Instrument
Ambient temperature	At bird level	Continuous	Automatic temperature monitor
Ambient air speed	At bird level	Continuous	Automatic Anemometer
Ambient Relative humidity g/m^3	At bird level	Continuous	Automatic relative humidity meter

Data Capture: Continuous recording of ambient temperature, relative humidity and air speed was achieved by use of programmed automatic data capture equipment. The data capture equipment were attached to a wire frame and clipped onto the front of the crates or just mounted next to the birds. The position of the equipment was to ensure that the conditions being monitored were at bird level. Start and end times were documented to establish experiment duration and average ambient conditions in terms of temperature, air

speed and humidity for the entire journey. The parameters and the instruments were as shown in Table 3.3

Table 3.3: Measurement of Physiological Response

Parameter	Point of Measurement	Frequency	Instrument
Body temperature	Rectum	*Time $_0$ and Time $_1$*	Thermistor
Blood pH	Blood samples	*Time $_0$ and Time $_1$*	Automated blood gas analyzer
Body weight	Live birds	*Time $_0$ and Time $_1$*	Weighting scale
Serum glucose	Blood samples	*Time $_0$ and Time $_1$*	Automated spectrophotometry
Cortisol	Blood samples	*Time $_0$ and Time $_1$*	ELISA Kits

Data Capture:

Temperature: Rectal temperatures of all the four (4) birds from each treatment were recorded immediately before and after transportation. An electronic temperature probe was inserted 3 cm into the cloaca of each bird until the temperature reading stabilized. The normal body temperature range of chicken of between 41- 42 ℃ was observed with variations between before and after treatment (Yosi et al., 2017).

Blood pH: As recommended by Owen (2011), blood for pH testing was drawn from the subclavian vein. An anticoagulant heparin (ethylene diamine tetra acetic acid (EDTA)) was added to the samples and kept in iceboxes for transportation to the lab for determination of pH values within 2 hours.

Weight: Birds were weighted using digital scales before and on arrival.

Cortisol and Serum glucose: Blood samples, containing anticoagulants heparin (EDTA) were placed in an icebox and transported to the lab for freezing within 2 hours for further serum analysis (hormonal and glucose tests). Illustrations on data captured and test equipment is as shown in Table 3.4.

Observations : Observation of the alignment of the bird plumage, lying position, level of agitation, refusal to move or jump on being released, presence of bruises or mortalities were recorded.

Table 3.4: Illustration of Data Capture & Analysis Equipment Specifications

Equipment	Specification
Thermohydrometer: Temperaure & Relative Humiduty **Make:** Sultron AT/RH Probes **Manufacturer**: Sultron Corporation (Virginia, USA)	**Accuracy:** RH: Up to 0.8 %RH, Temperature: Up to 0.1 °C (0.18 °F) Temperature measurement range: -70 ... +180 °C (-94 ... +356 °F) Probe and sensor warming functions minimize condensation on probe Sensor purge provides superior chemical resistance Traceable calibration certificate: 6 points for humidity, 1 point for temperature

Anemometer: Wind speed		
	Range	0 - 60 m/s (116 knots)
	Accuracy	±2% @12 m/s
Make: Gill Wind Sensors	Resolution	0.01 m/s (0.02 knots)
Manufacturer: Gill Instruments Ltd (Hampshire, UK)	Response Time	0.25 seconds
	Threshold	0.01 m/s
	Ultrasonic Output Rate	0.25, 0.5, 1, 2 or 4 Hz
	Parameters	Wind Speed & Direction or U and V (vec- tors)
	Units of Measure	m/s, knots, mph, kph, ft/min

Data Logger:	
Make: Sultron 9210 **Manufacturer**: Sultron Corporation (Virginia, USA)	**Measurement Interval:** 1.0 second to 24 hours (program-mable) **Measurements Supported**: Unlimited number **Analog Channels:** 10 **Range** **Single-Ended** 0-5 V, ±□78 mV (with respect to ground) **Differential** ± 2.5V, ±78 mV (+ input with respect to – input) **Accuracy**: 0.002% of 5V typ 0.003% of 78mV typ **Resolution:** 16 bit **Max Frequency:** Channel 1, 8KHz; other channels 1KHz **Output Type**: Open collector with 100-ohm current limiting resistor, 100 mA max, 15V max **Communications**: 4 Simultaneous **Operating Temperature** -40°C to +60°C

Equipment	Specification
	Power Requirements 10-16VDC (20VDC max)
Biochemistry Tests: Glucose, pH Make: Rayto RT 9200 semi auto chemistry analyser **Manufacturer**: RAYTO Life And Analytical Sciences Co., Ltd (Shenzhen, China)	**Absorbance Range**: -0.500-3.500 Abs **Light Source**: Halogen lamp **Wavelengths:** 340,405,500,546,620nm, **Wavelength Accuracy**: +/- 2nm **Resolution:** 0.001ABS (Displayed), 0.0001ABS (Calculated) **Band Width**: No More Than 10nm **Flow Cell: Metal** – quartz flow cell **Temperature Control**: 25°C, 30°C, 37°C; ±0.5°C and ambient temperature **Display**: LCD display **Outpu**t: Internal printer **Power Supply**: AC 110V/220V ± 10%, 50HZ/60Hz **Net Weight**: 7KG **Dimensions L x W x H (mm)**: 360 x 318 x 160
Weighing Scale: Weight **Make:** Von Hotpoint HESL05CS 50KG Luggage Weighing Scale **Manufacturer:** Hotpoint Appliances Limited (Nairobi, Kenya)	Electronic weighing Scale 50KGS capacity Accuracy: 0.01 Precision: ±0.005 Resolution: 0.01 Zero/Tare Weight Unit Conversion 50GMS division Blue Backlight Display for Button & LCD
Thermomistor: Body Temperature **Make:** Digital thermometer YB-009 **Manufacturer**: Oskyoo Technology Co. Limited (Shenzhen, Guangdong, China)	**Usage:** Orally, rectally, armpit Accuracy: ±0.1 ℃ Precision: 1 ℃ Range: 25 ° C to 50 ° C

Equipment	Specification
Assay Genie Chicken Cortisol (Cortisol) ELISA kit: Cortisol Make: **SKU:** CHEB0529 **Manufacturer:** Medical Supply Company LTD (Dublin, Ireland)	**Alias:** Cortisol, Cortisol, hydrocortisone **Sensitivity:** 0.23ng/ml **Range**: 1.56-100 ng/mL **Assay Type:** Competitive **ELISA Microplate (Dismountable):** 96 assays (8x12 strips)

3.7 Handling of Experimental Animals

In this study, four indigenous chickens were used per treatment group. That is, 4 subjects were used to test differences between treatments and control for effect of transportation on mean body temperature, blood pH, weight, stress hormones and behavioral responses between two groups at an α level, $p = 0.05$. Thus, the experimental unit comprised of specific indigenous chicken on which treatments were randomly assigned for data collection.

The test birds were rested, adequately fed and watered 24 hours before the experiment. According to Tamzil et al. (2019), giving chicken a resting time of 12 h after transportation restores hematological conditions and reduce the adverse effect of transportation stress. Treatment birds were allowed free movement. The treatment birds were gently caught weighed, temperature taken and blood samples taken before being placed either in the traditional transportation cage and loaded on the open roof top.

3.8 Treatment Procedure

The experiment involved subjecting the test birds to two conditions of transportation. One batch of 4 birds were packed in a traditional transportation cage while the other batch of 4 birds were bound and placed on open roof of transport vehicle.

Treatments: One batch of 4 birds were transported inside a traditional transportation cage (Plate 3.1 (a)). The other batch was tied on open vehicle top as shown in Plate 3.1 (b).

Plate 3.1 (a): Bird Transportation in the traditional cage

Plate 3.1 (b): Bird Transportation in the open roof top

Plate 3.1 (c): Transportation of birds using a "Matatu"

Plate 3.1 (c) is the public transportation system locally known as 'matatu' that is commonly used mode of bird transportation over long distances and was therefore used for this study.

3.8.1 Measurement of Ambient Condition

Measurement of Temperature & Relative Humidity was done using thermo-hydrometer mounted in the cage as shown in Plate 3.2 while anemometer was used to measure air speed as shown in Plate 3.3.

Plate 3.2: Placement of the Temperature gauge to determine the environmental temperature

Plate 3.3: Placement of Anemometer to determine air speed

3.8.2 Measurement of body temperature

Rectal temperatures of all the birds from each treatment were recorded immediately before and after transportation. An electronic temperature probe was inserted 3 cm into the cloaca of each bird until the temperature reading stabilized as shown in Plate 3.4.

Plate 3.4: Insertion of thermistor in the birds' cloaca to measure body temperature

Plate 3.5 picture is of a research assistant (veterinary doctor) taking body temperature of the test birds by inserting an electronic thermometer probe into the cloaca of the birds.

3.8.3 Measurement of Weight

Birds were weighed using digital scales before and on arrival as shown in Plate 3.5.

Plate 3.5: Taking of Weights using digital weighing scale

3.8.4 Drawing of blood for pH, glucose and hormone test:

Approximately 2 ml of blood was drawn from the subclavian vein using 3 ml syringes and 25-gauge needles. One ml was put into a serum vacutainer and another 1 ml into EDTA vacutainer for purposes of getting serum and plasma respectively. Blood extraction procedure is shown in Plate 3.6. approximately 2 ml of blood was drawn from the subclavian vein using 3 ml syringes and 25-gauge needles. One ml was put into a serum vacutainer and another 1 ml into EDTA vacutainer for purposes of getting serum and plasma respectively. The blood samples, were then placed in an icebox and transported to a lab in Chiromo for freezing within 2 hours for further serum analysis (hormonal and glucose tests) and plasma analysis (pH).

Plate 3.6: Drawing blood samples

Plate 3.6 is of a research assistant drawing sample blood from the ulna vein.

Plate 3.7: Sample handling

Plate 3.7 is a picture showing a research assistant (lab technician) putting blood samples into containers with anticoagulants (ethylene diamine tetra acetic acid (EDTA)) before transportation in a cool box. Banfi et al. (2007) recommed EDTA as the anticoagulant of choice for hematological testing because it allows the best preservation of cellular components and morphology of blood cells.

At the lab, the blood samples were centrifuged at 250 g force to obtain clear plasma and serum. About 0.3 -0.5 ml of serum or plasma was obtained. For cortisol, a validated cortisol kit for chicken was commercially obtained and plasma samples used for the test. The protocol was followed as the kit's manual. The procedure was carried out in a well-established lab for ELISA at University of Nairobi, Chiromo campus where all the necessary equipment (incubators, pipettes, distilled water, ELISA plate readers as well as ELISA program software) was readily available.

For glucose, standard glucometer was used and together with a positive sample/quality control sample from each bird was measured in duplicate and mean value obtained for each bird at each sampling point.

Plate 3.8: Glucometer

Plate 3.8 is a picture of glucometer used for reading glucose content in the blood while Plate 3.9 shows the pH measuring stripes used.

Plate 3.9: Reading of Plasma pH

3.8.5 Observations

Observation of the alignment of the bird plumage, lying position, level of agitation, refusal to move or jump on being released, presence of bruises or mortalities. These were recorded alongside the measures for more inferences.

3.9 Data Analysis Plan

Data analysis involved two models. One model testing the effect of each treatment (paired sample t-test) and the second model testing the difference between the treatments (independent sample t-test). Paired sample t-test entailed observations of the test variables before and after treatment on the same experimental units to test whether the mean difference between the two sets of observations (pre and post-treatments) is zero. The null hypothesis was that there was no difference in mean pre and post-treatments while the alternative hypothesis was that, there was a difference in mean for pre and post-treatments.

H_o:There is no difference in mean pre and post-treatments

H_a: There is a difference in mean pre and post-treatments

Evaluation criteria involved test of significance (p- value) at 95% level of confidence. The null hypothesis was rejected if p-value was less than 0.05 indicating strong evidence that the treatment caused a change in the mean of the test variables.

The second test model was a two-sample t-test with 2 types of treatments (**Treatment 1 and Treatment 2**). The null hypothesis was that there was no difference in mean between treatment 1 and treatment 2, while the alternative hypothesis was that, there was a significant difference between the treatments.

H_o:There is no significant difference between treatments

H_a: There is a significant difference between treatments

Evaluation criteria involved test of significance (p- value) at 95% level of confidence. The null hypothesis was rejected if p-value was less than 0.05 indicating strong evidence that there was a difference between the treatments.

3.10 Ethical Consideration

The study strived to handle the chicken as humanely as possible. No bird was subjected to treatments extraneous beyond the normal practice of transportation of chicken in the county. The birds were adequately watered, fed and rested prior to the journey and were released immediately on arrival to limit further discomfort post the journey. A permit was also sought from Kenya Society for the Protection and Care of Animals for oversight of the study.

4.0 RESULTS

4.1 Ambient Conditions

The results of the three ambient conditions of ambient temperature, relative humidity and wind speed are as follows.

4.1.1 Ambient Temperature

The temperature probe took continuous reading but reported average temperature for every 10 minutes and showed an average temperature of 30.1 ℃ during the journey. The graphical presentation is shown in Figure 4.1. The variations in the temperature were attributed to two factors. One was the time of the day. Between 1:26 pm and 2:26 pm, the position of the sun was directly above with the highest temperatures. This declined as the sun descended. However, drop in temperature was observed between 1:26 pm and 1:55 pm. This was attributed to terrain differences during the journey.
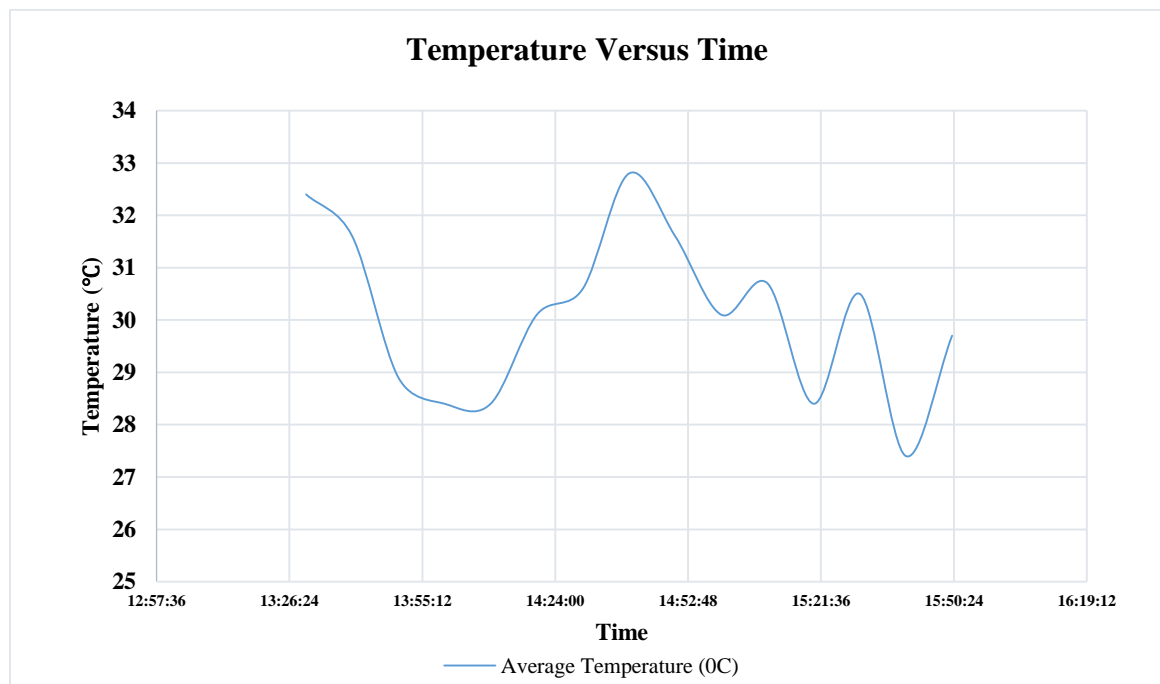
Figure 4.1: Graph of ambient temperature versus time during the journey

4.1.2 Ambient Relative Humidity

Relative humidity was continuously recorded with average relative humidity at 30.6% as shown in Figure 4.2.
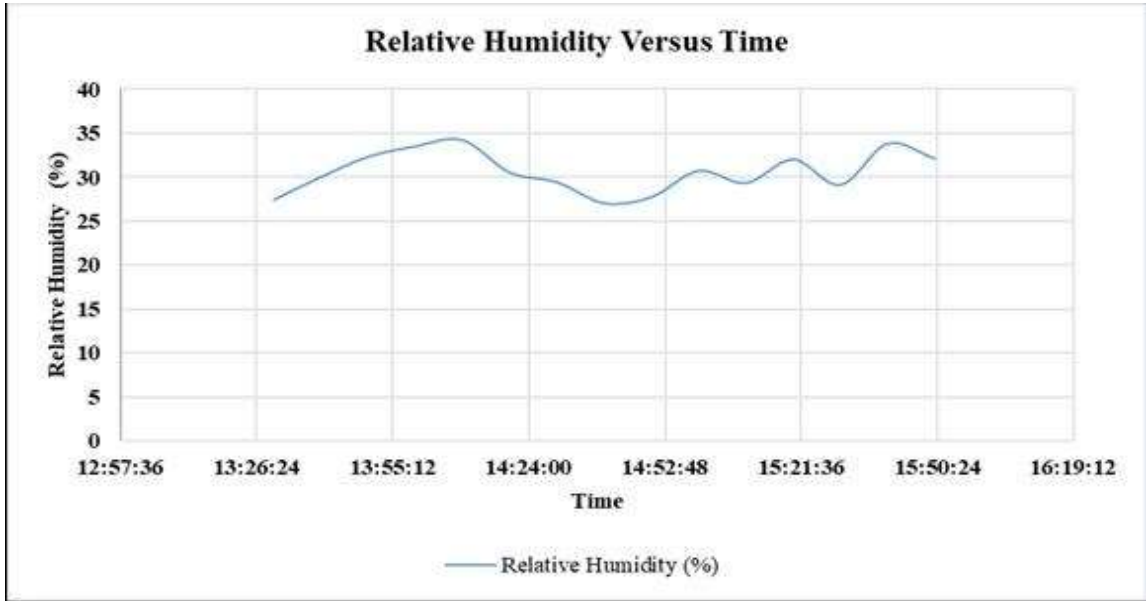

Figure 4.2: Graph of relative humidity versus time during the journey

4.1.3 Ambient Wind Speed

Wind speed reading was continuously measured by use of anemometer and 10 minutes averages were as shown in Figure 4.3. Average wind speed was 11.71 m/s
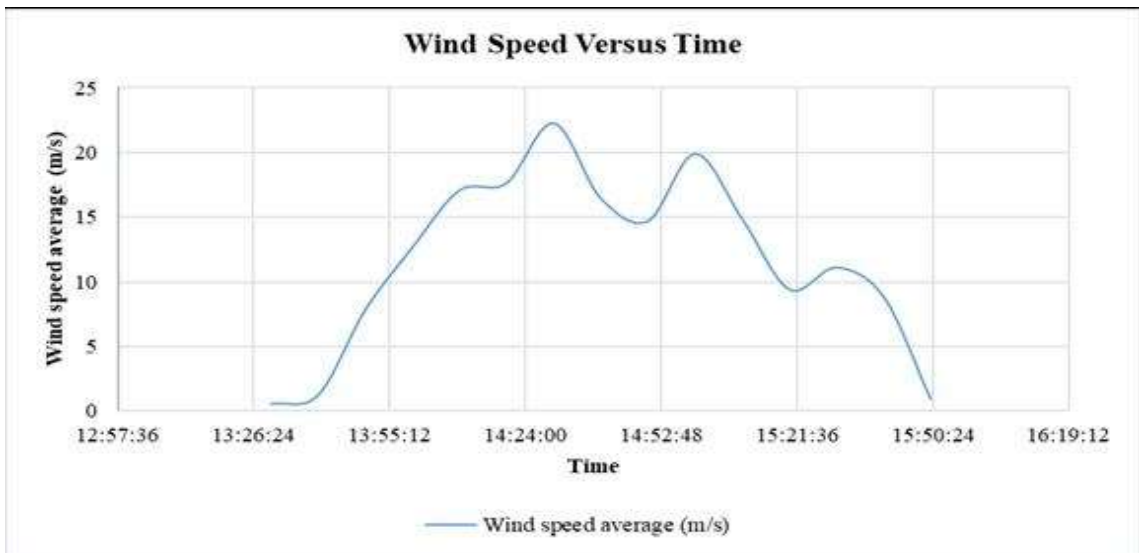
Figure 4.3: Graph of wind speed versus time during the journey

4.2 Effect of Transportation on Body Temperature

Table 4.1 shows that the mean temperature for treatment one (open roof top) increased by 0.05 °C while the mean temperature for treatment two (cage) increased by 0.6 °C. The finding shows an increase in temperature for both treatments however, treatment two had higher mean increase (0.6 °C) compared to treatment one (0.05 °C).

Table 4.1: Body temperature of the test birds before and after treatment

Parameter	Treatment 1 (No cage)		Treatment 2 (Caged)	
	T_0 (°C)	T_1 (°C)	T_0 (°C)	T_1 (°C)
Body Temperature	41.6	41.2	41.2	42.5
	41.6	41.1	41.8	42.7
	41.3	41.7	42.4	42.7
	41.1	41.8	42.5	42.4
Mean	**41.40**	**41.45**	**41.98**	**42.58**
Stdev	**0.24**	**0.35**	**0.60**	**0.15**
SEM (Stdev/(SQRT (N))	**0.12**	**0.18**	**0.30**	**0.08**
Coefficient of Variation	**0.6%**	**0.8%**	**1.4%**	**0.4%**

Student t-tests were run to determine whether the changes in observed mean temperature before transportation (T_0) and after transportation T_1 in the caged birds (Treatment 1) and the open birds (Treatment 2) were statistically significant. Table 4.2 shows that the mean difference within treatment one at time T_0 and T_1 was statistically insignificant, ($t = -0.1690$, $p = 0.08755$). Likewise, mean difference within treatment two at time T_0 and T_1 was statistically insignificant, ($t = -1.9298$, $p = 0.1492$). Further, mean difference between treatment one and treatment two at time T_0 was statistically insignificant, ($t = -1.7692$, $p = 0.1516$). However, there was a statistically significant difference between treatments one and two at time T_1 ($t = -5.8919$, $p = 0.0041$). Meaning, treatment two (cage) exhibited statistically significant higher mean change in body temperature at time T_1 (42.58 °C) compared to treatment one at time T_1 (41.55 °C). That is, while increase in body temperature was generally observed in the two treatments, birds transported in the traditional cage generally exhibited higher temperatures compared to the birds transported on the open roof top by an average of 0.55 °C.

Table 4.2: t-test for body temperatures between and within groups

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean (°C)	41.4	41.45	41.975	42.575	41.4	41.975	41.45	42.575
Variance (°C)	0.06	0.1233	0.3625	0.0225	0.06	0.3625	0.1233	0.0225
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	-0.9687		-0.0092		-	-	-	-
Hypothesized Mean Difference	0		0	0	0	0	0	0
df	3		3		4		4	
t Stat	-0.1690		-1.9298		-1.7692		-5.8919	
P(T<=t) two-tail	**0.8765**		**0.1492**		**0.1516**		0.0041	
t Critical two-tail	3.1824		3.1824		2.7764		2.7764	

4.3 Effect of Transportation on Blood pH

Findings in Table 4.3 indicates that only one subject under treatment one registered a decline in blood pH at time T_1 (from 8 to 7.5). Similarly, only one subject under treatment two registered a decline in blood pH at time T_1 (from 8 to 7.5).

Table 4.3: Blood pH values for the test birds before and after treatment

Parameter	Treatment 1 (No cage)		Treatment 2 (Caged)	
	T_0	T_1	T_0	T_1
Blood pH	8	7.5	8	8
	8	8	8	7.5
	8	8	8	8
	8	8	8	8
Mean	**8**	**7.875**	**8**	**7.875**
Stdev	**0**	**0.25**	**0**	**0.25**
SEM (Stdev/(SQRT (N))	**0.00**	**0.13**	**0.00**	**0.13**
Coefficient of Variation	**0.0%**	**3.2%**	**0.0%**	**3.2%**

Student t-tests were run to determine whether there was a statistically significant difference in the mean blood pH within and between treatments. The findings in Table 4.4 indicates

statistically insignificant (p>0.05) difference within and between treatments. This indicated that the transportation had no significant effect on the blood pH.

Table 4.4: t-test for blood pH values between and within groups

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean	8	7.875	8	7.875	8	8	7.875	7.875
Variance	0	0.063	0	0.063	0	0	0.063	0.063
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	-		-		-		-	
Hypothesized Mean Difference	0		0		0		0	
df	3		3		6		6	
t Stat	1		1		N/A		0	
P(T<=t) two-tail	**0.3910**		**0.3910**		**N/A**		**1**	
t Critical two-tail	3.1824		3.1824		N/A		2.447	

4.4 Effect of Transportation on Body Weight

Table 4.5 shows an overall decrease in the mean body weights for the test subjects after treatment one at time T_1 (0.04 kg) as well as after treatment two at time T_1 (0.03 kg).

Table 4.5: Body weight of the test birds before and after treatment

Parameter	Treatment 1 (No cage)		Treatment 2 (Caged)	
	T_0 (Kg)	T_1 (Kg)	T_0 (Kg)	T_1 (Kg)
Body Weight	2.05	1.9	2.3	2.15
	2.4	2.5	1.7	1.35
	2.5	2.45	1.5	1.5
	2.2	2.15	1.25	1.65
Mean	**2.29**	**2.25**	**1.69**	**1.66**
Stdev	**0.202**	**0.280**	**0.448**	**0.347**
SEM (Stdev/(SQRT (N))	**0.10**	**0.14**	**0.22**	**0.17**
Coefficient of Variation	**8.8%**	**12.4%**	**26.5%**	**20.9%**

Student t-tests were run to determine whether there was a statistically significant difference within and between treatments. Table 4.6 indicates statistically significant difference between the treatments one and two at time T_1 ($t = 2.6342$, $p = 0.0388$). Meaning, birds transported on an open roof top had a higher mean loss of body weight (0.04 kg) compared to birds transported in the cages (0.03 kg). That is, birds transported onboard an open roof top lose on average 10 grams more in body weight than birds transported in the traditional cages.

Table 4.6: t-test for body weight between and within groups

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean (Kg)	2.2875	2.25	1.6875	1.6625	2.2875	1.6875	2.25	1.6625
Variance (Kg)	0.0406	0.0783	0.2006	0.1206	0.0406	0.2006	0.0783	0.1206
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	0.9602		0.7084		-		-	
Hypothesized Mean Difference	0		0		0		0	
df	3		3		4		6	
t Stat	0.7276		0.1575		2.4431		2.6342	
P(T<=t) two-tail	0.5195		0.8849		0.0710		0.0388	
t Critical two-tail	3.1824		3.1824		2.7764		2.4469	

4.5 Effect of Transportation on Plasma Glucose

Table 4.7 shows that the mean plasma glucose after treatment one at time T_1 increased by 5.05 mmol/l while that for treatment two at time T_1 increased by 1.88 mmol/l. The significance of the differences is presented in Table 4.7.

Table 4.7: Plasma glucose values for the test birds before and after treatment

Parameter	Treatment 1 (No cage)		Treatment 2 (Caged)	
	T_0 (mmol/l)	T_1 (mmol/l)	T_0 (mmol/l)	T_1 (mmol/l)
Glucose	22.3	19	10.3	21.7
	21.3	20.3	20.4	18.6
	15.0	25.7	16.2	20.1
	3.9	17.7	22.3	16.3
Mean	15.63	20.68	17.30	19.18
Stdev	3.891	3.514	3.865	2.297
SEM (Stdev/(SQRT (N))	1.95	1.76	1.93	1.15
Coefficient of Variation	24.9%	17.0%	22.3%	12.0%

Table 4.8 showed statistically insignificant difference within and between treatment at alpha level of 0.05.

Table 4.8: t-test for plasma glucose between and within groups

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean (mmol/l)	15.625	20.675	17.3	19.175	15.625	17.3	20.675	19.175
Variance (mmol/l)	71.5425	12.3492	28.2733	5.2758	71.543	28.273	12.349	5.276
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	0.2067		-0.9511		-		-	
Hypothesized Mean Difference	0		0		0		0	
df	3		3		5		5	
t Stat	-1.1936		-0.4977		-0.3353		0.7146	
P(T<=t) two-tail	**0.3184**		**0.6529**		**0.7510**		**0.5068**	
t Critical two-tail	3.1824		3.1824		2.5706		2.5706	

4.6 Effect of Transportation on Serum Cortisol

Findings in Table 4.9 show an increase in the mean serum cortisol level for treatment one at time T_1 by 28.1 ng/ml and a mean increase in the serum cortisol level for treatment two at time T_1 by 2.05 ng/ml.

Table 4.9: Serum cortisol values for test birds before and after treatment

Parameter	Treatment 1 (No cage)		Treatment 2 (Caged)	
	T_0 (ng/ml)	T_1 (ng/ml)	T_0 (ng/ml)	T_1 (ng/ml)
Cortisol	27.1	132	28.8	47.1
	81.5	94.0	78.6	61.5
	86.3	91.7	60.5	76.3
	94.0	83.6	72.8	64.0
Mean	**72.23**	**100.33**	**60.18**	**62.23**
Stdev	**17.498**	**21.583**	**15.137**	**11.980**
SEM (Stdev/(SQRT (N))	**8.75**	**10.79**	**7.57**	**5.99**
Coefficient of Variation	**24.2%**	**21.5%**	**25.2%**	**19.3%**

Comparing the means, Table 4.10 indicates a statistically significant difference between treatment one and treatment two at time T_1 (t=3.0870, p=0.0273). The mean difference was 26.05 ng/ml. That is, treatment one resulted in higher values of serum cortisol compared to treatment two by an average of 26.05 ng/ml. Thus, while both treatments led to an increased quantity of serum cortisol in the test subjects, on average birds transported on open roof top recorded higher values by 26.05 ng/ml compared to birds transported in the traditional cages. The wide variation in cortisol value for bird number one under treatment 1 was attributed to individual characteristic with regards to response to stress.

Table 4.10: t-test for Serum Cortisol between and within groups

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean (ng/ml)	72.225	100.325	60.175	62.225	72.225	60.175	100.325	62.225
Variance (ng/ml)	931.52	465.81	494.46	143.52	931.516	494.456	465.809	143.516
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	-0.9987		0.6109		-		-	
Hypothesized Mean Difference	0		0		0		0	
df	3		3		5		5	
t Stat	-1.0790		-0.2319		0.6382		3.0870	
P(T<=t) two-tail	**0.3596**		**0.8315**		**0.5514**		0.0273	
t Critical two-tail	3.1824		3.1824		2.5706		2.5706	

4.7 Effect of Transportation on Behavior

Physical observations were carried out on the test birds during the experiment. For treatment 1, the birds were visibly tired with increased panting, rough feathers and closed eyes. Plate 4.1 shows panting of bird with open beak while Plate 4.2 shows a bird with closed eyes. Minor bruises were also observed on the legs where the birds were tied. Further, the birds appeared tired. No mortalities were recorded. For treatment two, the birds remained fairly alert and active compared to those in treatment one.

Plate 4.1: Panting bird

Plate 4.2: Bird with closed eye at the end of the journey

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Transportation on Body Temperature

Considering the normal range of normal chicken body temperature of between 40.5 and 42.5 ℃ (Dadgar et al., 2010), the study established that comparatively, birds transported on the open vehicle rooftop had lower body temperature post treatment than birds transported in the traditional cages. Although an increase in body temperature was generally observed in the two treatments, birds transported in the traditional cage generally exhibited higher temperatures compared to the birds transported on the open rooftop. This could be attributed to the fact that within the cage, the birds were much closer to each other and had comparatively lower exposure to the environmental elements such as wind and direct sunlight. This may have led to comparatively higher heat retention within the cage resulting in higher mean body temperatures for the birds in the cage.

On the contrary, birds transported on the open vehicle rooftop, were comparatively more exposed to wind and direct sunlight. This creates a condition for higher evaporative heat loss and cooling, leading to comparatively lower body temperature. It is postulated that under heat stress, the ability of the bird to lose heat from evaporative cooling is dependent on a gradient in temperature and (or) moisture between the bird and the surrounding environment (Cockram & Dulal, 2018). Thus, micro climate between the birds transported in the open was significantly different from those transpotted in the cage resulting into significant difference in mean body temperatures between the two treatments.

The findings are in concurrence with Dadgar et al. (2010) to the extent that different transport micro climates have significantly different effects on chicken body temperature. Hence, while transport within high ambient temperature ranges (> 29.05 ℃) is expected to result in higher mean body temperature (Aldridge, 2017), this is dependent on relative humidity, wind speed, exposure time and the birds' ability to lose heat from evaporative cooling.

Nonetheless, since both treatments lead to higher mean body temperature, this triggered discomfort, an indicator of an onset of stress in the birds. Indeed, the way the birds were transported violated the second animal welfare freedom i.e. freedom from thermal and physical discomfort (Webster, 2016). Hence, when transporting birds in traditional cages, adequate spacing should be considered to reduce heat accumulation. For warmer temperatures, recommended cage floor is 0.075 square meters per bird (Mangnale, Desai, Ranade, & Avari, 2019) with a head space of 0.356 meters in layers (Kiess, Hester, Mench, Newberry, & Garner, 2012).

The actual size of the traditional cage used was 0.0447 square meters per bird and height of 0.24 meters. This was so since the study was exploratory with respect to indigenous chicken in Machakos County. Hence, the actual practice by traders was tested by using the generic traditional cages normally used to establish the actual effects.

The findings also showed a positive change in the mean temperature of indigenous chicken transported on the open roof top from 41.2 ℃ to 41.45 ℃. This was within the normal range of normal chicken body temperature (40.5- 42.5 ℃) (Dadgar et al., 2010). Compared to mean temperature change in the birds transported in the cage, the observed change was minimal. Nevertheless, use of standard cages is still recommended.

5.2 Effect of Transportation on Blood pH

Transport induced stresses have been shown to have varying degrees of effects on muscle, blood and serum metabolites of chicken (Tang et al., 2013). The study indicated that transportation had no significant effect on the blood pH for both treatments (birds transported in the traditional cage and ones transported in the open vehicle roof top). It is believed that transportation particularly acute heat or cold stress during chicken transportation can provoke release of adrenaline in the blood causing significant change in the composition of blood and serum metabolites (Hasan, 2012). For instance, stressful transport conditions may lead to depletion of muscle glycogen reserves causing higher pH values (Tang et al., 2013).

It is noteworthy as observed by Sowiñska et al. (2013), that transport related change in chicken blood pH due to transportation is greatly dependent on exposure time. The study by Sowiñska et al. (2013) established an acidity decrease by 0.69 units in broiler chicken transported for 300 km, compared to 0.62, 0.60 and 0.62 units in broiler chicken groups not transported, transported for 100 km and transported 200 km respectively. Indicating that transport distance has a correlation with change in blood pH.

Transportation was expected to significantly affect blood plasma glucose level and glycogen level in blood and muscles (Zhang et al., 2009). This means that longer transportation time was expected to lead to depletion of glycogen reserves causing higher pH values. However, a study by Castellini et al. (2016) provided conflicting results by indicating a reduced pH of breast muscle for 4 hour transported chickens. Further to this, the reduction in pH seemed to be strain specific with naked neck birds exhibiting relatively lower breast muscle pH values post 4-hour transportation compared to Ross 308 strain of chicken (Castellini et al., 2016).

Although the current study that was done in under 2 hours did not demonstrate any significant change in the blood pH for both treatments. This is contrary to findings by Bonou et al. (2018) which established a significant difference in chicken blood pH after 1 hour and 2 hours transportation. Further, Li et al. (2017) also established a significant change in chicken blood metabolites and body weight after 2 hours transportation. On the contrary, Fernandez et al. (2011) reported that 30 to150 minutes transport duration had little influence on blood serum parameters and muscle pH.

Meaning, the time threshold for triggering change in blood metabolites is further moderated by the study context including prevailing ambient conditions, transport containers, vehicle type and road surface. Therefore, it is critical to take into account how long the birds are transported without rest to in conjuction with the other moderating variables to lessen the onset of pH change in the chicken blood. This calls for limiting transportation of chicken as much as possible to reduce potential impact on blood glucose, glycogen and pH levels.

Further, for long journeys, rests and recovery periods should be considered along the journey.

5.3 Effect of Transportation on Body Weight

Shrinkage in live weight during transportation has been reported in other breeds of chicken (Li et al., 2012; Sowinska et al., 2013; Aral, eta al., 2014; Arikan et al., 2017). A shrinkage in weight in broilers of up to 5.43 g was reported after an average of 349 minutes of transportation (Aral et al., 2014). Increased live weight shrinkages has also been reported with increase in the distance covered (Sowiñska et al., 2013). The current study showed an overall decrease in the mean body weights after transportation. However, treatment one (open roof) had a statitically significant higher mean loss of body weight compared to treatment 2 (cage), which had a lower and insignificant difference i.e. birds transported onboard an open roof top lost more weight than birds transported in the traditional cages.

These findings are in line with findings by Aral et al. (2014); Sowiñska et al. (2013) and Li et al. (2017), where open transportation causes body weight loss in chicken. This loss is attributed to evaporative loss of body moisture and dehydration. In response to heat stress due to high environmental temperatures, chicken like all other warm-blooded animals respond by initiating both physiological and behavioral responses seeking thermoregulation (Chikwa et al., 2019), including evaporative cooling. Evaporative cooling if excessive can lead to loss of body weight. Thus, this observed weight loss is linked to evaporative cooling.

Considering that birds on the roof top were more exposed to wind and direct sunlight, the evaporative cooling and weight loss was comparatively higher and significant compared to than birds transported in the traditional cages, which were somehow sheltered from the direct environmental elements (wind and direct sunlight). Nonetheless, the way the indigenous chickens were transported in this experiment triggered violation of the freedom from thirst and freedom from thermal and physical discomfort (Webster, 2016)).

5.4 Effect of Transportation on Cortisol

Studies on the effect of transportation on chicken blood cortisol are conflicting. Whereas some show increase in blood cortisol post transportation (Scanes, 2016; Zhang et al., 2009), others show little or no effect (Fernandez et al., 2011). The current study showed an increase in the mean serum cortisol level for treatment one (open roof top) and a mean increase in the serum cortisol level for treatment two (traditional cage). A comparison of the means showed statistically significant differences between treatment one and treatment two at the end of the journey. Thus, while both treatments led to an increased quantity of serum cortisol in the test subjects, birds transported on open roof top recorded higher values compared to birds transported in the traditional cages.

The findings of an overall increase in cortisol level on birds after transportation is in line with studies by Zhang et al. (2009); Kang and Kuenzel (2014), and Huth & Archer (2015). It has been shown that when chicken are exposed to stressful situation, particularly fear related discomfort, their nervous systems respond by releasing cortisol among other hormones from the adrenal gland (Qi et al., 2017). These hormones are to prepare the birds' response by stimulating glycogenesis for release of energy into the birds' muscles and blood (Tang, Yu, Zhang & Bao, 2013) in readiness for flight.

This means that transportatiuon stress in this experiment violated the fourth animal welfare freedom. That is, freedom from fear and distress (Webster, 2016). The fact that birds transported on the open vehicle roof top had higher cortisol levels compared to birds transported in the traditional cage signifies higher level of distress when birds are transported in the open. Thus, use of standard cages would be suitable to limit the level of this stress.

5.5 Effect of Transportation on Behavior

In response to adverse changes in the environmental conditions, birds utilize multiple ways for maintaining homeostasis (Chikwa et al., 2019). For instance, in response to heat stress,

the birds normally respond by increasing panting, elevating their wings, reducing movement or reducing feeding to seek thermoregulation of their bodies (Lara & Rostagno, 2013).

Physical observations for treatment one (open roof top) showed that the birds were visibly tired with increased panting, rough feathers and closed eyes. Minor bruises were also observed on the legs on the sections where the birds were tied. Other than appearing tired and slow in movement when released, there were no adverse difference in behavior between treatment one and two. No mortalities were recorded. For treatment two, the birds remained fairly alert and active compared to those in treatment one. These were signs of behaviour adjustments to cope with adverse enviromental conditions. The average ambient temperature of 30.1 ℃ was well above the normal room temperature. Likewise, average wind speeds of 11.71 m/s.

These changes in behavior are related to poor transport condition and heat stress. This means that the transport condition violated the animal welfare rights of freedom from thirst, thermal and physical discomfort, pain and injury, and freedom to express normal behavior. These observations were in line with observations by Chikwa et al. (2019) that birds on transit are subjected to stressful conditions due to varying temperatures and relative humidity. This transportation induced stress can cause stress to birds, ranging from mild discomfort, morbidity and aversion to death leading to adaptive behavior response such as changes such as increased panting, elevated wings and limited movements (lying-down) (Li, Wu & Chen, 2015).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study concludes that comparatively, as demonstrated by the changes in the response indicators of body temperature, body weight, blood cortisol and behaviour, open roof top transportation has an adverse effect on the welfare of the indigenous chicken. It leads to violation of animal welfare concerns of freedoms from thirst; thermal and physical discomfort; fear and distress; pain and injury; and freedom to express normal behavior.

Specifically, the study makes the following conclusions. First, transportation of indigenous chicken whether in cages or open vehicle roof top lead to increased chicken body temperature. However, in comparison, transportation in small unstandardized cages lead to statistically higher temperatures than on the open roof top. Secondly, transportation for 2 hours has minimal effect on blood pH for indigenous chicken under the transportation conditions for the experiment. Thirdly, transportation of indigenous chicken whether in traditional cages or on open vehicle top lead to decreased body weight. However, comparatively, the change in body weight for birds transported without the cage is higher than for birds transported in the cage.

Fourth, transportation of indigenous chicken triggers release of more cortisol in the blood but the effect is more pronounced in the birds transported in the open roof top. That is, while both treatments lead to an increased quantity of serum cortisol, birds transported on open roof top on average records significantly higher values compared to birds transported in the traditional cages. Lastly. transportation of indigenous chicken causes behaviour changes such as increased panting, roughening of feathers and closing of eyes with visible fatigue.

6.2 Recommendations for Practice

i. Transportation cages should be designed, constructed and fitted properly to ensure sufficient floor and head space to allow the chicken to sit comforably and evenly distributed during transportation as appropriate for the chicken size and weight

ii.	Care should be taken to protect the birds from adverse temperatures and direct sunlight as well as wind. Avoid loading and transportation during the hottest time of the day.

iii.	The transportation cages should be adequately ventilated to meet the thermoregulation conditions of the birds at all times during transportation.

iv.	Where possible, transportation time should be limited as much as possible to reduce potential adverse effects on blood glucose, glycogen and pH levels.

v.	For long journeys, recovery periods should be instituted along the journey.

vi.	Proper use, loading and fastening of cages onto the transport vehicle should be encouraged as the study showed that transportation on the open roof top leads to increased panting, rough feathers and closed eyes and visible fatigue compared to cage transportation.

vii.	For government agencies, the study recommends full enforcment of regulations on standard chicken transportation to safeguard on their welfare.

6.3 Future Research Areas

The study was carried out in the mid-afternoon (1:30 pm and 3:30 pm) when temperature was at its highest. Thus, comparative studies carried out at different times of the day such as early mornings and late afternoon are recommended to provide additional insights with respect to the time of day.

The study was cross-sectional; hence additional studies at various times of the year would also be insightful for drawing conclusions.

Further, the current study only focused on female indigenous chicken since it has been shown that indigenous chicken hens are more prone to stress than the cocks under humid tropical conditions due to the different physiological make-up of the female chickens (Abioja et al., 2020). Comparative studies using cocks is highly recommended.

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APPENDICES

Appendix A: Research Tool

<table>
<tr><th colspan="5">Transport/Control Microenvironment</th></tr>
<tr><td></td><td colspan="4">Average</td></tr>
<tr><td>Average Ambient Temperature</td><td colspan="4"></td></tr>
<tr><td>Average Ambient Relative Humidity</td><td colspan="4"></td></tr>
<tr><td>Average Ambient Air Speed</td><td colspan="4"></td></tr>
<tr><td>Average vehicle speed</td><td colspan="4"></td></tr>
<tr><th colspan="5">Response Indicators</th></tr>
<tr><td></td><td colspan="2">Treatment 1</td><td colspan="2">Treatment 2</td></tr>
<tr><td>Parameter</td><td>T_0</td><td>T_1</td><td>T_0</td><td>T_1</td></tr>
<tr><td>Body Temperature</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Blood pH</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Body weight</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">NB: Measurements will be disaggregated using sex (Male: Female)</td></tr>
</table>

Appendix B: Observation Form

Parameter	Description
Plumage	
Lying position	
Agitation	
Movement	
Bruises	
Mortalities	

Appendix C: Ambient Conditions Data Readings

a) Temperature Readings

Date	Time	Average (°C)	Max (°C)	Min (°C)
10/23/2020	13:30:00	32.4	34.3	31.4
10/23/2020	13:40:00	31.6	34	31.6
10/23/2020	13:50:00	28.9	30.7	26.8
10/23/2020	14:00:00	28.4	29.5	27
10/23/2020	14:10:00	28.4	30	28.2
10/23/2020	14:20:00	30.1	31.3	28
10/23/2020	14:30:00	30.6	31.7	30.3
10/23/2020	14:40:00	32.8	32.8	29.7
10/23/2020	14:50:00	31.6	32.4	29.7
10/23/2020	15:00:00	30.1	31.8	30.1
10/23/2020	15:10:00	30.7	31.4	29.3
10/23/2020	15:20:00	28.4	31.5	28.4
10/23/2020	15:30:00	30.5	30.9	29.4
10/23/2020	15:40:00	27.4	30.5	27.4
10/23/2020	15:50:00	29.7	29.7	27

b) Relative Humidity (RH) Readings

Date	Time	RH	Unit
10/23/2020	13:30:00	27.4	%
10/23/2020	13:40:00	30	%
10/23/2020	13:50:00	32.3	%
10/23/2020	14:00:00	33.5	%
10/23/2020	14:10:00	34.2	%
10/23/2020	14:20:00	30.5	%
10/23/2020	14:30:00	29.4	%
10/23/2020	14:40:00	27	%
10/23/2020	14:50:00	27.7	%
10/23/2020	15:00:00	30.7	%
10/23/2020	15:10:00	29.3	%
10/23/2020	15:20:00	32	%
10/23/2020	15:30:00	29.1	%
10/23/2020	15:40:00	33.8	%
10/23/2020	15:50:00	32.1	%

c) Wind Speed Average (WSA) Readings

Date	Time	WSA-Knots	WSA-m/s (1 knot =0.51444 m/s)
10/23/2020	13:30:00	1.1	0.566
10/23/2020	13:40:00	2.4	1.235
10/23/2020	13:50:00	15.2	7.819
10/23/2020	14:00:00	24.6	12.654
10/23/2020	14:10:00	33.2	17.078
10/23/2020	14:20:00	34.3	17.644
10/23/2020	14:30:00	43.3	22.274
10/23/2020	14:40:00	32	16.461
10/23/2020	14:50:00	28.6	14.712
10/23/2020	15:00:00	38.7	19.907
10/23/2020	15:10:00	28.9	14.866
10/23/2020	15:20:00	18.3	9.414
10/23/2020	15:30:00	21.6	11.111
10/23/2020	15:40:00	17.2	8.848
10/23/2020	15:50:00	1.8	0.926

Appendix D: Physiological Response Data Readings

a) **Body Temperature**

	Treatment 1	
	Before T_0 (^0C)	*After T_1 (^0C)*
Test Item 1	41.6	41.2
Test Item 2	41.6	41.1
Test Item 3	41.3	41.7
Test Item 4	41.1	41.8

	Treatment 2	
	Before T_0 (^0C)	*After T_1 (^0C)*
Test Item 5	41.2	42.5
Test Item 6	41.8	42.7
Test Item 7	42.4	42.7
Test Item 8	42.5	42.4

b) **Blood Ph**

	Treatment 1	
	Before T_0	*After T_1*
Test Item 1	8	7.5
Test Item 2	8	8
Test Item 3	8	8
Test Item 4	8	8

	Treatment 2	
	Before T_0	*After T_1*
Test Item 5	8	8
Test Item 6	8	7.5
Test Item 7	8	8
Test Item 8	8	8

c) Body Weight

	Treatment 1	
	Before T_0 (Kg)	*After T_1 (Kg)*
Test Item 1	2.05	1.9
Test Item 2	2.4	2.5
Test Item 3	2.5	2.45
Test Item 4	2.2	2.15
	Treatment 2	
	Before T_0 (Kg)	*After T_1 (Kg)*
Test Item 5	2.3	2.15
Test Item 6	1.7	1.35
Test Item 7	1.5	1.5
Test Item 8	1.25	1.65

d) Plasma Glucose

	Treatment 1	
	Before T_0 (mmol/L)	*After T_1 (mmol/L)*
Test Item 1	17.3	19
Test Item 2	20.3	20.3
Test Item 3	13	25.7
Test Item 4	11.9	17.7
	Treatment 2	
	Before T_0 (mmol/L)	*After T_1 (mmol/L)*
Test Item 5	12.3	21.7
Test Item 6	20.4	18.6
Test Item 7	16.2	20.1
Test Item 8	20.3	16.3

e) Serum Cortisol

	Treatment 1	
	Before T_0 (ng/ml)	*After T_1 (ng/ml)*
Test Item 1	47.1	132
Test Item 2	81.5	94
Test Item 3	86.3	91.7
Test Item 4	74	83.6
	Treatment 2	
	Before T_0 (ng/ml)	*After T_1 (ng/ml)*
Test Item 5	38.8	47.1
Test Item 6	68.6	61.5
Test Item 7	60.5	76.3
Test Item 8	72.8	64