EFFECTS OF VITAMINS C AND E ON SOME PHYSIOLOGICAL PARAMETERS OF SHIKA BROWN LAYERS TRANSPORTED BY ROAD DURING THE HOT-DRY SEASON

BY

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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY, FACULTY OF MEDICINE,

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ZARIA, NIGERIA

December, 2006

DECLARATION

I declare that the work in the thesis, entitled 'Effects of vitamins C and E on some physiological parameters of Shika Brown layers transported by road during the hot-dry season' has been carried by me in the Department of Physiology and Pharmacology under the supervision of Professors. J.O. Ayo, S.A. Ojo and K.A.N. Esievo.

The information derived from the literature has been duly acknowledge in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis, entitled: "Effects of vitamins C and E on some physiological parameters of Shika Brown layers transported by road during the hot-dry season" by **Ajakaiye**, Joachim Joseph meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to GOD the MOST HIGH, and to my late mother, Mrs. Veronica Iyetomola Ajakaiye, whose singular efforts delivered me from the clutches of illiteracy.

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ABSTRACT

Twenty-two weeks old Shika Brown layer (SBL) chickens administered with vitamins C (L-ascorbic acid), and E (α-tocopherol) were investigated for their physiological responses to 6-h road transportation, covering a distance of 280 km at a speed of 40-50 km/h, during the late (May, 2006) hot-dry season in the Northern Guinea Savannah zone of Nigeria. 90 SBLs were divided into three groups comprising 30 birds each. Birds in Group I served as control, and they were given 5 ml of sterile water only. Group II layer chickens were administered orally 200 mg/kg of vitamin C dissolved in 5 ml of sterile water individually. Group III birds were given *per os* vitamin E at the dose of 200 mg/kg dissolved in 5 ml of vegetable oil. The following environmental and physiologic parameters were recorded during the course of the study. They include the ambient temperature (AT), relative humidity (RH), rectal temperature (RT), some haematological parameters. In addition biochemical and behavioural responses of both treated and control SBLs were recorded before, during and after 6 hours of road transportation, using standard recording methods. Egg production and liveweight were also recorded pre- and post-transportation. The AT at the experimental site fluctuated between the minimum value of 25 and the maximum of 32^0C, while the RH ranged between 77 and 99%. During the journey, the AT values inside the vehicle fluctuated between 27.0 and 33.0^0C, while the RH ranged between 48 - 73%. The meteorological results showed that the weather conditions during the late hot-dry season were predominantly outside the thermoneutral zone for layer

chickens, indicating that the season was thermally stressful and did not favour transportation of the birds. There was no significant ($P>0.05$) difference between the RT of the control and treated groups pre-transportation. The RT values obtained in control layers were significantly ($P<0.05$) higher than those recorded for vitamins C and E-treated layers during and post-transportation. The RT values recorded in treated and control layers fluctuated with the hour of the day and ambient temperature. The fact that the RT responses of the transported layers increased significantly and immediately after loading demonstrated that the loading of layers into the vehicle was an unusual exercise, affecting the physiological parameters of the birds reared under the intensive management system. The Packed Cell Volume (PCV), red blood cell (RBC) count and haemoglobin (Hb) values of vitamins C- and E-treated layer chickens decreased significantly ($P<0.05$) from 31.4 ± 1.8 to $24.9 \pm 1.5\%$, 10.5 ± 0.5 to $7.1 \pm 1.1 \times 10^6/mm^3$, and 10.3 ± 0.6 to 8.3 ± 0.5 g/dl pre– and post-transportation, respectively. In control layers the haematological values obtained pre– and post–transportation did not differ significantly ($P>0.05$); with the exception of RBC values, which decreased significantly ($P<0.05$) from $10.1 \pm 0.3 \times 10^6/mm^3$ pre-transportation to $8.0 \pm 0.6 \times 10^6/mm^3$ post-transportation. In control layers, the heterophil/lymphocyte ratio rose significantly ($P<0.05$) from 0.26 ± 0.02 pre-transportation to 0.65 ± 0.22 post-transportation. The significant increase in total protein (TP) in all experimental groups, suggested that the layer chickens suffered dehydration due to road transportation. The alkaline phosphatase (ALP) showed a significant decrease in the vitamin C-treated and control groups, but a sharp increase

in the vitamin E-treated group. This may be due to its ability to maintain the supply of egg precursors in plasma of laying hens. Transportation decreased the liveweight of experimental layer chickens administered vitamins C or E by 9.1%, but in control layer chickens the decrease was 18.2%. Road transportation of layer chickens resulted in significant decrease in egg production in control birds, and the percentage reduction was as high as 44.7%. In vitamin E-treated layer chickens, the loss in egg production was lowest, 11%. The tonic immobility values indicated that the control birds showed significant signs of fear and fatigue as they exhibited greater responses to tonic immobility test than experimental birds. The results showed that pre-transportation administration of vitamin C or E ameliorated the adverse effects of road transportation stress in layer chickens, reduced the losses in liveweight and egg production. In conclusion, vitamins C and E reduced the adverse effects of road transportation stress during the late hot-dry season and enhanced productivity in transported layer chickens.

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LIST OF ABBREVIATIONS

EEG = Electroencephalogram
FR = Free radical
NADP = Nicotinamide adenine diphosphate
HOCl = Hypochlorous acid
H_2O_2 = Hydrogen peroxide
H_b = Haemoglobin
RBC = Red blood cells
WBC = White blood cells
NO_2Cl = Nitryl chloride
NO^{2-} = Nitrite
NO_2 = Nitrogen dioxide
O_2 = Oxygen
$\prod$ = pi or phi
in vivo = In live or in the animal
AO = Antioxidant
ROS = Reactive oxygen species
ATP = Adenosine triphosphate
PUFA = Polyunsaturated fatty acid
LPO = Lipid peroxidation
4-HNE = 4-hydroxyl nonenal
MDA = Malondialdehyde
in vitro = In experimental or in laboratory condition
DNA = Deoxyribonucleic acid
RNS = Reactive nitrogen species
in situ = In normal place or condition
CS = Chemiluminescent spectroscopy
NaCl = Soduim chloride
HPLC = High performance liquid chromatography
ELISA = Enzyme linked immunosorbent assay
8-ohdG = 8-Hydroxiguanosine
pH = Hydrogen ion index or hydrogen ion concentration
GSHPx = Glutathione peroxidase enzyme
SOD = Superoxide dismutase
CAT = Catalase
NAPRI = National Animal Production Research Institute
CAAS = College of Agriculture and Animal Science

ABU = Ahmadu Bello University
NAFDAC = National Food, Drug and Administration Control
EDTA = Ethylene diamine tetra acetic acid
MCV = Mean corpuscular volume
MCHC = Mean corpuscular haemoglobin concentration
MCH = Mean corpuscular haemoglobin
H/L = Heterophil/ Lymphocyte ratio
PCV = Packed cell volume
TP = Total protein
AST = Aspartate aminotransferase
ALP = Alkaline phosphokinase
RT = Rectal temperature
RH = Relative humidity
SBL = Shika Brown layers
AT = Ambient temperature
DBT = Dry bulb temperature
HPA = Hypothalamus pituitary axis
ACTH = Adrenocorticotropic hormone
GC = Glucocorticoids
CNS = Central nervous system
GAS = General adaptation syndrome
CS = Corticosteroids
AMP = Adenosine monophosphate
CRF = Corticotropic releasing factor
CBG = Corticosteroid binding globules
POMC = Precursor for ACTH, ß-endorphin
NS = Nervous system
EU = European Union
DFD = Dark, Firm and Dry
MAFF = Ministry of Agriculture, Fisheries and Food
FAWC = Food animal welfare council
RSPCA = Royal society for the prevention of cruelty to animals
PSE = Pale, Soft and Exudative
LD = longissimus dorsi muscle
SM = Semi membranous muscle
pHµ = Ultimate pH
AD = Abductor muscles
FFA = Free fatty acid

B-OHB = B-hydroxybutyrate
LDH-5 = Lactate dehydrogenase ISO enzyme-5
NO^* = Nitric oxide radical
ONOO* = Peroxynitrite radical
HO* = Hydroxyl radical
O_2* = Superoxide radical
APG = Anterior pituitary gland
CP = Crude protein
CPK = Creatine phosphokinase
SPSS = Statistical package for social science

CHAPTER ONE

1.0 INTRODUCTION

Animal breeders all over the world have exploited information obtained from animal geneticists to improve both quality and quantity of livestock production. The 1993/1994 total poultry production in Nigeria was put at 175,975,000, out of which chicken accounted for 84,986,000; ducks - 6,107,000; pigeons - 987,000; guinea fowls - 83,666,000; turkeys - 101,000 and geese - 41,000. There are also other species like quails and ostriches (Muhammad *et al.,* 2002). The increased poultry production has predicated the need for market sourcing by the farmers for their end-products, that is, egg and table meat. To meet this need, birds must be transported from the production site to sale outlets. In Nigeria, live birds transported are day-old-chicks, growers, pullets, point-of-lay and spent layers, often from the southern part of the country to the northern part. This journey covers an average distance of 12-14 h across the various ecological zones of the country.

Although a lot of data concerning livestock transportation are currently available, scientific knowledge on the effects of transportation stress in different animal species is scanty and often controversial. This is due to the influence of many individual stressors acting simultaneously during transportation (Ferlazzo, 2003). For example, in-transit birds are exposed to a wide variety of potential stressors, including the thermal demands of the transport microenvironment, motion, acceleration, vibration,

impact, withdrawal of food and water, noise, restriction of behaviour and social disruption. Other stressors include rough handling during loading and unloading, poor vehicle design, poor road conditions, extremes of ambient temperature, overcrowding, mixing different species and age groups, and high air velocity.The effects of these stressors singly and their combinations may range from mild discomfort, distress and aversion to death. It has been reported that 40% of transport mortalities are due to stress, with the rate increasing with journey length (Mitchell and Kettlewell, 1994). This is because stress reactions overtax the physiologic system and cause reduction in fitness of the animal by inducing dysfunctions of the pituitary, adrenal and thyroid glands (Anokhin *et al*., 1991; Hartung, 2003). Transportation also induces changes in the haematological values (Ayo and Oladele, 1996; Minka *et al.*, 2004), leading to increased morbidity and mortality, loss of liveweight, poor meat quality; and, consequently, substantial economic losses (Elrom, 2000; Fazio and Ferlazzo, 2003; Fallenberg and Speisky, 2006).

Information on the effect of transportation stress on the physiological parameters of poultry species in Nigeria is currently lacking in the available literature, despite the large number of birds transported by road from Southern to Northern Nigeria. The physiologic parameter of rectal temperature (RT) remains the most relevant on-the-spot diagnostic parameter of the state of an animal's health before any laboratory analysis is carried out. It is of great importance in evaluating the adaptability of domestic animals to various environmental factors, including transportation stress (Vihan and Sahni, 1981; Ayo *et al.*, 1998; Ayo and Minka, 2004).

The fluctuations in RT provide accurate information on the adverse effect of stress factors acting on animals (Ozkan *et al.,* 2003). Transportation also induces changes in the blood composition as well as other bodily parameters (Fazio and Ferlazzo, 2003). Thus, analysis of haematological and plasma chemistry parameters gives complementary information on the health status of the animal (Oladele *et al.*, 2005).

Vitamin C or L-Ascorbic acid is popularly used as a therapeutic agent against respiratory diseases, including common cold (Balz, 2003). Its administration has been shown to ameliorate the adverse effect of heat stress in livestock production (Ayo and Sinkalu, 2003; Minka *et al.,* 2004; Ayo *et al*., 2006). Thus, the available information in the literature strongly suggests that vitamin C is a potent antioxidant that may be of value in the prevention and therapy of adverse effects of stress factors (Altan *et al.*, 2003; Tauler *et al.*, 2003, Minka *et al.,* 2004), including road transportation.

Alpa-tocopherol or vitamin E is considered as a fertility factor (Kevin, 1982). This compound was found to be important not only for the fertility of rats, but also for that of poultry. Normal testicular function of cockerels and the hatchability of breeder eggs depend on vitamin E (Kevin, 1982). Vitamin E deficiencies may cause a variety of pathological conditions such as encephalomalacia, nutritional muscular dystrophy, and haemolysis. Together with selenium, which is an integral part of the enzyme glutathione peroxidase, it can prevent conditions like exudative diathesis, gizzard erosion and other diseases. It has also been reported that adequate supply of vitamin E stimulates the immune response of poultry, helps to detoxify dangerous organic and

inorganic compounds and ensures oxidative stability of meat (Mcmurray, 1980; Kevin, 1982; Hossain *et al*., 1998; Lin *et al*., 2006).

Animal Welfare Order during road transportation of food animals in Nigeria and many other countries in the West Africa sub-region is not taken into consideration.

There is a need to study the adverse effects of road transportation stress on some physiological parameters of food animals, and suggest preventive measures aimed at alleviating the adverse effects, with special reference to the peculiarities of the climatic conditions prevailing in the Northern Guinea Savannah zone of Nigeria.

1. To establish the effects of transport stress on the environmental, physiological, haematological, biochemical parameters and in addition liveweight, behavioural and egg production responses of SBL during the hot-dry season in Northern Guinea Savannah zone of Nigeria.
2. To evaluate the role of vitamins C and E on transported layer chickens.
3. To suggest possible measures of mitigating the adverse effects of road transportation stress on birds during the study season.

The hypothesis of the present study is:

H_o: Vitamins C and E have no effects on Shika Brown Layers transported by road.

H_a: Vitamins C and E have effects on Shika Brown Layers transported by road.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Stress as a Complex Adaptation Syndrome

2.1.1 Developing a definition of stress.

Among the early discussants on stress was Hippocrates (460 –375 BC), who in his essay *Vis medicatrix naturae*, wrote that 'balance' was the essential state of health, and that 'disharmony' was disease (Chrousos *et al*., 1988). Many years later, Claude Bernard (1813 –1878) described the 'milieu interieur' of animals and showed that it was characterized by relative constancy and balance. Cannon (1871 –1945) recognized this internal balance in a modern physiological context and called it homeostasis. He also implicated the autonomic nervous system in stress and described the fight – flight reaction. Hans Seyle (1907 –1982) proposed the general adaptation syndrome (GAS), the first comprehensive biological theory of stress. As part of the theory, like Hippocrates, he recognized that the persistent state of stress could lead to the pathological state (Griffin, 1989). Fraser *et al.* (1975) defined stress in a veterinary context as 'an abnormal or extreme adjustment in the physiology of the animal to cope with adverse effects of its environment and management. Others have refined the definition to describe the adverse stimuli that cause pathology as 'distress', while stimuli with no adverse effect cause 'eustress' (Griffin, 1989). Most recently,

stress was defined as the state of threatened balance, equilibrium, or harmony. The disturbing forces or threats to homeostasis were called 'stressors' and the

counteracting re-establishing forces were called ‘adaptive responses’ (Chrousos *et al.*, 1988). What all these definitions have in common are adaptive responses to challenges in homeostasis. The adaptive reactions to stressors may involve mobilization of a variety of physiological responses, including the immune response.

2.1.2 Causes and modifiers of stress

All life forms must respond to changing environmental conditions in order to maintain homeostasis. Threats to homeostasis are quite varied. They may be external stimuli such as thermal extremes or from within the animal itself e.g. infectious agent may elicit a stress response once inside a susceptible host. There are important modifiers of stressors – a stressor in one case may not be stressor in another situation. Modifiers of stressors include stimuli severity, duration, novelty, the genetic variability between or within a species or the immune status of a host animal (Griffin, 1989). Ultimately, a stressor must be defined by the adaptive response to reclaim homeostasis (stress). This determination or diagnosis of stress has been widely debated and is not a trivial determination. From the above statement, it is clear that there is no one test that identifies stress (Griffin, 1989).

2.1.3 Mechanisms of stress

The stress response is supposed to help the organism in “a stressful” situation when its life is at risk. The control of stress is simultaneously integrated by the *neural* and *endocrine* systems. There is evidence that the immune system also plays a significant

role in cotrolling stress (Hendricks *et al*., 1991). The concept of GAS proposed by Seyle (1963) was derived from observation and experimentation on laboratory animals. Using a variety of stressors (i.e. pain, thermal extremes and starvation), Seyle (1963) described a common non-specific stress response pathway. After initial perception of a stressor, the animal mounts an emergency 'alarm' or fight-flight response (neurogenic system). This catecholamine-driven reaction results in increased cardiovascular function and overall increase in metabolism. If the stressor persists, the resistance phase or 'conservation withdrawal reaction' is initiated, which is a physiological coping reaction to the increased demands of maintaining homeostasis. Thus chronic stress leads to the exhaustion phase and may lead to pathology. Seyle's theory (1963) unified the stress phenomena because it provided a common response pathway to all the varied stressors encountered. The pathway, the hypothalamic-pituitary-adrenal (HPA) axis, involves perception in the brain with release of hypothalamic corticotropin-releasing factor and vasopressin, which stimulate the anterior pituitary to secrete adrenocorticotropic hormone (ACTH). Circulating ACTH causes the adrenal cortex to produce glucocorticoids (GC). GC cause gluconeogenesis with conversion of lipid to glucose for central nervous system (CNS) and other functions. Thus, GAS allows the identification of stressors (and presumably the status of well-being of the animal) by measurement of glucocorticoid levels. This theory has become attractive because GC levels were relatively easy to measure (Griffin, 1989).

The GAS theory has proved too simplistic. Mason's experiments with Rhesus monkeys exposed to different types of stressors showed a disparity in neuroendocrine responses, specific stressors provoked specific responses. For instance, monkeys provided with emotional stress showed high circulating GC levels, yet those subjected to a heat-stress regime failed to show GC elevation. Other neuroendocrine mediators were characteristically produced to specific stressors (Moberg, 1985). Emerging information on the response to stressors suggests that there are at least four different avenues of neuroendocrine response. These involve the autonomic nervous system, the HPA axis, neuropeptides and neurotransmitters, and neuroimmunological peptides and receptors (Griffin, 1989).

The neurogenic system or autonomic nervous system:

Lymphoid tissues are innervated by sympathetic and parasympathetic divisions of the autonomic nervous system. These nerves release catecholamines in the fight-flight reaction. Activation of the neurogenic system is an effort to combat or avoid the offending stimulus, rather than adapt to it. It is manifested by rapid increase in blood pressure, muscle tone, nerve sensitivity, respiration rate and blood sugar. These immediate responses are mediated by neurogenic amines (catecholamines), notably *norepinephrine or noradrenaline* (from nerve synapses) and *epinephrine or adrenaline* (from the adrenal medulla). Catecholamines stimulate the hepatic adenyl-cyclase activity, which, in addition to catalyzation of ATP to cAMP (energy flux), directly increase the promotion of antibodies. In acute stress, birds increase the

concentrations of neurogenic amines in the blood and concomitant adrenal depletion (Siegell, 1995).

The changes produced by catecholamines in the cardiovascular system are augmented cardiac output (tachycardia and increased contractility), hypervolaemia (peripheral vasoconstriction of the skin, gastro-intestinal tract and spleen). Catecholamines also induce important metabolic changes such as increased lipolysis, glycogenolysis and gluconeogenesis (Ahrnes, 1996), which alter the thermoregulatory system of the body.

A supportive mechanism, which is directly connected with the neurogenic system, incorporates the use of naturally occurring opioid peptides in the brain and adrenal medulla. These peptides, mainly ß-endorphin, have an analgesic effect during pain or other unpleasant situations. ß-endorphin is released following activation of the neurogenic system, and it is increased due to elevation in catecholamines. It is responsible for "stress-induced analgesia". Some effects of ß-endorphin counteract some catecholamine influences, and may be regarded as additional controlling factor to prevent fatal deterioration due to extreme arousal and metabolic exhaustion. Endorphins directly decrease the release of catecholamines from the adrenal medulla (paracrine effect by *adrenorphine*) during stress (Robinzon, 1990).

The hypothalamic-pituitary-adrenal system:

Compared with the neurogenic system, the activation of hypothalamic-pituitary-adrenal (HPA) system is a long-term adjustment by the bird to environmental changes. Consequntly, it is expected that stressors induce neural and hormonal events, which

begin with the stimulation of the hypothalamus and lead to release of corticotropin releasing factor (CRF). This hormone stimulates the anterior pituitary (*adenohypophysis*) to produce ACTH. ACTH stimulates the adrenal cortex, which in turn releases corticosteroids (CS), primarily corticosterone in birds. In addition to effects on glucose and mineral metabolism, CS produces many symptoms associated with long-term stress, including cardiovascular disease (myocardial necrosis and arteriosclerosis), hypercholesteraemia, gastro-intestinal lesions (ulcers) and modification of immunological functions such as lymphatic involution (Robinzon, 1990; Siegell, 1995; Kostova, 1997; Grandin, 1998; Elrom, 1999; Plumb, 1999). In birds and vertebrate, 80-90% of circulating CS is specifically bound to corticosteroid binding globulins (CBG). This is important in controlling stress since only unbound CS can pass the cell membrane and exert their effects.

Siegell and Gold (1995) reported that during the embryonic life of a chick, between 10 - 12 days of incubation, the CBG can be found in the plasma. The half-life of bound CS is reported to be 90 min and that of free CS to be 7.5 min. CS in the blood decreases the synthesis of CBG in the liver and so increases their effects on the organism (Robinzon, 1990). It is known that in birds (especially in quails, pigeons and ducks) the link between different anatomical parts of the HPA system is not so essential to find a basal level of CS in the blood as in mammals. These findings suggest that the avian pituitary appears more autonomic and less subject to hypothalamic control. CS receptors too are present in the hypothalamus, and prior stress may increase the binding of these receptors in a way that will suppress the

release of CRF by a negative feedback mechanism. Thus end organ responses, such as heterophil and lymphocyte responses or lymphoid organ regression may represent better indicators of chronic stress, whereas plasma hormones (CS or catecholamines) concentration are effective indicators of acute stress. The heterophil/lymphocyte ratio might act as a bioassay for chronic stress (Gross and Siegell, 1983).

In the study by Siegell (1995), the adrenal gland was also found to be more autonomic and less subjected to the control of the pituitary gland. He showed that hypophysectomized birds respond to stress stimuli with elevated blood CS. He also proposed that there are extra-pituitary areas in the brain that are responsible for production of ACTH or ACTH-like substances. He later discovered that avian leucocytes (lymphocytes and macrophages) produce ACTH or ACTH-like substances which are stimulated by CRF *in vitro*. Injections of ACTH also increased the endogenous production of CS in the cytoplasm and nuclei of sensitive lymphatic tissues such as thymus and bursa of Fabricius (Siegell, 1995). He concluded that high levels of CS, reached at extreme environmental changes (heat or cold), decline as stress continues. This indicates that CS synthesis and release cannot be maintained and may lead to exhaustion of this system. Birds under consistent stress condition show lympholytic effect of ACTH. The regression includes depletion of lymphocytes from germinal centres, lymphopenia and heterophilia (Siegell, 1995).

Neuropeptides and neurotransmitters- extra-adrenal pathways:

There is interesting information on the immunomodulating role of neuropeptides, neurotransmitters and other compounds elevated by the nervous tissue stimulation of

extra-medullary pathways, but there is no complete understanding of the orchestration of the stress pathways involving nervous and lymphoid systems. A list of these compounds that are potential immune modulators includes serotonin; substance – P; vasoactive intestinal peptides; vasopressin and oxytocin; insulin; growth hormone; endorphins and enkephalins; acetycholine; norepinephrine; somastostatin; melatonin; thyroid hormone; histamine; thymosin; estradiol and testosterone. Receptors for these compounds are found either on lymphocytes and their exogenous applications have been found to alter immune responses (Golub and Gershwin, 1985; Griffin, 1989). The role of these compounds on immunocompetence is apparently related to the level and duration of the specific stressor and stressor modifiers.

Neuroimmunologic mediators:

It has become evident that stress responses by the nervous and immune systems have integrated and bi-directional activities. Both systems involve recognition of stimuli that threaten homeostasis and initiate integrated adaptive responses. Left, but not right, neocortical brain lesions produced in mice were shown specifically to suppress some but not all immunological responses (Biziere *et al*., 1985). Sympathetic innervation of primary and secondary lymphoid tissues and organs is extensive (Griffin, 1989). In the human gut and associated mucosal lymphoid tissue, the number of nerve cell bodies is nearly equivalent to that of spinal column (Bienenstock, *et al*., 1989). Mononuclear cells possess receptors for neuropeptides and neurotransmitters, and lymphocyte neuropeptide receptor densities have been shown to increase with increasing levels of immune activation. This fact suggests the ability of nervous responses to affect

modulations of immunocompetence. Evidence has accumulated that activated lymphoid cells can modulate CNS activity via cytokine release. Besedosky *et al*. (1975) first showed GC elevation during an immune response. Changes in firing rates of hypothalamic neurones occur during the immune response, and changing patterns of noradrenergic neuronal activities can be mimicked by injection of soluble mediators from immune cells (Besedovsky *et al*. 1977; 1983). Activated lymphocytes produce ACTH that directly stimulate pituitary-adrenal pathways. The monokine interleukin-1, in addition to its effect on T-helper cells, causes fever responses and the hypothalamic release of ACTH. Interleukin–2 replaces CRF in POMC (the precursor for ACTH , β–endorphin) synthesis and increases blood insulin levels. Thymosin fraction V can elevate pituitary ACTH, β–endorphin, cortisol and produces a hyperglycaemic state. Finally, molecules of the immunoglobulin supergene family are found on glial cells of the CNS. Theories concerning the significance of the bi-directional activity of these systems have been suggested (Blalock, 1988; Griffin, 1989). Beside the need for metabolic energy during the stress response, one idea emphasizes stress and inflammation. During fight-flight and conservation withdrawal, there may be a need to reduce inflammation from wound or damage resulting from sudden excessive muscular activity. GC, endorphin and enkephaline release may reduce inflammation and provide analgesic benefit. Inflammatory responses need critical control in animals. Inflammation has survival value as microorganisms are eliminated by the complex pathways resulting in healing of affected areas. However, generalized inflammation can cause widespread normal tissue damage and can result in severe disease and death.

The Important HPA axis anti-inflammatory pathway involves GC release. GC elevation, caused by 'the stress of infection', has been observed in Q fever and sandfly fever in man, as determined by rise in urinary 17 ketosteroids (Mims, 1982). In Addison's disease, where GC levels are chronically low, human and animal patients are more severely affected by inflammatory aspects of infections and are treated with corticosteroids. In contrast, among patients with Cushing's syndrome, where excessive GC are produced, the leading cause of death is bacterial infection resulting from the immunosuppressive aspects of chronically high GC levels. Thus when homeostasis is challenged by infection and inflammation, GC modulates this important host reaction. Prolonged elevation of inflammatory GC results in immunosuppression.

The bi–directional exchange between immune and nervous systems may provide different ways of recognizing challenges to homeostasis. External stressors are perceived by cognitive pathways, while more subtle microbial threats are recognized by the immune system (Blalock, 1984). The energy required to mobilize body defenses against microbial invasion is substantial. Both external and internal stressors once perceived produce responses in both neural and immune systems so that homeostasis is maintained through integrated responses.

Specific and non-specific regulatory processes:

Siegel (1995) demonstrated the regulatory processes can be categorized as specific or non-specific. According to his findings, specific processes are aroused in a particular condition that elicits specific responses related to that condition. Thus, a rise in environmental temperature raises the body temperature and the surface blood vessels

to dilate allowing rapid heat dissipation, while feathers are rearranged to reduce insulative value. In birds, panting is a very important homeothermic response to deal with hyperthermia. Behaviourally, wings are held away from the body and the birds seek cool surfaces for conductive heat loss.

Non-specific processes occur when, regardless of the stressor – heat or cold, hypoxia or toxin, the bird responds in a generalized manner, going into a state of general stress. Both regulatory processes may occur simultaneously and are known as the responses of NS and HPA systems (Siegell, 1995). Other environmental stimuli with non-specific stress response include reduced atmospheric pressure, muscular exercise – tread-mill running, food deprivation, water deprivation and handling (Frazer and Broom, 1990; Siegell, 1995; Jones, 1996). Other stressors include increased ambient ammonia, electric shock and heat. Overpopulation, human sight and handling are also potent stressors (Elrom, 2000).

2.1.4 Stress – mechanisms of immunosuppression in food animals.

Stress-induced modulation of the avian immune system is a topic of economic, social and scientific importance. In food animal production, it is widely believed that the negative economic impact of this perception in minimizing stress is paramount in developing disease control programmes. Stress in farm animals is generally understood as environmental conditions, adversely affecting the well-being of animals (Fazio and Ferlazzo, 2003), leading to a pathological loss or fall in production. Stress may be climatic, such as extensive cold or heat; nutritional, due to feed or water

deprivation; social resulting from a low rank in the pecking order; internal due to some physiological disorders, pathogens or toxins; physical/trauma stress due to unavoidable veterinary manipulations; for example, animal bleeding, castration, body weighing, cosmetic surgery, ducking of tail or debeaking; weaning; and transportation. (Hafez, 1968; Robert, 1983; Anokhin *et al.,* 1991). Stress in farm animals has been explained above with reference to Selye's (1955) concept as exposure to noxious environmental factors which elicit non-specific reactions. This reaction is characterized by enhanced pituitary-adrenal responses and facilitates the return to homeostasis. However, stress reactions may have detrimental effects on the health of animals. Such effects include gastric ulcers, decrease in immune functions, cessation of growth and reproduction, and even death from failure of adaptive mechanisms.

Farm animals are very susceptible to the unfavourable consequences of stress. This is because genetic selection and environmental pressure have oriented their metabolism towards anabolism instead of defence mechanisms that are by essence catabolic (Judge *et al*., 1966). Since farm animals cannot put into practice Selye's motto 'stress without distress,' they must be helped by man. Therefore, whenever necessary they are provided with wide variety of anti-stress drugs, including tranquilizers to decrease their reactions to environmental stressors, antibiotics to control bacterial diseases, and hormones and vitamins to compensate for falling physiological defence mechanisms (Dautzer and Mormede, 1983).

In recent times, increasing demand from the ever-growing world population for protein and its products has necessitated the intensification, and especially

industrialization of livestock production to meet high animal protein needs. In pursuit of these objectives, food animals are subjected to high technological manipulations, including changes in genetic composition, feeding on high-quality feeds, and transportation of livestock worldwide. Heat and transport stress are, therefore, the most common types of stress encountered today in the livestock industry (Anokhin *et al.,* 1991). Technologically, heat stress may be reduced, avoided or even eliminated completely, but transport stress remains unfavourable and a crucial problem in an ever-expanding and growing livestock industry worldwide, including Nigeria. Thus, transportation of livestock is a complicated multifactorial stressful and traumatic event (Anokhin *et al.,* 1991; Elrom, 2000).

2.2 Road transportation stress in food animals

Road transportation stress is the acquired stress condition of animals during their transportation by road in which the rules and regulations governing normal transport welfare are often broken. They include lack of adequate preparation before the journey, influence of unfavourable climatic factors, and management related problems during the process of transportation. The symptoms of road transport stress are virtually the same, regardless of the animal species being transported (Anokhin *et al.,* 1991; Ayo and Oladele, 1996; Parker *et al.,* 2003). However, highly productive, pregnant and young animals suffer more stress during road transportation than others (Broom, 2003; Ferlazzo, 2003).

2.2.1 Causes of road transportation stress

Road transport conditions are known to influence the physiological responses of animals either as a result of psychological stress or physical fatigue (Lambooij *et al.*, 1985; Brandshaw *et al.*, 1996). The causes of road transport stress are classified into three, viz: pre-transportation, transportation and post-transportation period.

Pre-transportation period: These include lack of adequate preparation before transportation, the very process of loading and confinement of animals into the vehicles are very strenuous. Chasing of animals with stick or club, pushing and kicking, prolonged standing while waiting for vehicle departures, overcrowding, improper positioning of a loading spot are very stressful right from the onset (Gracey, 1986, Ayo and Oladele, 1996; Hartung, 2003). Other pre-transportation cause is mixing different species of animals of different sizes and ages in one place as they await transportation. Transporting animals in vehicles not specifically designed for their transportation, with inadequate ventilation, bad engine and poor ramp design cause significant stress on the animals (Gracey, 1986; Ayo and Oladele, 1996).

Transportation period: Causes of transportation stress during the journey include the distance and duration of transport, climatic factors and changes in the accustomed daily routine (stereotypy), nature of road and speed of the vehicle. The most important cause of stress during the journey is the sudden change in climatic conditions to which the animals are subjected. The climatic conditions include high AT and RH that occur during the hot-dry period, and with little or no water supply to the animals, particularly in Northern Nigeria and neighbouring Northern countries in the West

Africa sub-region, from where food animals are imported to Nigeria by road. These climatic conditions aggravate transportation stress (Ayo and Oladele, 1996; Hartung, 2003). As a result of concomitant action of heat and transportation stress, loss of body weight may rise up to 10.6% (Plyaschenko and Sidorov, 1987). Since mortality increases during transportation in hot and strong sunshine (Smith and Allien, 1976), it is preferable that animals be transported at night or the early morning (Scholtyssek and Ehinger, 1976; Ehinger, 1977).

Post-transport period: They include rough unloading of animals from the transported vehicle, poor unloading ramp, lack of adequate food, water and rest in lairage after transportation, and lack of post-transportation medication complicate the conditions of the already stressed animals. Overstaying of animals in open lairage may expose them to serious heat or cold stress (Anokhin *et al.,* 1991; Warriss, 2003).

2.2.2 Clinical signs of road transportation stress in food animals

The symptoms of transportation stress are non-specific, and they depend on the stress factors, their duration of action, species and age of the animals (Anokhin *et al.,* 1991). They are manifested clinically during the journey and up to two days post-transportation. The symptons include prominent excitation that is gradually replaced by strong inhibition, uncoordinated gait, loss of appetite, increase in heart and respiratory rates, elevation of RT, increased urination and defaecation, loss in liveweight by about 6-10% (Knowles *et al.*, 1999a), decrease in body resistance to diseases, poor adaptability and poor meat and skin quality (Fazio and Ferlazzo, 2003). All these bodily changes impair the homeostatic mechanisms, resulting in metabolic

shift and dysfunction of the pituitary gland, adrenal cortex, thyroid gland and changes in the cellular and biochemical constituents of the blood (Plyaschenko and Sidorov, 1987; Anokhin *et al.*, 1991; Broom, 2003).

2.2.3 Diagnosis of road transportation stress in food animals

Transportation stress is easily diagnosed either at the time of journey or few days after. Diagnosis is made on the basis of the clinical signs mostly affecting the functions of the central nervous system, loss in body weight, increased RT and haematological dysfunctions, especially decrease in the number of eosinophils and lymphocytes, and a significant increase in neutrophils / heterophils. Indicators of welfare and stress status during handling and transport include behavioural, biochemical, functional, endocrine and pathological variables (Ferlazzo, 2003). It is well known that physiological measurements of stress are dependent on the interaction of many systems, and some stress responses can be measured through the evaluation of functional status of the primary system involved. The hypothalamo-pituitary-adrenal cortex, the sympathetic and adrenal medullary systems and thyroid functions are involved in stress response. Therefore, the opioid systems, changes in the level of hormones such as β-endorphin, ACTH, cortisol, total and free iodothyronines or reproductive hormones are used to quantify the responses of animals to stress (Rimbach *et al.*, 1999).

Changes in heart rate, electrolytes, hormones, metabolites, enzymes and liveweight are of great value in assessing the response of livestock to transportation (Fazio and Ferlazzo, 2003). Previous studies have examined the effects of road transportation on pigs (Smith and Allen, 1976; Brown *et al.*, 1999a; Warriss, 2003),

cattle (Atkinson, 1992; Fernandez *et al.*, 1996, Mackenzie *et al.*, 1997; Knowles *et al.*, 1999b; Arthington *et al.*, 2003; Parker *et al*, 2003), sheep (Broom *et al.*, 1996; Ruiz-de-la-Torre *et al.*, 2001; Minka, 2005), horse (Ferlazzo *et al.*, 1993, 1997; Cusumano *et al.*, 2001), goats (Nwe *et al.*, 1996; Aoyama *et al.*, 2003; Ayo *et al.*, 2006), and poultry (Freeman *et al.*, 1984; Warriss *et al.*, 1993; Minka *et al.*, 2005). The results of the studies demonstrated that road transportation has effects on various hormones, acid-base balance, body temperature, liveweight, glycogen reserves, meat and skin qualities. The effects varied with the duration of transportation, age and breed of the animal, previous experience, and the nature of vehicle and the roughness of the road. Transport stress should be differentiated from avitaminosis, anthrax, pasteurellosis, babesiosis, poisoning and heat stroke (Plyaschenko and Sidorov, 1987, Anokhin *et al.*, 1991; Fazio and Ferlazzo, 2003).

2.2.4 Treatment of transportation stress

There are two options to the treatment of transportation stress in food animals, viz: provision of a preconditioned environment or amelioration of the environment. Measures to prevent animals from exposure to transportation stress are the best approach, but they are not always practicable. They include well organized transportation procedures, taking into cognizance the welfare need of the animals. Following exposure to transport stress, therapeutic methods are aimed at enhancing the natural adaptability of the animals and reducing their nervous excitation by the administration of neuroleptics, which include analgesic given intramuscularly at a

dose of 0.5 – 2mg/kg of body weight, depending on the distances the animals are to be transported (Anokhin *et al.*, 1991). In order to increase body resistance to stress factors 2-3 days pre– and 3-5 days post-transportation, it is recommended that calves be given aminazine at a dose of 0.3 mg/kg/day for 4-7 days before and 10-14 days after transportation. Glucose administration is also recommended pre– and for 5 days post-transportation to help the animal build more energy to withstand the stress. The administration of vitamins 3-5 days before and 8-12 days after transportation has also been recommended (Plyaschenko and Sidorov, 1987). Drug administration in transported food animals increases the risk of consumption of meat with drug residues. Therefore, the search for more efficient but less toxic agents to reduce road transportation stress in food animals continues.

2.2.5 Prophylaxis of transportation stress

It has been recommended that 2-3 days before transportation, animals should be housed or kept in a clean environment and fed with good quality feed. Regular physical examination of the health status of animals should be done daily before transportation. The animals should be handled humanely. During transportation, severe climatic conditions such as hyperthermia, severe cold and high RH, should be avoided on the day of transport. If the weather conditions are unfavourable before the start of the journey, then the journey should be suspended. Vehicles to be used should be sound with less noise and vibrations. Over-speeding, multiple and sharp breaks in the journey itself should be avoided. Only healthy animals should be transported.

Animals of the same breed, species and age group should be transported together, and without overstocking. Animals in the last trimester of pregnancy should not be transported for a long distance on hot days (Fazio and Ferlazzo, 2003; Hartung, 2003). The effects of vitamins and glucose which are nutrients on transported food animals are not yet elucidated in the available literature. It is conceivable that the effects of antioxidant vitamins may shed light on their potential role in ameliorating road transport stress in food animals.

2.3. Adverse effects of vehicle design, handling, loading and unloading on food animals during transportation.

2.3.1 Vehicle design

Handling, loading, transportation and unloading of animals adversely affect their welfare. The major factors determining the well-being of farm animals in road transportation are vehicle design, stocking density, ventilation, standard of driving and quality of the road (Fazio and Ferlazzo, 2003). The to-and-fro movements induced in the animals by a moving vehicle can cause motion sickness (Nicol and Saville-Weeks, 1993). Transporting food animals by using vehicles not specifically designed for that purpose leads to injuries, and severe stress in food animals (Gracey, 1986; Javis and Cockrann, 1994; Ayo and Oladele, 1996). Thus, vehicles such as open trailers, trucks, tippers, pick-ups, dilapidated buses and other vehicles with poor ramp design or very narrow doors with unnecessary projections, primarily designed to transport goods are unsuitable for animal transportation and should be avoided (Kettlewell *et al*., 1999).

In order to reduce the effects of stress imposed by the nature of vehicle when transporting sheep, Broom *et al.* (1996) suggested the use of a 3.5-ton standard cattle lorry with ventilation louvres. The sheep were confined within a 1.8 x 2.3 pen that is 0.23 m^2/animal, whose floor was covered with wood shavings. In transporting pigs of liveweights ranging from 80-100 kg, Brown *et al.* (1999b) used an articulated lorry and by means of a loading ramp, loaded pigs at a stocking density between 0.50 and 0.54 m^2 /100 kg with straw as beddings. Knowles *et al.* (1999b) used a stocking density of 141 kg/m^2 bedded on straw in transporting young calves one to two weeks of age for a journey of 19 h. While studying development of calves aged 60 days during the milk-feed period, Steinhardt and Thielscher (1998) used a single-axle trailer platform of corrugated aluminium and wooden sidewalls 1.05 m high, canvas roof, loading ramp as rear wall. Ruiz-De-La-Torre (2001) used a modified vehicle with a tri-axial accelerometer linked to a multi-channel data-logger for recording any movement of the vehicles in transporting sheep with a stocking density of 0.26 m^2/animal. The stocking density was within the European Union (EU) recommendations. During 31 h transportation of cattle by road, Knowles *et al.* (1999a) suggested the usage of either a single-deck articulated cattle lorry with leaf spring suspension or a double-deck articulated cattle lorry with air suspension with a stocking density of 1.55 m^2/animal for transporting goats. Nwe *et al.* (1996) and Aoyama *et al.* (2003) suggested combining goats in crates that are in turn loaded into truck lorry for transportation. In transportation of horses, single straight deck and two-tiered “pot

belly" trailers were recommended at a stocking density of 1.14-1.54 m^2 / horse (Stull, 1999).

Despite various modifications of the transport vehicles and the standard journey speed of 25-50 km/h, it remains obvious that the stress produced by the vehicle and its stocking density cannot be eliminated completely. This is because during the journey through sharp bends and ascending or descending hills, unrestrained animals inside the vehicle often stagger from one side of the vehicle to the other. Such animals are often compressed against the sides of the vehicle, and sometimes they fall. Therefore during transportation, when animals are unrestrained, the strong ones often attack the weak ones in an attempt to occupy the best place in the vehicle (Ayo and Oladele, 1996). This results in fight, which is even more serious when the animals are constituted from different groups of pens. The outcome of the fight is a new hierarchical order within the groups, resulting into severe traumatic injuries (Grandin *et al.*, 1999; Stull, 1999). Also, the effort needed by the animals to keep their balance while the vehicle is in motion may be demanding in terms of energy requirements and, thus, increases the incidence of traumatic injuries resulting into dark firm dry (DFD), low meat quality and slaughter output (Tarrant and Grandin, 1993).

2.3.2 Handling, loading and unloading

The very process of loading animals into vehicles is very strenuous. Most of the changes in body parameters of the transported animals are as a result of handling, loading and unloading. Loading and the commencement of vehicle motion induce

large increases in cortisol and prolactin concentrations (Brown *et al*., 1999a; Knowles *et al.,* 1999b; Hartung, 2003). The sudden loading and confinement of animals reared predominantly in Nigeria or the neighbouring countries under the traditional extensive management system into a vehicle is an unusual exercise. They constitute a break in the dynamic stereotype of the animals, often accomplished by excessive noise, chasing with stick or club, pushing and kicking of the animals. Other stress factors acting upon the animals just before transportation include pressure exerted by ropes that are used to restrain the animals, the rough road along which the animals are chased, the prolonged standing of the animal while awaiting the vehicle or its departure. All these deleterious factors acting simultaneously make the journey right from the onset very stressful to the local animals (Ayo and Oladele, 1996).

Brown *et al.* (1999a) noted that the greatest stimulation of cortisol release was brought about by handling and transferring sheep from their individual loading pens to the communal pen on the vehicle. They observed that the start of driving produced an additional cortisol peak of 350% above the initial value. Osmolality and haematocrit decreased in response to loading and thereafter remained depressed.

2.3.2 Catching, handling and loading of poultry for transportation

Handling of poultry is recognized as a huge welfare problem throughout the developed world. In the UK the Ministry of Agriculture, Fisheries and Food (MAFF), the Farm Animal Welfare Council (FAWC) and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) are concerned that bird welfare is often compromised

during handling. Although an accurate definition of welfare is difficult (Duncan and Dawkins, 1983), the term can be considered as general well-being of the individual. Various states of suffering such as fear, frustration, pain and discomfort can be considered to have deleterious effects on the welfare of the animal (Duncan and Dawkins, 1983).

Handling is suggested to be the cause of a great deal of injury. In the UK there were approximately 500 million broilers slaughtered in 1988 (MAFF, 1989). Typically 5% of these are downgraded due to injury (Kettlewell and Turner, 1985). Handling injuries include bruising, fractures and dislocations. Gregory and Wilkins (1990) stated that the problem of fractures in transit is not a serious welfare problem for broilers since most of the damage occurs during processing rather than during handling and transport. However, they also report that up to 31% of battery hens had fractures immediately before stunning, most of these breaks occurring during the handling of the birds out of the house (Gregory *et al*., 1990). They showed that the keel and ischium are the principal bones broken in birds from battery cages, whereas it was the pubis and ischium in perchery and free range hens. While these figures apply to the UK industry, the same principles may also apply elsewehere.

Swarbrick (1986) noted that in a sampling period of 4 weeks at a particular broiler processing plant, nearly 80% of the recorded deaths on arrival were due to transport stress and injury. While commercial producers may be willing to accept that there may be up to about 0.1% dead on arrival at a processing plant, Swarbrick (1986) suggested a range from less than 0.2% to over 1% on particular loads. Handling is

defined as the process of removing birds from a building to a transport system (usually a truck or bus) and subsequently the reverse procedure. The main emphasis is on the stages leading up to the loading of poultry onto vehicles. In its simplest form the process consists of three basic components: (1) collection (including catching where appropriate); (2) portage (carrying the birds from the point of collection to the point of loading onto the transport vehicle); and (3) loading onto the transporter. In practice, handling also includes placing birds in containers (crates or other structures), which is necessary in order to keep the large numbers of birds normally involved under control, and to make both transportation and subsequent unloading easier and safer for the birds (Scott, 1993).

Containerization may occur close to the point of collection, after portage from the point of collection to the truck or, in the case of fixed crate systems, on the truck itself (Scott, 1993).

Manual handling:

During manual handling, all the handling components described are performed manually, except perhaps loading the transporter which may require the use of fork lift equipment (trucks). Swarbrick (1986) considered the loading and unloading of broilers to be the times when most transport damage occurs and that the major welfare problems occur at catching. Transportation itself can also elicite physiological stress responses. Duncan (1989) found higher plasma cortisol levels after a journey of 40 minutes duration compared with handling alone.

Animals may adapt to regular handling (Gross and Siegell, 1980; Podberscek *et al*., 1990). If this is the case, increasing the level of both visual and physical human contact prior to catching, as a form of training, might reduce the level of fear and stress for animals unused to handling. Preliminary investigations suggest that broilers subjected to gentle human contact during the growing stage exhibit longer periods of tonic immobility (TI) after catching compared with birds without prior human contact. This is an unexpected result. If birds are subjected to mild stressors such as having balloons rapidly inflated beside them, the handled birds are apparently less fearful. Duncan (1989) stated that manual handling alone causes stress to broilers, regardless of the degree of roughness of the handling, and that less stress results from herding or conveying. He found that none of the birds handled either manually or mechanically appeared to be 'extremely' stressed, based on the lack of significant increase in the plasma cortisol levels. Nevertheless catching and loading of birds, both broilers and hens, potentially result into a major source of injury (Table 2.1.).

Table 2.1. Principal bones broken in birds from battery, perchery and free range systems.

Free range	Sample size (numbers)	Proportion of birds with broken bones before stunning (%)
Battery	*172*	*31*
Free range	169	14
Perchery	456	10

Adapted from Gregory *et al*. (1990)

The degree of injury depends mainly upon the catchers and their equipment (Singh *et al*., 1979). Most of the damage causing downgrading occurs within the 12-h period before slaughter (Kettlewell, 1987).

Spent hens and pullets must also be handled between the truck and the cages. While the industry might consider spent hens to be of little economic value, an economic premium is put on pullets at point of lay. These two groups of birds are handled essentially in the same manner. The birds are inverted and each carried in groups of two in one hand and three in the other. During depopulation, birds are pulled by their legs from the cages. Breast bone and back injuries are prevalent (Gregory *et al*., 1990). The use of sliders which fit over the feed trough, enabling the birds to be pulled over a ramp, could reduce injury. Hens are often withdrawn from cages quickly and in a rough manner. Injury may result, particularly if the cage front is poorly designed so that birds are required to pass through small apertures or protrusions. It is likely that spent hens are more prone to injury than pullets because their bones are

extremely fragile following continuous egg production, whereas pullets are reasonably robust by comparison (Scott, 1990).

2.3.4. Factors of stress during catching, handling and loading in poultry

Kettlewell (1989) recommended to classify stress factors into 3 subgroups : Mental, physical and mixed (mental and physical) factors.

Another classification was suggested as psychological and physical stress (Grandin, 1998)

Mental stress factors:

Mental stressors in broilers are very complicated. During their relatively short life (5-8 weeks). The birds are exposed to very few external stimuli (Kettlewell, 1989; North and Bell, 1990). Sudden disruptions of their life-style are likely to be bewildering to broilers, as they have neither the time nor the experience to react to such disturbances. Fear is an important mental stressor that has a detrimental effect on the welfare of the bird.

Broilers are undoubtedly subjected to mental stress during catching, social group disruption, crating and transportation. This is mainly due to physical, visual, auditory and social factors. The mental stress caused by lifting the feed and water conveyors that results in starvation of animals a few hours before catching is significant as well (Siegell, 1995; Jones, 1996).

Physical stress factors:

Physical stressors are factors that usually cause injuries and mental stress. Common display of physical stress in broilers/layers are mainly from harvesting and transportation; catching by legs (in groups of 4-5), inversion of the bird, carrying them inverted to the crates, loading, transportation (vibration, environmental and microclimate conditions), unloading the crates and loading on shackles (Kettlewell and Mitchell, 1994; Randal *et al*.,1994; Kannan *et al*., 1997). Additional injury can occur a few hours before catching when feed conveyors and drinking bowls are removed (Kettlewell and Mitchell, 1994).

Mixed (mental and physical) stress factors:

Siegell (1995) suggested that the environment is a composite of interacting stressors that, in the broadest sense, can include all conditions in which the birds live – external (temperature, light, social or behavioural environments), as well as internal (pathogens and toxins). Therefore handling and transportation embrace mixture factors, which are mental (pain, fear, anxiety), and physical (environmental: heat, cold, wind, strokes wounds, *etc*.).

2.3.5 The consequences of stress

The responses of the stressed bird assist the bird to cope with the new condition and survive. Unfortunately when stress becomes chronic and last longer, then it affects the immune and reproductive system in addition to the impact on metabolism and energy balance (Elrom, 2000). Neurogenic amines, such as epinephrine and

norepinephrine potently activate the breakdown of glycogen to glucose in the liver. Subsequently, increased blood pressure and pulse with the supply of the "new energy", the free-living bird is enriched with resources to escape predation or deal with injury (Dohms and Metz, 1991).

2.4 Haematological and biochemical changes in food animals subjected to transport stress

The stress factors occurring during transportation, especially during the hot days cause physical and psychic exertions, which disrupt the homeostasis and, consequently, metabolism in animals (Plyaschenko and Sidorov, 1987; Oladele *et al.*, 2005). As a result of the exertion, the activity of hormones and enzymes increases. Also, blood levels of enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamic phosphatase (GPT), and substances such as glucose, creatine phosphokinase (CPK), cortisol, nitrogen urea, lactic acid, uric and free fatty acids (FFA) are increased (Apple *et al.,* 1993; Parker *et al.,* 2003; Gallipalli *et al.,* 2004).

During transportation of hens, Freeman *et al.* (1984) demonstrated that blood cortisol level and utilization of noradrenaline were highly increased, and the rough-handling, which is normal when hens were being removed from battery cages to vehicles, had much greater effect than gentle handling or a short period of transport. During road transportation of goats by Nwe *et al.* (1996), it was observed that the greatest stress was induced by handling and the start of the journey, which activated in

the first place the sympathetic nervous system, including the adrenal medulla, and finally the adrenal cortex.

Another indicator of welfare problems during handling, loading and unloading is the quality of the meat after slaughter. The biochemical changes in muscle, especially in glycogen metabolism are affected by the responses of the animals to various conditions during the hours before slaughter. In pigs such conditions cause the meat to be pale, soft and exudative (PSE) (Vanputten *et al.*, 1978). PSE meat arises when there is very rapid glycolysis, with consequent high production of lactic acid and a fall in pH immediately after death. Consequently, water-binding capacity of muscle proteins declines, water leaks out of the meat and the colour becomes more pale and grey. Another condition which can arise in cattle, pigs, sheep and goats is dry firm and dark (DFD) meat (Tarrant, 1981; Fazio and Ferlazzo, 2003). This occurs if muscle glycogen reserves is depleted before death due to poor unloading so that little lactic acid can be produced in muscle after death and the pH remains high. Both PSE and DFD meat cause substantial economic losses.

2.4.1 Glucocorticoids

Glucocorticoids are well known not only as hormones essential for maintaining life and normal growth, but also as stress hormones. When animals are subjected to stress conditions such as long transportation, electric shock, fasting (starvation), low or high ambient temperature, high relative humidity, *etc*, the hypothalamic-pituitary-adrenal axis is activated and glucocorticoids are released from the adrenal cortex (Klemeke *et al.,* 1989). Excessive concentrations of glucocorticoids result in catabolic effects in skeletal muscles (Tomas *et al.,* 1979; Odredra *et al.,* 1983). Elevated concentrations of glucocorticoids decrease the rate of synthesis (Hayashi *et al.,* 1992) and increase the rate of breakdown of myofibrillar proteins, which leads to muscle wasting and growth retardation (Ohtsuka *et al.,* 1992; Higuechi *et al.,* 1996). Thus, adrenal function intensely influences the performance of meat animals.

The adrenal cortical activity is increased in road transport stress. The increase in concentration of cortisol in the blood is often an indicator of psychological stress imposed on the animal even before transport is commenced. Such stress includes exposure of animals to a new environment, handling, loading, the start of vehicle and motion (Broom, 2003). Broom *et al.* (1996) noted that the greatest stimulation of cortisol release was induced by transferring sheep from their individual holding pens to vehicles, and that the commencement of vehicle motion produced an additional cortisol peak of 350% above the initial value. Glucocorticoid content in blood is generally considered to be a good index for the

reaction of animals to any environmental challenge. An elevation in plasma cortisol levels in response to transport has been widely documented in the calf (Warriss *et al.*, 1999; Alberghina *et al.*, 2003).

During transportation for up to 31 h, cattle that lied down had higher plasma cortisol levels than those that remained standing (Knowles *et al.*, 1999b). The transport of sport horses to the hippodrome produces either moderate or significant increases in cortisol levels (White *et al.*, 1991; Leadon 1995), equivalent to those detected in trained horses at rest before a competition. In transporting goats, Aoyama *et al.* (2003) found that plasma level of cortisol significantly increased during 1 h transportation, and that the values in females were significantly higher than those in males. Nwe *et al.* (1996) also observed an increase in plasma level of cortisol from an initial value of 42 $ng.ml^{-1}$ before transport to a peak of 166 $ng.m^{-1}$ within the first 1 h of a 6-h road transportation of Japanese goats. Generally speaking, cortisol is a useful indicator of short-term stresses from handling or husbandry procedures. It is a time-dependent measure that takes 10-20 min to reach peak levels (Lay *et al.*, 1992a). Cortisol levels in cattle were classified into three categories: 1) base-line level, 2) level that occurs during restraint, and 3) extreme stress (Dunn, 1990). Therefore, according to Grandin (1997), cortisol levels are highly variable and absolute comparisons should not be made between studies. The degree to which plasma cortisol increases during travelling depends on the initial concentration. Furthermore, in sheep sampled before and after 9-14 h journey, carried out under commercial conditions, cortisol increased by 19% in August (summer) and 65% in November (Knowles *et al.*, 1993). Also, in a similar study by Knowles *et al.* (1994), a significant increase (18%) in cortisol occurred after an 18 h journey (from 30 to 154 $mmol.l^{-1}$), whereas a

decrease (7%) was noted following 24 h transport (from 113 to 105 mmol.l^{-1}). Knowles *et al.* (1995) showed that sheep transported for 3 h had greater increase in cortisol (90%) than those transported for 9, 15, 18, or 24 h.

2.4.2 Haematocrit, leucocyte and erythrocyte counts

Packed Cell Volume (PCV) or haematocrit decreases in animals subjected to transport stress. Broom *et al.* (1996) found that haematocrit decreased abruptly after loading (time zero), and more gradually subsequently. The haematocrit was highest ($P<0.001$) in the stationary condition. Knowles *et al.* (1993) reported decreases in haematocrit following 9-14 h journeys, although the effect was considered to be due to recovery after initial rise induced by rounding up and handling. Increases in haematocrit values were also observed with different journey times (Knowles *et al.*, 1995), but Knowles (1999) noted a decrease in all transported calves. However, it should be noted that even when initial values are similar, restraint or isolation stress produced a greater fall in haematocrit than handling alone (Parrot *et al.*, 1988). Hence, it seems that stressors can produce an actual, rather than apparent, decrease in haematocrit values.

Erythrocyte and leucocyte counts increased by 5.3 and 3.9%, respectively in transported animals. The differential leucocyte count was altered as evidenced by decrease in eosinophil (eosinopenia) and lymphocyte (lymphopenia) counts by 300% and 6.1%, respectively; neutrophil count increased by 11.3%. Such changes in blood

picture also indicated body dehydration (Plyashenko and Sidorov, 1987; Nwe *et al.*, 1996). Transport combined with fasting of 48-72 h resulted in higher haematocrit than for fasting only, thereby demonstrating that the greater state of dehydration is as a result of water turn-over associated with the physical activity during transport as well as the stress of adjusting to a new environment (Cole *et al.,* 1987; Becker *et al.,* 1989). In horses, however, during transportation the PCV was found to increase after the journey (Ferlazzo *et al.*, 1993a).

2.4.3 Polyunsaturated fatty acid

Polyunsaturated fatty acid (PUFA) is a good indicator of stress as a result of lipid peroxidation (LPO). LPO end products are peroxidized lipids; when these are degraded, they generate new free radicals (FR) and several cytotoxic compounds such as aldehydes. For example 4-hydroxy-nonenal (4-HNE) and malondialdehyde (MDA) (Cheeseman and Slater, 1993; Morrisey and O'Brien, 1998; Romero *et al.,* 1998), though it has been proved that MDA has a low toxicity (Esterbauer *et al.,* 1982). The consequences of damage in the molecular structure of PUFA are more evident when these lipids are part of the cell and sub-cell membrane because it alters their cohesion, fluidity, permeability and metabolic function (Yu, 1994; Bandyopadhyay *et al.,* 1999). The value of PUFA increases in the blood when animals are deprived of food, and with the length of journey (Brown *et al.,* 1999b). As carcass weight decreases only after 24 h, fat breakdown, apparently, occurs in the deposits around the gut. A similar result was obtained by Knowles *et al.* (1999a), while studying the effect of 19 h road journey on 60 calves.

2.4.4 Creatine phosphokinase

The activity of creatine phosphokinase (CPK) increases in the blood after tissue damage, apparently as a result of increase in the permeability of muscle membrane that may be induced by transport stress through the process of lipid peroxidation. The increase occurs, especially when the animals are roughly driven and mishandled before and after transport (Moss and McMurray, 1979; Broom *et al.*, 1996). Similar results were recorded in calves, where after 4 h of journey the level of CPK gradually declined (Knowles *et al.,* 1999a). In some experiments when the Animal Welfare

Transport Order was abided with, the rise in concentration of CPK in the blood was either minimal or showed none at all, signifying low level of tissue damage (Knowles *et al.*, 1993, 1994).

2.4.5 Ultimate pH and muscle glycogen

Ultimate pH (pHu) of muscle and muscle glycogen are useful indicators of fatigue or stress in an animals. A high pHu and depletion of muscle glycogen can result from chronic stress. There were differences in some of these measures in pigs transported over different periods. Longer journeys led to a higher pHu in the *longissimus dorsi* (LD), semimembranosus muscles (SM) and adductor (AD) muscles, and the pHu remained high in the LD and AD muscles of the animals kept in lairage for 6 h (Brown *et al.*, 1999a). These high pH values are potentially associated with a greater incidence of DFD meat. The results are similar to those observed by Warriss (1982) in pigs transported for short periods and fasted. The SM and AD muscles are more susceptible to glycogen depletion because they have a higher proportion of oxidative, myoglobin rich fibres than the LD muscles. The liver is a major store of carbohydrate that can be readily mobilized for energy during fasting or exercise. In an attempt to make up for energy loss during transport, glucose is mobilized from glycogen in the liver and muscles into the systemic circulation. Liver glycogen concentrations decreased non-significantly during longer journeys (Warris and Brown, 1983). Brown *et al.*(1999b) recorded significant decrease in liver glycogen concentration in pigs deprived of food for similar periods. In transported birds, the longer period of deprivation reduced liver weight and glycogen content, and also elevated the pHu value in the biceps muscle and, by implication, reduced its glycogen content. With long journey times, liveweight and glycogen content decreased (Warriss *et al.*, 1993).

2.4.6 Plasma total protein, albumin and haemoglobin

These parameters were observed to increase when animals suffer dehydration as a result of long hour journeys. Brown *et al*. (1999a) noted that pigs subjected to 24 h road transportation suffered severe dehydration as the concentration of plasma total protein and albumin were increased significantly, and that the animals drank heavily on return to the lairage. During rest, in the lairage the concentration of plasma total protein, albumin and haemoglobin returned to normal. This result was similar to that observed in sheep (Knowles *et al.*, 1996) and calves (Knowles *et al.*, 1999a).

During a 19-h road transport of calves, the levels of total protein increased after the journey in all groups during both winter and summer periods (Knowles *et al.*, 1999a). Similarly, during a 31-h road transportation of cattle, the concentration of plasma total protein increased during the journey, but the increase was independent of the journey time. In journeys of 21, 26 and 31 h, increases of up to 80 to 82 g/l were

recorded, and in the 14 h group the values decreased from 80 to 78.5 g/l (Knowles *et al.,* 1999b). In the same study, it was observed that the concentration of plasma albumin increased independently of the journey time. The overall increases were from 39.8 to 43.1g/l. In control groups, however, there were no increases in albumin.

2.4.7 Plasma glucose

When broilers were subjected to food withdrawal and long hours of journey which model commercial transportation, they showed evidence of dehydration and exhibited depletion of body glycogen (Warriss *et al.,* 1993). Glycogen depletion may be related to the sensation of fatigue and could account for the apparent exhaustion exhibited by some birds after commercial transport. Plasma glucose concentrations increased from approximately 4.5 to 5.5 mmol/l after journeys of 21, 26 and 31 h, and there was a marginally greater increase in the cattle transported for 14 h from approximately 4.5 to 6.0 mmol/l. Glucose concentration was observed to remain constant at approximately 4.6 mmol/l in the 14, 21 and 26 h groups, but increased by about 1 mmol/l in the

31 h group of cattle transported (Knowles *et al.,* 1999b). The commonly used physiological indicators of stress during transport are presented in Table 2.2 (Broom, 2003).

Table 2.2. Physiological indices of stress in transported domestic animals.

Stressor	**Physiological variables measured in blood or other body fluids**
Food deprivation	↑FFA, ↑B-OHB , Glucose, ↓Urea
Dehydration	↑Osmolality, ↑Total Protein, PCV ↑Albumin.
Physical exertion, bruising	↑CK, ↑LDH5, ↑Lactate, ↑Cortisol, ↑PCV, ↑LDH5, ↑Heart rate
Fear / arousal	↑Heart rate, ↑Respiraatory rate

Motion sickness	↑ Vasopressin
Hypothermia / hyperthermia	Body temperature, ↑ Skin temperature

Increase in the value of the parameters Decrease in the value of the parameters FFA – Free fatty acids, B-OHB – B-hydroxybutyrate; PVC, Packed Cell Volume; CK-Creatine kinase, LDH5, Lactate dehydrogenase ISO enzyme 5.

(Adapted from Knowles and Warriss, 2000; modified after Broom, 2003).

2.5 Behavioural activities of transported poultry

Behaviour is the link between any animal's internal (physiologic, neurochemical, neuroanatomic and genetic components) and external environments (the ecosystem) (Aliu, 2004). It plays a critical role in biological adaptations. Individual animals may be excitable, nervous, aggressive, docile, or affectionate; the sum of these behavioural characteristics is called temperament (Takeuchi and Houpt, 2003). An animal's behaviour is an important and easily accessible indicator of its health and welfare. When an animal is organically ill or stressed, the most visible sign by which the client or experimenter recognizes the illness or stressed condition is change in behaviour (Overall, 2003; Aliu, 2004). All presenting signs are non-specific; they may be locomotory, oral, aggressive, destructive, vocalization or hallucinatory. Many ethologists capture their observations on video- or audio-tape. From these observations, they make a behavioural inventory- an **ethogram,** a description and documentation of the behavioural pattern under study. An effective management

system must take poultry behaviour into consideration, since behaviour is an indicator of welfare (Oluyemi and Roberts,1988).

Cashman *et al.* (1989) reported that duration of transportation was the major factor determining tonic immobility (TI) reactions in broilers, but acknowleged that although the TI response is frequently used as a measure of fear, fatigue engendered by a long journey might also prolong subsequent TI reactions. Although fear is adaptive in ideal circumstances, in reality it can seriously harm the welfare and performance of poultry and other farm animals, particularly if its arousal is sudden, intense, prolonged or inescapable. Fear is a very important behavioural trait, which may lead, if not evaluated properly, to high economic losses (Elrom, 2000).

2.5.1 *Fear and fatigue*

Fear is a major stressor that is widely regarded as an undesirable state of suffering by many people, the scientific community, welfare and policy groups, as well as growing numbers of farmers. In ideal conditions fear is adaptive behavioural functioning, whose biological role is to protect the animal from injury. Both acute and chronic fear can seriously harm the welfare and performance of poultry. Fear responses are seen as escape or panic reactions, which are inappropriate in intensive rearing systems and can cause injury, pain or even death (Jones, 1996; Etches *et al.*, 1995).

Jones (1996) claimed that the most efficient ways of reducing fearfulness and increasing adaptability in poultry (at least in laboratory conditions) are: environmental

enrichment, regular handling or related treatments, vitamin C (ascorbic acid) supplementation to the diet or the drinking water and genetic selection. He cited a few precursors of fear; peril, painful emotion, excitement by danger, a state of alarm or dread and more. Fear is usually listed among the emotions as love, hate, and anger and is regarded as an emotional – psychophysiological response to perceived danger. In ideal circumstances fear is an adaptive state. Fear behaviour acts to protect the animal from psychochemical damage and injury. High levels of fear decreases plumage condition, egg production, egg shell quality, growth and feed conversion efficiency. Fear impairs the adaptability of the birds to environmental changes, decreases their ability to interact between themselves and between the poultry-man. In poultry, the most frightening events encountered by poultry are sudden changes in their physical or social environment and exposure to humans, such as occur during transportation.

2.5.2 R*elation of fear to stress*

Fear may contribute to overall stress, particularly if the stimulation is intense or prolonged. Fear is a powerful stressor that can seriously damage the welfare, management and performance of poultry. It is important particularly in the present farming systems when birds are housed in systems, which prevent or restrict the expression of appropriate or adaptative responses to threatening stimuli, so leading to a more severe outcome. Today, it has been established that fear is not only a state of

suffering, but also a powerful and potentially damaging stressor (Craig and Adams, 1984; Jones, 1987; Nicol and Scott, 1990; Jones, 1996).

The intensity of the fear response is influenced by several cognitive, physiological and physical factors such as previous experience, assessment of the opportunity to escape, hormonal status and the size of a threatening animal or object. There are varieties of fear responses and the birds respond appropriately for coping with a particular danger (Jones, 1996).

2.5.3 The causes of fear in poultry

Many factors are known to be fearful for birds. Chickens often perceive contact with humans as an alarming predatory encounter. Two of the commonest and most potentially frightening events for domestic fowls are sudden changes in their social or physical environment and exposure to people. That is why gathering, crating and transportation of broilers are probably the most fearful events in their life (Jones, 1996; Elrom, 2000).

Factors that are fearful for birds are:

1. Transport from one place to another, for example from hatchery to brooding house.
2. Exposures to large open areas (neophobia) are considered as fearful. This is because domestic fowls descended from Red Jungle fowl, whose natural habitat is dense rainforest. Chicken may feel exposed and vulnerable to attack by predators in open areas.

3. Exposure to unfamiliar stimuli such as: object, food, noise or odour, are all potentially frightening. Examples are changes in diet formulation, lighting, thunder, sonic booms and banging of metal buckets (Jones, 1996).

4. Visual, auditory and/or physical contacts with people may be threatening to birds that are unaccustomed to their presence or have unpleasant experiences with them (Duncan, 1989). Birds may panic if untrained people enter the poultry house (Jones, 1992).

   Also sudden changes in the clothes (including colour and style) of the poultry-man, which may elicit fear and panic. The quality of the contact is also important where inversion and suspension by the legs are more frightening than being held upright.

5. Rapid approaching objects (humans, animals and machinery) are fear-provoking stimuli. These may be sensed at a distance of 25 m or more by layers.

6. Unpredicted appearance and movement of animate and inanimate objects (e.g. other birds, people, *etc*.), regardless of how familiar these may be.

7. Disruption of the social environment: Common changes are separation of familiar companions, presence of strangers or exposure to a strange group, destabilization of an established hierarchy, aggressive encounters, alarm vocalizations and displays (Jones, 1996; Elrom, 1999).

8. Exposure to potential predators or predator-related stimuli. It is interesting to stress out that cockerels show alarming behaviour when they are presented with computerized simulators such as view of raccoon and flying hawks. Though

feather pecking and cannibalism are induced by sight of blood, it was suggested that domestic chicks and pheasants avoid non-specific blood when presented in small dishes (Jones, 1996).

9. Large fast-moving stimuli: many poultry producers report serious losses when their birds are frightened by low flying aircraft.
10. Husbandry procedures (such as dust cleaning; preventive treatment against external parasites).
11. The transportation of poultry from the farm to the slaughterhouse or markets is a multi-factorial process, which may embrace all the above-mentioned factors and a number of potentially traumatic events. Harvesting, loading, disruption of social environment, motion, sudden acceleration and deceleration, exposure to unfamiliar environments; birds and noises, climatic changes, pre-slaugher processing and many other stressful factors (Knowles and Broom, 1990; Nicol and Scott, 1990; Elrom, 1999).

Transportation arouses relatively high levels of fear in broilers and layers, and is related to the duration of the journey (Bayliss and Hinton, 1990; Fraser and Broom, 1990; Nicol and Scot, 1990; Scot, 1994; Elrom, 1999). Also, social, genetic and environmental backgrounds such as group size, stocking density, social stability, genome housing system, the shape, dimensions and position of cages may induce fear in birds(Nicol & Scott, 1990; Jones, 1996).

2.5.4 The responses to fear

Responses which, in normal circumstances may be appropriate, tend to be devastating in intensive rearing systems. During catching before transportation to the slaughter house, panic, hysteria and violent attempts to escape may lead to a waste of energy, injury, pain or even death of birds. The injuries caused directly or indirectly by the elicitation of acute fear state represent a major welfare problem because they lead to chronic pain, infection, physical disability, and undesirable behavioural symptoms such as social withdrawal. Moreover, they also cause reduced profitability through the downgrading of carcasses at slaughter as well as through increased death on arrival (Jones *et al.*, 1996).

2.5.5 Measurement of fear

Jones (1997) stressed that neither the fear state nor underlying fearfulness can be measured directly in any species, including man. The following behavioural fear-ranking tests have been frequently used to measure fear in poultry.

1. *The open field or novel environment:* Birds are observed in a novel and generally barren enclosure which is often larger and more brightly lit than the home cage, after removal from their home environment and placed in sudden insulation. Silent, inactive birds are considered to be more frightened than those which vocalized, ambulate, eat, or explore the environment (Jones, 1997).

2. *Emergence or hole-in-wall test:* The test is based on the concept that a frightened bird will avoid emergence from a sheltered area, through a doorway, into an exposed brightly lit unfamiliar area than a less fearful one (Jones, 1997).

3. *Home cage avoidance test:* This test may be done without removing the bird from its cage. A stimulus such as nearby humans or an object (e.g. pencils, metronomes, coloured rods), which is placed in the feeder (trough), is estimated for its ability to cause the birds' withdrawal. This test is controversial since fear functions to protect the organism from harmful stimuli and so the avoidance, in this case, may not always reflect the bird's fear of the stimuli (Jones, 1997).

4. *Box plus experimenter:* The method is used in the laboratory to score the approach or avoidance tendencies of chicks towards a visible human, seated behind a wire mesh (net) at one end of an unfamiliar room. High avoidance is considered to reflect high fear of people. It should be noted that in this case, regular handling of the birds may influence the results of the test (Jones, 1997).

5. *Approaching human test:* The number of birds that remain close to or approach the experimenter as he walks slowly through the poultry shed estimates fear of human. This has been shown to be an important limiting factor of productivity in broilers. The experimenter can use a video camera, so video tapes can be analyzed later.

6. *Tonic immobility (TI):* This relates to an unlearned response, which is easily induced by placing a light manual restrain down on its back or side for approximately 15 seconds and then releasing. At first when fixed, the bird struggles and attempts to escape but soon it adopts an immobile posture, which may last from a few seconds to several hours. The state is characterized by temporary motor inhibition, rigidity and loss of righting response, which according to Craig and Adams (1984) is the return of an animal to sternal recumbency after being placed on its back or side. Their postural reaction turns a falling animal in space, so its feet or paws are pointed at the ground. The normal reaction is dependent on normal vestibular, visual and proprioceptive functions. TI is an indicator of fear and stress during transportation (Fraser and Broom, 1990; Knowles and Broom, 1990; Jones, 1992; Sherwin *et al*., 1993).

Additional characters of the state of TI are slow-wave deactivated electroencephalogram (EEG) pattern, sympathetic nervous activity and reduced overt responsiveness to external stimulation. This is an anti-predator reaction in which the more frightened bird will show a longer TI reaction (Scott, 1993).

Behavioural measures can also be used to determine fatigue caused by transport, but might be confounded by the progressive immobility normally correlated with increasing fearfulness (Jones, 1987). For instance, Cashman *et al.* (1989) reported that duration of transport was the major factor determining TI reactions in broilers, but acknowledged that although the TI response is frequently used as a measure of fear, exhaustion engendered by a long journey might also prolong subsequent immobility reactions. It is important to distinguish whether the primary response of transported

birds is fearfulness or fatigue, as this may determine priorities for improvements in transport design and driving practice (Jones, 1987).

2.6 Adverse effects of transportation stress on nutrition and disease susceptibility of food animals

Transportation exposes food animals to stress, resulting in increased morbidity and mortality. Deprivation of food and water for quite a long time during transportation further depresses the condition of the already stressed animal. Transportation during the hot-dry season for a long time, which is commonly done in Nigeria, aggravates the incidences of diseases in the transported food animals (Lefaucheur *et al.,* 1991). These deleterious factors have direct negative effects on optimum production by reducing heat production and the energy available for maintenance of production (Vertsegen, 1987). They also significantly weaken the body resistance to diseases by depressing the cellular and humoural immunity. Consequently, the virulence of opportunistic microorganisms such as *Escherichia coli*, *Pasteurella spp*, and *Salmonella spp* increases. The morbidity of respiratory and digestive diseases in such animals may also increase (Kent and Ewbank, 1983; Nikitchenko *et al.,* 1988; Hartung, 2003). Exposure of animals to other stress factors that often accompany transport stress such as changes in temperature, ventilation, noise levels, group size, density and fatigue may also aggravate other bacterial infections, which cause pneumonia and enteritis in young calves and erysipelosis in pigs (Scott, 1986). In each of these situations, stress occurs when individuals attempt

to adjust to the new but adverse environmental conditions. The link between stress and disease has been explored extensively in man, farm and laboratory animals (Gross and Siegel, 1981).

With lack of feeding, electrolytes are reduced. The extent of dehydration is measured by changes in plasma total protein and albumin concentrations, but there is some indication that giving water alone was detrimental in a journey time of 19 h, including a break during the journey (Knowles *et al.*, 1999a). In general, mid-transport feeding in a journey of 19 h with a one-hour rest was of limited benefit, and it was concluded that under commercial conditions mid-journey feeding was likely to be detrimental (Knowles *et al.*, 1999a). However, when the journey time is up to 24 h and above, mid-transport feeding has a greater effect and beneficial changes (Knowles *et al.*, 1999a). It has been observed that pigs transported for 24 h without food and water were metabolically stressed, hungry and thirsty. The lack of food for up to 24 h had by far the largest effect on the parameters measured, and transport in itself had little additive effect (Knowles *et al.*, 1999b).

The latest European Union (EU) rule (Anon, 1991) implemented in the UK as Welfare Order of Animals (Transport), 1997 allows mature cattle to be transported by road for up to 31 h provided they are given a 1 h rest with water; and if necessary, feed should be provided after the first 14 h (Knowles *et al.*, 1999b). The welfare tends to become poorer as journey distance and duration increase, when water and food supply are insufficient. A 6-h transport tends to develop an energy deficit. Abrasions and

injuries occur most often in short journeys. Bad road causes fatigue and the health of animal is easily impaired, especially if rough-driven (Hartung, 2003).

2.7 Journey time and its effect on liveweight of food animals

Transporting animals for a long distance is found to cause a loss in liveweight or carcass weight (Brown *et al.,* 1999). The current U.K. legislation (Welfare of Animals Transport Order (1997) addresses the issue on how long animals should be transported before resting or taking food and water. The Order recommends and allows a maximum journey time of 19 h, which must include a one-hour break for food and rest. After 19 h the journey time can be extended to 24 h, if the lorry meets additional requirements, one of which is to provide continuous access to drinking water (Knowles *et al.,* 1999b). Young and pregnant animals and birds suffer most during transportation within the recommended EU legislation on journey time (Freeman *et al.,* 1984).

The European parliament in November, 2001 adopted a detailed resolution on animal transport demanding that cattle, horses, goats, sheep and pigs not intended for specific breeding and sporting purposes be transported for a maximum duration of eight hours, or a distance of 25 km. In addition, the means of transport should have mechanical ventilation (irrespective of engine types), and its internal temperature must not fall below 5^0C or exceed 30^0C (Gavinellii, 2003).

In a study which examined the effects of transporting calves less than four weeks of age by road, in both summer and winter, for 19 h with a one-hour break on the lorry, it was found that the effects of the journey were greater during winter (a mean loss of 2 kg in winter and 1.4 kg in summer), when liveweight loss was greater and the journey was more prolonged. The transported calves suffered a depression in body temperature and pre-transport liveweight was regained by all the transported groups, within eight hours in summer and within 16 h in winter. However, the mean weight of the transported groups remained below that of their control groups for 48 h after the journey in summer and for 72 h in winter (Knowles *et al.,* 1999b).

Concern about how long pigs should be transported was also evaluated using pure Large White breed. The pigs were transported for 8, 16 or 24 and then either slaughtered immediately on arrival at the abattoir or kept in lairage for 6 h before slaughter. It was found that pigs travelling for 8, 16 or 24 h lost 2.2, 3.0 and 4.3% of liveweight, respectively. Both liveweight and carcass weight were regained during the lairage period, although there was a net liveweight loss in comparison with the control animals. Only transport for 24 h led to losses in carcass weight (Plyaschenko and Sidorov, 1987; Brown *et al.*, 1999).

During an international journey of 30 h, the losses in liveweight and carcass weight in cattle were slightly lower (Lambooij *et al.*, 1985), and were similar to those observed after food was withdrawn during a short journey, lasting less than three hours (Warriss, 1982). Similarly, during 15-h road transportation, sheep lost 5.5% of their initial liveweight, while in the stationary control group the loss was only 3.6%. This suggested that feed and water deprivation, alteration in metabolism and elimination of waste products which accounted for about 60% of liveweight loss, rather than stress-related effects, are responsible for liveweight loss during journeys (Brown *et al.*, 1999a). However, these effects were rather long-lasting because the animals did not regain their liveweights by the following day, despite the overnight provision of ample food and water. The findings suggested that the stress factors are also responsible for the loss of liveweight (Plyaschenko and Sidorov, 1987). Another factor that was reported to account for loss of liveweight during transportation was high air temperature on hot-dry periods. Such a loss is common in Nigeria during the

hot-dry season. The concomitant action of high air temperature and humidity results in water loss through enhanced respiratory evaporation and greater water turn-over leading to about 10.6% loss of liveweight (Plyaschenko and Sidorov, 1987; Ayo *et al.*, 1996; Hartung, 2003).

2.8 The effect of ambient temperature on rectal temperature in transported food animals

The physiological parameter of RT continues to remain the most relevant on- the-spot diagnostic parameter of the state of an animal's health before any laboratory analysis is done. This parameter is of great importance in evaluating the adaptability of domestic animals to various environmental factors (Vihan and Sahni, 1981; Ayo *et al.,* 1998), including transportation stress (Ayo and Minka, 2004). The fluctuations in the parameter, partly caused by variations in the ambient temperature above or below the zone of comfort, provide accurate information on the adverse effect of stress factors acting on the body (Ozkan *et al.,* 2003). It has been observed that the high air temperature and high humidity that occur during the hot-dry period, with little or no water supply to the animals during the long journey time, aggravate transport stress and are apparently responsible for the increase in RT values during road transportation (Ayo *et al.,* 1996; Altan *et al.,* 2003). Strong sunshine, with increase in day time and high ambient temperature, increases mortality rate in transported pigs (Smith and Allan, 1976). Similarly, in another study on pigs transported for 3-4 h, sharp increase in RT values were observed (Plyschenko and Sidorov, 1987).

Ambient temperature had been measured along the route during transport and was always higher inside than outside the lorry and higher at the front than at the back, and also very high during stop-overs. The ambient temperature fluctuations can cause increase in body temperature, and consequently heavy losses in livestock, particularly pigs and birds. During stop-over for a long time on hot days, the initial discomfort to transported animals increases with time and death may occur, especially if the relative humidity is high. Therefore, transportation of food animals at night or in the early morning is recommended (Plyschenko and Sidorov, 1987; Ayo *et al.,* 1996).

2.9 Effect of noise and vibration on transported food animals

Noise in acoustic biology is defined as any sound that the acoustic organ finds difficult to listen to and also causes increased stimulation of the nervous system, leading to dysfunction of normal physiological processes in the animal (Belanovsky, 1989; Warriss *et al.*, 1997). The normal quantity of noise that the animal can perceive without any dysfunction is between 30 – 55 dB (acoustic norm). Cattle are, however, more sensitive to noise than humans, the auditory sensitivity of cattle is at its greatest at 8000 Hz, sheep are visibly frightened by the barking of dogs and are upset (Gracey, 1986).

During road transportation of animals, noise can arise from different sources such as human voices, the use of whips, noisy machinery, thunder, barking dogs, compressed air brakes on vehicles, alarm bells, and vehicles. In Nigeria the use of vehicles not specifically designed for animal transportation of animals and birds, indiscriminate use of whips on the animal during loading and unloading, poor engine quality of the vehicles, unnecessary stop-overs, especially where many trailers are packed with ignited engines, aggravate the condition of the already stressed animals and birds (Ayo *et al.,* 1996; Ayo and Minka, 2004).

Intensive noise from many sources influences blood circulation of the transported animal and causes a rise in erythrocyte sedimentation rate, dysfunction of endocrine organs and cardiovascular diseases during the transport period. It has been reported that noise of up to 120 dB reduces body weight of transported animals, especially in birds, and reduces significantly egg laying ability of birds. It also impairs

digestion in the rumen and reduces lactation by 30% (Belanovsky, 1989). Excess noise increases defaecation and aggression in animals which may lead to injuries (Gracey, 1986).

Vibration from vehicle used in transportation, when the engine of the vehicle is ignited or the vehicle is in motion, causes a serious discomfort to the animals. This leads to depletion of glycogen stored in the body, and consequently causes fatigue in transported animals and birds (Randall *et al.*, 1996). Vibration for 3 h increases the body temperature of animals and decreases pH in both white *pectoralis superficial* and red *biceps femoris* muscles in birds. Higher vibration frequencies resulted in reduced glycogen concentration in liver and *biceps femoris* muscles of birds during transportation (Warriss *et al.*, 1995). Similar findings confirmed that birds show aversion to specific frequencies and magnitude of whole body vibration (Duggan and Randall, 1995). Warriss *et al.* (1993) showed that transport of broilers for up to 6 h depleted liver and muscle glycogen as did fasting for 10 h. The glycogen depletion observed during transport, particularly in the muscles, could be partly due to counteracting effects of vibration. Vibration during transport could, therefore, potentially contribute to fatigue and injuries. Cattle, sheep and pigs are all able to perceive higher frequency sounds than humans, and it is possible for the animals to become fearful of sounds inaudible to man (Fazio and Ferlazzo, 2003).

2.10 Vitamin C

Vitamin C or L-ascorbic acid in its pure form is a white crystalline solid substance that is freely soluble in water. Vitamin C is a very powerful reducing agent, an effective antioxidant. Even in

small amounts, it can protect indispensable molecules in the body, including proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and ROS. FR and ROS are generated during normal metabolism as well as through exposure to toxins, stress and environmental pollutants. Vitamin C is capable of regenerating other antioxidants such as vitamin E and carotenoids (Balz, 2003; Pedro *et al.,* 2003). It gives up two hydrogen atoms to become dehydroascorbic acid, the L-form of the vitamin. The oxidation is reversible in the animal body. However, in exceptional cases the reaction is irreversible, producing oxalic and diketogluconic acid (McDonald *et al.,* 1987).

The stability of Vitamin C is affected by pH, temperature, contact with oxygen and traces of metals, especially copper. The acid is easily destroyed by cooking and food processing. It is easily synthesized by certain moulds, fungi, higher plants and animals, with the exception of man, primate and guinea pig that lack the hepatic enzyme that synthesizes L-gulonolactone to vitamin C. Therefore, there is need for its supplementation in the daily food of these animals.

The biosynthetic pathway of the vitamin is from D-glucose $\longrightarrow$ D. glucoronolactone $\longrightarrow$ L-gulonolactone $\longrightarrow$ L-ascorbic acid (McDonald *et al*., 1987). In animals, Vitamin C is present in all body fluids and tissues. The adrenal glands have 150-200 mg of Vitamin C/100g of tissue. Also, organs like pituitary, corpus luteum, adrenal cortex and thymus are rich in the vitamin, while the liver, lungs and heart have the lowest levels. The skeletal muscle has only a trace of Vitamin C (George and Norman, 1970). In the body, the vitamin can be detected by subjecting it to a weak solution of silver nitrate, parts containing the vitamin are indicated by deposits of silver (McDonald *et al.,* 1987).

Vitamin C is commonly found in different fruits and vegetables. The content of Vitamin C in fruits and vegetables varies greatly, but its contents are very high in

oranges, strawberries, and especially in raw sweet red pepper, which contains up to 141 mg per ½ cup (Balz, 2003).

2.10.1 Antioxidant role of vitamin C

Vitamin C can act as an antioxidant by two mechanisms. Firstly, it can be readily oxidized to dehydroascorbic acid. This reaction is reversible, so the interconversion between these molecules forms a redox system. This interconversion forms the basis for many of the physiological activities of vitamin C, and so both vitamin C and dehydroascorbic acid show vitamin C activity. Vitamin C can also form an ascorbate radical giving another route to antioxidant activity by destroying free radicals derived from oxygen, including hydroxyl, singlet oxygen and superoxide. In this role it may show a synergistic action with other protective enzymes, including superoxide dismutase, glutathione peroxidase and catalase. This has been confirmed by Gecha and Fagan (1992), who showed that *in vitro* addition of vitamin C decreased the rate of H_20_2-induced proteolysis and also the destruction of exogenously added superoxide dismutase. Vitamin C is also involved in vitamin E antioxidant system, converting oxidized forms of α-tocopherol back to α-tocopherol.

Puthpongsiriporn *et al.* (2001) found that the antioxidant properties in egg yolk and plasma were improved in heat-stressed hens given supplemental vitamin E. Aydemir *et al.* (2000) studied effects of various nutrients, including vitamin C on activities of antioxidant enzymes and lipid peroxide levels in erythrocytes in mature

chickens. Vitamin C increased superoxide dismutase and glutathione peroxidase activity by 20 to 33%, respectively, but the combination of selenium plus vitamin E was most effective in depressing lipid peroxide levels.

Vitamin C has been demonstrated to enhance antioxidant activity of vitamin E by reducing the tocopheroxyl radicals back to their active form of vitamin E (Jacob, 1995) or by sparing available vitamin E (Retsky and Frei, 1995). A positive synergistic effect of antioxidant property of vitamins A and C has been reported (Thaxton and Pardue, 1984; Yin *et al*., 1993; Bendiche *et al*., 1994).

2.10.2 Physiological basis for the use of ascorbic acid in therapy and prophylaxis of diseases

> Ascorbic acid plays roles in cell respiration, growth and division. It is involved in calcium, amino acid, carbohydrate and fat metabolism. It regulates blood circulation and permeability of blood vessels. Vitamin C activates the functions of many enzymes, including amylase, lipase, protease and hormones, especially cortisol and adrenaline. It promotes immunological processes in the body. Consequently, it enhances antibody formation, increases bactericidal properties of the blood and phagocytic activities of leucocytes (Kondratyev, 1988). Thus, vitamin C increases the body resistance to environmental stressors (Hasanzadeh, 1997).

It is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments and bones. It plays a significant role in the synthesis of the neurotransmitters, neuropinephrine and 5-hydroxytryptamine, and in the control of brain function and mood. In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to mitochondria for conversion to energy. In general vitamin C is used in the prevention of many chronic diseases, when taken in large quantity than required for prevention of scurvy and for a relatively long duration of time (Bogolyubov 1987). It is also used to prevent heart diseases, stroke, cancer, cataracts, lead toxicity and infertility in cockerels. It retards the process of ageing (Byadevlatov *et al.*, 1992; Balz, 2003).

2.10.3 The therapeutic and prophylactic use of Vitamin C in poultry

Although vitamin C is mostly considered to have an antioxidant effect, there are situations in which it functions as a pro-oxidant. This occurs particularly in the interaction of vitamin C with transition metals, especially iron. Ferrous (Fe^{2+}) iron reduces H_20_2 to generate the OH radical and in the process becomes ferric (Fe^{3+}) iron. Vitamin C can convert ferric iron back to ferrous iron, itself being oxidized to dehydroascorbic acid. Continued supply of vitamin C can thus generate a series of cycles of vitamin C -driven free radical generation from iron (Herbert *et al.* 1996). This system can be used *in vitro* to provide oxidative stress. There is little direct information in poultry on whether adverse effects can occur *in vivo* from the combination of elevated dietary concentrations of iron and vitamin C. Since inorganic iron is relatively poorly absorbed, any such effects might be largely restricted to the gastro-intestinal tract. Vitamin C was not found to protect the rat intestine from iron-induced oxidative stress during repletion of iron-deficient animals, but supplementation with vitamin E did provide protection (Srigiridhar and Nair, 2000). Absorbed iron is usually bound to proteins (e.g. haemoglobin, ferritin) which may protect it from oxidative reactions. In humans, individuals with plasma vitamin C concentrations at the upper end of the normal range did not show any sign of oxidative damage to DNA when given supplemental iron (Proteggente *et al.* 2001). In view of these findings on other species, it is therefore probable that adverse effects of interactions between vitamin C and iron do not occur in poultry under normal nutritional conditions. However, tissue protein degradation resulting in release of

protein-bound iron may explain the observation of vitamin C -enhanced oxidation in raw poultry meat (Grau *et al.,* 2001).

There have been many studies over the years on the effects of feeding vitamin C to laying hens, with a great variability in the results reported: sometimes quantitative or qualitative responses have been observed, but sometimes they have not. These experiments have been carried out under a wide range of environmental and nutritional conditions and involving different rates of vitamin C. As with most other large sets of nutritional data, it is not always possible to reconcile conflicts between some individual observations. Nevertheless, it seems apparent that responses of laying hens to vitamin C have occurred most frequently in birds under stress. This trend seems to have been maintained in the more recent experimental observations (Abd-Ellah, 1995; Ubosi and Gandu, 1995; de Faria *et al.,* 2001).

Specific benefits of supplemental vitamin C have been established in climatically controlled conditions, where induction of heat stress can decrease feed intake, egg production and shell quality and increase mortality, depending upon the severity of the stress. Cheng *et al.* (1990) studied effects of vitamin C on experimentally induced continuous heat stress (31^0C, 60% RH). Dietary vitamin C (200 mg/kg) decreased mortality and had a small effect in improving shell quality. De Faria *et al.* (2001) reported that the imposition of chronic heat stress in climate chambers depressed egg production and shell quality. Dietary vitamin C at 200 and 400 mg/kg improved shell percent and egg specific gravity, but did not affect production. Balnave and Muheereza (1997) compared effectiveness of sodium

bicarbonate, organic zinc and vitamin C on shell quality of heat-stressed hens (30 and 35^0C ambient temperatures) in temperature-controlled rooms. Heat stress impaired egg production, feed intake and shell quality. Sodium bicarbonate (1%) improved shell quality, particularly shell strength, whereas effects of organic zinc or vitamin C (400 mg/kg) were not significant.

Benabdeljelik *et al.* (1990) carried out a series of trials on brown egg-layers over the course of a year to cover a range of environmental temperatures. Vitamin C (1 g/kg) was generally without effect on egg production or egg or shell quality. Kassim and Norziha (1995) studied hens under a hot (20-35^0C), humid tropical climate. They observed that Feeding 400 and 600 mg vitamin C/kg did not affect egg production or feed intake, but reduced egg weight and increased internal egg quality and shell thickness. Ubosi and Gandu (1995) studied dietary supplementation with 0, 100, 200, 300 or 500mg vitamin C/kg in hens under tropical semi-arid conditions. Vitamin C supplementation had no effect on egg quality or shell thickness, but some of the treatments improved egg production and feed intake. Behl *et al.* (1995) supplemented diets with 44 or 88 mg vitamin C/kg under hot tropical conditions (mean maximum temperature 42.5^0C). Egg production, feed intake and feed conversion were improved marginally by vitamin C. Shell thickness was also increased with 88mg/kg. Sahota and Gillani (1995) studied hens at high daytime ambient temperatures (31-45^0C), supplementation with vitamin C (50 and 100mg/kg) improved egg production, feed intake and feed conversion (Abd-Ellah, 1995). Vitamin C supplementation (1,125,250 and 500mg vitamin C/kg) during hot summer months,

where temperatures reached up to 43^0C in the experimental room, caused reduced mortality (28%). The higher levels of vitamin C markedly reduced mortality (8%), improved egg production, feed intake, egg weight, feed conversion, but not egg shell weight, 250 mg/kg gave optimum response. Oruwari *et al.* (1995) fed 0, 1.0, 1.5 or 2.0 g vitamin C/kg to Babcock hens under tropical condition where peak temperatures reached 36^0C, vitamin C supplements improved survivability, egg production, quadratic, with optimum effect at 1.5g vitamin C/kg. Sushil *et al.* (1998a, b) fed a diet supplemented with 800 mg vitamin C/kg to hens housed under average maximum ambient temperatures of 35.5 and 32.5 and 32.2^0C during the early (24-34 weeks) and peak (34-40 weeks) periods, respectively. Egg production and weight were higher and overall mortality was lower in the vitamin C -supplemented birds. Egg quality measurements indicated that shell thickness was lower during the peak period in the vitamin C -treated hens.

El-Gendi *et al.* (1999) fed 0, 200, 300 and 400 mg/kg of vitamin C to hens during the summer months in Egypt and observed that treated hens showed a reduction in egg production, but an increase in mean egg weight and shell weight per unit surface area. Plasma Ca concentration was increased by vitamin C progressively with time. Yolk total lipids and cholesterol were decreased, particularly at the higher vitamin C concentrations. Zapata and Gernat (1995) studied effects of moulted hens under relatively moderate (tropical) environmental temperature conditions of 25^0C and dietary supplementation with 100, 250 or 500 mg vitamin C/kg. They reported that

egg production was increased at the 2 higher doses of vitamin C, with egg weight unaffected.

Fear and other psychological stressors can activate the physiological responses to stress. Thus, fear induced in quails by catching and cooping considerably elevated plasma corticosterone concentrations (Satterlee *et al.*, 1993). Dietary treatment with vitamin C failed to affect corticosterone concentrations, but did attenuate the TI fear reactions of stressed and unstressed quails. Pretreatment with drinking water supplemented with vitamin C also reduced the fearfulness of quail. Lines of quails have been selected for low or high adrenocortical response to stress. The lines showed reduced fearfulness after vitamin C supplementation of water (Jones *et al.*, 1999). Similar responses have been observed in broilers. Thus, plasma corticosterone concentrations rose 5-fold in birds stressed by catching, blood sampling and cooping for 10 minutes, but this rise was unaffected by prior provision for 24 h of water containing 1 g AA/kg. However, vitamin C treatment did decrease non-specific, underlying fearfulness of birds in open field and TI tests (Satterlee *et al.*, 1994). In contrast, Campo and Carnicer (1994) did not find an effect of dietary vitamin C (400 mg/kg) on period of TI in laying hens.

The findings in both quails and broilers corticosterone responses to fear are not alleviated by vitamin C contrasts with the findings in heat stress, where rises of the same order of magnitude occur. This suggests that different factors may govern the release of corticosterone from the adrenal glands in animals subjected to different types of stress. Thus, the very rapid onset of stress induced by intense fear may trigger

a sudden release of corticosterone that is not influenced by the vitamin C content of the adrenal glands, perhaps because the adrenal vitamin C content does not become depleted over a short period. However, the more gradual onset of heat stress may allow a greater opportunity for the vitamin C -corticosterone interaction to develop. Likewise, the apparent lower intensity of fear associated with open field or TI tests might allow the effect of vitamin C on behaviour through an effect on neurotransmitter systems. Vitamin C is known to influence catecholamine synthesis and the conversion of dopamine to norepinephrine (Tolbert *et al.*, 1979).

Birds under stressful conditions seem to recognize the beneficial effects of vitamin C and can adjust feeding behaviour to increase their vitamin C intake. In a series of studies, Kutlu and Forbes (1993a, b) have shown that broilers under stress can select greater proportions of feed containing supplemental vitamin C. The diets need to be coloured to enable the birds to identify the supplemented diet. Thus, when presented with coloured diets supplemented with 0 and 200 mg vitamin C/kg, the birds consumed more of the supplemented diet. When returned to the thermoneutral environment, selection was reversed.

Both psychological and physiological stresses, including heat stress, can affect broilers during transportation. Zulkifi *et al.* (2001) have shown that provision of water containing 1200 mg/l of vitamin C for 24 h before crating and transportation increased H/L ratio and TI. Satterlee *et al.* (1989) also studied the effects of provision of vitamin C in the drinking water (1200 mg/l) to broilers 24 h before they were cooped, held in a cooled holding shed for 8 hours without food or water prior, then transported to an

abattoir. Plasma corticosterone concentration was elevated approximately 5-fold at the end of this procedure in birds not given vitamin C, but the rise was 30% less in birds given vitamin C. H/L ratio was also lowered by the vitamin C treatment. Thus vitamin C can alleviate the overall effects of the stresses involved in the course of catching and transportation. The lowering of plasma corticosterone concentration is likely to decrease the imbalances in plasma electrolyte profile and other metabolic disturbances induced by corticosterone, perhaps thereby reducing water loss or tissue breakdown. This may explain the reports of improved carcass yields seen in broilers given supplemental vitamin C prior to transportation (Farr *et al.*, 1988).

The effects of vitamin C in relation to multiple concurrent stressors has been studied by McKee and Harrison (1995). The stressors were beak trimming, exposure to coccidia and heat stress, applied from 10 to 17 days of age in a factorial design to birds fed diets containing 0, 150 and 300 mg vitamin C/kg. Vitamin C failed to alleviate depressed growth or feed efficiency associated with coccidiosis, but did alleviate depressed growth or feed efficiency, elevated H/L ratios in the heat stressed birds. Vitamin C also alleviated the rise in H/L ratio in the beak-trimmed birds. In the absence of vitamin C, there was an additive effect on performance and H/L ratio of imposing the stressors in relation to depressed growth and abolished the effect completely on feed efficiency and H/L ratio. It was concluded that 150 mg vitamin C/kg gave the optimum effect in protecting birds from these multiple stressors.

2.11 Vitamin E

Vitamin E was discovered in 1922 by Evans and Bishop as a soluble factor in vegetable oils which was found to be very important for normal reproduction in rats. This compound was found to be important not only for rats, but also for the fertility of poultry. Normal testicular function of cockerels as well as the hatchability of breeder eggs depend on α-tocopherol or vitamin E, but there are other functions, besides fertility, performed by the vitamin. Vitamin E deficiencies can cause a variety of pathological conditions, including well-known encephalomalacia, nutritional muscular dystrophy and haemolysis. Together with selenium which is an integral part of the enzyme glutathione peroxidase, it can prevent conditions like exudative diathesis, gizzard erosion and other diseases. On the other hand, vitamin E stimulates the immune response of poultry, helps to detoxify dangerous organic and inorganic compounds and ensures oxidative stability of meat. Thus, vitamin E performs many important functions in most body tissues (Macmurray, 1980).

2.11.1 Structure and analytical determination of vitamin E

Instead of vitamin E, the term 'α-tocopherol' has been used. From a chemical point of view, vitamin E comprises a variety of compounds, α-tocopherol being one of them.

Basically, two groups of the vitamin can be distinguished: tocopherols and tocotrienols. Both of these are characterized by a 2-ring hydrocarbon structure, the

"toco", and an isoprenoid side chain. The latter C16 structure can be either saturated in which case the compound is called tocopherol, or unsaturated with three bonds which denotes the tocotrienol. Depending on methylation (-CH3) in position 5, 7 and 8 of the ring structure, the resulting compounds are commonly called, α, β, γ, δ-tocopherols or tocotrienols. Biologically, all these substances have different activities and are present in feed-stuff in varying amounts. However, α-tocopherol is not only the dominating vitamin E active compound in feed ingredients, but also shows the highest biological activity.

Therefore, in analytical terms, α-tocopherol is considered as the general reference standard (Kevin, 1982).

2.11.2 The interactions of vitamin E as an antioxidant

The whole process of the degeneration of tissues intimately involves the tripartite interaction of PUFA, selenium (Se), and vitamin E. Lipid as an antagonist and Vitamin E and selenium as protagonists. The components of PUFA are linoleic, linolenic and arachidonic acids. Selenium is a chemical compound of the enzyme Glutathione Peroxidase (GSHPx) (Rotruck *et al.*, 1973).

Oxygen, during metabolism, produces an unpleasant set of compounds (the toxic oxygen metabolities) such as hydrogen peroxide, superoxide, hydroxyl radical and singlet oxygen (Chance *et al.*, 1979). These compounds, once produced, can initiate damage pathways leading to molecular damage of lipids, nucleic acids and proteins. This damage at the molecular level alters the functional capacity of these molecules

resulting in alteration of membrane structure with a subsequent loss of transport and compartment functions. Damage to DNA induces alteration of the information it carries so that misreading or alteration may result, for instance, in carcinogenesis. Damage to proteins in loss of enzyme activity with subsequent loss of catalytic function. These far-reaching effects can then, either singly or in concert, result in damage of tissue with the subsequent appearance of disease in the animal. Other components external to the animal, for example, some drugs, toxins and nutrients, under certain conditions, can either enhance the capacity of the cell to produce these toxic oxygen metabolites or produce compounds by transformation during the course of their metabolism. Consequently, products that can result in similar damage pathways are generated (Bus and Gibson, 1979).

Nature has not left the animal completely unprotected and enzymes have been provided to remove these toxic products from the cell. For instance: Catalase (iron dependent) removes hydrogen peroxide; GSHPx removes hydrogen peroxide and lipid peroxides; vitamin E quenches free radicals and also can destroy some of the other toxins; superoxide dismutase (copper) removes superoxide ion, and carotene is a particularly effective quencher of singlet oxygen (Foote, 1979). It is interesting that most of these detoxifying pathways are under some form of nutritional control whether it be a trace metal or a vitamin. The role, therefore, of any particular protective mechanism will depend on how the damage was initiated and sustained.

A suggested specific interaction between polyunsaturated lipid, vitamin E and selenium is shown in Fig. 2.2. and 2.3. Polyunsaturated lipid can undergo either *in*

vitro or in vivo peroxidation resulting in the production of hydroperoxides. The hydroperoxide breaks down to a free radical, which causes damage to tissue. This sequence can be interrupted firstly by selenium in the form of glutathione peroxidase with the removal of hydroperoxide and secondly by vitamin E, which removes the free radicals, thus, preventing damage from occurring (Hoekstra, 1974; Scott *et al.*, 1974).

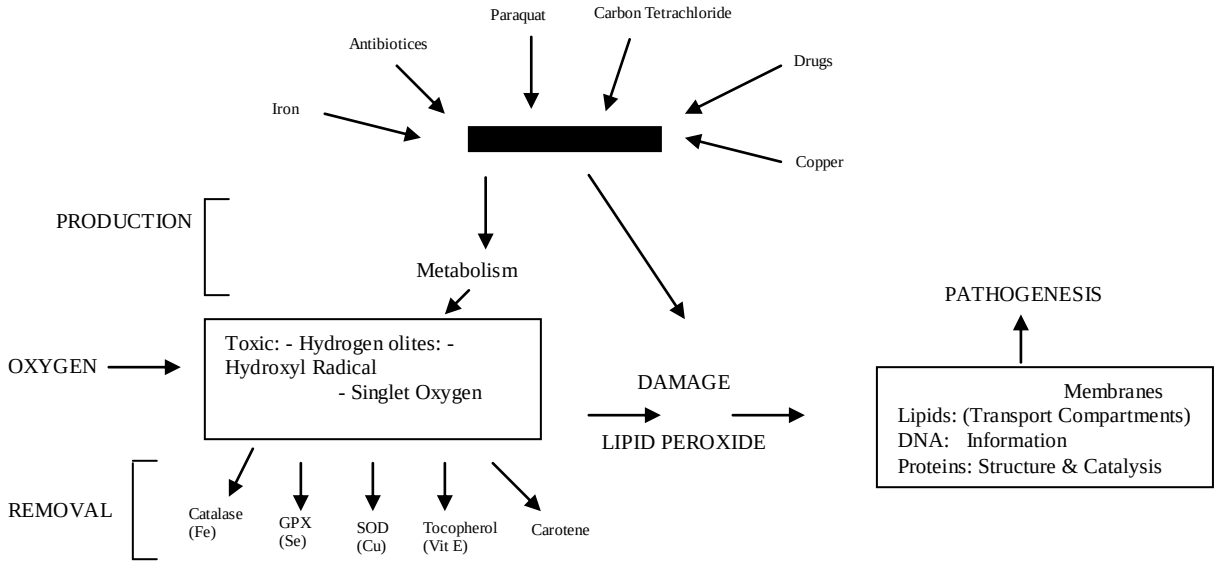


Fig 2.2 interaction between polyunsaturated lipid, vitamin E and selenium

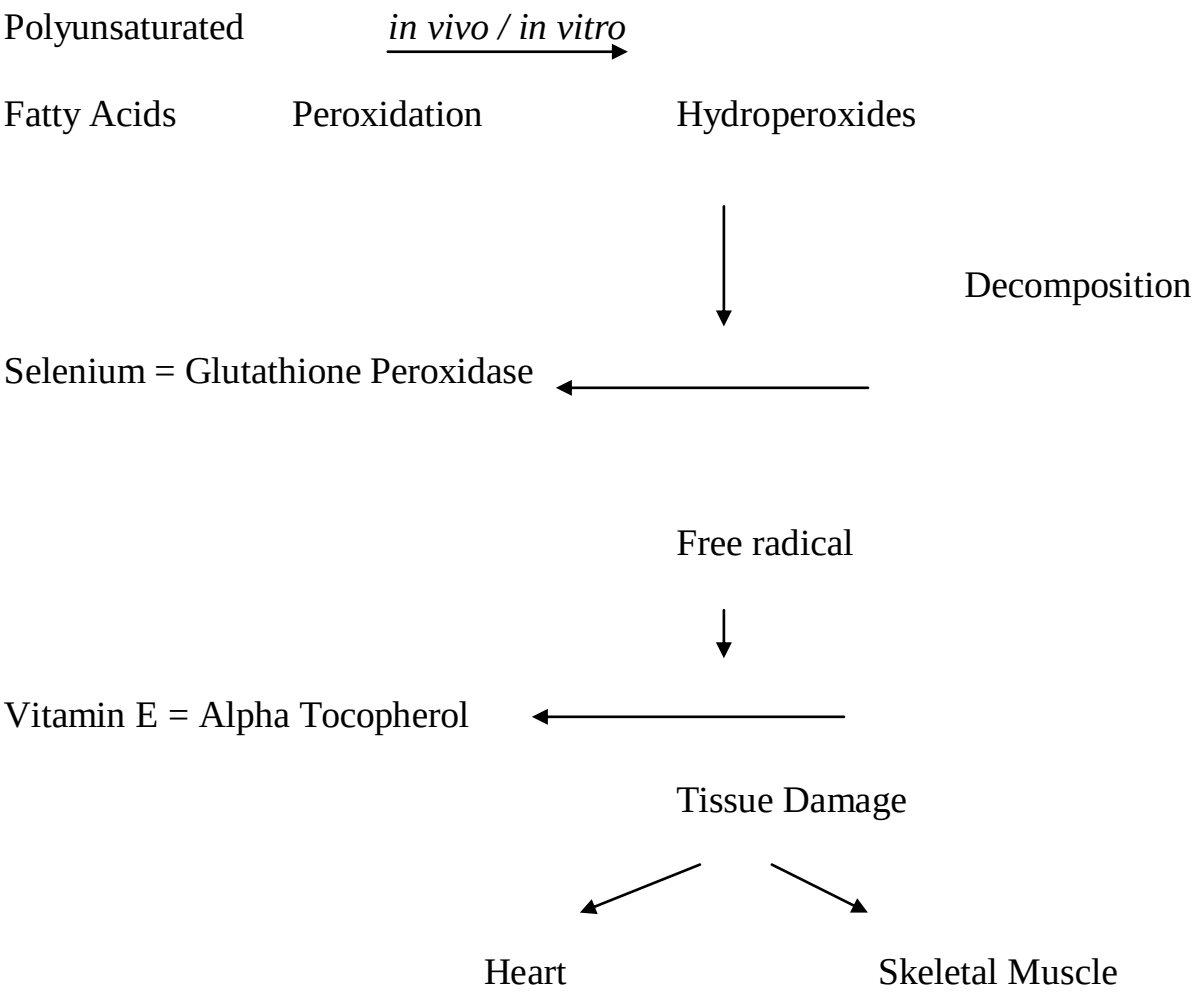


Fig. 2.3 Specific interaction between polyunsaturated fatty acids, Selenium and Vitamin E.

It should be noted that the capacity of the defensive mechanism to protect the organism depends on the rate at which the hydroperoxide is being produced. Should the capacity for protection be swamped, then damage will still result.

2.11.3 Physiological basis for the use of vitamin E in therapy and prophylaxis of diseases

A study was undertaken to determine the amount of vitamin E and the route of administration which would correct vitamin E deficiency and improve immunity. Hostein heifer calves were allotted at birth to one of four vitamin E treatments (1) 0 mg; (2) 1400mg; (3) 2,800mg DL-α-tocopheryl acetate fed orally weekly for 12 weeks; (4) 1,400IU DL- α-tocopherol was injected intramuscularly weekly (Reddy *et al*., 1985; Reddy *et al*., 1986). The calf starter contained 0.44 mg kg^{-1} selenium. Lymphocyte stimulation to pituitary hypothalamus axis (PHA) was increased ($P<0.05$) at 4 and 8 weeks in calves injected with vitamin E (Reddy *et al*., 1986). Serum from calves fed 2,800 mg of vitamin E and those injected with the vitamin had lower ($P<0.05$) viral replication titres. Serum IgM was increased ($P<0.05$) at week 6 by feeding the highest vitamin E amount. When averaged over the 12-week period, serum creatine kinase was reduced ($P<0.05$) by oral and injected vitamin E supplementation compared to controls. The authors pointed out that the higher creatine kinase content in unsupplemented calves was preclinical indication of muscular dystrophy which was not prevented by selenium alone, and that both vitamin E and selenium are necessary for prevention. In an additional experiment, 2,000IU of DL- α-tocopherol was injected into yearling heifers and the serum α-tocopherol and lymphocyte stimulation indices were increased ($P<0.01$) 7 days later (Reddy *et al*., 1986)

Following shipping, calves weighing 242 kg were each supplemented with 800 IU vitamin E daily for the first 21 days and 400 IU for the next 7 days (Hicks, 1985). During this 28-day period, average daily gain and feed conversion were increased significantly ($P<0.01$) and the number of days sick, percent morbidity and mortality were reduced by vitamin E fortification. In another study, newly arrived stressed feeder cattle were fed either 0 or 1,600 IU vitamin E kg^{-1} diet (Gill *et al*., 1986). The cattle fed vitamin E gained weight 22.2% faster ($P<0.05$), had 11.7% less morbidity and 12.5% fewer sick days than controls.

Following the arrival of stressed calves, ten were injected with 1,500 IU D- α-tocopherol and ten received the carrier of the vitamin (Nockels *et al*., 1993). All the calves were injected with keyhole limpet hemocyanin and hen egg ovalbumin. Antibody development to the two antigens was increased ($P<0.05$) in those given the vitamin E.

2.12 The Shika Brown breed of layer

Following a proposal by National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University Zaria, Nigeria, to carry out a National Poultry Breeding Project (Buvanendran, 1979), the institute was mandated to initiate the project. This led to the importation of two strains of grand-parent lines (A and B) in 1985 to fulfill the mandate. The overall objective of the project was to develop genetic materials for the supply of parent-stock lines as a step towards self-sufficiency in supply of commercial layers. Selection was practised for the single trait, egg

production (No of eggs) up to 280 days of age. Other traits considered are age at sexual maturity, egg weight and mature body weight. In general, the average performance of the strain at different locations in Nigeria is in close agreement with published values for brown egg shell stocks. (Working, 1994; Nuru, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was performed at the Livestock Farm of the College of Agriculture and Animal Science (CAAS), Kaduna (11^{o} $10^{/}$ N , 07^{o} $38^{/}$ E), located in the Northern Guinea Savannah zone of Nigeria. The CAAS is a campus of the Ahmadu Bello University, Zaria.

3.2 Meteorological data from the study area

The ambient temperature (AT), dry bulb temperature (DBT) and relative humidity (RH) were recorded at the experimental site at 07:00, 14:00 and 18:00 h daily for three consecutive days before transportation. Values of the meteorological parameters were recorded at 1, 2, 3, 4, 5 and 6 h of the journey, and at 1, 2 and 3 h after unloading the layer chickens from the vehicle. For three days after the journey, the meteorological data were recorded daily at 07:00, 14:00 and 18:00 h.

3.3 Animals and management

Ninety apparently healthy SBL, of about 22 weeks old and weighing between 1.1 - 1.2 kg, were used for the study. The layer chickens were obtained from the Livestock Farm of CAAS, Ahmadu Bello University (ABU), Mando Road, Kaduna, and were pre-conditioned in three separately disinfected pen in the same farm two weeks before the commencement of the experiment. The pen was made of concrete floor and corrugated metal zinc walls and roofing, with a pen size of 5 m x 4 m wide

and 2.2 m high, with upper half of the length to the roof covered with wire mesh, which provided adequate ventilation. They were kept on deep litter system and were fed three times daily with a standardized layer mash ration formulated by CAAS, Kaduna (Table 3.1). Ample amount of water was provided. The chickens were routinely dewormed with morantel tartrate (Banmith F, Pfizer Ltd., USA) orally, at a dosage of 0.8 g/kg. They were also given coccidiostat (Amprolium hydrochloride, Amcox Agrar B. V., Holland) at a dosage of 100 g/200L of water orally. Vaccination against Newcastle disease was also administered. Maximum biosecurity was adhered to strictly, and the pen was kept clean every day.

Table 3.1 Layer mash ration (17.10% crude protein (CP) Layer mash) given to layer chickens during the experimental period (ton).

S/no	Items	KG
1	Maize	345
2	*Soya bean meal*	*100*
3	Groundnut meal	150
4	Wheat offal	100
5	Brewer's dried grain	100
6	Perm kernel cake	80
7	Limestone	80
8	Salt (Sodium Chloride)	2.5
9	Methionine	1
10	Lysine	1
11	Fish meal	8
12	Bone meal	30
13	Premix	2.5

Source : Poultry unit, CAAS, ABU, Mando road, Kaduna.

3.4 Experimental design

Ninety SBL layers were used for the study. They were obtained from the commercial layers of the school farm at the age of twenty-two weeks. Before the commencement of the study they were kept on deep litter system at the school farm for a period of two weeks when basic metereological and physiological parameters on each bird were observed. Such parameters were AT, DBT, RH, RT, behavioural, liveweight and egg production. In addition the following haematological information were obtained e.g. Red Blood Cells (RBC) counts, Haemoglobin (Hb), White Blood Cells (WBC) and the differential leucocytes counts. The birds were then divided at random into three groups of thirty each. Group (I} were each orally administered with vitamin C (Juhel Nig. Ltd., Enugu, Nigeria, NAFDAC No. 04-0170) at a dose of 200 mg/kg, dissolved in 5ml (w/v) of sterile water. Group (II} were each administered orally with vitamin E, (Pharco Pharmaceuticals, Sidi Gaber Alex – Egypt, NAFDAC No. 04-4635) at a dose of 200 mg/kg, dissolved in 5 ml (w/v) vegetable oil (Turkey Brand, NGO Chew Hong Edible Oil, PTE Limited, Singapore, Malaysia, NAFDAC No. 01-5780). Group (III} which served as the control group were each orally given 5ml (v/v) of sterile water. All the treatments were administered just (thirty minutes) before loading. The travel distance was between Kaduna and Birnin-gwari and back to Kaduna all within Kaduna State in the Northern part of Nigeria. The travel was undertaken on the 17th of May, 2006. This represent the late hot-dry and humid season of the year. The travel was undertaken at 10: 00 am to 16:00 pm, the distance covered was 280 km, and took about 6 hr. for completion. Food and water were withdrawn just

before the journey and throughout the journey period. On completion of the journey, the layers were unloaded at the same spot where they were loaded into the vehicle just before the start of the journey. Feeds were given as done before the commencement of the journey, and ample quantity of water was provided.

3.5 Vehicle design, loading and stocking of birds

A standard Liteace bus (made in Japan) was used for the transportation of the layers. The floor was made with flat metal iron, and the inner compartment of the vehicle measured 2.2 m x 1.5 m and 1.2 m high. The side walls of the vehicle from the floor to a height of 0.6 m was completely covered with corrugated aluminium. Both sides of the vehicle length were provided with sliding windows without curtains to allow for cross ventilation. A door was provided at the rear end. The roof top of the vehicle was provided with corrugated iron sheet, which held the body of the vehicle firmly. The vehicle was previously disinfected with 2% formol and 5% potassium permanganate solutions (Knowles *et al.,* 1999b), respectively. The transportation procedure was carried out in accordance with the guidelines governing animal transport welfare by road (Knowles *et al.,* 1999b). The floor of the vehicle was covered with wood shavings and the loading of the birds was done by nine people divided into three groups of three persons each. In a relatively calm condition, the first person caught one chicken at a time and calmly gave it to a standing intermediary, who passed the bird to the last person, already inside the vehicle. The last person in turn kept the chicken inside the plastic carrier. Six standard plastic carriers were used.

The dimensions of each carrier was 0.8 x 0.6 x 0.3 m. The layer chickens were stocked at a density of 0.05 m^2 per bird. The loading period was thirty minutes.

3.6 Measurements of rectal temperature of layer chickens

Three days before commencement of the journey, RT of the layer chickens was measured daily at 07:00, 14:00 and 18:00 h. On the day of departure, the RT was taken at 09:00 h; that is, just before loading the birds into the vehicle and immediately after loading at 10:00 h just before the vehicle kicked off. The RTs were measured at 1, 2, 3, 4, 5 and 6 h during the journey period. The RTs were measured immediately after unloading the birds from the vehicle and hourly for the first 3 h post-transportation, and subsequently daily for the first three days post-transportation (07:00, 14:00 and 18:00 h). All RT measurements were carried out in a relatively calm situation, and each bird was easily and calmly restrained for the measurements.

The RT was recorded, as an indicator of body temperature, using a manual clinical thermometer of 50^0C grade (Hospilabs Investment Ltd. ChongQing, China). The thermometer was inserted about 1-2 cm through the colorectum of each layer chicken by tilting (Zaytsev *et al*., 1971), and kept for 45 seconds before the readings were taken.

3.7 Measurements of liveweight of layer chickens

Liveweight of each layer was recorded using a standard dial spring scale (Camry IS09002 Model SPR : capacity 20 kg/44 lb, Shanghai, China) before loading,

immediately after the journey and, subsequently, daily for three consecutive days post-transportation. A previously perforated and weighed brand new carton was placed on the weighing scale and each layer chicken was calmly placed inside the clean carton and the weight quickly recorded. The weight of the layer chicken was taken as the total weight recorded minus the weight of the perforated carton.

3.8 Measurement of behavioural activities

3.8.1 Tonic immobility test

The following tests Tonic immobility (TI), Regrouping tests and awareness test associated with behavioural activities of the birds during transportation were carried out as enumerated by various researchers (Gallup, 1977; Jones, 1986; Elrom, 2000b). TI test has been used as an indicator of the extent of fearfulness and fatigue, this relates to an unlearned response, which is easily induced by light manual restraint. Immediately prior to the test, birds were removed individually from the transport crates and held down on its back or side for approximately 15 s and then released.At first when fixed, the bird struggles and attempted to escape but soon it adopted an immobile posture, which may last from a few seconds to several hours. The number of inductions required to produce TI (maximum of 5), and the duration of TI (maximum of 20 min) were recorded.

3.8.2 Regrouping test

Regrouping test was carried out as described by Sherwin *et al*. (1993). During the process, birds were removed individually from the transport crates and placed at one end of a 2 m runway constructed with wooden sides, 0.8 m high and 9.4 m apart, on a concrete floor covered with wood shavings. At the other end of the runway, four non-experimental birds were confined with wire mesh so as to be easily visible to the experimental bird. The time taken for the experimental bird to move half way (1 m) and fully (2 m) along the runway towards the confined birds was recorded. Birds

which had not moved either 1 m or 2 m down the runway after 300 s were removed, and their time recorded as 300 s. Latency that it took the birds to leave the starting box (maximum of 180 s) was also recorded.

3.8.3 Awareness test

One sub-group of birds (n=15) was conditioned to become more aware of their surroundings upon hearing a signal, and their reactions upon hearing this signal after transport/fasting were observed and recorded.

Each bird was placed individually on a plastic crate in the centre of an empty room and a variable period (10-60 s) was allowed for the bird to settle. The bird was then exposed to a taped auditory-conditioned stimulus (15 s duration piano scale of 2 octaves), followed by a midly aversive unconditioned stimulus (a fine water spray applied from a distance of 3 m). A further variable settling period was allowed to elapse and the procedure repeated. Each bird received two of these training trials on four of the seven days prior to transportation. After transportation, the birds were placed individually on a crate and exposed to the conditioned stimulus. The behaviour of the birds during this 15 s period was recorded from a hidden view point at a distance of 3 m from the birds. The following indicators of awareness were considered: headshaking, feeding, drinking, standing but not feeding, walking, lying, panting, crouching, croaking. The latency to first headshake was taken as a measure of awareness to the conditioned stimulus (Sherwin *et al.*, 1993; Elrom, 2000b).

3.9 Haematologic and biochemical analysis

After the completion of measurements of the physiological parameters on the experimental day, just before loading into the vehicle and immediately after unloading the birds post-transportation, 3 ml of blood sample was collected quickly and calmly from five birds selected at random in each treated group, through basilic venipuncture (*Vena basilica*), using sterile 18 gauge needle fitted into 10 ml sterile syringe. The collected blood was at once emptied into a 5 ml sterile test tube containing ethylenediaminetetra acetic acid (EDTA) as an anticoagulant. Another 5 ml of blood was collected from five different layer chickens selected at random, using the same procedure into lithium heparinised anticoagulant test tubes. The anticoagulants used were 5 mg/ml of blood. The tubes containing blood were kept on ice pack and quickly transferred to the Departments of Haematology, and Clinical Pathology of Ahmadu Bello University Teaching Hospital, Shika-Zaria for haematological and biochemical analyses, respectively.

3.9.1. Haematological analysis

Before centrifugation, two blood smears were manually prepared on microscope glass slides spreader, from each of the samples containing anti-coagulants. The blood smears were dried at room temperature and at an angle less than 45^{o} and stained with Leishmann's (Bayer Corp., Diagnostic Division, Elkhart, IN, USA) using an automatic slide stainer. After drying, the total and differential leucocyte counts were carried out under the microscope oil-immersion objective and 100 cells per slide were counted using the straight-edged method as described by Brown (1980).

In addition the differential white blood cell counts, the erythrocytes and their indices were carried out with an automatic haematolgy analyzer (ADVIA 60 Haematology Analyser, RAB 046 IND. 4, Bayer Health Care, Lyon – France) for both blood samples taken before and after transportation.

3.9.2 Biochemical analysis

Biochemical analysis carried out were for the following; creatine phosphokinase (CPK), total protein (TP), alkaline phosphatase (ALP) and aspartate aminotransaminase (AST). Standard methods as described by the following (Rosalki, 1967; King, 1960; Kind and King, 1954 and Reitman and Frankel, 1957) were used for each test respectively.

3.10 Statistical Analysis

All data obtained were subjected to Student's paired *t*-test and the statistical package for social science (SPSS) was used for the correlation analysis. Data were expressed as mean plus or minus standard error of the mean (Mean ± S.E.M.). Values of $P<0.05$ were considered significant. Frequencies of behavioural activities like walking, lying, crouching and others, recorded were summed-up for each 30 min observation period. The data from four replicates were averaged and the results presented as simple percentile.

CHAPTER FOUR

4.0 RESULTS

4.1 Meteorological data

The meteorological data from the study period are shown in Tables 4.1 - 4.3. Table 4.1 shows metereological data from the experimental site three days before transportation. The maximum AT was 32.0^{0}C, while the minimum AT recorded was 25.0^{0}C, with a diurnal range of 7.0^{0}C. The AT was higher inside the pen than outside, with the values of 31.2 $\pm$ 1.9^{0}C and 25.3 $\pm$ 1.7^{0}C, respectively. The overall mean AT was 33.7 $\pm$ 0.8^{0}C (Table 4.2). The RH from the study period followed the same pattern, with the value of 98.0 $\pm$ 3.3 % inside the pen and that of 77.0 $\pm$ 3.0 % outside. There was no rainfall during the experimental period, and the wind direction was predominantly south-west.

During the journey period, the DBT was highest at the 4 and 5 h into the journey, with a value of 36.0^{0}C, and lowest at the 6 h with a value of 32.0^{0}C. The highest RH of 73% was recorded at the 6 h of transportation while the lowest value of 48% was recorded at 5 h of transportation. The RH range was 25%.

The metereological data at the experimental site after transportation (Table 4.3) showed a maximum DBT of 33.0^{0}C and a minimum of 25.0^{0}C, demonstrating a narrow diurnal range of 8.0^{0}C. The DBT was higher outside the pen than inside, with the values of 31.5 $\pm$ 0.8^{0}C and 25.5 $\pm$ 0.3^{0}C, respectively. The RH had a maximum of 98.7 $\pm$ 1.3^{0}C and minimum of 80.0 $\pm$ 5.0^{0}C. There was no rainfall during this period

and the wind direction was predominantly south-west. The AT values were not significantly ($P>0.05$) different before and after transportation. The pre- and post-transportation DBT, just as the RH, values outside and inside the pen were not significantly ($P>0.05$) different (Table 4.3).

Table 4.1: Meteorological Data at the Experimental Site Three Days before Transportation of the SBL chickens.

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

SEM = Standard error of the mean.

^{NS} = Non-significant difference (P> 0.05)

Table 4.2: Ambient temperature and relative humidity inside the vehicle during the journey period.

Hour of Journey	Dry-bulb temperature (^{o}C)	Relative humidity (%)
1	32.0	67
2	32.0	67
3	34.0	62
4	36.0	52
5	36.0	48
6	32.0	73
Mean ± SEM	33.7 ± 0.8	61.5 ± 3.9

SEM = Standard error of the mean

Table 4.3: Meteorological data at the experimental site for three days post–transportation of the layer chickens.

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

SEM = Standard error of the mean.

^{NS} = Non-significant difference (P> 0.05)

For three days post-transportation, the RT rose significantly in all the groups especially in the control layers. The RT rise was highest just as it was before transportation at 14:00 h in all the birds. The minimum RT of treated groups of layer chickens before transportation was lowest at 18:00 h and highest at 14:00 h, with the corresponding values of 40.0 ± 0.0^0C and 40.3 ± 0.3^0C for vitamin C-treated birds, while 39.9 ± 0.1^0C and 40.3 ± 0.3^0C were obtained for vitamin E-treated birds, respectively. The control group followed the same pattern with RT values of 39.9 ± 0.0^0C and 40.3 ± 0.2^0C at 18:00h and 14:00h, respectively (Table 4.4 / Appendix II). The minimum RT of treated groups of layer chickens after transportation was lowest at 07:00 h and highest at 14:00 h, with the corresponding values of 40.6 ± 0.3^0C and 41.2 ±0.5^0C for vitamin C, while 40.5 ± 0.3^0C and 41.0 ± 0.6^0C were obtained at 18:00 h and 14:00 h for vitamin E, respectively. The control group followed the same pattern as vitamin C-treated layer chickens, with RT values of 40.7 ± 0.4^0C and 41.0 ± 0.6^0C at 07:00 h and 14:00 h, respectively (Table 4.4 / Appendix II). The maximum RT of treated groups of layer chickens before transportation was lowest at 18:00 h and highest at 07:00 h, with the corresponding values of 41.4 ± 0.4^0C and 41.7 ± 0.2^0C for vitamin C-treated birds, while 41.4 ± 0.1^0C and 41.7 ± 0.2^0C were obtained for vitamin E-treated birds, respectively. The control group also followed the same pattern with RT values of 41.4 ± 0.2^0C and 41.7 ± 0.2^0C at 18:00 h and 07:00 h, respectively (Table 4.5 / Appendix III). The mean minimum and maximum RT values in treated and control groups before transportation did not differ significantly ($P>0.05$), but after

transportation, the mean minimum RT and maximum RT values rose significantly ($P<0.05$) from the corresponding pre-transportation values.

The maximum RT of treated groups of layer chickens after transportation was lowest at 18:00 h and highest at 14:00 h, with the corresponding values of 41.6 ± 0.1^0C and 42.0 ±0.5^0C for vitamin C-treated group, while 41.7 ± 0.2^0C and 41.9 ± 0.4^0C were obtained for vitamin E C-treated group, respectively. The control group followed the same pattern with RT values of 41.9 ± 0.0^0C and 42.7 ± 0.9^0C at 18:00h and 14:00h, respectively (Table 4.5 / Appendix III). The difference in mean minimum RT values before and after transportation in vitamin C-treated layer chickens was highly significant ($P<0.01$), very significant ($P<0.05$) for vitamin E C-treated birds and very highly significant ($P<0.001$) for control group respectively (Table 4.4 / Appendix II). The difference between the mean maximum RT values before and after transportation recorded in vitamin C-treated layer chickens was very significant ($P<0.05$), not significant ($P>0.05$) for vitamin E-treated birds and very highly significant ($P<0.001$) for control groups respectively (Table 4.5 / Appendix III).

4.2 Rectal temperature before transportation period.

The mean range RT values in treated and control layer chickens before transportation were not significantly ($P>0.05$) different. However, post- transportation the values were significantly higher than those obtained before transportation in layer chickens administered vitamin E (Table 4.6 / Appendix IV).

The mean values of hourly fluctuations in minimum RT of layer chickens during transportation were significantly ($P<0.05$) different between all the groups (Table 4.7 / Appendix V). The mean values of hourly fluctuations in maximum RT of layer chickens during transportation were not significantly ($P<0.05$) different between the treated groups, but significantly ($P<0.05$) different when compared with the control group (Table 4.8 / Appendix VI). The highest range in minimum and maximum hourly RT fluctations was recorded in layer chickens administered vitamin E, with the values of 1.7 and 2.2^{0}C, respectively.

4.3 Range and mean rectal temperature during transportation period

The mean hourly fluctuations in the range RT values of layer chickens during transportation were significantly ($P<0.05$) different between vitamin C-and E-treated groups, and vitamin C-treated and control groups. There was no significant ($P>0.05$) difference between Vitamin E-treated and control groups (Tables 4.9).

During transportation period, the overall mean RT values obtained from the experimental and control groups were significantly ($P<0.05$) different fron each other. The highest range of mean RT values was obtained in birds treated with vitamin C, with the values of 2.0^0C. The lowest mean RT value of 41.4 ± 0.1^0C was recorded also in layer chickens at the 6 h of transportation, when the journey was completed (Table 4.10).

Table 4.4: Hourly fluctuations in range values of rectal temperature (^{o}C) of SBL chickens during transportation period (Mean ± SEM, n = 45).

Hour (Time)	$^{++}$Vitamin C	Vitamin E	Control
1 (10:00)	1.2	1.3	1.5
2 (11:00)	1.7	1.0	0.8
3 (12:00)	1.2	0.7	1.1
4 (13:00)	1.7	1.2	0.8
5 (14:00)	1.2	0.7	1.2
6 (15:00)	1.9	0.8	0.6
Mean ± SEM	***1.5 ± 0.1 ^{a}***	***1.0 ± 0.1 ^{b}***	***1.0 ± 0.1 ^{b}***

Means with different superscript alphabets are significantly (P<0.01) different.
++ n = 15 across the row

Table 4.5: Hourly fluctuations in mean rectal temperature (^{o}C) values of SBL chickens during the transportation period (Mean ± SEM, n = 45).

Hour (Time)	++Vitamin C	Vitamin E	Control
1 (10:00)	40.3 ± 0.1	43.4 ± 0.1	43.1 ± 0.2
2 (11:00)	41.3 ± 0.1	42.0 ± 0.1	43.7 ± 0.1
3 (12:00)	41.3 ± 0.1	42.2 ± 0.1	44.8 ± 0.1
4 (13:00)	41.4 ± 0.1	42.2 ± 0.1	44.7 ± 0.1
5 (14:00)	41.3 ± 0.1	42.2 ± 0.1	44.5 ± 0.1
6 (15:00)	41.6 ± 0.1	41.4 ± 0.1	44.2 ± 0.1
Overall Mean ± SEM c	***41.2 ± 0.2 a***	***42.2 ± 0.3 b***	***44.2 ± 0.3***

Means along the same row with different superscript alphabets are significantly ($P < 0.05$) different.
++ n = 15 across the row

4.4 Fluctuations in rectal temperature in layer chickens three days pre- and three days post-transportation

There was no significant difference (Table 4.11 / Appendix VII) between the overall mean RT values in the control and treated chickens three days before transportation. However, the overall values before and after transportation period showed a significant ($P<0.05$) difference between control groups, a highly significant ($P<0.01$) difference between vitamin C-treated groups and a very highly significant ($P<0.001$) difference between vitamin E treated groups respectively (Table 4.11 / Appendix VII).

The RT values recorded in both treated and control chickens for three consecutive days post-transportation. The physiological parameters fluctuated with the hours of the day, with maximum RT recorded at 14:00 h, while the minimum was recorded at 06:00 h in both treated and control chickens.

The RT of 41.0 ± 0.1^0C obtained immediately after loading in vitamin C-treated group was significantly ($P<0.05$) higher than the value of 40.4 ± 0.2^0C, recorded just before loading. However, the RT values recorded immediately after loading in vitamin E-treated and control layer chickens respectively of 41.5 ± 0.1^0C and 41.3 ± 0.2^0C were highly significant ($P<0.01$), than the values corresponding of 40.3 ± 0.1^0C and 40.3 ± 0.1^0C, recorded just before loading (Table 4.12). There were significant ($P<0.05$) differences between vitamin C-nd vitamin E-treated groups and control group immediately after loading, but there was no significant ($P>0.05$) difference between Vitamin E-treated and control groups.

Table 4.6: Effect of loading on rectal temperature (^{o}C) values of SBL chickens (Mean ± SEM, n = 45).

Period	[++] Vitamin C	Vitamin E	Control
Just before Loading (9:00 am)	*40.4 ±0.2*	*40.3 ±0.1*	*40.3 ±0.1*
Immediately after Loading (10:00 am)	41.0 ± 0.1*	41.5 ± 0.1**	41.3 ± 0.2**

*** = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference**

++ n = 15 across the row

The correlation coefficients of the metereological and rectal parameters during and after transportation are shown in Table 4.13. Before transportation there was a negative correlation between the hour of the day, meteorological parameters and rectal temperature of the layer chickens. But after the transportation, the relationship was positive and significant ($P<0.01$) between the DBT, minimum AT, maximum AT and RT values of all the transported layer chickens. The relationship between the hour of the day, RH and RT values were positive but insignificant ($P>0.05$).

Table 4.8 shows the correlation coefficients of the metereological and rectal parameters during the journey period. The DBT rose with the values of RT in control layer chickens ($r=0.734$, $P<0.001$), while the correlation coefficient value of 0.312 obtained for vitamin C-treated group was insignificant. There was a negative relationship between the DBT and RT values in layer chickens treated with vitamin E. The RH was negatively and significantly correlated with the RT in control layer chickens ($r= -0.546$, $P<0.05$). In layer chickens treated with vitamin C and E, the relationship between the RH and RT was negative and insignificant ($r= -0.136$ and -0.118), respectively.

Table 4.7: The relationship between the meteorological and rectal temperature parameters SBL chickens pre-and post- transportation (n=45).

Correlated Parameters	++Vitamin C-treated Pre-transportation	Post-transportation	Vitamin E-treated Pre-transportation	Post-transportation	Control Pre-transportation	Post-transportation.
Hour of the day and rectal temperature	0.778**	0.156^{NS}	-0.778**	0.156^{NS}	-0.778**	0.156^{NS}
Dry-bulb temperature and rectal temperature	-0.582*	0.679**	-0.582*	0.679**	-0.582*	0.679**
Minimum ambient t temperature and rectal temperature	-143^{NS}	0.731**	-0.143^{NS}	0.731**	-0.143^{NS}	0.731**
Maximum ambient temperature and rectal temperature	-189	0.822***	-0.189^{NS}	0.822***	-0.189^{NS}	0.822***
Relative humidity and rectal temperature	-0.109	0.038^{NS}	-0.109^{NS}	-0.38^{NS}	-109^{NS}	0.38^{NS}

^{NS} = Non-significant difference (P>0.05); * = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference; *** = P<0.001 = Very highly significant difference. ++ n = 15 across the row

Table 4.8: The relationships between hour of the journey, dry-bulb temperature, relative humidity and rectal temperature during 6-h road transportation of SBL chickens.

	Correlated Parameters	Vitamin C-treated	Vitamin E-treated	Control
a.	Hour of the journey and rectal temperature	0.774**	0.773**	0.635*
b.	Dry bulb temperature and rectal temperature	0.312^{NS}	-0.052^{NS}	0.734**
c.	Relative humidity and rectal temperature	-0.136^{NS}	-0.118^{NS}	-0.546*

^{NS} = Non-significant difference (P>0.05); * = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference; *** = P<0.001 = Very highly significant difference.

4.5 Effects of road transportation on total red blood cell counts, platelets, and red blood cell indices of Layer chickens.

The erythrocyte values and their indices in layer chickens exposed to road transportation are presented in Table 4.15. Post-transportation, the haemoglobin in the layer chickens significantly ($P<0.01$) decreased from the pre-transportation values in birds administered vitamin E, vitamin C, but more especially in control birds. Similarly, after the journey, values of the layer chickens were significantly ($P<0.01$) reduced from the pre-transportation values. There was no significant difference between the pre- and post-transportation RBC values in layers administered vitamin C and E; rather in the control birds, the difference was significant ($P<0.05$). The values of platelets rose significantly ($P<0.01$) in control, vitamin C and vitamin E administered layer chickens. The mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) values pre-transportation did not differ from those obtained post-transportation in the control, vitamin C and vitamin E administered layer chickens. However, after transportation the mean corpuscular volume (MCV) in layer chickens administered vitamin C and those administered vitamin E increased significantly ($P<0.05$) from their corresponding pre-transportation values. In control layer chickens, the pre- and post-transportation MCV values were not significantly ($P<0.05$) different. The heterophil/lymphocyte (H/L) ratio increased considerably by about 0.2 in layer chickens treated with vitamin C and those treated with vitamin E while in the control birds, the ratio only increased by 0.10.

Table 4.9: Total erythrocyte and Platelets counts, erythrocyte dependable factors and indices in Transported Layer chickens (Mean ± SEM, n=30).

Group	H_b (g/dl)	PCV (%)	RBC ($x10^{6}/mm^3$)	Platelets ($x10^3/mm^3$)	MCHC (g/dl)	MCV(μm^3)	MCH (pg)
BeforeTransportation							
$^{++}$Vitamin C-treated	8.8 ± 0.1	27.1 ± 0.3	2.7 ± 0.1	70.2 ± 0.6	37.1 ± 0.3	135.7 ± 1.8	46.3 ± 0.7
$^{++}$Vitamin E -treated	8.8 ± 0.1	27.4 ± 0.2	2.8 ± 0.1	70.1 ± 0.8	37.1 ± 0.4	136.8 ± 1.6	46.0 ± 0.9
$^{++}$Control	8.9 ± 0.1	26.8 ± 0.3	2.9 ± 0.1	71.0 ± 0.8	36.6 ± 0.3	136.4 ± 1.8	47.1 ± 0.8
After Transportation							
$^{++}$Vitamin C-treated	7.1 ± 0.1**	22.0 ± 0.4**	2.5 ± 0.0 ^{NS}	63.6 ± 0.7**	36.3 ± 0.3 ^{NS}	126.3 ± 1.4*	46.5 ± 0.5 ^{NS}
$^{++}$Vitamin E-treated	7.0 ± 0.1**	22.6 ± 0.2***	2.6 ± 0.0^{NS}	61.8 ± 0.8***	36.7 ± 0.2 ^{NS}	126.6 ± 1.4*	46.1 ± 0.3 ^{NS}
$^{++}$Control	5.9 ± 0.1***	17.0 ± 0.1***	2.3 ± 0.1*	46.6 ± 0.7***	36.1 ± 0.3 ^{NS}	131.0 ± 0.3 ^{NS}	45.6 ± 0.5^{NS}

^{NS} = Non-significant difference (P>0.05); * = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference; *** = P<0.001 = Very highly significant difference;. Hb = haemoglobin; PCV = packed cell volume; RBC = red blood cell; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; ++ n = 10 across the row.

4.6 Effects of road transportation on total leucocyte and differential value of layer chickens.

Leucocytes and the differential white blood cell counts are presented in Table 4.16. The WBC counts significantly ($P<0.05$) decreased following the transportation in control layer chickens from pre-transportation values of 3.7 ± 0.1 to 3.4 ± 0.0 x $10^3/mm^3$ post-transportation.

In layer chickens administered vitamins C and E respectively, pre- and post-transportation WBC values did not differ significantly ($P>0.05$). Heterophils in the 3 groups (Vitamins C, E and Control) rose significantly ($P<0.01$) following transportation of layer chickens. The lymphocytes increased ($P<0.05$) following post transportation in control, vitamin C– and vitamin E-treated groups. Alas, it was observed that the values of basophil count in birds treated with Vitamins C and E pre- and post-transportation did not change when compared to that of the control. Howerver, eosinophil rose significantly ($P< 0.05$) from the pre-transportation value (1.8 ± 0.1) to 2.1 % post-transportation in the control.

Table 4.10: Total leucocyte and differential leucocyte counts in Transported SBL chickens (n=30).

Group	WBC ($X10^3/mm^3$)	Heterophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)	H/L ratio
BeforeTransportation							
$^{++}$Vitamin C-treated	3.8 ± 0.1	30.9 ± 0.2	55. 6 ± 0.6	1.9 ± 0.1	1.9 ± 0.0	6.3 ± 0.2	0.56
$^{++}$Vitamin E-treated	3.8 ± 0.1	30.1 ± 0.3	55. 5 ± 0.6	1.9 ± 0.1	1.9 ± 0.1	6.4 ± 0.2	0.54
$^{++}$Control	3.7 ± 0.1	30.9 ± 0.3	55. 7 ± 0.7	1.9 ± 0.1	1.8 ± 0.1	6.3 ± 0.2	0.55
After Transportation							
$^{++}$Vitamin C-treated	3.7 ± 0.1 ^{NS}	40.9 ± 0.2***	53. 8 ± 0.2*	1.7 ± 0.0^{NS}	2.0 ± 0.0 ^{NS}	3.9 ± 0.1**	0.76
$^{++}$Vitamin E –treated	3.7 ± 0.0 ^{NS}	39.5 ± 0.2***	52. 5 ± 0.4*	1.7 ± 0.0 ^{NS}	2.0 ± 0.0 ^{NS}	4.1 ± 0.1***	0.75
$^{++}$Control	3.4 ± 0.0*	32.6 ± 0.1**	50. 4 ± 0.5**	1.8 ± 0.0 ^{NS}	2.1 ± 0.0*	5.3 ± 0.0**	0.65

^{NS} = Non-significant difference (P>0.05); * = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference; *** = P<0.001 = Very highly significant difference; WBC = white blood cell ; ++ n = 10 across the row.

4.7 Effects of road transportation on clinical chemistry of blood parameters of layer chickens.

The clinical chemistry of blood parameters measured are shown in Table 4.17. The CPK value of layer chickens administered vitamin C decreased in all the groups, but the decrease in the value was significant ($P<0.05$) after the journey in layer chickens administered with vitamin C (from 84.4 ± 2.4 before and after the journey, to 73.2 ± 2.2 μmol/l respectively). However in vitamin E-treated and control groups, the difference between the values obtained pre- and post-transportation was not significant ($P>0.05$).

The TP values post-transportation were significantly ($P<0.05$) higher than pre-transportation values in both the treated and control groups. The ALP activity decreased significantly ($P<0.01$) following transportation in vitamin C-treated and control groups, but the activity increased in vitamin E-treated layer chickens post-transportation. After transportation the AST activity considerably decreased in vitamin E-treated layer chickens value obtained from the pre-transportation . However, the activity pre- and post-transportation did not differ significantly in both vitamin C-treated and control layer chickens.

Table 4.11: Clinical Chemistry of blood parameters of SBL chickens before and after transportation (Mean ± SEM, n=15).

Test	Before Transportation			After transportation		
	++Vitamin C-treated	Vitamin E-treated	Control	Vitamin C-treated	Vitamin E-treated	Control
Creatine (CPK, μ mol/l) Phosphatase Kinase	84.4 ± 2.4	75.4 ± 2.7	75.4 ± 2.7	73.2 ± 2.2**	73.2 ± 5.7^{NS}	73.2 ± 2.2 ^{NS}
Total Protein (Tp, g/dl)	46.4 ± 2.2	44.6 ± 1.7	42.8 ± 1.9	54.0 ± 2.0*	55.2 ± 4.0*	48.6 ± 0.8*
Alkaline Phosphatase (ALP, IU/L)	102.2 ± 7.7	133.8 ± 18.8	170.8 ± 25.2	85.0 ± 15.9**	169.0 ± 71.4**	70.2 ± 2.4**
Aspartate aminotransferase (AST, IU/L)	40.2 ± 6.5	53.4 ± 3.2	56.4 ± 2.2	42.0 ± 2.4 ^{NS}	42.0 ± 2.1*	52.4 ± 8.4 ^{NS}

^{NS} = Non-significant difference (P>0.05); * = P<0.05 = Significant difference; ** = P<0.01 = Very significant difference; *** = P<0.001 = Very highly significant difference n = 15 across the row.

4.8 Effects of road transportation on the liveweights of layer chickens

Liveweight of layers administered with vitamin C, vitamin E and control groups are shown in (Table 4.18). Three days pre-transportation, there was no significant difference in the liveweights of layer chickens in all groups. However three days post-transportation, the liveweights of experimental layer chickens decreased by 9.1% for both vitamins C and E groups, when compared with the corresponding pre-transportation values, while that of the control chickens decreased by 18.2%.

Table 4.12: Liveweights of SBL chickens three days before and three days after transportation. (Mean ± SEM, n = 45)

	Before Transportation, kg			After transportation, kg		
Day	***++Vitamin C-treated***	***Vitamin E-treated***	***Control***	***Vitamin C-treated***	***Vitamin E-treated***	***Control***
1	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.0± 0.0	1.0± 0.0	0.8± 0.0
2	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0± 0.0	1.0± 0.0	0.9± 0.0
3	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0± 0.0	1.1± 0.0	1.0± 0.0
Mean ± SEM	1.1± 0.0	1.1 ± 0.0	1.1± 0.0	1.0± 0.0^{NS}	1.0 ± 0.0 ^{NS}	0.9± 0.0 ^{NS}

^{NS} = Means are not significantly (P>0.05) different ++ n = 15 across the row

4.9 Effect of road transportation on egg production

Table 4.19 shows changes in egg production of layer chickens administered with vitamin C, vitamin E and control groups. Three days post-transportation, the decrease in egg production of layer chickens administered with vitamins C and E were 40.5% and 13.6% respectively, while the control group was 59.6%. The level of the decrease in control group post-transportation was very highly significant ($P<0.001$), but only significant ($P<0.05$) in vitamin C-treated group. In Vitamin E-treated group, the decrease was insignificant ($P>0.05$).

Table 4.13: Number of eggs collected from SBL chickens (n=90) three days before and three days after transportation.

	Before Transportation			After Transportation		
Day	***[++]Vitamin C-treated***	***Vitamin E-treated***	***Control***	***Vitamin C-treated***	***Vitamin E-treated***	***Control***
	No (% Production)	No (% Production)	No (% Production)	No (% Decrease)	No (% Decrease)	No (% Decrease)
1	22 (73.3)	22 (73..3)	20 (66.7)	7.0 (23.3)	15 (50.0)	04 (13.3)
2	26 (86.7)	26 (86.7)	25 (83.3)	15 (50.0)	22 (73.3)	10 (33.3)
3	26 (86.7)	25 (83.3)	27 (90.0)	22 (73.3)	26 (86.7)	15(50.0)
Mean ± SEM	24.7± 1.3 (82.3)	24.3 ± 1.2 (81.0)	24.0± 2.1 (80.0)	14.7± 4.3 (49.0)*	21.0± 3.2 (70.0)[NS]	9.7± 3.2 (32.3)***

[NS] = Non-significant

* = P<0.05

*** = P<0.001

[++] n = 30 across the row

4.10 Effect of road transportation on behavioural indices of layer chickens.

The behavioural indices of TI, regrouping and awareness test for all experimental chickens are shown in Table 4.14. Three days before transportation, all values obtained in the experimental groups were not significantly different from those recorded in control layer chickens. However, post-transportation the TI duration, duration to move 2-m and latency to headshake significantly ($P<0.05$) rose from the pre-transportion values in control chickens.

Table 4.14: Behavioural indices of SBL chickens before and after transportation (n=45).

Test	Before Transportation			After transportation		
	++*Vitamin* C-treated	*Vitamin* E-treated	*Control*	*Vitamin* C-treated	*Vitamin* E-treated	*Control*
1 Tonic immobility (n=10)						
Number of inductions	1.8 ± 0.3	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
Duration(s)	220.4 ± 16.1	225.8 ± 21.8	234.0 ± 14.7	270.3 ± 17.1^{NS}	250.1 ± 14.0^{NS}	410.9 ± 26.6**
2. Regrouping (n=5)						
Latency to leave box (s)	48.8 ± 1.4	48.4 ± 0.9	46.8 ± 1.6	52.2 ± 2.9^{NS}	53.4 ± 3.2 ^{NS}	46.4 ± 3.9 ^{NS}
Time to move 1m (s)	47.0 ± 1.9	50.6 ± 1.1	47.2 ± 1.7	51.4 ± 1.3 ^{NS}	51.6 ± 3.3 ^{NS}	42.8 ± 1.9 ^{NS}
Time to move 2m (s)	93.2 ± 4.2	92.2 ± 2.8	94.6 ± 1.7	89.4 ± 3.2 ^{NS}	98.2 ± 3.6 ^{NS}	87.6 ± 2.3*
3. Awareness (n=5)						
Latency to headshake (s)	8.6 ± 0.5	8.2 ± 0.6	8.4 ± 0.5	8.0 ± 0.6 ^{NS}	8.4 ± 0.5 ^{NS}	5.2 ± 0.9*

^{NS} = Non-significant

* = P<0.05

** = P<0.01 ++ n = 15 across the row

CHAPTER FIVE

5.0 DISCUSSION

5.1 Meteorological data from the study period

The results obtained in the present study showed that the transported chickens were subjected to the influence of high ambient temperature and high relative humidity, characteristic of the late hot-dry and early rainy seasons in the Northern Guinea Savannah zone of Nigeria. The high AT values recorded during the study period were predominantly outside the established thermoneutral zone for chickens, which ranges between 12-24^{0}C for birds reared in the temperate region (Selyansky, 1975), and 22-28^{o}C for tropical region (Donkoh, 2002). Similarly, during the journey period, the DBT (33.7 ± 0.8^{0}C), and RH (61.5 ± 3.9%) values obtained were relatively high, which indicated that the meteorological conditions prevailing during the hot-dry season generally did not favour the transportation of chickens during the season, even in the early hours of the day. Furthermore, after completion of the transportation, the meteorological values recorded were predominantly outside the established zone of comfort for the layers. The meteorological results demonstrated that transportation of layers during the early rainy season was thermally stressful to the layers and may impair their homeostatic control mechanisms. Such responses adversely affect health status and productivity of layers (Jindal, 1980; West, 2003). The results of the present study agreed with the findings of Igono *et al.* (1982) and Ayo *et al*. (1998) that the hot-dry season is thermally stressful to animals. Consequently, additional stress factors

acting upon layers should be reduced to minimum, especially at the onset of the rainy season, when both the AT and RH values are particularly high.

5.2 Rectal temperature values of the transported layers

The RT values recorded in both the treated and control birds showed that the values fluctuated with the hour of the day. The result was in agreement with the findings in goats (Igono *et al.,* 1982; Ayo *et al.,* 1998) and sheep (Mittal and Gosh, 1979), which showed that RT fluctuated with the hour of the day and AT (Picciona and Caola (2002). However, RT obtained in both experimental and control chickens before transportation fell within the established normal range of 41.5 ± 1.5^{0}C for the chicken (Zaytsev *et al.,* 1971). This indicated that the chickens were healthy and fit for the journey. However, immediately after loading and just before the commencement of the journey, even in the stationary vehicle, the values of RT in both experimental and control layers increased significantly ($P<0.05$). Such an increase indicated that the loading of the layers into the vehicle was an unusual exercise that altered sharply the RT of the birds.

Similar observation that the highest physiological and biochemical reactions occurred during handling, loading and commencement of transportation was made by Broom *et al*. (1996) and Hartung (2003). It is worth noting that during the loading exercise, the differences in the RT values in both the treated (administered with vitamins C or E) and control layers (administered with sterile water only) were not significant ($P>0.05$). This indicated that neither vitamin C nor vitamin E administration to the layers exerted any significant effect on the RT during loading.

This could be due to the fact that more time was needed for the effect of the vitamins to be manifested following their administration.

The RT values recorded during the journey period clearly showed that transportation increased significantly the values of RT in all the groups of transported layers, especially in the control layers. This confirmed the findings of Ayo and Oladele (1996) and Hartung (2003) that the period of transportation of an animal is one of the most traumatic in its life. The results also showed that vitamins C and E reduced the increased RT values in layers, transported by road during the season. This finding demonstrated that both vitamins reduced significantly the adverse effects of transportation stress on the RT, and, therefore, their administration to layers may ameliorate the adverse effects of road transportation stress.

The mechanisms of action of vitamins C and E on the body temperature were not elucidated in the present study. However, both vitamins may have some ability to normalize the impaired homeostatic mechanisms of the transported layers. It is known that both vitamins are antioxidants, which stabilize the membrane integrity of the cells and, therefore, decrease membrane susceptibility to lipid peroxidation by donating free molecule of hydrogen (Koshebekov, 1991; Altan *et al.*, 2003). Thus, the vitamins are capable of increasing the favourable adjustment of layers to the stressful environmental and transportation conditions during the peak of the hot-dry season. The results also indicated that the RT values rose significantly with the duration of the journey especially in th control layers. This demonstrated that road transportation was

more stressful to layers, especially in control group, than those administered with the antioxidants vitamins C and E.

The results further demonstrated that vitamins C and E ameliorated the adverse effects of the unfavourable meteorological and transportation conditions prevailing during the thermally stressful hot-dry season in the zone. Although it is true that in order to reduce the adverse effects of transportation on animals, it has been recommended that they should be transported during the morning and evening hours (Ayo and Minka, 2004), long journeys started in the morning extend inevitably to the afternoon hours, are known to be stressful to animals. The results of the present study demonstrated that vitamin C or E administration just before the onset of the journey was beneficial because it reduced the adverse effects of multiple stressors acting upon the transported layers during the hot hours of the day.

Further studies are required in order to evaluate the beneficial effect of vitamins C and E on layers transported by road for more than 6 h and covering long distance. The results of the present study also demonstrated clearly that RT measurements could be used to assess the health status and, consequently, the stressful nature of road transportation on the SBLs, which are often transported to and from areas where laboratory diagnostic facilities may be lacking, especially in the rural areas of Northern Nigeria. Therefore, measurements of RT may be beneficial and handy under such conditions, and the values obtained may indicate the need to subject layers that are distressed to compulsory slaughter at the designated site for such purpose in order to reduce economic losses.

The RT values obtained in the transported layers indicated that road transportation altered their RT values throughout and after the journey period. Such findings were obtained by Plyaschenko and Sidorov (1987), Knowles *et al*. (1999), and Ayo *et al.* (2006) who demonstrated that the excitability of the nervous system in animals remained high up to 3-4 hours after the journey. Apparently, such responses by layers were due to the stimulatory effect of the various stressors (extraneous stimuli), acting upon the chickens during the journey period. Although the journey had been completed, the after-effect of the stimuli continued to induce excitation of the nervous system, and consequently activate the body responses, manifested in increased RT values of the transported layers. It is worth noting that in vitamin C-or E-treated layers such post-transportation effect was reduced to the minimum or completely abolished. Similar findings were also reported by Tauler *et al*. (2003) in humans treated with vitamin C and subjected to intense exercise. Vitamins C and E alleviated the post-transportation stress in layers, and, apparently, restored rapidly the impaired homeostasis of the birds after the journey.

The RT values obtained in the transported chickens for three days after the journey were not significantly different from those values obtained for three days before transportation of the layers. This finding showed that the increase in RT values recorded during the 6 h journey period was transient. Consequently, at the third day after the transportation, the RT values were restored to the pre-transportation level. The results demonstrated that 6 h road transportation of layers during the late hot-dry

season in the zone may not have adverse effects on the thermoregulatory ability, other physiological parameters, and, by extension, the productivity and health status of SBLs, especially if the layers are administered vitamins C and/or E prior to the commencement of the journey. Such transient effect of road transportation stress demonstrated that the SBL has successfully adapted to the various stress factors prevailing in the Northern Guinea Savannah Zone of Nigeria. However, further studies are required on the effect of prolonged hours of transportation on the physiological responses of the SBL during the various seasons prevailing in the zone. The effect of road transportation stress on other breeds of layers in the zone and the comparison of such effect with that observed in the SBL may be of value in identifying the most resistant breed of layers to road transportation stress in the zone. The results showed that not only were the post-transportation RT values restored to their pre-transportation values, but that they also manifested diurnal fluctuations as reported by Zaytsev *et al.* (1971), Ayo *et al*. (1998), and Picciona and Caola (2002).

5.3 Haematology

The haematological results showed that exposure of layer chickens to transportation stress caused a siginificant decrease in RBC values. Consequently, Hb, PCV and RBC decreased in all experimental groups after transportation (Table 4.15). The decrease observed in the Hb, PCV and RBC post-transportation, compared to pre-

transportation, were within the normal range for birds especially in the vitamins C-and E-treated groups but outside the range in the control group.

The observed reduction in Hb, PCV and RBC demonstrated and suggested an anaemic condition in all the experimental birds. This observation disgreed with the findings of Singh and Sirisinghe (2005) on the acute effect of an 18-kilometer run on haematological and plasma electrolyte parameters of human subject under conditions of high ambient temperatures and humidity, who observed very highly significant ($P<0.001$) increases in PCV and Hb concentrations.

The results indicated that leucocytosis occurred as a result of 6-h road transportation in control layer chickens. However, the administration of the antioxidants with Vitamin C or E resulted in the maintenance of WBC counts at the value obtained pre-transportation. The result suggests that the vitamins might have prevented the release of leucocytes from their pool in the body into the peripheral circulation, apparently due to the inhibitory role of Vitamin C on circulating corticosteroids in chickens under stress. Besides, decrease in vitamin C in the body tissues, especially in adrenal gland is known to be associated with corticosteroid release (Whitehead and Keller, 2003). Depletion of vitamin C in the birds was prevented in the present study by its exogenous supply prior to transportation of the birds.

Road transportation of layer chickens did not alter basophil counts in all experimental and control groups. However, eosinophilia was observed following transportation of the birds in the control group but not in experimental groups. The

results perhaps is due to the antioxidant effects of the vitamins which might have inhibited the mobilization of eosinophils from the body pools into the peripheral circulation. The mechanism involved in this mobilization was not elucidated in this study. However, Whitehead and Keller (2003) observed that the release of corticosteroids occurring in stressed layer chickens may enhance eosinophilia. The eosinophilia obtained in the control group in the present study contradicted the findings of Alexandra *et al.* (2002), who observed no significant changes in eosinophil count in racing pigeons transported for 3 h under temperate environmental conditions. Lymphocytes and monocytes were found to be significantly ($P<0.05$) and highly significantly ($P<0.01$) depressed in vitamins C-and E-treated and control groups, respectively. Similar results were obtained by Thompson and Lippman (1974) as well as Sudakov (1992), who showed that ACTH and glucocorticoids cause the regression of lymphoid tissues due to stress.

The recorded decrease in lymphocytes and monocytes could be attributed to the effects of transportation on lymphoid tissues, which could result in antibody depression, impaired migration of phagocytic cells (Spain, 1975).

Road transportation of layer chickens induced monocytosis in control and experimental layer chickens. This finding disagreed with the result of Alexandra *et al.* (2002), who did not observe any significant changes in monocyte count in transported racing pigeons for 3 h by road. The increase in H/L observed in all the transported birds in the present study is in agreement with the established fact that H/L ratio is a good measure of responses of chickens to environmental stress and a reliable index for

determining stress in poultry (Gross and Siegel, 1983; Gross *et al*., 1984; Mcfarlane and Curtis, 1989; Maxwell and Robertson, 1998).

The findings supported the results of Parga *et al.* (2001) on transported hawks, who observed a significant increase in H/L ratio and proposed that the H/L ratio is the most sensitive measure of stress response in the blood picture of raptors.

Increase in the H/L and the decrease in lymphocytes is consistent with the finding that heterophilia occurs in birds during stress state, which stimulates the anterior pituitary gland (APG) to secret ACTH. Circulating ACTH in turn induces the adrenal cortex to produce glucocorticoids involved in the mobilization of heterophils from the body pool into the peripheral circulation. The increase in the H/L ratio was more pronounced in experimental group than control groups, which indicated that antioxidant vitamins enhanced heterophilia necessary for the increase in body resistance to stress situations (Dohms and Metz, 1991). The H/L ratio is an indication of the activity of the hypothalamus – adreno-hypophyseal adreno-cortical axis and increases with degree of stress. This ratio is one of the most sensitive and lasting indicator of physiological stress in poultry (Whitehead and Keller, 2003).

The primary function of WBC appears to be to defend the body against (irritants) foreign bodies; which is achieved by leucocytosis and antibody production (Robbin and Angel, 1976). Total WBC count decreased post-transportation when compared with the pre-transportation values (Table 4.16). The results also agreed wuth the findings of Oladele and Ayo (1999), who observed that the PCV, Hb and TP values obtained from clinically sick chickens (CSC) were significantly ($P<0.05$) lower

compared to apparently healthy chickens (AHC), and they suggested that this might have been due to adverse effects of stress factors triggered by a depressed immune system of the chickens. Similarly, platelets were observed to decrease post-transportation compared with pre-transportation values.

There was a decrease in CPK value of all experimental groups, with only the vitamin-C treated group being highly significant ($P<0.01$). This finding disagreed with that reported by Lumeij (1985), Kerr and snow (1983) and Alexranda *et al*. (2002) where after transportation in macaws, equine endurance rides and eventing, and poultry transportation and handling, AST and CPK activities were markedly increased. The result in this investigation may be due to the anti-oxidants activities of vitamins C and E-treated groups and in addition to such factors as breed, genetic and individual variability of birds.

There were significant ($P<0.05$) increase in TP post-transportation in all experimental groups. Brown *et al* (1999a) noted that pigs subjected to 24 h road transportation suffered severe dehydration as the concentration of plasma total protein and albmin were increased significantly. Similar results were observed in sheep (Knowles *et al*. 1996) and calves (Knowles *et al.* 1999a).

The ALP showed a significant decrease in birds belonging to vitamin C-treated and control groups, but a sharp increase in the vitamin E-treated group. This, apparently may be due to its ability to maintain the supply of egg precursors in plasma of laying hens (Bollengier-lee *et al*. 1998).

5.4 Transportation time and its effects on liveweight of layers chickens.

Although transportation decreased the liveweight of the layer chickens in experimental and control groups, the decrease was more significant in control layer chickens not treated with antioxidants, vitamin C and vitamin E. The decrease of 18.2% in liveweight obtained in control layer chickens was 200% higher than the value of 9.1% recorded in birds treated with vitamin C or E. The fact that the liveweight of layers treated with vitamin C or E did not alter significantly after the journey showed that both vitamins decreased the negative effects of transportation on the layer chickens. In control, unlike in experimental chickens, road transportation resulted in liveweight loss of the layers after the journey. This finding agreed with that of Knowles *et al*. (1999) and Brown et al. (1999), who demonstrated that road transportation resulted in decrease in liveweight of animals. The fact that vitamin C or E administration in transported layers did not reduce significantly the liveweight of the birds showed that losses due to liveweight, often encountered during transportation of layers for marketing and pre-slaughter exercises, may be reduced or completely eliminated by administering either of the vitamins, and thus enhance the productivity and profitability of the SBL in the zone.

Similar findings that supplementation of vitamins C and E in birds during heat stress have a positive effect on liveweight of the birds was demonstrated by Thaxton and Pardue (1984, 1986) and Kazim *et al*. (2002).

The proximate cause of loss in liveweight during transportation has been described to be dehydration of tissue as a result of intensification of energy degradation of carbohydrate and fat through the hormonal pathways of cortisol and glucagons (Knowles *et al.*, 1999b). The loss in liveweight recorded in the present study was similar to that obtained by Graham (1993), who reported that poor handling, loading and unloading caused a great deal of injury and reduction in liveweight of birds. Kannan *et al.* (2000), also observed that a two-hour transportation, combined with 18 h feed deprivation resulted in 10% liveweight loss in goats. Based on the findings of Plyashenko and Sidorov (1987) in Europe that about 0.5% of liveweight is lost per hour through road transportation of livestock, the liveweight loss of 9.1% recorded for vitamins C and E treated groups and the 18.2% for the control group showed that losses due to road transportation of birds in the Northern Guinea Savannah zone of Nigeria were higher than in the temperate region.

5.5 Transportation and its effects on egg laying of layer chickens.

Road transportation of layer chickens resulted in significant decrease in egg production; especially in control birds where the percentage reduction was as high as 47.7%. In vitamin E-treated layer chickens the loss in egg production was the lowest (11%). The result confirmed the findings of Whitehead and Keller (2003) that vitamin E posses both anti-stress and fertility effect. Similar finding that supplementation of vitamin E in birds during heat stress has a positive effect on egg production of the birds in the temperate region was reported by Bollengier-Lee *et al.* (1998). In this

study there was a significant ($P<0.05$) decrease in egg laying in vitamin C treated chickens and very highly significant ($P<0.001$) drop in the control group post-transportation, when compared with the numbers laid before transportation. However, there was no significant ($P>0.05$) decrease in number of eggs laid during the same period in the vitamin E-treated group. This finding agreed with that of Bollienger-lee *et al*. (1998) and Kazim *et al*. (2002) that dietary supplementation of vitamins C and E decreased serum lipid peroxidation. They showed that the administration of the two vitamins enhanced the laying performance of birds at high temperatures. In addition, the results suggested that dietary supplementation of vitamins C and E may alleviate the adverse effects of acute and chronic heat stress in laying hens, perhaps by maintaining the supply of egg precursors in plasma, while at the same time desreasing serum ACTH concentration. Furthermore, Kevin (1982) showed that dietary supplementation of vitamin E increased the fertility of poultry, normal testicular functions of cockerels, layability of laying hens as well as the hatchability of breeder eggs. Therefore, the results of the present study further demonstrated that vitamin E administration to transported layer chickens during the hot-dry season did not only alleviate the adverse effect of road transportation stress, but also reduced significantly egg production losses unavoidably associated with road transportation stress in birds.

5.6 Behavioural responses of transported layer chickens.

The behavioural activity of the layer chickens was not significantly ($P>0.05$) different pre– and post-transportation in experimental birds. This finding showed that antioxidant vitamins significantly reduced the stressful behavioural responses of layer chickens to road transportation during the early rainy season. In control birds the TI duration and regrouping time to move 2 m as well as latency to head shake (awareness) significantly increased post-transportation.

The results agreed with those of Jones *et al.*, (1996, 1999) and Whitehead and Keller (2003), who observed that antioxidant vitamin C attenuated the TI and fear reaction of stressed quails. The finding is also in agreement with that of Chervyakov (1977) who reported that vitamin C activated the adrenergic system.

Similar findings were obtained in transported goats administered with vitamin C during hot-dry conditions in the Northern Guinea Savannah zone of Nigeria by Ayo *et al.* (2006). TI duration of transported birds were also reported to increase during road transportation as well as latency to headshake in broiler chickens by Sherwin *et al.* (1993); Cockram *et al.* (1999) and Jackson *et al.* (1999). The results suggested that behavioural and physiological responses can complement one another in assessing the welfare of transported layer chickens and they could be of value in determining on-the-spot response of transported layer chickens to stress situations. If need be, they could be used to determine the fitness of the birds to continue or the need to terminate the journey. The tests, especially the behavioural test, could be applied and interpreted

easily, yet they give in-depth evaluation of the physiological fitness of the transported birds.

In this study, the increased lying behaviour of the control group was observed post-transportation as an evidence of fatigue and a depressive effect due to the 6 h road transportation in the layer chickens, which was sustained for more than 48 h post-transportation immediately after the journey. The TI responses of layer chickens were higher in both treated than control groups. This confirmed that road transportation was stressful to layer chickens, the activity of the central nervous system, following a transit excitation (Plyashenko and Sidorov, 1987), normal vestibular, visual and proprioceptive functions (Elrom, 2000), and thus inducing fear and stress (Frazer and Broom, 1990; Knowles and Broom, 1990 and Jones, 1992).

The durations and number of inductions of TI recorded in the present study were considerably lower than those observed after commercial transportation (Cashman *et al*. 1989), suggesting that the stimuli experienced by the birds in the present study were less severe than those experienced by commercially transported birds. The journey was relatively short. In Nigeria, more than 80% of commercially transported birds are transported over longer journey period. However, Cashman *et al. (*1989) Showed that increased journey duration resulted in increased TI reactions.

Regrouping and awareness test followed the same pattern as the TI for the experimental birds in the present study. Several possible indicators of awareness were considered, but only headshaking occurred sufficiently and frequently. It has been reported that chickens display increased awareness of their environment by

headshaking (Hughes, 1983; Savory and Hughes, 1988). Administration of vitamin C and E eliminated the adverse effect of depression, fear and fatigue as evidenced by the significant decrease in the number of birds that were lying immobile, not regrouping and not aware of their environments.

This finding was further further consolidated by the fact that the number of birds found standing with high sense of arousal after the journey rose almost by 80% in treated birds when compared with the control birds. Thus, the production gain increased an almost 100% layability 72 h post-transportation, in vitamin E- treated group and closely followed by vitamins C-treated group with 49% layability..

The results agreed with the findings of Chervyakov (1977) and Kondratyev (1988) that vitamin C activates the nervous system, especially the sympathetic nervous systems. Similarly, results were obtained by Kazim *et al*. (2002) who reported that dietary supplementation of vitamin C and E in laying hens reared at high ambient temperature decreased serum ACTH, and glucocorticoids while at the same time it increased serum protein concentrations, decreased lipid peroxidation and subsequently normalised vestibular, visual and proprioceptive functions. Ayo *et al.* (2006) also reported the replacement of depression with excitation in goats administered with vitamin C shortly after transportation.

The fact that vitamin C and E decreased the duration of TI in transported birds, after the journey showed that the vitamins rapidly abolished the depressed status, often observed in transported animals after transoportation. Furthermore, the present

study showed that vitamins C and E increased agonistic behaviour of head pecking, steps, pushes, threats and chases after the journey.

Similarly, vitamins C and E increased the numbers of birds involved in quacking, scolding, running, cawing, strutting around, even a few martially locked hen fights were observed, although this increase was not statistically significant. Further studies are, however, required to elucidate the neurogenic mechanism of action of vitamins C and E in ameliorating the adverse effects of road transportation in layer chickens. From the results obtained in the present study, the 6 h road transportation of layer chickens during the late hot-dry season in the Northern Guinea Savannah zone of Nigeria induced stress in the layer chickens. The stress response in the layer chickens were apparently linked to HPA axis route of GAS described by Selye (1976).

Vitamins C and E-treated groups manifested the first two stages of stress response mechanism of adaptation, that is, the alarm reaction and quickly the stage of resistance; while the response in the control group progressed further to the third stage of stress called exhaustion, and were restored only after 72 h post-transportation, apparently due to good management and balanced feed after the journey.

The results obtained in the present study, for the first time, showed the adverse effects of road transportation, even though transient, on the physiological parameter of SBL in the Northern Guinea Savannah zone of Nigeria, which are currently lacking in the available literature. The results also demonstrated, as a pioneer work, the ameliorative roles of vitamins C and E on the physiological parameters of SBL transported by road during the hot dry season in the Guinea Savannah zone of Nigeria.

CHAPTER SIX

6.0 SUMMARY

The results obtained from the present study clearly demonstrated that road transportation of SBL during the hot-dry season in the Northern Guinea Savannah zone of Nigeria impaired the homeostatic mechanisms of the birds.

This transient stress causes hormonal imbalance leading to increased metabolic reactions, lipid peroxidation, fatigue, reduced liveweights and poor production performance and the attendant economic losses.

6.1 CONCLUSION

1. The RT of all transported birds increased during road transportation.
2. Multiple stressors, including climatic factors act upon layers during road transportation.
3. Tonic immobility was manifested greatly as a result of fatigue than fear in control birds than treated.
4. Road transportation of layers for 6 h reduced their liveweights by 18.2% in control layers.
5. The heterophil-lymphocyte ratio and total protein increased in transported layers in the treated than control groups respectively.
6. Antioxidants, vitamins C and E, which are relatively common to find and not too expensive, but, above all, easy to administer should be given to layers before embarking on long journey in order to ameliorate the road transportation-induced stress and economic losses.

6.2 Recommendations

1. Further studies are required in order to evaluate the effects of different seasons, long journey periods, age, and breed differences on the physiological parameters of chickens during road transportation.
2. There is a need to evaluate the possible synergistic effects of administration of vitamins C and E in layer chickens transported by road.

3. The administration of vitamin C or E is recommended in layers before transportation in order to reduce the risk of adverse effects of road transportation stress, and economic losses incurred by poultry farmers.

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APPENDIX I

Physiological parameters of blood in poultry

1. RBC m/mm³ = 2.5 – 3.5
2. Haemoglobin = 11 g/dl
3. WBC / mm³ = 23,000 – 35,000
4. WBC differential expressed as percentage of the composite leucocytes
    a. Lymphocytes 60 (50-70)
    b. Heterophils 30 (20-50)
    c. Eosinophils 2
    d. Basophils 2
    e. Monocytes 6 (2-10)
5. Blood platelets or thrombocytes

    In mm3 40,000 – 210,000

(thrombopenia, and thrombocytopenia occurs majorly in hemorrhagic diathesis

Adapted from (Marek – Mocsy, 1979).

APPENDIX II

Table 4.4 Minimum rectal temperature (^{o}C) values of SBL chickens three days before and three days after road transportation (Mean ± SEM, n = 45)

	Before Transportation			After Transportation		
Hour	***++Vitamin C***	***Vitamin E***	***Control***	***Vitamin C***	***Vitamin E***	***Control***
07:00	*40.3 ±0.3*	*40.1 ±0.1*	*40.2 ±0.3*	*40.6 ±0.3*	*40.7 ±0.3*	*40.7 ±0.4*
14:00	40.3 ± 0.3	40.3 ± 0.3	40.3 ± 0.2	41.2 ± 0.5	41.0 ± 0.6	41.0 ± 0.6
18:00	40.0 ± 0.0	39.9 ± 0.1	39.9 ± 0.0	40.8 ± 0.2	40.5 ± 0.3	40.8 ± 0.5
Mean ± SEM	40.2 ± 0.1	40.1 ± 0.1	40.1± 0.1	40.9 ± 0.2**	40.7 ± 0.2*	40.8 ± 0.1***

* = P<0.05

** = P<0.01

*** = P<0.001 ++ n = 15 across the row.

APPENDIX III

Table 4.5: Maximum rectal temperature (^{o}C) values of SBL chickens three days before and three days after transportation (Mean ± SEM, n = 45)

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

^{NS} = Non-significant (P>0.05) difference

* = P<0.05

*** = P<0.001 ++ n = 15 across the row.

APPENDIX IV

Table 4.6: Range of rectal temperature (^{o}C) values of SBL chickens three days before and three days after transportation (Mean ± SEM, n = 45)

	Before Transportation			After Transportation		
Hour	***++Vitamin C***	***Vitamin E***	***Control***	***Vitamin C***	***Vitamin E***	***Control***
07:00	1.4 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.8	1.0 ± 0.4	1.7 ± 0.4
14:00	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	1.7 ± 0.5
18:00	1.4 ± 0.4	1.4 ± 0.1	1.5 ± 0.2	0.8 ± 0.1	1.1 ± 0.3	1.4 ± 0.4
Mean ± SEM	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.0 ± 0.2^{NS}	1.0 ± 0.1**	1.6 ± 0.1^{NS}

^{NS} = Non-significant (P>0.05) difference

** = P<0.01

++ = n= 15 across the row

APPENDIX V

Table 4.7: Hourly fluctuations in minimum rectal temperature (^{o}C) values of SBL chickens during transportation period (Mean ± SEM, n = 45).

Hour (Time)	++Vitamin C	Vitamin E	Control
1 (10:00)	39.9	42.7	42.5
2 (11:00)	41.0	41.6	43.0
3 (12:00)	41.0	41.9	44.1
4 (13:00)	40.9	41.8	44.3
5 (14:00)	40.9	41.9	43.9
6 (15:00)	41.0	41.0	43.9
Mean ± SEM	***40.8 ± 0.2 ^{a}***	***41.8 ± 0.2 ^{b}***	***43.6 ± 0.3 ^{c}***

Means along the same row with different superscript alphabets are significantly (P<0.05) different.

++ n= 15 across the row

APPENDIX VI

Table 4.8: Hourly fluctuations in maximum rectal temperature (°C) values of SBL chickens during transportation period (Mean ± SEM, n = 45).

Hour (Time)	++Vitamin C	Vitamin E	Control
1 (10:00)	44.1	44.0	44.0
2 (11:00)	42.8	42.6	43.8
3 (12:00)	42.2	42.6	43.8
4 (13:00)	42.6	43.0	45.1
5 (14:00)	42.1	42.6	45.1
6 (15:00)	42.9	41.8	44.5
Mean ± SEM	***42.3 ± 0.3 [a]***	***42.8 ± 0.3 [a]***	***44.6 ± 0.3 [b]***

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

++ n=15 across the row

APPENDIX VII

Table 4.11: Fluctuations in mean rectal temperature (°C) values of SBL chickens three days before and three days after transportation (Mean ± SEM, n = 45).

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

* = P < 0.05

** = P < 0.01

***=P<0.001 ++ n = 15 across the row