THE EFFECT OF DIETARY SELENIUM AND ORGANIC ASCORBIC ACID IN AMELIORATING HEAT STRESS, THERMOREGULATORY, GROWTH, BLOOD INDICES AND MORPHOMETRICS IN BROILER CHICKENS

BY

David Ikechukwu NDUBUISI

DEPARTMENT OF ANIMAL SCIENCE,

FACULTY OF AGRICULTURE,

AHMADU BELLO UNIVERSITY,

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David Ikechukwu NDUBUISI

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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,

FACULTY OF AGRICULTURE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

SEPTEMBER, 2021

DECLARATION

I hereby declare that this dissertation entitled "**THE EFFECT OF DIETARY SELENIUM AND ORGANIC ASCORBIC ACID IN AMELIORATING HEAT STRESS, THERMOREGULATORY, GROWTH, BLOOD INDICES AND MORPHOMETRICS IN BROILER CHICKENS**" has been written by me under the supervision of Dr. M. Abdulrashid and Dr. (Mrs) O.M. Daudu. It is the result of my investigation and has not been presented for any other qualification in any institution. All literature has been duly acknowledged and a list of references provided.

_____ _____

David Ikechukwu NDUBUISI Date

CERTIFICATION

This dissertation entitled "**THE EFFECT OF DIETARY SELENIUM AND ORGANIC ASCORBIC ACID IN AMELIORATING HEAT STRESS, THERMOREGULATORY, GROWTH, BLOOD INDICES AND MORPHOMETRICS IN BROILER CHICKENS**" by David Ikechukwu NDUBUISI meets the regulations 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.

_____ _____

Dr. M. Abdulrashid Date
Chairman, Supervisory Committee

_____ _____

Dr. (Mrs) O.M. Daudu Date
Member, Supervisory Committee

_____ _____

Prof. M. Kabir Date
Head, Department of Animal Science
Ahmadu Bello University, Zaria

_____ _____

Prof. Sani A.Abdullahi Date
Dean, School of Postgraduate studies
Ahmadu Bello University, Zaria

DEDICATION

To Chief and Mrs. Samuel Ndubuisi

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ABSTRACT

Two studies were conducted to evaluate the effect of selenium and organic ascorbic acid in ameliorating heat stress on growth, haematological, biochemical indices and morphometrics of broiler chickens. A total of five hundred and twelve(*Cobb 500*) broiler chickens were randomly distributed into four experimental treatments (64 birds each) for each study with selenium supplementation at 0, 0.1, 0.2 and 0.3 mg/kg and ascorbic acid supplementation at 0, 68, 136 and 204 mg/kg each with four replicates in a Completely Randomized Design. Data generated during the experiment was analyzed using general linear model of statistical analysis system and means were compared using Tukey procedure. The study comprised the starter (1-28 days) and finisher (29-49 days) phases of each study. Resultsof the selenium study revealed thatbirds fed the diet supplemented with 0.2 mgSe/kg had an increase ($P<0.05$) in daily water intake (149.86 ml). Higher ($P<0.05$) band cell count was observed for birds fed 0.2 and 0.3 mgSe/kg. Cortisol was lowered ($P<0.05$) in broilers fed 0.1 mgSe/kg.Increased($P<0.05$) in packed cell volume (33.88%), haemoglobin (11.28g/dl) and erythrocytes (5.50 $x10^{12}$/l) was observed for birds fed 0.1 mgSe/kg supplemented diet. Increased ($P<0.05$) cholesterol level was observed for birds fed control diet and 0.1 mgSe/kg, Superoxide dismutase (SOD) was elevated ($P<0.05$) in broilers fed 0.2 and 0.3 mgSe/kg, higher ($P<0.05$) catalase (CAT) was observed in birds fed control diet and 0.3 mgSe/kg and increased ($P<0.05$) serum phosphorus level was observed in birds fed 0.2 mgSe/kg. Broilers fed control diet, 0.1 and 0.2 mgSe/kg had lowered ($P<0.05$) cortisol level. Dressing percentage and back weight was improved ($P<0.05$) in birds fed 0.3 mg selenium supplemented diet, thigh was higher in birds fed 0.1 mgSe/kgwhile wing was higher ($P<0.05$) in broilers fed 0.1 and 0.3 mgSe/kg. Villus area was improved by 0.1 and 0.2 mgSe/kg and the ratio of crypt depth/height was higher ($P<0.05$) in broilers fed control diet, 0.1 and 0.2 mgSe/kg.

Result for the ascorbic acid study indicated a higher (P<0.05) feed conversion ratio in birds fed the control diet and 68 mg/kg ascorbic acid. Low density lipoproteins (LDL) was higher (P<0.05) in broilers fed 68 and 136 mg/kg ascorbic acid, birds fed 136 mg/kg ascorbic acid had higher (P<0.05) level of ALT. Cortisol level was lower (P<0.05) in birds fed 136 and 204 mg/kg ascorbic acid. At the finisher phase, broilers fed control diet and 136 mg/kg ascorbic acid had higher (P<0.05) LDL, Glutathione Peroxidase activity was improved (P<0.05) at 68 and 136 mg/kg ascorbic acid. Higher (P<0.05) phosphorus was observed in the tibia bones of broilers fed control, 68 and 136 mg/kg ascorbic acid and the ascorbic acid supplemented groups had higher (P<0.05) ash than the control group. Higher (P<0.05) faecal calcium was observed in 204 ascorbic acid supplemented group while phosphorus was similar in all the groups. Weight of wing was higher (P<0.05) in broilers fed the control, 68 and 136 mg ascorbic acid/kg groups. Higher (P<0.05) crypt depth and villus area was observed in broilers fed 204 mg/kg ascorbic acid. Tibia length was higher (P<0.05) in broilers fed 204 mg/kg ascorbic acid. Higher (P<0.05) ratio of tibia weight/length index was observed in the control group and improved (P<0.05) robusticity index was observed in the control group and 68 mg ascorbic acid supplemented group. It was concluded that selenium (0.1 mg/kg) and organic ascorbic acid (204 mg/kg)improved thermoregulatory parameters,growth, blood indices and morphometrics of broiler chickens.It is therefore recommended that the feed of broilers be supplemented with either 0.1 mgSe/kg or 204 mg/kg ascorbic acid during period of heat stress.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

There is increasing demand for poultry products globally (USDA, 2012). This is due to the perceived healthiness of poultry being high in beneficial unsaturated fatty acids, high return over a short period, low capital for investment, high acceptability in many culinary traditions and increase in human population. The demand is expected to be more in developing countries due to increase in human population in the region (Guyomard *et al*., 2013). However, the current poultry production in the developing nations is incapable of meeting the current protein needs of the masses let alone meeting future demand (Guyomard *et al*., 2013).

The major problem facing poultry industry in the developing countries is inadequate and expensive feedstuffs as well as heat stress, particularly during dry season. Heat stress is so important because all classes of poultry are susceptible to it. High ambient temperature and high relative humidity prevailing in the micro-environment of the poultry house in the tropics are unfavourable for efficient poultry production (Ayo *et al*., 2011). The environment under which birds are reared under intensive production system in the tropical countries is far from the ideal. Stress management is low and this can lead to reduction in production performance in poultry farming.

Global warming which can lead to heat stress (HS) is considered one of the most important challenges affecting the performance of the birds (Gregory, 2010; Ali *et al*., 2010). Heat exposure and intensity at high levels affects poultry production on a worldwide basis and has a significant impact on well-being and production of poultry birds. Heat stress (HS) occurs when the amount of heat produced by an animal surpasses the animal's capacity to dissipate the heat to

its surrounding environment. This imbalance may be as a consequence of variation in a complex formof environmental factors (sunlight, thermal irradiation, air temperature, humidity, and movement) and characteristics of the animal (species, gender, and rate of metabolism) (Lara and Rostagno, 2013). The physiological consequences of HS are numerous and deleterious to normal body functionsand are usually manifested as an adaptive measure for survival, and these include: increased body core temperature, reduced voluntary feed intake, depressed immunity, alteration of the electrolyte balance and blood pH, impairment in endocrine and reproductive functions, decreased energy availability to cells, alteration in the digestibility and metabolism of various nutrients, disruption in the structure and function of intestinal epithelium, alteration of the normal and protective microbiota, and increased circulatory cortisol and corticosterone levels (Yahav, 2009; Syafwan *et al*., 2011; Renaudeau *et al*., 2012; Lara and Rostagno, 2013).

More recently, HS has been said to be one of the major causes of oxidative stress (OS) (Ayo *etal*., 2011). Oxidative stress is defined as the presence of reactive species (RS) in excess of the available antioxidant capacity of animal cells (Halliwell and Whiteman, 2004). Many radicals and metabolic substances are described as potentially toxic and are defined as "reactive oxygen/nitrogen/chlorine species" (Halliwell and Whiteman, 2004). These substances are highly reactive and can modify several biologically cellular macromolecules, such as proteins, lipids, and nucleic acids (DNA and RNA) (Davies, 1995). These phenomena contribute to the development of several metabolic dysfunctions, including cell death by causing "oxidative stress" and "oxidative damage" (Halliwell and Whiteman, 2004). Oxidized molecules extract electrons from other molecules, resulting in a chain reaction and, if not controlled, this reaction can cause extensive tissue damage, since these high energy molecules are highly diffusible

through membrane thereby changing membrane organization and integrity by disrupting the lipid structure(Halliwell and Whiteman, 2004).

Heat stress causes an increase in free radicals and reactive oxygen species (ROS) by initiating lipid peroxidation in cell membranes and releasing stress hormones that directly influence glucose and lipid metabolism as well as protein catabolism, hence oxidative damage (Zhang *et al*., 2014; Imik *et al*., 2012; Hosseini-Mansoub *et al*., 2010). Under such adverse conditions (>30 ^{0}C), the body is not able to synthesize the enzymes required to destroy ROS or repair the damage, due to inhibitory effects on sensitive enzymes. The hot-dry season, characterized by high atmospheric temperature (AT) and high relative humidity (RH), in the Northern Guinea Savannah zone has been shown to be thermally stressful to poultry (Sinkalu *et al*., 2015). Therefore, poultry production suffers significant economic losses every year due to stress such as high morbidity and mortality, reduced growth rate and immune suppression (Utomo *et al*., 1994; Star *et al.,* 2008). Their higher production performance and feed conversion efficiency makesimproved chickens more susceptible to heat stress than even before (Lin *et al.,* 2004). High mortality, decreased feed intake, lower body weight gain and poor feed efficiency are common adverse effects of stress often observed in meat-type poultry flocks (Yegani and Korver, 2008).

Antioxidants are substances which, when present in feed or food at a concentration lower than that of an oxidizable substrate, will significantly interrupt or prevent the oxidation of the substrate (Halliwell and Gutteridge, 1999). Selenium (Se) is an essential metalloid trace element that is critical to the normal physiology of a wide range of species, including birds and has a very narrow margin of safety between the toxic and deficient doses in animals and humans (Khanal, and Knight, 2010). It possesses critical roles in immune function, growth, productivity and anti-

stress. It plays an important role in elevating production of immunoglobulin (Hoffmann, 2007). Selenium research has attracted tremendous interest because of its main function as an antioxidant action involved in protection against damage caused by free radicals and oxidative stress. Selenium is considered an important antioxidant because it is critical to the formation of the glutathione peroxidase (GHPx). Selenium can accept or give out electrons due to its existence in basal biological pH as an Anion (Abdel-Azeem, 2010). Selenium exists in two chemical forms, organic and inorganic forms (Abdel-Azeem, 2010).

Ascorbic acid commonly known as vitamin C is a 6-carbon lactone (carbohydrate compound) that is synthesized from glucose and found in considerable amount in citrus fruits, green vegetables (Abdulrashid *et al*., 2010) and many plant parts such as baobab fruit (*Adansonia digitata*). Ascorbic acid is an organic substance which is an essential dietary nutrient required as a co-factor of many enzymes (Youssef, 2004) and is needed in minute quantity. Under heat stress condition, ascorbic acidsupplementation in poultry feeds has been reported to have positive effects such as weight gain, egg production, egg shell strength, fertility, hatchability and improved immune response of poultry birds (Abdulrashid *et al*., 2010). Ascorbic acid also promotes resistance to some infectious and contagious diseases (Gross, 1988). Kutlu and Forbes (1993) observed reduction in rectal and skin temperatures in broilers and layers given ascorbic acidand also reduced panting rates.

The baobab(*Adansonia digitata*) fruit pulp is composed of a hard external coatterm epicarp, the endocarp is floury, dry and powdery with thread-like fibrous materials that enclose seeds and filaments (Nour *et al*., 1980). The fruit pulp is powdery, whitish coloured and dries naturally with slight sour taste. The pulp can be separated from the shell and seed using a knife or pounded slightly in a mortar; its processing does not require chemical treatment (Magdi, 2004). Baobab

(*Adansonia digitata*) alsocalled African tree is knownto be a valuable source of nutrient and a non-conventional feedstuff. Baobab trees are tolerant to drought. The leaves, bark and fruits of baobab are known for its medicinal purposes and as food-stuff (Etkin and Ross, 1982). In Africa, baobab fruit is used in the preparation of refreshing drink due to its nutritional properties (Lockett *etal.*, 2000). Baobab (*Adansonia digitata*) fruit pulp has been found to contain a significant amount of ascorbic acid. Adeosun (2012)reported 299.75mg/100g of ascorbic acid in the Baobab dry fruit pulp. Addition of this natural source of vitamin C to poultry diets could be beneficial in alleviating stress problems associated with poultry production.

1.2 Statement of the Research Problem

Poultry production is one of the fastest growing sectors of livestock industry in developing countries. It is estimated that the world population in 2025 will reach abouteight billion inhabitants, and in 2050, up to nine billion inhabitants (Gangadoo *et al*., 2016). The Food and Agriculture Organization of the United Nations predicts that annual meat production of 200 million tonnes will be required by 2050 to meet up to this increase in human population (Ghasemzadeh, 2012). Consequently, there was a forecast that global broiler meat production was going to rise by1% to a record of about 89.5 million tonnes in 2017, and that this trend will continue in the coming years (Anon, 2017). The constant need for more foods of animal origin, especially chicken, has led to development of research aimed at improving production as well as producing healthy animals.

In recent years, feed formulations were manipulated using different additives (various sources, forms, formulations) with the aim of improving poultry growth and conversion ratios; obtaining better quality and value-added products, and therefore, achieving cost-effectiveness of production (Gangadoo *et al*., 2016).The efficiency of contemporary poultry production is based

on well-balanced nutrition and highly productive lines of birds. Successful production involves a number of impedements such as stress factors to which broilers and hens are exposed.Heat stress causes an increase in free radicals and reactive oxygen species (ROS) by initiating lipid peroxidation in cell membranes and releasing stress hormones that directly influence glucose and lipid metabolism as well as protein catabolism, hence oxidative damage (Imik *et al*., 2012). Under such adverse conditions, the body is not able to synthesize the enzymes required to destroy ROS or repair the demage, due to inhibitory effects on sensitive enzymes. During such adverse conditions, the positive effect of antioxidants on the animals must not be neglected(Surai, 2002a; Surai, 2002b). Hence, it is imperative to supplement the diets of broiler chickens with antioxidants (selenium or ascorbic acid) to alleviate the deleterious effect of heat stress on broiler birds.

1.3Justificationof the Study

In tropics and subtropics, one of the major challenges facing the poultry industry is the adverse effects of chronic heat stress on growth, physiological and economic traits of chickens (Attia *etal*., 2009; Varasteh *et al*., 2015). Heat stress has a negative impact on immune, growth and productivity of poultry. (Quinteiro-Filho *etal*., 2010; Ghazi *et al*., 2012). During heat stress, as the bird attempt to maintain its thermal homeostasis, increased levels of reactive oxygen species (ROS) occur and the body may enter a state of oxidative stress (Droge, 2002; Gu *etal*., 2012).Reducing stress in poultry remains a topic of concern amongst producers and scientists. Considering the high cost and difficulty of cooling animal buildings especially in the tropics, dietary manipulation is the main focus (Konca *etal*., 2009). Remarkably, the activity of supplemental antioxidants such as selenium and ascorbic acid has been ascertained to boost the capacity of the biological antioxidant barrier in combating excess free radicals in heat stressed

broiler chickens (Celi and Chauhan, 2013). Ascorbic acid have the capacity to accept or give out electrons, lower plasma corticosterone and adrenocorticotropic hormone level which stabilizes the free radicals thereby allowing the birds to utilize it energy in building tissues rather than combating heat stress. Effect of various sources and levels of selenium in poultry diet on productiveperformance has been the subject of a number of studies but results are not consistent, with both negative and positive responses being reported (De Medeiros *et al*., 2012; Salah-Eldin *et al.,* 2015; Baltić *et al*., 2016). The use of organic Ascorbic acid in ameliorating the adverse effect of heat stress on broiler chickens especially in the tropics has not been extensively exploited as most researches had been tilted towards the use of the inorganic forms of ascorbic acid. Similarly, studies on the morphometric functions, haematological and serum biochemical indices in poultry under the influence of adverse weather condition (heat stress) at the early growth phase using dietary organic form of ascorbic acid has not been fully elucidated. Therefore it is imperative to investigate the possible role of Selenium and organic Ascorbic acid in ameliorating the impact of heat stress on haematological, biochemical indices and morphometrics of broiler chickens.

1.4Objective of the Study

The broad objective of this study was to evaluate the effect of dietary selenium and organic ascorbic acid in ameliorating heat stress,growth, blood indices and morphometrics in broiler chickens.

The specific objectives of the study include:

- Evaluation of selenium on thermoregulation,growth performance, tibia geometry, mineral retention, haematology and serum biochemistry of (*Cobb 500*) broiler chickens.

- Evaluation of organic ascorbic acid on thermoregulation, growth performance,tibia geometry, mineral retention, haematology and serum biochemistry of (*Cobb 500*) broiler chickens.

1.5Research Hypotheses

- **H_o:** Dietary selenium has no effect on thermoregulation, growth performance, tibia geometry, mineral retention, haematology and serum biochemistry of broiler chickens.
- **H_1:** Dietary selenium has effect on thermoregulation, growth performance, tibia geometry, mineral retention, haematology and serum biochemistry of broiler chickens.
- **H_o:** Dietary organic ascorbic acid has no effect on thermoregulation, growth performance, tibia geometry, haematologyand serum biochemistryof broiler chickens.
- **H_2:** Dietary organic ascorbic acid has effect on thermoregulation, growth performance, tibia geometry, haematology andserum biochemistryof broiler chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Industry in Nigeria

Agriculture, including poultry production is the most important sector of the Nigeria economy, providing livelihood for most Nigerians (FAO, 2020). In Nigeria, poultry production is a major contributor to animal protein(FAO, 2020). Nigeria has poultry population of 180 million, comprising 130 million chickens and 50 million of other species (FAOSTAT, 2017). The predominant systems of poultry production in Nigeria aresubsistent and commercial systems. The commercial system is further sub-divided into small (100 to 20,000 birds), medium (20,001 to 50,000 birds) and large (> 50,000 birds) scale poultry farming. These birds are kept under intensive system of management at a stocking density of 22 to 25 birds/m^2 (Dafwang, 2002). However, subsistence farming comprises indigenous breeds that are allowed to roam about to scavenge for feeds (Adeyemo and Onikoyi, 2012). In the tropical and subtropical regions of the world, including Nigeria, performance of broiler chickens is adversely affected by high ambient temperature (Ayo *et al*., 2011). Heat stress results in decreased feed intake, feed efficiency, body weight and flockactivities.

2.2 Thermo-Neutral Zone of Broiler Chickens

The thermo-neutral zone is the range of ambient temperature, which does not affect regulatory changes in metabolic heat production or evaporative heat loss in birds (Kingma *et al*., 2012). In the tropics, the diurnal ambient temperature fluctuations usually exceed the thermo-neutral zone of chickens, resulting in heat stress (Dei and Bumbie, 2011). Ambient temperatures outside the thermo-neutral zone of birds, irrespective of age, may negatively affect their energy balance and fitness (Ardia, 2013).

Metabolic disorders such as ascites and sudden death syndromes may occur in broilers reared above the temperature of thermal comfort in the first week of life of the birds (Fernandes *et al*., 2013). Broiler chickens subjected to heat stress show elevated corticosterone levels and lower levels of thyroid hormones (Mahmoud *et al*., 2014). Elevated temperature negatively affects production, reproductive potential, immune responses and health status of livestock (Nardone *et al.,* 2010). Holik (2009) reported that the thermo-neutral zone for poultry in the tropics is between 18 to 24 ^{0}C and between 12 to 26 ^{0}C in temperate regions, while Kingori (2011) showed that the most favourable temperature range for poultry is between 12 to 26 ^{0}C. Broiler chickens exhibit different behavior in response to daily variations in the thermal environment. Wing-spreading and panting are some important adaptations to thermal environments (Fernandes *et al*., 2013). Since adaptation of animal (including birds) to their thermal environment requires regulation of body temperature, measurement of such adaptation through the energy the animal expends, provides an indicator of the extent and energy cost of adaptation (Nienaber *et al.,* 2009).

2.3 Causes of Stress in Poultry Production

Poultry are constantly subjected to conditions that induce stress. Such as high ambient temperatures, relative humidity, nutritional imbalances, diseases, exposure to pathogenic organisms, transportation and management (Njoku, 1990). Similarly Ayo *et al* (2011) stated that the concept of stress embraces numerous deleterious factors such as biologically active substances, toxins, pathogenic and physical factors.

2.4 Effect of Heat Stress on Poultry Production

Stress, a response to adverse stimuli, is difficult to define and understand because of its nebulous perception (Lara and Rostagno, 2013). According to Selye (1976) and Lara and Rostagno (2013)

"stress is a non-specific response of the body to any demand which force flocks to adapt to a new or abnormal situation", whereas stressor can be defined as "an agent that produces stress at any time". Stress represents the reaction of an organism (*i.e.*, a biological response) to stimuli that disturb its normal physiological equilibrium or homeostasis. Heat stress results from a negative balance between the net amount of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal. This imbalance may be caused by variation of a combination of environmental factors (sunlight, thermal irradiation, and air temperature, humidity and movement), and characteristics of the animal (species, metabolism rate, and thermoregulatory mechanisms).

Two major categories of heat stress (acute heat stress and chronic heat stress) has been distinguished (Abdollah *et al*., 2016). Acute heat stress refers to a short and rapid rise in ambient temperature. Chronic heat stress refers to high ambient temperature over a long period of time (days to weeks), permitting acclimatization to the environment. Further, chronic heat stress is categorized as either cyclic chronic heat stress which refers to a limited period of heat exposure followed by comfortable temperature for the rest of the day, or as "constant chronic heat stress" whereby the animal is continuously confronted with high ambient temperature. Environmental stressors, such as heat stress, are particularly detrimental to animal agriculture (Renaudeau *et al*., 2012). Under acute heat stress, reactive oxygen specie (ROS) level in the body is rapidly increased and the antioxidant enzyme system also responds rapidly, by which the activity of catalase (CAT), super oxide dismutase (SOD), and glutathione peroxidase (GSHPx) are increased significantly to remove excessive free radicals. Pamok *et al*. (2009) reported that after four days of acute heat stress (38 + 2 ^{0}C), the GSHPx activity and serum malondialdehyde (MDA)was increased, which can reflect the degree of oxidative damage in poultry. Del-Vesco

etal. (2017) also reported that acute heat stress (38 ^{0}C for 24 h) increased the expression of the GSHPx gene. However, chronic heat stress can weaken the antioxidant enzyme system and cause ROS accumulation in the body to induce oxidative stress by decreasing the activity of CAT, SOD and GSHPx. Lu *et al.* (2017) reported that chronic heat stress (32 ^{0}C for seven days) increased the muscle ROS level and MDA content with reduced SOD and GSHPx activity in poultry.

The importance of animal responses to environmental challenges applies to all species. However, poultry seems to be particularly sensitive to temperature-associated environmental challenges, especially heat stress. It has been suggested that modern poultry genotypes produce more body heat, due to their greater metabolic activity (Deeb and Cahaner, 2002).

Heat stress adversely affects the efficiency of broiler production and meat quality (Ranjan *et al.*, 2019). High environmental temperature and temperature humidity index (THI) value above the critical threshold level lead to reduced feed intake, lower body weight, and lower feed conversion efficiency (Sohail *et al.*, 2012).

2.4.1 Effect of Heat Stress on Behaviour and Physiology of Broiler Chickens

Thermoregulation is important in maintaining homeostasis and it is controlled by central, metabolic and endocrine systems. The body mass, conformation and morphological parameters such as fur colour are related to basal metabolic rate and can be used for behavioral adjustments (Cooper *et al.*, 2008). Thermoregulatory capacities of animals plays an adaptive role in their survival in adverse environment. Under high temperature conditions, birds alter their behaviour and physiological homeostasis seeking thermoregulation, thereby decreasing body temperature (Lara and Rostagno, 2013). In general, different types of birds react similarly to heat stress,

expressing some individual variation in intensity and duration of their responses(Lara and Rostagno, 2013). Birds subjected to heat stress conditions spend less time feeding, more time drinking and panting, as well as more time with their wings elevated, less time moving or walking, and more time resting, squatting near the ground, slow and lethargic with closed eyes, reduced body weight, and increased cannibalism(Lara and Rostagno, 2013).

Animals utilize multiple ways for maintaining thermoregulation and homeostasis when subjected to high environmental temperatures, including increasing radiant, convective and evaporative heat loss by vasodilatation and perspiration (Mustaf *et al*., 2009). Birds have an additional mechanism to promote heat exchange between their body and the environment, which are the air sacs. Air sacs are very useful during panting, as they promote air circulation on surfaces contributing to increased gas exchange with the air, and consequently, the evaporative loss of heat (Mustaf *et al.,* 2009). However, it is worth noting that increased panting under heat stress conditions leads to increased carbondioxide levels and higher blood pH (alkalosis), which decreases free calcium levels in the blood. Also, negative effects caused by heat stress in males have been shown in different studies(McDaniel *et al*., 2004; Ayo *et al*., 2011). Semen volume, sperm concentration, number of live sperm cells and motility decreased when males were subjected to heat stress (McDaniel *et al*., 2004).

High environmental temperatures alter the activity of the neuroendocrine system of poultry, resulting in activation of the hypothalamic-pituitary-adrenal (HPA) axis, and elevated plasma corticosterone concentrations (Garriga *et al.,* 2006; Star *et al*., 2008; Quinteiro-Filho *et al*., 2010; Quinteiro-Filho *et al*., 2012). Body temperature and metabolic activity are regulated by the thyroid hormones, triiodothyronine (T3) and thyroxine (T4). Geraert *et al.* (1996) reported that

endocrinological changes caused by chronic heat stress in broilers stimulate lipid accumulation through increased *de novo* lipogenesis, reduced lipolysis, and enhanced amino acid catabolism.

2.4.2 Effect of Heat Stress on the Immune Response of Broiler Chickens

Modulation of the immune response by the central nervous system (CNS) is mediated by a complex network that operates bi-directionally between the nervous, endocrine and immune systems. The hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal medullar (SAM) axes constitute the main pathways through which the immune response can be altered (Ademu, 2018). It has been shown that lymphocytes, monocytes or macrophages, and granulocytes exhibit receptors for many neuroendocrine products of the HPA and SAM axes, such as cortisol and catecholamines, which can affect cellular proliferation, cytokine secretion, antibody production and cytolytic activity (Padgett and Glaser, 2003; Butts and Sternberg, 2008; Marketon and Glaser, 2008). In general, all studies showed an immunosuppressing effect of heat stress on broilers and laying hens.

Lower relative weights of thymus, bursa, spleen and lymphoid organ has been found in broilers and laying hens subjected to stress (Bartlett and Smith, 2003; Niu *et al*., 2009; Ghazi *et al*., 2012; Ademu *et al.,* 2018). Additionally, Felver-Gant *et al.* (2012) observed reduced liver weights in laying hens subjected to chronic heat stress conditions. Bartlett and Smith (2003) observed that broilers subjected to heat stress had lower levels of total circulating antibodies, as well as lower specific IgM and IgG levels, during primary and secondary humoral responses. Aengwanich (2008) also demonstrated the occurrence of reduced bursa weight in broilers subjected to heat stress, as well as decreased numbers of lymphocytes in the cortex and medulla areas of the bursa. Similarly, Bozkurt *et al*. (2012) reported reduced systemic humoral immune response. Bartlett and Smith, (2003) and Niu, (2009) also reported reduced antibody response, as well as reduced

phagocytic ability of macrophages, in broilers under heat stress. Moreover, reduced macrophages performing phagocytosis, as well as induced oxidative burst were observed in heat-stressed broilers (Quinteiro-Filho *et al*., 2012). Recent studies have also demonstrated that heat stress can alter levels of circulating lymphocytes cells(Prieto and Campo, 2010; Ademu, 2018). It has been shown that heat stress causes an increase in heterophil:lymphocyte ratio, due to reduced numbers of circulating lymphocytes and higher numbers of heterophils (Prieto and Campo, 2010).

2.4.3 Impact of Heat Stress on the Antioxidant System in Poultry

Poultry is more sensitive to heat stress than other domestic animals, because they do not have sweat glands, their metabolism is rapid and they have high body temperatures (Lara and Rostagno, 2013). Increased environmental temperature caused increased lipid peroxidation (in addition induced the formation of malondialdehyde (MDA), which is an indicator for lipid peroxidation). Therefore, the antioxidant defence system is altered (Lara and Rostagno, 2013; Chauhan *et al*., 2014).

In general, it can be concluded that a large amount of ROS causes disruption of mitochondrial function, increases lipid peroxidation, decreases vitamin concentrations, induces stress gene expression, leads to dysfunction in antioxidant enzymes and also causes DNA damage. Yang *et al.* (2010), studied short term heat stress (35°C for 3 h/day) in broilers and reported that the activity of the mitochondrial respiratory chain is reduced by heat stress, leads to over-production of ROS and results in lipid peroxidation and oxidative stress in the birds. Lipid peroxidation and SOD activity was measured in broilers under heat stress (32°C for 6 h/day) and SOD concentration was seen to be increased by heat stress (Lin *et al*., 2006a). The result showed that high temperature disrupt the equilibrium between the synthesis and catabolism of ROS production.

Birds can consume dietary antioxidants or up-regulate their endogenous antioxidant systems to mitigate the effects of the increased amount of ROS (Cooper-Mullin & McWilliams, 2016).In response to oxidative stress, organs and tissues possess distinct antioxidant systems. The knowledge of antioxidant defense systems (GSHPx, SOD, selenium, vitamin E, and A) serves as a guide for establishing the most effective nutrient supplementation to reduce or rather alleviate oxidative stress. Such approach enhances bird's health and welfare, product quality and increases economic returns of broiler production (Panda and Cherian, 2013). The body protects itself against the negative effects of ROS by two mechanisms, namely through the regulation of membrane permeability and its antioxidant potentials (Lushchak, 2011). Superoxide dismutase enzyme is an important cellular defense against ROS (Klooppel *et al.,* 2010), through the process of dismutation.

The suppression of nuclear factor erythroid 2-related factor 2 (Nrf 2), an important transcription factor in antioxidant regulation system, occurs during oxidative stress in poultry as evident by changes in levels of activities of superoxide dismutase, catalase, glutathione and thiobarbituric acid-reactive substances (Kim *et al*., 2012; Liu *et al.,* 2013). The skeletal muscle mitochondria of broilers produce superoxide anions during heat stress which is detrimental to the animal (Mujahid *et al*., 2005). The life-span of erythrocytes, which may function in antioxidant defenses, decreases by 50%, when the cells are exposed to excessive ROS production(Olszewska *et al*., 2012). The decrease in the longevity of erythrocytes may be due to protein and/or amino acid degradation (such as tryptophan) in the cytoskeleton of broilers (Olszewska *et al*., 2012). It may also be due to post-translational modification of proteins, destroying the fate and functions of the erythrocytes (Pandey and Rizvi,2013).

2.4.4 Effect of Heat Stress on Intestinal Integrity of Broiler Chickens

Under heat stress conditions, some morphological and physiological changes had been observed in the gastrointestinal tract on account of functionality and integrity of intestinal epithelium (Soderholm and Perdue, 2001). Heat induced stress leads to changes in intestinal microflora (Suzuki, 1983), disruption in blood flow of gastrointestinal tract (Wolfenson, 1986) and deterioration in intestinal morphological traits, such as villus height, crypt depth, villus width, ratio between villus height and crypt depth and villus absorptive surface area (Burkholder, 2008). Deng (2012) reported a decline in villus height and ratio between villus height/crypt depth under heat stress. In another study,Burkholder (2008) reported that villus height decreased to 18.8% in birds exposed to 30^0C for 24 hours, compared to the control birds raised at 23^0C. It is well known that the intestinal mucosa protects the organism from pathogenic bacteria and is also necessary for digestion and absorption of nutrients in the intestine (Fagarasan, 2006; Keita and Soderholm, 2010). Under heat stress condition, the integrity of the intestinal barrier is affected (Song *et al.*, 2014), when this happens, intestinal permeability and subsequently intestinal inflammation can increase (Quinteiro-Filho *et al.*, 2012). Stress conditions cause a decline in protective functionality of intestinal epithelium and increase susceptibility of birds to some diseases, such as *Salmonella* spp. infections (Quinteiro-Filho *et al*., 2010).

2.4.5 Indicators of Heat Stress in Broiler Chickens

Modern commercial broilers appear to have a compromised immune status, higher mortality and lower resistance to stressors such as heat stress (Khan *et al*., 2012). Heat stress induces hyperthermia in poultry (Syafwan *et al*., 2011). Skin temperature, measured by thermography could be used as an index of welfare for domestic birds (Marelli *et al*., 2012). Erythrocyte osmotic fragility (EOF) may be used as an indirect measure of lipid peroxidation (Minka and

Ayo, 2013). The EOF is an important biomarker of oxidative stress, which may be utilized as an indicator for heat stress in laying hens (Sinkalu *et al.,* 2014). It may be used in broiler chickens to diagnose oxidative stress, having been demonstrated to serve as a biomarker of transport-induced oxidative stress under high ambient temperature conditions, and as a reliable method for diagnosis of oxidative stress in quails (Minka and Ayo, 2013) and rabbits (Ayo *et al*., 2014). Heterophil/lymphocyte ratio is also used as an indicator of heat stress (Prieto and Campo, 2010). Furthermore, high levels of corticosterone and heat-shock protein 70 may indicate levels of heat stress, and could result in high intensity of fear responses, evident by long tonic immobility in heat-stressed broiler chickens (Al-Aquil *et al*., 2009; Zulkifli *et al*., 2009).

2.4.6 Effect of Heat Stress on Hormone Secretion

Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma glucocorticoid concentrations are two of the most important responses of animals to heat stress. The short and long-term environmental heat affects endocrine glands and in turn the release of hormones, such as thyroxine, cortisol, growth hormone and catecholamines (Aggarwal and Upadhyay, 2013). An initial increase due to acute stressors and a decline in plasma levels after prolonged exposure to stressors has been observed. Acclimation to thermal stress is a homeorhetic process under endocrine control. The process of acclimation (coordinated phenotypic response developed by the animal to a specific stressor in the environment, e.g. increased water intake, reduced feed intake, altered physiological functions) occurs in two phases, that is, acute and chronic, and involves changes in hormone secretion as well as receptor populations in target tissues (Collier *et al.,* 2006). The time required to complete both phases vary from species to species and may take days to weeks.

Thyroid hormones are important in an animal's adaptation to a hot environment. The thyroid gland secretes triiodothyronine (T3) and tetraiodothyronine/thyroxine (T4) which provide a major mechanism important for growth and acclimation during heat stress (Pusta *etal*., 2003). Both triiodothyronine (T3) and thyroxine (T4) are associated with metabolic homeostasis and are susceptible to climatic changes (Aggarwal and Upadhyay, 2013). The plasma thyroxine (T4) and triiodothyronine (T3) levels have been observed to decline under heat stress as compared to thermoneutral conditions. The decline in thyroid hormones along with decreased plasma growth hormone (GH) level has a synergistic effect to reduce heat production.The reduced secretion of growth hormone (GH) is required for survival of the homeotherm during heat stress (Aggarwal and Upadhyay, 2013).

Adrenal corticoids, mainly cortisol, elicit physiological adjustments that enable animals to tolerate stress.Plasma cortisol levels increased significantly due to increase in ambient temperature in cattle and buffaloes to different levels. Exposure of non-pregnant female buffaloes for 2-3 hours to solar radiation at 42.1^0C increased plasma cortisol concentration rapidly for 30 min, followed by a gradual fall and the cooled animals had lower plasma cortisol concentration than the non-cooled animals (Aggarwal and Singh, 2010) . The elevated plasma cortisol concentrations of non-cooled animals may reflect the stress due to high temperatures (Chaiyabutr *et al.*, 2008).

2.4.7 Effect of Heat Stress on Tibia Bone

Heat stress can negatively affect bone development in broiler chickens by initiating mineral extraction from the bone (Sgavioli *et al*., 2016). Calcium and Phosphorus is the most abundant mineral in the body with immense importance in poultry diets for efficient growth and development (Shahid *et al*., 2018). Heat stress can led to decreased binding of minerals to protein

for proper intestinal absorption and utilization. Persia *et al*. (2003) reported a reduction in the available calcium and phosphorus during chronic heat stress due to reduced growth. Reduction in feed intake during heat stress in poultry compromises the nutrient intake hence decreases the mineral intake as well (Persia *et al*., 2003). Modern poultry has been bred for superior meat production, possibly overlooking the consequences on bone quality (Rath *et al*., 2000).

Bone problems have been identified as one of the main production and health concerns in meat-type poultry (Rath *et al*., 2000; Ademu, 2018). Rapid growth results in skeletal deformities including problems caused by leg weakness that result in lameness and consequently poor animal welfare (Sanotra *et al*., 2001). Deficiency and loss in bone mineral structure is a function of skeletal problem (osteoporosis) in modern highly productive laying chickens. Osteoporosis is the major reason for bone loss and subsequent fractures in laying hens due to decreased nutrient absorption (Ogunwole *et al*., 2018). Ash is used as an index of the overall mineral content of bones. Robusticity index is an indication of bone strength, and a low robusticity index indicates a strong bone structure. (Reisenfeld, 1972). Ademu (2018) reported that dexamethasone; a glucocorticoid decreased tibia length and weight of broiler birds. Rath *et al*. (2000) reported that bones from younger turkeys were more susceptible to corticosteroid-induced stunting of growth.

2.5 Relationship between Heat Stress and Oxidative Stress

Oxidative stress is defined as the excess production of reactive oxygen species (ROS) in excess of the available antioxidant capacity of animal cells (Shankar and Mehendale, 2014) while heat stress is a rise in temperature within an animal's body more than the level it can handle and can lead to the production of ROS, hence oxidative stress. Many radicals and metabolic substances are described as potentially toxic and are defined as "reactive oxygen/nitrogen/chlorine species" (Shankar and Mehendale, 2014). These substances are highly reactive and can modify several

biologically cellular macromolecules, such as proteins, lipids, and nucleic acids (DNA and RNA) (Davies, 1995).

These phenomena contribute to the development of several metabolic dysfunctions, including destruction of biomolecules and "oxidative stress" and (Shankar and Mehendale, 2014). Oxidized molecules extract electrons from other molecules, resulting in a chain reaction and, if not controlled, this reaction can cause extensive tissue damage. In addition, ROS may alter the redox equilibrium of several cellular redox couples (e.g. reduced glutathione (GSH) and glutathione disulfide (GSSG) and reduced and oxidized thioredoxin) leading to an altered expression of key enzymes in detoxification, antioxidant defense, cell transitions, inflammatory responses, etc. Cells have evolved defense systems to control the production of ROS. These include both non-enzymatic low-molecular weight (vitamin C, GSH, and uric acid) and enzymatic high molecular weight (e.g. superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and arylesterase) compounds. They limit the rate and progression of oxidation and thereby protect cells from oxidative damage. Under environmental stressful conditions, as the bird's body attempts to maintain its thermal homeostasis, increased levels of reactive oxygen species (ROS) occur. As a consequence, the body enters a stage of oxidative stress, and starts producing and releasing heat shock proteins (HSP) in attempt to protect itself from the deleterious cellular effects of ROS (Droge, 2002). Higher concentrations of HSP70 were found in broilers and laying hens exposed to heat stress (Gu *et al*., 2012).

2.6 Reactive Oxygen Species and their Roles in Broiler Chickens during Heat Stress

In the eukaryotic cells, mitochondria is the site of aerobic energy production. Electron transfer from respiratory substrates is coupled to oxygen to produce ATP, however, this transfer may lead to the formation of radicals and other ROS (Venditti *et al*., 2013). ROS are also generated in the

mitochondria of skeletal muscles of heat-stressed broiler chickens due to a rise in mitochondrial membrane potential (Azad *et al*., 2010; Kikusato *et al*., 2010). The continuous generation of ROS and the inability to manage the burden results in oxidative stress and cellular damage due to lipid peroxidation, in cell membranes (George *et al*., 2012; Singh *et al*., 2013). ROS-mediated damages of erythrocyte membrane results in haemolysis and haemolysis causes erythrocyte fragility (Eroglu *et al*., 2013; Toplan *et al*., 2013). Damage to the cytoskeleton is common in neuronal cell death and this is an early event in oxidant-induced cell injuries (Tiogo *et al*., 2011).

Oxidative stress causes depletion in energy, accumulation of cytotoxic mediators and cell death (Lee *et al*., 2012). Excessive ROS production is involved in the pathogenesis of contractile dysfunction in heart-failure (Kubin *et al*., 2011) and in carcinogenesis (Quan *et al*., 2011), because cancer cells (as seen in Marek's disease), require elevated levels of ROS to maintain their high multiplication rate (Sosa *et al*., 2013). Acute heat stress-induced over-production of mitochondrial ROS may depend on mitochondrial membrane potential and consequently results from increased substrate oxidation and a decrease in the mitochondrial avian uncoupling protein (avUCP) content (Kikusato and Toyomizu, 2013). There is a link between oxidative stress and mitochondrial dysfunction which leads to cytotoxin-mediated muscle pathology (Ramadasan-Nair *et al*., 2014). It is also implicated in intermittent hypoxia-induced hypertension (Del Rio *et al*., 2010). Animals that live long are established to have low rate of ROS generation and low oxidation damage to their mitochondrial DNA (Sanchez-Roman and Barji, 2013).

Excessive oxidative destruction and alterations in amino acid (serine, tyrosine and isoleucine) concentrations in the diencephalon may contribute to the physiological, behavioral and thermoregulatory responses of thermally-exposed chicks (Chowdhury *et al.*, 2012). Heat stress can cause increased expression of hypothalamic neuropeptides (Chowdhury *et al*., 2012).

Similarly, heat/oxidative stress can cause the destruction of biomolecules, cells and tissues leading to decreased immunity and antioxidant enzyme, poor growth rate, and decreased production in broiler chickens (Keshavamurthy *et al*., 2013).

2.7 Management Approaches to Reduce Heat Stress in Poultry

Reduction of the heat stress in poultry requires a multi-disciplinary approach, such as modification of surrounding environment, ventilation system, reduction of bird density and nutritional management (Dayyani and Bakhtiyari, 2013).Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature in poultry (Mahmoud*et al*., 2004).

2.7.1 Nutritional management in Reducing Heat Stress in Poultry

To reduce the effect of heat stress, poultry will decrease feed intake and increase water intake to control the body temperature (Gous and Morris, 2005; Sohail *et al.,* 2012). Feed intake will be reduced by 1.2% for every 1 ^{0}C rise in the temperature range of 22-32 ^{0}C and 5% for 1 ^{0}C rise in the temperature range of 33-38 ^{0}C. Heat stress increases excretion of mineral from body and decreases the serum and liver concentrations of vitamins and minerals (Sahin *et al.,* 2009). Vitamin and mineral supplementation has been used to decrease mortality and improve growth performance of poultry birds during heat stress. Vitamin C supplementation in the diet of broilers or layers during heat stress is effective in reducing mortality rate and improving performance (Ahmed *et al.,* 2005).

Vitamin C acts as anti-stress and growth stimulant in commercial broiler production due to maintenance of normal collagen metabolism (Mahmoud *et al.,* 2004).Several methods

(biological or technological) are available to alleviate the negative effects of heat stress in poultry. As it is usually more expensive to cool buildings, most methods focus on nutritional tools, hence the need to explore a cheaper or rather cost effective technique. Different nutritional strategies are known to reduce the negative effects of heat stress, e.g. decreased protein level and different amino acid composition in the diet, increased fat intake, electrolytes in water, probiotics and betaine supplementation (Balnave & Brake, 2005; Gous & Morris, 2005; Lin *et al*., 2006b; Daghir, 2009).

2.7.2 Modification of Surrounding Environment

Environmental temperature and relative humidity of the surrounding environment affects the evaporative cooling mechanism in birds. Evaporative heat loss increases in high temperature with wind speed but decreases with increasing humidity (Sinha *et al.,* 2017). The surrounding environment is controlled by using various ventilators such as fans, fogger with fan, cooling pads, curtain, static pressure controllers and thermostats(Sinha *et al.,* 2018). The orientation of building, insulation and roof overhang also influence the temperature inside the poultry house. Air movement inside the house is important for efficient ventilation(Sinha *et al.,* 2018). Use of sprinkler and fogger with fan reduces the temperature inside the house in hot climatic condition (Sinha *et al.,* 2018).

Good ventilation system is essential for heat stress management. It aids proper flow of air and reduces the CO_2 load within a poultry building. Ventilation system should be maximized as the air movement assists removal of ammonia, moisture and carbon dioxide from the poultry house and inflow of fresh oxygen from outside (Butcher and Miles, 2012). Proper ventilation houses can provide consistent airflow patterns. Tunnel ventilation connects moving air of building from inlets to exhaust fans, providing high airflow speed. This fast air movement increases convective

heat loss, reducing the body temperature of birds. Evaporative cooling pads works with the same cooling principle as foggers, air is cooled inside the house when it passes through the cooling pads. Circulation fans are recommended for proper ventilation in a poultry house for maximum air movement over the birds to increase convective cooling (Daghir, 2008).

2.8 Water intake of Birds

Water is one of the most important nutrients in animal nutrition, and plays an essential physiological role related to the thermal homeostasis of birds and other animals during heat stress (Bruno *et al*., 2011). At high temperatures, chickens consume more water than feed. Water intake increases in order to maintain thermoregulatory balance as heat stress induces high water loss through the respiratory tract as a means to achieve efficient thermoregulation through evaporative cooling (Bruno and Macari, 2002). Water is more important for thermoregulation in modern broiler chickens compared to other animal species (Bruno and Macari, 2002), especially under hot environment. Broilers subjected to early water deprivation present inadequate development of the intestinal mucosa (Maiorka *et al*., 2003). Water intake of birds increases about 7% for every 1°C increase above 21°C (NRC, 1994). Birds' performance depends upon water temperature, drinker type, shape and height of water trough under heat stress (Daghir, 2009; Bruno *et al*., 2011). The most important factor influencing broiler water intake pattern is environmental temperature (Bruno *et al*., 2011). Broilers subjected to cyclic temperatures developed different patterns of water intake, and different acclimation temperatures may be responsible for the development of different water intake and excretion capacities to maintain hydro-electrolytic balance (Bruno *et al*., 2011).

2.9 The Role of Antioxidants in Alleviating Heat Stress in Broiler Chickens

Anti-oxidants are chemical substance that limit reactive oxygen species (ROS) production, scavenge for existing free radicals and promote the repair of cell structures damaged by ROS (Argarwal and Allamaneni, 2004). Similarly, Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the ignition or propagation of oxidizing chain reactions(Argarwal and Allamaneni, 2004).

Antioxidants improved erythrocytic indices of broiler chickens subjected to heat stress (Majekodunmi *etal*., 2013). Antioxidants decrease the deterioration of broiler meat quality due to lipid peroxidation and stabilize meat oxidation after slaughter (Yasin *et al*., 2012). At any point in time, one antioxidant molecule can react with a free radical and neutralizeit by donating one of its own electrons, ending the carbon "stealing" reaction(Sujogya, 2012). Antioxidants prevent cell and tissue damage as they act as scavengers. A variety of endogenous and exogenous components neutralize free radicals within the body (Sujogya, 2012), these include endogenous enzymatic and non-enzymatic antioxidants.

Oxidative stress results when the endogenous antioxidants are overwhelmed by the rate and extent of free radical generation, therefore, an increase in the exogenous supply of antioxidants improves the capacity of the tissue to cope with high antioxidant demand (Ambali *et al*., 2010). In response to oxidative stress, organs and tissues possess distinct antioxidant systems. An understanding of these systems helps to develop strategies to protect the body against oxidative damage due to the destruction of lipids, proteins and DNA (Al-Gubory *et al*., 2010). The body protects itself against the negative effects of ROS by two mechanisms, regulation of membrane permeability (less membrane permeability to influx of H_2O_2, ie. ROS) and the endogenous antioxidant system potential (which function by sensing of reactive species and transduction of the signal to transcription and translation machineries) (Lushchak, 2011).

2.9.1 The role of antioxidant enzymes in counteracting heat stress in broiler chickens

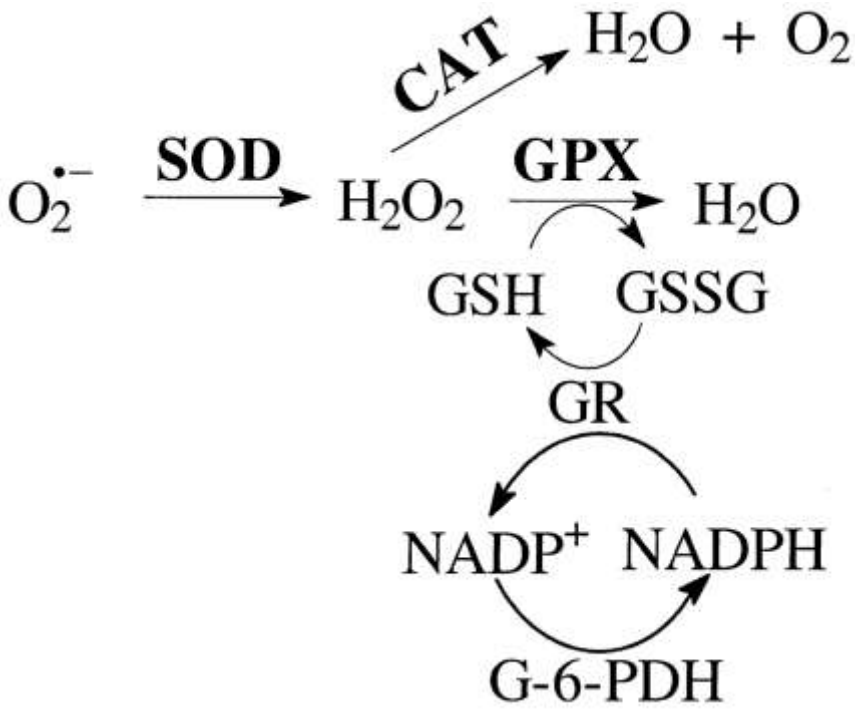
Cells, tissue and body fluids are equipped with powerful defense systems that help counteract oxidative challenge. To maintain a steady state of metabolites and functional integrity in the aerobic environment, antioxidant defense is organized at three principal levels of protection, prevention, interception and repairs (Klimczack *et al*., 2007). The biological mechanism of defense against oxidative stress includes antioxidant enzymes such as Superoxide dismutase (SOD) and Catalase (CAT) (Edem *et al*., 2012).Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) play a vital role in protecting cells from harmful effects of ROS (Altan *et al.*, 2003). Synthesizing these enzymes is an important regulation, in terms of animal response to stress conditions. The SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical (O_2^-), a highly potent ROS, into a less reactive specie, hydrogen peroxide (H_2O_2) (Edem *et al*., 2012). CAT is an ubiquitous enzyme present in cells of aerobic organisms. It converts two molecules of hydrogen peroxide (H_2O_2) to molecular oxygen (O_2) and two molecules of water (H_2O). Glutathione peroxidase (GSHPx) also inactivates free radicals by breaking down the hydrogen peroxide (H_2O_2) formed from superoxide radical anion (O_2^-) into water (H_2O). The conversion of H_2O_2 into H_2O precedes the conversion of glutathione from reduced form to oxidized form (Figure 2.1).

2.9.2 The Role of Non-enzymatic Antioxidants in Alleviating Heat Stress in Broiler Chickens

Non-enzymatic antioxidants are divided into metabolic and nutrient antioxidants. Metabolic antioxidants are the endogenous antioxidants, which are produced by metabolic activity in the body, like lipoid acid, glutathione, L-arginine, co-enzyme Q10, melatonin, uric acid, bilirubin

and metal-chelating proteins(Sujogya, 2012). Nutrient antioxidants belong to exogenous antioxidants, which cannot be produced in the body, but are provided through diet or supplement, viz. trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, ascorbic acid (Sujogya, 2012).

The high environmental temperature decrease the concentrations of vitamins and micro minerals in serum and increase its excretion (Khan *et al*., 2012); therefore, supplementation of direct or



SOD- superoxide dismutase
CAT- catalase
GPx- glutathione peroxidase
GR- glutathione reductase
GSH- glutathione
GSSG- oxidized glutathione
NADP- nicotinamide adenine dinucleotide phosphate
NADPH- reduced nicotinamide adenine dinucleotide phoshate

Figure 2.1: Transformation pathways of superoxide radical anion (O_2^-) in the body and role of Glutathione metabolism in neutralization of reactive oxygen species(Adapted from Kwiecient *et al*., 2004).

indirect antioxidant compounds (e.g. vitamins and micro nutrients) at higher levels is commonly recommended (Yun *et al.,* 2012). These additives support mechanisms against lipid peroxidation, improve immune status and performance. Several nutritional remedies have been recommended to relieve the detrimental effects of chronic heat stress and adjust dietary levels of energy, proteinand/or amino acids (Daghir, 2008; Attia *et al.,* 2011; Attia and Hassan, 2017).

2.10 Selenium as an Antioxidant in Broiler Chickens

Selenium (Se) is an essential trace element that was discovered by Jacob Berzelius in 1817 and initially considered toxic to humans and animals (Shi *et al*., 2011). The word "*selenium*" originates from the Greek word *Selene*, which means moon goddess.Later on, selenium was recognized as a nutritionally essential trace element that is important in many biological processes in mammals and birds (Jlali *et al.,* 2013). Selenium fulfils a number of significant functions by means of specific selenium enzymes, including antioxidant protection of the organism against free radicals, maintenance and strengthening of natural immunity of the organism, support for correct function of the thyroid gland and reproductive organs.

Heat stress induced oxidative stress can be partially ameliorated by supplementing selenium in poultry due to its cofactor properties. Performance (Niu *et al*., 2009; Harsini *et al*., 2012) and

antioxidant status (Harsini *et al*., 2012) of broilers under heat stress were both improved by 0.2 - 1 mg/kg selenium supplementation. It is suggested that the metabolic role of selenium is to protect cells against oxidation and tissue damage (Zoidis *et al.,* 2018). Rapid oxidation of glutathione (GSH) to oxidized glutathione (GSSG) is necessary to compensate for ROS production caused by heat stress. However, selenium supplementation increases the level of available NADPH to promote the activation of glutathione reductase, leading to increased GSSG reduction to GSH (Suchý *et al*., 2014). Therefore, selenium supplementation affects GSHPx activity and GSHPx/GSH ratio.Its role as an important component of antioxidant enzymes such as glutathione peroxidase(GSHPx), thioredoxin reductase (TrxR) and iodothyronine deiodinase (IDD), which destroys free radicals in the cytoplasm, has created an increased interest in the study of other Selenium containing proteins (selenoproteins) or enzymes (selono-enzymes) (Ujang, 2008).

Selenium is an integral part of a large number of seleno-proteins such as glutathione peroxidase, thioredoxin reductases, and iodothyronine deiodinases, which participate in the regulation of various functions of the body, including redox balance maintenance and antioxidant defense, of which glutathione peroxidase (GSHPx) was the first identified in 1973 (Boostani *et al*., 2015). Selenium-dependent GSHPx is considered as the second most important factor in antioxidant defense after vitamin E. Hence, dietary selenium as GSHPx and as thioredoxin reductase enzymatic cofactor, participates in two levels of antioxidant cell defense,detoxification of H_2O_2resulting from SOD activity (Surai, 2002a).In poultry, a delicate antioxidant/pro-oxidant balance in the body is an important determinant of chicken health, embryonic and postnatal development, immunity, muscle function, sperm quality and probably productive and reproductive characteristics of poultry (Jlali *et al*., 2013).

2.10.1Different Forms of Selenium Bioavailability and Application in PoultryNutrition

Sources of Selenium can be divided into two groups basedon their efficiency, inorganic and organic selenium(Schrauzer, 2000). InorganicSe (sodium selenite) is lessbiologicallyactive. Itaccelerates oxidization processes inorganismandmaycausehealthproblems as they are toxic at higher doses. Organicseleniumcompoundsperformakeyroleinbiological processes,they are more active than inorganic salts. They are part of proteins and include seleno-methionine (Se-Met)andseleno-cysteine(Se-Cys).Se-Metexistsin two isomer forms, d and l, and was identified in plant proteins (Schrauzer, 2000). Only the l-form occurs naturally, d-form may only be prepared synthetically. This form makes up to 50% of the total Se content in vegetarian food and higher organisms are unable to synthesize it (Schrauzer 2000).

Selenium enzymes are often formed from Se-Cys. Se-Cys aremainly found in foodof

Selenium enzymes are often formed from Se-Cys. Se-Cys aremainly found in food of animal origin and in plants able toaccumulatehighlevelsofSelenium(Hartikainen,2005). Se-Metisquicklyabsorbedwiththeconsequence ofhigherbloodlevelsincomparisontoinorganic Selenium.Most of Selenium in the inorganic form is excreted via urine, as to avoid selenium accumulation and toxicity (Groce *et al*. 1971). Selenium in its organic form shows higher bioavailability (75.7%) than Selenium bound in the inorganic form (49.9%) (Mahan *et al*., 1999). This is manifested by higher levels of organic Se in all tissues and anatomies.

The absorption mode of various selenium forms is different, leading to different digestibility and bioavailability. Organic selenium is absorbed in the small intestine via the transport mechanism

for amino acids, inorganic selenium is absorbed by passive transport, while the nanoparticles have a high specific surface area, small particles, and form nano emulsion droplets that are well absorbed in the intestines (Hu *et al*., 2012). Inorganic forms of selenium can lead to production of selenocysteine, which is incorporated specifically into selenoproteins, whereas organic selenium sources can lead to the production of selenomethionine as well as selenocysteine (Jlali *et al*., 2013; Briens *et al*., 2013). The cell can non-specifically incorporate selenomethionine into the structural proteins and, thus, increase the selenium deposit in all tissues(Surai, 2002a; Jlali *et al*., 2013).

2.10.1.1 Nano-Selenium Compound

Products of nanotechnology have begun to be applied in the area of nutritional supplements and have become largely available and usable now (Suchy *et al*., 2014). A good example may be nano-elements, including nano-selenium (nano-Se), with noted significant increase of chemical reactivity. The consequences of nanotechnology include changed efficiency as well as potential toxicity of the elements. Cai *et al*. (2012), reported significant effects of nano-Se (0.3 – 0.5 mg/kg) on GSHPx activity, free radical inhibition, and serum levels of IgM.

Zhang *et al*. (2001) reported that nano-Se hadseven-times lower acute toxicity than sodium selenite in mice. In Se-deficient rat, both nano-Se and selenite can increase tissue Se and GSHPxactivity.Nano-Seandseleniteare effective instimulating the synthesis of GSHPx, phospholipid hydroperoxideglutathione peroxidase, and thioredoxin reductase. Nano-Se shows less pro-oxidative effects than selenite, as measured by cell growth. These results revealedthatnano-Sehasasimilarbioavailabilityin rat and antioxidant effect oncells.

In recent years, the possibility of using nanoparticles as supplements in poultry feed has developed. The particle size of minerals as feed additives in nanoparticle form is typically between 0.2 - 100.0 nm and this property distinguishes them with respect to their physical, chemical, and biological properties (great specific surface area, high surface activity, many surface active centers, high catalytic efficiency and strong adsorbing ability) from non-nano, larger particle sizes (Baltić, 2013; Suchý, 2014). Nano additives can also be incorporated in micelles or capsules of protein or other natural food/feed ingredient (Bunglavan*et al.*, 2014).

Nanoparticles such as nanoselenium, can be used to improve performance (Gangadoo *et al*., 2016). Numerous studies have shown that nano selenium, possessed higher efficiency than selenite, selenomethionine, and methylselenocysteine in up-regulating selenoenzymes, higher bioavailability, and exhibited a lower toxicity (Shi *et al*., 2011).Nanoparticles have been used in poultry feed to decrease number of harmful bacteria in broilers digestive systems (silver, gold, zinc, copper, metal oxides – Al_2O_3, Fe_3O4, CeO_2, ZrO_2, MgO).

2.10.2 Selenium and the Activity of Glutathione-Peroxidase in Broiler Chickens

Glutathione-peroxidase (GSHPx) is an enzyme transforming the toxic and carcinogenic hydrogen peroxide to harmless water and oxygen. Its activation requires small amount of Selenuim, probably substituting Sulphur in the glutathione molecule and causing development of modified enzyme GSHPx. The basic function of GSHPx is elimination of excessive peroxide and hydrogen peroxide of fatty acids resulting from oxidative elimination of lipid (De Almeina *et al.,* 2012).

Rama-Rao *et al.* (2013) reported decreased lipid peroxidation in plasma while activities of $GSHP_X$ and glutathione reductase in plasma increased linearly with selenium concentration (100

- 400 µg/kg) in broiler chicken diet. The selenium source (selenium-enriched yeast and selenium-enriched alga *Chlorella*), level (0.15 - 0.30 mg/kg), including sodium selenite, significantly influenced the GSHPx activity in breast and thigh meat (Heindl *et al.,* 2010). Boostani *etal*. (2015) demonstrated that supplementation with 0.3 mgSe/kg of diet of different sources increased the antioxidative capacity of broiler chicken under oxidative stress, where the nanoselenium effect was higher than an organic or inorganic source. Baltić *et al*. (2015) reported that organic selenium- enriched yeast (0.2 - 0.6 mgSe/kg of diet) increased GSHPx activity in plasma of 49 days old ducks.Low selenium (0.032 mg/kg)diet caused a decrease in the activities of total antioxidant capacity, superoxide dismutase, GSHPx, and an increase in xanthine oxidase (XOD) activity and malondialdehyde content (Zhang *et al.,* 2012).The activityofglutathioneperoxidase (GSHPx) in the serumremainsthesame both in the organic and the inorganicform.MaximumactivityofGSHPxisachievedattheSeleniumlevelof0.1mg/kgoffodderinthecaseofboth organic and inorganic form. Thisactivityisindependentofthechemicalform(Xia*etal*.,1992).Giving the fact that glutathione peroxidase gets saturated in the body system at certain level.

2.10.3 The Role of Different Sources of Selenium on Growth and Productivity of Poultry

Effects of various sources and levels of Selenium in the diet of poultry have been a subject of a number of studies. The results were not uniform, both negative and positive responses being reported (Table 2.1). Dlouha *et al.* (2008) supplemented the diet of broiler chickens with 0.3 mg/kg sodium selenite and there was no significant difference in body weight while higherselenium (1.58 mg/kg DM) was seen in the excreta. Similarly, Attia *et al*. (2010) fed

inorganic selenium (0.15 and 0.3 mg/kg sodium selenite) and organic selenium (0.15 and 0.3 mg/kg seleno-methionine as Se-yeast) to 30 weeks old dual purpose breeding hens of Gimmizah strain and reported a decrease in piped embryos and spleen percentage, plasma cholesterol concentration. Most levels of organic and inorganic supplementation significantly increased tibia calcium and phosphorus percentages and yolk selenium concentration (Attia *et al*., 2010). The duodenum and ileum mucosa was negatively affected by 0.3 mg/kg of inorganic selenium while the organic form was less toxic on spleen and hepatic tissues of chickens (Attia *et al*., 2010). Generally, organic and inorganic selenium supplementation at 0.3 mg/kg diet improved the productive and reproductive performance of the birds (Attia *et al*., 2010). Heindle *et al*. (2010) reported an increase in body weight of Ross 308 cockerel fed 0.15 mg/kg selenium-Plex and 0.30 mg/kg selenium-enriched algae chlorella. Selenium content in breast muscle was increased by both levels (0.6 and 0.85 mg/kg DM Se-plex and 0.6 and 0.82 mg/kg DM Se chlorella), while most forms of selenium increased GSHPx activity in breast and high meat (Heindle *et al*. 2010).

Dietary supplementation with organic selenium (0.3 ppm Se-enriched yeast) increased daily weight gain and feed intake by 8.92 and 3.99% respectively while survival rate and feed conversion was decreased by 0.93 and 4.48% respectively(Yang *et al*., 2012). Similarly glutathione peroxidase activity in the birds fed the organic selenium were 155.83% higher than that of the inorganic group indicating that the effect of organic selenium on broiler growth performance were better than the inorganic selenium except for survival rate (Yang *et al*., 2012). Chen *et al*. (2013) supplemented broiler diet with organic selenium (0.3, 0.5, 1.0, 2.0 mg/kg) for 42 days and the result showed no significant difference in growth performance, slaughter performance, immune status, drip loss and flesh of birds. The activities of glutathione peroxidase, total superoxide dismutase, the ability to inhibit hydroxyl radical, total antioxidant

capacity and the content of GSH were significantly increased along the selenium level whereas the content of malondialdehyde (MDA) was significantly decreased with organic selenium levels.

Habibian *et al*. (2014) supplemented broiler chickens diet with dietary selenium (0.5, 1 mg/kg) and vitamin E (125, 250 mg/kg) and observed that body weight and feed intake were not influenced significantly, whereas feed conversion was improved significantly by 125 mg/kg vitamin E. Rama-Rao *etal*. (2013) reported that dietary supplementation with organic selenium (100, 200, 300, 400 µg/kg) did not significantly influence body weight gain, feed efficiency, relative weight of liver and abdominal fat. Lipid peroxidation in plasma decreased linearly with selenium concentration in diet but there was no significant difference in the ratio between heterophils and lymphocytes and relative weight of lymphoid organs (bursa, spleen and thymus). Different levels of dietary selenium (0.15 mg/kg inorganic and 0.50 mg/kg organic) and vitamin E (40, 100, 150, 200 mg/kg) was fed to broiler chickens for 35 days and final weight of birds were not significantly different (Zdunczyk *et al*., 2011). Feeding of selenized yeast (0.25 mg/kg diet) increased the live body weight of chickens compared with the controls (Rozbicka-Wieczorek *et al*. 2012). Poultry diets deficient in selenium result in poor growth and development, increased mortality, reduced egg production, decreased hatchability, nutritional encephalomalacia, nutritional pancreatic atrophy, exudative diathesis, and muscle myopathy (Tufarelli *et al*., 2016).

2.10.4 The Effect of Selenium on the Immune System of Broiler Chickens

Selenium is an essential mineral that aids numerous physiological activities of the immune system. Se deficit damaged both cellular and humoral immunity; impaired humoral immunity resulting in reduced levels of IgG and IgM antibodies (Arthur *et al*., 2003).

Selenium stimulates the immune system, strengthening proliferation of activated T-lymphocytes (Rayman, 2000). Daily intake of 200 µg of selenium increased the activities of natural killers, increased lymphocyte activities such as stimulation of antigens and it maturation to cytotoxic lymphocytes which destroyed tumour cells. The mechanism behind the increased antibodies activity is closely connected with increased numbers of receptors for interleukin-2 on the surface of the activated lymphocytes and natural killers (Rayman, 2000; Arthur *et al*., 2003).

Rama-Rao*etal.*(2013) reported thatcell-mediatedimmunity(lymphocyte proliferation ratio) of broiler chickens increased linearly with dietary organic Se concentration. Liao *et al.* (2012) reported that Se yeast was more effective than sodium selenite (Na_2SeO_3) or Se protein in increasing tissue selenium retention; however, Se protein was more effective than Na_2SeO_3 or selenium yeast in improving immune functions of heat-stressed broilers. The study demonstrated that chickens fed diets deficient in selenium exhibited lesions in immune organs, decreased serum interleukin-1β, interleukin-2 and serum tumour necrosis factor content, which reveals that oxidative stress inhibited the development of immune organs, hence impaired the immune function of chickens (Zhang *et al.,* 2012).

2.10.5 SeleniumToxicity in Poultry

Generally speaking, inorganic compounds are more toxic than organic ones. In the order ofdecreasing toxicity the compounds may be sorted as follows,selenite >selenate >

selenocysteine>methylatedseleniumcompounds. Selenite is the most toxic form of selenium (Hu *et al*., 2012; Mohapatra *et al*., 2014).BartikandPiskac(1974)defined three

types of intoxication with selenium: acute, sub-acute,andchronicpoisoning(alkalidisease). Acute intoxication is manifested with respiratory disorders, ataxia, diarrhoea or death. The signs include garlic odour in the breath caused by the presence of methyl selenide. The chronic form of intoxication caused by long-term supply of high selenium levels in the diet and can lead to reduced feed intake, retarded growth, hair loss, liver cirrhosis or anaemia. Chronic poisoning called selenosis, most often occurs in regions with high selenium levels in soil and drinkingwater (Suchy *et al*., 2014).

Therangeofseleniumintakesufficientandnon-toxicfortheorganismisverynarrow,dependinghowever onthechemicalformofSe.Rats fed 5mgSe/kg body weighthad retarded growthwhile6.4mgSe/kgbodyweightcausedliverchangesand 8 mg/kg of body weight caused anaemia and increasedmortality (Suchy *et al*., 2014).Thereasonforgrowthretardation isreducedsecretionofthegrowthhormone(WHO,1996).Complexpoultryfoddermixesarerecommended to include Se supplement of 0.5 mg/kg of the fodder mix (Suchy *et al*., 2014).Higher seleniumlevels in the rations can negatively affect animalhealth.Selenium doses lower than 3 - 5 mg/kg feed are usually not associated with toxicity, but

Table 2.1: Effect of Selenium Supplementation in the Diet of Poultry during Heat Stress

Spices	Duration of the study	Environmental temperature	Amount of selenium supplementation (per kg diet)	Effects on performance	Other effects	References
Broiler	1-49 d	37 °C (8h/day)	1 mg	↑ BW, FI ↓FCR	φZn concentration ↑GPx	Harsini *et al*. (2012)
Broiler	1-28 d	33 °C (24h/day for 4weeks)	0.46 ppm		↑GSH, GSSG, GPx, GPx/GR ratio	Mahmound & Edens (2003)
Broiler	22-42 d	33 ± 1°C (8h/day) 27 ± 1°C (8h/day)	0.30 mg	↑FI	↑ GPx, selenium concentration	Liao *et al* (2012)
Broiler	1-42 d	38°C (5h/day)	0.2	φFI, WG ↓FCR		Niu *et al* (2009)
Japanese quail	10-40 d	34°C (24h/day)	0.1/0.2mg	↑FI, WG ↓FCR	↑Serum Vitamin E, A, zinc ↓MDA	Sahin *et al* (2002)

BW = body weight (kg), FI = feed intake (kg), FCR = feed conversion ratio (kg gain/kg feed) MDA = malondialdehyde concentration in blood, GSSG = glutathione disulphide concentration in blood, GHS = reduced glutathione concentration in blood, GPx = glutathione peroxidase concentration in blood, GR = glutathione reductase concentration in blood, ↑ = increase, ↓ = decrease, φ = no change.

selenium is toxic to poultry when used in high doses (when dose exceeds the physiological requirement by at least 10 fold), especially inorganic compounds, which are more toxic than organic ones (Mohapatra *et al*., 2014). The molecular mechanisms of selenium toxicity can be explained by substitution of selenium for sulfur, which could result in weakened protein structure, and by reaction between selenite and glutathione with the production of free radicals (i.e. a pro-oxidant effect) (Raisbeck, 2000).

Hepatic degeneration, diffuse tubulo-nephrosis, myocardial and skeletal myodegeneration, damage to the bursa Fabricius and cerebellar edema are some signs of chronic poisoning (selenosis) which could be caused by prolonged feeding of high doses of selenium (0.5 – 1.0 mg/kg) to chickens (Surai, 2002a). Supplementing 0.3 to 0.5 mg/kg of nanoselenium seemed to be effective and advantageous in improving oxidation resistance, the maximum supplementation of nanoselenium should not exceed 1.0 mg/kg(Cai *et al*., 2012).

In poultry as well as in other animal species, selenium can be added to diets in different forms, as the form determines it mode of it action (Suzuki, 2005). Daily selenium requirement during intensive broiler growth is 0.15 mg/kg (NRC, 1994). Regardless of selenium source, the maximum amount of supplemental selenium that can be added to animal diets is limited to 0.3 mg/kg of diet in the United States (Anon 1987), while in the European Union, the maximum amount of selenium allowed in animal diets is 0.5 mg/kg of diet (Anon 2012), and in Serbia, the minimum amount of selenium allowed in animal diets is 0.15 mg/kg of diet (Anon 2010-2014).

2.11 The Role of Ascorbic Acid as an Antioxidant in Broiler Chickens

Ascorbic acid (Vitamin C) is a water soluble antioxidant that protects animals under heat stress conditions and also protect against oxidation (Lin *et al.*, 2006a). It is an antioxidant which is

normally synthesized by the chicken (Khan, 2011). It is a simple compound which was initially detected in the mammalian adrenal gland. It was given the name hexuronic acid and later it was also known as cevitaminic acid, scorbutamin and ant scorbutamin vitamin (Elkheir *et al*., 2008). Vitamin C has an important metabolic role as a result of its reducing properties and function as an electron donor. The biosynethesis of ascorbic acid in birds and mammals occurs in the liver and kidneys, however in chickens, biosynthesis mainly occur in kidneys (Elkheir *et al*., 2008), but its ability is impeded under stress conditions such as high or low atmospheric temperature (AT), relative humidty (RH), high productive rate and parasite infestation (Gursu*et al*., 2004; Abdulrashid *et al*., 2010).

Sugars such as glucose, fructose and mannose serve as precursors for the synthesis of ascorbic acid. Ascorbic acid can donate two electrons and in the process is converted to dehydro-L-ascorbic acid. Ascorbic acid is not an essential nutrient for avian species because they possess enzyme gulonolactone oxidase which is required for the biosynthesis of this vitamin but is lacking in humans and some other species (Lin *et al.*, 2006b; Khan, 2011). No recommended requirement for ascorbic acid has been established by NRC for birds (NRC, 1994). However it has been recommended in poultry feeds as a supplement to alleviate stress, on the assumption that during stress the requirement may exceed the synthesizing ability (Gouis and Morris, 2005; Abdulrashid *et al*., 2010). Physiological stressors like heat, disease may increase the chicken's requirement for ascorbic acid (Nockels, 1984). Moreover, high ambient temperature impairs the absorption of ascorbic acid and increase the dietary requirement of this vitamin (Klasing, 1998).

Supplementation with ascorbic acid, both singly and in combination with vitamin E, is beneficial to heat-stressed layer hens (Ajakaiye *et al*., 2011). Ascorbic acid may be supplemented at 40 mg/bird/day in drinking water to reduce significantly the impact of heat stress and improve the

productivity of broiler chickens (Vathana *et al.*, 2002), due to its ability to improve the breast meat of broilers under heat stress (Abioja *et al.*, 2010). During heat stress, endogenous ascorbic acid, produced by the kidneys of birds is not sufficient to mitigate the negative effects of thestress. The adverse effects, resulting from heat stress, include reduction in immunity, feed intake, weight gain, egg production, number of chicks per hen, hatchability of fertile eggs, and carcass quality (Abidin and Khatoon, 2013). It may also cause mineral imbalance, increase in panting and mortality, hence, necessitating the administration of supplemental ascorbic acid (Abidin and Khatoon, 2013). It has been shown that the body temperature, which is the net effect of heat production and heat loss, is reduced in chickens administered ascorbic acid during exposure to high environmental temperature (Mckee and Harrison,2013).

The physiological and biochemical potentials of ascorbic acid is due to its ability to donate one or two electrons, making it a potent reducing agent and an antioxidant (Du *et al*., 2012). Its supplementation alleviates the negative effects of oxidative stress (Sujatha *et al.*, 2010). Ascorbic acid has been demonstrated to decrease lipid peroxidation, improve protein concentration and iron status of broilers (Wang *et al*.,2011). Its supplementation in broiler chickens enhances the metabolic response to heat stress (Imik *et al*., 2012); improves performance and antioxidant status during heat stress (Sahin and Kucuk, 2003). Glutathione activity is important for the maintenance of ascorbic acid metabolism by regulating the expression of ascorbic acid transporter and function (Mardones *et al*., 2012).

Chickens require ascorbic acid for amino acid and mineral metabolism, as well as for the synthesis of hormones like testosterone (Mcdowell, 1989). Ascorbic acid is involved in the production of white blood cells and thus enhances the immune status of birds. High environmental temperatures may increase the requirement of ascorbic acid, decrease the

biosynthesis of this vitamin and affect the endocrine system responsible for the retention and proper metabolic functioning of this vitamin (Sobayo, 2005; Abdulrashid *et al*., 2010). Broilers under heat stress utilized more feed when ascorbic acid fortified feed was coloured (Kutlu and Forbes, 2000).

2.11.1 Ascorbic Acid Storage Organs

The renal threshold and other tissues largely determines the level of ascorbic acid in blood plasma (Abdurahid *et al*., 2010). Hediger (2002) reported that the biological tissues which accumulate over 100 times the level of ascorbic acid in blood plasma are the adrenal glands, thymus, pituitary, retina and corpus luteum. Tissues with 10 to 50 times the concentration present in blood plasma include the brain, lung, spleen, testicles, liver, lymph nodes, thyroid, pancreas, small intestinal mucosa, leukocytes, kidney and salivary glands.

2.11.2 Performance of Poultry Fed Ascorbic Acid

Although chickens are known to synthesize ascorbic acid, increased supplementation has proved to have beneficial effects in broilers reared under heat stress (Mahmoud *et al*., 2004). Ascorbic acid is actively transported into tissues and their requirement increases under heat stress. Under high environmental conditions, the bird's synthesizing capacity may become inefficient thereby reducing plasma ascorbic acid concentrations (Abdurashid *et al*., 2010).

Ascorbic acid has a pro-oxidant and anti-oxidant property (Ahmadu *et al.*, 2016). Ascorbic acid plays an important role in the biosynthesis of corticosterone during stress and improves performance of birds by lowering the plasma corticosterone level and adrenocorticotropic hormone (Sahin*et al*., 2003a; Mahmoud *et al.*, 2004, Lin *et al.*, 2006b). Kutlu(2001) have shown the beneficial effect of ascorbic acidsupplementation on urea, glucose, triglycerides, albumin, cholesterol, total protein and alkaline phosphatase. According to Kumar *et al*. (2017) ascorbic

acid supplementation (200 mg/kg feed) caused significant increases in plasma ascorbic acid levels in broilers under heat stress.Adenkola*et al*. (2016) fed 500 mg/kg ascorbic acid to broiler chickens and reported increasedvalues of PCV, haemoglobin and total erythrocyte count as compared to the control group. On the contrary, Muhammad *etal*. (2016) reported that supplementing the diets of broilers with 0.07-0.30 g of ascorbic acid per kg did not affect haematological parameters. Similarly, Alaeldein *et al.* (2018) demonstrated that addition of natural vitamin C to the drinking water of broilers during heat stress with either 100 or 200 mg did not affect the Hb nor erythrocyte.

Production parameters of broilers improved under various high temperature (35^0C and 75-80% RH) conditions with Vitamin C supplementation (250 mg/kg diet) which can be explained by its function of scavenging free radicals (Attia *et al*., 2011). Ascorbic acid supplementation is also efficient in increasing egg production, egg weight and egg mass in layers (Asli *et al*., 2007; Ajakaiye *et al*., 2011). Similarly, under heat stress condition, ascorbic acid supplementation at 300 mg/kg in poultry feeds had positive effect on weight gain, egg production, egg shell strength, fertility, hatchability and improved immune response of poultry birds (Puthpongsiriporn *et al.*, 2001; Lin *et al.,* 2003). Kutlu and Forbes (1993) observed reduction in panting rate, rectal and skin temperatures in broilers and layers given ascorbic acid (250 mg/kg diet). There was a significant increase in growth rate and immunity of broiler chickens fed 200 mg/kg dietary Vitamin C (Youssef *et al*., 2017) while Tuleun *et al*. (2010) reported increased weight gain, feed conversion ratio, reduced feed cost with supplementation of dietary ascorbic acid at 250 mg/kg. This suggests that ascorbic acid may decrease the heat load of birds, increase heat loss or improve the birds' tolerance to high atmospheric temperature (Tuleun *et al.*, 2010).

Peebles and Brake (1985) reported that supplemental ascorbic acid holds promise for increased production during high atmospheric temperature or for nutritionally marginal diets. Sahin and Kucuk (2001) reported that supplemental ascorbic acid at 250 mg/kg, improved performance and carcass quality in broiler birds reared under heat stress conditions (32 ^{0}C). Attia *et al*. (2009) reported that 250 mg/kg ascorbic acid relieved the birds from the negative effects of heat stress and increased feed intake, protein digestibility, dressing percentage and feed conversion ratio (FCR). Sahin and Kucuk (2001) recorded better body weight and feed intake in heat stressed broiler chickens in response to 200 mg/kg of ascorbic acid.

Dietary supplementation of ascorbic acid (200 mg/kg) improved antioxidant status in White Leghorn layer during summer stress (Panda *et al*., 2007). Sahin *et al*. (2003a) supplemented the diet of heat stressed laying Japanese quails with 250 mg/kg dietary of ascorbic acid and observed a decrease in concentration of Malondialdehyde (MDA) in the serum.Jena *etal*. (2013) reported that dietary supplementation of broiler feed at 400 mg/kg ascorbic acid for a period of 8 weeks significantly lowered Malondialdehyde (MDA) leveland increased activities of Superoxide dismutase (SOD), Catalase (CAT) enzymes and Ferric Reducing Antioxidant Power (FRAP). Mckee and Harrison (1995) observed that ascorbic acid at 150 ppm, enhanced performance of broiler chicks exposed to multiple concurrent environmental stressors. In addition, ascorbic acid at 40 - 200 ppm improved growth parameters of broiler chicks exposed to multiple environmental stressors (Vathana *et al*., 2002; Attia *et al*., 2009; 2011). Sahin *et al.* (2002) reported that dietary supplementation of ascorbic acid at 250 mg/kg in birds reduced the synthesis of corticosteroid hormone and adrenocorticotrophic hormone (ACTH), and improved performance.

However, Miraei-Ashtiani *et al.* (2004) and Abioja *et al*. (2012) reported that ascorbic acid inclusion (0.5 g/L) in broiler drinking water did not result in any difference in cloacal temperature compared to chickens receiving diet without ascorbic acid supplementation. Ascorbic acid supplementation in poultry feeds between 100 to 400 mg/kg has been reported to have positive effect such as weight gain, improved immune response, resistance to some infectious and contagious diseases, reduction in rectal and skin temperatures and reduction in panting rate (Puthpongsiriporn *et al.,* 2001; Lin *et al.,* 2003a). Literature data on the effect on the performance of poultry with different ascorbic acid supplementation is presented in Table 2.2.

2.11.3 Effect of Ascorbic Acid on Immune System of Chickens

A high concentration of ascorbic acid is found in immune cells and is consumed readily during infections. However, it is not certain how ascorbic acid interacts with the immune system; but it has been hypothesised to modulate the activities of phagocytes, the production of lymphocytes and cytokines, and the number of cell adhesion molecules in monocytes (Preedy *et al.,* 2010).

2.11.4 Ascorbic Acid Absorption, Transport and Excretion

Vitamin C is absorbed in the body by both simple diffusion and active transport. Sodium-Dependent Active Transport Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs) are the two transport systems required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbic acidacross the plasma membrane (Savini *et al.,* 2008). Sotiriou *etal.* (2002) reported GLUT1 and GLUT3 as the two glucose transporters and transfer only the dehydroascorbic acid form of ascorbic acid. Although dehydroascorbic acid is absorbed in higher rates than ascorbic acid, the amount of dehydroascorbic acid found in plasma and

tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to ascorbic acid (May *et al.,* 2003). Thus, SVCTs appear to be the predominant system for ascorbic acid transport in the body.

SVCT2 is involved in ascorbic acid transport in almost every tissue (May *et al.,* 2007), the notable exception being red blood cells, which lose SVCT proteins during maturation (Savini *et al.,* 2008).

Table 2.2: Effects of Ascorbic Acid supplementation under heat stress based on different studies.

Spices	Duration of the study	Environmental temperature	Recommended Amount of Vitamin C supplementation (per kg diet)	Effects on performance	Other effects	References
Layers	39 week	35.9°C (24h/day)	150 mg	↑ egg weight		Ajakaiye *et al*. (2011)
Broiler	21-84 d	38°C (4h/day)	250 mg	↑FI, protein digestion ↓FCR		Attia *et al.* (2011)
Broiler	10-32 d	35°C (24h for first week) 32.5°C (24h for second week)	400 mg (20 g/5kg)	↑ BW, FI		Farooqi*et al.* (2005)
Broiler	1-28 d	36°C (6-10 h/day)	250 mg	↑BW, FI ↓FCR		Kutlu& Forbes (1993)
Broiler	1-45 d	37 ± 5°C	200 mg		↑plasma ascorbic acid concentration	Kumar *et al.* (2017)
Japanese quail	10-40 d	34°C (24h)	200 mg	↑BW, FI ↓FCR		Sahin *et al.* (2003a)

BW = body weight (kg), FI = feed intake, FCR = feed conversion ratio (kg gain/kg feed) MDA = malondialdehyde concentration in blood, ascorbic acid = ascorbic acid, ↑ = increase, ↓ = decrease, φ = no change.

With regular intake, the absorption rate of ascorbic acid varies between 70 to 95%, however, the degree of absorption decreases as intake increases.

2.11.5 Ascorbic Acid Overdose and its Adverse Effects

Vitamin C is water soluble, with dietary excesses not absorbed. An excess of ascorbic acid in the blood is rapidly excreted and thereby, exhibits remarkably low toxicity. The LD50 (the dose that will kill 50% of a population) in rats is accepted to be 11.9 g/ kg body weight when given by forced gavages (orally). The mechanism of death from such doses is unknown but may be more mechanical than chemical and relatively large doses (200 g/kg) of ascorbic acid may cause indigestion, particularly when taken on an empty gizzard(Safety (MSDS) data for ascorbic acid, 2005).

However, taking ascorbic acid in the form of calcium ascorbate and sodium ascorbate may lower this effect (Ahmadu *et al*., 2016). Large doses of ascorbic acid (2000 g/kg) causes diarrhea, nausea and fatigue in healthy subjects and enhances iron absorption (Fleming *et al.*, 2002), but might cause iron poisoning in subjects with poor utilization of iron, a genetic condition known as hemochromatosis and can result in decreased levels of enzyme glucose-6-phosphate dehydrogenase (G6PD) which causes patients to develop heamolytic anaemia (Cook and Reddy, 2001).

Vitamin C, at concentrations above the renal re-absorption threshold, is passed freely and excreted. At high dietary doses, ascorbic acid is accumulated in the body until the plasma levels reach the renal re-absorption threshold. Traxer *et al.* (2003) and Massey *et al.* (2005) reported that a high intake could increase the risk of oxalate (an ascorbic acid metabolite) kidney stones. Some studies have reported that supplemental ascorbic acid increases urinary oxalate levels

(Auer *et al.,* 1998). Choi *et al.* (2009) found that total daily ascorbic acid intake was inversely associated with risk of gout, with higher intakes being associated with greater risk reductions. High doses of ascorbic acid have also been found to interfere with the interpretation of certain laboratory examination such as serum creatinine, serum bilirubin, and the guaiac assay for occult blood (Ahmadu*et al*., 2016).

2.11.6 Baobab (*Adansonia digitata L.*)

In an attempt to counteract the detrimental effects of oxidative damages to the biological system caused by Reactive Oxygen Species (ROS), researchers have supplemented human diets with antioxidants, especially those derived from natural sources. The ethnobotanical researches have highlighted the antioxidant capacity of natural antioxidant phyto extracts, with particular attention on *Adansonia digitata L*. (Yazzid *et al.,* 1994; and Phyto Trade Africa, 2006).

The Baobab plant, known as African tree is said to be a valuable source of energy, protein and a non-conventional feedstuff. It is grown in the arid and semi-arid regions of the world. Baobab trees are tolerant to high ambient temperatures and prolong drought. The leaves, bark and fruits of baobab tree are used in several African regions as foodstuff and for medicinal purposes. For this reason, Baobab is named “the small pharmacy” or Chemist tree” (Kerharo and Adam 1974; Etkin and Ross, 1982). Similarly, Yazzid *et al*.(1994) reported that Baobab leaves are used for making soup. Obizoba and Amaechi (1983) stated that the pulp is used as a beverage and in food preparation. In Africa, Baobab fruit is used as famine food to prepare decoctions, sauces and natural refreshing drink due to its nutritional properties (Obizoba and Anyika, 1994; Lockett *etal.*, 2000).

The baobab fruit pulp is embedded in a hard resistant external shell or capsule called epicarp, the internal ripe fruit endocarp is floury, dry and powdery with thread-like fibrous materials that enclose seeds and filaments (Nour *et al*., 1980). The fruit pulp is powdery, whitish coloured and dries naturally with slight sour taste. The pulp can be separated from the shell and seed using a knife or pounded slightly in a mortar; its processing does not require chemical treatment (Magdi, 2004). Furthermore, Addy and Eteshola (1984) reported that fermented seeds are used to flavour soup, while the roasted seeds are used in a dish to substitute pea nut. The pulp is therapeutically employed as febrifuge, analgesic, anti-diarrhoea or anti-dysentary and for the treatment of small pox and measles (Kerharo and Adam, 1974). The Baobab tree and fresh pod (*Andansonia digitata L.*) is presented in Figure 2.2 while the cross- section of Baobab pod is presented in Figure 2.3.The documented chemical compositions indicated that baobab pulp is a good source of energy, minerals, vitamins and good quality protein (Magdi, 2004; Phyto Trade Africa 2006; Chadare *et al.,* 2009).

2.11.7 Chemical Composition of Baobab Fruit Pulp

2.11.7.1 Crude Protein Contents of Baobab Fruit Pulp

Berker (1983) reported that baobab pulp from Senegal contains 2.7% crude protein, in Malawi, 3.1% crude protein was recorded by Saka (1995). Similarly, Phyto Trade Africa (2006) gave a range of 2.03 to 3.24% crude protein for baobab pulp obtained from different locations. Magdi (2004) also indicated 3.2% crude protein for baobab fruit pulp while Adeosun (2012) reported crude protein content of 3.31% for baobab fruit pulp.

2.11.7.2 Carbohydrate Content of Baobab Fruit Pulp

The major component of baobab fruit pulp is the carbohydrate, an energy source. Berker (1983) indicated 73.3% carbohydrate, while Saka (1995) reported 79.4%. A range of total carbohydrate as obtained by Phyto Trade Africa (2006) showed 78.3 to 78.9% of which the available

Fig. 2.2: Baobab tree and pod

Fig. 2.3: Cross section of baobab fruit pulp

carbohydrate ranges between 25 to 33% while Adeosun (2012) reported 79.20% of carbohydrate in baobab fruit pulp. The differences recorded in the carbohydrate content were attributed to regions and maturity of the plants.

2.11.7.3 Crude Fibre Content of Baobab Fruit Pulp

Crude fibre values of 9.9, 8.3, 5.4% and 9.27% was reported by Berker (1983); Saka *et al.* (1994); Magdi (2004) and Adeosun (2012) respectively. On the other hand, Phyto Trade Africa (2006) gave a range of total dietary fibre content of between 45 to 54%. This could be the reason why some authors further reported that out of about 79% carbohydrate content of baobab pulp, only about 33% is available (Adeosun, 2012).

2.11.7.4 Fatty Acid Content of Baobab Fruit Pulp

Berker (1983) reported 0.2% fat, Saka *et al.,* (1994) reported 4.30% while Glew *et al.,* (1997) reported 2.3% and Adeosun (2012) reported an ether extract content of 2.12% in Baobab fruit pulp. On the other hand, Magdi (2004) reported a value as low as 0.31% fat. The Phyto Trade Africa (2006) gave a range of 13.3 to 20.4% fat content.

2.11.7.5 Ash Content of Baobab Fruit Pulp

Phyto Trade Africa (2006) indicated a value of 5.5g/100g. Magdi (2004) reported 4.5g/100g while Adeosun (2012) reported an ash content of 6.29%.

2.11.8 Ascorbic Acid Content of Baobab Fruit Pulp

Baobab fruit pulp is known to contain a high amount of ascorbic acid which makes it a good source of antioxidants. The ascorbic acid content of baobab fruit pulp was reported to be between 150 to 499 mg/100g (Manfredini *et al.,* (2002). Agbessi (1987) reported 169 mg/100g ascorbic acid in baobab pulp, while Eromosele *et al.,* (1991) reported 337 mg/100g Ascorbic acid. The Phyto Trade Africa (2006) indicated 300 mg/100g pulp while Adeosun (2012) reported an ascorbic acid content of 299.85 mg/100g.

2.11.9 Anti-Nutritional Factors in Plants

Among the major factors limiting the use of many tropical plants as feedstuffs or feed supplement in livestock production is the presence of diverse range of compounds capable of precipitating deleterious effects in man and animals although the presence of this compounds in little quantity is tolerable (Table 2.3).

Compounds which reduce feed intake and nutrient utilization are referred to as Anti-Nutritional Factors (ANF) or food toxicants (Umoh, 1998). The significance of the toxicity ranges from reduction in feed consumption and nutrient utilization to profound neurological effects or even death. Some of these anti-nutritional factors include; phytates, tannins, hydrogen cyanide, oxalates, saponins and trypsin inhibitors. Adeosun (2012) reported the presence of anti-nutrient factors in baobab fruit pulp, tannin, phytic acid, oxalate, saponin and trypsin inhibitors at 0.26, 0.12, 0.48, 0.048 and 1.06 mg/100g respectively.

Table 2.3: Permissible levels of Anti-nutrients in animal

Anti-nutritional factors	Permissible level (mg/kg body weight)
Phytate	6.4
Oxalate	10-20
Tannin	15
Saponin	0.54
Hydrogen cyanide	5-50

Source: Ogwu, 2010

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Experimental Site

The experiment was conducted at a private facility within Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria. Zaria is located in the Northern Guinea Savannah zone of Nigeria on Latitude 11^0 09' 01.78" N and Longitude 7^0 39' 14.79" E, at an altitude of 671 m above sea level (IARMS, 2019). The area has three distinct seasons; namely the hot dry season from March to May, the warm rainy season from June to September, and a cool dry season from November to February with a mean annual rainfall of about 700-1400 mm. The area has an average relative humidity of 36.0% during the dry season and 78.5% for the wet season and an ambient temperature ranging from 26-32^0 C (IARMS, 2019).

3.2 Experiment 1: The Effect ofDietary Selenium in Ameliorating Heat Stress in Broiler Chickens

3.2.1Source of inorganic selenium

The inorganic selenium that was used during this experiment was bought from a retail outlet at Lagos state, Nigeria. It was weighed into 0.1, 0.2 and 0.3 mg/kg selenium using a sensitive digital scale at the Department of micro-biology, Ahmadu Bello University, Zaria.

3.2.2Experimental Design, Diets and Management of Birds

Two hundred and fifty six, day old (*Cobb 500*)broiler chicks were randomly allotted to four experimental treatments (0, 0.1, 0.2 and 0.3 mgSe/kg) each having 64 birds, with four replicates each in a Completely Randomized Design. A maize/soybean meal based broiler starter and finisher diets (Tables 3.1 and 3.2)was formulated according to NRC (1994) nutrient requirement. Feed and water were provided *ad libitum*. All standard routine management practices were strictly adhered to.

Initial weight was taken using a measuring scale at the beginning of the experiment, while feed intake and weight gain were taken weekly using a digital weighing scale. Measured volume of water was offered daily to the birds, leftover was also measured at the end of each day, measured volume of water was poured into an open container and placed at intervals along the passage within the building and evaporation records was accounted for daily by measuring the quantity of water left in the container and subtracted from the initial quantity measured using a calibrated measuring cylinder. The leftover and evaporation losses was then subtracted from the quantity of water offered to the birds to determine the amount of water consumed daily. Mortality were recorded as they occurred.

3.2.3 Thermoregulatory Measurements

Indoor temperature and relative humidity readings were recorded twice daily (8.00 am and 1.00 pm) using an electronic digital thermo-hygrometer throughout the experimental period and were used to calculate the morning and afternoon THI. Using the same chickens (2 birds/rep)rectal temperaturewas measured by placing a digital thermometer in the rectum at 1cm depth, body temperaturewas measured by placing the digital thermometer below the feathers under the wing web while respiratory ratewas measured by counting of respiration (breath/minute) with the aid of a stopwatch. Heart ratewas measured by placing a stethoscope at the breast region and count taken with the aid of a stop watch (beat/minute). Temperature-humidity index (THI) was calculated using the standard formula by Tao and Xin (2003) for poultry.

$$THI = 0.85\ Tdb + 0.15\ Twb$$

Where,

THI = temperature-humidity index in ^0C

Tdb = dry-bulb or ambient temperature in ^0C

Twb = wet-bulb temperature in ^0C

Table 3.1: Ingredient Composition of Selenium Supplemented Diets Fed to BroilerStarter Chickens (day 1-28)

	Dietary levels of Selenium (mg/kg diet)			
Ingredients (%)	**0**	**0.1**	**0.2**	**0.3**
Maize	57.00	57.00	57.00	57.00
Soyabean cake	25.00	25.00	25.00	25.00
Groundnut cake	13.50	13.50	13.50	13.50
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (KCal/kg)	2953.00	2953.00	2953.00	2953.00
Crude protein (%)	23.04	23.04	23.04	23.04
Ether extract (%)	4.77	4.77	4.77	4.77
Crude fibre (%)	3.76	3.76	3.76	3.76
Calcium (%)	1.34	1.34	1.34	1.34
Available phosphorus (%)	0.58	0.58	0.58	0.58
Lysine (%)	1.26	1.26	1.26	1.26
Methionine (%)	0.54	0.54	0.54	0.54
Selenium (%)	0.72	0.82	0.92	1.02
Cost (₦/kg)	119.47	119.72	119.97	120.22

Vitamin-mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2,000,000 IU; Vit. E, 20,000 UI; Vit. K, 2,250mg; Vit. B1, 1,750mg; Vit. B_2, 5,000mg; Vit. B_6, 2,750mg; Vit. B_{12}, 15mg; Niacin, 27,500mg; Panth. Acid, 7,500mg; Folic acid, 7,500mg; Biotin, 50mg; Choline Chloride, 400g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg

Table 3.2: Ingredient Composition of Selenium Supplemented Diets Fed to Broiler Finisher Chickens (day 29-49)

	Dietary levels of Selenium (mg/kg diet)			
Ingredients (%)	**0**	**0.1**	**0.2**	**0.3**
Maize	59.00	59.00	59.00	59.00
Soyabean cake	20.00	20.00	20.00	20.00
Groundnut cake	9.50	9.50	9.50	9.50
Maize offal	7.00	7.00	7.00	7.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (KCal/kg)	2994.00	2994.00	2994.00	2994.00
Crude protein (%)	20.05	20.05	20.05	20.05
Ether extract (%)	4.52	4.52	4.52	4.52
Crude fibre (%)	3.86	3.86	3.86	3.86
Calcium (%)	1.32	1.32	1.32	1.32
Available phosphorus (%)	0.56	0.56	0.56	0.56
Lysine (%)	1.09	1.09	1.09	1.09
Methionine (%)	0.50	0.50	0.50	0.50
Selenium (%)	0.71	0.81	0.91	1.01
Cost (₦/kg)	112.07	112.32	112.57	112.82

Vitamin-mineral premix provide per kg of diet: Vit. A, 8,000,000 IU; Vit. D_3, 1,600,000 IU; Vit. E, 5,000 UI; Vit. K, 2000mg; Vit. B1, 1,500mg; Vit. B_2, 4,000mg; Vit. B_6, 1,500mg; Vit. B_{12}, 10mg; Niacin, 15,000mg; Panth. Acid, 5,000mg; Folic acid, 500mg; Biotin, 20mg; Choline Chloride, 200g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg

Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression function by Stull (2011). Heat stress was classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

3.2.4 Haematological and Serum Analyses

Brachial vein blood samples (2 ml) was collected from two birds per replicate on day 28 and 49 for haematological and serum assay,1 ml was collected into tubes containing EDTA (Ethylene di-ammine tetra-acetic acid) anticoagulant to analyze for haematological parameters and 1 ml was collected in tubes containing no EDTA for serum assay. Concentration of Calcium and Phosphorus in the blood was also analyzed at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Haematological parameters such as Packed cell volume (PCV) was determined by the microhaematocrit method, haemoglobin concentration (Hb) was determined photometrically at the wavelength of 540 nm, the erythrocyte (RBC) and leucocyte (WBC) counts were determined using the improved Neubauer haemocytometer and Differential counts were determined by the thin slide method (Lamb, 1991). Liver function and lipid profile test was determine according to the procedure of (Lamb, 1991).

3.2.4.1 Determination of Bio-markers

Malondialdehyde, catalase, superoxide dismutase and glutathione peroxidase were determined at the Physiology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.2.4.1.1 Malondialdehyde (MDA) concentration

This was measured according to the method described by Atawodi, (2011), 2 cm^3 of 15% trichloroacetic acid (TCA) was measured into a test tube, 2 cm^3 of thiobabitutric acid (TBA) and 100 µl of serum were added. The mixture was incubated at 80^oC for 30 minutes in a water bath, allowed to cool for 30 minutes andcentrifuged at 3000 rpm for 10 minutes. The clear supernatant was collected and the absorbance was measured at 535 nm using a spectrophotometer.

Thiobarbituric acid reactive substances (TBARS) concentration was expressed in nmol/mg protein calculated as follows:

$$\text{Concentration (nmol/mg) protein} = \frac{\text{Absorbance of sample}}{\text{1.5x10-5 x protein concentration (mg/ml)}}$$

Protein concentration (mg/ml) was done as described by Atawodi, (2011), 5 cm^3 of serum was mixed with 4 cm^3 of biuret reagent and incubated at room temperature for 30 minutes, and the absorbance was measured at 540 nm. The protein concentration was determined from the calibration curve, which is a plot of the absorbance of egg albumin (standard) against reaction mixture.

3.2.4.1.2 Catalase (CAT) activity

Catalase activity was measured using the method of Abebi, (1974). Micro-pipette was used to aspirate 10 µl of serum and was added to a test tube containing 2.80 cm^3 of potassium phosphate buffer (50 mM, pH 7.0). The reaction was initiated by adding 0.1 cm^3 of freshly prepared 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 minutes using a spectrophotometer. A molar extinction coefficient (Ɛ) of 0.041 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity.

$$\text{Catalase Concentration} = \frac{\text{Absorbance of sample}}{\varepsilon}$$

$$\text{Catalase activity} = \frac{\text{Catalase concentration}}{\text{Protein concentration (mg/ml)}}$$

3.2.4.1.3 Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by the method described by Fridovich, (1989). The principle of the method is the ability of superoxide dismutase to inhibit auto oxidation of adrenaline.Carbonate buffer (pH 10.2) (0.05 M) and Adrenaline (0.3 mM) solution were prepared. Micro-pipette was used to aspirate 0.2 cm^3 of serumand was added to 2.5 cm^3 of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 cm^3 of 0.3 mM adrenaline. The reference mixture contained 2.5 cm^3 of 0.05 M carbonate buffer, 0.3 cm^3 of 0.3 mM adrenaline and 0.2 cm^3 of distilled water. The absorbance was measured at 480 nm at 30 seconds as initial and 180 seconds as final absorbance respectively.

$$\text{Increase in absorbance per minute} = \frac{A2 - A1}{2.5}$$

Where A1 = Initial absorbance
A2 = Final absorbance

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{(increase in absorbance for sample)}}{\text{increase in absorbance of blank}} \times 100\right]$$

SOD activity of 1 unit is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute.

3.2.4.1.4 Glutathione (GSHPx) concentration

This measurement was carried out as described by Rajagopalan *et al.* (2004). Micro-pipette was used to aspirate 150 µl of serum and was added to 10 % TCA and centrifuged at 1500 rpm for 5 minutes and 1 cm^3 of the supernatant was treated with 0.5 cm^3 of Ellman's reagent and 3 cm^3 of

phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm. The quantity of glutathione concentration was deduced as:

$$\text{GSHPx Concentration} = \frac{\text{Absorbance}}{\varepsilon}$$

Where Ɛ = Molar extinction coefficient

3.2.4.2 Hormonal Assay

Thyroxine: It was determined according to the procedure of Chopra *et al*. (1971). Micro pipette was used to collect 25µl of the control, serum reference and sample serum into the microwells and 100µl of T4 enzyme reagent was then added to the wells. The plates was swirl gently for 20-30 seconds for proper mixing and was then covered and incubated for 60 minutes. The content of the microwells was discarded by decanting using absorbent paper. 350µl of wash buffer was added to the wells and decanted, the washing was done three times. Micro-pipette was used to aspirate 100µl of working substrate solution was then added to the wells and was incubated at room temperature for 15 minutes. Micro-pipette was used to aspirate 50µl of stop solution,it was added to the wells and gently mix for 15-20 seconds,within 30 minutes of adding the stop solution, the plate was subjected to a microplate reader to read the absorbence of each well at a wavelength of 450nm.

Cortisol: this was determined according to the procedure of (Foster and Dunn 1974). Micro pipette was used to collect 25µl of the control, serum reference and sample serum into the microwells and 50µl of cortisol enzyme reagent was then added to the wells. The plates were swirled gently for 20-30 seconds for proper mixing and 50µl of cortisol biotin was also added to all the wells. The microplate was again swirled for 20-30seconds to mix properly and the plate was then covered and incubated for 60 minutes at room temperature. The content of the

microwells was discarded by decanting method using an absorbent paper to dry. Wash buffer (350µl) was added to the wells and decanted, the washing was done three times. Working substrate solution (100µl) was then added to the wells and incubated at room temperature for 15 minutes. Stop solution (50µl) was added to the wells and gently mixed for 15-20 seconds.After incubating for 30 minutes at room temperature, the plate was subjected to a microplate reader to read the absorbence of each well at a wavelength of 450nm.

3.2.5 Organ Collection and Examination

On day 50 of the experiment, two birds per replicate with an average weight of the group were selected and fasted overnight but with provision of water. The birds were slaughtered, dressed and eviscerated. Live, dressed, eviscerated weights, as well as weight of cut parts and organs including liver, kidneys, spleen, thymus and bursa of Fabricius were measured using a sensitive digital scale (Satorius ENTRIS). Relative weight of each organ was expressed as percentage of live weight. The carcass analysis was carried out at the Meat Product Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria.

3.2.6 Histomorphometric Analysis

Jejunum measuring 1 cm length was harvested and fixed in 10% buffered formalin solution for 24 hours after which ten villi of each jejunum were selected according to its integrity (well inserted in the submucosa base, presenting neither discontinuity nor folds, but with simple columnar epithelium at the tip). Villus height, width, crypt depth, absorption area as well as villus parameter were measured. Villus height/crypt depth was determined by dividing villus height by crypt depth. Pictures of the jejunum were taken with an OlympusC-7070[3]camera, coupled to trinocular OlympusCX40[3]microscopy, with 40x (length of villus), 200x (depth of crypt, thickness of wall) and 400x (width of villus) of magnification. The equipment was

calibrated before each use and the MoticImagens Advanced (Motic) was used for measurements (Bancroft and Stevens, 2008; Gava *et al*., 2015). Histomorphometrics of the small intestine was carried out at the anatomy Laboratory of the Faculty of Medicine, Ahmadu Bello University, Zaria.

3.2.7 Determination of Tibia Geometric Properties

During carcass analysis at day 50, the left tibia of two birds per replicate were removed. Tibia was labeled and immersed in boiling water (100 °C) for 15 minutes according to the procedure described by Applegate and Lilburn, (2002) to completely remove the tissue. Length of each bone was measured using a meter rule. The tibia length measurement was the distance from proximal to distal extremities of each tibia. The bone weights were obtained using a digital precision weighing balance (Satorius ENTRIS). The bone weight/length index was computed by dividing the tibia weight by its length (Seedor *et al*., 1991), while the Robusticity index was determined using the formula described by Reisenfeld, (1972).

$$\text{Robusticity Index} = \frac{\text{Bone length}}{\text{Cube root of bone weight}}$$

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to the procedure described by AOAC (1990). The percentage ash was then determined relative to dry weight of the tibia. Calcium and Phosphorus in bone and faeces were also analysed after digestion of the bone and faeces and read in a spectrophotometer (AOAC, 1990).

3.2.8 Statistical Analysis

All data obtained from this experiment were statistically analyzed using General Linear Model Procedure of Statistical Analysis System software package while significant difference among

means were compared using the Tukey Procedure (SAS, 2002). The model used for this experiment is as follows:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where:

Y_{ij} = Observation as influenced by i^{th} levels of Selenium

μ= Overall mean

S_i= the effect of i^{th} level of Selenium

e_{ij}= Randon error (which is assumed to be independently identical with normal distribution with zero mean and standard deviation).

3.3Experiment 2: The Effect of Organic Ascorbic Acid in Ameliorating Heat Stress in Broiler Chickens

3.3.1 Source of Organic Ascorbic Acid

Baobab fruit pulp (*Adansonai digitata*) was purchased from Giwa market in Zaria and used as source of organic Ascorbic acid.The pulp was separated from the seeds by mild pounding in a mortar to prepare the baobab fruit pulp meal (BFPM) and supplemented in the diet of the broiler chickens at 2, 4 and 6 kg.

3.2.2 Chemical Analysis of Baobab Fruit Pulp

Chemical analysis of the dry baobab fruit pulp was carried out according to AOAC (1990) methods to determine the proximate composition (dry matter, crude protein, crude fibre, ether extract and ash contents). The ascorbic acid (vitamin C) content was also determined in the Biochemistry Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria.

3.2.2.1 Determination of Anti-Nutritional Factors in Baobab Fruit Pulp

3.2.2.1.1 Determination of Tannin Content of Baobab Fruit Pulp

The method used was based on the reaction of Folin-Denis reagent with filtered sample solution and orthophosphoric acid that gives a colour which can be determined colorimetrically, using the procedure of Price *et al.* (1978).Baobab fruit pulp (1 g) was weighed into a 100ml conical flask then 50cm3 of distilled water was added and allowed to boil gently for 1 hour using a hot plate. The warm solution was filtered into a 50cm^3 volumetric flask and made to mark when cooled. Tannic acid (0.3 cm^3) and 3cm^3 of aliquot of the sample was pipetted into a 50cm^3 volumetric flask. Water was added to both standard and sample until half-full. Folin-Denis reagent (2.5 cm^3) was added to each flask and 10ml of 17% (W/v) sodium carbonate solution was also added and diluted to 50cm^3 mark mixed and allowed to stand in water bath at 25^{0}C for 5 minutes. The absorbance was read at 760nm wave length using distilled water as blank, the calibration curve, was then used to determine the concentration of tannins

3.2.2.1.2 Determination of Oxalate Content of Baobab Fruit Pulp

Baobab fruit pulp weighing 1 g was weighed into a 250ml volumetric flask and digested with 190ml distilled water and 10ml of 6N Hydrochloric acid. It was allowed to stand in a water bath at 90^{0}C for 5 hours. The mixture was centrifuged and filtered into a 250ml volumetric flask and the filtrate made up to mark with distilled water, 50ml aliquot of the extract was mixed with 25ml 6N HCL and the mixture was placed on a hot plate to evaporate to about 25ml. A brown precipitate formed and was filtered off and washed with hot distilled water. The mixed solution was titrated with concentrated ammonia until pink-yellow colour was observed. The solution was then heated on a hot plate to about 90^{0}C. The oxalate was precipitated with 10% (W/v) calcium chloride solution. After keeping the mixture over night, it was filtered and precipitated and

washed with distilled water until calcium free as tested with bench sodium hydroxide. The precipitate was then washed with 25% (V/V) sulphuric acid solution and diluted with 125ml distilled water. It was warmed to 90^0C and titrated with 0.05N potassium permanganate solution. Oxalate was then determined according to the procedure of Oke (1966).

3.2.2.1.3 Determination of Phytate Content of Baobab Fruit Pulp

Five grams of powdered baobab fruit pulp meal was weighed out and soaked in 1000 ml of 2% hydrochloric acid, it was allowed to stand for four hours and filtered, 25mls of the filtrate was taken into a conical flask and 5cm^3 of 0.3% ammonium thiocynate solution was added. The mixture was titrated with a standard solution of iron (III) chloride until a brownish-yellow colour was observed for 5 minutes. The phytic acid was then determined by the procedure of Reddy *et al.,* 1989.

3.2.2.1.4 Determination of Saponin Content of Baobab Fruit Pulp

The procedure of Hudson and El-Difrawi, (1979) was used to determine saponin concentration in baobab fruit pulp meal. Dry baobab fruit pulp (5 grams) was weighed into 100 cm^3 of 20% aqueous ethanol in water and was agitated. The solution was filtered using filter paper. The residue was re-extracted with 300 cm^3 of 20% aqueous ethanol, the extract was combined and reduced to about 40 cm^3 under vacuum using rotary evaporator. The extract and 20cm^3 diethyl ether were transferred into 250cm^3 separatory funnel and agitated vigorously. The aqueous layer was then discarded. The process of purification was repeated until a colourless aqueous extract was obtained. The pH of the remaining aqueous solution was adjusted to about 4.5 by adding 4grams of sodium dichloride and the solution was agitated with 60ml N-butanol. The combined Butanolic extract was washed twice with 10ml of 5% aqueous NaCl and evaporated to dryness in a fume cupboard to give a crude saponin.

3.2.3 Experimental Design, Diets and Management of Birds

Two hundred and fifty six, day old (*Cobb 500*)broiler chicks were randomly allotted to four experimental treatments (0, 68 136 and 204 mg/kg ascorbic acid) each having 64 birds, with four replicates each in a Completely Randomized Design. A maize/soybean meal based broiler starter and finisher diets (Tables 3.3 and 3.4) was formulated according to NRC (1994) nutrient requirement. Feed and water were provided *ad libitum*. All standard routine management practices were strictly adhered to.

Initial weight was taken at the beginning of the experiment, while feed intake and weight gain were taken weekly using a digital sensitive scale. Known amount of water was offered daily to the birds, left-over was also measured at the end of each day, known amount of water was measured into an open container and placed at intervals along the passage within the building and evaporation losses was accounted for daily by measuring the quantity of water left in the container and subtracted from the initial quantity measured using a calibrated measuring cylinder. The left-over and evaporation loss was then subtracted from the quantity of water offered to the birds to determine the amount of water consumed daily. Mortality records were taken as they occurred.

All procedures are same as described in experiment 1.

The model used is as follows:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where:

Y_{ij} = Observation as influenced by i^{th} levels of Ascorbic acid

μ= Overall mean

A_i= the effect of i^{th} level of Ascorbic acid

e_{ij}= Random error (which is assume to be independently identical with normal distribution with zero mean and standard deviation)

Table 3.3: Ingredient Composition of Ascorbic Acid Supplemented Diets Fed to Broiler Starter Chickens (Day 1-28)

	Ascorbic acid content of diets (mg/kg diet)			
Ingredients (%)	**0**	**68**	**136**	**204**
Maize	57.00	54.50	52.30	50.00
Soyabean cake	25.00	25.50	25.70	26.00
Groundnut cake	13.50	13.50	13.50	13.50
BFPM	0.00	2.00	4.00	6.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (KCal/kg)	2953.00	2943.00	2934.00	2924.00
Crude protein (%)	23.04	23.08	23.01	23.00
Ether extract (%)	4.77	4.74	4.71	4.68
Crude fibre (%)	3.76	3.92	4.06	4.22
Calcium (%)	1.34	1.34	1.34	1.34
Available phosphorus (%)	0.58	0.58	0.58	0.58
Lysine (%)	1.26	1.27	1.27	1.27
Methionine (%)	0.54	0.53	0.53	0.53
Ascorbic acid (%)	163.44	166.14	167.08	168.60
Cost (₦/kg)	119.47	128.57	136.63	145.17

Vitamin-mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2,000,000 IU; Vit. E, 20,000 UI; Vit. K, 2,250mg; Vit. B1, 1,750mg; Vit. B_2, 5,000mg; Vit. B_6, 2,750mg; Vit. B_{12}, 15mg; Niacin, 27,500mg; Panth. Acid, 7,500mg; Folic acid, 7,500mg; Biotin, 50mg; Choline Chloride, 400g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg; BFPM= Baobab Fruit Pulp Meal

Table 3.4: Ingredient Composition of Ascorbic Acid Supplemented Diets Fed to Broiler Finisher Chickens (Day 29-49)

	Ascorbic acid content of the diet (mg/kg diet)			
Ingredients (%)	**0**	**68**	**136**	**204**
Maize	59.00	56.50	54.00	51.50
Soyabean cake	20.00	20.50	21.00	21.50
Groundnut cake	9.50	9.50	9.50	9.50
Maize offal	7.00	7.00	7.00	7.00
BFPM	0.00	2.00	4.00	6.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (KCal/kg)	2994.00	2982.00	2971.00	2962.00
Crude protein (%)	20.05	20.09	20.09	20.10
Ether extract (%)	4.52	4.50	4.47	4.45
Crude fibre (%)	3.86	4.01	4.17	4.32
Calcium (%)	1.32	1.33	1.33	1.33
Available phosphorus (%)	0.56	0.56	0.56	0.56
Lysine (%)	1.09	1.10	1.10	1.11
Methionine (%)	0.50	0.50	0.50	0.50
Ascorbic acid (%)	131.73	134.43	137.13	139.83
Cost (₦/kg)	112.07	120.77	129.47	138.17

Vitamin-mineral premix provide per kg of diet: Vit. A, 8,000,000 IU; Vit. D_3, 1,600,000 IU; Vit. E, 5,000 UI; Vit. K, 2000mg; Vit. B1, 1,500mg; Vit. B_2, 4,000mg; Vit. B_6, 1,500mg; Vit. B_{12}, 10mg; Niacin, 15,000mg; Panth. Acid, 5,000mg; Folic acid, 500mg; Biotin, 20mg; Choline Chloride, 200g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg; BFPM= Baobab Fruit Pulp Meal.

CHAPTER FOUR

4.0 RESULTS

4.1 The Effect of Dietary Selenium in Ameliorating Heat Stress in Broiler Chickens

4.1.1 Thermoregulatory Parameters

The indoor temperature humidity index (THI) during the experimental period is shown in Fig. 4.1. THI in the morning averaged 26.85 and 35.65 at afternoon. In general, the results obtained indicated that THI in the afternoons was higher than THI in the mornings which revealed the absence of heat stress in the morning, and the presence of severe heat stress in the afternoon. Respiratory rate, heart rate, rectal temperature and body temperature (Fig. 4.2to4.5) of broiler chickens fed diets supplemented with varying levels of selenium were similar (P>0.05) in this experiment. All the parameters measured were similar (P>0.05), Respiratory and heart rate of chickens range was from(76.66-86.91 cpm) and (193.94-199.75 bpm) respectively. Rectal temperature (42.01-42.31^{0}C) and body temperatures (42.01-42.38^{0}C) increased with increase in selenium supplementation.

4.1.2 Effect of Selenium Supplementation on Performance of Broiler Starter Chickens (day 1-28)

The growth performance of broiler chickens fed varying levels of selenium supplemented diet is presented in Table 4.1. Initial weight, daily feed intake, daily weight gain, final weight, feed conversion ratio and mortality were similar (P>0.05) across the treatments. Daily water intake ranged from 134.70 to 149.86 ml. Broilers fed 0.2 mgSe/kg diet had higher (P<0.05) daily water intake (149.86 ml/b/d) than the other treatment groups. Birds fed the control diet had the least feed cost (119.47₦/kg) per kg gain.

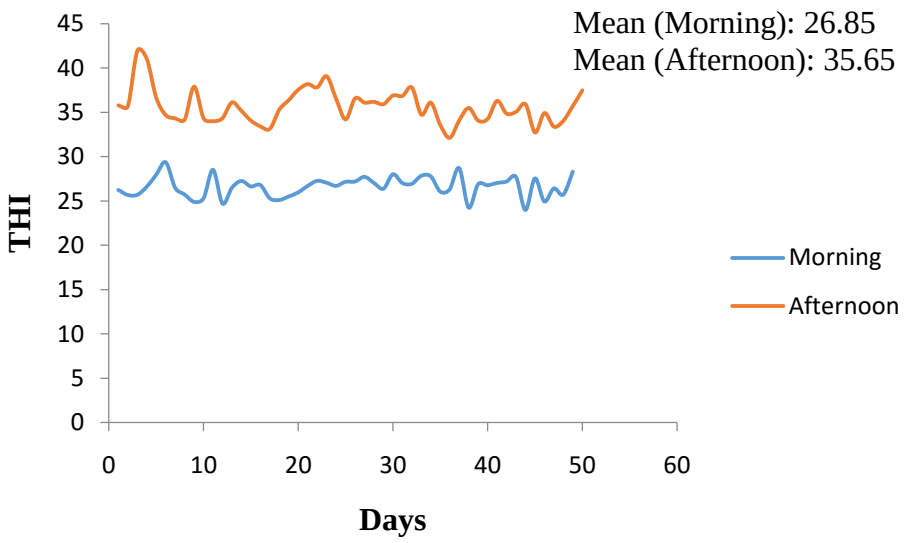


Fig. 4.1: Daily Temperature-Humidity Index inside the Poultry House during the Experimental Period

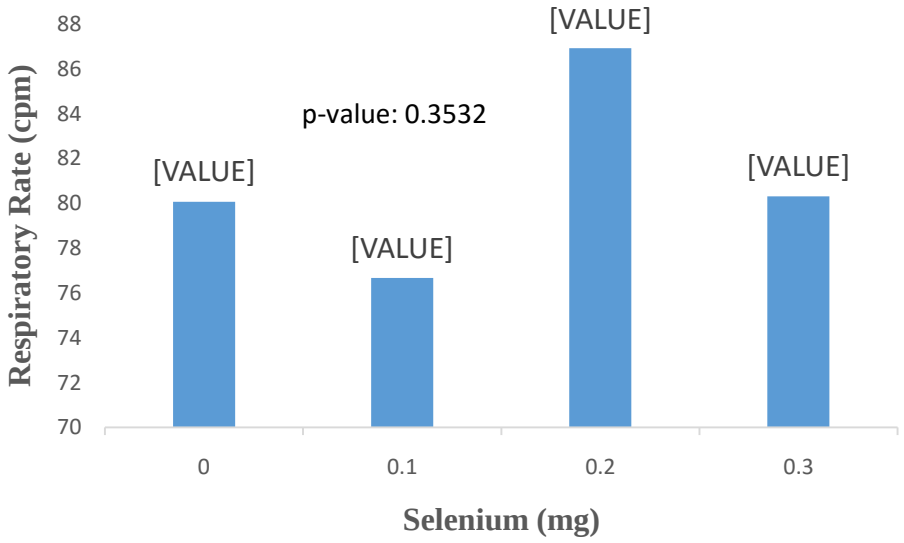


Fig. 4.2: Effect of Varying Levels of Selenium on Respiratory Rate of Broiler Chickens

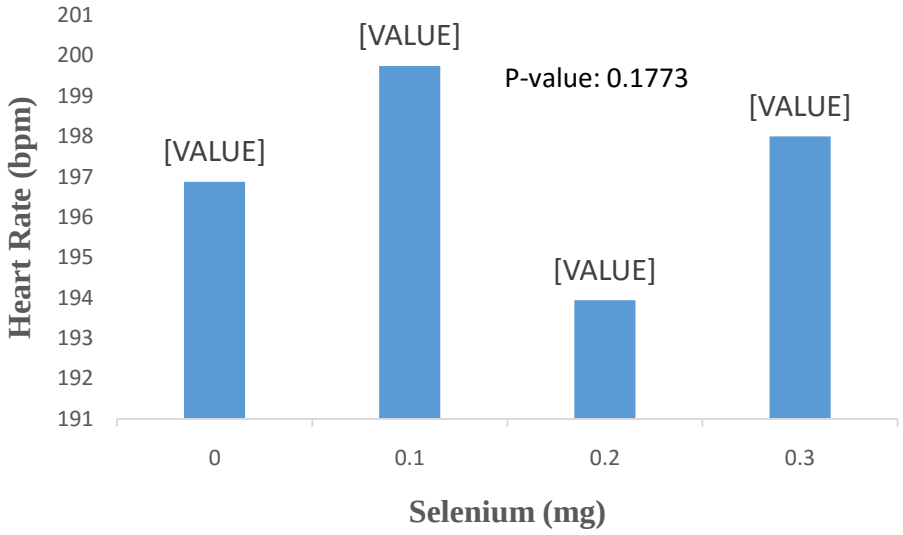


Fig. 4.3: Effect of Varying Levels of Selenium on Heart Rate of Broiler Chickens

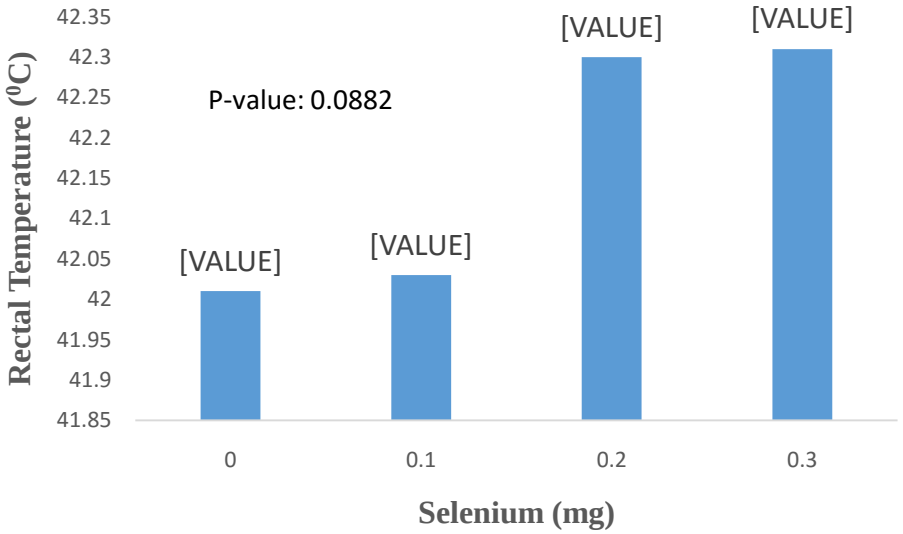


Fig. 4.4: Effect of Varying Levels of Selenium on Rectal Temperature of Broiler Chickens

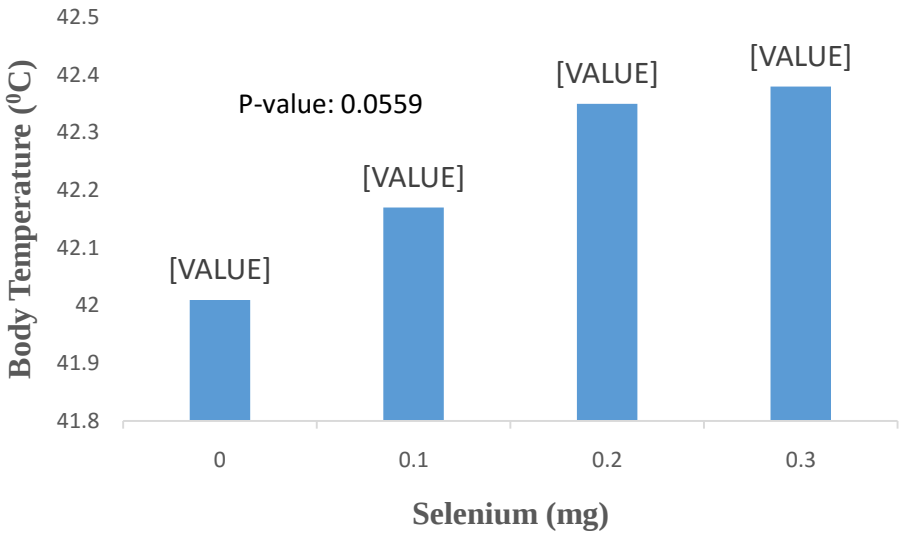


Fig. 4.5: Effect of Varying Levels of Selenium on Body Temperature of Broiler Chickens

4.1.3 Effect of Selenium Supplementation on Haematological Parameters of BroilerStarter Chickens (day 28)

Haematological parameters of broiler chickens fed diets supplemented with varying levels of selenium is presented in Table 4.2. All parameters were similar (P>0.05) except band cell count.The values of PCV and Hb rangewas from 26.50-29.75% and 8.81-9.84 g/dL respectively, erythrocytes and leucocytes values range was from (4.56-5.05 $x10^{12}$/L) and (9.98-13.25 $x10^{9}$/L) respectively. Heterophils (17.88%) and H:L (0.22) were lower for broilers fed 0.1 mgSe.Lymphocytes, monocytesand eosinophils count range was from (76.50-81.50, 0.13-1.63, and 0.00-0.13%).Higher band cell was observed in broilers fed 0.2 and 0.3 mgSe/kg.

4.1.4: Effect of Selenium Supplementation on Serum Biochemical Indices of Broiler Starter Chickens (Day 28)

Table 4.3 shows the result of serum biochemical indices of broiler chickens fed varying levels of selenium supplemented diet at starter phase. All parameters were similar (P>0.05). Broilers fed the control diet had more (P>0.05) glucose (238.05mg/dL), alanine-amino transferase (ALT) (31.50µ/L), alkaline phosphatase (ALP) (32.00µ/L), albumin (2.73 g/dL), cholesterol (131.75 nmol/L), low density lipoprotein (LDL) (162.75 mg/dL) and calcium (8.55 mg/dL). Aspartate-amino transferase (AST) (36.25µ/L), superoxide dismutase (SOD) (5.10µmol/mL) and phosphorus (7.18 mg/dL) were higher for chickens fed 0.2 mg selenium supplemented diet. Broilers fed 0.1 mg selenium supplemented diet had higher values of total protein (4.03 g/dL), globulin (1.45 mg/dL), malondialdehyde (MDA) (46385.00 nmol/mL), glutathione peroxidase (GSHPx) (1.85µmol/mL) and triglyceride (100.25 mg/dL) while catalase (CAT) was higher (P>0.05) in broilers fed 0.3 mg selenium supplemented diet and ranged from 3.17 – 4.03 U/ml.

Table 4.1: Effect of Selenium Supplementation on Performance of Broiler Starter Chickens (day 1-28)

Parameters	Dietary levels of Selenium (mg/kg diet)					
	0	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Initial weight (g/bird)	41.41	39.69	43.44	39.54	1.13	0.0982
Daily feed intake (g/b/d)	51.53	50.54	55.88	51.79	1.38	0.0785
Daily weight gain (g/b/d)	32.67	32.23	33.72	32.47	0.90	0.6641
Final weight (g/bird)	956.25	942.19	987.75	948.75	25.42	0.6094
Feed conversion ratio	1.58	1.57	1.66	1.60	0.02	0.0777
Daily water intake (ml/b/d)	134.70[b]	136.78[b]	149.86[a]	140.54[b]	3.44	0.0395
Feed cost/kg gain (₦/kg)	119.47	119.72	119.97	120.22	-	-
Mortality (%)	4.69	3.13	0.00	4.69	2.30	0.4592

[a,b] Means with different superscript on the same row differ significantly (P<0.05)

Table 4.2: Effect of Selenium Supplementation on Haematological Parameters of Broiler Starter Chickens (day 28)

	Dietary levels of Selenium (mg/kg diet)						
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**	**Ref-value**
PCV (%)	29.00	26.50	28.50	29.75	2.71	0.6683	24.00-40.00[w]
Haemoglobin (g/dl)	9.65	8.81	9.46	9.84	0.91	0.6979	7.00-15.00[w]
Erythrocytes ($x10^{12}$/l)	5.05	4.56	4.89	4.95	0.47	0.7520	1.59-4.10[w]
Leucocytes ($x10^{9}$/l)	9.98	13.25	12.69	10.60	1.89	0.2601	1.90-9.50[x]
Heterophils (%)	19.38	17.88	20.50	18.63	3.19	0.8630	15.00-40.00[x]
Lymphocytes (%)	78.88	81.50	76.50	79.50	3.15	0.4755	40.00-100.00[y]
H:L	0.25	0.22	0.27	0.23	0.09	0.9061	-
Monocytes (%)	1.13	0.38	1.63	0.13	0.98	0.4128	1.00-7.00[z]
Eosinophils	0.00	0.13	0.00	0.00	0.09	0.4074	1.50-6.00[x]
Bands (%)	0.25[bc]	0.13[c]	1.38[ab]	1.75[a]	0.60	0.0233	-

[abc] Means with different superscript on the same row differ significantly (P<0.05), PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993, H:L= Heterophils-lymphocytes ratio.

Table 4.3: Effect of Selenium Supplementation on Serum Indices of Broiler Starter Chickens (Day 28)

	Dietary levels of Selenium (mg/kg diet)						
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**	**Ref**
Glucose (mg/dL)	238.05	229.05	193.95	207.90	23.65	0.5611	137-363[w]
Total Protein (g/dL)	3.85	4.03	3.98	3.78	0.24	0.8807	3.60-5.50[x]
Albumin (g/dL)	2.73	2.58	2.55	2.40	0.40	0.9510	1.10-2.20[x]
Globulin (g/dL)	1.13	1.45	1.43	1.38	0.49	0.9624	-
Cholesterol (nmol/L)	131.75	121.25	93.25	122.00	15.66	0.3794	120-237
Low Density Lipoprotein (mg/dL)	162.75	124.75	103.25	149.00	20.25	0.2219	<130.00
Triglyceride (mg/dL)	58.25	100.25	66.50	75.50	15.22	0.2838	<135.00
Alanine-Amino Transferase (µ/L)	31.50	25.25	24.50	19.00	4.40	0.3046	-
Aspartate-Amino Transferase (µ/L)	19.00	35.50	36.25	23.75	6.96	0.2587	10-40[y]
Alkaline Phosphatase (µ/L)	32.00	25.50	19.00	27.25	5.33	0.4187	10-106[z]
Glutathione Peroxidase (µmol/mL)	1.55	1.85	1.77	1.68	0.30	0.9071	
Superoxide Dismutase (µmol/mL)	4.65	4.35	5.10	3.90	0.96	0.8421	
Malondialdehyde (nmol/mL)	18201.00	46385.00	16523.00	22092.00	13249.56	0.3856	
Catalase (U/ml)	3.29	3.17	3.41	4.03	1.18	0.9569	
Calcium (mg/dL)	8.55	6.95	7.18	8.05	0.72	0.3909	
Phosphorus (mg/dL)	6.13	4.65	7.18	4.88	0.74	0.1076	

Reference values: [w]Goodwin *et al*. (1994), [x]Ross *et al*. (1976), [y]LAVC (2009), [z]Bounous and Stedman (2000), Clinical Diagnostic Division (1990), Collins (2018).

4.1.5: Effect of Selenium Supplementation on Serum Cortisol and Thyroxine of Broiler Starter Chickens (day 28)

Figures 4.6 and 4.7 show the cortisol and thyroxine levels of broiler chicks fed varying levels of selenium supplemented diets during 28 day of the study. Cortisol was higher ($P<0.05$) in broilers fed the control diet (10.40 ng/mL) and 0.3 mgSe/kg (12.20 ng/mL) but broilers fed 0.1 mgSe/kg (5.00 ng/mL) had the least cortisol level. Thyroxine was similar ($P>0.05$) for all groups and range from (35.05-41.68 nmol/L).

4.1.6: Effect of Selenium Supplementation on Performance of BroilerFinisher Chickens (day 29-49)

Growth performance of broiler chickens fed diets supplemented with varying levels of selenium during day 29-49 (Table 4.4) shows that daily feed intake, daily weight gain, final weight, feed conversion ratio, daily water intake and mortality were similar ($P>0.05$) for all treatment groups. Broilers fed diet supplemented with 0.3 mg of selenium had the least feed cost (236.42₦/kg) per kg gain.

4.1.7: Effect of Selenium Supplementation on Performance of Broiler Chickens (day 1-49)

The growth performance of broiler chickens fed diets supplemented with different levels of selenium from day 1-49 of the study (Table 4.5) shows that daily feed intake, daily weight gain,final weight, feed conversion ratio, daily water intake and mortality were similar ($P>0.05$) for all treatment groups. Broilers fed 0.3 mg selenium supplemented diet has the least feed cost per kg gain.

4.1.8: Effect of Selenium Supplementation on Haematological Parameters of Broiler Finisher Chickens (day 49)

Haematological parameters of broiler chickens fed diets containing varying levels of selenium supplementation at day 49 is presented in Table 4.6. PCV, Hb and Erythrocyte ranged from

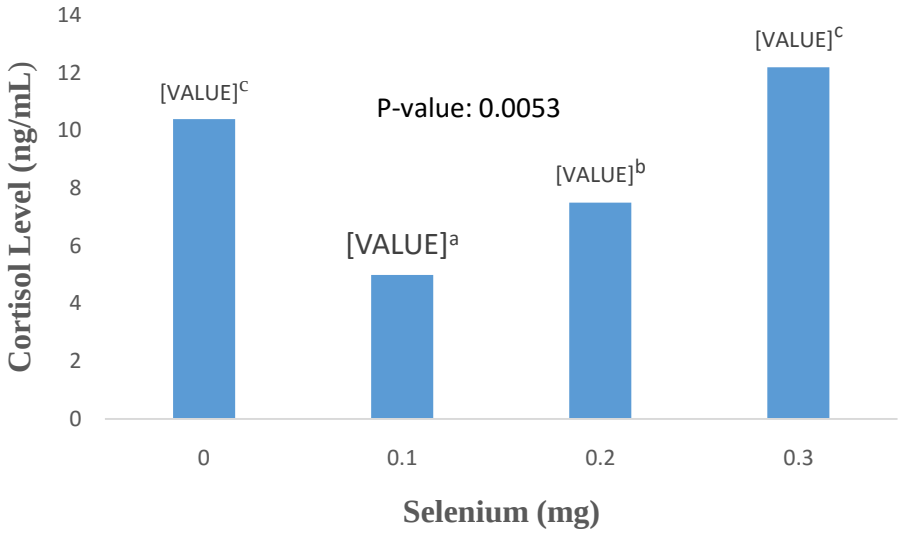


Fig. 4.6: Effect of varying levels of Selenium on Cortisol level of Broiler Starter Chickens (Day 28)

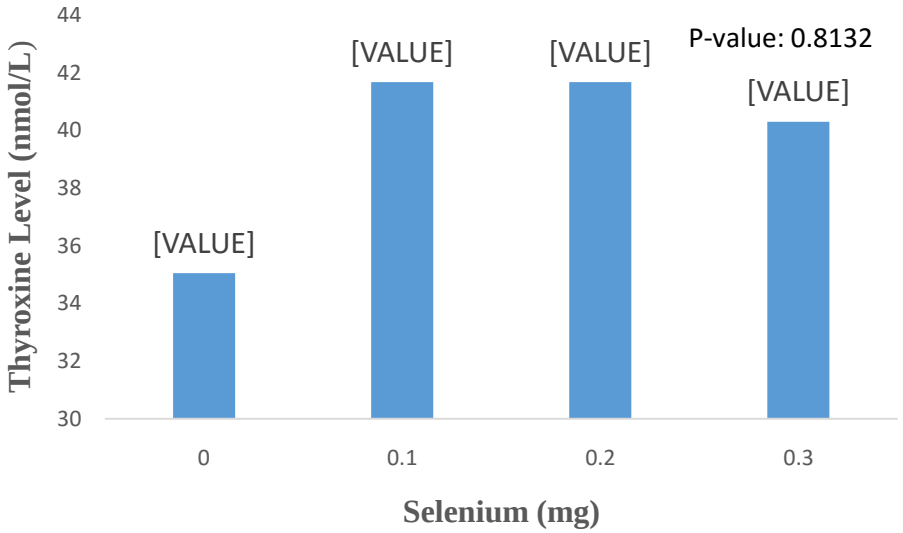


Fig. 4.7: Effect of varying levels of Selenium on Thyroxine level of Broiler Starter Chicks (Day 28)

Table 4.4: Effect of Selenium Supplementation on Performance of Broiler Finisher Chickens (day 29-49)

	Dietary levels of Selenium (mg/kg diet)					
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Initial weight (g/bird)	956.25	942.19	987.75	948.75	25.42	0.6094
Daily feed intake (g/b/d)	122.93	121.21	128.25	118.50	3.95	0.3930
Daily weight gain (g/b/d)	60.50	59.98	62.34	60.37	1.77	0.7880
Final weight (g/bird)	2226.80	2201.75	2296.89	2216.46	46.81	0.5108
Feed conversion ratio	2.03	2.02	2.06	1.97	0.05	0.6626
Daily water intake (ml/b/d)	421.94	427.93	423.74	440.17	13.86	0.7893
Feed cost/kg gain (₦/kg)	242.81	242.10	246.83	236.42	-	-
Mortality (%)	9.38	9.38	10.94	15.63	4.58	0.7437

Table 4.5: Effect of Selenium Supplementation on Performance of Broiler Chickens (day 1-49)

	Dietary levels of Selenium (mg/kg diet)					
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Initial weight (g/bird)	41.41	39.69	43.44	39.54	1.13	0.0982
Daily feed intake (g/b/d)	82.13	80.08	86.90	80.08	2.26	0.1646
Daily weight gain (g/b/d)	44.60	44.12	45.99	44.43	0.96	0.5434
Final weight (g/bird)	2226.80	2201.75	2296.89	2216.46	46.81	0.5108
Feed conversion ratio	1.84	1.82	1.89	1.80	0.03	0.2437
Daily water intake (ml/b/d)	257.93	261.92	267.64	269.33	6.90	0.6401
Feed cost/kg gain (₦/kg)	219.91	217.43	226.63	216.68	-	-
Mortality (%)	14.06	12.50	10.94	20.31	4.96	0.5777

(25.25-33.88%), (8.36-11.28g/dL) and (4.29-5.50x10^{12}/l) respectively. Chickens fed diet containing 0.1 mgSe/kg had higher (P<0.05) PCV (33.88%), Hb (11.28 g/dL) and erythrocytes (5.50x10^{12}/l) than other groups. Leucocytes (13.15%), monocytes (2.63%) and band cells (0.88%) were higher (P>0.05) for chickens fed 0.2 mgSe/kg diet. Chickens fed 0.3 mg selenium supplemented diet had lower (P>0.05) heterophils (14.88%) and H:L (0.19) while lymphocytes (83.50%) and eosinophils (0.50%) were higher (P>0.05) for birds fed 0.3 mgSe/kg diet. The result also reveal that among the selenium supplemented groups, heterophils and H:L decreased while lymphocytes increased with increase in selenium supplementation.

4.1.9: Effect of Selenium Supplementation on Serum Biochemical Indices of Broiler Finisher Chickens (Day 49)

Serum biochemical indices of broiler chickens fed varying levels of selenium supplemented diet during their finisher phase(Table 4.7). Broilers fed the control diet and 0.1 mgSe/kg diet had higher (P<0.05) Cholesterol values of 144 and 179.25 nmol/L respectively, cholesterol range from 93.50-179.25 nmol/L.Broilers fed 0.2 mgSe/kg diet had more (P>0.05) glucose (136.80mg/dL), ALT (45.50µ/L), total protein (5.33 g/dL), globulin (2.30 g/dL), and LDL (215.75 mg/dL). Similarly, chickens fed 0.1 mgSe/kg diet had higher (P>0.05) AST (40.50µ/L), triglyceride (95.25 mg/dL). Higher (P<0.05) values of SOD was recorded for broilers fed 0.2 (5.85µmol/mL) and 0.3 mgSe/kg (6.00µmol/mL) supplemented diet respectively, SOD ranged from 3.30-6.00µmol/mL. CAT was higher (P<0.05) for broilers fed control diet (5.61 U/mL) and 0.3 mg selenium/kg diet (5.98 U/mL) and ranged from 1.71-5.98 U/mL. MDA was lower for broilers fed 0.1 mgSe/kg, broilers fed 0.3 mgSe/kg had higher (P>0.05) ALP (73.25µ/L) and GSPHx (1.55µmol/mL). Calcium ranged from 8.53-9.70 mg/dL and was higher (P>0.05) in birds

fed the control diet.Phosphorus was higher ($P<0.05$) in birds fed 0.2 mgSe/kg diet and ranged from 3.78-7.70 mg/dL.

Table 4.6: Effect of Selenium Supplementation on Haematological Parameters of Broiler Finisher Chickens (day 49)

	Dietary levels of Selenium (mg/kg diet)						
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**	**Ref-value**
PCV (%)	27.75[b]	33.88[a]	26.88[b]	25.25[b]	2.20	0.0030	24.00-40.00[w]
Haemoglobin (g/dl)	9.25[b]	11.28[a]	8.93[b]	8.36[b]	0.74	0.0031	7.00-15.00[w]
Erythrocytes ($x10^{12}$/l)	4.76[b]	5.50[a]	4.45[b]	4.29[b]	0.37	0.0140	1.59-4.10[w]
Leucocytes ($x10^{9}$/l)	12.08	9.96	13.15	12.64	1.62	0.2388	1.90-9.50[x]
Heterophils (%)	18.00	21.50	19.00	14.88	3.39	0.2914	15.00-40.00[x]
Lymphocytes (%)	80.50	77.38	76.63	83.50	3.72	0.2546	40.00-100.00[y]
H:L	0.23	0.29	0.22	0.19	0.04	0.3959	-
Monocytes (%)	0.88	1.00	2.63	1.13	1.35	0.5384	1.00-7.00[z]
Eosinophils	0.38	0.00	0.25	0.50	0.46	0.7285	1.50-6.00[x]
Bands (%)	0.13	0.13	0.88	0.00	0.36	0.0841	

[ab] Means with different superscript on the same row differ significantly (P<0.05), PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993, H:L= Heterophils-lymphocytes ratio.

Table 4.7: Effect of Selenium Supplementation on Serum Indices of Broiler Finisher Chickens (Day 49)

	Dietary levels of Selenium (mg/kg diet)						
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**	**Ref**
Glucose (mg/dL)	89.55	107.10	136.80	124.65	16.83	0.2638	137-363[w]
Total Protein (g/dL)	4.55	4.68	5.33	4.20	0.27	0.0741	3.60-5.50[x]
Albumin (g/dL)	3.45	2.85	3.03	2.78	0.61	0.8655	1.10-2.20[x]
Globulin (g/dL)	1.10	1.83	2.30	1.43	0.72	0.6787	1.20-3.20[y]
Cholesterol (nmol/L)	144.00[a]	179.25[a]	100.50[b]	93.50[b]	17.98	0.0181	120-237
Low Density Lipoprotein (mg/dL)	184.25	102.25	215.75	165.00	34.29	0.1757	<130.00
Triglyceride (mg/dL)	52.50	95.25	81.00	97.00	22.34	0.4934	<135.00
Alanine-Amino Transferase (µ/L)	28.75	39.25	45.50	40.00	11.62	0.7812	-
Aspartate-Amino Transferase (µ/L)	29.75	40.50	28.00	20.00	10.95	0.6309	10-400[y]
Alkaline Phosphatase (µ/L)	38.25	67.25	45.00	73.25	12.73	0.2063	10-106[z]
Glutathione Peroxidase (µmol/mL)	1.40	1.40	1.28	1.55	0.15	0.6814	
Superoxide Dismutase (µmol/mL)	3.30[b]	3.30[b]	5.85[a]	6.00[a]	0.63	0.0115	
Malondialdehyde (nmol/mL)	9985.00	6435.00	17624.00	15981.00	4457.68	0.2999	
Catalase (U/ml)	5.61[a]	1.71[b]	2.93[b]	5.98[a]	1.13	0.0543	
Calcium (mg/dL)	9.70	9.20	8.53	9.10	1.57	0.9621	
Phosphorus (mg/dL)	4.10[c]	5.10[b]	7.70[a]	3.78[c]	0.40	0.0001	

[abc] Means with different superscript on the same row differ significantly (P<0.05), Reference values: [w]Goodwin *et a*l. (1994), [x]Ross *et al*. (1976), [y]LAVC (2009), [z]Bounous and Stedman (2000), Clinical Diagnostic Division (1990), Collins (2018).

4.1.10: Effect of Selenium Supplementation on Serum Cortisol and Thyroxine of Finisher Broiler Chickens (day 49)

The result of cortisol and thyroxine levels of broiler chickens fed varying levels of selenium supplemented diets during 49 days of the study is presented in Figures 4.8 and 4.9. Serum cortisol level was lower ($P<0.05$) in broilers fed the control diet (7.10 ng/mL), 0.1 (6.30 ng/mL) and 0.2 (7.30 ng/mL) mgSe/kg and was higher (11.40 ng/mL) in broilers fed 0.3 mg selenium supplemented diet. Thyroxine was higher ($P>0.05$) in broilers fed selenium supplemented diet than the control group and ranged from 22.36 – 34.50 nmol/mL.

4.1.11: Effect of Selenium Supplementation on Calcium, Phosphorus and Ash content in Bone and Faeces of Broiler Chickens

Table 4.8 shows the calcium, phosphorus and ash of bone and faeces of broiler chickens fed varying levels of selenium supplemented diet during the study. Calcium, phosphorus and ash content of the tibia bones of the various groups ranged from 30.83 – 33.75, 10.04 – 10.42 and 0.37 – 0.46% and were similar ($P>0.05$). Calcium and phosphorus discharged via faeces ranged from 16.67 – 22.50 and 0.89 – 1.12%. Broilers fed the control diet had higher ($P<0.05$) fecal calcium deposition while higher ($P<0.05$) phosphorusdeposition in faeces was observed with chickens fed the control diet, 0.1 mgSe and 0.3 mgSe/kg diet respectively.

4.1.12: Effect of Selenium Supplementation on Carcass Cut Parts and Organ Weights of Broiler Chickens

The result of carcass and cut parts of broiler chickens fed varying levels of selenium is presented in Table 4.9. Chickens fed 0.3 mgSe/kg diet had a higher ($P<0.05$) dressing percentage. Dressing percentage varied from 69.24 – 74.49%. Similarly, the weight of thigh was higher ($P<0.05$) for chickens fed the control diet. Wings weight of broiler chickens ranged between 10.01– 11.83%.

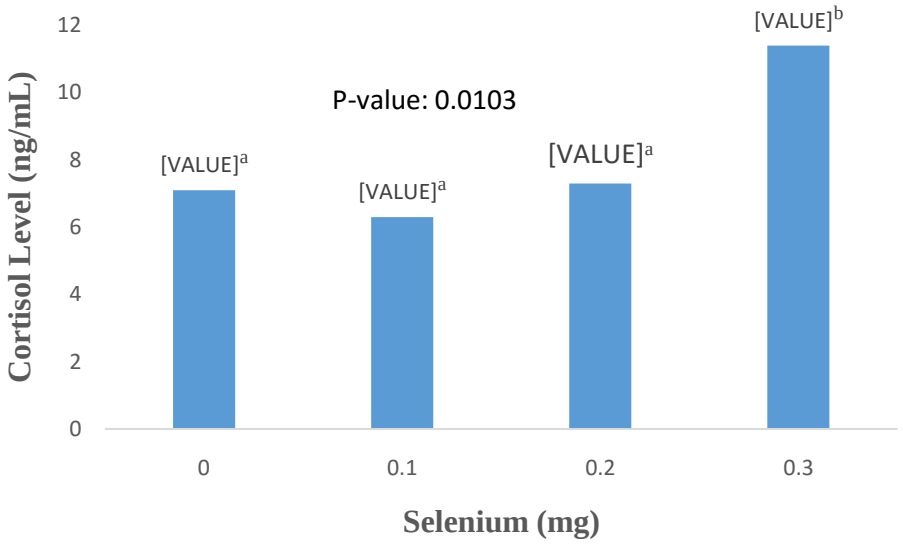


Fig. 4.8: Effect of varying levels of Selenium on Cortisol level of Broiler Finisher Chickens (Day 49)

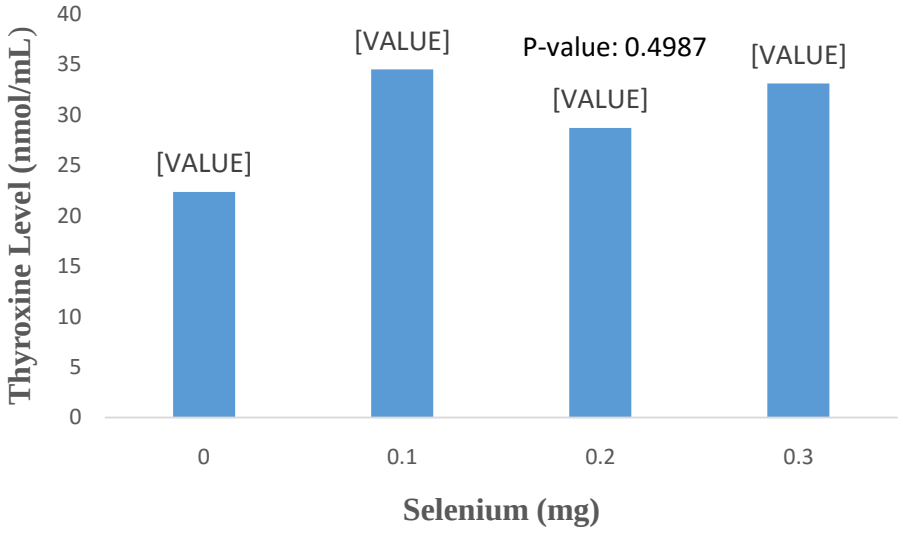


Fig. 4.9: Effect of varying levels of Selenium on Thyroxine level of Broiler Finisher Chickens (Day 49)

Table 4.8: Effect of Selenium Supplementation on Calcium, Phosphorus and Ash content in Bone and Faeces of Broiler Chickens

	Dietary levels of Selenium (mg/kg diet)					
Parameters (%)	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Bone						
Calcium	33.67	33.75	30.83	33.75	2.84	0.8443
Phosphorus	10.42	10.04	10.32	10.27	0.18	0.4995
Ash	0.46	0.42	0.37	0.39	0.03	0.1327
Faeces						
Calcium	22.50^{b}	18.75^{a}	17.92^{a}	16.67^{a}	1.34	0.0483
Phosphorus	1.06^{b}	1.12^{b}	0.89^{a}	1.07^{b}	0.05	0.0421

[ab] Means with different superscript on the same row differ significantly (P<0.05)

Broilers fed the control diet and 0.3 mg selenium supplemented diet had higher (P<0.05) wing weight. Birds fed 0.3 mgSe/kg had higher (P<0.05) back cut than the other groups. The organs of broiler such as the liver, gizzard, heart, kidney, lungs, intestines spleen, thymus and bursa were similar (P>0.05) in this study.

4.1.13: Effect of Selenium Supplementation on Jejunum Mucosal Morphology of Broiler Chickens

The jejunum mucosal integrity of broiler chickens fed varying levels of selenium supplemented diet is presented in Table 4.10. Villus height, width, crypt depth and villus perimeter were similar (P>0.05). Broilers fed 0.1 mgSe and 0.2 mgSe/kg diet had higher (P<0.05) villus area while the control, 0.1 and 0.2 mg selenium supplemented groups had higher (P<0.05) villus height/crypt depth, as compared to 0.3 mgSe/kg group.

4.1.14: Effect of Selenium Supplementation on Tibia Measurements of Broiler Chickens

The tibia geometry measurement of broiler chickens fed varying levels of selenium supplemented diets is presented in Table 4.11. Tibia weight, length, weight/length and robusticity index among chickens in all treatment groups were similar (P>0.05).

4.2 The Effectof Organic Ascorbic Acid in Ameliorating Heat Stress in Broiler Chickens

4.2.1 Results of the Proximate Analysis and Anti-Nutritional Factor Contents of Baobab Fruit Pulp

The result of the proximate composition (Table 4.12) indicates that Baobab Fruit Pulp Meal (BFPM) contains 95.95% dry matter, 6.38% crude protein, 4.31% crude fibre, 3.94% ether extract, 73.87% Nitrogen Free Extract and 11.50% Ash. The ascorbic acid content was 340 mg/100g of BFPM. Table 4.13 shows the results of the Anti-Nutritional Factors present in the Baobab Fruit

Table 4.9: Effect of Selenium Supplementation on Carcass Cut Parts and Organ Weights of Broiler Chickens

	Dietary levels of Selenium (mg/kg diet)					
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Live weight (g/b)	2256.25	2306.25	2350.00	2256.25	41.55	0.3341
Dressed weight (g/b)	1589.88	1636.50	1626.13	1680.38	39.42	0.4567
Dressing percent (%)	70.47^{b}	70.95^{b}	69.24^{b}	74.49^{a}	1.24	0.0341
Cut parts expressed as percentage of dressed weight (%)						
Breast	33.70	34.16	32.01	34.22	0.72	0.1257
Back	11.41^{c}	13.66^{b}	13.52^{b}	14.57^{a}	0.41	0.001
Thigh	16.96^{a}	15.26^{b}	15.99^{b}	15.26^{b}	0.39	0.0128
Drum Stick	14.19	13.67	14.37	13.53	0.62	0.7391
Wing	11.83^{a}	10.01^{c}	10.08^{bc}	10.99^{ab}	0.47	0.0316
Organs weights expressed as percentage of live weight (%)						
Liver	1.74	2.01	1.89	2.04	0.13	0.3633
Gizzard	1.77	1.80	1.74	1.76	0.09	0.9695
Heart	0.37	0.37	0.37	0.41	0.02	0.4536
Kidney	0.52	0.55	0.50	0.57	0.02	0.1334
Lungs	0.65	0.62	0.54	0.61	0.03	0.1641
Intestine	2.01	1.95	1.93	2.03	0.04	0.2827
Spleen	0.06	0.07	0.06	0.06	0.01	0.6048
Thymus	0.13	0.14	0.16	0.17	0.02	0.1730
Bursa of Fabricius	0.02	0.02	0.24	0.03	0.00	0.9343

[abc] Means with different superscript on the same row differ significantly ($P<0.05$)

Table 4.10: Effect of Selenium Supplementation on Jejunum Mucosal Morphology of Broiler Chickens

	Dietary levels of Selenium (mg/kg diet)					
Parameters	**0**	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Villus height (μm)	298.98	359.34	390.71	286.04	31.11	0.0683
Villus width (μm)	101.26	129.05	105.11	105.85	14.38	0.5149
Crypt depth (μm)	70.39	76.27	82.89	87.79	5.07	0.0954
Villus area (μm^2)	12015^b	21312^a	21516^a	11714^b	2593.77	0.0075
Villus perimeter (μm)	710.03	840.74	879.75	686.13	63.51	0.0946
Villus height/Crypt depth ratio	4.58^a	4.69^a	4.79^a	3.28^b	0.39	0.0310

[ab] Means with different superscript on the same row differ significantly (P<0.05)

Table 4.1.11: Effect of Selenium Supplementation on Tibia Measurement of Broiler Chickens

Tibia compositions	Dietary levels of Selenium (mg/kg diet)					
	0	**0.1**	**0.2**	**0.3**	**SEM**	**P value**
Tibia Weight (g)	4.63	4.76	5.14	4.84	0.22	0.4396
Tibia Length (cm)	7.13	7.08	7.38	7.20	0.12	0.3354
Tibia Weight/Length Index (g/cm)	0.65	0.67	0.70	0.67	0.02	0.6402
Robusticity Index ($cm/g^{1/3}$)	4.64	4.47	4.35	4.47	0.16	0.6641

Pulp. The Baobab Fruit Pulp contains 3.5% Tannin, 0.28% Phytate, 1.14 mg/100g Oxalate and 22.6 mg/100g Saponin.

4.2.2 Thermoregulatory Parameters

The indoor temperature humidity index (THI) during the experimental period is shown in Fig. 4.6. THI in the morning average 26.83 and 35.81in the afternoon. Thermo-regulatory responses of broiler chickens fed diet supplemented with varying levels of ascorbic acid is presented in Fig. 4.7-4.10. Respiratory rate, heart rate, rectal temperature and body temperature were similar ($P>0.05$) during the study. The Respiratory rate of broilers range from 79.69 – 87.94 cpm, heart rate range from 189.31 – 192.94 bpm, rectal temperature was 42.05 – 42.30^0C and body temperature of 42.17 – 42.27^0C.

4.2.3 Effect of Ascorbic Acid Supplementation on Performance of Broiler Starter Chickens (Day 1-28)

The growth performance of starter broiler chicks fed varying levels of ascorbic acid is shown in Table 4.14. All the parameter measured were similar ($P>0.05$) except for feed conversion ratio ($P<0.05$). Chicks fed the control diet and 68 mg/kg ascorbic acid diet had a better feed conversion ratio (1.39) than the other groups, values range from 1.39 to 1.56. Chickens fed the control diet had least feed cost per kg gain.

4.2.4: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Starter Chickens (day 28)

Table 4.15 shows the haematological parameters of broiler chickens fed varying levels of ascorbic acid supplemented diets during 28 days of the study. All the parameters measured were similar ($P>0.05$). PCV, haemoglobin and erythrocytes values range from 25.00 – 29.00%, 8.27 –

9.60g/dL and 4.16 – 4.78x10^{12}/l respectively. Leucocytes ranged from (10.68 – 13.08 x10^{9}/l). Heterophils,

Table 4.12: Proximate and Ascorbic Acid Composition of Baobab Fruit Pulp

Component	**% (DM Basis)**
Dry Matter	95.95
Crude Protein	6.38
Crude Fibre	4.31
Ether extract	3.94
Nitrogen Free Extract	73.87
Ash	11.50
Ascorbic acid	340 mg/100g

Table 4.13: Anti-Nutritional Factors in Baobab Fruit Pulp

Anti-nutrient compound	Baobab fruit pulp
Tannin (%)	3.5
Phytate (%)	0.28
Saponin (mg/100g)	22.6
Oxalate (mg/100g)	1.14

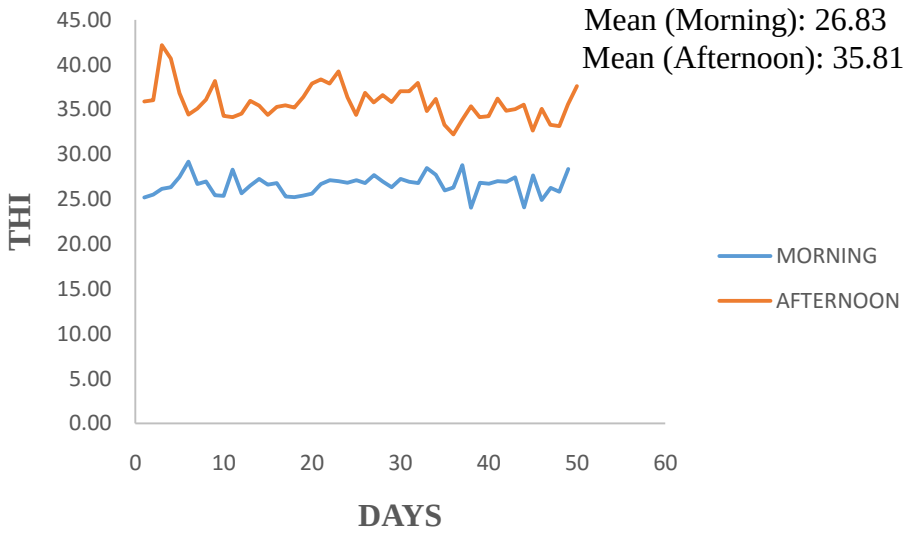


Fig. 4.10: Daily Temperature-Humidity Index inside the Poultry House during the Experimental Period

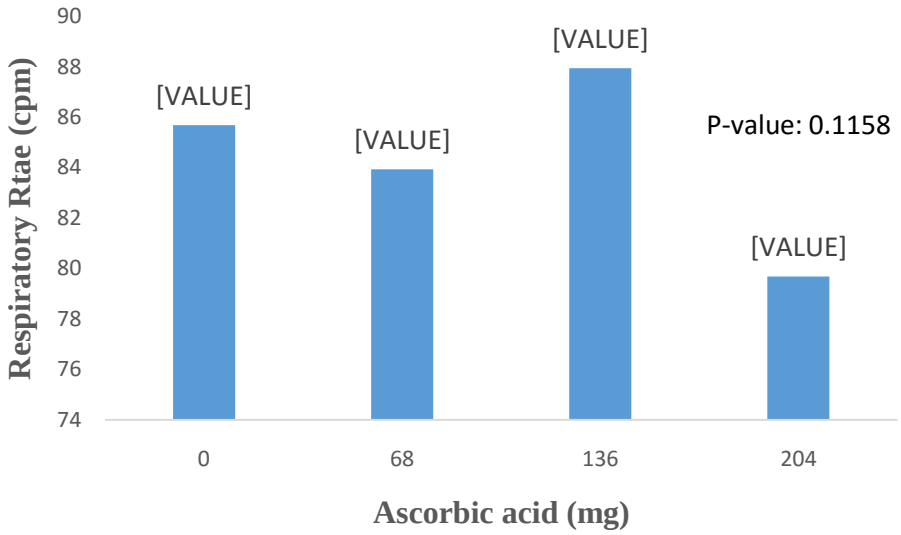


Fig. 4.11: Effect of Varying Levels of Ascorbic Acid on Respiratory Rate of Broiler Chickens

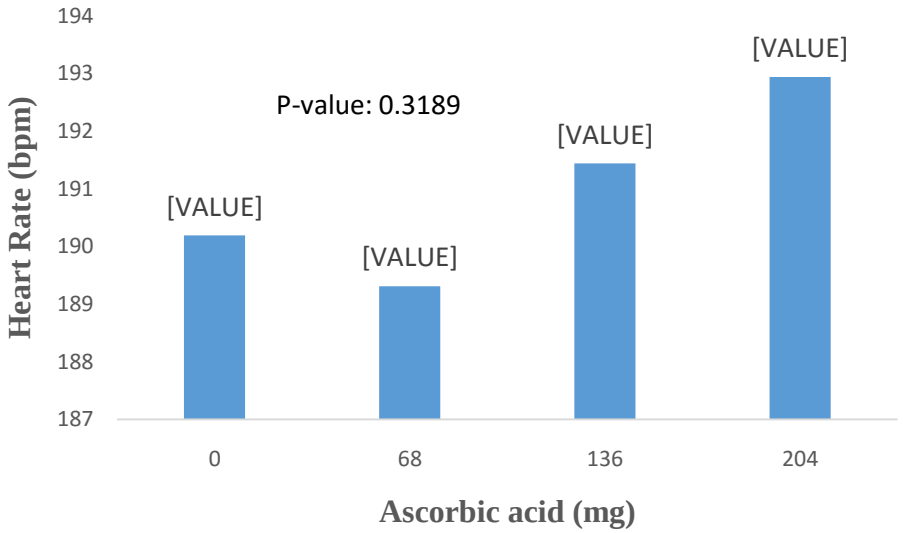


Fig. 4.12: Effect of Varying Levels of Ascorbic Acid on Heart Rate of Broiler Chickens

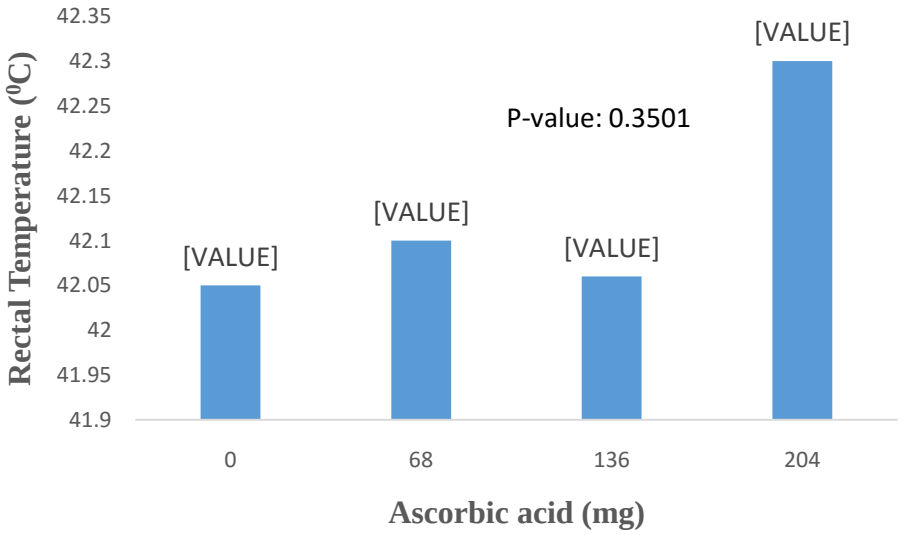


Fig. 4.13: Effect of Varying Levels of Ascorbic Acid on Rectal Temperature of Broiler Chickens

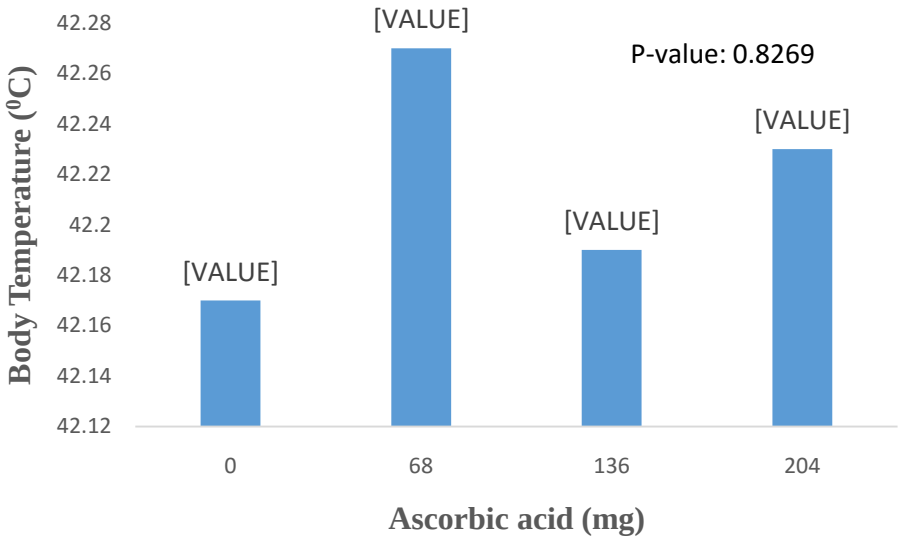


Fig. 4.14: Effect of Varying Levels of Ascorbic Acid on Body Temperature of Broiler Chickens

lymphocytes and H:L range from 14.50 – 17.25%. 80.25 – 84.00% and 0.18 – 0.22. Monocyte and eosinophils ranged from (0.38 – 1.38 and 0.00 – 0.63%) respectively.

4.2.5: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Starter Chickens (Day 28)

Serum indices of starter broiler chickens fed diet supplemented with varying levels of ascorbic acid is presented in Table 4.16.All the parameters were similar except for LDL and ALT. Glucose, total protein, albumin and globulin rangedfrom 193.95 – 252.90 mg/dL, 3.65 – 4.70 g/dL, 2.25 – 2.95g/dL and 1.30 – 2.20g/dL.Cholesterol range from 46.00 – 94.25 nmol/L. LDL range from 55.25 – 123.50 mg/dL and was higher ($P<0.05$) in broilers fed 68 mg and 136 mg of ascorbic acid supplemented diet. ALT varied from 8.25 – 55.50µ/L and was higher ($P<0.05$) for broilers fed diet supplemented with136 mg of ascorbic acid per kg. AST and ALP range from 13.00 – 34.25µ/L and 23.50 – 43.50µ/L. GSHPx, SOD, MDA and CAT ranged from 1.64 – 2.56µmol/mL, 1.50 – 4.35µmol/mL, 17452 – 60526 nmol/mL and 3.78 – 9.45 U/mL. Calcium and phosphorus ranged from 6.45 – 9.25 and 2.40 – 2.85 mg/dL.

4.2.6: Effect of Ascorbic Acid Supplementation on Serum Cortisol and Thyroxine level of Broiler Starter Chickens (day 28)

Figures 4.15 and 4.16 show the cortisol and thyroxine levels of broiler chicks fed varying levels of ascorbic acid supplemented diets during 28 days of the study. Cortisol was lower ($P<0.05$) in broilers fed control diet (6.00ng/ml) and 68 mg/kg ascorbic acid (4.80 ng/ml) and higher in broilers fed 136 (12.40 ng/ml) and 204 (11.90 ng/ml) mg ascorbic acid supplemented group. Numerical increase level of thyroxine was recorded in broilers fed 68 (50.51 nmol/L) and 204

(46.92 nmol/L) mg/kg ascorbic acid , although there was a decrease in broilers fed 136 mg (35.88 nmol/L) ascorbic acid supplemented diet compared to the control group (38.36 nmol/L).

Table 4.14: Effect of Ascorbic acid Supplementation on Performance of Broiler Starter Chickens (day 1-28)

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Initial weight (g/bird)	39.85	40.63	41.10	42.50	0.72	0.1178
Daily feed intake (g/b/d)	43.70	42.27	45.76	44.65	1.93	0.6350
Daily weight gain (g/b/d)	31.33	30.27	29.42	28.79	1.01	0.3525
Final weight (g/bird)	916.98	888.03	864.76	848.48	27.74	0.3684
Feed conversion ratio	1.39 [a]	1.40 [a]	1.56[b]	1.55[b]	0.04	0.0143
Daily water intake (ml/b/d)	128.07	127.50	123.19	136.01	6.50	0.5840
Feed cost/kg gain (₦/kg)	166.06	179.99	213.14	225.01	-	-
Mortality (%)	4.69	6.25	4.69	10.94	2.89	0.4066

[a,b] Means with different superscript on the same row differ significantly (P<0.05).

Table 4.15: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Starter Chickens (Day 28)

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
PCV (%)	25.00	25.13	28.25	29.00	1.71	0.2399	24.00-40.00[w]
Haemoglobin (g/dl)	8.26	8.34	9.39	9.60	0.58	0.2493	7.00-15.00[w]
Erythrocytes ($x10^{12}$/l)	4.16	4.18	4.53	4.78	0.29	0.3894	1.59-4.10[w]
Leucocytes ($x10^{9}$/l)	13.08	12.16	12.75	10.68	1.18	0.4969	1.90-9.50[x]
Heterophils (%)	17.25	14.50	15.38	17.25	2.18	0.7653	15.00-40.00[x]
Lymphocytes (%)	80.88	84.00	83.88	80.25	2.18	0.4985	40.00-100.00[y]
H:L	0.22	0.18	0.19	0.22	0.03	0.7811	-
Monocytes (%)	0.38	1.38	0.38	1.38	0.68	0.5476	1.00-7.00[z]
Eosinophils (%)	0.25	0.00	0.38	0.63	0.31	0.5520	1.50-6.00[x]
Bands (%)	1.25	0.13	0.00	0.63	0.39	0.1212	-

PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993, H:L= Heterophils-lymphocytes ratio

Table 4.16: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Starter Chickens (Day 28)

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
Glucose (mg/dL)	193.95	211.50	203.40	252.90	19.65	0.2103	137-363[w]
Total Protein (g/dL)	3.65	4.08	4.70	4.48	5.06	0.4646	3.60-5.50[x]
Albumin (g/dL)	2.25	2.78	2.50	2.95	0.32	0.4526	1.10-2.20[x]
Globulin (g/dL)	1.40	1.30	2.20	1.53	5.18	0.4722	1.20-3.20[y]
Cholesterol (nmol/L)	53.25	89.50	94.25	46.00	16.14	0.1258	120-237
Low Density Lipoprotein (mg/dL)	73.25[b]	111.00[a]	123.50[a]	55.25[b]	13.40	0.0119	<130.00
Triglyceride (mg/dL)	58.00	38.50	62.75	71.75	12.66	0.3415	<135.00
Alanine-Amino Transferase (µ/L)	14.75[c]	8.25[c]	55.50[a]	33.25[b]	3.59	0.0001	-
Aspartate-Amino Transferase (µ/L)	13.00	34.25	29.00	17.50	5.59	0.0663	10-40[y]
Alkaline Phosphatase (µ/L)	23.50	43.50	28.00	38.75	5.93	0.1145	10-106[z]
Glutathione Peroxidase (µmol/mL)	1.64	2.56	1.82	1.79	0.37	0.3270	
Superoxide Dismutase (µmol/mL)	3.60	1.50	3.90	4.35	0.84	0.1327	
Malondialdehyde (nmol/mL)	60526.00	24138.00	28184.00	17452.00	14008.82	0.1886	
Catalase (U/ml)	3.78	4.39	9.45	7.49	1.69	0.1108	
Calcium (mg/dL)	8.55	6.45	9.25	7.55	1.67	0.6673	
Phosphorus (mg/dL)	2.45	2.75	2.85	2.40	0.36	0.7652	

[abc] Means with different superscript on the same row differ significantly (P<0.05), Reference values: [w]Goodwin *et a*l. (1994), [x]Ross *et al*. (1976), [y]LAVC (2009), [z]Bounous and Stedman (2000), Clinical Diagnostic Division (1990), Collins (2018).

4.2.7: Effect of Ascorbic acid Supplementation on Performance of Broiler Finisher Chickens (day 29-49)

The growth performance of finisher broiler chickens fed varying levels of ascorbic acid is presented in Table 4.17. Daily weight gain, final weight, daily feed intake, feed conversion ratio, daily water intake and mortality were similar (P>0.05) for all treatment groups. Chickens fed 68 mg/kg ascorbic acid diet had the least mortality record. Birds fed 68 mg/kg ascorbic acid diet had least feed cost per kg gain.

4.2.8: Effect of Ascorbic Acid Supplementation on Performance of Broiler Chickens (day 1-49)

The growth performance of broiler chickens fed varying levels of ascorbic acid from day 1-49 is presented in table 4.18. All the parameters measured were similar except for feed cost per kg gain. Feed cost per kg gain ranged from 207.83 to 253.29,birds fed the control diet and 68 mg/kg ascorbic acid diet had least (P<0.05) feed cost per kg gain than the other groups. Chickens fed 68 mg/kg ascorbic acid diet had the least mortality record.

4.2.9: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Finisher Chickens (Day 49)

Table 4.19 shows the haematological parameters of heat stressed broiler chickens fed varying levels of ascorbic acid supplemented diets on day 49 of the study. All the parameters measured were similar (P>0.05). PCV and haemoglobin decreased numerically with increase in ascorbic acid supplementation, ranging from 26.88 – 30.71% and 8.51 – 10.21 g/dL respectively. Broilers fed the control diet had higher numerical PCV (30.71%) value, haemoglobin (10.21 g/dL) and eosinophils (0.86%). Leucocytes ranged from 10.14 – 13.16 $x10^9$/L and was numerically higher in chickens fed 204 mg/kg ascorbic acid diet. Broilers fed 68 mg ascorbic acid supplemented diet

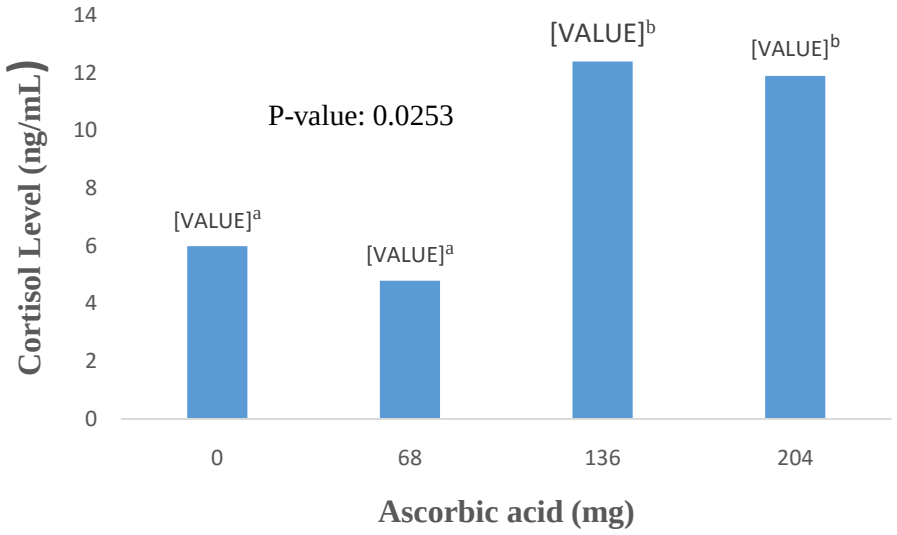


Fig. 4.15: Effect of varying levels of Ascorbic acid on Cortisol level of Broiler Starter Chickens (Day 28)

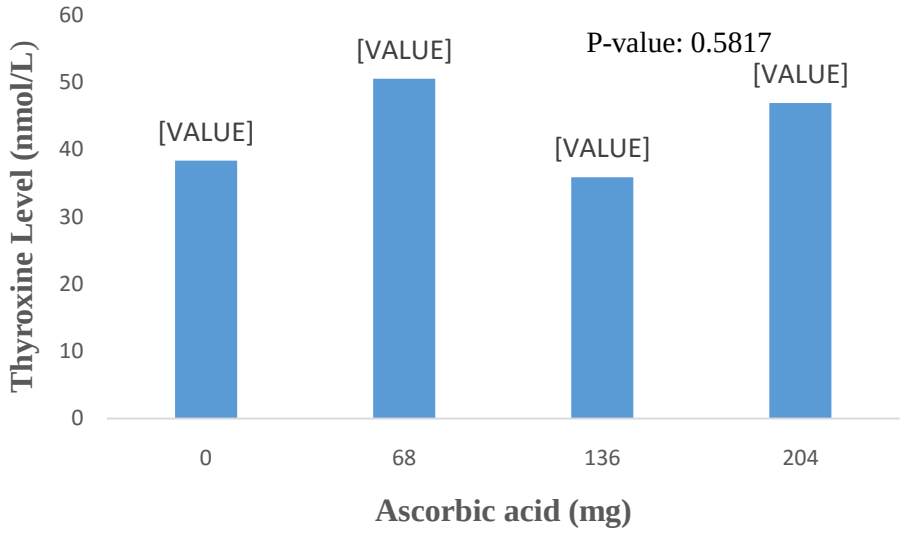


Fig. 4.16: Effect of varying levels of Ascorbic acid on Thyroxine level of Broiler Starter Chickens (Day 28)

Table 4.17: Effect of Ascorbic acid Supplementation on Performance of Broiler Finisher Chickens (day 29-49)

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Initial weight (g/bird)	916.98	888.03	864.76	848.48	27.74	0.3684
Daily feed intake (g/b/d)	133.08	127.51	122.12	133.09	5.61	0.4795
Daily weight gain (g/b/d)	61.44	64.61	64.55	66.66	2.71	0.6071
Final weight (g/bird)	2207.20	2245.00	2220.40	2248.40	73.87	0.9740
Feed conversion ratio	2.17	1.98	1.89	2.00	0.08	0.1292
Daily water intake (ml/b/d)	426.55	435.85	409.77	473.49	22.60	0.2840
Feed cost/kg gain (₦/kg)	242.92	238.97	245.22	276.65	-	-
Mortality (%)	6.25	1.56	9.38	6.25	2.16	0.1388

Table 4.18: Effect of Ascorbic acid Supplementation on Performance of Broiler Chickens (day 1-49)

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Initial weight (g/bird)	39.85	40.63	41.10	42.50	0.72	0.1178
Daily feed intake (g/b/d)	82.01	78.82	78.48	82.55	3.30	0.7493
Daily weight gain (g/b/d)	44.23	44.99	44.48	45.02	1.52	0.9773
Final weight (g/bird)	2207.20	2245.00	2220.40	2248.4	73.87	0.9740
Feed conversion ratio	1.85	1.75	1.77	1.83	0.05	0.4686
Daily water intake (ml/b/d)	255.99	259.65	246.01	280.63	12.95	0.3305
Feed cost/gain (₦/kg)	207.83[a]	211.71[a]	228.82[b]	253.29[c]	6.7376	0.0018
Mortality (%)	10.94	7.81	14.06	17.19	4.33	0.4837

[a,b,c] Means with different superscript on the same row differ significantly (P<0.05)

had numerically increased erythrocytes (4.93 $x10^{12}$/L) and lymphocytes (82.83%) with a decrease in heterophils (15.00%) and H:L (0.18). Monocyte and band cells range from 0.14 – 2.00% and 0.29 – 2.14%.

4.2.10: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Finisher Chickens (Day 49)

Serum indices of starter broiler chickens fed diet supplemented with varying levels of ascorbic acid is presented in Table 4.20. All the parameters measured were similar except LDL and GSHPx. Broilers fed diet supplemented with 204 mg of ascorbic per kg had numerically higher glucose (175.95 mg/dL), ALT (57.50µ/L), AST (52.00µ/L), globulin (2.30 g/dL), cholesterol (132.75 nmol/L). LDL ranged from 56.00 – 198.75 mg/dL, chickens fed the control diet and diet supplemented with 136 mg of ascorbic acid had higher ($P<0.05$) LDL. Triglyceride, ALT, AST and ALPranged from 44.00-96.25 mg/dL, 23.75-57-50µ/L, 23.00-52.00µ/L and 44.50-68.50µ/L. Broilers fed 68 mg and 136 mg of ascorbic acid supplemented diet had higher ($P<0.05$) GSHPx. SOD, MDA amd CAT ranged from 4.05-5.55µmol/mL, 4686-20548 nmol/mL and 4.15-6.10 U/mL and calcium and phosphorus ranged from 9.03-11.85 mg/dL and 4.07-5.95 mg/dL.

4.2.11: Effect of Ascorbic Acid Supplementation on Serum Cortisol and Thyroxine level of BroilerFinisher Chickens (day 49)

The result of cortisol and thyroxine levels of broiler chickens fed varying levels of ascorbic acid supplemented diets on day 49 of the study is presented in Figures 4.17 and 4.18. Numerically decreased ($P>0.05$) cortisol level was observed in broilers fed diet supplemented with 68 mg/kg ascorbic acid, while 136 and 204 mg ascorbic acid supplemented group had slightly numerically higher cortisol level than the control group. Cortisol ranged from 6.80 – 8.00 ng/mL. Thyroxine

Table 4.19: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Finisher Chickens (Day 49)

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
PCV (%)	30.71	29.83	28.86	26.88	1.31	0.2456	24.00-40.00[w]
Haemoglobin (g/dl)	10.21	9.92	9.59	8.51	0.44	0.0705	7.00-15.00[w]
Erythrocytes ($x10^{12}$/l)	4.83	4.93	4.73	4.56	0.24	0.7658	1.59-4.10[w]
Leucocytes ($x10^{9}$/l)	11.56	10.73	10.14	13.16	1.32	0.4479	1.90-9.50[x]
Heterophils (%)	20.00	15.00	18.43	15.88	2.16	0.4276	15.00-40.00[x]
Lymphocytes (%)	78.71	82.83	76.43	82.50	2.06	0.1496	40.00-100.00[y]
H:L	0.26	0.18	0.25	0.20	0.03	0.4026	-
Monocytes (%)	0.14	0.50	2.00	0.75	0.57	0.1826	1.00-7.00[z]
Eosinophils (%)	0.86	0.33	0.57	0.25	0.32	0.5861	1.50-6.00[x]
Bands (%)	0.29	1.33	2.14	0.63	0.57	0.1619	-

PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993, H:L= Heterophils-lymphocytes ratio.

Table 4.20: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Finisher Chickens (Day 49)

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
Glucose (mg/dL)	134.55	168.30	135.90	175.95	15.69	0.1861	137-363[w]
Total Protein (g/dL)	4.75	4.58	3.88	4.45	0.38	0.4265	3.60-5.50[x]
Albumin (g/dL)	2.75	2.70	2.68	2.15	0.37	0.6413	1.10-2.20[x]
Globulin (g/dL)	2.00	1.88	1.20	2.30	0.45	0.3974	1.20-3.20[y]
Cholesterol (nmol/L)	130.25	123.25	92.75	132.75	13.74	0.2003	120-237
Low Density Lipoprotein (mg/dL)	198.75[b]	81.00[a]	192.25[b]	56.00[a]	35.47	0.0270	<130.00
Triglyceride (mg/dL)	96.25	70.50	86.25	44.00	18.85	0.2743	<135.00
Alanine-Amino Transferase (µ/L)	34.75	41.75	23.75	57.50	12.88	0.3500	-
Aspartate-Amino Transferase (µ/L)	23.00	33.00	31.25	52.00	9.09	0.1974	10-40[y]
Alakaline Phosphatase (µ/L)	68.50	58.00	44.50	48.50	8.29	0.2269	10-106[z]
Glutathione Peroxidase (µmol/mL)	1.24[c]	2.14[ab]	2.53[a]	1.84[b]	0.28	0.0405	
Superoxide Dismutase (µmol/mL)	4.05	4.05	4.80	5.55	0.83	0.5413	
Malondialdehyde (nmol/mL)	14132.00	4686.00	20548.00	10280.00	5877.21	0.3234	
Catalase (U/ml)	5.49	4.15	6.10	4.39	1.19	0.6299	
Calcium (mg/dL)	11.85	9.03	10.73	11.05	2.23	0.8347	
Phosphorus (mg/dL)	5.50	4.70	5.23	5.95	0.78	0.7210	

[abc] Means with different superscript on the same row differ significantly (P<0.05), Reference values: [w]Goodwin *et a*l. (1994), [x]Ross *et al*. (1976), [y]LAVC (2009), [z]Bounous and Stedman (2000), Clinical Diagnostic Division (1990), Collins (2018).

wasnumerically higher (P>0.05) in broilers fed the control diet than the ascorbic acid supplemented group and range from 24.29 – 30.36 nmol/L.

4.2.12: Effect of Ascorbic acid Supplementation on Calcium, Phosphorus and Ash content in Bone and Faeces of Broiler Chickens

Table 4.21 shows the percentage of calcium, phosphorus and ash of tibia bone and faeces of broiler chickens. Calcium ranged from 25.58 – 50.00 mg/dL. Phosphorus content of bone ranged from 9.47 – 11.08 mg/dL, broilers fed the control diet and diet supplemented with 68 mg and 136 mg of ascorbic acid had a higher (P<0.05) phosphorus content in their bones. Similarly, the ascorbic acid supplemented groups had higher (P<0.05) ash content in their bones than the control group.Fecal deposition of calcium was higher (P<0.05) in broilers fed 204 mg of ascorbic acid supplemented diet while fecal phosphorus was similar (P>0.05) for all groups.

4.2.13: Effect of Ascorbic Acid Supplementation on Carcass Cut Parts and Organ Weight of Broiler Chickens

The carcass cut parts and organ weights of broiler chickens fed varying levels of ascorbic acid supplemented diets is presented in Table 4.22. Carcass cut parts and organ weight were similar (P>0.05). Broilers fed the control diet, 68 mg and 136 mg of ascorbic acid supplemented diet had higher (P<0.05) weight of wings, wing weights range from 9.80 – 10.76%.

4.2.14: Effect of Ascorbic acid Supplementation on Jejunum Mucosal Morphology of Broiler Chickens

The jejunum mucosal morphology of broiler chickens fed varying levels of ascorbic acid supplemented diet is presented in Table 4.23. Villus height, width, perimeter and villus height/crypt depth were similar (P>0.05). Crypt depth range from 79.45 – 118.27 (μm) and villus

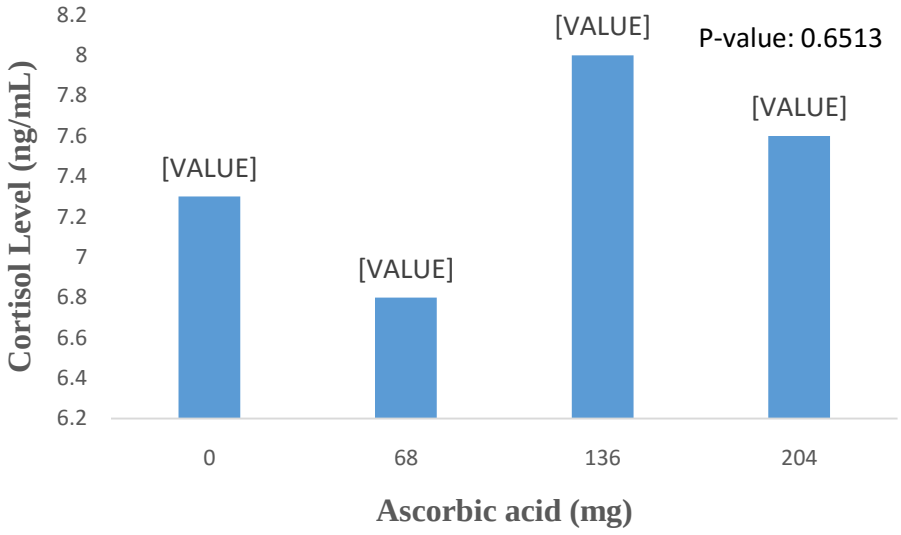


Fig. 4.17: Effect of varying levels of Ascorbic acid on Cortisol level of Broiler Finisher Chickens (Day 49)

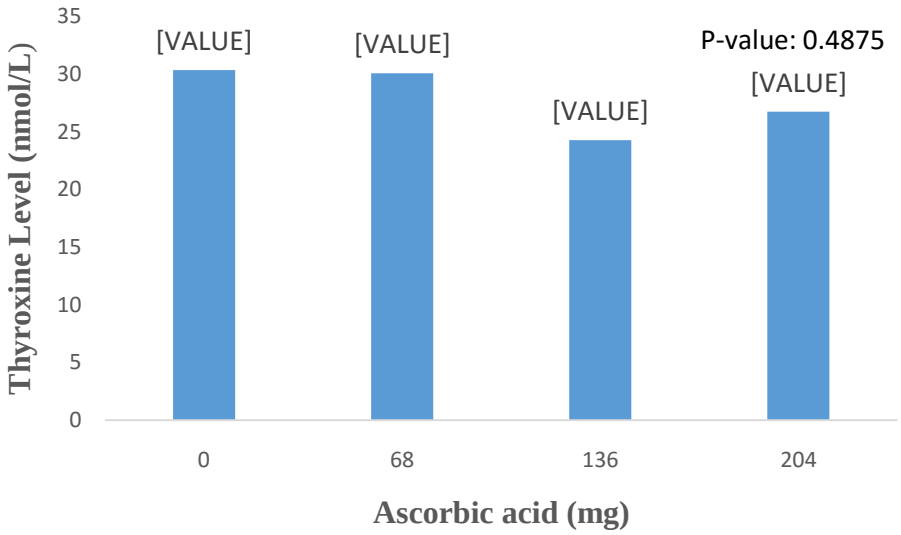


Fig. 4.18: Effect of varying levels of Ascorbic acid on Thyroxine level of Broiler Finisher Chickens (Day 49)

Table 4.21: Effect of Ascorbic acid Supplementation on Calcium, Phosphorus and Ash Content in Bone and Faeces of Broiler Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters (%)	**0**	**68**	**136**	**204**	**SEM**	**P value**
Bone						
Calcium	25.58	29.58	42.50	50.00	8.24	0.1854
Phosphorus	10.51[a]	10.46[a]	11.08[a]	9.47[b]	0.36	0.0509
Ash	0.32[b]	0.37[a]	0.40[a]	0.39[a]	0.02	0.0179
Faeces						
Calcium	14.50[c]	19.58[b]	18.75[b]	24.17[a]	1.51	0.0059
Phosphorus	0.93	1.01	0.97	0.88	0.07	0.5650

[abc] Means with different superscript on the same row differ significantly (P<0.05).

area range from 8665 – 22451(μm^2). Crypt depth (118.27μm) and villus area (22451μm^2) were higher (P<0.05) in chickens fed diet supplemented with 204 mg of ascorbic acid.

4.2.15: Effect of Ascorbic acid Supplementation on Tibia Measurements of Broiler Chickens

Tibia geometry measurements of broiler chickens fed varying levels of ascorbic acid is presented in Table 4.24. Tibia weight and length was similar (P>0.05) for all groups. Chickens fed the control diet had higher tibia weight/length index (0.75 g/cm) while birds fed diets supplemented with 136 mg/kg ascorbic acid diet (4.55 cm/g$^{1/3}$) and 204 mg/kg ascorbic acid diet (4.71 cm/g$^{1/3}$) had higher robusticity index.

Table 4.22: Effect of Ascorbic Acid Supplementation on Carcass Cut Parts and Organ Weight of Broiler Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Live weight (g/b)	2343.75	2300.00	2268.75	2350.00	45.17	0.5477
Dressed weight (g/b)	1699.38	1651.50	1672.88	1696.38	34.18	0.7330
Dressing percent (%)	72.54	71.99	73.72	72.25	1.23	0.7643
Cut parts expressed as percentage of dressed weight (%)						
Breast	34.28	32.45	35.86	33.91	0.95	0.1107
Back	12.29	12.87	12.48	13.06	0.33	0.3618
Thigh	17.14	16.56	16.51	16.05	0.44	0.3897
Drum Stick	13.61	13.51	12.80	13.37	0.29	0.2072
Wing Weight	10.49[a]	10.76[a]	10.65[a]	9.80[b]	0.16	0.0007
Organs weights expressed as percentage of live weight (%)						
Liver	1.64	1.80	1.60	1.78	0.11	0.4667
Gizzard	1.75	1.75	1.75	1.84	0.08	0.8235
Heart	0.38	0.40	0.35	0.36	0.02	0.1907
Kidney	0.55	0.58	0.53	0.48	0.03	0.1905
lungs	0.58	0.63	0.58	0.59	0.04	0.7718
Intestine	2.09	2.14	2.19	2.08	0.05	0.4078
Spleen	0.06	0.06	0.05	0.06	0.01	0.8598
Thymus	0.19	0.24	0.21	0.18	0.02	0.2110
Bursa of Fabricius	0.03	0.03	0.02	0.02	0.00	0.3618

[ab] Means with different superscript on the same row differ significantly (P<0.05).

Table 4.23: Effect of Ascorbic Acid Supplementation on Jejunum Mucosal Morphology of Broiler Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Villus height (μm)	311.71	257.07	314.44	271.28	33.36	0.5295
Villus width (μm)	82.38	78.78	90.79	143.14	24.73	0.2351
Crypt depth (μm)	98.14^{b}	79.45^{c}	98.05^{b}	118.27^{a}	6.87	0.0032
Villus area (μm^2)	11914^{b}	8665^{b}	14014^{b}	22451^{a}	3112.39	0.0212
Villus perimeter (μm)	699.49	613.85	721.11	740.03	62.97	0.5101
Villus height/Crypt depth ratio	3.32	3.18	3.47	2.30	0.38	0.1410

^{abc} Means with different superscript on the same row differ significantly ($P<0.05$).

Table 4.24: Effect of Ascorbic Acid Supplementation on Tibia Measurements of Broiler Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)					
Tibia compositions	**0**	**68**	**136**	**204**	**SEM**	**P value**
Tibia Weight (g)	5.47	5.04	4.89	4.79	0.29	0.2155
Tibia Length (cm)	7.28	7.38	7.40	7.48	0.14	0.7967
Tibia Weight/Length Index (g/cm)	0.75^{a}	0.68^{b}	0.66^{bc}	0.64^{c}	0.02	0.0256
Robusticity Index ($cm/g^{1/3}$)	4.01^{a}	4.41^{ab}	4.55^{bc}	4.71^{c}	0.15	0.0283

^{abc} Means with different superscript on the same row differ significantly ($P<0.05$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 The Effect of Dietary Selenium in Ameliorating Heat Stress in Broiler Chickens

5.1.1 Thermoregulatory Parameters

Heat is one of the most important stressors affecting poultry production, leading to high loss of investment on capital each year because modern broiler breeds are more susceptible to heat stress than earlier genotypes, high ambient temperature have been observed to reduce feed intake, live weight gain, feed efficiency, and immune response of broilers chickens (Melesse *et al*., 2011). The results obtained in this study showed that THI in the afternoon were higher by 24.68% than THI in the morning which revealedreduced heat stress in the morning than in the afternoon.

Under heat stress, normal physiological behaviours of birds were observed to be altered such as lying down, increase their respiratory rate, heart rate, body temperature and spread their wings in an attempt to lose heat to the environment (Lara and Rostagno, 2013). The rectal and body temperatures that were observed to have increased with increased in selenium supplementation which revealed that selenium supplementation up to 0.3 mg/kg did not reduce the rectal temperature of the chickens. Similarly, the respiration rate of chickens increased when dietary supplementation of selenium was above 0.1 mg/kg and could be due to saturation effect of GSHPx at levels above 0.1 mg of selenium. Thermoregulatory parameterswere within normal range and are in accordance with the report of Majid *et al*. (2018). During severe heat stress, chickens cannot dissipate their body heat and physiological disorders appear following multi-organ dysfunction which can lead to death (Sohali *et al*., 2012). However, the body temperatures observed among broilers could be due to the fact that

the feathers contributed to the regulation of the thermal equilibrium. According to Melo *et al.* (2016), the bird parts covered with feathers favour some thermal insulation and make it difficult to exchange heat with the medium. The result of the body temperature observed during the study is in accordance to the report of Adrian and Dumitru (2017) who reported no significant difference in the body temperature of broiler chickens fed diet supplemented with 0.5 ppm of organic selenium. Chickens, as homeotherms, maintain constant body temperature within the thermoneutral zone (TNZ) of 18 to 24°C (Cahaner *et al.*, 2008; Soleimani *et al.*, 2008). This, however, requires the loss of excessive body heat and heat exchange could be assessed directly from physiologicalmeasurements, e.g. rectal and skin temperatures, respiratory rate, panting and heat production. Rectal temperature, respiratory rate and heart rate can be used, in conjunction with temperature-humidity index (THI) as indices of heat stress in birds. The THI is an example of indices developed to assess the impact of the thermal environment on the thermoregulatory status of animals (Purswell *et al.*, 2012), from the THI of the present study it is pertinent to say that the birds were under severe stress especially during the afternoon which may be due to the increased thermal insulation of the building.

5.1.2 Effect of Selenium Supplementation on Performance of Broiler Starter Chickens (day 1-28)

Under stress, birds tend to change their behavior such as reduced feed intake and increased water intake to ensure their survival in the face of adversity (Lara and Rostagno, 2013). The higher water consumption that was recorded at 0.2 mg selenium supplementation could be due to intestinal health of the birds as selenium has potency in maintaining the integrity of the intestinal mucosa, improved assimilation of water via the intestine and reduced stress (Jan-Dirk and Loek, 2013). The higher numeric values for daily feed intake, daily weight gain,

final weight, FCR and zero mortality that was observed for birds fed 0.2 mgSe/kg could be due to their higher ability to combat heat stress and return to homeostasis. Yang *et al*. (2012) reported that inorganic selenium increased survival rate than the organic forms of selenium and could be due to the passive diffusion of the inorganic selenium.

5.1.3: Effect of Selenium Supplementation on Haematological Parameters of Broiler Starter Chickens (day 28)

Blood is a pertinent and reliable medium for determining the physiological and well-being of various animals (Egbe-Nwiyi *et al.,* 2000). The value of PCV obtained in this study fell within the normal physiological range and is an indication that the diet was not toxic to the chickens (Isikwenu and Omeje, 2007). The higher value of PCV and Hb observed in broilers fed diets supplemented with 0.3 mg of selenium per kg diet agrees with the report of El-Sheikh *et al*. (2010) who reported that supplementation of broilers diet with organic selenium at 0.2 and 0.3 ppm significantly increased Hb concentration. Erythrocytes and leucocyte counts were slightly above the normal range but monocytes and eosinophils were below the normal range. This could be due to underlying disease conditions or evidence of heat stress. Altan *et al.* (2000) concluded that a temperature high enough to cause increased body temperature also changes circulating leucocyte components in chickens. PCV and Hb are indicators of erythrocyte integrity, the higher PCV and Hb in broilers fed diet supplemented with 0.3 mgSe/kg is an indication of proper erythrocyte production and improved oxygen carrying capacity of the blood (Kuhn *et al*., 2017). The similarity among the haematological parameters measured during the study is in line with the report of Selim *et al*. (2015) who fed different forms and varying levels of selenium (0.15-0.3mg/kg of diet) to broiler birds. The increase in erythrocytes observed during the study could be due to polycythemia vera (a blood

disorder in which the bone marrow produces too many red blood cells) (Raduta *et al*., 2011) or heat stress. Leucocyte and erythrocyte are indicators of the immune integrity of an animal as they protect the animal during adverse conditions and improve the health statues of an animal. Selenium has an antioxidant effect on the red blood cells membrane, it prevents the degradation of mature erythrocytes (Selim *et al*., 2015), and hence selenium may have aided more erythrocytes production in the birds during this study.

H:L is used as an indication of physiological stress in poultry. It has been reported that during conditions of extreme stress as seen in the THI value of this study, heteropenia and basophilia may develop, the H/L ratio cannot be used as a reliable measurement of stress (Altan *et al.*,2000). The heterophil, H:L and monocyte observed in this study was not consistent but were within the normal physiological range (Jain, 1986; Simrak *et al*., 2004) and is in contrast with the report of Abdelhady *et al.* (2017) who observed a significant difference in heterophil, H:L and monocytes of quails subject to heat stress and fed diets supplemented with 0.3 mg of selenium only or in combination with 1400 µg of Chromium. Lymphocyte (%) was within the normal physiological range and similar across the treatment groups and is in contrast to the report of Selim *et al*. (2015) who observed a significant difference in the lymphocyte of broiler birds when their diet was supplemented with 0.15 and 0.3 ppm of selenium. The result of eosinophil obtained in this study was below the normal range reported by Simrak *et al*. (2004). The low eosinophil count could be due to the period of the day when the blood samples was collected, as this blood component is known to be low at morning and high at evening and is not considered as a threat if other leucocyte component are normal (Corinna, 2018),the result of eosinophilis in contrast to the report of Abdelhady *et al.* (2017) who

observed a significant difference in eosinophil of quails subject to heat stress and fed selenium and Chromium supplemented diets.

5.1.4: Effect of Selenium Supplementation on Serum Biochemical Indices of Broiler Starter Chickens (Day 28)

Broilers fed the control diet had higher numeric values of glucose, ALT, cholesterol and calcium which agrees with the work of El-Samra *et al.* (2014) who fed laying hens with 0, 0.3 and 0.5 mg of organic selenium supplemented diet,he also reported a higher ($P<0.05$) value of albumin in layers fed the control diets.

Metabolic alterations in poultry during stress seems to cause mobilization or synthesis of glucose for energy required to maintain homeostasis during stress (Ademu, 2018). The glucose value recorded in the selenium treated groups was within normal physiological range and is an evidence that selenium exhibited its antioxidant property in ameliorating heat stress in broilers fed the selenium supplemented diets.Under conditions of chronic stress, there is synthesis of higher blood glucose level (Lin *et al.,* 2007). Also, the similarity in glucose observed in the selenium treated groups showed that selenium mitigated the effect of heat stress, hence glucose remained unchanged. According to the current result, supplemented dietary selenium alleviated the adverse effect of heat stress by lowering the activity of ALT in the blood plasma thereby promoting protein synthesis than cellular energy production. El-Mallah *et al.* (2011) observed a beneficial effect of selenium yeast on ALT by lowering its level. Abdelhady *et al.* (2017) observed that plasma cholesterol and triglyceride concentration was significantly increased in heat stressed quails.Cholesterol and triglyceride were within the normal range, cholesterol was lower in the selenium supplemented groups and can be attributed to its role in down-regulating fat metabolism. Changes in enzymes responsible for

regulating cholesterol synthesis, oxidation or elimination may be responsible for lowering the cholesterol synthesis in mature as well as immature chickens (El-Samra, 2014). The similar results obtained for total protein and globulin agrees with the report of Salem *et al.* (2018) who supplemented selenium and zinc in the drinking water of broilers.

Antioxidant enzymes and MDA are involved in the regulation of the activities of free radicals (Dalia *et al*., 2017). The values of biomarkers (GSHPx, SOD, MDA and CAT) observed agrees with the report of Guoshun *et al.* (2013). The antioxidant potential of selenium was generally influenced by GSHPx because selenium is an active component of this enzyme, GSHPx activity depends on the selenium content of a feed. About 30-40% of selenium exists in the form of GSHPx in animal body tissue, and many animal diseases and dysfunction are caused by GSHPx deficiency (Pilarczyk *et al.*, 2012). The higher values of GSHPx recorded with selenium treated groups revealed that selenium is an important component in GSHPx formation and that the birds were stressed.

The values of AST and LDH that were below the normal range might be due to free radical production, which induced oxidative damage to cellular membranes and lipid peroxidation which caused hepatocellular injury and release of intracellular enzymes (Tan *et al*., 2010)**.** In the present study, triglycerides, total proteins and albumin concentrations were within the normal range.

5.1.5: Effect of Selenium Supplementation on Cortisol and Thyroxine level of Broiler Starter Chickens (day 28)

Glucocorticoids (cortisol, corticosterone, dexamethasone, prednisolone) are released from the adrenal cortex in response to stress, which affects metabolic pathways, immune function, and

endocrine systems (Fan *et al*., 2009; Shokraneh *et al.,* 2019). The decreased level of cortisol in broilers fed 0.1 and 0.2 mg supplemented diet is an indication of the antioxidant role of selenium against ROS and pro-oxidants during heat stress. This is consistent with Shokraneh *et al.* (2019) who reported that in-ovo injection of 40 µg of nano selenium in the eggs of broiler during late incubation decreased the level of corticosterone and cortisol. Similarly, Fan *et al.* (2009) reported a decrease in corticosterone level in broilers fed diets supplemented with 0.1 and 0.4 mg of sodium selenite compared to the control. The high level of cortisol in broilers fed 0.3 mgSe/kg diet might be due to a pro-oxidant effect of selenium caused by some chemical interaction with meal ions. Antioxidants or polyphenols can act as pro-oxidants under certain conditions, such as the presence of metal ions (potassium, sodium, calcium, iron, zinc, magnesium), the concentration of antioxidant in matrix environment and its redox potential (Sotler *et al.,* 2019).

Thyroid hormone plays a significant role on metabolism and growth (Fan *et al.,* 2009; Shokraneh *et al.,* 2019). Preter (2000) revealed that selenium deficiency can lead to a decrease in T3 synthesis and this may be due to the fact that selenium is an important auxiliary factor for the key enzyme of 5- deiodinase. The iodothyroxine deiodinase enzymes convert the pro-hormone thyroxine (T4) to the active form triiodothyronine (T3) (Selim *et al*., 2015). T3 is the main hormone that regulates growth by controlling the body's energy and protein anabolism (Preter, 2000) and protect the thyroid gland from oxidative stress. The higher thyroxine level observed in selenium supplemented group is an indication that selenium protects thyroid gland (site for thyroxine production) from ROS and improve growth and this is further justified by the higher level of thyroxine in broilers fed 0.3 mg supplemented diet. The result

of thyroxineobserved in this study is consistent with Fan *et al*. (2009) and El-Samra *et al.* (2014) but in contrast to Shokraneh *et al.* (2019).

5.1.6: Effect of Selenium Supplementation on Performance of Broiler Finisher Chickens (day 29-49)

Selenium is the cofactor and activator of 5'deiodinase a key enzyme of Triiodothryonine (T3) synthesis, and T3 isa growth control components of animals particularly poultry by controlling the body's energy and protein assimilation, and thus could regulate animal growth (Ozbal *et al.*, 2008). The similarity recorded for daily feed intake, daily weight gain, final weight, feed conversion ratio, daily water intake, feed cost/kg and mortality is in agreement with the work of Guoshun*et al*. (2013) who fed finisher broiler chickens with 0.3-2 mg/kg of selenium yeast.

5.1.7: Effect of Selenium Supplementation on Performance of Broiler Chickens (day 1-49)

The similarity observed in growth performance of broiler chickens throughout the experimental period could be due to the ability of broiler chicken to adapt and return to homeostasis during prolong (chronic) stress. The result obtained in this study is in agreement with the report of Guoshun*et al.* (2013) and Bakhshalinejad *et al*. (2018). The mortality observed could be due to chronic respiratory disease, Newcastle disease and severe heat stress as shown by the report of the post mortem examination. Diets are shown to be safe for poultry when they contain 0.15 - 4.0 mg/kg selenium (NRC, 1994).

5.1.8: Effect of Selenium Supplementation on Haematological Parameters of Broiler Finisher Chickens (day 49)

The increased PCV, Hb and erythrocytes that was observed in the present study at 0.1 mg/kg selenium supplementation can be attributed to increased synthesis, stability, and activity of enzymes involved in haematopoietic pathway and improved antioxidant and immune status of the birds (Shlig, 2009; Raza*et al.,* 2018). It could also be due to the recommended optimum selenium requirement (0.1 mgSe/kg) that was met in the diet although 0.4 mgSe/kg is also adequate for broiler chickens(NRC, 1994). The result of these parameters agrees with the report of Raza*et al*. (2018) who fed inorganic selenium at 0.15-0.45 mg/kg to broiler chickens under heat stress and recorded significant difference in Hb and RBC but is in contrast with the report of (Okunlola *et al*., 2015). The significance observed in PCV, Hb and erythrocyte among the different groups agrees with the report of El-Samra *et al.* (2014); Raza *et al*. (2018), who supplemented the diet of layers and broiler chickens with selenium. PCV and Hb were within the normal physiological range described by Mitruka and Rawnsely (1997).

Lymphocytes, monocytes, eosinophils and basophils are components of the leucocytes and depict the immune status of an animal and a deviation from the normal range could be as a result of disease condition or stress (Simrak *et al*., 2004). The higher leucocyte and monocyte observed at 0.2 mg selenium supplementation agrees with the report of Raza *et al*. (2018). Leucocyte was slightly above the normal range described by Simrak *et al*. (2004) which could be as a result of chronic respiratory disease (CRD), leukemia and severe stress as evidence by the THI of this study.

At the finisher phase, increasing Se concentration from 0.1-0.3 mg/kg decreased heterophils (%), H:L ratio and increased lymphocytes which is an indication of the antioxidant property of Se against heat stress. Yalçýn *et al*. (2003) reported an increased H:L ratio in broiler chicken exposed to heat. The H:L ratio is said to be a reliable indicator of stress in poultry, because during environmental or physiological stress the number of lymphocytes decreases and the heterophils and H:L increases (Gross and Siegel, 1983), the result of this study is also in accordance with the report of Ademu (2018). Habibian *et al*. (2014) reported a non-significant difference in the percentages of heterophils and lymphocytes and the H:L ratio at day 32. The lower value of Heterophil and H:L observed at 0.3 mg of selenium supplementation agrees with the report of Selim *et al.* (2015) who reported a significant and lower value of these parameters in broilers fed 0.3 mg of selenium supplemented diet at 40 days of the study.

Birds fed corticosterone and ACTH supplemented diet had been observed to have decreasing values of lymphocytes with increase in these stressors in their diet (Gross and Seigel, 1983). Higher value of lymphocyte was recorded at 0.3 and agrees with the report of Raza*et al*. (2018) where a higher value of lymphocyte was observed with increase in selenium supplementation (0.15-0.45 mgSe/kg diet) in broiler diet. The values recorded for eosinophil was below the normal range which could be due to severe heat stress. Broilers given very little feed showed only a small decrease in eosinophil and monocyte numbers, while eosinophils disappear from circulation and basophils increase in circulation during stress, particulary acute stress (Altan *et al*., 2000). The higher numeric value of eosinophil in broilers fed 0.3 mg selenium supplemented diet agrees with the report of Abdelhady *et al*. (2017) who

reported a higher (P<0.05) value of eosinophil at 0.3 mg of selenium supplementation in the diet of quails.

5.1.9: Effect of Selenium Supplementation on Serum Indices of BroilerFinisher Chickens (Day 49)

Acute and chronic induced heat stress is a precussor of oxidative stress and causes metabolic changes. According to Yang *et al.* (2010), birds subjected to stress by high temperatures may have reduced activity of the mitochondrial respiratory chain, followed by increased production of reactive oxygen species (ROS). The higher numeric values observed for glucose, ALT, total protein, globulin and LDL in broilers fed diet supplemented with 0.2 mg of selenium could be due to an adequate antioxidant activity of selenium which lowered the rate of metabolic reactions. These result is similar to that of Salem *et al*. (2018) who supplemented the drinking water of broilers with sodium selenite and Zinc and reported no significant difference in these parameters. Many chemical and biochemical reaction rate increase with temperature via the accelerated metabolic reactions in the cells and tissues (Lin *et al.*, 2006b). Although AST, triglyceride and MDA were within normal range and similar in all treatments, broilers fed 0.1 mgSe/kg diet had higher values of AST and triglyceride with lower MDA while ALP and GSHPx were increased at 0.3 mgSe/kg diet.This agrees with the report of El-Samra *et al*. (2014) and Selim *et al.* (2015) who fed selenium supplemented diet to layers and broilers respectively and recorded higher AST, GSHPx and lower MDA at 0.3 mgSe/kg.

The value of cholesterol was within normal physiologic range. The higher cholesterol level observed in broilers fed the control diet and 0.1 mgSe/kg diet agrees with the report of

Abdelhady *et al*. (2017). Heat stress enhances lipid metabolism associated with the increase in serum total cholesterol and triglyceride (Hosseini-Mansoub *et al.,* 2010; Tawfeek *et al.,*2014). This rise in blood lipids under heat stress was explained by Rashidi *et al*. (2010) who pointed out that since heat stress reduced feed intake of broilers, the energy required by birds during this periodis derived from lipolysis. In addition, Hajati *et al*. (2016) reported that the higher levels of stress hormones due to heat stress stimulate lipolysis and increase circulating cholesterol and triglyceride levels.

Animal antioxidant system is greatly influenced by animal nutrition, and dietary Se supplementation is necessary to up-regulate the body's glutathione pool and its selenium containing antioxidant enzymes (Jiang *et al*., 2017). The higher values of SOD and CAT recorded for broilers fed 0.2 and 0.3 mgSe/kg and those fed the control diet and 0.3 mg of selenium per kg diet respectively agrees with the report of Dalia *et al*. (2017) who fed different forms of selenium to broiler chickens and recorded higher values for SOD and CAT in broilers fed diet supplemented with 0.3 mg of sodium selenite. The increased SOD and CAT observed with selenium supplementation at 0.3 mg could be due to the antioxidant property associated with selenium, this is in accordance with the report of Selim *et al*. (2015) who supplemented the diet of broilers with 0.15 and 0.3 mg of selenium.

The significant differences in the values of phosphorus reported in this study agrees with the report of El-Samra *et al.* (2014). Shahid *et al*. (2018) reported that 0.2 mgSe/kg supplementation improved antioxidant capacity during heat stress and the higher level of serum phosphorus observed in broilers fed 0.2 mgSe/kg could be due to the ability of the birds to maintain homeostasiswhich could have elevated circulating serum phosphorus for bone

mineralization.Usayran (2001) reported a decrease in concentration of plasma phosphorus content of heat stressed poultry.

5.1.10: Effect of Selenium Supplementation on Cortisol and Thyroxine level of Broiler Finisher Chickens (day 49)

The elevation of blood corticosterone level could be considered as a sign of stress (Fan *et al.*, 2009). The body in a normal state produces antioxidant enzyme (SOD and GSHPx) which attenuate the activities of ROS although this enzymes are overwhelmed during heat stress (Shokraneh *et al.,* 2019). During prolonged period of stress the body tends to adjust and return to homeostasis (acclimatization) and this could be the reason for the lower cortisol level in broilers fed the control diet (Collier *et al*., 2019). Selenium suppresses glucocorticoid (cortisol) secretion (Sathya *et al*., 2007) and is responsible for the decreased level of cortisol in broilers fed 0.1 and 0.2 mgSe/kg. The higher cortisol level in broilers fed 0.3 mgSe/kg diet could be due to pro-oxidant activity of selenium at higher level(Sotler *et al.,* 2019). This is in contrast to Fan *et al*. (2009) who reported an increased level of corticosterone in broilers fed the control diet than the selenium supplemented group during induced stress. Similarly, (Shokraneh *et al.,* 2019) reported increased cortisol level in the control group than broiler eggs injected with 40 µg of selenium under high temperature. Thyroxine was higher in all the selenium supplemented groups than the control groups. This could be due to selenium involvement in the synthesis and transformation of T4 to T3 (Ozbal *et al*., 2008). Fan *et al*.(2009), El-Samra *et al*.(2014) and Selim *et al.*(2015) revealed increased thyroxine level in broilers fed different forms and levels of selenium supplemented diet. In contrast, Shokraneh *et al.* (2019) reported that in-ovo selenium injection into the eggs of broilers at high temperature during incubation decreased thyroxine level.

5.1.11: Effect of Selenium Supplementation on Calcium, Phosphorus and Ash content in Bone and Faeces of Broiler Chickens

Phosphorus is the second most abundant mineral in the body with immense importance in poultry diets for efficient growth and development (Shahid *et al*., 2018). Although, calcium, phosphorus and ash content of tibia bones of broiler chickens in the different groups were similar, the higherexcretion of calcium in the faeces of broilers fed the control diet and phosphorus in the faeces of broilers fed the control diet, 0.1 and 0.3 mgSe/kg of feed respectivelycould be due to the impact of heat stress which led to decreased binding of minerals to protein for proper intestinal absorption and utilization (Shahid *et al*., 2018).Sahin *et al*. (2009) further stated that heat stress increases mineral excretion and decreases serum and liver concentration of minerals. Persia *et al*. (2003) also reported that there is a reduction in the available calcium and phosphorus utilization during chronic heat stress due to reduced growth, reduction in feed intake during heat stress in poultry compromises the nutrient intake hence decreases the mineral intake as well.

5.1.12: Effect of Selenium Supplementation on Carcass Cut Parts and Organ Weight of Broiler Chickens

The result obtained revealed that broilers fed 0.3 mgSe/kg had higher dressing percentage and back cut. The difference observed could be due to higher antioxidant activity of selenium that reduced the deleterious effect of heat stress thereby allowing the birds to concentrate their energy in building up tissue instead of combating heat stress. This is in contrast to the work of Heindl *et al*. (2010) who fed different selenium sources and levels (0.15 and 0.3 mg/kg) to broiler chickens and reported non-significant difference in dressing percentage. Tayeb and

Qader (2012) also showed that selenium and vitamin E had no significant effect on carcass weight, carcass parts and dressing percentage at 42 and 49 days of age.

Broilers fed the control diet had higher thigh weight while those fed the control diet and 0.3 mgSe/kg of diet had higher wing weight. Heindl *et al.* (2010) reported that 0.15 and 0.3 mg of selenium did not affect the weight of the thigh muscle of broiler chickens. The non-significant differences observed in the weight of other partsand organsweight of heat stressed broiler birds fed selenium supplemented diets is in agreement with Heindl *et al.* (2010) and Salem *et al*. (2018) who supplemented the feed of broilers with varying levels of selenium and reported similar result for cut parts and organ weights across the treatments respectively.

5.1.13: Effect of Selenium Supplementation on Jejunum Mucosal Morphology of Broiler Chickens

Olsen *et al*. (2005) demonstrated that stress could affect intestinal activity of animals and disrupt nutrient absorption (Albin *et al.* 2007). The similar result obtained for villus height, width, crypt depth and villus parameter is an indication that selenium did not negatively affect the intestinal integrity nor nutrient absorption of broilers under heat stress.Villus absorption area was improved and the ratio of villus height/crypt depth was higher with broilers fed the control diet, 0.1 and 0.2 mg selenium supplemented diet. Li *et al*. (2009) stated that villus height and the mucosa area are indicators of the ability of the intestine to absorb nutrients and deeper crypt depth signifies higher maturation of the intestinal epithelium. The result obtained for the above parameters agrees with the report of Kasaikina *et al.* (2011), who revealed that selenium as an antioxidant can have positive effect on the morphology of the intestine.

5.1.14 Effect of Selenium Supplementation on Tibia Measurements of Broiler Chickens

Zeng *et al.* (2013) reported that insufficient Se intake can lead to retarded growth and modification of bone metabolism;the involvement of Se in bone health has mainly been its role against oxidative stress and inflammation, boost of immune system and involvement in cell division, proliferation and apoptosis. The increase in bone weight observed in the selenium treated groups is an indication of selenium's involvement in growth. This agrees with Moreno-Reyes *et al.* (2009) who reported that selenium deficiency impaired bone metabolism and osteopenia in rats. The decrease observed in tibia development of broilers fed diet supplemented with 0.3 mg of selenium could be due to the increased cortisol level which may have led to increased ROS activity and reduced bone metabolism.

Robusticity index is an indication of bone strength, and a low robusticity index indicates a strong bone structure. The decrease in robusticity index reveals that selenium is involved in bone turgidity.The similar results obtained in this study agrees with the work of Ademu (2018) who observed non-significant values for robusticity index in broilers fed betaine supplemented diet.

5.2 The Effect of Organic Ascorbic Acid in Ameliorating Heat Stress in Broiler Chickens

5.2.1: Chemical Composition and Anti-Nutritional Factor Contents of Baobab Fruit Pulp Meal

The values of the chemical analysis fell within the range of values earlier reported (Magdi, 2004; Adeosun, 2012). Similarly the ascorbic acid content of 340 mg/100g obtained from the chemical analysis fell within the range of 150 to 499 mg/100g reported by Manfredini *et al.* (2002) and its high content of ascorbic acid makes it a good source of organic ascorbic acid

for utilization in animal nutrition. The result of the chemical analysis of Baobab Fruit Pulp Meal showed no indication that it contains high levels of anti-nutritional factors that can pose any serious threat to its utilization in poultry diets especially if it is used at low levels of inclusion.

5.2.2 Thermoregulatory Parameters

As global warming is getting severe, environmental temperature has been rising faster in the last ten years (Richter*et al.,* 2010). Seasonal variation in ambient temperature is an important stressor that affects broiler production in many regions of the world and huge losses on capital of investment may accrue due to reduced growth, depressed immunity and mortality (Melesse *et al*., 2011). The result obtained in this study shows that THI in the afternoon was higher by 25.08% than THI in the morning which revealedreduced heat stress in the morning and the presence of very severe heat stress in the afternoon (Ademu *et al*., 2018), hence the birds were severely stressed. Lin *et al*. (2004) had earlier revealed that sensitivity to high temperature increases with body weight. The respiratory rate of broilers was not consistent but was within the normal range,a surge in respiratory rate was observed in broilers fed diet supplemented with 136 mg/kg ascorbic acid but was lower in broilers fed 204 mg/kg ascorbic acid supplemented diet. Broilers fed 136 and 204 mg ascorbic acidsupplemented diet had an increased heart rate than the other groups. The inconsistency observed in these parameters could be as a result of birds attempt to resist restraint at the point of measuring these parameters which could had increased the metabolic rate of the birds. Although broilers fed 68 and204 mg ascorbic acidsupplemented diet had lower heart beatand respiratory rate respectively. This is in contrast with the report of Abdelrafea*et al*. (2013), who reported that

increasing ascorbic acidcontent from 0.5-1 gram alone or in combination with ethylenediaminetetrascorbic acidcetic acid (EDTA) in the diet of broilers increased respiration. Similarly, Olukomaiya *et al*. (2015) reported that increasing the ascorbic acidcontent of broilers feed from 150-300 mg did not lower their respiratory rate. Chaiyabutr (2004)reported that one of the physiological responses to heat-stress in birds is increase inrespiratory frequency and that panting occurs whenthe deep body temperature of poultry reaches 41-43^{0}Cbecause birds have no sweat glands like ruminants. Rectal and body temperatures of broilers were similar and within the range reported by (Abdelrafea*et al*., 2013; Olukomaiya *et al*., 2015; Jahejo*et al*., 2016). The higher rectal temperature observed in broilers fed the diet supplemented with 204 mg of ascorbic acidcould be due to the more severe chronic respiratory disease (CRD) observed in this group. The similar result observed for rectal and body temperatures reveals that ascorbic acid had no negative impact on these parameters since theywere within the normal range (40.6 – 43.00^{0}C) reported by Robertshaw (2004). Broiler chickens are homeotherms and their body system may allow certain variations in temperature range without considerable disturbance within their system (St-Pierre *et al*., 2003). The increase in thermoregulatory parameters (except respiratory) rate as ascorbic acid supplementation increases agrees with the report of Abdelrafea*et al*. (2013), who reported a significant increase in cloacal, skin, feather temperature and respiratory rate of broilers fed 1 g of ascorbic acid. Thermobalance is the stability attained between the heat produced and heat given off by living organism and this is at its maximal physiological level within the thermoneutral range of any given specie Olukomaiya *et al*. (2015).

5.2.3 Effect of Ascorbic acid Supplementation on Performance of Broiler Chickens

The similar performance of broilers in this study except for FCR is consistent with the report of Muhammad *et al*. (2016) but in contrast with the report of (Lohakare, 2005; Talebi and Khademi, 2011; Adeosun, 2012; Jahejo *et al*., 2016; Youssef *et al*., 2017) who reported that ascorbic acidsignificantly improved the performance of broiler chickens during heat stress although the THI indicated that their birds were not as stressed as those of the present study. FCR was better only at the starter phase when chicks were fed the control diet and 68 mg/kg ascorbic acid diet. Adeosun (2012) reported a better FCR in broiler chicks fed BFPM at 3.5 and 7% inclusion levelsand 3% at finisher phase during the dry-hot season and3.5% inclusion level for both starter and finisher phases during the wet-cool season. Similarly, Jahejo *et al*. (2016) reported a lower FCR in broilers fed 200 mg/kg ascorbic acid. Furthermore, Talebi and Khademi (2011) reported a lower FCR in heat stressed broilers fed varying levels of glucose combined with either 200 or 300 ppm of ascorbic acidin drinking water during a 42 days period.

5.2.4: Effect of ascorbic acid Supplementation on haematological parameters of starter broiler chickens (day 28)

The haematological parameters measured were within the normal physiological range, except for leucocytes which were slightly above the normal range and eosinophils which were below the normal range reported by Simrak *et al*. (2004). This could be due to heat stress or underlying disease conditions that might have led to an alteration in the production of the components of white blood cells (Altan *et al*., 2000). The non-significant result observed in all the parameters measured is an indication that the ascorbic acid levels and anti-nutritional factors contained in the baobab fruit pulp meal posed no negative impact on the blood profile

of the birds (Eggum, 1986). PCV, Hb and erythrocyte were higher in birds fed 204 mg of ascorbic acidsupplemented diet, this is an indication that the feed was safe for consumption and improved the health status of broilers during heat stress. Isikwenu and Omeje (2007), reported that PCV and Hb are nutritional indicators which are largely influenced by the diets fed to the animals. The result of this study agrees with Adenkola *et al*. (2016), who fed broilers 500 mg of ascorbic acidsupplemented diet and reported that PCV, Hb and erythrocyte were similar**.** Similarly, Alaeldein*et al.* (2018) demonstrated that addition of either 100 or 200 mg natural vitamin C to the drinking water of broilers during heat stress did not affect the Hb nor erythrocyte. Leucocyte and band cells were higher in broilers fed the control diet. Leucocyte, depicts the well-being of an animal and increases with stress or during a disease condition (Louis and Caplan, 2009). Ascorbic acid supplementation lowered the leucocyte count and was close to the normal range reported by Simrak *et al.* (2004).Sorensen *et al.* (2010) reported that the presence of ascorbic acidmay limit free radical damage and free radicals may play a complex role in the healing response.

H:Lis an indicator of stress and increases during stress (Gross and Seigel, 1983). The lower heterophil, H:L and increased lymphocytes recorded in broilers fed 68 mg of ascorbic acid supplemented diet are indications that ascorbic acidmitigated the deleterious impact of heat stress on the birds. Youssef *et al*. (2017), reported that vitamin C at 200 mg lowered the concentrations of heterophils, H:L and increased lymphocyte countduring heat stress. Broilers fed diets supplemented with 68 and 204 mg of ascorbic acidhad higher number of monocyte and within normal physiological range while broilers fed 204 mg supplemented diet had higher number of eosinophil. This is consistent with the work of Youssef *et al*. (2017)

who reported higher monocyte and eosinophils at 200 mg vitamin C supplementation. The higher monocytes and eosinophils recorded could be an indication of ascorbic acidinvolvement in repair of tissues damage caused by free radicals or ROS (Sorensen *et al.,*2010). Monocytes and eosinophils are component of leucocyte. Monocytes remove dead, damaged cells as well as microorganism and are often found in higher numbers during a stress response or inflammation and eosinophils are increased during hypersensitivity responses especially parasitic infections (Melissa *et al*., 2015).

5.2.5: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Starter Chickens (day 28)

Glucose values were similar among the groups and within the normal range reported by Goodwin *et al*. (1994). This implies that heat stress did not negatively affect the energy level of the birds during this period. Lin *et al*. (2007) stated that physiological stress induces a higher glucose level in the blood. Ismail *et al.* (2013), supplemented the diet of heat stressed broilers at two, four and six weeks with 1 g of vitamin C and reported non-significant difference in the glucose level among the groups.

The significant increase observed in ALT of broilers fed 136 mg of ascorbic acidsupplemented diet might be due to increased synthesis of ALT by the liver and could be as a result of inflammation of the liver observed during the post mortem examination.Alteration in liver enzymes activity under stress conditions occurs due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm (Khan and Sardar, 2005). Oxygen free radicals can damaged liver tissue and cause lipid peroxidation which is revealed by increase in serum ALT concentration, indicating the

inability of liver to metabolize ALT (Bharavi *et al.,* 2010) and attributed to the outflow of these enzymes from the liver cytosol to the blood stream (Cinar *et al.,* 2011). In contrast, El-Habbak *et al.* (2011), argued that heat stress significantly decreased ALT level in the serum of broilers while Ismail *et al*. (2013) reported that ALT was non-significant when the diet of broilers was supplemented with 1 g of ascorbic acid or its combination with zinc bacitracin. Similarly, Adenkola *et al*. (2016) reported a non-significant effect in ALT of broilers fed 500 mg ascorbic acid supplemented diet. The increased ALT concentration observed as a result of higher ascorbic acidsupplementation agrees with the report of Youssef *et al*. (2017) who reported higher ($P<0.05$) value of ALT in broiler fed 200 mg of ascorbic acidsupplemented diet.

The similar result obtained for ALP, total protein, albumin, globulin, cholesterol, triglyceride, calcium and phosphorus which were within the normal range implies that ascorbic acid improved the biochemical indices of broilers during heat stress. The result obtained for these parameters agrees with the report of Konca *et al* (2009); Rashidi *et al.* (2010); Ismail *et al*. (2013) andAlaeldein*et al.* (2018).Rashidi *et al*. (2010) reported that heat stress increased levels of plasma glucose, triglyceride and cholesterol and reduced protein level. The increase in blood lipids under heat stress is due to the fact that high temperature reduces feed intake and broilers compensate their need for energy by lipolysis of body lipid which causes increase in blood cholesterol and triglycerides (Rashidi *et al*. (2010). Ascorbic acidhas been reported to increase serum calcium and phosphorus level Abdelrafea *et al*. (2013).

Higher value of LDL was recorded in broilers fed 68 and 136 mg/kg ascorbic acid diet but was lower in broilers fed the control diet and 204 mg/kg ascorbic acid. This could be due to

the fact that ascorbic acid reduced corticoid secretion and prevented the stimulation of lipoproteins and tissue lipases (Seyrek *et al.,* 2004). The synthesis and metabolism of LDL is similar to cholesterol and ascorbic acid is necessary for the conversion of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation, this reaction limit cholesterol catabolism in the liver, but the deficiency of ascorbic acid slow down the rate of this reaction, leading to cholesterol accumulation in liver and blood (Seyrek *et al.,* 2004). Ascorbate reduces corticoid secretion and prevent lipoprotein and tissue lipases, hence lipids are not transported from tissue to liver and this also leads to reduced serum LDL level. Seyrek *et al*. (2004) reported that supplementing ascorbic acid at 150 – 500 mg/kg in the diet of quails during heat stress significantly lowered the level of serum lipoproteins.

GSHPx was higher in broilers fed 68 mg of ascorbic acidsupplemented diet, while 204 mg of ascorbic acidsupplementation increased SOD activity and lowered MDA concentration. Broilers fed diet supplemented with 136 mg of ascorbic acid had higher CAT level than the other groups. In general ascorbic acidin the diet of broiler improved the stress enzymes and lowered the MDA level of broilers. This is an evidence of the antioxidant property of ascorbic acidagainst free radical and ROS. The result obtained in this study agrees with the report of Jena *et al.* (2013);Adenkola *et al*. (2016).

5.2.6: Effect of Ascorbic Acid Supplementation on Cortisol and Thyroxine level of Broiler Starter Chickens (day 28)

High environmental temperatures causes an increase in plasma corticosterone and reduction in the activity and performance of lymphoid organs and total leukocytic count, but ascorbic acid has been found to cause a reduction in high plasma corticosterone levels with subsequent maintenance of normal leukocytic count (Abidin and Khatoon, 2013). This was observed

when the diet of broilers was supplemented with 68 mg/kg ascorbic acid, the lower cortisol level of the control birds could be due to the basal ascorbic acid (163.44 %) content of the control diet. The higher cortisol recorded in broilers fed 136 and 204 mg/kg ascorbic acid could be due to the involvement of ascorbic acid in the bio-synthesis of corticosterone (Abidin and Khatoon, 2013) or it pro-oxidant activity at high doses, matrix environment or chemical interaction. Sotler *et al*. (2019) reported that vitamin C is a potent antioxidant but can intervene as a pro-oxidant depending on the dose (antioxidant; 30-100 mg/kg body weight and pro-oxidant; 1000 mg/kg body weight) or its interaction with iron or copper. The report of the present study agrees with Mahmoud *et al.* (2014) who reported an elevated corticosterone level in broilers fed 250 mg/kg ascorbic acid during heat stress but is in contrast to Poungpong *et al.* (2019) who reported a decreased cortisol level in broilers fed diet supplemented with 286 ppm ascorbic acid during heat stress. Mosleh *et al*., (2018) reported a significantly similar corticosterone level in broilers administered control or 12 g ascorbic acid/100L of drinking water during heat stress.

Thyroid hormones are known to be negatively influenced by stress, lowering plasma concentrations of T3 and T4. The higher thyroxine level reported in broilers fed 68 mg/kg ascorbic acid diet could be due to the attenuating role of ascorbic acid against the deleterious effect of heat stress while the decrease observed in broilers fed 136 mg/kg ascorbic acid could be as a result of elevated cortisol level in the birds which might have interrupted thyroid activity. Mahmoud *et al*. (2014) reported that supplementing the diet of broilers with 250 mg/kg ascorbic acid during heat stress elevated thyroxine level.

5.2.7: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Finisher Chickens (Day 49)

Broilers fed the control diet had higher PCV, Hb and eosinophils levels. PCV and Hb were within the normal range described by Mitruka and Rawnsely (1997). This revealed that ascorbic acid supplementation had no negative effect on the birds, also it was earlier stated that these parameters are indicators of the adequacy of feeds (Isikwenu and Omeje, 2007). Eosinophils was slightly below the normal range in all the groups and could be as a result of heat stress (Altan *et al*., 2000). This agrees with the work of Muhammad *et al.* (2016), who reported that supplementation of the diet of broilers with 0.07, 0.15, 0.22 and 0.30 g of ascorbic aciddid not have any significant effect on the PCV and Hb count. Leucocyte was above the normal range reported bySimrak *et al*. (2004) and higher leucocyte count was recorded in broilers fed diet supplemented with 204 mg/kg ascorbic acid. This agrees with the work of Muhammad *et al.* (2016), who recorded higher leucocyte number in broilers fed 0.22 g of ascorbic acid.

Higher erythrocyte and lymphocyte count with a decreased heterophil and H:L was recorded in birds fed 68 mg/kg ascorbic acid supplemented diet. Ascorbic acidshowed its potency in ameliorating heat stress as indicated by increase in lymphocyte and decrease in heterophils and H:L than the control group. This agrees with the report of Youssef*et al*. (2017), who revealed that vitamin C supplementation increased lymphocyte and lowered heterophil and H:L during chronic heat stress than vitamin E and probiotics.

5.2.8: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Finisher Chickens (Day 49)

The increase in glucose concentration is directly responsive to an increase in glucocorticoids (Borges *et al*., 2007), which can result from various stressors including heat stress. Glucocorticoids have primary effects on metabolism, stimulating gluconeogenesis from muscle tissue proteins. Glucose values was within the normal range reported by Goodwin *et al*. (1994). The glucose level observedin ascorbic acid supplemented groups could be due to the fact that cortisol secretion was higher in broilers fed 204 mg and might had led to gluconeogenesis from muscle tissue or it could also be due to the higher quantity of BFPM which is a carbohydrate source. The increased ALT and AST could be as a result of higher synthesis of these enzymes in the liver due to inflammation of the liver caused by poor digestion of feed as seen during the post mortem examinatiom. AST was below the normal range reported by LAVC (2009). Globulin was within the normal range reported by LAVC (2009). The lowered cholesterol (136 mg/kg ascorbic acid),LDL (68 and 204 mg/kg ascorbic acid) and triglyceride(204 mg/kg ascorbic acid) might be due to the antioxidant property of ascorbic acid in preventing lipid peroxidation and metabolism. Seyrek *et al.* (2004) reported thatascorbate is necessary for the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation and its deficiency induces a marked slowing down of this reaction, leading to cholesterol accumulation in liver and in blood. The higher cholesterol level reported for broilers fed 204 mg/kg ascorbic acid could be due to liver inflammation caused by poor digestion of feed as a result of heat stress observed during the post mortem.

GSHPx activity was higher in broilers fed 68 and 136 mg of ascorbic acidsupplemented diet. The result of this study is in contrast to the findings of Jena *et al*. (2013) and Adenkola *et al*.

(2016), who reported that ascorbic aciddid not improve the level of serum GSHPx in broilers.Glutathione annihilates oxygen toxicity by interrupting the reaction involving oxygen conversion to oxygen radicals (Yoda *et al*., 1986) in its reduced form, it metabolizes hydrogen peroxide and hydroxyl radicals. A healthy body is marked by a balance between free radicals and antioxidants,when this balance is disrupted by over-abundance of free radicals, oxidative stress (OS) occurs.

SOD was higher in all ascorbic acidsupplemented groups than the control, this might be due to the fact that SOD scavenges for both intra and extracellular superoxide radicals and annihilate their deleterious activities by acting in conjugation with catalase and glutathione peroxide (Agarwal and Prabhakaran, 2005) and ascorbic acid is known to improve the activities of antioxidant enzymes. CAT level was higher in 136 mg ascorbic acidsupplemented broilers with broiler fed the control diet being next and better than that of broilers fed 68 and 204 mg/kg ascorbic acid group. Adenkola *et al*. (2016) reported that broilers fed control diet during heat stress had higher GSHPx, SOD and CATthan the ascorbic acidgroup. Production of MDA *in vivo* increases with exposure to environmental oxidants (Draper and Hadley, 1990). MDA was lowered by 68 mg of ascorbic acid supplemented diet but was higher in broilers fed 136 mg/kg ascorbic acid diet. The higher MDA at 136 mg/kg ascorbic acid supplementation could be due to the increased serum cortisol level. This is in contrast with the work of Jena *et al.* (2013) who revealed that supplementation of ascorbic acid at 200 and 400 mg in the diet of broiler breeder hen during summer lowered their MDA level (4.96 + 0.61 and 4.71 + 0.59 nmol/mg) Similarly, Adenkola *et al*. (2016) reported that supplementing the diet of broilers with 500 mg/kg ascorbic acid lowered serum MDA level

(0.93 + 0.009 ng/mL).Both enzymatic (catalase and superoxide dismutase) and non-enzymatic (MDA) antioxidants play important roles in combating oxidative stress. Catalase detoxifies hydrogen produced during different metabolic processes and also in stressful condition by reducing it to hydrogen peroxide and oxygen. Superoxide dismutase is known to catalyze dismutation of superoxide radicals into water and oxygen (Kwiecient *et al*., 2004).

5.2.9: Effect of Ascorbic Acid Supplementation on Cortisol and Thyroxine level of Broiler Finisher Chickens (day 49)

Ascorbic acid is important for different biochemical reactions as well as regulation of corticosterone secretion, body temperature and activation of immune system (Sahin and Kucuk, 2003). The decreased cortisol level reported in broilers fed 68 mg/kg ascorbic acid is consistent with that of the starter phase and is an evidence of ascorbic acid involvement in interrupting the activity of ROS and lowering of plasma cortisol level as reported by other studies (Abidin and Khatoon, 2013; Poungpong *et al*., 2019). The higher cortisol level in broilers fed 136 and 204 mg/kg ascorbic acid than the control group could be due to the pro-oxidant activity of ascorbic acid. The similar result reported among the different groups in the present study is consistent with (Mosleh *et al*., 2018; Poungpong *et al*., 2019). Mahmoud *et al*. (2014) reported a decreased corticosterone level in broilers fed 250 mg/kg ascorbic acid during heat stress.

In general, thyroxine was higher in broilers fed the control diet compared to the ascorbic acid supplemented group. This could be due to the elevated cortisol level in broilers fed diet supplemented with 136 and 204 mg of ascorbic acid. The decrease in thyroxine observed in this study is consistent with Hassanzadeh *et al*. (1997), who reported that supplementing the diet of broilers with 500 ppm of vitamin C significantly reduced thyroid hormones (T3 and

T4) levels. In contrast, Mahmoud *et al*. (2014) reported an increased thyroxine and a decrease in triiodothyronine levels in broilers fed 250 mg ascorbic acid supplemented diet.

5.2.10: Effect of Ascorbic acid Supplementation on Calcium, Phosphorus and Ash content in Bone and Faeces of Broiler Chickens

Bone problems have been identified as one of the main production and health concerns in broiler production and modern poultry has been bred for superior meat production, possibly overlooking the consequences on bone quality(Rath *et al*., 2000). Ascorbic acidplays a significant role in collagen formation, nutrient absorption and anti-oxidant functions (Bhattacharya, 2010; Cheeke and Derenfeld, 2010; Vansudevan *et al*., 2011). Although the calcium content in tibia bone of broilers was similar across the groups, ascorbic acidsupplementation increased the calcium levels in bone up to 204 mg/kg ascorbic acid. Similarly, phosphorus was higher in both the control and ascorbic acidsupplemented groups but declined in broilers fed 204 mg/kg ascorbic acid. This agrees with the report of Olugbenga (2015) who supplemented the diet of broilers with 100-500 ppm of ascorbic acidand reported that ascorbic acidsignificantly increased the bone calcium and phosphorus content of heat stressed broilers up to 300 and 400 ppm respectively and declined afterwards. The increase in calcium observed with ascorbic acidsupplementation could be due to cardinal roles of ascorbic acid in bone metabolism primarily in osteoblasts and in the formation of osteoid, the bone intercellular substance without which bone salts deposition is arrested (Bhattacharya, 2010; Cheeke and Derenfeld, 2010). Lohakare *et al.* (2005) further enunciated that dietary ascorbic acid affects bone resorption marker enzymes. Also, vitamin C influences the conversion of vitamin D into its metabolite active form calcitriol which is essential for calcium and phosphorous regulation and calcification processes. Although the decline

observed in phosphorus at 204 mg ascorbic acidsupplementation is not clear. Ash is used as an index of the overall mineral content of bones, the higher ash content observed in all the ascorbic acidsupplemented groups is an indication of ascorbic acidinvolvement in mineral synthesis and metabolism. The higher ash content recorded in the bones of broilers fed diet supplemented with ascorbic acid is consistent with the report of Olugbenga (2015). Contrary to the report of this study, Pointillart *et al*. (1997) reported that vitamin C supplementation did not modify bone mineral content, mineral absorption or bending movement in growing pigs fed 500 or 1000 mg vitamin C/kg. Although there are direct evidence thatdeficiency of vitamin C alters bone formation, but the background ascorbic acidcontent of the control diet is usually above 100 mg/100kg as reported in the present study and that of Pointillart *et al*. (1997). Hence, the disparity in the report of previous authors could be due to the fact that there are no data demonstrating that dietary intake of ascorbic acid over the minimal requirement stimulates bone metabolism or mineral retention (Pointillart *et al*., 1997).

Heat-stressed birds have been said to develop metabolic acidosis, which first stimulates mineral dissolution and then the cell-mediated bone resorption due to the increased calcium excretion by the kidneys (Rions, 2001; Oliveira *et al.*, 2010). Calcium loss results in reduced bone mineralization and may affect mechanical quality of the bone. The increased excretion of calcium via the faeces of broilers fed ascorbic acidsupplemented diets than those of the control could be due to the increased synthesis of calcium in broilers fed ascorbic acidsupplemented diet as evident in the calcium levels of their bones. This is in contrast to the report of Pointillart *et al*. (1997) who reported that ascorbic acidsupplementation (500 and 1000 mg/kg) in the diet of young pigs did not alter the calcium and phosphorus levels in their faeces.Some studies suggest that vitamin C interacts with vitamin D metabolism by protecting

or stimulating renal 1α-hydroxylase activity (Cantatore and Carrozzo 1990; Weiser *et al*. 1992), hence increased synthesis of minerals.

5.2.11: Effect of Ascorbic acid Supplementation on Carcass Cut Parts and Organ Weights of Broiler Chickens

Konca *et al*.(2009); Abdelrafea *et al*.(2013) andYoussef *et al*.(2017) reported that dietary ascorbic acidsupplementation did not improve the weight of broilers cut parts nor the weight of its organs. Broilers fed the control diet, 68 and 136 mg of ascorbic acid supplemented diet had higher weight of wing than those fed 204 mg/kg ascorbic acid. The decrease observed in broilers fed 204 mg of ascorbic acidsupplemented diet could be due to post mortem losses that might had occurred due to the time lag in measuring the different group (FSAMISS, 2002). The result of this study is in contrast to the work of Konca *et al*. (2009) who reported that there was no significant difference in the weight of broilers wing fed diet supplemented with either 150 or 300 mg of ascorbic acid. Lohakare *et al*. (2005) also reported that gradually supplementing the diet of broilers with ascorbic acid from 10 - 200 mg/kg improved the carcass yield.

5.2.12: Effect of Ascorbic acid Supplementation on Jejunum Morphology of Broiler Chickens

Hajati *et al*. (2016) revealed that nutrient absorption and blood flow to the gastrointestinal tract was reduced during heat stress condition. Jahejo *et al*. (2016) reported that ascorbic acidwaseffective in reducing stress and improving the jejunum environment through increased nutrient absorption. The similar villus height and width observed in this study is an indication that ascorbic aciddid not increase the surface area of absorption in the luminal capillaries, improved digestive enzymes production and nutrient transport on the surface of the villi

(Ariyadi *et al*., 2019). Broilers fed diet supplemented with 204 mg of ascorbic acidhad higher crypt depth and villus absorption area. Villus height and the mucosa area are indicators of the ability of the intestine to absorb nutrients and crypt depth is an indicator of the maturity of intestinal epithelium with a deeper crypt depth signifying higher maturation of the intestinal epithelium (Li *et al*., 2009). The higher crypt depth and absorption area observed during this study could be due to the antioxidant property of ascorbic acidin limiting ROS which improve blood movement to the gut and maturation of intestinal epithelium.Soltani *et al*. (2019) reported that in-ovo injection of ascorbic acid during incubation improved the intestinal morphology of broiler chicks. Villus height/crypt depth was higher in broilers fed 136 mg/kg diet but decreased at 204 mg/kg ascorbic acid supplemented birds which may be due to the intensed stress as a result the persistent CRD caused by the more dusty feed. Xu *et al*. (2003) reported that a lower villus height/crypt depth ratio is associated with the presence of toxins, poor nutrient absorption and increased secretion of bile in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance.

5.2.13 Effect of Ascorbic acid Supplementation on Tibia Measurements of Broiler Chickens

Tibia weight and length were similar in this study but the length of tibia increased with increase in ascorbic acidsupplementation which could be due to the role of ascorbic acid in bone growth. The result obtained agrees with the work of Olugbenga (2015). Sgavioli*et al*. (2016) also demonstrated that the tibia weight and length of broilers injected with ascorbic acid in-ovo during incubation and reared under heat stress until 42 days of age were not significantly improved. Lohakare *et al.* (2005) revealed that vitamin C have positive influence in the conversion of vitamin D into its metabolite active form calcitriol which is essential for

calcium and phosphorous regulation and calcification processes,therefore ascorbic acid is involved in mineralization and bone growth. Broilers fed the control diet had higher tibia weight/length index while robusticity index decreased in broilers fed the control diet and 68 mg of ascorbic acidsupplementation. The decreased bone weight/length index observed in this study with supplementation of ascorbic acid is in contrast to earlier report (Ogunwole*et al*., 2018) that ascorbic acid increased bone weight/length index,with higher index indicatinghigher bone density and increased bone strength. Lower robusticity index has been associated with increased bone strength (Reisenfeld, 1972),thus suggesting that ascorbic acidsupplementation above 68 mg did not favour the formation of strong bone. On the other hand, Olugbenga (2015) reported that the physical properties (weight, length, tibiotarsal index and robusticity) of broiler bones fed diet supplemented with ascorbic acid up to 500 mg/kg were similar during heat stress.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Two studies were carried out to investigatethe role of selenium and organic ascorbic acid in ameliorating heat stress on growth, haematological, biochemical indices and morphometrics of broiler chickens.

The first experiment to determine the role of selenium in ameliorating the impact of heat stress in broiler chickenswas conducted using 256 day-old *Cobb* 500 broiler chicks. There was four treatments (0, 0.1, 0.2 and 0.3 mgSe/kg), each having four replicates with sixteen birds per replicate arranged in a completely randomized design. Results showed that the supplementation of selenium in the diet of broilers did not alleviate the deleterious impact of heat stress on the thermoregulatory parameters and growth performance, but 0.1 mgSe/kg increased PCV, haemoglobin and erythrocyte concentrations. Some serum indices (cholesterol, SOD, catalase, cortisol and phosphorus)were improved and mineral retension was higherin selenium supplemented groups. Carcass traits especially dressing percentage and back weight was improved by 0.3 selenium supplementation. 0.1 and 0.2 mgSe/kg positively improved villus area and villus height/crypt depth ratio. Tibia measurement was the same for all the treatment groups.

The second experiment that involvesthe role of organic ascorbic acid in ameliorating the impact of heat stress in broiler chickenswas conducted using 256 day-old *Cobb* 500 broiler chicks. There were four treatments (0, 68, 136 and 204 mg/kg ascorbic acid), each having four replicates with sixteen birds per replicate arranged in a completely randomized design.

Results showed that the supplementation of organic ascorbic acid at 204 mg/kgin the diet of broilers lowered respiratory rate and improved heart rate although rectal and body temperature were higher. The control and 68 mg ascorbic acid supplemented groups positively improved FCR and had the least cost/kg gain. Ascorbic acid supplementation improved haematological indices especially at the starter phase and 68 mg ascorbic acid group had a better H:L. Serum biochemical indices was improved by 68 and 136 mg ascorbic acid supplementation and was in consonance with those of the control group forcortisol and low density lipoprotein at the starter and finisher phases respectively.Phosphorus level of tibia bone was positively improved by 68 and 136 mg ascorbic acid supplementation and was in consonance with that of the control group, ash content of the tibia bones for all the ascorbic acid supplemented groups were in consonance and better than those of the control group, and fecal calcium level was lower in the control groups.Findings from carcass analysis revealed that the weight of wings of broiler birds fed 68 and 36 mg ascorbic acid supplemented diet was higher and in consonance with those of the control. Higher crypt depth (118.27 µm) and villus area (22451 μm^2) was observed in broilers fed 204 mg ascorbic acid supplemented diet. Broilers fed the control diet had a better tibia weight/length index (0.75 g/cm) and robusticity index (4.01 $cm/g^{1/3}$).

6.2 Conclusions

This study concludes as follows:

- Selenium had no effect on thermoregulatory parameters and growth performance of broiler chickens except for water intake during the starter phase with higher values recorded for broilers fed 0.2 mgSe/kg diet.

- Selenium supplementation at 0.1 mg/kg increased PCV, haemoglobin and erythrocyte concentrations as compared to the control and other selenium treated groups.
- Selenium supplementation improved serum indices especially of cholesterol, SOD, catalase, cortisol and phosphorus.
- Selenium increased mineral (calcium and phosphorus) assimilation and utilization in the body as evident by reduced mineral excretion via faeces, thus selenium might have the potency in improving calcium metabolism of broiler chickens.
- Dietary supplementation of selenium at 0.1 mg/kg improved carcass quality especially of dressing percentage and back weight.
- Supplementation of broilers diet with 0.1 and 0.2 mg of selenium positively improved villus area and villus height/crypt depth ratio.
- Organic ascorbic acid when supplemented at 204 mg/kg diet, improved thermoregulatory parameters via lowered respiratory rate and increased heart beat.
- Growth performance was improved especially of FCR in broiler starters fed 68 mg/kg ascorbic acid and was similar with the control group and least feed cost/kg gain was recorded in the control and 68 mg ascorbic acid supplemented groups
- Serum indices at both starter and finisher phase (low density lipoprotein, Alanine-Amino Transferase, GSHPx and cortisol) was also improved at 68 and 136 mg/kg ascorbic acid supplementation.
- Minerals of tibia bone was better in the ascorbic acid supplemented groups, but did not decreasecalcium losses through faeces. Ascorbic acid supplementation at 68, 136 and 204 mg/kg increased carcass quality (wing weight), crypt depth and villus area.

- Tibia weight/length index was inversely proportional to ascorbic acid supplementation upto 204 mg/kg, thus ascorbic acid supplementation tends to increase the tibia length rather than the weight.Robusticity index was higher in broilers fed both the control and 68 mg ascorbic acid supplemented diet.

6.3 Recommendation

The study recommends as follows:

- During periods of heat stress, supplementation of 0.1 mgSe/kg or 68 mgAA/kg in the form of BFPM was cost effective (1.49 and 1.83% respectively) than the control.
- At finisher phase, supplementing the diets of broilers with 0.1 mgSe/kg diet lowered cortisol (12.70%) and increased thyroxine (35.19%), hence improved growth (0.29%) than the control.
- Selenium promoted Ca and P utilization than those of the control group by 34.97% and 19.10% respectively, hence its involved in bone strength and development.
- Supplementing the diets of broiler chicks with 68 mgAA/kg improved growth performance (7.74%) via lowered serum cortisol (25%) and elevated thyroxine level (24%) than the control group.

REFERENCES

A.O.A.C. (1990). Association of Official Analytical Chemists, Official Methods of Analysis. 15th Ed Washington DC.

Abdel-Azeem, S.A. (2010). Improving Reproductive Efficiency of Rabbit Does using Hormonal, Nutritional or Acetic Intra-Vaginal Washing Treatments, A PhD thesis. Faculty of Agriculture, Fayoum University, Egypt.

Abdelhady, D.H., El-Abasy, M.A., Atta, M.S., Ghazy, E.W., Abuzed, T.K. and El-Moslemany, A.M. (2017). Synergistic Ameliorative Effects of Organic Chromium and Selenium Against Heat Stress in Japanese Quails: Performance, Immunological, Hematological, Biochemical And Antioxidant Studies. *Alexandria Journal of Veterinary Science*, 55(2): 113-123.

Abdelrafea, A., El-Shafei, A., Arafa, M., Abdelrahman, M., Gihan, A. S., Elsakkar, H. (2013). Physiological, Biochemical and Histopathological Changes of Ethylenediaminetetrascorbic acidcetic acid (EDTA) and Vitamin C Supplementation in Broiler Chicks Diets. *Journal of Animal Sci*ence, 9(2):316-333. (ISSN: 1545-1003). http://www.jofamericanscience.org.

Abdollah, A., Joris, M., Jeroen, D., Maryam, M., Abolghasem, G. and Stefascorbic acidn, D.S. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology*, 7: 37. DOI 10.1186/s40104-016-0097-5.

Abdulrashid, M., Agwunobi, L.N. and Hassan, M.R. (2010). Ascorbic Acid and Heat Stress in Domestic Chicken Nutrition: A Review. *Journal of Agriculture, Forestry and the Social Sciences*, 8(2): 249-257.

Abebi, H. (1974). Catalase in methods of enzymatic analysis Ed. By bergmeyer, HU Verlag Chemie, New York, Pp. 673-684.

Abidin, Z. and Khatoon, A. (2013). Heat stress in poultry and the beneficial effects of ascorbic acid (vitamin C) supplementation during periods of heat stress. *World's Poultry Science Journal*, 69: 135-152.

Abioja, M.O., Ogundimu, K.B., Akibo, T.S., Odukoya, K.E., Ajiboye, O.O., Abiona, J.A., Williams, T.J., Oke, E.O. and Osinowo, O.A. (2012). Growth, Mineral Deposition, and Physiological Responses of Broiler Chickens Offered Honey in Drinking Water during Hot-Dry Season. *International Journal of Zoology*, vol. 2012, Article ID 403502, 6 pages. https://doi.org/10.1155/2012/403502.

Abioja, M.O., Osinowo, O.A., Smith, O.F., Enirbetine, D. and Agiono, J.A. (2010).

Evaluation of cold water and vitamin C on broiler growth during hot-dry season in South-West, Nigeria. *ArchZootechnology*, 60: 1095-1103.

Addy, E.O. and Eteshola, E. (1984). Nutritive value of a mixture of tiger nut tubers (*Cyperus esculentus* L.) and baobab seeds (*Adansonia digitata* L.). *Journal Science Food Agriculture*, 35: 437-440.

Ademu, L.A. (2018). Responses of broiler chickens fed betaine hydrochloride supplementation underdexamethasone induced stress condition. A PhD thesis, Ahmadu Bello University, Zaria.

Adenkola, A.Y., Carew, N.S., Ojabo, L.D. and Angani, M.T. (2016). Comparative Studies Ascorbic Acid and *Hibiscus sabdariffa* Calyces against Heat-Stress Inclusion in Broiler Chicken. *Alexandria Journal of Veterinary Sciences*, 5(2): 17-23.

Adeosun, S.L. (2012). Effects of synthetic ascorbic acid and baobab Fruit pulp meal supplementation as sources of Ascorbic acid in layer and broiler diets during Cool-wet and hot-dry seasons. A PhD thesis submitted to the Department of Animal Science, Ahmadu Bello University, Zaria. Pp. 74.

Adeyemo, A.A. and Onikoyi, M.P. (2012). Prospects and challenge of large-scale commercial poultry production in Nigeria. *Journal of Agriculture*, 7(6): 388-393.

Adrian, R. and Dumitru, C. (2017). Organic selenium effect on body temperature and body weight in broilers. *European Journal of Biotechnology*, 1(4): 332-336.

Aengwanich, W. (2008). Pathological changes and the effects of ascorbic acid on lesion scores of bursa of Fabricius in broilers under chronic heat stress. *Research Journal of Veterinary Science*, 1: 62-66.

Agbessi, D. (1987). Manual de Nutrition Africaine: Elements de Base Appligue Tome 1, Acct, 1PD et editions.

Agarwal, A. and Allamaneni, S.R. (2004). Role of free radicals in female reproductive diseases and assisted reproduction. *Reproductive Biomedicine*, 9(3): 338-347.

Agarwal, A. and Prabakaran, S. (2005). Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology*, 43: 963-974.

Aggarwal, A. and Upadhyay, R. (2013). Heat Stress and Animal Productivity. Springer India. Pp. 2. DOI 10.1007/978-81-322-0879-2.

Aggarwal, A.and Singh, M. (2010). Hormonal changes in heat stressed Murrah buffaloes under two different cooling systems. *Buffalo Bull*, 29:1-6.

Ahmadu, S., Mohammed, A.A., Buhari, H. and Auwal, A. (2016). An Overview of Vitamin C

as an Antistress in Poultry. *Malaysian Journal of Veterinary Research*, 7(2): 9-22.

Ahmed, N., Haldar, S., Pakhira, M.C. and Ghosh, T.K. (2005). Growth performances, nutrient utilization and carcass traits in broiler chickens fed with a normal and a low energy diet supplemented with inorganic chromium and ascorbic acid. *Journal of Agricultural Science*, 143: 427-439.

Ajakaiye, J.J., Perez-Bello, A. and Mollineda-Trujillo, A. (2011). Impact of heat stress on egg quality in layer hens supplemented with L-ascorbic acid and DL-tocopherol acetate. *VeterinarskiArchive*, 81(1): 119-132.

Alaeldein, M., Abudabos, A.N., Al-Owaimer, E., Hussein, O.S. and Mutahar, H.A. (2018). Effect of Natural Vitamin C on Performance and Certain Haemato-Biochemical Values in Broiler Chickens Exposed to Heat Stress. *Pakistain Journal of Zoology*, 50(3): 951-955.

Al-Aquil, A., Zulkifli, I., Sazili, A.Q., Omar, A.R. and Rajion, M.A. (2009). The effects of the hot, humid tropical climate and early age feed restriction on stress and fear responses, and performance in broiler chickens. *Asian Australas Journal of Animal Science*,22: 1581-1586.

Albin, D.M., Wubben, J.E., Rowlett, J.M., Tappenden, K.A. and Nowak, R.A. (2007). Changes in small intestinal nutrient transport and barrier function after lipopolysaccharide exposure in two pig breeds. *Journal of Animal Science*, 85: 2517-2523.

Al-Gubory, K.H., Fowler, P.A. and Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidant in pregnancy outcomes. *The International Journal of Biochemistry and Cell Biology*, 42: 1634-1650.

Ali, M.N., Qota, E.M.A and Hassan, R.A. (2010). Recovery from adverse effects of heat stress on slow-growing chicks using natural antioxidants without or with sulphate. *International Journal of Poultry Science*, 9(2): 109-117.

Altan, O., Altan, A., Cabuk, M. and Bayraktar, H. (2000). Effects of heat stress on some blood parameters in broilers. *Turkish Journal of Veterinary Animal Science*, 24: 145-148.

Altan, Ö., Pabuccuoglu, A., Altan, A., Konyalioglu, S. and Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science,* 44: 545-550.

Ambali, S.F., Dayo, O.A., Muftaʻu, S., AbdulGaniyu, G., Olushola, O.O. and Joseph, O.A. (2010). Chlopyrifos- induced clinical haematological and biochemical changes in Swiss albino mice- Mitigating effect by co-administration vitamins C and E. *Life Science Journal*, 7(3): 37-44.

Anon, (2017). Livestock and Poultry: World Markets and Trade. Foreign Agricultural Service/USDA, Office of Global Analysis, Despite HPAI Outbreaks, Global Broiler Meat Production and Trade Forecast to Rise in2017. *United States Department of Agriculture*, Pp.

1-28.

Anon. (1987). FDA Food additives permitted in feed and drinking water of animals. *Federal Register,* 52:10887.

Anon. (2010 2012 2014). Regulation on quality for animal feed. *Official Gazette of the Republic of Serbia,* 4:113-127.

Anon. (2012). European Food Safety Authority (EFSA) Scientific opinion on safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R646 (Selmax 1000/2000) as feed additive for all species. *Journal of EuropeanFoodSafety Authority*, *10*: 2778.

Applegate, T.J. and Lilburn, M.S. (2002). Growth of the femur and tibia of a commercial broiler line. *Poultry Science,* 81: 1289-1294.

Ardia, D.R. (2013). The effects of nestbox thermal environment on fledging success and haematocrit in tree swallows. *Avian Biology Research*, 6: 99-103.

Ariyadi, B., Sudaryati, S., Harimurti, S., Wihandoyo, H., Sasongko, H., Habibi, M.F. and Rahayu, D. (2019). Effect of feed form on small intestine histomorphology of broilers. *Earth and Environmental Science,* 387: 012047.

Arthur,J.R.,McKenzie,R.C. andBeckett,G.J.(2003).Seleniumintheimmunesystem.*JournalofNutrition,*133:1457-1459.

Asli, M.M., Hosseini, S.A., Lotfollahian, H. and Shariatmadari, F. (2007). Effect of probiotics, yeast, Vitamin E and Vitamin C supplements on performance and immune response of laying hen during high environmental temperature. *International Journal of Poultry Science,* 6(12): 895-900.

Atawodi, S.E. (2011). Evaluation of the hypoglycemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-ofa" polyherbal tea (A-Polyherbal) in alloxan-induced diabetic rats. *Drug Invent Today.* 3: 270-276.

Attia, Y.A. and Hassan, S.S. (2017). Broiler tolerance to heat stress at various dietary protein and energy levels. *European Poultry Science,* 81, 171. https://doi.org/10.1399/eps.

Attia,Y.A.,Abdalah,A.A.,Zeweil,H.S.,Bovera,F.,TagEl-Din,A.A. and Araft, M.A. (2010). Effect of inorganicororganicselenium supplementation onproductiveperformance,eggqualityandsomephysiologicaltraitsofdual-purposebreeding hens. *Czech Journal of Animal Science*, 55, 505-519.

Attia, Y.A., Hassan, R.A. and Qota, M.A. (2009). Recovery from adverse effect of heat stress on slow growing chicks in the tropics 1: Effect of ascorbic acid and different levels of betaine. *Tropical AnimalHealth and Production*, 41: 807-818.

Attia, Y.A., Hassan, R.A., Tag El-Din, A.E. & Abou-Shehema, B.M. (2011). Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. *Journal of Animal Physiology and Animal Nutrition,* 95(6): 744-755.

Auer, B.L, Auer, D. and Rodgers, A.L. (1998). The effect of ascorbic acid ingestion on the biochemical and physicochemical risk factors associated with calcium oxalate kidney stone formation. *Clinical ChemistryLaboratory Medicine,* 36(3): 143-147.

Ayo, J.O., Minka, N.S. and Hussein, K.A. (2014). Effect of ascorbic acid administration on erythrocyte osmotic fragility in rabbits (*Oryctolagus cuniculus*) subjected to road transportation. *Journal of Applied. Animal Research,* 43(1): 26-32.

Ayo,J.O., Obidi,J.A. and Rekwot, P.I.(2011). Effects of Heat Stress on the Well-Being, Fertility, and Hatchability of Chickens in the Northern Guinea Savannah Zone of Nigeria: A Review. *International ScholarlyResearch Noticesof Veterinary Science,* Pp.10. http://dx.doi.org/10.5402/2011/838606.

Azad, M.A.K., Kikusato, M., Sudo, S., Amo, T. and Toyomizu, M. (2010). Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken. *Comparative Biochemistry and Physiology*, 157: 266 -271.

Bakhshalinejad.R.,Akbari,M.K.R. andZoidisE. (2018). Effectsofdifferentdietarysources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. *BritishPoultryScience*, 59: 81-91.

Balnave, D. and Brake, J. (2005). Nutrition and management of heat-stressed pullets and laying hens. *World's Poultry Science Journal*, 61(3): 399-406.

Baltić, M.Ž, Bošković, M., Ivanović, J., Dokmanović, M., Janjić, J., Lončina, J. and Baltić, T. (2013). Nanotechnology and its potential applications in meat industry. *Tehnologija mesa*, 54(2): 168-175.

Baltić, M.Ž., Dokmanović Starčević, M., Bašić, M., Zenunović, A., Ivanović, J., Marković, R., Janjić, J., Mahmutović, H. and Glamočlija, N. (2016). Effects of dietary selenium-yeast concentrations on growth performance and carcass composition of ducks. *Animal Production Science,* 7: 2-6.doi.org/10.1071/AN16126

Baltić, M.Ž., Dokmanović-Starčević, M., Bašić, M., Zenunović, A., Ivanović, J., Marković, R., Janjić, J. and Mahmutović, H. (2015). Effects of selenium yeast level in diet on carcass and meat quality, tissueseleniumdistributionandglutathioneperoxidaseactivityinducks.*AnimalFeedScience and Technology,* 210: 225-233.

Bancroft, J.D. and Stevens, A. (2008). Theory and practice of histological techniques, 7th Edition, Churchill Livingstone. Edinburg, London, Melbourne and New York, 725 pages.

Bartik, M. and Piskac, A. (1974). Veterinary Toxicology. Státní zemědělské nakladatelství, Prague, Czech Republic. (in Czech). 24-27.

Bartlett, J.R. and Smith, M.O. (2003). Effects of different levels of zinc on the performance and immune competence of broilers under heat stress. *JournalofPoultry Science,*82: 1580-1588.

Becker, B. (1983). The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal). *Agroforestry Systems*, 1, 257-267.

Bharavi, K., Reddy, A.G., Rao, G.S., Reddy, A.R. and Rao, S.V.R. (2010). Reversal of cadmium inducedoxidative stress in chicken by herbaladaptogens *Withania Somnifera* and *Ocimum* Sanctum.*Toxicology*, 17: 59-63.

Bhattacharya, L. (2010). Biochemistry of nutrition. Discovery Publishing House PVT. Ltd,New Delhi-110 002. 74-77.

Boostani, A., Sadeghi, A.A., Mousavi, S.N., Chamani, M. and Kashan, N. (2015). Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. *Journal*of *Livestock Science,* 178: 330-336.

Borges, S., Fischer, A., Da, A., Silva, V. and Maiorka, A. (2007). Acid-base balance in broilers. *World's Poultry Science Journal*, 63: 73-81.

Bounous, D.I. and Stedman, N.L. (2000). Normal Avian Haematology, Chicken and Turkey. In Feldman, B.F., Zinkl, J.G. and Jain, N.C.: Schalm's Veterinary Haematology. Philadelphia, Lippincott Williams and Wilkins, 1145-1154.

Bozkurt, M., Kucukvilmaz, K., Catli, A.U., Cinar, M., Bintas, E. and Coven, F. (2012). Performance, egg quality, and immune response of laying hens fed diets supplemented with manna-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. *JournalofPoultry Science,*91: 1379-1386.

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V. and Geraert, P.A. (2013). Comparative study of a new organic selenium source, seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. *British Journal of Nutrition,*110(4): 617- 624.

Bruno L.D.G. and Macari, M. (2002). Ingestão de água: mecanismos regulatórios. In: Macari M., Furlan R.L. and Gonzales, E, editors. Fisiologia aviária aplicada à frangos de corte. Jaboticabal: FUNEP; Pp. 201-208.

Bruno, L.D.G., Maiorka, A., Macari, M., Furlan, R.L., Givisiez, P.E.N (2011). Water intake behavior of broiler chickens exposed to heat stress and drinking from bell or and nipple drinkers. *Brazilian Journal of Poultry Science,* 13(2): 147-152.

Bunglavan, S.J., Garg, A.K., Dass, R.S. and Sameer, S. (2014). Use of nanoparticles as feed additives to improve digestion and absorption in livestock. *International Journal of Livestock Research,* 2(3):36-47.

Burkholder, K.M. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. *JournalofPoultry Science,* 87(9): 1734-1741.

Butcher, G.D. and Miles, R. (2012). Heat stress management in broilers. VM65 series of the Veterinary Medicine Large Animal Clinical Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Butts, C.L. and Sternberg, E.M. (2008). Neuroendocrine factors alter host defense by modulating immune function. *Cell Immunology,*252: 7-15.

Cahaner, A., Ajuh, J.A., Siegmund-Schultze, M., Azoulay, Y., Druyan, S., and Valle-Zarate, A. (2008). Effects of the genetically reduced feather coverage in naked neck and featherless broilers on their performance under hot conditions. *Journal Poultry Science,* 87: 2517-2527.

Cai, S.J., Wu, C.X., Gong, L.M., Song, T., Wu, H. and Zhang, L.Y. (2012). Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. *JournalofPoultry Science,* 91(10):2532-2539.

Cantatore, F.P. and Carrozzo, M. (1990). The action of ascorbic acid in increasing serum $(OH)_2D_3$ and osteocalcin in human. *Calcified Tissue International,* 46(1): 25.

Celi, P. and Chauhan, S.S. (2013). Oxidative stress management in farm animals: opportunities and challenges. In Proceedings of the 4th international conference on sustainable animal agriculture for developing countries, Pp. 95-109.

Chadare, F.J., Linnmann, A.R, Hounhouigan J.D. Nout, M.J. and Van Boeke, M.A. (2009). Badal Food Products. A review on their composition and nutritional value. In Critical Revised *Food Science Nutr*ition, 49(3): 254-274.

Chaiyabutr, N., Chanpongsang, S. and Suadsong, S. (2008). Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *International Journal of Biometeorology,* 52: 575–585. DOI: 10.1007/s00484-008-0151-x.

Chaiyabutr, N. (2004). Physiological reactions of poultry to heat stress and methods to reduce its effects on poultry production. *Thai Journal of Veterinary Medicine,* 32(2):17-30.

Chauhan, S.S., Celi, P., Leury, B.J., Clarke, I.J. and Dunshea, F.R. (2014). Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *Journal of Animal Science,* 92(8): 3364-3374.

Cheek, A. and Derenfeld, E. (2010). Comparative animal nutrition and metabolism. CABI, UK. Pp. 218-219.

Chen, G., Wu, J. and Li, C. (2013). The effect of different selenium levels on production performance and biochemical parameters of broilers. *Italian Journal of Animal Science,* 12: 486-491.

Choi, H.K., Gao, X. and Curhan, G. (2009). Vitamin C intake and the risk of gout in men: a prospective study. *Archive of Internal Medicine,* 169(5): 502-507.

Chopra, I.J., Solomon, D.H. and Ho, R.S. (1971). A radioimmunoassay of thyroxine. *Journal of Clinical Endocrinology,* 33: 865.

Chowdhury, V.S., Tomonaga, S., Ikegami, T., Erwan, E., Ito, K., Cockrem, J.F. and Furuse, M. (2012). Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature. *Comparative Biochemistry and Physiology,* 169: 70-76.

Cinar, M., Yigit, A.A., Yalcinkaya, I., Oruc, E., Duru, O. and Arslan, M. (2011). Cadmium inducedchanges on growth performance, some biochemicalparameters and tisse in broiler: Effects of vitamin Cand vitamin E. Asian Journal of Animal and Veterinary Advances, 6:923-934.

Clinical Diagnostic Division (1990). Veterinary Reference Guide, Eastman Kodak Company, Rochester, New York.

Collier, R.J., Baumgard, L.H., Zimbelman, R.B. and Xiao, Y. (2019). Heat stress: physiology of acclimation and adaptation. *Animal Frontier,* 9(1): 12-19.

Collier, R.J., Stiening, C.M., Pollard, B.C., VanBascorbic acidle, M.J., Baumgard, L.H., Gentry, P.C. and Coussens, P.M. (2006). Use of gene expression microarrays for evaluating environmental stress tolerance at the cellular level in cattle. *Journal of Animal Science,* 84:1-13.

Collins, A. (2018). Cholesterol in chicken. Retrieved 3/10/2018 from *http://www.annecollins.com/* Solomons, G.T.W. Organic Chemistry (4th ed.) John Wiley and Sons, New York. Pp. 270-273.

Cook, J.D. and Reddy, M.B. (2001). Effect of ascorbic acid intake on non-heme-iron absorption from a complete diet. *American Journal of ClinicalNutrition,* 73(1): 93-98.

Cooper, C.E. and Geiser, F. (2008). The "minimal boundary curve for endothermy" as a predictor of heterothermy in mammals and birds: a review. *Journal of Comparative*

Physiology B-Biochemical Systemic and Environmental Physiology, 178(1): 1-8.

Cooper-Mullin, C. and McWilliams, S.R. (2016). The role of the antioxidant system during intense endurance exercise: lessons from migrating birds. *Journal of Experimental Biology*, 219(23): 3684-3695.

Corinna, U. (2018). Eosinophil count: what it is and what it means. Healthline. https://www.heathline.com/health/eosinophil-count-absolute.

Dafwang, I.I. (2002). Broilers and broiler production in Nigeria. Poultry Production in Nigeria. National Training Workshop on Poultry Production in Nigeria, 1st -6th September, 2002. Pp. 95-114.

Daghir, N.J. (2008). Broiler feeding and management in hot climates, in: Daghir, N.J. (Ed.) Poultry production in Hot Climate, CAB International. Cromwell Press, *Trowbridge*, Pp. 227-260.

Daghir, N.J. (2009). Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Lohmann information.* 44: 6-15.

Dalia, M.A., Loh, T.C., Sazili, A.Q., Jahromi, M.F. and Samsudin, A.A. (2017). The effect of dietary bacterial organic selenium on growth performance, antioxidant capacity, and Selenoproteins gene expression in broiler chickens. *BMC Veterinary Research*, 13: 254.

Davies, J.A. (1995). Oxidative stress: the paradox of aerobic life. Free Radicals and Oxidative Stress: *Environmental, Drugs and Food Additive,* 61:1-13.

Dayyani, N. and Bakhtiyari, H. (2013). Heat stress in poultry: background and affective factors. *International journal of Advanced Biological and Biomedical Research,* 1(11): 1409-1413.

De-Almeida,J.N.,Dos-Santos,G.R.,Beteto,F.M.,De-Medei-rosL.G.,Oba,A.,Shimokomaki,M. andSoares,A.L.(2012). Dietary supplementation of chelated selenium and broiler chickenmeatquality. *Semina-CienciasAgrarias*, 33(2): 3117-3122.

Deeb, N. and Cahaner, A. (2002). Genotype-by-environment interaction with broiler genotypes differing in growth rate. 3. Growth rate and water consumption of broiler progeny from weight-selected versus non-selected parents under normal and high ambient temperatures. *Journal of Poultry Science,81*: 293-301.

Dei, H.K. and Bumbie, G.Z. (2011). Effect of wet feeding on growth performance of broiler chickens in a hot climate. *British Poultry Science*, 52: 82-85.

Del Rio, R., Moya, E.A. and Hurriage, R. (2010). Carotid body and cardio respiratory alterations in intermittent hypoxia: the oxidative link. *European Respiratory Journal*, 36: 143-150.

Del-Vesco, A.P., Gasparino, E., Zancanela, V., Grieser, D.O., Stanquevis, C.E., Pozza, P.C. and Oliveira-Neto, A.R. (2017). Effects of selenium supplementation on the oxidative state of acute heat stress-exposed quails. *Journal of Animal Physiology and Animal Nutrition.* 101: 170-179.

De-Medeiros, L.G., Oba, A., Shimokomaki, M., Pinheiro, J.W., Da-Silva, C.A., Soares, A.L., Pissinati, A. and De-Almeida, M. (2012). Performance, broiler carcass and meat quality characteristics, supplemented with organic selenium. *Semina: Ciências Agrárias,* 33(2):3361-3370

Deng W. (2012). The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. *JournalofPoultry Science,* 91(3): 575-582.

Dlouha, G., Sevcikova, S., Dokoupilova, A., Zita, L., Heindl, J. and Skrivan, M. (2008). Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers. *Czech Journal of Animal Science,* 53: 265-269.

Draper H. H. and Hadley M. (1990). Malondialdehyde determination as index of lipid peroxidation. Methods in enzymology, 186, 421-431.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews,*82: 47-95.

Du, J., Gullen, J.J. and Buettner, G.R. (2012). Ascrobic acid: chemistry, biology and the treatment of cancer. *Biochemistry and Biophysics Acta*, 182: 443-457.

Edem, V.F., Kosoko, A., Akinyoola, S.B., Owoeye, O., Rahamon, S.K. and Arinola, O.G. (2012). Plasma antioxidant enzymes, lipid peroxidation and hydrogen peroxide in wista rats exposed to Dichlorvos insecticide. *Archives of Applied Science Research,* 4(4): 1778-1781.

Egbe-Nwiyi, T.N., Nwaosu, S.C. and Salami, H.A. (2000). Haematological values of apparently healthy sheep and goats as influenced by age and sex in arid zone in Nigeria. *African Journal of Biomedical Research,* (32): 109-115.

Eggum, B.O. (1986): Blood urea Cheesbrough M. (1999): District Laboratory Practice in Tropical Countries. 1999 Ed Cambridge University Press, Edinburgh Building Cambridge, U.K. Measurement as a technique for assessing protein quality. *British Journal of Nutrition,* 4: 983-988.

El-Habbak, M.M., El-Ghamry, A.A., El-Mallah, G.M., Younis, H.H and El-Komy, E.M. (2011). Influence of dietary vitamin E and C supplementation on the performance and some metabolic response of broiler chicks subjected to heat stress. *World Journal of Agricultural Science,* 7: 258-269.

Elkheir, S., Mohammed, M.K., Ahmed, M.M. and Abdelgadir, S.M. (2008). Effect of feed restriction and ascorbic acid supplementation on performance of broiler chicks reared under heat stress. *ResearchJournal of Animal and Veterinary Science*, 3: 1-8.

El-Mallah, G.M., Yassein, S.A., Abdel-Fattah, M.M. and El-Ghamry, A.A. (2011). Improving performance and some metabolic response by using some antioxidants in laying diets during summer season. *Journal of Animal Science*, 7: 217-224.

El-Samra, H.A.A., Kalaba, Z.M., Tolba, A.A.H. and El-Deeb, M.A.I. (2014). Alleviating adverse effects of heat stress by using organic selenium and chromium for local laying hens. *Journal Animal and Poultry Production, Mansoura University*, 5(7): 397-411

El-Sheikh, A.M.H., Abdalla, E.A. and Hanafy, M.M. (2010). The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. *Egyptian Poultry Science Journal*, 30(2): 517-533.

Eroglu, S., Pandir, D., Uzun, F.G. and Bas, H. (2013). Protective role of vitamins C and E in dichlorivos induced oxidative stress in human erythrocyte *in vitro*. *Biological Research*, 46: 3338.

Eromosele, I.C., Eromosele, C.O. and Kuzhkuzha, D.M. (1991). Evaluation of mineral elements and ascorbic acid content in some wild plants. *Plant Foods and Human Nutrition*, 41:151-154.

Etkin, N.L. and Ross, P.J. (1982). Food as medicine and medicine as food. *Social Science Medicine*, 16:1559-1573.

Fagarasan, S. (2006). "Intestinal IgA synthesis: a primitive form of adaptive immunity that regulates microbial communities in the gut". *Current Topics in Microbiology and Immunology*, 308: 137-153.

Fan, C., Yu, B. and Chen, D. (2009). Effects of different sources and levels of selenium on performance, thyroid function and antioxidant status in stressed broiler chickens. *International Journal of Poultry Science*, 8(6): 583-587.

Farooqi, H.A.G., Khan, M.S., Khan, M.A., Rabbani, M., Pervez, K. and Khan, J.A. (2005). Evaluation of betain and vitamin C in alleviation of heat stress in broilers. *International Journal for Agricultural Biology,* 5: 744-746.

Felver-Gant, J.N., Mack, L.A., Dennis, R.L., Eicher, S.D. and Cheng, H.W. (2012). Genetic variations alter physiological responses following heat stress in 2 strains of laying hens. *JournalofPoultry Science,91*: 1542-1551.

Fernandes, J.M., Scapini, L.B., Gottardo, E.T., Burin, A.B., Marques, F.E. and Gruchouskei, L. (2013). Thermal conditioning during the first week on performance, heart morphology and carcass yield of broilers submitted to heat stress. *Acta Science*, 35: 311-313.

Fleming, D.J., Tucker, K.L., Jacques, P.F., Dallal G.E., Wilson P.W. and Wood R.J. (2002). Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort. *AmericanJournal of Clinical Nutrition,* 76(6): 1375-1384.

Food and Agricultural Organization (FAO) (2020). Nigeria at a glance. Food and Agricultural Organization of the United Nations. Available from: http://www.fao.org/nigeria/fao-in-nigeria/nigeria-at-a-glance/en/ Search in Google Scholar

Food and Agricultural Organization of the United Nations (FAOSTAT). (2017). Available from: http://www.fao.org/statistics/en/ Search in Google Scholar

Food Science Australia Meat Industry Services Section (FSAMISS). (2002). The causes of drip in meat. www.meatupdate.csiro.au

Foster, L. and Dunn, R. (1974). Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry*, 20: 365.

Fridovich, I. (1978). Free radicals in biology (Ed. W. Q. Proyer). 1: 239 (Acad Press, New York).

Fridovich, I. (1989). Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal Biology and Chemistry,* 264: 7761-7764.

Gangadoo,S.,Stanley,D.,Hughes,R.J.,Moore,R.J.andChapman,J. (2016).Nanoparticlesinfeed: Progress and prospects in poultry research. *Trends Food Science Technology*, 58: 115-126.

Garriga, C., Hunter, R.R., Amat, C., Planas, J.M., Mitchell, M.A. and Moreto, M. (2006). Heat stress increases apical glucose transport in the chicken jejunum. *American Journal of Physiology*, 290: R195-R201.

Gava, M.S., Moraes, L.B., Carvalho, D., Chitolina, G.Z., Fallavena, L.C.B., Moraes, H.L.S., Herpich, J. and Salle, C.I.P. (2015). Determining the best sectioning method and intestinal segment for morphometric analysis in broilers. *Brazilian Journal of Poultry Science*, 17(2): 145-150.

George, A., Pushkaran, S., Konstantinidis, D.G., Koochaki, S., Malik, P., Mohandas, N., Zheng, Y., Joiner, C.H. and Kalfa, T.A. (2012). Erythrocyte NADPH oxidase activity modulated by rac GTpase, PKC and plasma cytokines contributes to oxidative stress in sickle cell disease. *Journal of American Society of Haematology*, 121(11): 2099-2107.

Geraert, P.A., Padilha, J.C. and Guillaumin, S. (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: biological and endocrinological variables. *British Journal of Nutrition*,75: 205-216.

Ghasemzadeh, A. (2012). Global issues of food production. *Agrotechnology*, 1: (102)

http://dx.doi.org/10.4172/2168-9881.1000e102.

Ghazi, S.H., Habibian, M., Moeini, M.M. and Abdolmohammadi, A.R. (2012). Effects of different levels of organic and inorganic chromium on growth performance and immune competence of broilers under heat stress. *Biological Trace Element Research*, 146: 309-317.

Glew, H.R., Jaget, V., Lockette, J. D., Grivetti, T. C., Smith, E. L., Pastuszyh, C.G. and Milsom, M. (1997). Amino acid, fatty acid and mineral composition of 24 indigenous plants of Burkina Faso. *Journal of Food Comprehensive Analysis*, 10: 205-217.

Goodwin, M.A., Bounous, D.I., Brown, J., McMurray, B.L., Ricken, W.L. and Magee, D.L. (1994). Blood glucose values and definations for hypoglycemia and hyperglycemia in clinically normal broiler chicks. *Avaian Disease,* 38(4): 861-865.

Gous, R.M. and Morris, T.R. (2005). Nutritional interventions in alleviating the effects of high temperatures in broiler production. *JournalofWorld's Poultry Science,* 61: 463-475.

Gregory, N.G. (2010). How climatic change could affect meat quality. *Food Research International*, 43(7): 1866-1873.

Groce, A.W., Miller, E.R., Keahey, K.K., Ullrey, D.E., Ellis, D.J. (1971). Selenium supplementation of practical diets for growing-finishing swine. *Journal of Animal Science,* 32: 905-908.

Gross, W.B. (1988). Effects of ascorbic acid on the mortality of leghorn-type chickens due to overheating. *Avian Diseases,* 32: 561-562.

Gross, W.B. and Siegel, H.S. (1983). Evaluation of the heterophil/Lymphocyte Ratio as a measure of stress in chickens. *Journal of Avain Diseases,* 27(4): 972-979.

Gu, X.H., Hao, Y. and Wang, X.L. (2012). Over-expression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. *JournalofPoultry Science*,91: 790-799.

Guoshun, C., Jinfeng, W. and Chong, L, (2013). The effect of Different Selenium Levels on Production Performance and Biochemical Parameters of Broilers. *ItalianJournal of Animal Science,* 12(4): 79. DOI: 10.4081/ijas.2013.e79.

Gursu, M.F., Onderci, M., Gulcu, F., and Sahin, K. (2004). Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress *in vivo*. *NutrientResearch*, 24: 157-164.

Guyomard, H., Manceron, S. and Peyraud, J. (2013). Trade in feed grains, animals, and animal products: Current trends, future prospects, and main issues. *Animal Frontiers*. 3(1): 14-18.

Habibian, M., Ghazi, S., Moeini, M.M, and Abdol-Mohammad, A. (2014). Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers

reared under thermoneutral or heat stress conditions. *International Journal of Biometeorology*, 58: 741-752.

Hajati, H., Hassanabadi, A., Golian, A, Nassiri, M.H. and Nassiri, M.R. (2016). The effect of in ovo injection of grape seed extract and vitamin C on hatchability, antioxidant activity, yolk sac weight, performance and ileal microflora of broiler chickens. *Research Opinions in Animal and Veterinary Sciences*, 4: 633-638.

Halliwell, B. and Gutteridge, J.M.C. (1999). Free Radicals in Biology and Medicine. In: Halliwell, B. and Gutteridge, J.M.C., Eds., Free Radicals in Biology and Medicine, 3rd Edition, Oxford University Press, Oxford, 1-25.

Halliwell, B. and Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean. *British Journal ofPharmacology*, 142: 231-255.

Harsini,S.G.,Habibiyan,M.,Moeini, M.M. andAbdolmohammadi, A.R.(2012):Effectsofdietaryselenium,vitaminE,andtheir combinationongrowth,serummetabolites,andantioxidant defense system in skeletal muscle of broilers under heat stress.*BiologicalTraceElementResearch,*148:322-330.

Hartikainen,H.(2005).Biogeochemistryofseleniumandits impact on food chain duality and human health. *Journal ofTraceElementsMedicineandBiology*,28:309-318.

Hassanzadeh, M.L., Buys, N. Dewil, E., Rahimi, G. and Decuypere, E. (1997). The prophylactic effect of vitamin C supplementation on broiler ascites incidence and plasma thyroid hormone concentration. *Avian Pathology,* 26(1): 33-44. DOI:10.1080/03079459708419191.

Hediger, M.A. (2002). New view at C. *Natural Medicine*, 8(5): 445-446.

Heindl, J., Ledvinka, Z., Englmaierova, M., Zita, L. andTumova, E. (2010). The effect of dietary selenium sources and levels on performance, selenium content in muscle and glutathione peroxidase activity in broiler chickens. *Czech Journal ofAnimal Science*, 55:572-578.

Hoffman, P.R. (2007). Mechanisms by which selenium influences immune responses. *Archivum Immunologiae et Therapiae Experimentalis*, 55(5): 289-97. DOI: 10.1007/s00005-007-0036-4

Holik, V. (2009). Management of laying hens to minimize heat stress. *Lohmann Information*, 44:16-29.

Hosseini-Mansoub, N., Chekani-Azar, S., Tehrani, A.A., Lotfi, A, Manesh, M.K. (2010). Infleunce of dietary vitamin E and zinc on performance, oxidative stability and some blood measures of broiler chickens reared under heat stress (35 ^{0}C). *Journal Agribiology*, 27(2): 103-110.

Hu, C.H., Li, Y.L., Xiong, L., Zhang, H.M., Song, J. and Xia, M.S. (2012). Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. *Animal Feed Science Technology,* 177(3): 204-210

Hudson, B.J. and El-Difrawi, E.A. (1979). The sapogenins of the seeds of the four Lupin spp. *Journal of Plants and Food* 3:181-186.

Hudson, B.J. and EL-Difrawi, E.A. (1979). The sapogenins of the seeds of four lupin species. *Journal of PlantFood,* 3: 181-186.

Imik, H., Atasever, M.A., Urgar, S., Ozlu, H., Gumus, R. and Atasever, M. (2012). Meat quality of heat stress exposed broilers and effect of protein and vitamin E. *British Poultry Science,* 53: 689-698.

Institute for Agricultural Research Metrological Stations, (IARMS). (2019). Metrological data from IAR Metrological Station, Ahmadu Bello University, Samaru, Zaria, Nigeria.

Isikwenu, J.O. and Omeje, S.I. (2007). The effects on the blood, carcass and organs of finisher broilers fed groundnut cake diets replaced with urea-treated and fermented brewer's dried grains. *Agricultural Journal,* 2(1): 64-70

Ismail, I.B., Al-Busadah, K.A and El-Bahr, S.M. (2013). Oxidative stress biomarkers and biochemical profile in broilers chicken fed Zinc Bacitracin and Ascorbic acid under hot climate. *American Journal of Biochemistry and Molecular Boilogy,* 3(2): 202-214.

Jahejo, A.R., Rajput, N., Mohammad, N. Pirzado, S.A., Nizamani, Z.A., Tarique, M. and Memon, A.A. (2016). Effect of Ascorbic Acid in Heat Stressed Broiler Chickens Of Sindh Zone. *Science International (Lahore),* 28(4): 4089-4093.

Jain, N.C. (1986). Schalm Veterinary Haematology, 4th edition Philadelphia, Lea and Ferbinger. *Journal of Applied Poultry Research,*Vol.5, Pp. 203-209.

Jain, N.C. (1993). Essentials of veterinary hematology. 1st Ed. Lea and Febiger Publication, Philadelphia, ISBN: 9780812114379, Pp: 589-595.

Jan-Dirk, V.D.K. and Loek, D.L. (2013). Water intake of poultry. 19th European Symposium on Poultry Nutrition, Potsdam, Germany. 1-11.

Jena, B.P., Panda, N., Patra, R.C., Mishra, P.K., Behura, N.C. and Panigrahi, B. (2013). Supplementation of Vitamin E and C Reduces Oxidative Stress in Broiler Breeder Hens during Summer. *Journal ofFood and Nutrition Sciences,* 4: 33-37.

Jiang, Z., Lin, Y., Zhou, G., Luo, L., Jiang, S, and Chen, F. (2017). Effects of dietary selenomethionine supplementation on growth performance, meat quality and antioxidant property in yellow broilers. *Journal Agriculture, Food and Chemistry,* 57(20): 9769–9772.

Jlali, M., Briens, M., Rouffineau, F., Mercerand, F., Geraert, P.A. and Mercier, Y. (2013). Effect of 2-hydroxy-4-methylselenobutanoic acid as a dietary selenium supplement to improve the selenium concentration of table eggs. *Journal of Animal Science*, 91(4):1745-1752

Kasaikina, M.V., Kravtsova, M.A., Lee, B.C., Seravalli, J., Peterson, D.A., Walter, J., Legge, R., Benson, A.K., Hatfield, D.L. and Gladyshey, V.N. (2011). Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *Natural Sciences*, 25(7): 2492-2499. https://doi.org/10.1096/fj.11-181990.

Keita, A.V. and Soderholm, J.D. (2010). "The intestinal barrier and its regulation by neuroimmune factors". *Neurogastroenterology andMotility*, 22(7): 718-733.

Kerharo, J. and Adam, J.G. (1974). "La Pharmacopee Senegalis trationalle plantes medicales Toxiques Edition Vigot Freres Paris.

Keshavamurthy, R.S., Kumar, S., Manohar, C.B. and Sharadamma, K.C. (2013). Effect of antioxidant formulation supplementation through water on antioxidant status of broiler chicken. *International Journal of Advanced Biological Research*, 3(3): 470-474.

Khan, R., Naz, S., Nikousefat, Z., Selvaggi, M., Laudadio, V. and Tufarelli, V. (2012). Effect of ascorbic acid in heat-stressed poultry. *World's Poultry Science Journal*, 68(3): 477-490.

Khan, S.H and Sardar, R. (2005). Effect of vitamin supplementation on the performance of Desi, Fayoumi and commercial white leghorn chicken exposed to heat stress. *Pakistan Veterinary Journal*, 25: 163-166.

Khan, R.U., Naz, S., Nikousefat, Z., Tufarelli, V., Javadani, M.
Rana, N., and Laudadio, V. (2011). Effect of vitamin E in heat-stressed poultry. *World's Journal Poultry Science*, 67: 469-478.

Khanal, D. and Knight, A. (2010). Selenium: Its Role in Livestock Health and Productivity. *Journal of Agriculture and Environment*, *11*, 101-106. https://doi.org/10.3126/aej.v11i0.3657

Kikusato, M. and Toyomizu, M. (2013). Heat stress-induced over-production of mitochondrial reactive oxygen species is down-regulated in laying-type chickens. *Energy and Protein Metabolism and Nutrition in Sustainable Animal Production*, 134: 267-268.

Kikusato, M., Ramsey, J.J., Amo, T. and Toyomizu, M. (2010). Application of modular kinetic analysis of mitochondria oxidative phosphosylation in skeletal muscle of birds exposed to acute heat stress. *Federation of European Biochemistry Society Letters*, 584: 3143-3148.

Kim, D.K., Lillehoj, H.S., Lee, K.W., Jang, S.I., Neumann, A.P., Siragusa, G.R., Lillehoj, E.P. and Hong, Y.H. (2012). Genome-wide differential gene expression profiles in broiler chickens with gangrenous dermatitis. *Avian Diseases*, 56: 670-679.

Kingma, B., Frijns, A. and Lichtenbalt, W.V. (2012). The thermoneutral zone: implication for metabolic studies. *Front Bioscience*, 4: 1975-1985.

Kingori, A.M. (2011). Review of the factors that influence egg fertility and hatchability in poultry. *International Journal of Poultry Science*, 10: 483-492.

Klasings, K.C. (1998). Nutritional modulation of resistance to infectious diseases. *Poultry Science*, 77: 1119-1125.

Klimczack, I., Malecka, M., Szlachta, M. and Gliszcynska- Swiglo, A. (2007). Effects of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition*, 20: 312-322.

Klooppel, C., Michels, C., Zimmer, J., Hermann, J.M. and Riemer, J. (2010). Yeast redistribution of sod1 to the mitochondrial intermembrane space provides protection against respiration derived oxidative stress. *Biochemistry and Biophysic Research*, 403: 114-119.

Konca, Y., Kirkpinar, F., Mert, S. and Yurtseven, S. (2009). Effects of dietary ascorbic acid supplementation on growth performance, carcass, bone quality and blood parameters in broilers during natural summer temperature. *Asian Journal of Animal Veterinary Advance*, 4: 139-147.

Kubin, A., Skoumal, R., Tavi, P., Konyi, A., Perjes, A., Leskine, H., Ruskoaho, H. and Szokodi, I. (2011). Role of reactive oxygen species in the regulation of cardiac contractility. *Journal of Molecular and Cellular Cardiology*, 50(5): 884-893.

Kuhn, V., Diederich, L., Stevenson, T.C., Christian, M.K., Wiebke, L., Christina, P., Tatsiana, S., Isakson, B. E., Kelm, M. and Cortese-Krott, M.M. (2017). Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. *Antioxidants & Redox Signaling*, 26: 13.

Kumar, K., Mishra, A., Sheikh, A.A., Patel, P., Ahirwar, M.K., Bashir, S.M. and Ali, A. (2017). Effect of ascorbic acid on some biochemical parameters during heat stress in Commercial broilers. *International Journal of Current Microbiology and Applied Sciences*, 6(11): 5425-5434.

Kutlu, H.R. (2001). Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature. *Arch Tierernahrung*, 54: 127-139.

Kutlu, H.R. and Forbes, J.M. (1993). Changes in Growth and Blood Parameters in Heat-Stressed Broiler Chicks in Response to Dietary Ascorbic Acid. *Livestock Production Science*, 36: 335-350.

Kutlu, H.R. and Forbes, J.M. (2000). Effects of environmental temperature and dietary ascorbic acid on the diurnal feeding pattern of broilers. *Turkish Journal of Veterinary*

AnimalScience, 24: 479-491.

Kwiecient, S., Brzozowski, T., Konturek, P.C., Pawlik, M.W. and Pawlik, W.W. (2004). Gastro protection by pennxyfilline against stress induced gastric damage. Role of lipid peroxidation, antioxidizing enzymes and pro-inflammatory cytokines. *Journal of Physiology and Pharmacology*, 55(2): 337-355.

Lamb, G.N. (1991). Manual of Veterinary Laboratory Technique. CIBA-GEIGY, Kenya, Pp. 96-107.

Lara, L.J. and Rostagno, M.H. (2013). Impact of Heat Stress on Poultry Production. *Journal of AnimalScience*, 3: 356-369.

Latin American Veterinary Congress. (2009). Clinical Pathology in Avian Species. Latin American Veterinary Congress (LAVC), Lima, Peru.

Lee, J., Giordano, S. and Zhang, J. (2012). Autophagy, mitochondria and oxidative stress: cross talk and redox signaling. *Biochemical Journal*, 441: 523-540.

Li, Y., Cai, H.Y., Liu, G.H., Dong, X.L. and Chang, W.H. (2009). Effects of stress simulated by dexamethasone on jejunal glucose transport in broilers. *Poultry Science*, 88: 330-337.

Liao, X., Lu, L., Li, S., Liu, S., Zhang, L., Wang, G., Li, A., Luo, X.(2012).Effectsofseleniumsourceandlevelongrowth performance, tissue selenium concentrations, antioxidation, and immune functions of heat-stressed broilers. *BiologicalTrace Element Research*, 150: 158-165.

Lin, H., Buyse, J., Sheng, Q., Xie, Y. and Song, J. (2003). Effects of Ascorbic Acid Supplementation on the Immune Function and Laying Performance of Heat-Stressed Laying Hens. *Feed Agricultural Environment*, 1(2): 103-107.

Lin, H., Decuypere, E. and Buyse, J. (2004). Oxidative stress induced by corticosterone administration in broiler chickens (*Gallusgallus domesticus*). Chronic exposure. *Comparative Biochemistry and Physiology*, 139: 737-744.

Lin, H., Decuypere, E., Buyse, J., (2006a). Acute heat stress induces oxidative stress in broiler chickens. *Comparative Biochemistry and Physiology Part A: Molecular Integrated Physiology*, 144: 11-17.

Lin, H., Jiao, H.C., Buyse, J. and Decuypere, E. (2006b). Strategies for preventing heat stress in poultry. *World's Poultry Science Journal*, 62: 71-86.

Lin, H., Sui, S.J., Jiao, H.C., Jiang, K.J., Zhao, J.P. and Dong, H. (2007). Effects of diet and stress mimicked by corticosterone administrationon early post mortem muscle metabolism of broiler chickens. *Poultry Science*, 86: 545-554.

Liu, R., Chen, H., Bai, H., Zhang, W., Wang, X., Qui, X., Zhang, X., Li, W., Liang, X. and

Hay, C. (2013). Suppression of nuclear factor erythroid 2-related factor 2 via extracellular signal-regualted kinase contributes to bleomycin-induced oxidative stress and fibrogenesis. *ToxicologyLetter*, 220: 15-25.

Lockett, C.T. Calvert, C.C. and Grivetti, L.E. (2000). Energy and micro-nutrient. Composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani Northern Nigeria. *InternationalJournal of Food Science Nutrition,* 51:195-108.

Lohakare, J.D., Kim, J.K., Ryn, M.H., Hahn, T.W. and Chae, B.J. (2005). Effects of vitamin C and vitamin D interactions on the performance, immunity and bone characteristics of commercial broilers. *Journal of AppliedPoultry Research*, 14(4): 670-678.

Louis, R. and Caplan, M.D. (2009). Imaging and Laboratory Diagnosis. Caplan's Stroke Ed. Fourth edition. Pp. 87.

Lu, Z., He, X.F., Ma, B.B., Zhang, L., Li, J.L., Jiang, Y., Zhou, G.H. and Gao, F. (2017). Chronic Heat Stress Impairs the Quality of Breast-Muscle Meat in Broilers by Affecting Redox Status and Energy-Substance Metabolism. *Journal of Agriculture and Food Chemistry*, 65: 11251-11258.

Lushchak, V. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals *Comparative Biochemistry and Physiology,* 153: 175-190.

Magdi, A.O. (2004). Chemical and nutrient of Baobab (*Adansonia digitata*). Fruit and seed protein solubility. *In Plant Food for Human Nutrition*, 59: 29-33.

Mahan,D.C.,Cline,T.R. andRichert,B.(1999):Effectofdietary levels of selenium-enriched yeast and sodium selenite, serum glutathione activity, carcass characteristics and loinduality.*JournalofAnimalScience*,77,2172-2179.

Mahmoud, K.Z. and Edens, F.W. (2003). Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). *Comprehensive Biochemical Physiology Part B: Biochemistry and Molecular Biology*, 136(4):921-34.

Mahmoud, K.Z., Edens, F.W., Eisen, E.J. and Havenstein, G.B. (2004). Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers subjected to cyclic heat stress. *Comparative Biochemistry and Physiology Part B: Biochemistry and molecular Biology,* 137(1): 137-142.

Mahmoud, U.T., Mootaz, A.M., Abdel-Rahman, M. and Darwish, H.A. (2014). Effects of propolis, ascorbic acid and vitamin e on thyroid and corticosterone hormones in heat stressed broilers. *Journal of Advanced Veterinary Research*,4(1): 18-27.

Maiorka, A., Santin, E., Dahlke, F., Boleli, I.C., Furlan, R.L. and Macari, M. (2003). Posthatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. *Journal of Applied Poultry Research,* 12: 483-492.

Majekodunmi, B.C., Sokunbi, O.A, Ogunwole, O.A. and Adebiyi, O.A. (2013). Influence of erythrocytes and ascorbic acid supplementation on serum and erythrocytic indices of broiler chickens reared in a hot environment. *African Journal of Agricultural Research*, 18:701-706.

Majid, S., Jeremy, J.C., Stuart, W., Mitchell, R., John, B.F. and Frank, R.D. (2018). Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. *Animals*, 8: 162.

Manfredini, S., Vertuani, S., Braccioli, E. and Valentin, B. (2002). Anti-oxidant capacity of Adansonia digitata fruit pulp and leaves In "*ACTA PHYTOTHERAPEUTICA*, 5(2): 2-7.

Mardones, L., Zuniga, F.A., Villagrans, M., Sotomayor, K., Mendoza, P., Esobar, D., Gonzales, M., Ormazabal, V., Maldonado, M., Onate, G., Angulo, C., Concha, I.I., Reyes, A.M., Carcamo, J.G., Barra, V., Vera, J.C. and Rivas, I. (2012). Essential role of intracellular glutathione in controlling ascorbic acid transporter expression and function in rat hepatocytes and hepatoma cells. *Free Radical BiologicalMedicine*,52: 1874-1887.

Marelli, S.P., Redaelli, V., Naini, C.L., Cozzi, M.C. and Luzi, F. (2012). Thermography, a non-invasive method to investigate thermo regulation as welfare indicator in naked neck broiler chickens. 11th International Conference on Quantitative Information for Red Thermography, 11-14 June. Naples, Italy, pp. 2.

Marketon, J.I.W. and Glaser, R. (2008). Stress hormones and immune function. *Cell Immunology*, 252, 16-26.

Massey, L.K., Liebman, M. and Kynast-Gales, S.A. (2005). Ascorbate increases human oxaluria and kidney stone risk. *Journal of Nutrition*, 135(7): 1673-1677.

May, J.M., Qu, Z.C., Neel, D.R. and Li, X. (2003). Recycling of vitamin C from its oxidized forms by human endothelial cells. *Biochemistry Biophysics Acta*, 1640(23): 153-161.

May, J.M., Qu, Z.C., Qiao, H. and Koury, M.J. (2007). Maturational loss of the vitamin C transporter in erythrocytes. *Biochemistry Biophysics Research*, 360(1): 295-298.

McDaniel, C.D., Hood, J.E. and Parker, H.M. (2004). An attempt at alleviating heat stress infertility in male broiler breeder chickens with dietary ascorbic acid. *International Journal of Poultry Science*,*3*: 593-602.

Mcdowell, L.R. (1989). Vitamins in animal nutrition: vitamin C, Folacin, in: Mcdowell, L.R. (ed.). *Comparative Aspect to Human Nutrition*, 289-322.

McKee, J.S. and Harrison, P.C. (2013). Supplemental ascorbic acid does not affect heat loss in broiler chickens exposed to elevated temperature. *Journal of Thermal Biology*, 38: 159-162.

McKee, J.S. and Hurrison P.C. (1995). Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. *Poultry Science*, 74: 1772-1785.

Melesse, A., Mascorbic acidk, S., Schmidt, R. and Von-Lengerken, G. (2011). Effect of long-term heat stress on key enzyme activities and T3 levels in commercial layer hens. *International Journal of Livestock Production*, 2: 107-116.

Melissa, G. (2015). White blood cells in Dogs and Cats. University of Illinois. Urbana Champaign. Vetmed.illinois.edu/pet.

Melo, A.S., Fernandes, R.T.V., Marinho, J.B.M., Arruda, A.M.V., Figueiredo, L.C. and Fernandes, R.T.V. (2016). Relationship between temperature and nutrition on laying performance. *Public Veterinary Medicine*,10: 855-860.

Minka, N.S. and Ayo, J.O. (2013). Physiological and behavioural responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid. *Journal ofVeterinary Behaviour*, 8: 349-356.

Miraei-Ashtiani, S.R, Zamani, P., Shirazad, M. and Zare-shahned, A. (2004). Comparison of the effect of different diets on acute heat stressed broilers. In *Proceeding of the 22nd World Poultry Congress*, Pp. 552, Istanbul, Turkey.

Mitruka, B.M. and Rawnsley, H.M. (1997). Clinical, Biochemical and Haematological Reference Value in Normal Experimental Animals. Mason Publishing Company, New York. Pp. 35-50.

Mohapatra, P., Swain, R.H., Mishra, S.K., Behera, T., Swain, P., Mishra, S.S., Behura-Sabat, S.C., Sethy, K., Dhama, K. and Jayasankar, P. (2014). Effects of dietary nano-selenium on tissue selenium deposition, antioxidant status and immune functions in layer chicks. *Internatinal Journal of Pharmacology,* 10(3): 160-167

Moreno-Reyes, R., Egrise, D., Neve, J. and Pasteels, J.L. (2009). Selenium deficiency induced growth retardation is associated with an impaired bone metabolism and osteopenia. *Journal of Bone and Mineral Research*, 18(8): 1-7.

Mosleh, N., Shomali, T., Nematollahi, F., Ghahramani, Z., Khafi, M.S.A and Namazi, F. (2018). Effect of different periods of chronic heat stress with or without vitamin C supplementation on bone and selected serum parameters of broiler chickens. *Avian Pathology*, 47(2): 197-205. DOI:10.1080/03079457.2017.1401212.

Muhammad, A.S., Abubakar, M., Adamu, S., Shehu, N.S. and Adamu, G. (2016). Effects of ascorbic acid on weight gain and haematological profile of broiler chickens. *ATBU, Journal of Science, Technology & Education (JOSTE),* 4 (1): 127-133.

Mujahid, A., Yoshiki, Y., Akiba, Y. and Toyomizu, M. (2005). Superoxide radical production

in chicken skeletal muscle induced by acute heat stress. *Journal of PoultryScience*, 84: 307-14.

Mustaf, S., Kahraman, N.S. and Firat, M.Z. (2009). Intermittent partial surface wetting and its effect on body-surface temperatures and egg production of white brown domestic laying hens in Antalya (Turkey). *Journal of British Poultry Science, 50*: 33-38.

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S. and Bernabucci, U. (2010). Effects of climate changes on animal production and sustainability of livestock systems. *JournalLivestock Science,130*: 57-69.

National Research Council (NRC). (1994). Nutrient requirement of poultry, National Academy Press, Washington, D.C. Ninth Edition.

Nienaber, J.A., Deshazer, J.A., Xin, H., Hillman, P.E., Yen, J. and Ferrel, C.F. (2009). Measuring energetic of biological processes. *Agriculture Biosystem Engineering*, 233: 73-112.

Niu, Z.Y., Liu, F.Z., Yan, Q.L. and Li, W.C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. *JournalofPoultry Science,*88: 2101-2107.

Njoku, P.C. (1990). The role of vitamin C in Livestock production. A workshop organized by Messr Roche Nig. Ltd. Lagos Sheraton Hotel. Sept 24.

Nockels, C.F. (1984). Effect of ascorbic acid on chicken metabolism, in: Wegger, I., Tagwerker, F.J. and Moustgascorbic acidrd, J. (eds). Ascorbic acid in domestic animals. *RoyalDanish Agricultural SocietyCopenhagen*, 23-31.

Nour, A.A., Magboul, B.I. and Kheiri, N.H. (1980). Chemical composition of Baobab (*Adansonia digitata L.*) *Tropical Science*, 22: 383-388.

Obizoba, T.C. and Amaechi, N.A. (1983). The effect of processing methods on the chemical composition of Baobab (*Adansonia digitata*) pulp and seed. *Ecology of Food Nutrition*, 29: 199-205.

Obizoba, T.C. and Anyika, J.U. (1994). Nutritive value of baobab milk (gubdi) and mixtures of baobab (*Adansonia digitata L*) and hungry rice, acha (*Digitaria exilis*) flours. *Plant Food* and *Human Nutrition,* 46:157-165.

Ogunwole, O.A., Adedeji B.S., Olumide, M.D., Mosuro A.O. and Adewemimo, I.E. (2018). Effects of Dietary Supplemental Ascorbic Acid and Cholecalciferol on Bone Characteristics of Hens at the Late Laying Stage. *International Journal of Food Science and Nutrition Engineering,* 8(6): 142-150. DOI: 10.5923/j.food.20180806.02.

Ogwu, J. (2010). Proximate Composition, Anti-Nutritional Factors and quantification of vitamin E (α–Tocophenol of Aloe vera (A barbadensis Leaf Gel.) Thesis. Dept. of Biochemistry ABU, Zaria Pp 66.

Oke, O.L. (1966). Chemical studies of some Nigerian vegetables. *Tropical Science, Pp.* 3.

Okunlola, D.O., Akande, T.O. and Nuga, H.A. (2015). Haematological and serum characteristics of broiler birds fed diets supplemented with varying levels of selenium powder. *Journal of Biology, Agriculture and Healthcare*, 5(1): 107-110.

Oliveira, M.C., Arantes, U.M., Stringuini, J.H. (2010). Efeito do balanco eletrolitico da racao sobre parametros osseos e da cama de frango. *Biotemas*, 23 (1): 203-209.

Olsen, R.E., Sundell, K., Mayhew, T.M., Myklebust, R. and Ringo, E. (2005). Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture*, 250: 480-495.

Olszewska, M., Wiatrow, J., Bober, J., Stachowska, E., Golembiawska, E., Jakubowska, K., Stanczyk-Dunaj, M. and Pietreak-Wowacka, M. (2012). Oxidative stress modulates the organization of erythrocyte membrane cytoskeleton. Postepy Hig Medecine Dosw, 66: 534-542.

Olugbenga, A.O (2015). Bone characteristics of broiler chickens fed diets supplemented with graded levels of vitamin C.*Agriculture and Biology Journal of North America*, 2151-7517. doi:10.5251/abjna.2015.6.1.30.33.

Olukomaiya, O.O., Adeyemi, O.A., Sogunle, O.M., Abioja, M.O. and Ogunsola, I.A. (2015). Effect of Feed Restriction and Ascorbic Acid Supplementation on Growth Performance, Rectal Temperature And Respiratory Rate of Broiler Chicken. *Journal of Animal and Plant Sciences*, 25(1): 65-71.

Ozbal, S., Erbil, G., Kocdor, H., Tugyan, K., Pekcetin, C. and Ozogul, C. (2008). The effects of selenium against cerebral ischemiareperfusion injury in rats. *Neuroscience Letters*, 438: 265-269.

Padgett, D.A. and Glaser, R. (2003). How stress influences the immune response. *Trends Immunology*,24: 444-448.

Pamok, S., Aengwanich, W. and Komutrin, T. (2009). Adaptation to oxidative stress and impact of chronic oxidative stress on immunity in heat-stressed broilers. *Journal of Thermoregulatory Biology*, 34: 353-357.

Panda, A.K. and Cherian, G. (2013). Role of vitamin E in counteracting oxidative stress in poultry. *Journal of Poultry Science*, 51(2): 10-15.

Panda, A.K., Ramarao, S.V. and Raju, M.V.L.N. (2007). Effect of Vitamin C Supplementation on Performance, Immune Response and Antioxidant Status of Heat Stressed White Leghron Layers. *Indian Journal of Poultry Science*, 42(2): 169-173.

Pandey, K.B. and Rizvi, S.I. (2013). Resveratrial upregulates erythrocyte plasma membrane

redox system and mitiages oxidation induced alterations in erythrocytes during aging in humans. *Rejuvenation Research*, 16: 232-240.

Peebles, E.D. and Brake, J. (1985). Relationship of dietary ascorbic acid to broiler breeder performance. *Poultry Science,* 64: 2041-2048.

Persia, M.E., Utterback, P.L., Biggs, P.E., Koelkebeck, K.W. and Parsons, C.M. (2003). Interrelationship between environmental temperature and dietary non-phytate phosphorus in laying hens. *Poultry Science,* 82: 1763-1768.

Phyto Trade Africa (2006). Boabab dried fruits pulp application for novel foods approvals in the EU as Food ingredient.

Pilarczyk, B., Jankowiak, D., Tomza-Marciniak, A., Pilarczyk, R., Sablik, P., Drozd, R., Tylkowska, A. and Skolmowska, M. (2012). Selenium concentration and glutathione peroxidase (GSH-Px) activity in serum of cows at different stages of lactation. *Biological Trace Element Research*, 147:91-96.

Pointillart, A., Denis, I., Colin, C. and Lacroix, H. (1997). Vitamin C Supplementation Does Not Modify Bone Mineral Content or Mineral Absorption in Growing Pigs. *American Society for Nutritional Sciences*, 28: 1514-1518.

Poungpong, K., Sumiyoshi, E. and Bunchasak, C. (2019). Effect of gallotannoids on the productive performance and stress index of broiler chickens under stress conditions. *International Journal of Poultry Science,* 18: 63-68.

Preedy, V.R., Watson, R.R. and Sherma, Z. (2010). Dietary Components and Immune Function. *Nutrition and Health*, Totowa, NJ: Humana Press, Pp. 36-52.

Preter, F.S. (2000). Organic selenium benefits to animal and humans. A biochemist view. In: Proceedings of Alltech's 16th symposium. Nottingham University Press, Nottingham, Pp. 205-213.

Price, M.L., Scoyoc, V.S. and Butter, L.G. (1978). A critical evaluation of reaction as assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214-1218.

Prieto, M.T. and Campo, J.L. (2010). Effect of heat and several additives related to stress levels on fluctuating asymmetry, heterophil:lymphocyte ratio, and tonic immobility duration in White Leghorn chicks. *JournalofPoultry Science*,89: 2071-2077.

Purswell, J.L., Dozier, I.I.W., Hammed, A., Olanrewaju, J., Davis, D. and Xin, H. (2012).Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. *Proceedings of the 9th International Livestock Environment Symposium*, July, 8-12. Valencia. Spain.

Pusta, D., Odagiu, A., Ersek, A. and Pascal, I. (2003). The variation of triiodothyronine (T3) level in milking cows exposed to direct solar radiation. *Journal of Cent Euro Agriculture*, 4:

308-312.

Puthpongsiriporn, U., Scheideler, S.E., Sell, J.L. and Beck, M.M. (2001). Effects of Vitamin E and C Supplementation on Performance, *in Vitro* Lymphocyte Proliferation, and Antioxidant Status of Laying Hens during Heat Stress. *Poultry Science*, 80(8): 1190-1200.

Quan, X., Lim, S. and Jung, G. (2011). Reactive oxygen species downregulate catalase expression via methylation of a CpG Island in the oct-1 promoter. *Federation of European Biochemical Society Letters*, 585: 3435-3441.

Quinteiro-Filho, W.M., Gomes, A.V., Pinheiro, M.L., Ribeiro, A., Ferraz-de-Paula, V., Astolfi-Ferreira, C.S., Ferreira, A.J. and Palermo-Neto, J. (2012). Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella* Enteritidis. *Avian Pathology,* 41: 421-427.

Quinteiro-Filho, W.M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M.L., Sakai, M., As, L.R., Ferreira, A.J. and Palermo-Neto, J. (2010). Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Journal ofPoultry Science,89*: 1905-20101914.

Raduta, A., Curca, D. and Constantinescu, C. (2011). Variation of some haematological indices and white blood cell count in chicks fed with organic selenium supplemented fodder. *Veterinary Medicine,* 57: 39-44.

Raisbeck, M.F. (2000). Selenosis. *Veterinary Clinical North American Food Animal Practice*, 16: 465-80.

Rajagopalan, R., Kode, A., Penumatha, S.V., Kallikat, N.R., and Venugopal, P.M. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmaceutical Sci*ence, 83: 2747-2752.

Ramadasan-Nair, R., Gayathri, N., Mishra, S., Sunitha, B., Mythri, R.B., Nalini, A., Subbannayya, Y., Harsha, H.C., Kolthur-Seetharam, U. and Bharath, M.M.S. (2014). Mitochondrial alterations and oxidative stress in an acute transient mouse model of muscle degeneration. *Journal of Biological Chemistry*, 289: 485-509.

Rama-Rao, S.V., Prakash, B., Raju, M.V.L.N., Panda, A.K., Poonam, S. and Murthy, O.K. (2013). Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. *Asian Australasian Journal of Animal Sciences*, 26: 247-252.

Ranjan, A., Sinha, R., Devi, I., Rahim, A. and Tiwari, S. (2019). Effect of Heat Stress on Poultry Production and their Managemental Approaches. *International Journal of Current Microbiology and Applied Science,*8(2): 1548-1555.

Rashidi, A.A., Ivari, Y.G., Khatibjoo, A. and Vakilia, R. (2010). Effects of dietary fat, vitamin E and zinc on immune response and blood parameters of broiler reared under heat stress.

Research Journal of Poultry Science, 3(2): 32-38.

Rath, N.C., Huff, G.R., Huff, W.E. and Balog, J.M. (2000). Factors regulating bone maturity and strength in poultry. *Poultry Science*, 79: 1024-1032.

Rayman,M.P.(2000).Theimportanceofseleniumtohuman health. *Lancet*, 356:233-241.

Raza, M., Deo, C., Mandal, A.B., Tyagi, P. and MIR, N.A. (2018). Effect of dietary supplementation of selenium and vitamin E on growth performance and hematology of broiler chicken under heat stress. *Indian Journal of Poultry Science*, 53(2): 171-176. DOI: 10.5958/0974-8180.2018.00033.8.

Reddy, N.R., Pierson, M.D, Sathe, S.K. and Salunkh, D.K. (1989). The Phytate in Cereal and Legumes. Boca Raton, F.L. Press CRC. Pp. 154.

Reisenfeld, A. (1972). Metatarsal robusticity in bi-pedal rats. *American Journal of Physiology and Anthropol*ogy, 40: 229-234.

Renaudeau, D., Collin, A., Yahav, S., de Basilio, V., Gourdine, J.L. and Collier, R.J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Journal of Animal production*, *6*: 707-728.

Richter, K., Haslbeck, M. and Buchner, J. (2010). The heat shock response: life on the verge of death. *Molecular Cell*, 40(2): 253-266.

Rions, J.L. (2001) Animal nutrition and acid-base balance. *European Journal of Nutrition*, 40:245-254.

Robertshaw, D. (2004). Temperature regulation and thermal environment, in Dukes' *Physiology of Domestic Animals*, Reece, W.O Ed. 12th edition.

Ross, J.G., Christie, G., Hascorbic acidlliday, W.G. and Jones, R.M. (1976). Determination of haematology and blood chemistry values in healthy six-week old broiler hybrids, *Avian Pathology*, 5(4): 273-281. DOI: 10.1080/03079457608418196.

Rozbicka-Wieczorek, A.J., Szarpak, E., Brzoska, F., Sliwinski, B., Kowalczyk, J. and Czauderna, M. (2012). Dietary lycopenes, selenium compounds and fish oil affect the profile of fatty acids and oxidative stress in chicken breast muscle. *Journal of Animal Feed Sciences*, 21: 705-724.

Sahin, K. and Kucuk, O. (2003a). Heat stress and dietary vitamin supplementation of poultry diets. Department of Animal Nutrition and Nutritional Diseases, School of Veterinary Medicine, University of Firat, Elazig, 23119: 37.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F. and Kucuk, O. (2003b). Dietary vitamin C and Folic acid supplemention ameliorates the detrimental effect of heat stress in Japanese Quail. *Journal of Nutrition*, 133: 1882-1886.

Sahin, K., Sahin, N. and Yaralioglu, S. (2002). Effect of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites, and mineral concentrations of laying hens reared at high ambient temperature. *Biological Trace Element Research*, 85: 35-45.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. and Prasad, A.S. (2009). Role of dietary zinc in heat-stressed poultry: A review. *Poultry Science*, 88: 2176-2183. Doi: 10.3382/ps.2008-00560.

Sahin, K., Sahin, N., Onderci, M., Gursu, M.F. and Issi, M. (2003). Vitamin C and E Can Alleviate Negative Effects of Heat Stress in Japanese Quails. *Food, Agriculture and Environment*, 1(2): 244-249.

Sahin, N. and Kucuk, O. (2001). Effect of vitamin C and vitamin E on performance, digestion on nutrients, and carcass characteristics of Japanese quails reared under chronic heat stress (34^0C). *Journal of AnimalPhysiology and Animal Nutrition*, 85: 335-342.

Saka, J.D.K. (1995). The nutritional value of edible indigenous fruits. Present research status and future directions. In Mayhembe, J.A., Ntupanyama, Y. and Chirwa P.W. (eds). Improvement Miombo Woodlands of South Africa, ICRAF, 50-57.

Salah-Eldin, T.A., Hamady, G.A.A., Abdel-Moneim, M.A., Farroh, K.Y. and El-Reffaei, W.H.M. (2015).NutritionalevaluationofSelenium-methioninenanocompositeasanoveldietary supplement for laying hens. *Journal Animal Health Production,* 3(3): 64-72.

Salem, A., Fayza, M., Amal, M., Hassan, A.A. El-Dayem, A. and El-Galil, K. (2018). The Effective Role of Selenium and Zinc on Broilers Performance. *Egyptian Journal of Nutrition and Feeds*, 22(1): 167-178.

Sanchez-Roman, I. and Barjai, G. (2013). Regulation of longevity and oxidative stress by nutritional intervention: Role of methionine restriction. *Experimental Gerontology*, 48(10): 1030-1042.

Sanotra, G.S., Lund, J.D., Ersboll, A.K., Petersen, J.S. and Vestergascorbic acidrd, K.S (2001). Monitoring leg problems in broilers: A survey of commercial broiler production in Denmark. *World's Poultry Science Journal,* 57(1): 55-69. DOI: 10.1079/WPS20010006

Sathya, A., Prabhakar, S., Sangha, S.P.S. and Ghuman, P.S. (2007). Vitamin E and selenium supplementation reduces plasma cortisol and oxidative stress in dystocia-affected buffaloes. *Veterinary Research Communications,* 31: 809-818.

Savini, I., Rossi, A., Pierro, C., Avigliano, L. and Catani, M.V. (2008). SVCT1 and SVCT2: key proteins for vitamin C uptake. *Amino Acids,* 34(3): 347-355.

Schrauzer, G.N. (2000). Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *Journal of Nutrition*, 130: 1653-1656.

Seedor, J.G., Quarruccio, H.A. and Thompson, D.D. (1991). The bio-phosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *Journal of Bone Mineral Research,* 6: 339-346.

Selim, N.A., Radwan, N.L., Youssef, S.F., Salah-Eldin, T.A. and Abo-Elwafa, S. (2015). Effect of inclusion inorganic, organic or nano selenium forms in broiler diets on: 2-physiological, immunological and toxicity statuses of broiler chicks. *International Journal of Poultry Science,* 14(3): 144-155.

Selye, H. (1976). Forty years of stress research: principal remaining problems and misconceptions. *Journal of Canadian Medical Association,115*: 53-56.

Seyrek, K., Yenisey, C., Serter, M., Kargın, Kıral, F., Ulutas, P.A. and Bardakcıoglu, H.E. (2004). Effects of dietary vitamin C supplementation on some biochemical parameters of laying Japanese quails exposed to heat stress (34.8 ^{0}C). *Revue Medical Veterinary*, 156: 339-342.

Sgavioli, S., Domingues, C.H.F., Santos, E.T., Quadros, T.C.O., deBorges, L.L., Garcia, R.G., Louzada, M.J.Q.L. and Boleli, I.C. (2016). Effect of In-Ovo Ascorbic Acid Injection on the Bone Development of Broiler Chickens Submitted to Heat Stress During Incubation and Rearing. *Revista Brasileira de Ciência Avícola*, 18(1): 153-162.

Shahid, H.M., Ravi, P.P., Veena, M., Tariq, A.M., Lamella, O. and Shimla, Y. (2018). Role of dietary minerals in heat-stressed poultry: A review. *Journal of Entomology and Zoology Studies,* 820-826.

Shankar, K. and Mehendale, H.M. (2014). Oxidative Stress. Encylopedia of Toxicology (third Edition), 735-737.

Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Shi, L., Wang, Q., Yang, R. and Lei, F. (2011). Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. *Small Ruminant Research,* 96(1): 49-52.

Shlig, A.A. (2009). Effect of vitamin E and selenium supplement in reducing aflatoxicosis on performance and blood parameters in broiler chicks. *Iraqi Journal of Veterinary Science*, 23: 97-103.

Shokraneh, M., Sadeghi, A.A., Mousavi, S.N., Esmaeilkhanian, S. and Chamani, M. (2019). Effects of *in ovo* injection of nano-selenium and nano-zinc oxide and high eggshell temperature during late incubation on antioxidant activity, thyroid and glucocorticoid hormones and some blood metabolites in broiler hatchlings. Acta Scientiarum. *Animal Sciences,* 42: 1-9.

Simrak, S., Chinrasari, O. and Aegwanich, W. (2004). Haematological, electrolyte and serum biochemical value on the thai indigenous chicken. *Journal of Science and technology,* 26(5): 425-430.

Singh, S., Brocker, C., Koppaka, V., Chen, Y., Jackson, B.C., Matsumoto, A., Thompson, D.C. and Vasiliou, V. (2013). Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress: a review. *Free Radical Biology and Medicine,* 56: 89-101.

Sinha, R., Kamboj, M.L., Lathwal, S.S. and Ranjan, A. (2018). Effect of housing management on production performance of crossbred cows during hot-humid season. *Indian Journal of Animal Research,* 52(7): 1091-1094.

Sinha, R., Lone, S.A., Ranjan, A., Rahim, A., Devi, I. and Tiwari, S. (2017). The impact of climate change on livestock production and reproduction: ameliorative management. *International Journal of Livestock Research,* 7(6): 1-8.

Sinkalu, V.O., Ayo, J.O., Abimbola, A.A and Ibrahim, J.E. (2015). Effect of melatonin on clocal temperature and erythrocyte osmotic fragility in layer hens during the hot-dry season. *Journal of Applied Animal Research,* 43(1): 52-60.

Sinkalu, V.O., Ayo, J.O., Abimbola, A.A. and Ibrahim, J.E. (2014). Effects of melatonin on cloacal temperature and erythrocyte osmotic fragility in layer hens during the hot-dry season. *Journal of Applied Animal. Research,* 59(1): 23-29. DOI: 10.1080/09712119.2014.888003

Sobayo, R.A. (2005). Effect of ascorbic acid supplementation on the performance and haematological profile of pullets and layers(Ph.D. thesis), Department of Animal Nutrition, University of Agriculture, Abeokuta, Nigeria.

Soderholm, J.D. and Perdue, M.H. (2001). Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *American Journalof Physiology Gastrointestinal and Liver Physiology,* 280(1): 7-13.

Sohail, M.U., Hume, M.E., Byrd, J.A., Nisbet, D.J., Ijaz, A., Sohail, A., Shabbir, M.Z. and Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *JournalofPoultry Science,* 91: 2235-2240.

Soleimani, A.F., Kasim, A., Alimon, A.R. and Zulkifli, I. (2008). Durability of induced heat tolerance by short term heat challenge at broilers marketing age. *Pakistan Journal of BiologicalSciences,* 11: 2163-2166.

Soltani, T., Salarmoini, M., Afsharmanesh, M. and Tasharrofi, S. (2019). The Effects of *In ovo* Injection of Ascorbic Acid on Hatchability, Growth Performance, Intestinal Morphology, and Tibia Breaking Strength in 36h Post Hatch Fasted Broiler Chickens. *Poultry Science Journal,* 7(1): 43-49.

Song, J., Xiao, K., Ke, Y.L., Jiao, L.F., Hu, C.H., Diao, Q.Y., Shi, B. and Zou, X.T. (2014). Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poultry Science,* 93(3): 581-588.

Sorensen, L.T., Toft, B.G. and Rygascorbic acidrd, J. (2010). Effect of smoking, smoking cessation, and nicotine patch on wound dimension, vitamin C, and systemic markers of collagen metabolism. *Surgery*, 148: 982-990.

Sosa, V., Moline, T., Somaza, R., Paciuian, R., Kondoh, H. and lleonart, M.E. (2013). Oxidative stress and cancer: an overview. *Ageing Research Reviews*, 12: 376-390.

Sotiriou, S., Gispert, S., Cheng, J., Wang, Y., Chen, A., Hoogstraten-Miller, S., Miller, G.F., Kwon, O., Levine, M., Guttentag, S.H. and Nussbaum, R.L. (2002). Ascorbic-acid transporterSlc23a1 is essential for vitamin C transport into the brain and for perinatal survival. *Natural Medicine*, 8, 514-517.

Sotler, R., Poljšak, B., Dahmane, R., Jukić, T., Jukić, D.P., Rotim, C., Trebše, P. and Starc, A. (2019). Prooxidant activities of antioxidants and their impact on health. *Acta Clinica Croatica*, 58(4): 726-736.

Star, L., Decuypere, E., Parmentier, H.K. and Kemp, B. (2008). Effect of single or combined climatic and hygienic stress in four layer lines: 2. Endocrine and oxidative stress responses. *Journal ofPoultry Science,87*: 1031-1038.

Statistical Analysis System (S.A.S). (2002). Statistical Analysis System Institute, User's Guide. Version 9 for Windows. North Carolina, U.S.A.

St-Pierre, N.R., Cobanov, B. and Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries. *Journal of Dairy Science*, 86: 52-77.

Stull, R. (2011). Wet-Bulb Temperature from Relative Humidity and Air Temperature. *Journal of applied Meteorology and Climatology,* 50: 2267- 2269.

Suchý,P.,Strakova,E.andHerzig,I. (2014).Seleniuminpoultrynutrition:areview*CzechJournal ofAnimal Science,* 59: 495-503

Sujatha, V., Korde, J.P., Rastogi, S.K., Maini, S., Ravikanth, K. and Rekhe, D.S. (2010). Amelioration of heat stress induced disturbances of the antioxidant defenses system in broilers. *Journal of Veterinary Medicine and Animal Health*, 2(3): 18-28.

Sujogya, K.P. (2012). Assay guided comparison of enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. http:// dx.doi.org/10.5772/50782.

Surai, P.F. (2002a). Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. *JournalWorld's Poultry Science,* 58(3):333-347.

Surai, P.F. (2002b). Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. *Journal ofWorld's Poultry Science,* 58(4):431-450.

Suzuki, K. (1983). Effect of crowding and heat stress on intestinal flora, body weight gain, and feed efficiency of growing rats and chicks. *Nippon Juigaku Zasshi*, 45(3): 331-338.

Suzuki,K.T. (2005).Metabolomicsofselenium:Semetabolitesbasedonspeciationstudies. *Journal of Health Science,* 51(2): 107-114.

Syafwan, S., Kwakkela, R.P. and Verstegena, M.W. (2011). Heat stress and feeding strategies in meat-type chickens. *Journal World's Poultry Science,* 67(4): 653-674.

Talebi, E. and Khademi, M. (2011). Combination Effects Of Ascorbic Acid And Glucose In Drinking Water On The Broiler Performance Under Acute Heat Stress. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(1): 92-96.

Tan, G.Y., Yang, L., Fu, Y.Q., Feng, J.H. and Zhang, M.H. (2010). Effects of different acute high ambient temperatures on function of hepatic mitochondrial respiration, antioxidative enzymes, and oxidative injury in broiler chickens. *Physiology, endocrinology and Reproduction,* 89(1) 115-122.

Tao, X. and Xin, H. (2003). Acute synergistic effects of air temperature, humidity and velocity on homeostasis of market-size broilers. *Transactions of the ASAE,*46(2): 491-497.

Tawfeek, S.S., Hosseinia, K.M.A. and Youssef, I.M.I. (2014). The effect of dietary supplementation of some antioxidants on performance,oxidative stress and blood parameters in broilers under natural summer conditions. *Journal World's Poultry Research*, 4(1): 10-19.

Tayeb, I.T. and Qader, G.K. (2012). Effect of feed supplementation of selenium and vitamin E on production performance and some hematological parameters of broiler. *KSÜ Doğa Bil. Derg*, 15(3): 46-56.

Tiogo, T., Marques-da-Silva, D., Samhan-Arias, A.K., Aurehand, M. and Gutero-Merino, C. (2011). Early disruption of the actin cytoskeleton in cultured cerebellar granule neurons exposed to 3-morpholino sydnonimine-oxidative stress is linked to alterations of the cytosolic calcium concentration. *Cell Calcium,* 49: 174-183.

Toplan, S., Dariyerli, N., Ozdemir, S., Ozcelik, D., Zengin, E.L. and Akyolcu, M.C. (2013). Lithium-induced hypothyradism: oxidative stress and osmotic fragility status in rats: *Biological Trace Element Research*, 152(3): 373-378.

Traxer, O., Huet, B., Poindexter, J., Pak, C.Y. and Pearle, M.S. (2003). Effect of ascorbic acid consumption on urinary stone risk factors. *Journal of Urology,* 170(2):397-401.

Tufarelli, V., Ceci, E. and Laudadio, V. (2016). 2-Hydroxy-4-methylselenobutanoic acid as new organicseleniumdietarysupplementtoproduceselenium-enrichedeggs.*BiologyTraceElement Research,* 171(2): 453-458.

Tuleun, C.D., Njoku, P.C. and Abel, I.O. (2010).Effect of Dietary Ascorbic Acid on Performance of Broiler Chickens Exposed to Different Lighting Regime. *International Journal of Poultry Science*, 9(2): 56-63. DOI: 10.3923/ijps.2010.118.125.

Ujang, T. (2008). Selenium: Its role as antioxidant in human health. *Environmental Health and Preventive Medicin*e, 13(2): 102-108.

Umoh, I.B. (1998). Commonly Used fruits in Nigeria. In Nutritional Quality of Plant Foods. Post Harvest Research Unit. Eds. Osagie and Ekah (1999).

Usayran, N., Farran, M.T., Awadallah, H.H., Al-Hawi, I.R., Asmar, R.J. and Ashkarian, V.M. (2001). Effects of added dietary fat and phosphorus on the performance and egg quality of laying hens subjected to a constant high environmental temperature. *Poultry Science*, 80: 1695-701.

USDA. (2012). Livestock and poultry: World markets and trade. USDA Foreign Agricultural Service, Washington, DC.

Utomo, D.B., Mitchell, M.A. and Whitehead, C.C. (1994). Effects of alpha-tocopherol supplemention on plasma egg yolk precursor concentrations in laying hens exposed to heat stress. *British Poultry Science*, 35: 828-829.

Vansudevan, D.M., Sreekumari, S. and Kannan, V. (2011). Textbook of Biochemistry for Medical Students. 6th edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi – 110002, India.

Varasteh, S., Braber, S., Akbari, P., Garssen, J. and Fink-Gremmels, J. (2015). Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. *PLoS One*, 10(9), 0138975. https://doi.org/10.1371/journal.pone.0138975.

Vathana, S., Kang, K., Loan, C.P., Thinggascorbic acidrd, G., Kabasa, J.D. and Ter-Meulen, U. (2002). Effect of Vitamin C Supplementation on Performance of Broiler Chickens in Cambodia. Conference on International Agricultural Research for Development, Deutscher Tropentag 2002, Witzenhausen, Hesse, Germany. October 9-11, Pp.7.

Venditti, P., Stefand, L.D. and Meo, S.D. (2013). Mitochondrial metabolism of reactive oxygen species: a review. *Mitochondrion*, 13: 71-82.

Wang, J.P., Yan, L., Lee, J.H., Zhou, T.X. and Kim, I.H. (2011). Effects of dietary delta-amino levulinic acid and vitamin C on growth performance, immune organ weight and ferrous status in broiler chicks. *Livestock Science*, 135: 148-152.

Weiser, H., Schlachter, M., Probst, H.P. and Kormann, A.W. (1992). The relevance of ascorbic acid for bone metabolism. In: Ascorbic Acid in Domestic Animals. Proc. 2nd Symp. Kartause Ittingen, Switzerland, 9-12 October 1990 (Wenk, C., Fenster, R. & Vo¨ lker, L., eds.), Pp. 73-95. ETH, Zu¨ rich, Switzerland.

WHO(1996).Seleniumindrinkingwater.In:Guidelinesfor Drinking-Water Quality, Volume 2: Health Criteria and Other Supporting Information. *World Health Organization,* Geneva,Switzerland.

Wolfenson, D. (1986). The effect of acclimatization on blood flow and its distribution in normothermic and hyperthermic domestic fowl. *Comparative Biochemistry and Physiology*, 85(4): 739-742.

Xia,Y.,Zhao,X.,Zhu,L. andWhanger,P.D.(1992).Metabolism of selenate and selenomethionine by a selenium-deficient population in China. *Journal of Nutritional Biochemistry*, 3:202-210.

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A. and Wang, M.Q. (2003). Effect of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science*, 82: 1030-1036.

Yahav, S. (2009). Alleviating heat stress in domestic fowl: Different strategies. *World's Poultry Science Journal*, 65(04): 719-732. DOI: 10.1017/S004393390900049X

Yalçýn, S., Ozkan, S., Çabuk, M.C. and Siegel, P.B. (2003). Criteria for evaluating husbandry practices to alleviate heat stress in broilers. *Journal of Applied Poultry Research*, 12: 382-388.

Yang, L., Tan, G.Y., Fu, Y.Q., Feng, J.H. and Zhang, M.H. (2010). Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ROS production and lipid peroxidation in broiler chickens. *Comparative Biochemistry and Physiology-Part C*, 151:204-208.

Yang, Y., Meng, F., Wang, P., Jiang, Y., Yin, Q. and Chang, J, (2012). Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. *African Journal Biotechnology,* 11(12): 3031-3036.

Yasin, M., Asghar, A., Anjum, F.M., Bult, M.S., Khan, M.I., Arshad, M.S., Shallidm, M., El-Ghorab, A.H. and Shibamota, T. (2012). Oxidative stability enhancement of broiler bird meats with α-lipoic acid and α-tocopherol acetate supplemented feed. *Food Chemistry*, 131:768-773.

Yazzid, D., Vander-Jaget, D.J., Pastuszen, A. Okolo, A. and Glwu, R.H. (1994). The amino acid and mineral content of Baobab (Adansonia digitata L) *Leaves. Journal of Food Comprehensive Annal*. 7: 189-193.

Yegani, M. and Korver, D.R. (2008). Factors Affecting Intestinal Health in Poultry. *Poultry Science*, 87(10): 2052-2063.

Yoda, Y., Nakazawa, M. and Abe, T. (1986). Prevention of doxorubicin myocardial toxicity in mice by glutathione.*Cancer Research*, 46: 2551-2555.

Yousef, M.I. (2004). Protective role of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. *Toxicology,* 207(1): 81-89. DOI: 10.1016/j.tox.2004.08.017

Youssef, A., Attia, M., Al-Harthi, A., El-Shafey, A.S., Rehab, Y.A. and Kim, W.K. (2017). Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. *Annual Animal Science,* 17(4): 1155-1169 DOI: 10.1515/aoas-2017-0012.

Yun, S.H., Moon, Y.S., Sohn, S.H. and Jang, I.S. (2012). Effects of cyclic heat stress or Vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines, and the antioxidant defense system in Sprague Dawley rats. *Experimental Animals,* 61(5): 543-553.

Zdunczyk, Z., Gruzauskas, R., Semaskaite, A., Juskiewicz, J., Raceviciute-Stupeliene, A. and Wroblewska, M. (2011). Fatty acid profile of breast muscle of broiler chickens fed diets with different levels of selenium and vitamin E. *Archiv fürGeflugelkunde,* 75: 264-267.

Zeng, H., Cao, J.J. and Combs, G.F. (2013). Selenium in bone health: Role in antioxidant protection and cell proliferation. *Nutrients,* 5(1): 97-110.

Zhang, B., Guo, X., Chen, J., Zhao, Y., Cong, X., Jiang, Z., Cao, R., Cui, K., Gao, S. and Tian, W. (2014). Saikosaponin-D attenuates heat stress-induced oxidative damage in 11c-pk1 cells by increasing the expression of anti-oxidant enzymes and hsp72. *American Journal of Chinese Medicine,* 42(5): 1261-1277.

Zhang, J.S., Gao, X.Y., Zhang, L.D. and Bao, Y.P. (2001). Biological effects of a nano red elemental selenium. *Biofactors,* 15: 27-38.

Zhang, Z.W., Wang, Q.H., Zhang, J.L., Li, S., Wang, X.L. and Xu, S.W. (2012). Effects of oxidative stress on immune suppression induced by selenium deficiency in chickens. *Biological Trace Element Research,* 149: 352-361.

Zoidis, E., Seremelis, I., Kontopoulos, N. and Danezis, G.P. (2018). Selenium-dependant antioxidant enzymes: Actions and properties of selenoproteins. *Multidisciplinary Digital Publishing Institute,* 7(5): 66.

Zulkifli, I., Al-Aqil, A., Omar, A.R., Sazili, A.Q. and Rajion, M.A. (2009). Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short and long tonic immobility reactions. *Poultry Science,* 88: 471-476.