EFFECTS OF BETAINE AND ASCORBIC ACID ON BIOMARKERS OF STRESS IN BROILER CHICKENS DURING THE HOT-DRY SEASON IN ZARIA, NIGERIA

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BY

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DEPARTMENT OF VETERINARY PHYSIOLOGY,
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JANUARY, 2015

DECLARATION

I declare that the work reported in this thesis, titled **'Effects of Betaine and Ascorbic Acid on Biomarkers of Stress in Broiler Chickens During the Hot-Dry Season in Zaria, Nigeria'** was carried out by me in the Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria. The information derived from literature has been duly acknowledged in the text and in the list of references provided. No part of this thesis has been presented for another degree or diploma at any university.

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Name of Student Signature Date

CERTIFICATION

This thesis, entitled **'EFFECTS OF BETAINE AND ASCORBIC ACID ON BIOMARKERS OF STRESS IN BROILER CHICKENS DURING THE HOT-DRY SEASON IN ZARIA, NIGERIA'** carried out by Ifeanyichukwu Chukwuemeka EGBUNIWE 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 knowledge and literary presentation.

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DEDICATION

I dedicate this project to the Almighty God and to my parents.

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ABSTRACT

High ambient temperature and high relative humidity adversely affect poultry. They result in heat stress, and, consequently, oxidative stress, especially during the hot-dry season in the Northern Guinea Savannah zone. The experiment was aimed at evaluating the effects of betaine and ascorbic acid (AA) on biomarkers of stress in broiler chickens during the hot-dry season in Zaria, Nigeria. Eighty White Ross breed of broiler chickens at day-old allotted into four groups of 20 birds each, were used. Group I (control) was given only sterile water *per os*, while Group II, Group III and Group IV were administered betaine (250 mg/kg), AA (50 mg/kg) and betaine+AA (250 mg/kg + 50 mg/kg), respectively, using gavage for forty-two days. The cloacal temperature (CT) of the birds, and dry-bulb temperature (DBT), relative humidity (RH) as well as temperature-humidity index (THI) in the pen, were measured bi-hourly, from 06:00 – 18:00 h, on days 28, 35 and 42. Blood samples were collected from birds in each group on days 21 and 42, through venopuncture, with and without anticoagulant, sodium ethylenediaminetetraacetate. Erythrocyte osmotic fragility (EOF) was determined. Serum obtained from blood samples were assayed for malondialdehyde (MDA) concentration and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). The mean DBT, RH and THI recorded (32.52 ± 0.61 ^{o}C, 77.81 ± 0.61 % and 31.98 ± 0.58, respectively) were predominantly outside the thermoneutral zone for broiler chickens. The results of this study showed that the mean CT value in group III broiler chickens (41.60 ± 0.02 ^{o}C) was significantly lower ($P < 0.05$) than that in group I (41.79 ± 0.03 ^{o}C), but mean CT values in group IV birds (41.95 ± 0.03 ^{o}C) were significantly higher ($P < 0.001$) than those in control group. Groups II, III and IV birds recorded significant decreases ($P < 0.05$) in EOF (6.66 ± 1.51%, 7.17 ± 1.31% and 7.00 ± 1.29%, respectively), when compared with that of group I (13.65 ± 2.30%). Betaine

(1.37 ± 0.038 nmol/L), AA (1.41 ± 0.039 nmol/L) and betaine+AA (1.41 ± 0.040 nmol/L) significantly ($P < 0.05$) decreased MDA concentration when compared with that in control (1.54 ± 0.043 nmol/L) broiler chickens. A highly significant ($P < 0.01$) increase in SOD activity in group IV broiler chickens (1.76 ± 0.06 IU/L) was observed, when compared with those in group I (1.44 ± 0.05 IU/L). GPx activity was significantly higher in birds of groups II (44.30 ± 1.00 IU/L; $P < 0.01$), III (43.10 ± 0.66 IU/L; $P < 0.05$) and IV (46.60 ± 1.61 IU/L; $P < 0.001$), when compared with those in group I (39.60 ± 1.09 IU/L). The results showed that betaine and/or AA exerted antioxidant properties. Administration of betaine and/or AA enhanced ability of broiler chickens to thrive under heat stress conditions occurring during the hot-dry season. It is concluded that, the administration of betaine and/or ascorbic acid is beneficial to broiler chickens in ameliorating the adverse effects of heat stress.

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ABBREVIATIONS

AA	Ascorbic acid
ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of Variance
CT	Cloacal temperature
DBT	Dry-bulb Temperature
DNA	Deoxyribonucleic acid
DTNB	5, $5^{/}$ diothio-bis-2-nitrobenzoic acid
EOF	Erythrocyte osmotic fragility
GPx	Glutathione peroxidase
H_0	Null Hypothesis
Hsp 90	Heat shock protein 90
IGF-1	Insulin-like growth factor-1
INT	2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride
kg	kilogram
MAPK	Mitogen-activated protein kinases
MDA	Malondialdehyde
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
PON-2	Paraoxase 2
RH	Relative Humidity
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
THI	Temperature-humidity Index
TNB	$5^{/}$ thio-2-nitrobenzoic acid

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Meteorological factors, such as high ambient temperature and high humidity, exert adverse effects on poultry production in many tropical countries, including Nigeria (Chen *et al*., 2013). They are known to cause heat stress in poultry during the hot-dry season (Minka and Ayo, 2007; Rhoads *et al*., 2013). Heat stress is a major danger facing poultry production in tropical and sub-tropical countries owing to increasing changes in climate resulting from global warming (Anna *et al*., 2014). The production of broiler chickens under heat stress condition results in reduction of feed intake (Azad *et al*., 2010a). The decrease in feed intake is an attempt to reduce heat production in the body (Mujahid, 2011). The decrease in live performance of birds reared under high ambient temperature, exceeding the comfort zone, is due to decreased feed conversion to meat (Lagana *et al*., 2007). Heat stress damages the intestinal barrier in broilers due to oxidative stress (Gu *et al*., 2012), and negatively influences the welfare of broilers kept under pre-slaughter conditions (Vieira *et al*., 2011). It induces a rise in serum corticosterone concentration, mortality, and a reduction in the percentage of phagocytizing macrophages (Quinteiro-Filho *et al*., 2012). Body temperature measurement is regarded as indicator for the development of both hypothermia and hyperthermia (Knezacek *et al*., 2010). However, practical and physiological obstacles make it irrelevant as a source of information to determine the thermal status of commercial poultry flocks (Giloh *et al*., 2012). Christensen *et al*. (2012), stated that core body temperatures decrease at night, when feeding activity is expected to be reduced. Osmotic fragility has been described as a potential biomarker of oxidative membrane

damage in pathologic conditions, as well as toxicant/xenobiotic/pesticide-induced oxidative membrane damage to erythrocytes (Sharma *et al*., 2010). It has been demonstrated that erythrocyte osmotic fragility increases with age (Kumar, 2011).

Broiler chickens utilise some behavioural responses, such as posture, orientation, shelter seeking, huddling and dispersion, as means by which they regulate their body temperature under hot ambient conditions (Kadzere *et al*., 2002). They also enhance heat tolerance by increasing sensible heat loss, which is determined by the difference between surface body temperature and ambient temperature. It is more useful than the evaporative heat loss, which causes dehydration (Yahav *et al*., 2005). In addition to the damages that occur at cellular level due to oxidative stress, a number of signaling pathways, consisting of certain proteins (for example: apoptosis signal-regulating kinase 1, C-jun NH2-terminal protein kinase, signal transducers and activators of transcription) are modulated by heat stress protein 90 (Hsp 90), to improve tolerance to heat stress (Padmini and Rani, 2011). Heat tolerance in broiler chickens may be assessed using biochemical parameters such as serum malondialdehyde (MDA) levels and antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] activities, especially during the early periods of heat stress, owing to the close association between heat tolerance and these biochemical indices (Run-Shen *et al*., 2011).

During heat stress, endogenous ascorbic acid, produced by the kidneys of birds is not sufficient to mitigate the negative effects of the stress. 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, egg and carcass quality. Heat stress may also cause mineral imbalance, increase in panting and

mortality; hence, necessitating the supplementation of ascorbic acid (Abidin and Khatoon, 2013). Antioxidants, including ascorbic acid (vitamin C) and betaine, are required for the sustenance of animals because they protect the cells from adverse effects of reactive oxygen species (ROS). They are present in fruits, vegetables, meat and fish (Hamid *et al*., 2010). Ascorbic acid (vitamin C), as an antioxidant, defends the body against the deleterious effects of ROS, protects the immune system, plays a role in converting the amino acid, tryptophan to serotonin, which acts as a neuro-transmitter, and assists the activation of B-vitamin and folic acid (Igbal *et al*., 2004). Betaine, an osmolyte and methyl-donor, is obtained either from diet or oxidation of choline. It is beneficial to the health of animals, including poultry (Lever and Slow, 2010; Mitsuya *et al*., 2013). It has the ability to replace methionine in some physiologically-important body processes, such as protein and fat metabolism (Fernandez *et al*., 2009), and it partially reduces the need for choline and methionine, as methyl donors in feeds (Zhan *et al*., 2006). It improves carcass yield (Esteve-Garcia and Mack, 2000), and meat quality (Alirezaei *et al*., 2012). Its supplementation in diet improves the digestive and absorptive potentials of the gastro-intestinal tract and nutrient utilisation (Honarbakhsh *et al*., 2007). It is conceivable that the negative effects of heat stress on the sustainable growth of broiler chicken production in the tropical and sub-tropical regions may be alleviated by betaine administration in drinking water (Mahmoudnia and Madani, 2012). The hot-dry season in the Northern Guinea Savannah zone of Nigeria, where Zaria is located, extends from March to May (Dzenda *et al*., 2013). The season has been demonstrated to be thermally stressful to poultry (Sinkalu and Ayo, 2008).

1.2 Statement of Research Problems

Heat stress induces oxidative stress in broiler chickens (Panok *et al*., 2009), which results in decrease in performance and productivity, owing to the decreases in feed intake, nutrient utilisation, growth rate, feed efficiency, immunity and lowered antioxidant status in birds (Khan *et al*., 2011). In broiler chickens, heat stress exerts significant adverse effects because of their high growth rate, and, consequently, high production rate and high metabolic heat production. Therefore, they are confronted with high demand for heat loss to the environment, especially at high ambient temperatures (MacLeod, 2004; Renaudeau *et al*., 2012). The high ambient temperature and high relative humidity, characteristic of the hot-dry season in Zaria, located in the Northern Guinea Savannah zone of Nigeria, further aggravates thermal load in broiler chickens. Heat stress weakens the body resistance to diseases; thus, causing very high morbidity and mortality in birds yearly (Ayo *et al*., 2011). Heat stress decreases efficient poultry production, resulting in a significant economic loss to poultry farmers (Rhoads *et al*., 2013). Finally, in domestic chickens, the thermo-neutral zone is narrow and this makes them succumb easily to thermal stress (Ramnath *et al.,* 2008). To date, measures to improve microclimatic conditions in poultry houses in the tropics are inadequate, especially during the hot-dry season.

1.3 Justification

Heat stress inflicts heavy economic losses in broiler chickens (Hassan and Reddey, 2012). Mortality rate may rise as high as 100% in poultry farms in Nigeria (Obeng, 1985). The mechanism underlying the aetiology of some diseases in broiler chickens has been shown to involve oxidative stress (Mokhtar *et al*., 2011). Ascorbic acid is an important antioxidant molecule in the brain. It is also involved in other activities

including, participating as a cofactor in several enzyme reactions, catecholamine synthesis, collagen production, and regulation of hypoxia-inducible factor (HIF)- 1α. (HIF)- 1α is a transcription factor that regulates many genes responsible for tumor growth, energy metabolism, and neutrophil function and apoptosis (Traber and Stevens, 2011). Betaine supplementation enhances both anabolic endocrine profile and the corresponding anabolic signaling environment, suggesting that it enhances protein synthesis (Apicella *et al*., 2013). The administration of antioxidants, especially their combination may be beneficial in reducing the losses in broiler chickens (Onu, 2009). This is because combination of antioxidants might be helpful in enhancing the oxidative stability (Hwanga *et al*., 2015). Betaine and ascorbic acid are readily available, cheap and accessible to farmers. Investigation into their ameliorative role, in reducing adverse effects of heat stress on physiological parameters, may improve health and productivity of broiler chickens reared in the zone. The co-administration of betaine and ascorbic acid gives the best economic efficiency in laying hens in terms of egg production, when compared to their individual supplementation during heat stress (Ezzat *et al*., 2011). The combination of both betaine and ascorbic acid improved semen quality and blood parameters in rabbit bucks under heat stress (Hassan *et al*., 2012). Bai *et al*. (2014) demonstrated that combined administration of antioxidants is believed to be more effective than single antioxidant, because their combined administration act synergistically to produce a magnified effect. The evaluation of physiological parameters may be used to measure the responses of poultry to stressful environmental conditions (Earley *et al*., 2010). Further investigations into methods of ameliorating the adverse effects of heat stress may improve productivity (Ognik and Sembratowicz, 2012). There is paucity of information on the physiologic responses of broilers administered with both betaine and ascorbic acid during the hot-dry season in Nigeria.

1.4 General Aim of the Study

The aim of the study was to determine the effects of betaine and ascorbic acid on biomarkers of heat stress in broiler chickens during the hot-dry season in Zaria, Nigeria.

1.5 Objectives of the Study

The specific objectives of the study were to determine:

a. Cloacal temperature (CT), erythrocyte osmotic fragility (EOF) and serum biomarkers of oxidative stress [malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx)] of broiler chickens during the hot-dry season.

b. Effects of betaine, ascorbic acid and their co-administration on the CT, EOF and biomarkers of oxidative stress (MDA, SOD and GPx) broiler chickens during the hot-dry season.

1.6 Statement of Research Hypotheses (H_1)

a. **H_1**: Heat stress alters CT, EOF and some biomarkers of heat stress (MDA, SOD and GPx) in broiler chickens during the hot-dry season.

b. **H_1**: Administration of betaine and ascorbic acid, either singly or in combination, alters CT, EOF and some biomarkers of heat stress (MDA, SOD and GPx) in broiler chickens exposed to heat stress during the hot-dry season.

CHAPTER TWO

LITERATURE REVIEW

2.1 Poultry Production

Production of chickens (*Gallus gallus*) for human consumption dates backs as far as 4000 years ago, and there has been a continuous selection for specific desired traits through selective breeding of parent stock to achieve the desire results (Kalmar *et al*., 2013). *Gallus gallus* is the major ancestor species, but *Gallus sonneratii* has also contributed to the genetic make-up of the domestic chicken. Furthermore, the knowledge of gene sequencing has accelerated the identification of causal mutations determining major morphological differences between wild *Gallus* and domestic breeds (Job *et al*., 2011). The advantages of poultry production are: ease of management, high turnover, fast returns on investment and wide acceptance for consumption (Haruna and Hamidu, 2004). Domestic chickens are also considered important biological model for researches in biomedical field (Rubin *et al*., 2010).

Poultry enterprise is becoming complex by the day, given the rapid strides in technology, changing market dynamics and growing scale of production. Due to some factors, starting from the procurement of chicks to their final disposal, entrepreneurs are faced with numerous constraints (Swu *et al*., 2012). Factors such as acute heat stress at marketing age, especially in broiler chickens raised in open houses with poor ventilation, and impaired heat exchange, result in economic losses (Hassan and Reddey, 2012). Some considerations should therefore be given to the microclimate within the broiler houses as birds experience heat stress (Lallo *et al*., 2012). Some of the challenges faced in poultry production, especially in developing countries, include poor

government support, poor management practices, high mortality and high cost of feed (Amos, 2006). Evidence has shown that the critical issues of low production and inefficiency in resource allocation and utilisation in poultry production have adversely affected farmers in Nigeria (Ezeh *et al.*, 2012). Some of these constraints could be overcomed by forming cooperative groups to obtain credit facilities from the government and financial institutions (Olaniyi *et al.*, 2008, Tijjani *et al.*, 2012).

2.2 Importance of Broiler Production

There is a shift in emphasis to broiler chickens for poultry meat, from spent layers in developing countries (Oluyemi and Roberts, 2000). There have also been significant improvements in poultry meat production in Nigeria due to efforts made in the use of improved breeds for production and the intensification of management systems of poultry (Ikani and Annalte, 2000). Poultry meat is affordable because it is relatively cheap for purchase by consumers (Damisar and Hassan, 2009). Broiler chicken production is profitable because it has a positive net return on investment (Heidari *et al.,* 2011). Broiler meat has gained wide acceptance because it is a healthier alternative to red meat (Shini *et al.*, 2010). The population in Nigeria, which is on the increase, has children between the ages of 0 - 14 years, constituting 37% of the population (NBS, 2005). This has resulted in increased demand for protein intake, which poultry meat reliably supplies (Owen and Dike, 2013). Owing to rising world population and demand for animal-based protein, there is increased pressure on animal production, such as broiler chickens, maximizing the yield permitted by the genetic make-up to meet the high demand (Koknaroglu and Akunah, 2013).

2.3 Management Systems in Poultry Industry

Domestic chickens are prone to infectious and zoonotic diseases (Madsen *et al*., 2013). Furthermore, the emergence and spread of diseases have negative impact on poultry production, and it poses a serious threat to human health (Wang *et al.,* 2013a). In Nigeria, good management practices are being advocated to meet the desires of customers (Kalio and Okafor, 2012). Despite the popularity of backyard poultry system, diseases easily spread in this setting (Hamilton-West *et al.,* 2012). The most crucial limitations affecting village chicken production are diseases, predators, shortage of supplementary feeds, problems with the housing and lack of veterinary services (Mamo *et al*., 2013). Outbreaks of diseases in poultry mostly result in areas where the locations of farms or trade overlap with habitats for wild birds (Si *et al*., 2013).

An integrated system of production is now canvassed in today's poultry industry which takes into consideration animal health and product safety in a sustainable way. It puts together factors such as an improved diet, biosecurity and innovative processing and packaging to improve the safety of poultry products (Cherian, 2013). Intensive system of production and some practices, though result in affordable products, have created serious health concerns for humans. Such practices are antibiotic usage, denseness of animals in enclosed units and movement of animals (Fasina *et al*., 2012; Liverani *et al*., 2013). Industry-driven researches into poultry welfare should be emphasized due to factors bordering on human health, environmental impact and cost (Dawkins, 2012). The combination of genetic improvement in leg soundness and advanced husbandry practices may improve broiler welfare with no negative impact on the efficiency of production (Rekaya *et al*., 2013).

2.4 Environmental Factors and the Welfare of Broiler Chickens

The Northern Guinean Savannah zone of Nigeria (11°, 12$^{/}$N; 7°E, 38$^{/}$E) has annual ambient temperature ranging between 18.0 ± 3.7 ^{o}C and 31.8 ± 3.2 ^{o}C. Its seasons are mainly harmattan (November-February), hot-dry (March-May) and rainy (June-October) seasons (Ayo *et al*., 2011; Dzenda *et al*., 2013). Production of broiler chickens is directly influenced by meteorological factors, such as ambient temperature and relative humidity (particularly during the hot-dry months), and their physiology (Genc and Portier, 2005). During hot conditions, characterized by high ambient temperature, relative humidity and radiant energy, there is decreased ability of animals to dissipate heat. This initiates compensatory and adaptive mechanisms to return the body to homeostasis (Daramola *et al*., 2012). When this persists, the difference between ambient temperature and body temperature of broiler chickens decreases, causing reduced rate of sensible heat loss, which further results in mortality due to hyperthermia (Azoulay *et al*., 2011).

It is necessary to assess the environmental parameters of rearing broilers because they affect performances of the birds (Sunil-Kumar *et al.*, 2011). For instance, heat stress, resulting from high ambient temperature and relative humidity negatively affects poultry performance in the tropical and subtropical regions (Sohail *et al*., 2010). The temperature-humidity index, an index of thermal comfort integrating the effects of ambient temperature and relative humidity (Purswell *et al*., 2012), may be used to evaluate the degree of thermal stress in livestock (Dikmen and Hansen, 2008).

2.5 Thermo-Neutral Zones of Broiler Chickens

The thermo-neutral zone can be defined as the range of ambient temperature during which regulatory changes in metabolic heat production or evaporative heat loss in birds is not induced (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). Elevated temperature negatively affects production, reproductive potentials, immune responses and health status of livestock (including broilers) (Nardone *et al.,* 2010). Holik (2009) reported that the thermo-neutral zone for poultry in the tropics is between 18 – 24 ^{o}C and between 12 – 26 ^{o}C in temperate regions, while Kingori (2011) established that the most favourable temperature range for poultry is between 12 – 26°C. The cardinal factor in understanding thermodynamic responses of homeotherms to their environments is by evaluating the energy involved in biological processes. This may serve as a measure of animal adaptation to its thermal environment (Nienaber *et al*., 2009).

2.6 Effects and Responses of Broiler Chickens to Heat Stress

Elevated ambient temperature is a limiting factor to poultry production in hot regions (Melesse *et al*., 2011). Though broiler chickens, to some extent, may acclimatize to some levels of oxidative stress, resulting from heat stress (Pamok *et al*., 2009), they experience some organ damage due to lipid peroxidation, caused by hyperthermia (Mete *et al*., 2012). Furthermore, the state of well-being of animals could be assessed by the health, physiological and behavioural responses (Earley *et al.,* 2010). Biochemical

responses may be used to evaluate their welfare under hot conditions (Wang *et al*., 2013b). Physiological responses to heat stress, which may be determined by their genetic make-up (Felvet Gant *et al*., 2012), include decrease in antioxidant capacity, increased respiratory rate and rectal temperature (Ali *et al*., 2010). Heat stress decreases feed consumption (Chowdhury *et al*., 2014), and consequently decreasing weight gain and growth. This further constitutes a major challenge in broiler production in the tropics (Widjastati and Hernawan, 2012). Heat stress also causes high water and electrolyte excretion, which impairs the ability for heat dissipation and alters acid-bases homeostasis (Sayed and Downing, 2011). It could further, result in increased haemolysis in broilers (Alhassan *et al*., 2010).

Certain hormones like glucocorticoids and catecholamines are released in response to stressful conditions to enable the body cope with stress (Mostl and Palme, 2002). Two neuronal hormonal regulators, arginine-vasotocin and adreno-corticotrophic hormones (ACTH), and plasma corticosterone are also involved in stress responses (Cornett *et al*., 2013). High-affinity mineralocorticoid receptors ensure the maintenance of homeostasis, while the low-affinity glucocorticoid receptors mediate the recovery from stress. Hence, it is important to maintain balance between these systems for cellular homeostasis, mental performance and health (Dekloet, 2004). Glucocorticoids cross the blood-brain barrier to interact with certain receptors, located mainly at the hippocampus and the frontal lobes, consequently influencing learning and memory (Lupien *et al*., 2007). Acute heat stress activates corticotrophic-releasing hormone, resulting in impairment of memory consideration (Roozendaal *et al*., 2002). Increased plasma corticosterone may down-regulate testosterone and progesterone concentration in plasma of chickens, impairing reproduction in breeders (Rettenbacher *et al*., 2013).

Finally, heat stress may result in redistribution of body resources (such as protein and energy) at the expense of growth, reproduction, production and health, to ensure survival (Gupta *et al*., 2013).

2.7 Biomarkers Used for the Evaluation of Heat Stress in Broiler Chickens

The health status of animals could be evaluated by measuring haemotological parameters (Talebi *et al*., 2005). Skin temperature, measured by thermography could be used as an index of welfare for domestic birds (Marelli *et al*., 2012). Erythrocyte osmotic fragility may be used as an indirect measure of lipid peroxidation (Uchendu *et al*., 2011; Minka and Ayo, 2011). Heterophil/lymphocyte ratio may also be 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 (Zulkifli *et al*., 2009), and could result in high levels of fear responses, evidenced by long tonic immobility in heat-stress broiler chickens (Al-Aquil *et al*., 2009). Oxidative stress may result in reduced expression of protein, mRNA and the contents of bcl-2, while increasing expression of the contents of bax and caspase-3 in the cecal tonsil. This results in decreased local intestinal immunity and increased apoptosis (Wu *et al*., 2014). Markers of endoplasmic reticulum stress such as proteins (GRP78/BiP and IRE1α), as well as the phosphorylation and expression of three mitogen-activated protein kinases (MAPKs) (JNK, p38 and ERK1/2) (Repoa *et al*., 2014), may be used to evaluate oxidative stress. Behaviour could also serve as an index for evaluating the welfare of broiler chickens and this could be observed using image analysis and data mining (Pereira *et al*., 2013).

2.8 Reactive Oxygen Species and their Roles in Broiler Chickens During Heat Stress

In the eukaryotic cells, mitochondria are 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*., 2010b; Kikusato *et al*., 2010). The continuous generation of ROS and the inability to manage the burden result in oxidative stress and cellular damage due to lipid peroxidation, in cell membranes (Singh *et al*., 2013). Disease ensues due to decrease in immune function of the body (Ambrozova *et al*., 2011), and lowered paraoxase 2 (PON 2) gene expression (Giordano *et al*., 2013). ROS-mediated damages of erythrocyte membrane results in haemolysis (Eroglu *et al*., 2013). This is responsible for erythrocyte fragility (George *et al*., 2012; 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 overproduction of mitochondrial ROS may depend on mitochondrial membrane potential. This 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 longer 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. Heat stress can cause increased expression of hypothalamic neuropeptides (Chowdhury *et al*., 2012). This results in 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).

2.9 Beneficial Roles of Reactive Oxygen Species Signaling in Broiler Chickens

The ROS, which may be generated by the activities of mitochondria respiratory chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, lipoxygenase, uncoupled nitric oxide synthase and myeloperoxidase enzymes, play both physiological (such as cell growth and stress adaptation) and pathological roles (such as cellular damage and attenuation of cell function) at different levels in the body (Sugarmura and Keaney (Jr), 2011). They are regarded as signaling molecules, propagating cellular pathways and the overall redox and cell metabolic activities in the mitochondria (Ghouleh *et al*., 2011). The ROS, under pro-oxidant conditions are regarded as an essential trigger and modulator of cell-signaling and cell behaviour. Hence, an antioxidant compound may interfere with cell signal transduction by interrupting ROS at critical levels of signaling pathways (Lee *et al*., 2009; Leonarduzzi *et al*., 2010). Studying the mechanism by which excess ROS production results in

improved lifespan may assist in the development of mimetics, which trigger that pathway without inducing oxidative stress (Lamming and Sabatini, 2011).

The ROS, hitherto considered a toxic by-product of aerobic respiration, is now known to be cardinal in cell-to-cell signaling, because they play numerous signaling roles in living organisms, from bacteria to mammalian cells (Mittler *et al*., 2011). ROS signaling explains the mechanotransduction of calcium ion (Ca^{2+}) discharge in the heart, both in healthy and pathological states, depending on the rate of discharges. This offers a possibility for new therapies (Prosser *et al*., 2011). The interaction of the splingosine - 1- phosphate (SIP) and its receptors controls the assembling of progenitor cells through the stimulation of ROS signaling on bone marrow stromal cells, haematopoietic progenitors and SDF-1 release (Golan *et al*., 2012).

2.10 Antioxidant Systems of the Body

In response to oxidative stress, organs and tissues possess distinct antioxidant systems. Understanding these systems help to develop strategies to defend against oxidative damage due to the destruction of lipids, proteins and DNA (Al-Gubory *et al*., 2010). The body protects against the negative effects of ROS by two mechanisms, namely: regulation of membrane permeability and the antioxidant system potential (Lushchak, 2011). Copper-zinc superoxide dismutase enzyme is an important cellular defence against ROS (Klooppel *et al*., 2010). The tumour necrotic factor α raises the basal levels of glutathione by up-regulating γ-glutamyl cystein synthetase synthesis and stabilizing potentials in cells (Persson and Vainikka, 2010).

The suppression of nuclear factor erythroid 2-related factor 2 (Nrf 2), an important transcription factor in antioxidant regulatory system, occurs during oxidative stress, as evidenced by changes in levels of activities of superoxide dismutase, catalase, glutathione and thiobarbituric acid-reactive substance (Liu *et al*., 2013). The skeletal muscle mitochondria of broilers produce superoxide anions during heat stress (Mujahid *et al*., 2005). Erythrocytes, which may function in antioxidant defenses, show decreased life-span by 50%, when exposed to excessive ROS production. This may be due to protein and/or amino acid degradation (such as tryptophan) in their cytoskeleton (Olszewska *et al*., 2012). It may be due to post-translational modification of proteins, destroying the fate and functions of erythrocytes (Pandey and Rizvi, 2013).

2.11 Antioxidant Supplementation in Broiler Chicken Production

Supplementation with anti-stress agents in poultry is useful in alleviating negative effects of stressors such as heat stress (Pandurang *et al*., 2011). Antioxidants decrease the deterioration of meat quality due to lipid peroxidation and stabilise meat oxidation after slaughter (Yasin *et al*., 2012). They also improve erythrocytic indices of broiler chickens subjected to heat stress (Majekodunmi *et al*., 2013). Khan *et al*. (2011), described vitamin E as a chain-breaking antioxidant because it was found to improve feed consumption, liveweight gain, feed conversion rate, nutrient digestibility, immunity and antioxidant status of poultry birds. Addition of vitamin C to water improves the breast meat of broilers under heat stress (Abioja *et al*., 2010). The supplementations of vitamin A and E have been shown to alleviate the adverse effects of heat stress in pullets (Sinkalu *et al*., 2009). Methionine supplementation has also been used to ameliorate the negative consequences of heat stress in poultry by improving amino acid balance, elevating protein synthesis, increase growth, feed

efficiency and enhanced immunity (Bunchasak, 2009). Certain plants, such as brocuoli's stem and leaf meal, also show some antioxidant properties in broiler chickens under hot conditions (Hu *et al*., 2012).

2.12 Physiologic Roles of Ascorbic Acid

Ascorbic acid is an important water-soluble antioxidant and enzyme cofactor in plants and animals. However, ascorbic acid is not synthesized by humans because they lack of the enzyme catalyzing the final step of the biosynthetic pathway (Mandl *et al*., 2009). AA serves as a neuromodulator of glutamatergic, dopaminergic, cholinergic, and GABAergic transmission and related behaviors (Harrison and May, 2009). Ghonim *et al*., (2009) demonstrated that spraying fertile Muscovy duck eggs with AA solution (30 g/L) twice times daily during the last 3 weeks of incubation period enhanced the percentage hatchability, immunity of hatched ducklings, economic efficiency of hatching process, as well as growth performance traits and viability through the first two weeks after hatch. Dietary AA supplementation to broiler chickens improved their meat composition, colour, serum aspartate transferase and alanine transferase levels during natural summer temperature (Konca *et al*., 2009). Vitamins C and E administration has been shown to ameliorate the adverse effect of road transportation stress on layering birds during the hot dry season in the Northern Guinea Savannah zone of Nigeria (Ajakaiye *et al*., 2010).

The physiological and biochemical potentials of ascorbic acid, an electron donor, are due to its ability to donate one or two electron(s), making it a potent reducing agent and an anti-oxidant (Du *et al*., 2012). Its supplementation alleviates the negative effects of oxidative stress (Sujatha *et al*., 2010). L-ascorbic acid stimulates synthesis of DNA and

cell multiplication by interacting with insulin-like growth factor-1 (IGF-1) receptors and by activating receptor tyrosine kinase/nitrogen activation phosphate pathway (Motekis *et al*., 2012). Ascorbic acid has been established to decrease lipid peroixidation, improves protein concentration and iron status of broilers (Wang *et al*., 2011). Ascorbic acid deficiency results in lack of hydroxylation of prolines and lysines, leading to the formation of looser triple helix and scurvy. Its blood level has an indirect relationship to disease state (Matsuoka *et al*., 2012). 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). Its supplementation to broiler chickens corrects metabolic response to heat stress (Imik *et al*., 2013), improving performance and decreased antioxidant status due to heat stress (Sahin, 2003). 2-0-α-glucopyranosyl-L-ascorbic acid, a derivative of ascorbic acid protects dermal fibroblast from oxidative stress and cellular senescence hence, may be used for its anti-ageing potentials (Taniguchi *et al*., 2012).

2.13 Uses of Betaine in Livestock Production

Betaine, widely found in animal tissues, plants (wheat bran, spinach), micro-organisms and seafood (from marine invertebrates), has been reported to protect cells, protein and enzymes from environmental stress (like high ambient temperature, low water, high salinity), because it is an osmolyte (Craig, 2004). It is also a metabolite of choline degradation and exerts an osmoregulatory role in cells (Hruby *et al*., 2005), especially in intestinal cells during heat stress (Metzler-Zebeli *et al*., 2009). As an osmolyte, betaine regulates water balance, resulting in the stability of tissue metabolism, especially in the gastrointestinal tract (Lipinski *et al*., 2012). It may also exert free radical-scavenging

ability against lipid peroxidation, as well as maintains myocardial energy status (ATP) via sustaining the enzyme activities in the Krebs cycle (Ganesan *et al*., 2007).

Betaine supplementation reduces the abdominal fat and facilitates the even distribution of lipid in geese (Su *et al*., 2009), and carcass fat in pigs (Sales, 2011). The effects of betaine on fat may be due to its influence on mRNA expression and the promoter CpG dinucleotide methylation profiles of chicken lipoprotein lipase gene (Yi *et al*., 2009; Xing *et al*., 2011). Furthermore, it influences levels of plasma homocysteine which could be used as a marker of methyl deficiency (Lever and Slow, 2010). Plasma homocysteine is reduced with betaine administration (Atkinson *et al*., 2009). In the transfer of methyl group, methionine, a key intermediate, is converted to S-adenosyl methionine, and subsequently to homocysteine. Homocysteine and methionine accumulate during methyl-donor deficiency (like in betaine deficiency), making these metabolites potentially toxic to the body system (Waldroup *et al*., 2006). It also induces expression of spot-14 (S_{14}) which responds to thyroid hormone, located in hepatic nuclei, and functions to relay hormone and nutrient-related signals to genes involved in lipid metabolism (Su *et al*., 2009). Methionine, a limiting amino acid in poultry (Kalbande *et al*., 2009), is involved in physiological processes such as methylation reactions of DNA (Swennen *et al*., 2011), and histones (Tesseraud *et al*., 2011). Osmolytes, like betaine, decrease inflammatory responses due to hyperosmolarity. This is because high osmolytic state triggers pro-inflammatory cytokine release and inflammation (Brocker *et al*., 2010). Betaine also prevents the up-reglation of heat shock protein 70 (Oliva *et al*., 2011). Accumulation of osmolytes is necessary for the viability of cells of renal medulla. This is because renal medulla is exposed to diverse

ionic and osmotic composition in their environment, which may result in ROS production (Rosab-Rodriguez and Valenzaela-Soto, 2010).

2.14 Physiologic Roles of Methylation in Broiler Chickens

Traits for growth are vital in the poultry industry. These have been shown to be related to DNA methylation of chicken muscle (Hu *et al*., 2013). DNA methylation down-regulates the expression of growth factor receptor, a growth factor for tumour formations (Juan *et al*., 2013). Furthermore, brain-derived neutrotropic factor, which determines the susceptibility or resistance of chickens to Marek's disease, plays some roles in neuronal survival, cholesterol metabolism, cellular differentiation and tumour formation (Yu *et al*., 2009). Tumour formation in chickens could be mediated by epigenetic alterations and genetic variation via the modification of DNA methylation, catalysed by DNA methyltransferase (Yu *et al*., 2008; Luo *et al*., 2012), and manifest in fluctuation in mRNA expression damage to CD_4 genes (Luo *et al*., 2011).

Generally, DNA methylation is the key factor of gene suppression (Useni *et al*., 2009). It functions in gene silencing by methylation of specific gene promoter defences of the host genome against retrovirus and transcriptional suppression of transgenes (Jang *et al*., 2013). Difference in the expression of gene-mediated epigenetic processes could result in broad phenotypic expression in animals. DNA methylation may influence the extent to which the gene expression varies and also the modification of epigenetic mechanism (Natt *et al*., 2012). Furthermore, methyl-donor deficiency results in liver steatosis and consequently, metabolic syndrome due to hypomethylation of the organic cation transporter PGC-1α, reduced binding with peroxisome proliferators-activated receptor γ (PPAR-γ), co-activator α and hepatic nuclear oxidation. This links methyl

donor deficiency and epigenomic deregulation of energy metabolism (Pooya *et al*., 2012). This deficiency may also result in increased risk of cardiovascular diseases due to hyper-homocysteinaemia. Consequently, it increases prohibin and decreases α-crystalline β, which indicates mitochondrial injury and stress to the endoplasmic reticulum (Martinez *et al*., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site and Meteorological Conditions

The experiment was carried out at a poultry house in Ahmadu Bello University, Zaria (11°10′N, 07°38′E), located in the Northern Guinea Savannah zone of Nigeria. It was carried out from March to May, 2013 during the hot-dry season (Dzenda *et al.*, 2013).

3.2 Experimental Animals, their Management and Antioxidant Administration

A total of 100 broilers belonging to the White Ross breed, were purchased at day-old from a reputable commercial farm in Ibadan (6°31′ N, 2°46′ E), Nigeria. All birds were identified using tags tied to their legs. The broiler chickens were fed with broiler pre-starter (day 0 - 14), broiler starter (day 15 - 28) and broiler finisher (day 29 - 42) manufactured by Animal Care Services Konsult (Nigeria) Limited, Iperu-Remo (6° 56′ N; 3° 43′ E), Ogun State, Nigeria. They were vaccinated against infectious bursal disease (at day 7 and 14) using Gumboro vaccine, and against Newcastle disease (at day 21) using Lasota vaccine. All vaccines were administered via drinking water. Prophylactic antibiotic (20% Enrofloxacin at 20 ml/100 L of water) and anticoccidial (Coccisul K at 100 g/200 L of water) medications were also administered to all the groups.

The birds were housed in the same pen, littered with wood shavings on concrete floor and zinc roof, having cardboard ceiling. The dimension of the pen was 8.4 m × 5.6 m × 1.91 m and the birds were stocked at 4 birds/m^2, lower than the density of 10 – 15 birds/m^2 described by Muniz et al. (2006). The pen was partitioned using plywood into four cubicles to house each group. Biosecurity measures were carried out by providing a

footbath and ensuring that the pen was not accessible to animals, birds and rodents. Farm wears such as footwares and laboratory coats were provided for persons, assisting in carrying out the experiments. Eighty birds were selected based on simple randomisation. They were divided into four groups (Groups I – IV). Group I, which served as the control group, was given only sterile water. Group II was administered with betaine hydrochloride only at 250 mg/kg (Pillai *et al*., 2006), Group III was given ascorbic acid only at 50 mg/kg (Sinkalu *et al*., 2008). Group IV was co-administered with both betaine hydrochloride (250 mg/kg) and ascorbic acid (50 mg/kg). Both betaine hydrochloride and ascorbic acid were given daily to each bird by oral route, using gavage and starting from day-old, for 42 days, when the experiment was terminated.

3.3 Experimental Measurements

3.3.1 Ascorbic acid and betaine administration

The ascorbic acid (100mg) was dissolved in 5 ml of distilled water and administered at the dose of 50 mg/kg, while the content of betaine hydrochloride capsules (648 mg) (Twinlab, Isi Brands Incorporated, American Fork, Utah, USA) was dissolved in 5 ml of distilled water and administered at 250 mg/kg.

3.3.2 Thermal environmental parameters

On each day of the recording, ambient temperature was obtained using the dry- and wet-bulb thermometer (Esal Scientific Industries, Delhi, India). The relative humidity (RH) was obtained using Osmon's hydrometric table (Narindra Scientific Industries, Haryana, India). The temperature-humidity index (THI) was determined using the following formula (Tao and Xin, 2003):

$$THI_{broiler} = 0.85\ T_{db} + 0.15\ T_{wb}$$

Where, $THI_{broiler}$ = temperature-humidity index for broilers, T_{db} = dry-bulb temperature and T_{wb} = wet-bulb temperature

.

The dry- and wet-bulb temperatures, relative humidity and temperature-humidity index was recorded every two hours on experimental days 28, 35 and 42.

3.3.3 Measurement of cloacal temperature

The cloacal' temperature, as an indication of the body temperature, was obtained using digital thermometer (Kruuse Incorporated, Denmark). The thermometer was inserted about 3 cm into the cloaca and inclined to make contact with the wall of the cloaca. The cloacal temperature was obtained every two hours from 06:00 h to 18:00 h on day 28, 35, 42 of the study period (Sinkalu *et al*., 2014).

3.3.4 Collection of blood samples

Blood sample (3 ml) was collected from the wing vein of each bird with and without anticoagulant, sodium ethylenediaminetetraacetate. The blood samples without anticoagulant was centrifuged at 3000 *g* for 10 minutes and the serum harvested and stored at 4 ^{o}C until assayed (Ramnath *et al.,* 2008). The blood collection was done on days 21 and 42 of the study period as described by Maini *et al*. (2007) using 23 gauge needle and immediately transferred to the Physiology Research Laboratory, Department of Veterinary Physiology, Ahmadu Bello University, Zaria, for analysis.

3.3.5 Determination of erythrocytic osmotic fragility

The erythrocyte osmotic fragility (EOF) test was performed as described by Oyewale (1992). Briefly, 0.02 ml of blood was added to tubes containing increasing

concentrations (0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%) of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4. The tubes was gently mixed and incubated at room temperature (26 ± 2 ^{o}C) for 30 minutes. The content of each tube was centrifuged at 150 x *g* for 10 minutes and the supernatant decanted. Optical density (OD) of the supernatant was determined using spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England) at wavelength of 540 nm. Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%:

$$\text{Percentage (\%)} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 100$$

3.3.6 Determination of biomarkers of oxidative stress

3.3.6.1 Evaluation of malondialdehyde concentration

Serum lipid peroxidation was evaluated as described by Janero (1990). Briefly, serum sample (100 μL) was mixed with sodium dodecyl sulfate-acetate buffer (pH 3.5) and aqueous solution of thiobarbituric acid. After heating at 95 ^{o}C for 60 minutes, the red pigment produced was extracted with n-butanol-pyrdine mixture and estimated by measuring spectrophotmetrically (Spectrolab 23A, Labomed Incorporated, Carlifornia, USA), the absorbance at 532 nm. Tetramethoxy-propane was used as an external standard and lipid peroxidation level was expressed in nmol/L.

3.3.6.2 Glutathione peroxidase assay

Glutathione Peroxidase activity (GPx) was evaluated using the Northwest Life Science Specialties (NWLSS™) glutathione peroxidase assay kits protocol NWK-GPX01, adapted from the method described by Paglia and Valentine (1967). Briefly, GPx

catalyzes the reduction of hydrogen peroxide and oxidizes reduced glutathione to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+. This results in decreased absorbance at 340 nm and the recycling of GSH. Decrease in absorbance at 340 nm is directly proportional to the GPx concentration. The GPx activity was expressed as IU/L.

3.3.6.3 Superoxide dismutase assay

The activity of superoxide dismutase (SOD) was measured using the Northwest Life Science Specialties SOD kit (NWLSS™ NWK-SOD02) based on the method of monitoring the auto-oxidation rate of hematoxylin originally described by Martin (Jr) *et al*. (1987) and modified to enhance reliability. Briefly, in the presence of SOD enzyme at specific assay pH, the rate of auto-oxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity is determined by measuring ratios of auto-oxidation rates in the presence and absence of the sample and expressed as McCord-Fridovich "cytochrome c" units.

3.4 Statistical Analysis

Data obtained were expressed as mean ± standard error of the mean (mean ± SEM). The values obtained were subjected to statistical analysis and compared using one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test and Pearson's correlation analysis. The analysis was done using Graphpad 4.0 for Windows (San Diego, California, USA). Values of $P < 0.05$ were considered significant (Snedecor and Cochran, 1994).

CHAPTER FOUR

RESULTS

4.1 Thermal Environmental Parameters in Poultry Pen

During the study period, the DBT ranged from 28 – 37 ^{o}C, while RH was from 69 – 93%. THI ranged from 27.85 – 36.1. The overall mean value of DBT recorded during the study period was 32.52 ± 0.63 ^{o}C, while those of the RH and THI were 77.86 ± 1.68% and 31.98 ± 0.59, respectively (Table 4.2). The lowest DBT (28.33 ± 0.33 ^{o}C) was recorded at 06:00 h, and rose with the hour of day to attain its peak (35.67 ± 0.67 ^{o}C) at 14:00 h. Thereafter the DBT decreased to 33.33 ± 0.33 ^{o}C, at 18:00 h (Table 4.1).

The lowest RH, 69.67 ± 0.33%, was obtained at 16:00 h, while that of the THI (28.18 ± 0.33) was recorded at 06:00 h. The highest RH (93.00 ± 0.00 %) and THI (34.92 ± 0.59) were recorded at 06:00 h and 14:00 h, respectively (Table 4.1). On day 28 of the study period, the highest values of DBT (33.71 ± 1.11 ^{o}C) and THI (33. 11 ± 1.01) where obtained, while the lowest RH (77.00 ± 3.25 %) was recorded (Table 4.2). Day 35 of the study period recorded the lowest DBT (31.71 ± 1.13 ^{o}C) and THI (31.22 ± 1.04), as well as the highest RH (79.0 ± 3.90 %) values (Table 4.2).

Table 4.1: Diurnal Fluctuations of Thermal Environmental Parameters During the Study Period

Hour of Day (h)	Dry-bulb Temperature (^oC)	Relative Humidity (%)	Temperature-humidity Index
6:00	28.33 ± 0.33 (28.00 – 29.00)	93.0 ± 0.00 (93.00 – 93.00)	28.18 ± 0.33 (27.85 – 28.85)
8:00	29.13 ± 0.88 (28.00 – 31.00)	84.00 ± 4.51 (79.00 – 93.00)	28.98 ± 0.81 (27.85 – 30.55)
10:00	31.67 ± 1.20 (30.00 – 34.00)	76.00 ± 3.51 (69.00 – 80.00)	31.12 ± 1.11 (29.55 – 33.25)
12:00	34.33 ± 0.88 (33.00 – 36.00)	71.33 ± 1.86 (69.00 – 75.00)	33.63 ± 0.84 (32.4 – 35.25)
14:00	35.67 ± 0.67 (35.00 – 37.00)	74.00 ± 2.00 (70.00 – 76.00)	34.92 ± 0.59 (34.25 – 36.1)
16:00	35.00 ± 0.58 (34.00 – 36.00)	69.67 ± 0.33 (69.00 – 70.00)	34.25 ± 0.58 (33.25 – 35.25)
18:00	33.33 ± 0.33 (33.00 – 34.00)	77.00 ± 2.00 (75.00 – 81.00)	32.78 ± 0.31 (32.4 – 33.4)
Mean ± SEM	32.52 ± 0.63 (28.00 – 37.00)	77.86 ± 1.68 (69.00 – 93.00)	31.98 ± 0.59 (27.85 – 36.10)

Values in parenthesis are minimum – maximum

Table 4.2: Variations in Thermal Environmental Parameters During the Study Period

Day	Dry-bulb Temperature (^oC)	Relative Humidity (%)	Temperature-humidity Index
28	33.71 ± 1.11 (29.00 – 37.00)	77.00 ± 3.25 (69.00 – 93.00)	33.11 ± 1.01 (28.85 – 36.10)
35	31.71 ± 1.13 (28.00 – 35.00)	79.0 ± 3.90 (68.00 – 93.00)	31.22 ± 1.04 (27.85 – 34.10)
42	32.14 ± 1.08 (28.00 – 35.00)	77.43 ± 2.98 (70.00 – 93.00)	31.61 ± 1.01 (27.85 – 34.25)

Values in parenthesis are minimum – maximum

4.2 Cloacal Temperature Responses of Broiler Chickens During the Study Period

On day 28 of the study period, the CT values was significantly higher ($P < 0.01$) in birds administered with betaine (41.62 ± 0.04 ^{o}C) than those co-treated with betaine and AA (41.70 ± 0.04 ^{o}C), when compared with the control group (41.50 ± 0.03 ^{o}C). On the same day of the study period, the mean CT value of the AA group did not differ ($P > 0.05$) from that of the control group (Table 4.3). On days 35 and 42 of the study period CT values decreased significantly ($P < 0.01$) in the broiler chickens administered with AA (41.52 ± 0.04 ^{o}C and 41.75 ± 0.00 ^{o}C, respectively), when compared with the corresponding values in the control group (41.75 ± 0.05 ^{o}C and 42.13 ± 0.05 ^{o}C, respectively) (Table 4.3). On day 35 of the study period, the CT value of the betaine group did not differ (41.69 ± 0.06 ^{o}C), while the co-administered group showed a significant ($P < 0.001$) increase (41.96 ± 0.06 ^{o}C), when compared with the value obtained in the control group (41.75 ± 0.05 ^{o}C) (Table 4.3). The CT values of both the betaine and co-administered groups did not differ on day 42 of the study period (42.19 ± 0.04 ^{o}C and 42.19 ± 0.04 ^{o}C, respectively), when compared with that of control (42.13 ± 05 ^{o}C) (Table 4.3).

The overall mean of CT values in the betaine and/or AA treated groups showed that in AA group, the CT significantly ($P < 0.001$) decreased (41.60 ± 0.02^{o}C), but in the co-administered group, the CT value increased (41.95 ± 0.03 ^{o}C; $P < 0.001$). In the group administered with betaine, the CT values did not differ significantly ($P > 0.05$), when compared to the value of 41.79 ± 0.03 ^{o}C recorded in the control group (Figure 4.1 and Table 4.3). There was no significant ($P > 0.05$) difference in both overall minimum CT values on days 28, 35 and 42 of the study period, in the betaine and/or AA groups, when compared respectively with the control group. However on day 42,

the mean minimum CT value of AA group (41.75 ± 0.03 ^{o}C) was significantly lower ($P < 0.01$), when compared with that of the control group (42.13 ± 0.05 ^{o}C) (Table 4.4). The mean value of the extreme maximum CT on day 42 also showed that the CT in AA group was significantly lower (42.34 ± 0.17 ^{o}C, $P < 0.01$) compared with that of the control group (42.91 ± 0.27 ^{o}C). The overall mean maximum and range CT values, as well as the mean maximum and range CT values on days 28, 35 and 42, did not show any significant difference ($P > 0.05$) in the betaine and/or AA groups when compared with the control group (Table 4.5 and Table 4.6).

In the present study, the control group showed continuous rise in CT from 41.27 ± 0.04 ^{o}C (06:00 h) to 42.23 ± 0.06 ^{o}C (18:00 h). However, the groups administered with betaine and/or AA exhibited decline in CT after attaining peak during the hot periods of the day (14:00 h – 16:00 h) (Table 4.7). The relationship between the DBT and CT values in the betaine ($r = 0.421$), AA ($r = 0.270$) and co-administered ($r = 0.347$) groups were positive, but not significant ($P > 0.05$). The relationship between DBT and CT value in the control group ($r = - 0.358$) was negative and insignificant (Table 4.8).

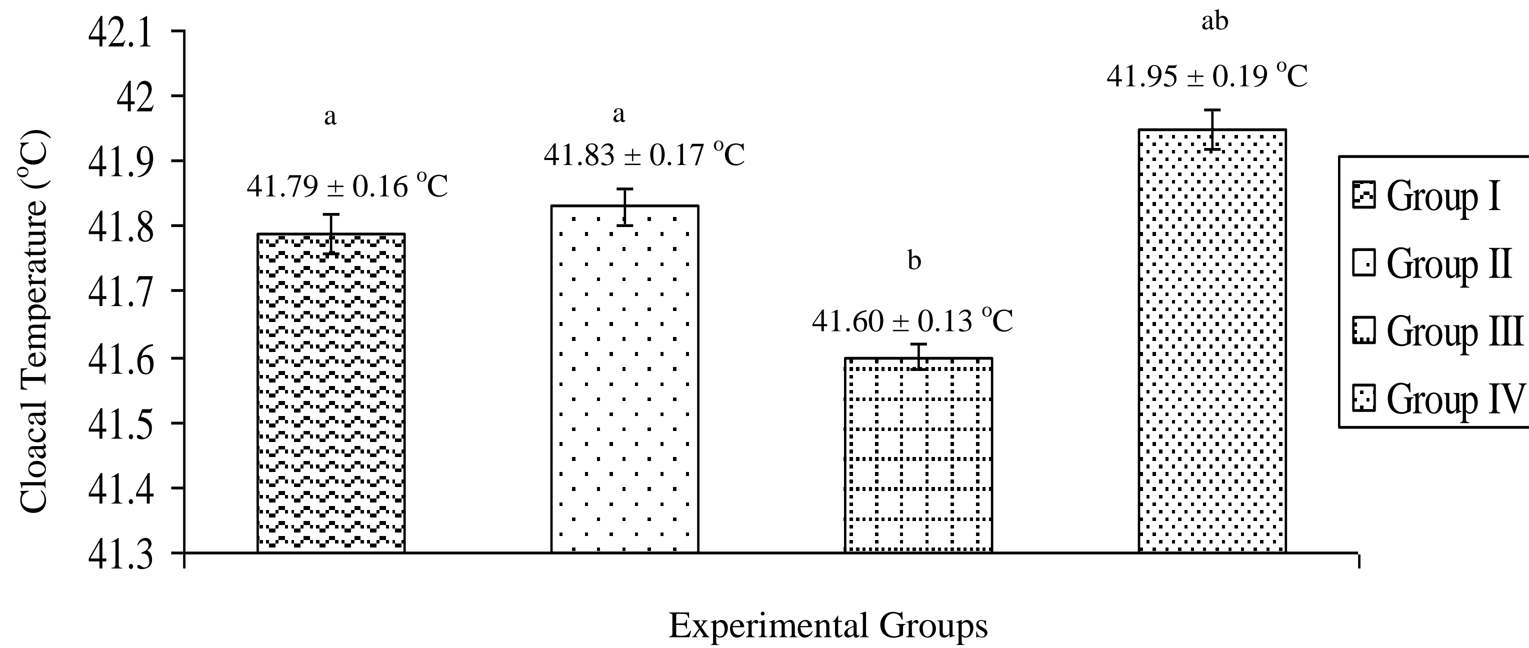


Figure 4.1: Variations in Cloacal Temperature of Broiler Chickens During the Study Period (n = 20)

[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.3: Cloacal Temperature of Broiler Chickens During the Study Period (n = 20)

	Mean Cloacal Temperature (°C)			
Day	Group I	Group II	Group III	Group IV
28	41.50 ± 0.03 [a] (40.50 – 42.40)	41.62 ± 0.04 [b] (40.40 – 42.60)	41.54 ± 0.04 [ab] (40.40 – 42.80)	41.70 ± 0.04 [b] (40.70 – 42.80)
35	41.75 ± 0.05 [a] (40.70 – 43.20)	41.69 ± 0.06 [a] (40.10 – 43.00)	41.52 ± 0.04 [b] (40.70 – 42.50)	41.96 ± 0.06 [ab] (40.50 – 43.60)
42	42.13 ± 0.05 [a] (41.00 – 43.70)	42.19 ± 0.04 [a] (41.00 – 43.50)	41.75 ± 0.03 [b] (40.80 – 42.70)	42.19 ± 0.04 [a] (41.00 – 43.40)
Mean ± SEM	41.79 ± 0.03 [a] (40.50 – 43.70)	41.83 ± 0.03 [a] (40.10 – 43.50)	41.60 ± 0.02 [b] (40.40 – 42.80)	41.95 ± 0.03 [ab] (40.50 – 43.60)

[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Values in parenthesis are minimum – maximum; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.4: Minimum Cloacal Temperature Changes of Broiler Chickens During the Study Period (n = 20)

	Mean Minimum Cloacal Temperature (°C)			
Day	Group I	Group II	Group III	Group IV
28	40.93 ± 0.14 (40.50 – 41.50)	41.09 ± 0.14 (40.40 – 41.40)	40.89 ± 0.22 [a] (40.30 – 41.60)	41.29 ± 0.17 [b] (40.70 – 41.80)
35	41.31 ± 0.20 (40.70 – 41.90)	41.10 ± 0.26 (40.10 – 41.90)	41.01 ± 0.09 (40.70 – 41.30)	41.16 ± 0.17 (40.50 – 41.60)
42	41.53 ± 0.12 [a] (41.00 – 41.80)	41.56 ± 0.36 [a] (41.00 – 41.90)	41.31 ± 0.12 [b] (40.80 – 41.60)	41.60 ± 0.15 [a] (41.00 – 42.10)
Mean ± SEM	41.26 ± 0.12 (40.50 – 41.90)	41.25 ± 0.16 (40.40 – 41.90)	41.07 ± 0.12 (40.30 – 41.60)	41.35 ± 0.13 (40.50 – 42.10)

[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Values in parenthesis are minimum – maximum; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.5: Maximum Cloacal Temperature Variations of Broiler Chickens During the Study Period (n = 20)

Day	Mean Maximum Cloacal Temperature (°C)			
	Group I	Group II	Group III	Group IV
28	42.06 ± 0.12 (41.60 – 42.40)	42.17 ± 0.13 (41.60 – 42.60)	42.11 ± 0.22 (41.30 – 42.80)	42.24 ± 0.20 (41.50 – 42.80)
35	42.37 ± 0.26 (41.60 – 43.20)	42.30 ± 0.27 (41.40 – 43.00)	42.07 ± 0.14 [a] (41.50 – 42.50)	42.54 ± 0.31 [b] (41.60 – 43.60)
42	42.91 ± 0.27 [a] (41.80 – 43.70)	42.91 ± 0.19 [a] (42.00 – 43.50)	42.34 ± 0.17 [b] (41.60 – 42.20)	42.76 ± 0.19 [a] (42.20 – 43.40)
Mean ± SEM	42.45 ± 0.25 (41.60 – 43.70)	42.46 ± 0.23 (41.40 – 43.50)	42.17 ± 0.08 (41.30 – 42.80)	42.51 ± 0.15 (41.50 – 43.60)

[a,b] = Means with different superscript letters are significantly (P < 0.05) different; Values in parenthesis are minimum – maximum; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.6: Range Temperature Variations of Broiler Chickens During the Study Period (n = 20)

	Mean Range Cloacal Temperature (°C)			
Day	Group I	Group II	Group III	Group IV
28	1.13 ± 0.09 (0.80 – 1.60)	1.09 ± 0.07 (0.80 – 1.30)	1.23 ± 0.19 (0.70 – 2.00)	0.96 ± 0.06 (0.80 – 1.20)
35	1.06 ± 0.06 (0.80 – 1.30)	1.20 ± 0.07 (0.90 – 1.40)	1.06 ± 0.08 (0.80 – 1.40)	1.39 ± 0.23 (0.70 – 2.00)
42	1.39 ± 0.16 (0.80 – 2.00)	1.36 ± 0.08 (1.00 – 1.60)	1.03 ± 0.06 (0.80 – 1.20)	1.16 ± 0.09 (0.80 – 1.50)
Mean ± SEM	1.19 ± 0.10 (0.80 – 2.00)	1.22 ± 0.08 (0.80 – 1.60)	1.11 ± 0.06 (0.70 – 2.00)	1.17 ± 0.12 (0.70 – 2.00)

^{a,b} = Means with different superscript letters within rows are significantly (P < 0.05) different; Values in parenthesis are minimum – maximum; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.7: Diurnal Fluctuations in Cloacal Temperature Values of Broiler Chickens During the Study Period (n = 20)

	Mean Cloacal Temperature (^{o}C)			
Hour of Day (h)	Group I	Group II	Group III	Group IV
6:00	41.27 ± 0.04^{a} (40.60 – 42.20)	41.24 ± 0.04^{a} (40.40 – 42.00)	41.11 ± 0.03^{b} (40.40 – 41.60)	41.30 ± 0.05^{a} (40.50 – 42.30)
8:00	41.24 ± 0.04 (40.50 – 41.80)	41.27 ± 0.06 (40.10 – 42.60)	41.17 ± 0.04^{a} (40.60 – 41.60)	41.36 ± 0.04^{b} (40.60 – 42.20)
10:00	41.55 ± 0.06 (40.70 – 42.70)	41.59 ± 0.07 (40.50 – 42.80)	41.54 ± 0.04 (40.40 – 42.60)	41.68 ± 0.04 (40.90 – 42.20)
12:00	41.98 ± 0.06^{a} (40.70 – 43.20)	42.00 ± 0.05^{a} (41.20 – 43.10)	41.75 ± 0.04^{b} (41.20 – 42.60)	42.04 ± 0.04^{a} (41.60 – 43.00)
14:00	42.13 ± 0.06^{a} (41.20 – 43.50)	42.34 ± 0.05^{b} (41.40 – 43.50)	41.82 ± 0.04^{ab} (41.10 – 42.50)	42.42 ± 0.06^{b} (41.60 – 43.20)
16:00	42.16 ± 0.06^{a} (41.20 – 43.40)	42.19 ± 0.05^{a} (41.30 – 43.10)	41.94 ± 0.04^{b} (41.20 – 42.70)	42.47 ± 0.06^{ab} (41.30 – 43.40)
18:00	42.23 ± 0.06^{a} (41.50 – 43.70)	42.19 ± 0.06^{a} (41.40 – 43.30)	41.88 ± 0.04^{b} (41.30 – 42.80)	42.37 ± 0.07^{a} (41.50 – 43.60)
Mean ± SEM	41.79 ± 0.16 (41.24 – 42.23)	41.83 ± 0.17 (41.24 – 42.34)	41.60 ± 0.13 (41.11 – 41.94)	41.95 ± 0.19 (41.30 – 42.47)

^{a,b} = Means with different superscript letters within rows are significantly (P < 0.05) different; Values in parenthesis are minimum – maximum; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Table 4.8: Relationship between Thermal Environmental Parameters and Cloacal Temperature of Broiler Chickens During the Study Period (n = 20)

Correlation Parameters	Group I	Group II	Group III	Group IV
Dry-bulb Temperature and Rectal Temperature	0.358 ^{NS}	0.241 ^{NS}	0.270 ^{NS}	0.347 ^{NS}
Relative Humidity and Rectal Temperature	0.194 ^{NS}	- 0.159 ^{NS}	- 0.023 ^{NS}	- 0.220 ^{NS}
Temperature-Humidity Index and Rectal Temperature	0.370 ^{NS}	0.247 ^{NS}	0.279 ^{NS}	0.354 ^{NS}
Hour of Day and Rectal Temperature	0.698 **	0.139 ^{NS}	0.361 ^{NS}	0.086 ^{NS}
Hour of Day and Minimum Rectal Temperature	0.931 ***	0.905 ***	0.772 **	0.829 **
Hour of Day and Maximum Rectal Temperature	0.866 **	0.760 **	0.961 ***	0.919 ***
Hour of Day and Range Rectal Temperature	- 0.278 ^{NS}	- 0.316 ^{NS}	0.184 ^{NS}	0.817 **
Maximum Rectal Temperature and Minimum Rectal Temperature	0.902 ***	0.936 ***	0.747 ***	0.819 ***
Minimum Rectal Temperature and Range Rectal Temperature	0.433 ^{NS}	0.216 ^{NS}	- 0.273 ^{NS}	0.178 ^{NS}
Maximum Rectal Temperature) and Range Rectal Temperature	0.780 **	0.546 *	0.435 *	0.711 ***

* = Significant (P < 0.05), ** = Highly significant (P < 0.01), *** = Very highly significant (0.001) and ^{NS} = Non-significant values (P > 0.05)

4.3 Changes in Erythrocyte Osmotic Fragility of Broiler Chickens During the Study Period

On day 21, there was significant decrease in EOF values recorded in AA-treated (2.26 ± 0.77%; $P < 0.01$) and the co-administered group (3.87 ± 1.12%; $P < 0.05$) when compared with the control group (14.39 ± 4.04%) at 0.9% sodium chloride (NaCl) concentration. At 0.7% NaCl concentration, there was a significant decrease ($P > 0.05$) in EOF values in all the treated groups (9.35 ± 2.71%; 6.98 ± 2.47% and 7.78 ± 1.66% for betaine, AA and combination groups respectively), when compared with the control (16.27 ± 9.35%). There were no significant decreases in percentage erythrocyte fragility at 0.5% NaCl concentration in the experimental groups when compared with the control (Figure 4.3).

On day 42, and at 0.7% NaCl concentration, there was significant decrease ($P < 0.05$) in EOF value in the betaine group (3.97 ± 1.05%) when compared with the control (11.04 ± 2.09%). EOF values in betaine+AA (6.23 ± 2.01%) and the AA treated (7.36 ± 0.97%) groups showed non-significant decreases ($P > 0.05$) when compared with the control at 0.7% NaCl concentration. At 0.5% NaCl concentration, there was significant decrease ($P < 0.05$) in EOF value in the betaine group (15.14 ± 4.38%) when compared with the control (33.58 ± 4.48%) (Figure 4.4).

At 0.7%, the overall EOF decreased significantly ($P < 0.05$) in the betaine, AA and co-administered groups (6.66 ± 1.51%, 7.17 ± 1.31% and 7.00 ± 1.29%, respectively), when compared with the control (13.65 ± 2.30 %) (Figure 4.2). At 0.5%, the treated groups betaine and/or AA) showed non-significant ($P > 0.05$) decreases in EOF, when compared with the control group (Figure 4.2).

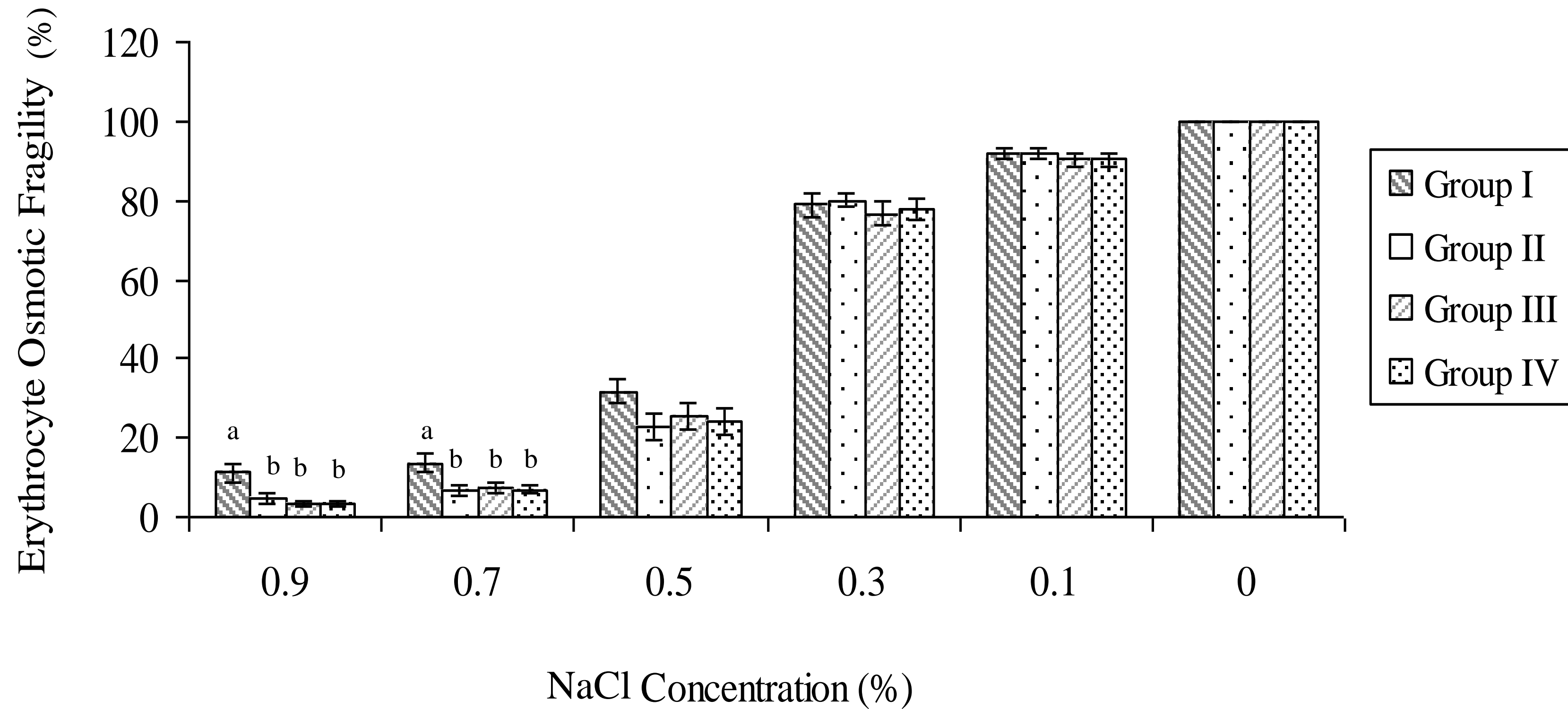


Figure 4.2: Percentage Erythrocyte Osmotic Fragility of Broiler Chickens in Response to Heat Stress (n = 15)
[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

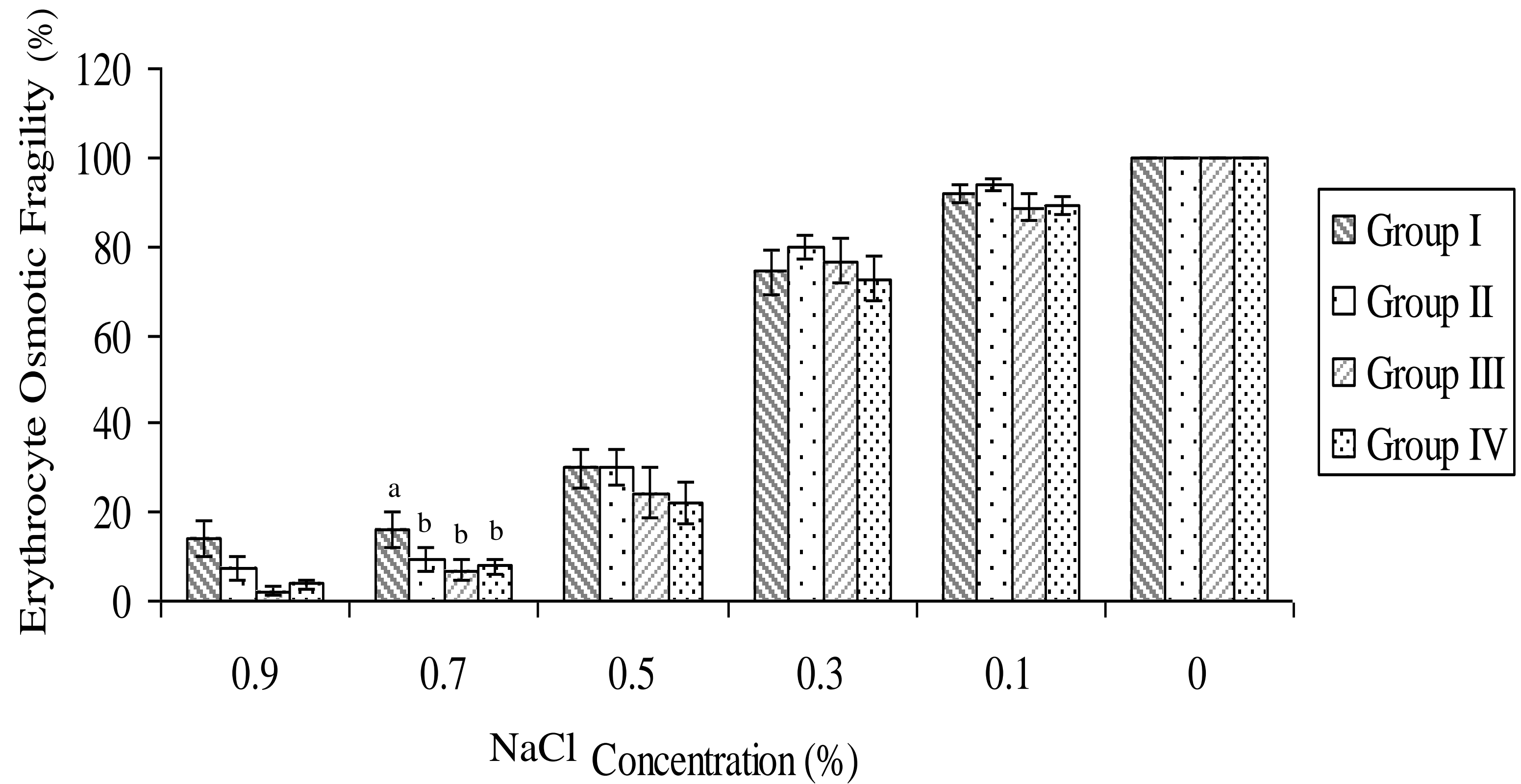


Figure 4.3: Variations in Erythrocyte Osmotic Fragility of Broiler Chickens on Day 21 of the Study Period (n = 15)
[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

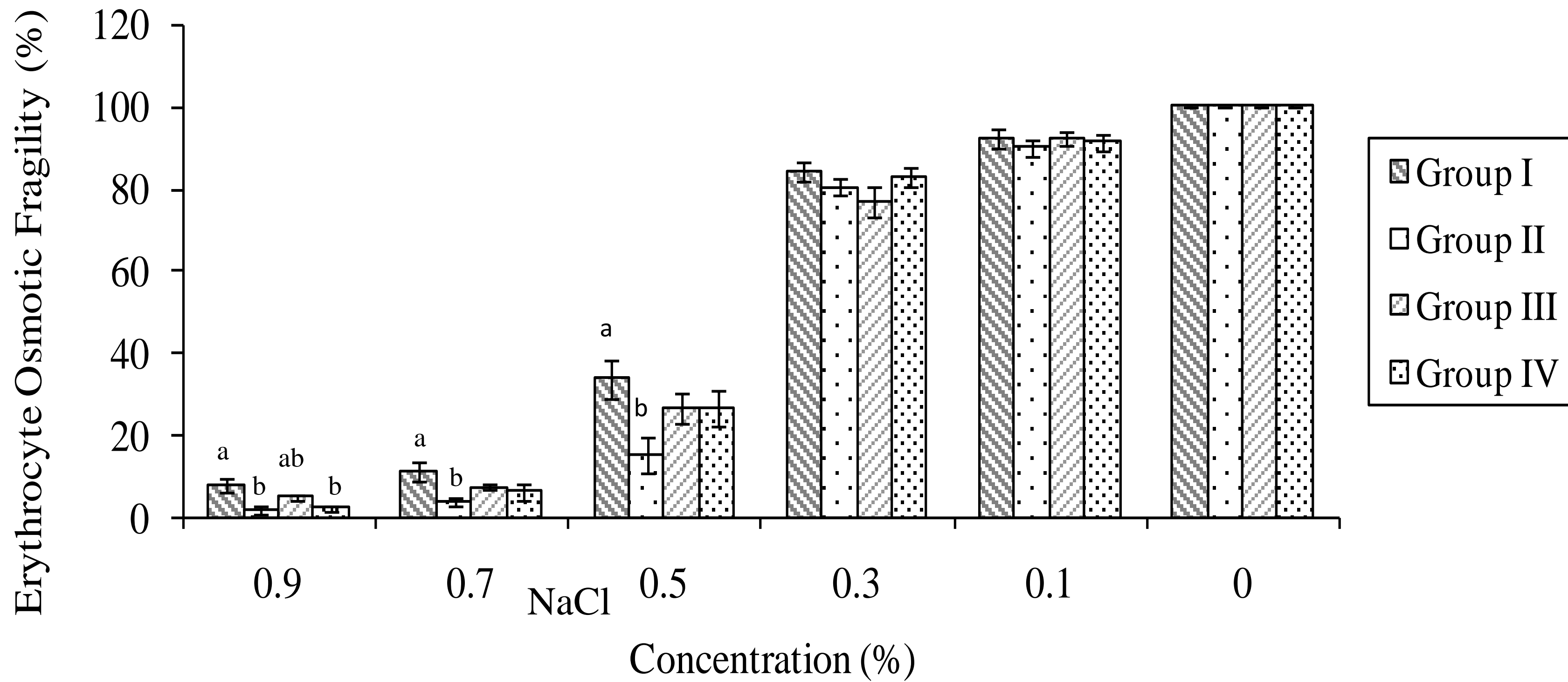


Figure 4.4: Variation in Erythrocyte Osmotic Fragility of Broiler Chickens on Day 42 During the Study Period (n = 15)
[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

4.4 Serum Biomarkers of Oxidative Stress in Broiler Chickens During the Study Period

4.4.1 Variations in malondialdehyde concentration

The MDA concentration in broiler chickens administered with AA was significantly ($P < 0.05$) lower than that recorded in the control group, and in betaine-treated group at day 21 of the experimental period (Table 4.9). At day 42, MDA concentration was lower ($P < 0.05$) in the betaine-treated group than in the control and in any of the treatment groups. The MDA concentration in betaine+AA group was significantly ($P < 0.05$) lower than that of the control or AA-treated group (Table 4.9). Overall, MDA concentration of broiler chickens in any of the treatment groups during the experimental period was significantly ($P < 0.05$) lower when compared with that of the control group (Figure 4.5)

Table 4.9: Changes in Malondialdehyde Concentration and Antioxidant Enzyme Activities of Broiler Chickens Exposed to Heat Stress (n = 7)

	Day	Group I	Group II	Group III	Group IV
Malondialdehyde/Protein (nmol/L)	Day 21	1.50 ± 0.066[a]	1.43 ± 0.036	1.31 ± 0.040[b]	1.40 ± 0.062
	Day 42	1.59 ± 0.055[a]	1.31 ± 0.063[b]	1.51 ± 0.040[a]	1.41 ± 0.055[b]
	Mean ± SEM	1.54 ± 0.043[a]	1.37 ± 0.038[b]	1.41 ± 0.039[b]	1.41 ± 0.040[b]
Superoxide Dismutase (IU/L)	Day 21	1.51 ± 0.08	1.53 ± 0.06	1.54 ± 0.07	1.70 ± 0.08
	Day 42	1.44 ± 0.05 [a]	1.45 ± 0.06 [a]	1.61 ± 0.19 [ab]	1.83 ± 0.10 [b]
	Mean ± SEM	1.48 ± 0.04 [a]	1.49 ± 0.04 [a]	1.57 ± 0.09 [ab]	1.76 ± 0.06 [b]
Glutathione Peroxidase (IU/L)	Day 21	39.00 ±1.93	44.70 ± 2.01	43.10 ± 1.04	47.30 ± 2.71
	Day 42	40.10 ± 1.32 [a]	44.00 ± 0.85	43.10 ± 0.86	46.00 ± 1.93 [b]
	Mean ± SEM	39.60 ± 1.09 [a]	44.33 ± 1.00 [b]	43.10 ± 0.66 [ab]	50.00 ± 1.61 [b]

[a,b] = Means with different superscript letters within rows are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

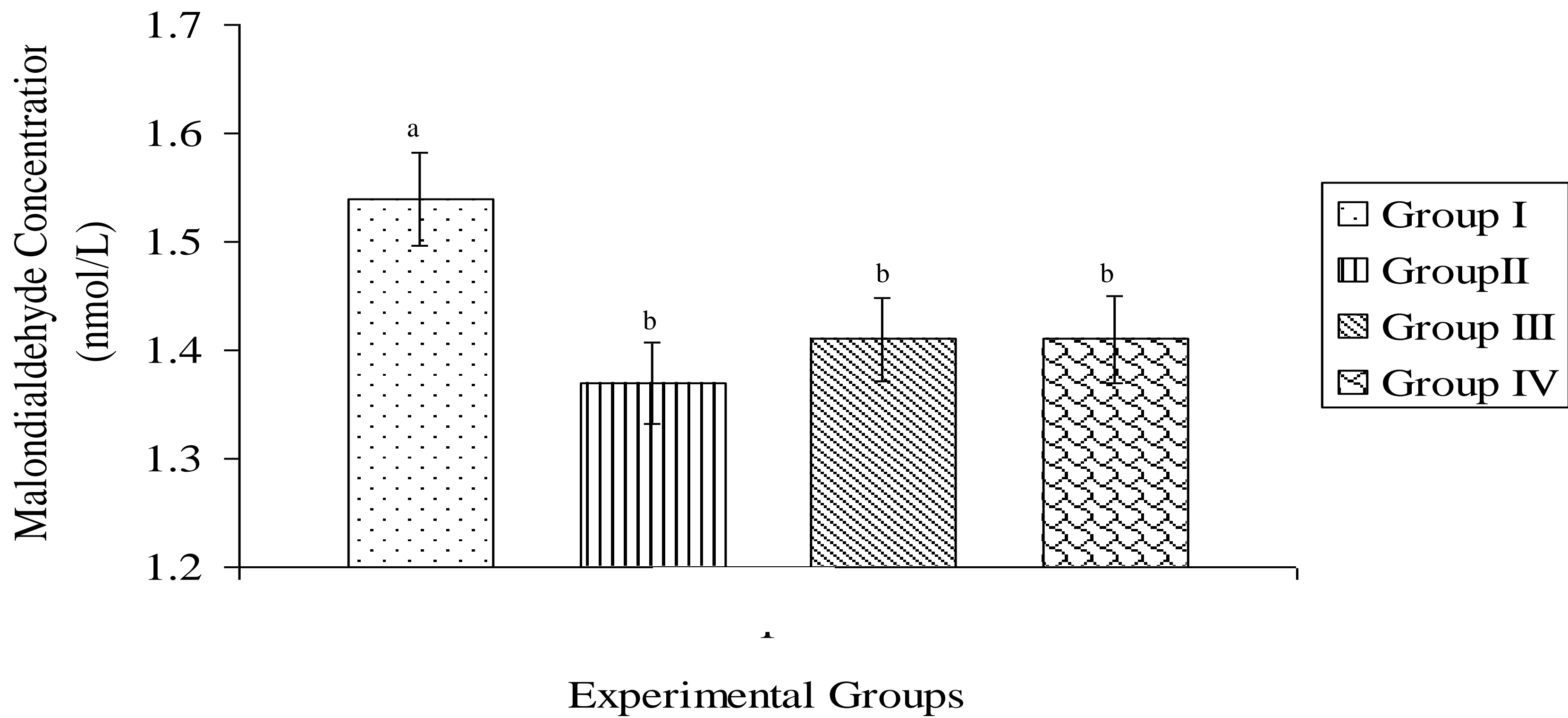


Figure 4.5: Fluctuations in Malondialdehyde Concentrations of Broiler Chickens During the Study Period (n = 7)
[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

4.4.2 Variations in antioxidant enzymes

4.4.2.1 Superoxide dismutase activity

On day 21 of the experimental period there was no significant ($P > 0.05$ differences in the activity of SOD in the betaine, AA and co-administered groups, when compared with that of the control group (Table 4.9). However, on day 42 the SOD activity rose ($P < 0.05$) in the co-administered group (1.83 ± 0.10 IU/L), when compared with the control group (1.44± 0.05 IU/L) (Table 4.9). On the overall, the co-administered group showed a highly significant ($P < 0.01$) increase in SOD activity (1.76 ± 0.06 IU/L), when compared with the control group (1.48 ± 0.04 1 IU/L). The SOD activity in betaine and AA groups did not differ significantly (1.49 ± 0.04 1 IU/L and 1.57 ± 0.09 1 IU/L; ($P > 0.05$), respectively) (Figure 4.6).

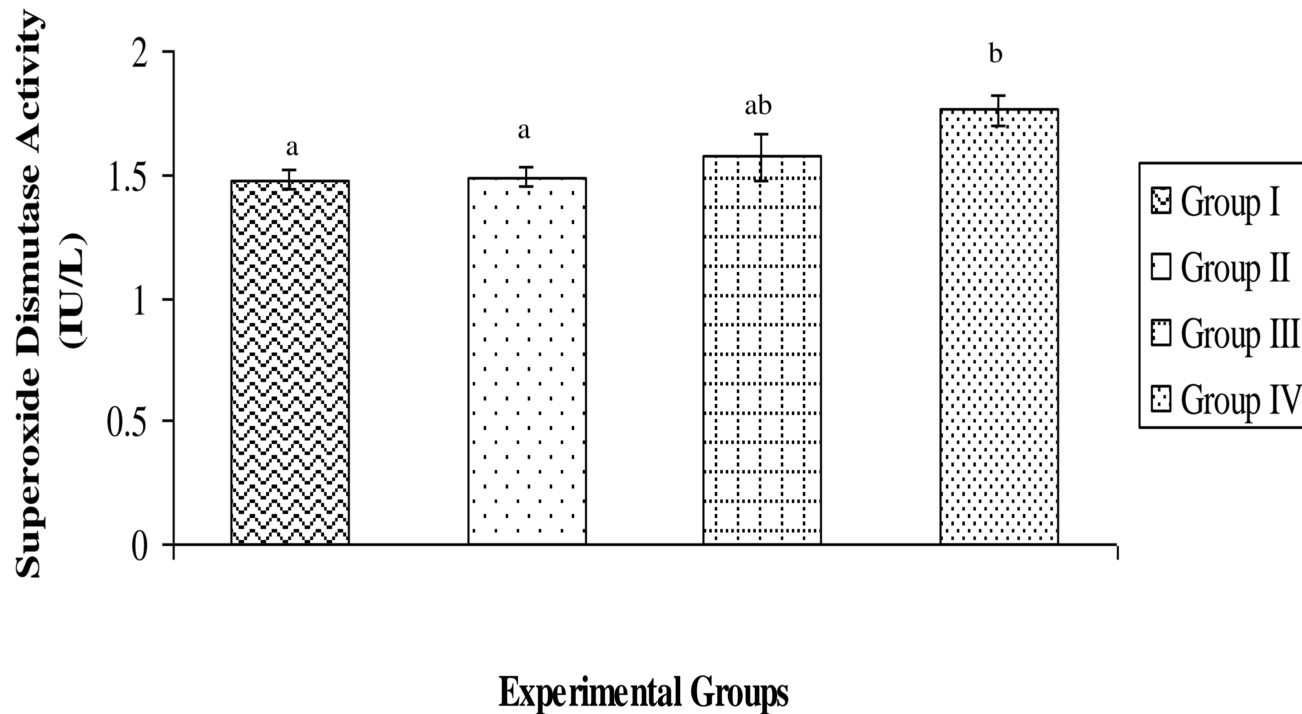


Figure 4.6: Changes in Superoxide Dismutase Activity of Broiler Chickens Exposed to Heat Stress (n = 7)
[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

4.4.2.2 Changes in glutathione peroxidase activity

There was no significant ($P > 0.05$) difference in the activity of GPx on day 21 of the experimental period (Table 4.9). On day 42, the co-administered group showed a significant ($P < 0.05$) increase in the activity (46. 0 ± 1.93 IU/L), when compared with that of the control group (40.10 ± 1.31 IU/L). The broiler chickens administered with betaine or AA showed non-significant ($P > 0.05$) increases (44.0 ± 0.85 IU/L and 43.10 ± 0.86 IU/L, respectively), in the activity of the enzyme, when compared with that of the control (Table 4.9).

On the overall, the betaine group demonstrated a highly significant ($P < 0.01$) increase in GPx activity (44.30 ± 1.001 IU/L), when compared with that of the control group 39.60 ± 1.09 IU/L) (Table 4.9). Broiler chickens treated with AA showed a significant ($P < 0.05$) increase in GPx activity (43.10 ± 0.66 IU/L), while the co-administered group indicated a very highly significant ($P < 0.001$) increase in enzymatic activity (46.60 ± 1.61 IU/L), when compared with that of the control (Figure 4.7).

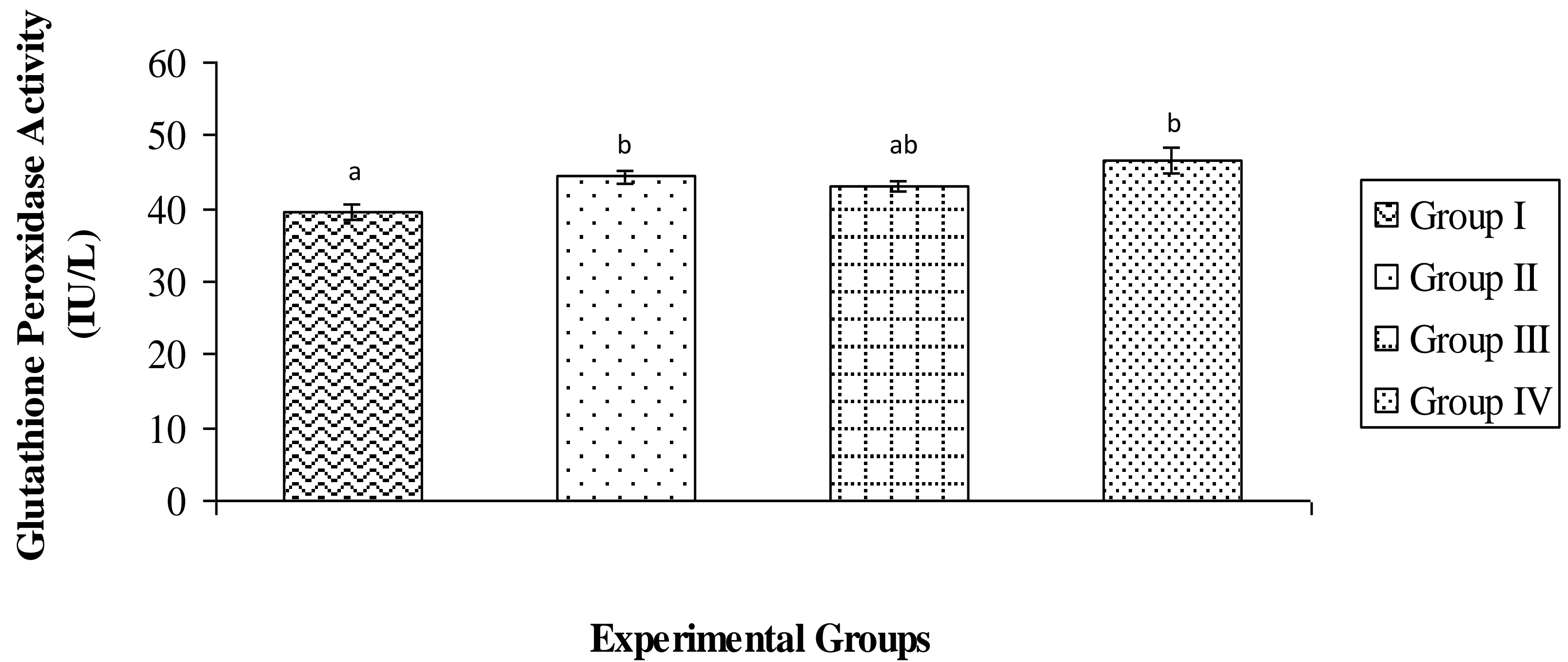


Figure 4.7: Fluctuations in Glutathione Peroxidase Activity in Broiler Chickens Exposed to Heat Stress (n = 7)
[a,b] = Means with different superscript letters are significantly different ($P < 0.05$); Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

CHAPTER FIVE

DISCUSSION

5.1 Thermal Environmental Measurements During the Study Period

The result of the present study showed that the DBT values (28.33 – 35.67 ^{o}C) obtained was outside the thermoneutral zone for broiler chickens above 4 week old, which is 18 – 21 ^{o}C (Aengwanich and Simaraks, 2004). The RH obtained (69.00 – 93.00%) in the present study was also predominantly outside the optimal range of 65 – 70% for broilers between the ages of 3 – 7 week old (Akyuz and Boyaci, 2010). This finding agrees with that of de Oliveria *et al*. (2013), who showed that the optimum AT and RH values for broiler chickens are 21 ^{o}C and 74%, respectively. Thus, the hot-dry season (March – May) in the Northern Guinea Savannah Zone of Nigeria, when the study was carried out, had high ambient temperatures (Adeniyi *et al*., 2009), and the season was thermally stressful to birds raised during this season (Sinkalu *et al*., 2009).

The THI revealed the impact of dry and wet-bulb temperatures on animals (Tao and Xin, 2003). From the present study, the overall mean value of THI recorded was 31.98 ± 0.58, which is stressful to the birds. This is because Purswell *et al.* (2012) showed that THI values greater than 20.8 ^{o}C induce heat stress in broiler chickens. Thermal stress, prevailing during the hot-dry season may adversely affect energy balance and the fitness of birds (Ardia, 2013). This may further result in poor performance, immune suppression and high mortality (Mujahid, 2011), hence the administration of antioxidants, such as betaine and AA, could be useful during the hot seasons. This measure may ameliorate the risk of the adverse effect of heat stress on the health and performance of broiler chickens, reared during the hot-dry season.

5.2 Fluctuations in Cloacal Temperature of Broiler Chickens During the Study Period

In the present study, diurnal fluctuation in mean CT values was recorded in both the treated and control groups. This result is in agreement with the finding of Sinkalu and Ayo (2008) that the CT fluctuates with the hour of day. The observation that CT values significantly decreased at 06:00 – 18:00 h in the AA group showed that AA administration exerted a significant hypothermic effect on the broiler chickens, especially during the hot period of the day (12:00 – 18:00 h). This finding is in agreement with the findings of Ihsan *et al.* (2011); Abidin and Khatoo, (2013), who reported that CT decreases with increase in hour of the day in birds administered AA. This finding indicates that AA administration to birds, subjected to heat stress, may have shown some improvement in the central thermoregulatory system, resulting in lower RT (Tanizawa *et al*, 2014).

Betaine and its co-administration with AA exerted a significant ($P < 0.05$) increase in CT at 14:00 h. At 16:00 h, the co-administered group showed a significant ($P < 0.001$) increase in CT. This finding indicates that betaine, and its co-administration with AA may increase the metabolic rate, as evidenced by higher CT values, when compared with the control group at 14:00 h and 16:00 h. This is because Lott *et al.* (1998), demonstrated that increased metabolic process generates more body heat. This may results in the birds reaching market weight in time, despite the adverse effects of high ambient temperature and RH. Betaine has been shown to enhance production level in broiler chickens by improving carcass yield (Metzler-Zebeli et al., 2009) and meat quality Alirezaei et al. (2012). However, this finding contradicts that of Hassan *et al.* (2011), who reported that betaine reduced RT in rabbits subjected to heat stress. The

difference in the latter report may be due to breed variation, as Hassan *et al*. (2011) performed the study in rabbits, while the present study was carried out in broiler chickens. The result of the present study also showed that betaine and its co-administration with AA may exert some hyperthermic effect during the early phase of growth; but at maturity, the broiler chickens may have adapted to heat exposure. Betaine administration, both singly and in combination with AA, increased the CT values in broiler chickens. This may be because betaine could have increased the thermoregulatory set-point in hypothalamus. This may facilitate the ability of the birds to adjust to the prevailing high AT during the hot-dry season, especially starting from the early age of the birds, and consequently, increase the resilience of the broiler chickens to the adverse effects of heat stress at maturity. This finding agrees with that of Sayed and Downing (2011), who demonstrated that betaine administration to chicks resulted in hyperthermia at an early age of 0 – 4 day old, but adaptation to heat stress may occur after this age. The study of Sayed and Downing (2011) was carried out in Egypt, while the present study was performed during the hot-dry season in the Northern Guinea Savannah zone of Nigeria. From the result of the present study, the decline in CT after peaking during the hot period of the day (14:00 h – 16:00 h) may further be evidence that betaine promotes resilience of broiler chickens to heat stress at maturity.

5.3 Erythrocyte Osmotic Fragility of Broiler Chickens During the Study Period

The present study showed that betaine administration to broiler chickens maintained the integrity of erythrocyte membrane during the hot-dry season. This implies that betaine may have an antioxidant effect on cellular membrane, protecting the cells from the adverse effects of lipid peroxidation, due to heat stress. This finding agrees with Ganesan *et al*. (2010), who demonstrated that betaine exerts an antioxidant defense potential on cell membranes, protecting myocardial membranes from the damage of free

radicals due to lipid peroxidation in myocardial infarction - induced rats. Furthermore, betaine may exert its osmolytic potential by stabilizing cell osmotic balance (Hruby *et al*., 2005; Lipinski *et al*., 2012). It has also been shown to improve hepatic metabolism of protein and lipid (Craig, 2004), which are the main constituents of cellular membrane. The present study also demonstrated that AA improved erythrocyte membrane stability in broiler chickens during the hot-dry season. This is in agreement with the result obtained by Azeez *et al*. (2011), who demonstrated that AA and/or E countered the adverse effect of oxidative stress on erythrocyte membrane of the domestic chicken. The findings of the present study also agrees with the finding of Olaifa *et al*. (2012), who reported that AA exerted an antioxidant potential on erythrocyte membranes of packed donkeys by decreasing oxidative stress - induced haemolysis.

This study also showed that the percentage EOF of broiler chickens in co-administered group showed the lowest value of haemolysis at 0.7% NaCl concentration, compared with the other experimental (betaine and AA) and control groups. This may indicate a synergistic effect between both agents. The synergy may attenuate apoptosis and haemolysis due to their free-radical scavenging abilities (Zhang *et al*., 2013). Heat stress, a major cause of oxidative stress, results from a total heat load (internal heat production and heat gain from environment) in excess of the capacity to dissipate heat (Ganaie *et al*., 2013). Oxidative stress decreases erythrocyte diapole potential (a biophysical determinant of membrane function) and results in the formation of spectin-haemoglobin complexes, which stiffen the membrane (Jewell *et al*., 2013), and impairing erythrocyte functions. The impairment induced by oxidative stress may necessitate the administration of antioxidants such as betaine and/or AA, to broiler chickens exposed to heat stress during the hot-dry season in the Northern Guinea

Savannah zone of Nigeria. The measure may protect the erythrocytes from the adverse effects of heat stress, including the prevention of anemia due to haemolysis, from impaired erythrocyte membrane integrity.

5.4 Evaluations of Serum Biochemical Parameters of Broiler Chickens During the Study Period

The findings of the study also showed that betaine decreased serum MDA concentration, but significantly increased SOD and GPx activities, especially on day 42. This observation indicates that betaine conferred some protection on the birds against the negative effects of oxidative stress, resulting from heat stress. This result agrees with that of Alirezaei *et al*. (2012), who reported that betaine decreased lipid peroxidation and enhanced SOD and GPx enzyme activities in meat of broiler chickens, subjected to oxidative stress due to methionine (a methyl donor) deficiency. This effect may be due to the ability of betaine (a methyl donor), to block the induction of mitochondrial lipid peroxidation (Ganesan *et al*., 2007).

Similarly, findings from the present study showed that AA exerted some positive effects by improving the antioxidant enzyme activities of SOD and GPx during heat stress in broiler chickens. This finding agrees with Halici *et al*. (2012), who reported that AA is an antioxidant, restoring the enzymatic activity of SOD in Japanese quail during heat stress condition. This finding may be due to the enhancing effect of AA on the body's antioxidant status (Orhana *et al*., 2013) under high AT. Furthermore, administration of AA decreased serum MDA concentration of the heat-stressed birds in the present study. This finding is in agreement with that of Olaifa *et al*. (2012), who reported that AA decreased MDA concentration in donkeys subjected to packing-induced oxidative stress.

There is evidence from this study that the co-administration of betaine and AA significantly decreased MDA concentration and also significantly increased activities of antioxidant enzymes SOD and GPx. These results indicate that both betaine and AA may exhibit a synergistic effect (Kambouh *et al*., 2013), by improving the antioxidant status of the broiler chickens during heat stress. Furthermore, the apparent synergistic effect between the two antioxidant agents in this study may confer better protection in the birds against the adverse effects of heat stress. This observation agrees with the finding of Jena *et al*. (2013), who reported that the synergistic action of AA and vitamin E gave better results in terms of reducing MDA concentration, at the same time increasing SOD activity, compared to the individual effect obtained following the administration of each antioxidant to broiler breeders. The co-administration of betaine and AA may have exerted it cytomembrane protection against the adverse effects of heat stress to the birds by modulating heat shock proteins and nuclear transcription factor (Sahin *et al*., 2012). This requires further investigation. Heat shock proteins have been shown to protect the intestinal mucosa from damage due to heat stress, by improving the SOD and GPx activities and inhibiting lipid peroxidation (Gu *et al*., 2012). This in turn stabilizes intestinal architecture and activity during periods of high AT and RH, resulting in improved digestibility, nutrients utilization and increased weight gain. This could ensure higher profitability for broiler farmers in the Northern Guinea Savannah zone of Nigeria. The co-administration of betaine and AA is also important in reducing oxidative process in birds due to heat stress by reducing the deleterious effects of ROS and RNS (Hosseini-Vashan *et al*., 2012; Kambouh and Zhu, 2013), and preventing lipid peroxidation (Kim *et al*., 2012) in broiler chickens during the hot-dry season.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

It can be concluded that:

1) Heat stress adversely affects physiological parameters of broiler chickens during the hot-dry season

2) The administration of betaine and/or ascorbic acid to broiler chickens modulated physiological responses to thermal environmental conditions, evidenced by alterations in the biomarkers of heat stress, during the hot-dry season.

6.2 Recommendations

It is recommended that:

1) Betaine and/or ascorbic acid should be administered to broiler chickens when they are exposed to heat stress, to improve their health, and consequently, productivity.
2) Further investigations may be carried out to ascertain the optimal doses for combined administration of betaine and AA to broiler chickens during the hot-dry season.
3) In addition to cloacal temperature, other indices of oxidative stress, such as erythrocyte osmotic fragility, malondialdehyde levels and antioxidant enzyme levels may be useful diagnostic tools for evaluating the effects of heat stress in broiler chickens during the hot-dry season.
4) Further studies may be conducted to elucidate the mechanism by which betaine administration, both singly and in combination with ascorbic acid, modulate cloacal temperature in broiler chickens subjected to heat stress.

REFERENCES

Abidin, Z. and Khatoo, A. (2013). Heat stress in poultry and the beneficial effects of ascorbic acid (ascorbic acid) supplementation during periods of heat stress. *World's Poultry Science Journal*, 69(1): 135 – 152.

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, *Archivos de Zootechnia*, 60(232): 1095 – 1103.

Adeniyi, M. O., Ogunsola, O. E., Nymphas, E. F. and Oladiran, E. O. (2009). Food security measures during uncertain climatic conditions in Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, 9(2): 625 – 676.

Aengwanich, W. and Simaraks, S. (2004). Pathology of heart, lung, liver and kidney in broiler under chronic heat stress. *Songklanakarin Journal of Science Technology*, 26: 417 – 424.

Ajakaiye, J. J., Ayo, J. O. and Ojo, S. A. (2010). Effects of heat stress on some blood parameters and egg production of Shika Brown layer chickens transported by road. *Biological Research*, 43: 183 – 189.

Akyuz, A. and Boyaci, S. (2010). Determination of heat and moisture balance for broiler house. *Journal of Animal and Veterinary Advances*, 9(14): 1899 – 1901.

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-Australasian Journal of Animal Science*, 22(11): 1581 – 1586.

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.

Alhassan, A. W., Adenkola, A. Y., Yusuf, A., Buachi, Z. M., Saleh, M. I. and Ochigbo, V. I. (2010). Erythrocyte osmotic fragility of Wistar rats administered ascorbic acid during the hot-dry season. *Journal of Cell and Animal Biology*, 4(2): 029 – 033.

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.

Alirezaei, M., Gheisari, H. R., Ranjbar, V. R. and Hajibemani, A. (2012). Betaine a promising antioxidant agent for enhancement of broiler meat quality. *British Poultry Science*, 53(5): 699 – 707.

Ambrozova, G., Pekarova, M. and Lojek, A. (2011). The effect of lipid peroxidation products on reactive oxygen species formation and nitric oxide production in lipopolysaccharide stimulated RAQW 264.7 macrophagers. *Toxicology In-vitro*, 25: 145 – 152.

Amos, T. T. (2006). Analysis of backyard poultry production in Ondo State, Nigeria. *International Journal of Poultry Science*, 5(3): 247 – 250.

Anna W., Arango, J., Settar, P., Fulton, J. E., Fernando, R. L., Garrick, D. J., Dekkers, J. and O'Sullivan, P. O. (2014). Genome wide association study for heat stress mortality in a white egg layer line. *International Plant and Animal Genome Conference*, 11 – 15 January, 2014. San-Diego, Califonia, USA, 641 pp.

Apicella, J. M., Lee, E. C., Bailey, B. L., Saenz, C., Anderson, J. M., Craig, S. A. and Kraemer, W. J., Volek, J. S. and Maresh, C. M. (2013). Betaine supplementation enhances anabolic endocrine and akt signaling in response to acute bouts of exercise. *Journal European Journal of Applied Physiology*, 113(3): 793 – 802.

Ardia, D. R. (2013). The effects of nestbox thermal environment on fledging success and haematocrit in tree swallows. *Avian Biology Research*, 6(2): 99 – 103.

Atkinson, W., Slow, S., Elinshie, J., Lever, M., Chambers, S. T. and George, P. M. (2009). Dietary and supplementary betaine: effects on betaine and homocysteine concentrations in male. *Nutrition, Metabolism and Cardiovascular Diseases*, 19: 767 – 773.

Ayo, J. O., Obidi, J. A. and Rekwot, P. I. (2011). Effects of heat stress on the well-being, fertility and hatchability of chicken in the Northern Guinea Savannah zone of Nigeria: a review *International Scholarly Research Network Veterinary Science*, vol. 2011, Article ID 838606, 10 pp.

Azad, M. K. A., Kikusato, M., Maekawa, T., Shirakawa, H. and Toyomizu, M., (2010a). Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. *Comparative Biochemistry and Physiology*, 155: 401 – 406.

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

Azeez, O. I., Oyagbemi, A. A. and Oyewale, J. O. (2011). Erythrocyte membrane stability after transportation stress in the domestic chicken as modulated by pretreatment with vitamins C and E. *Journal of Animal and Veterinary Advances*, 10(10): 1273 – 1277.

Azoulay, Y., Druyan, S., Yadgary, L., Hadad, Y. and Cahaner, A. (2011). The ability and performance under hot conditions of feather loss broilers versus fully feathered broilers. *Poultry Science*, 10(1): 19 – 29.

Bai, H., Liu, R., Chen, H., Zhang, W., Wang, X., Zhang, X. and Li, W. (2014). Enhanced antioxidant effect of caffeic acid phenethyl ester and trolox in combination against radiation induced-oxidative stress. *Chemico-Biological Interactions*, 207: 7 – 15.

Brocker, C., Thompson, D. C. and Vasilion, V. (2010). The role of hyperosmotic stress inflammation and disease. *Biomolecular Concepts*, 3(4): 345 – 364.

Bunchasak, C. (2009). Role of dietary methionine in poultry production. *The Journal of Poultry Science*, 46(3):169 – 179.

Chen, X. Y., Wei, P. P., Xu, S. Y., Gen, Z. Y. T and Jiang, R. S. (2013). Rectal temperature as an indicator for heat tolerance in chickens. *Animal Science Journal*, 84(11): 737 – 739.

Cherian, G. (2013). Keynote symposium: Tomorrow's poultry: sustainability and safety. *Poultry Science*, 92(2): 492 – 495.

Chowdhury, V. S., Tomonaga, S. S., Nishimura, S., Tabata, S. and Furuse, M. (2014). Physiological and behavoural responses of young chicks to high ambient temperature. *The Journal of Poultry Science*, 49(3): 212 – 218.

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. .

Christensen, K., Vizzier Thaxton, Y., Thaxton, J. P. and Scanes, C. G. (2012). Changes in body temperature during growth and in response to fasting in growing modern meat type chickens. *British Poultry Science*, 53(4): 531 – 537.

Cornett, L. E., Kang, S. W. and Kuenzel, W. J. (2013). A possible mechanism contributing to the synergistic action of vasotocin (VT) and corticotropin-releasing hormone (CRH) receptors in corticosterone release in birds: a review. *General and Comparative Endocrinology*, 188: 46 – 53.

Craig, S. A. S. (2004). Betaine in human nutrition. *The American Journal of Clinical Nutrition*, 80(3): 539 – 549.

Damisar, M. A. and Hassan, M. B. (2009). Analysis of factors influencing the consumption of poultry meat in the Zaria emirate of Kaduna State, Nigeria. *European Journal of Education Studies*, 1(11): 1 – 5.

Daramola, J. O., Abioja, M. O. and Onagbesan, O. M. (2012). Heat stress impact on livestock production. *In*: *Enviromental Stress and Amelioration in Livestock Production*, Springer Berlin, Heidelberg, Germany, Pp. 53 – 73.

Dawkins, M. S. (2012). Commercial scale research and assessment of poultry welfare. *British Poultry Journal*, 54(1): 1 – 6.

de Olivieira, W. P., de Oliveira, R. F., Donzele, J., Neto, A. R., Gomes, P. C., Maia, A. P., Campos, P. H.R. and Gasparino, E. (2013). Dietary crude protein reduction on growth and carcass performance of 22 to 42-day-old broilers reared under different temperatures. *Revista Brasileira de Zootecnia*, 42(8): 599 – 604.

Dei, H. K. and Bumbie, G. Z. (2011). Effect of net feeding on growth performance of broiler chickens in a hot climate. *British Poultry Science*, 52(1): 82 – 85.

Dekloet, E. R. (2004). Hormones and stressed brain. *Annals of New York Academy of Sciences*, 1018: 1 – 15.

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

Dikmen, S. and Hansen, P. J. (2008). Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in sub-tropical environment? *Journal of Diary Science*, 92: 109 – 116.

Du, J., Gullen, J. J. and Buettner, G. R. (2012). Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochinica et Biophysica Acta*, 182: 443 – 457.

Dzenda, T., Ayo, J. O., Lakpini, C. A. M. and Adelaiye, A. B. (2013). Seasonal, sex and liveweight variations in feed and water consumptions of adult captive African Giant rat (*Cricetomys gambianus*, Waterhouse-1840) kept individually in cages. *Journal of Animal Physiology and Animal Nutrition*, 97: 465 – 474.

Earley, B., Buckham-Sporer, K., Gupta, S. and Pang, W. (2010). Biologic responses of animals to husbandry stress with implications for biomedical models. *Open Access Animal Physiology*, 2: 25 – 42.

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: 33 – 38.

Esteve-Garcia, E. and Mack, S. (2000). The effect of D-methionine and betaine on growth performance and carcass characteristics in broilers. *Animal Feed Science and Technology*, 87(1-2): 86 – 93.

Ezeh, C. I., Anyiro, C. O. and Chukwu, J. A. (2012). Technical efficiency in poultry broiler production in Umuahia capital territory of Abia State, Nigeria. *Greener Journal of Agricultural Sciences*, 2(1): 1 – 7.

Ezzat, W., Shoeib, M. S., Mousa, S. M. M., Bealish, A. M. A. and Ibrahiem, Z. A. (2011). Impact of betaine, ascorbic acid and folic acid supplementations to the diet on productive and reproductive performance of Matroch poultry strain under Egyptian summer conditions. *Egyptian Poultry Science*, 31(3): 521 – 537.

Fasina, F. O., Ali, A. M., Yilma, J. M., Thieme, O. and Ankors, P. (2012). The cost benefit of biosecurity measures on infectious diseases in the Egyptian household poultry. *Preventive Veterinary Medicine*, 103(2-3):178 – 191.

Felvet-Gant, J. N., Mack, L. A., Dennis, R. I., Eicher, S. D. and Cheng, H. W. (2012). Genetic variations alter physiological responses following heat stress in two strains of laying hens. *Poultry Science*, 91(7): 1542 – 1551.

Fernandez, C., Mata, C., Piquer, O., Bacha, F. and de la Fuente, J. M. (2009). Influence of betaine on goat milk yield and blood metabolites: short note. *Tropical and Subtropical Agro Ecosystems*, 11: 209 – 213.

Ganaie, A H., Gauri Shanker, G., Nazir A. Bumla, N. A., Ghasura R.S., Mir, N. A., Wani, S. A. and Dudhatra, G. B. (2013). Biochemical and physiological changes during thermal stress in bovines. *Journal of Veterinary Science and Technology,* 4: 126 – 132.

Ganesan, B., Rajesh, R, Anandan, R. and Dhandepani, N. (2007). Biochemical studies on the protective effects of betaine on mitochrondrial function in experimentally induced myocardial infection in rats. *Journal of Health Science*, 53(6): 671 – 681.

Ganesan, B., Buddhan, S., Anandan, R., Sivakumar, R. and Anbin-Ezhilan, R. (2010). Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. *Molecular Biology Reports*, 37(3): 1319 – 1327.

Genc, L. and Portier, K. M. (2005). Sensible and latent heat productions from broilers in laboratory conditions. *Turkish Journal of Veterinary and Animal Science*, 29: 635 – 643.

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.

Ghonim, A. I. A., Awad, A. L., Fattouh, M. H. A. and El-Shhat, A. M. (2009). Comparative study of ascorbic acid treatment methods on hatchability traits and growth performance of ducklings. *Journal of Egyptian Poultry Science Journal*, 29(4): 1085 – 1099.

Ghouleh, I. A., Khoo, N. K. H., Knaus, U. G., Griending, K. K., Toayz, R. M. Thannickal, V. J., Barchowsky, A., Nauseef, W. M., Kelley, E. E., Bauer, P. M., Darley-Usman, V., Shira, S., Cifuentes-Pagano, E., Freeman, B. A,. Gladwin, M. T. and Pagan, P. J. (2011). Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling. *Free Radical Biology and Medicine*, 51: 1271 – 1288.

Giloh, M., Shinder, D. and Yahav, S. (2012). Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. *Poultry Science*, 91: 175 – 188.

Giordano, G., Tait, L., Furlong, C. E., Cole, T. B., Kavanagh, T. J. and Costa, L. G. (2013). Gender differences in brain susceptibility to oxidative stress and mediated by levels of paraoxonase-expression. *Free Radical Biology and Medicine*, 58: 98 – 108.

Golan, K., Vagina, Y., Kudin, A., Itkin, J., Cohen-Gur, S., Kalinkorich, A., Kollet, O., Kim, C., Schajnovitz, A., Ovadya, Y., Lapid, K., Shivtiel, S., Morris A. J. Ratajczk, M. Z. and Lapidot, T. (2012). SIP promotes immune progenitor cell egress and mobilization via SIP mediated ROS signaling and SDF-1 release. *Blood*, 11(11): 2478 – 2488.

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: intestinal oxidative stress. *Poultry Science*, 91(4): 790 – 799.

Gupta, M., Kumar, S., Dangi, S. S. and Jangir, B. C. (2013). Physiological, biochemical and molecular responses to thermal stress in goats. *International Journal of Livestock Research*, 3(2): 27 – 28.

Halici, M., İmik, H., Koç, M. and Gümüş, R. (2012). Effects of α-lipoic acid, vitamins E and C upon the heat stress in Japanese quails. *Journal of Animal Physiology and Animal Nutrition*, 96(3): 408 – 415.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., and Lawal, A. (2010). Antioxidants: its medicinal and pharmacological applications – a review. *African Journal of Pure and Applied Chemistry*, 4(8): 142 – 151.

Hamilton-West, C., Rojas, H., Pinto, J., Orozco, J., Harve-Claude, L. P. and Urecelay, S. (2012). *Research in Veterinary Science*, 93(1): 121 – 124.

Harrison, F. E. and May, J. M. (2009). Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2: review. *Free Radical Biology and Medicine*, 46(6): 719 – 730.

Haruna, U. and Hamidu, B. M. (2004). Economic analysis of turkey production in the Western agricultural zone of Bauchi. *Proceedings of the 9th Annual Conference of Animal Science Association of Nigeria*, September 13 – 16, 2004. Pp 166 – 168.

Hassan, A. M. and Reddy, P. G. (2012). Early age thermal conditioning improves broiler chick's response to acute heat stress at marketing age. *American Journal of Animal and Veterinary Sciences,* 7(1): 1 – 6.

Hassan, R. A., Ebeid, T. A., Abd El-Lateef, A. I. and Ismail, N. B. (2011). Effect of dietary betaine supplementation on growth, carcass and immunity of New Zealand White rabbits under high ambient temperature. *Livestock Science*, 135: 103 – 109.

Hassan, R. A., Morsy, W. A. and Abd El-Lateif, A. I. (2012). Effect of dietary ascorbic acid and betaine supplementation on semen characteristics of rabbits bucks under high ambient temperature. *Proceedingsof the 10th World Rabbit Congress*, September 3-6, 2012, Sharm EC-Sheikh-Egypt, Pp. 273 – 277.

Heidari, N. D., Omid, M. and Akram, A. (2011). Energy efficiency and econometric analysis of broiler production farmers. *Energy*, 36: 5538 – 6541.

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

Honarbakhsh, S. Zaghari, M. and Shivazad, M. (2007). Can exogenous betaine be an effective osmolyte in broiler chicks under water salinity stress? *Asian-Australasian Journal of Animal Science*, 20(11): 1729 – 1737.

Hosseini-Vashan, S. J., Golian, A., Yaghobfar, A., Zarban, A., Afzali, N. and Esmaeilinasab, P. (2012). Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress. *African Journal of Biotechnology*, 11(94): 16118 – 16125.

Hruby, M., Ombabi, A. and Schlagheck, A. (2005). Natural betaine maintains layer performance in methionine/choline chloride reduced diets. *Proceedings of the 15th European Symposium on Poultry Nutrition*, Balatonfured, Hungary, 25 – 29 September, 2005, pp. 507 – 509.

Hu, C. H., Wang, D. G., Pantt, Y., Zheng, W. B., Zuo, A. Y., and Liu, J. X. (2012). Effects of coli stem and leaf on broiler performance, skin pigmentation, antioxidant function, and meat quality. *Poultry Science*, 91(9): 2229 – 2234.

Hu, Yi, Xu, H., Li, Z, Zheng, X., Jia, X., Nie, Q. and Zhang, X. (2013). Comparison of the genome-wide DNA methylation profiles between fast growing and slow-growing broilers. *Public Library of Science ONE*, 8(2): e56411 – e56425.

Hwanga, K., Kima, H., Songa, D., Kima, Y., Hama, Y., Leeb, J., Choic, Y. and Kima, C. (2015). Effects of antioxidant combinations on shelf stability of irradiated chicken sausage during storage. *Radiation Physics and Chemistry*, 106: 315 – 319.

Igbal, K., Khan, A. and Khattak, M. M. A. K. (2004). Biological significance of ascorbic acid (ascorbic acid) in human health – a review. *Pakistan Journal of Nutrition*, 3(1): 5 – 13.

Ihsan, T. T., Yokhana, J. S. and Amedy, V. J. A. (2011). Effect of ascorbic acid and potassium chloride supplementation on performance and some physiological parameters in broiler chicks reared under summer condition. *International Journal of Poultry Science*, 10(3): 247 – 250.

Ikani, E. I. and Annalte, A. I., (2000). Improving the performance of local chickens. *Poultry Series (Extension Bulletin) National Agricultural Extension and Research Liaison Services*, Ahmadu Bello University, Zaria, 6(62): 30 pp.

Imik, H., Kaynar, O., Ozkanlar, S., Gumus, R., Polat, H. and Ozkanlar, Y. (2013). Effects of vitamin C and α-lipoid acid dietary supplementations on metabolic adaptation of broilers to heat stress. *Revue de Medecine Veterinaire*, 164(2): 52 – 59.

Janero, D. R.. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnositic indexes of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine*, 9: 515 – 540.

Jang, H. J., Lee, M. O., Kin, S., Kim, S. K., Song, G., Womack, J. E. and Han, J. Y. (2013). Biallelic expression of the L-arginine; glycine amidino transferase gene with different methylation status between male and female primordial germ cells in chickens. *Poultry Science*, 92(3): 760 – 769.

Jena, B. P., Panda, N.,2, 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. *Food and Nutrition Sciences*, 4: 33 – 37.

Jewell, S. A., Petrov, P. G. and Winlove, C. P. (2013). The effect of oxidative stress on the membrane dipole potential of human red blood cells. *Biochimica et Biophysica Acta – Biomembranes*. 1828(4): 1250 – 1258.

Job, D., Pelletier, G. and Pernollet, J. (2011). Chicken domestication: from archeology to genomics: review. *Comptes Rendus Biologies*, 334(3): 197 – 204.

Juan, L., Shuang, C., Huanmin, Z., Bichun, L. and Jiuzhou, S. (2013). DNA methylation down-regulates epidermal growth factor receptor (EGFR) expression in chicken. *Avian Diseases,* 57(2): 366 – 371.

Kadzere, C. T., Murphy, M. R. Silanikove N. and Maltz, E. (2002). Heat stress in lactating cows: a review. *Livestock Production Science*, 77: 59 – 91.

Kalbande, N. H., Ravikanth, K., Maini, S. and Rekhe, S. S. (2009). Methionine supplementation options in poultry. *International Journal of Poultry Science*, 8(6): 581 – 591.

Kalio, G. A. and Okafor, B. B. (2012). Response of broilers to two management systems of housing in Etche Local Government Area of Rivers State, Nigeria. *Asian Journal of Agricultural and Rural Development*, 2(2): 184 – 188.

Kalmar, I. D., Vanrompay, D. and Janssens, G. P. I. (2013). Broiler ascites syndrome: collateral damage from efficient feed to meat conversion: review. *The Veterinary Journal*, 197(2): 169 – 174.

Kambouh, A. A. and Zhu, W. (2013). Individual and combined effects of genistein and hesperidin supplementation on meat quality in meat-type broiler chickens. *Journal of the Science of Food and Agriculture*, 93(13): 3362 – 3367.

Kambouh, A. A., Hang, S. Q.,, Bakhetgul, M. and Zhu, W. Y. (2013). Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. *Poultry Science*, 92(9): 2411 – 2418.

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. U., Naz, S., Nikousefat, Z., Tufarelli, V., Jardani, M., Rana, N. and Laudadio, V. (2011). Effect of vitamin E in heat-stressed poultry: review. *World's Poultry Science Journal*, 67(3): 469 – 478.

Kikusato, M. and Toyomizu, M. (2013). Heat stress-induced overproduction 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, K., Yoon, K., Hong, H. and Lee, B. (2012). *Schisandra chinensis* prevents hepatic lipid peroxidation and oxidative stress in rats subjected to heat environmental stress. *Phytotherapy Research*, 26(11): 1674 – 1680.

Kingma, B., Frijns, A. and Lichtenbalt, W. V. (2012). The thermoneutral zone: implication for metabolic studies. *Frontiers in 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(6): 483 – 492.

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. *Biochemical and Biophysical Research Communication*, 403: 111 – 119.

Knezacek, T. D., Olkowski, A. A., Kettlewell, P. J., Mitchell, M. A. and Classen, H. L. (2010). Temperature gradients in trailers and changes in broiler rectal and core body temperature during winter transportation in Saskatchewan. *Canadian Journal of Animal Science*, 90(3): 321 – 330.

Koknaroglu, H. and Akunah, T. (2013). Animal welfare on animal science approach. *Meat Science*, 95(4): 821 – 827.

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 and Veterinary Advances*, 4(3): 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.

Kumar, A. (2011). Biomedical studies on lipid peroxidation and erythrocyte fragility during the process of ageing. *Asian Pacific Journal of tropical Biomedicine*, 1(1): 6 – 7.

Laganá, C., Ribeiro, A. L. M., Kessler, A. M., Kratz, L. R. and Pinheiro, C. C. (2007). Effects of the reduction of dietary heat increment on the performance, carcass yield, and diet digestibility of broilers submitted to heat stress. *Revista Brasileira de Ciência Avícola,* 9(1): 45 – 51.

Lallo, C. H. O., Williams, M., Campbell, M. and Palmer, D. W. (2012). The effect of stocking density on the performance and economic implications for broilers grown to 42 days in open sided house in Trinidad. *Tropical Agricultural Science*, 89(3): 170 – 180.

Lamming, D. W. and Sabatini, D. M. (2011). A radical role for target of rapamycin (TOR) in longevity. *Cell Metabolism*, 13: 617 – 618.

Lee, M. S., Yaar, M., Eller, M. C., Runger, T. M., Geo, Y. and Gilchrest, B. A. (2009). Telemetric DNA induces p53-depednent reactive oxygen species and protects against oxidative damage. *Journal of Dermatological Science*, 56: 154 – 162.

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

Leonarduzzi, G., Sottero, B. and Poli, G. (2010). Targeting tissue oxidative damage by means of cell signaling modulators: The antioxidant concept revised. *Pharmacology and Therapeutics,* 128: 336 – 374.

Lever, M. and Slow, S. (2010). The clinical significance of betaine, an osmolyte with a key-role in methyl group metabolism. *Clinical Biochemistry*, 43: 732 – 744.

Lipinski, K., Szramko, E., Jerochi, H. and Matasexicius, P. (2012). Effects of betaine on energy utilization in growing pigs - a review. *Annals of Animal Science*, 12(3): 291 – 300.

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-regulated kinase contributes to bleomycin-induced oxidative stress and fibrogenesis. *Toxicology Letters*, 220(1): 15 – 25.

Liverani, M., Waage, J., Barnett, T., Pferffar, D. U., Rushton, J., Rudge, J. W., Loevinsohn. M. E., Scoones, I., Smith, R. D., Cooper, B. S., White, L. J., Goh, S., Horby, P., Wren, B., Gundogy, O. J., Woods, A. and Coker, R. J. (2013). Understanding and managing zoonotic risk in the new livestock industries. *Environmental Health Perspectives*, 121: 873 – 877.

Lott, B. D., Simmons, D. and May, J. D. (1998). Air velocity and high temperature effects on broiler performance. *Poultry Science,* 77: 391 – 393.

Luo, J., Yu, Y., Zhang, H., Tian, F., Chang, S., Cheng, H. H. and Song, J. (2011). Down-regulation of promoter methylating level of CD4 gene after MDU infection in MD-susceptible chickens line. *Biomedical Centered Proceedings,* 5(4): 57 – 62.

Luo, J., Yu, Y., Chang, S., Tian, F., Zhang, H. and Song, J. (2012). DNA methylation fluctuation included by virus infection differs between MD-resistant and susceptible chickens. *Frontiers of Genetics*, 23: 20 – 35.

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A. and Schramer, T. E. (2007). The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. *Brain and Cognition*, 65: 209 – 237.

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

MacLeod, M. G. (2004). Climate-nutrition interactions in poultry, *First Science Conference*, Faculty of Veterinary Medicine, Zagazig University (Benha Branch), Moshtohor, Egypt. September 1 – 4, 21 pp.

Madsen, J. M., Zimmermann, N. G., Timmons, J. and Tablante, W. L. (2013). Evaluation of Maryland backyard flocks and biosecurity practices. *Avian Diseases*, 57(2): 233 – 237.

Mahmoudnia, N. and Madani, Y. (2012). Effect of betaine on performance and carcass composition of broiler chicken in warm weather - a review. *International Journal of AgriScience*, 2(8): 675 – 683.

Maini, S., Rastogi, S. K., Korde, J. P., Madan, A. K. and Shukla, S. K. (2007). Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. *The Journal of Poultry Science*, 44: 339 – 347.

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 chicken s reared in a hot environment. *African Journal of Agricultural Research*, 18(8): 701 – 706.

Mamo, W., Melaku, M. and Tamir, B. (2013). Characterization of urban poultry production system in Northern Gondar, Amhara Regional State, Ethiopia. *Agricultural and Biology Journal of North America*, 4(3): 192 – 198.

Mandl, J., Szarka, A. and Bánhegyi, G. (2009). Vitamin C: update on physiology and pharmacology. *British Journal of Pharmacology*, 157(7): 1097 – 1110.

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 Biology and Medicine*, 52: 1874 – 1887.

Marelli, S. P., Redaelli, V., Naini, Costa, 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, 2012, Naples - Italy, 2 pp.

Martin, J. P. Jr., Dailey, M. and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, 255: 329 – 336.

Martinez, E., Gerard, N., Gracia, M. M., Mazur, A., Gueant-Rodriguez, R., Comte, B., Gueant, J. and Brachet, P. (2013). Myocardium proteome remodeling after nutritional deprivation of methyl donors. *Journal of Nutritional Biochemistry*, 24(7): 1241 – 1250.

Matsuoka, Y., Yamato, M., Yamusaki, T., Mito, F. and Yamada, K. (2012). Rapid and convenient detection of ascorbic acid using a fluorescent introxide switch. *Free Radical Biology and Medicine*, 53: 2112 – 2118.

Melesse, A., Maak, S., Schmid R. and Von Lenger Ken, G. (2011). Effect of long-term heat stress on key enzyme activities and T_3 levels in commercial layer hens. *International Journal of Livestock Production*, 2(7): 107 – 116.

Mete, F., Kilic, E., Somay, A. and Yilmaz, B. (2012). Effects of heat stress on endocrine functions and behaviour in the pre-pubertal rat. *Indian Journal Medical Research*, 135: 233 – 239.

Metzler-Zebeli, B. U., Eklund, N. and Mosenthin, R. (2009). Impact of osmoregulatory and methyl donor functions of betaine on intestinal health and performance in poultry: review. *World's Poultry Science Journal*, 65(3): 419 – 442.

Minka, N. S. and Ayo, J. O. (2007). Haematology and behaviour of pullets transported by road and administered with ascorbic acid during the hot-dry season. *Research in Veterinary Science*, 85(2): 389 – 393.

Minka, N. S. and Ayo, J. O. (2011). Modulating role of vitamins C and E against transport-induced stress in pullets during the hot-dry conditions. *International Scholarly Research Network Veterinary Science*, Volume 2011, Article ID 497138, 7 pages.
\
Mitsuya, S.; Kozaki, K. and Takabe, T. (2013). Tissue localization of the glycine betaine biosynthetic enzymes in barley leaves. *Plant Production Science*, 16(2): 117 – 122.

Mittler, R., Vanderauwera, S., Surulei, N., Miller, G., Togtetti, V. B., Vandepoele, K., Gollery, M., Shutaev, V. and Breusegen, F. V. (2011). ROS signaling: the new wave? *Trends in Plant Science*, 16(6): 300 – 309.

Mokhar, F., Kambiz, N., Yahaya, E. N., Habib, A. S., Mohsen, D. and Taimor, T. (2011). The role of oxidative stress in the development of congestive heart failure (CHF) in broilers with pulmonary hypertension syndrome (PHS). *Journal of cell and Animal Biology*, 5(8): 176 – 181.

Mostl, E. and Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, 23: 67 – 74.

Motekis, H., Shimamura, Y., Kimura, M., Ogibara, M. (2012). Signal transduction pathway of L-ascorbic acid-and L-ascorbic acid 2 glucose-induced DNA syntheses and cell proliferation in primary cultures of adult rat hepatocytes. *European Journal of Pharmacology*, 683: 276 – 284.

Mujahid, A. (2011). Nutritional strategies to maintain efficiency and production of chickens under high environmental temperature: review. *The Journal of Poultry Science*, 48(3): 145 – 154.

Mujahid, A., Yoshiki, Y., Akiba, Y. and Toyomizu, M. (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poultry Science*, 84(2): 307 – 314.

Muniz, E. C., Fascina, V. B., Pires, P. P., Carrijo, A. S. and Guimarães, E. B. (2006). Histomorphology of bursa of Fabricius: effects of stock densities on commercial broilers. *Brazilian Journal of Poultry Science*, 8(4): 217 – 220.

Nardone, A., Ronchy, B., Lacetera, N., Ranieri, M. S. and Bernabucci, U. (2010). Effects of climatic changes on animal production and sustainability of livestock system. *Livestock Science*, 130(1-3): 57 – 69.

National Bureau of Statistics (NBS), (2005). *Poverty profile for Nigeria*. Federal Republic of Nigeria, Abuja, 98 pp.

Natt, D., Rubin, C., Wright, D., Johnsson, M., Belteky, J., Andersson, L. and Jensen, P. (2012). Heritable genome-wide variation of gene expression and promoter methylation between wild and domesticated chickens. *Biomedical Central Genomics*, 13: 59 – 61.

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

Obeng, J. E. (1985). The Secrets of Poultry Production, *Macmillian*, Nigeria, 104 pp.

Ognik, K. and Sembratowicz, I. (2012). Stress as a factor modifying the metabolism in poultry: a review. *Annales Universitatis Mariae Curie-Skk Odowska Lublin-Polonia, Zootechnica,* 30(2): 34 – 43.

Olaifa, F., Ayo, J. O., Ambali, S. F. and Rekwot, P. I. (2012). Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid. *Onderstepoort Journal of Veterinary Research*, 79(1): 1 – 5.

Olaniyi, A. O., Adesiyan, I. O. and Ayoade, R. A. (2008). Constraint to utilization of poultry production technology among farmers in Oyo State, Nigeria. *Journal of Human Ecology*, 24(1): 305 – 309.

Oliva, J., Bardag-Gorce, F., Tillman, B. and French, S. W. (2011). Protective effect of querceti, EGCG, catechin and betaine against oxidative stress induced by ethanol in vitro. *Experimental and Molecular Pathology*, 90(3): 295 – 299.

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 Higieny i Medycymy Doswiadczalnei*, 66: 534 – 542.

Oluyemi, J. A. and Roberts, F. A. (2000). Poultry Production in wet climate. *Macmillan Publishers*, London. 197 pp.

Onu, P. N. (2009). Growth performance, carcass characteristics and economic benefits of supplemental ascorbic acid on broiler starters exposed to heat stress. ARPN Journal of Agricultural and Biological Sciences, 4: 19 – 24.

Orhana, C., Sahina, N., Akdemirb, F., Markiewicz-Zukowskac, R., Borawskac, M. H., Isidorovd, V., Hayirlie, A. and Sahina, K. (2013). The effect of *Cirsium arvense* extract on antioxidant status in quail. *British Poultry Science*, 54(5): 620 – 626.

Owen, O. J. and Dike, U. A. (2013). Japanese quail (*Coturnix coturnix japonica*) husbandry: a means of increasing animal protein base in developing countries. *Journal of Environmental Issues and Agriculture in Developing Countries*, 5(1): 1 – 4.

Oyewale, J. O. (1992). Effects of temperature and pH on osmotic fragility of erythrocytes of domestic fowl (*Gallus domesticus*) and guinea fowl (*Numbia meleagris*). *Research in Veterinary Science*, 52: 1 – 4.

Padmini, E. and Rani, M. U. (2011). Heat-shock protein 90 alpha (HSP90α) modulates signaling pathways towards tolerance of oxidative stress and enhanced survival of hepatocytes of *Mugil cephalus*. *Cell Stress and Chaperones*, 16(4): 411 – 425.

Paglia, D. E. and Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine,* 70: 158 – 169.

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 Thermal Biology*, 34(7): 353 – 357.

Pandey, K. B. and Rizvi, S. I. (2013). Resveratrol upregulates erythrocyte plasma membrane redox system and mitigates oxidation-induced alterations in erythrocytes during aging in humans. *Rejuvenation Research*, 16(3): 232 – 240.

Pandurang, L. T., Kul Karni, G. B., Gangare, G. R., More, P. R., Ravihanth, K., Maini, S., Deshmukh, V. V. and Yeotikar, P. V. (2011). Overcrowding stress management in broiler chickens with verbal antistressor. *Iranian Journal of Applied Animal Science*, 1(1): 49 – 55.

Panok, S., Aengwanichy, W. and Komutrin, T. (2009). Adaption to oxidative stress and impact of chronic oxidative stress on immunity in heat stressed broilers. *Journal of Thermal Biology*, 34: 353 – 357.

Pereira, D. F., Miyamoto, B. C. B., Maia, G.D.B., Sales, G. T., Magalhães, M. M. and Gates, R. (2013). Machine vision to identify broiler breeder behavior. *Computers and Electronics in Agriculture*, 99: 194 – 199.

Persson, H. L. and Vainikka, L. K. (2010). TNF alpha preserves lysosomal stability in macrophages: a potential defense against oxidative lung activity. *Toxicology Letters*, 192: 261 – 267.

Pillai, P. B., Fanatico A. C., Beers, K. W., Blair, M. E. and Emmert, J. L. (2006). Homocysteine remethylation in young broilers fed varying levels of methionine, choline, and betaine. *Poultry Science*, 85: 90 – 95.

Pooya, S., Blaise, S., Garicia, M. M., Giudielli, J., Alberto, J., Gueant-Rodriguez, R., Jeonnesson, E., Gueguen, N., Bressenot, A., Nicolas, B., Malthiery, Y., David, J., Peyrin-Biroulet, L., Bronowicki, J. and Gueant J. (2012). Methyldonor deficiency inpairs fatty acid oxidation through PGC-1α hypomethylation and dereased ER-α and HNF-4α in the rat liver. *Journal of Hepatology*, 57: 344 – 351.

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. *Poultry Science*, 89(10): 2071 – 2077.

Prosser, B. L., Ward, C. W. and Lederer, W. J. (2011). X-ROS Signaling: Rapid mechano-chemotransduction in heart. *Science*, 333(6048): 1440 – 1445.

Purswell, J. L., Dozier C. D., Olarenwaju, A. A., Davis, J. D., Yin, H. and Gates, R. S. (2012). Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. *An American Society of Agricultural Engineers Conference Presentation,* 9th International Livestock Environment Symposium, Valencia, Spain. July 8-12, 2012. Pp. 1 – 9.

Quan, Xi, 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., Rodvigues, M. U., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Sa, L. R. M., Ferreira, A. J. P. and Palermo-Neto, J. (2012). Acute heat stress in paios performance parameters induces mild intestinal enteritis in broiler chickens: role of acute hypothalamic-pituitary-adrenal axis activation. *Journal of Animal Science*, 90(6): 1986 – 1994.

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.

Ramnath, V., Rekha, P. S. and Sujatha, K. S. (2008). Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by *Brahma rasyana*. *Evidence-Based Complementary and Alternative Medicine*, 5(1): 77 – 84.

Rekaya, R., Sapp, R. L., Wing, T. and Aggreyi, S. E. (2013). Genetic evaluation for growth, body composition, feed efficiency and leg soundness. *Poultry Science*, 92(4): 923 – 929.

Renaudeau, D., Collin, A., Yahav, S., de Basilio, V., Gourdine, V. L. and Colliera, R. J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*, 6(5): 707 – 728.

Repoa, J. K., Pesonen, M., Mannelli, C., Vähäkangas, K. and Loikkanen, J. (2014). Exposure to ethanol and nicotine induces stress responses in human placental BeWo cells. *Toxicology Letters*, 224(2): 264 – 271.

Rettenbacher, S., Henriksen, R., Groothuids, T. G. and Lepschy, M. (2013). Corticosterone Metabolism by chicken follicle cells does not affect ovarian reproductive hormone synthesis in vitro. *General and Comparative Endocrinology*, 184: 67 – 74.

Rhoads, R. P., Baumgard, L. H. and Suagee, J. K. (2013). Metabolic priorities during heat stress with an emphasis on skeletal muscle. *Journal of Animal Science*, 91(6): 2492 – 2503.

Roozendaal, B., Brunson, K.L ., Holloway, B. L., McGaugh, J. L. and Baran, T. Z. (2002). Involvement of stress-released corticotropin-releasing hormone in the basal lateral amygdala in regulating memory consolidation. *Proceedings of the New York Academy of Sciences*, 99(21): 13908 – 13913.

Rosab-Rodrigue, J. A. and Valenzuela-Soto, E. M. (2010). Enzymes involved in osmolyte synthesis: how does oxidative stress affect osmoregulation in renal cells? *Life Science*, 87(17-18): 515 – 520.

Rubin, C., Zody, M. C., Eriksson, J., Meadows, J. R. S., Sherwood, E., Webster, M. T., Jiang, L., Ingman, M., Sharpe, T., Ka, S., Hallbo¨o¨k, F., Besnier, F., Carlborg, O., Bed'hom, B., Tixier-Boichard, M., Jensen, P., Siegel, P., Lindblad-Toh, K. and Andersson, L. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, 464: 587 – 592.

Run-shen, J. I. A. N. G., Wen-tao, X. I. A., Xing-yong, C. H. E. N. and Zhao-yu, G. E. N. G. (2011). Evaluation of chicken heat tolerance using the changing of serum biochemical indices in the early stage of heat stress. *Journal of China Agricultural University*, 2: 017 – 021.

Sahin, K., Onderai, M., Sahin, N., Gursu, M. F. and Kucuk, O. (2003). Dietary vitamin C and folic acid supplementation ameliorate the detrimental effects of heat stress in Japanese quail. *The Journal of Nutrition*, 133: 1852 – 1886.

Sahin, K., Orhan, C., Akdemir, F. , Tuzcu, M., Iben, C. and Sahin, N. (2012). Resveratrol protects quail hepatocytes against heat stress: modulation of the Nrf2 transcription factor and heat shock proteins. *Journal of Animal Physiology and Animal Nutrition*, 96(1): 66 – 74.

Sales, J. (2011). A meta-analysis of the effects of dietary bataine supplementation on finishing performance and carcass characteristics in pigs. *Animal Feed Science and Technology*, 165: 68 – 78.

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.

Sayed, M. A. M. and Downing, J. (2011). The effects of water replacement of oral rehydration fluids with or without betaine supplementation on performance, acid-base balance, and water retention of heat-stressed broiler chickens. *Poultry Science*, 90(1): 157 – 167.

Sharma, B., Rai, D. K., Rai, P. K., Rizvi, S. I. and Watal, G. (2010). Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. *Advanced Protocols in Oxidative Stress II*, 594: 123 – 128.

Shini, S., Huff, G. R., Shini, A. and Kaiser, P. (2010). Understanding stress-induced immune suppression: Exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes. *Poultry Science*, 89(4): 841– 851.

Si, Y., de Boer, W. F. and Gong, P. (2013). Different environmental drivers of highly pathogenic avian influenza H5N1 outbreaks in poultry and wild birds. *Public Library of Science ONE*, 8(1): e53362 – e53370.

Singh, S., Brocker, C., Koppaka, V., Chen, Y., Jackson, B. C., Matsumoto, A., Thompson, D. C. Vasiliou, V. (2013). Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress: a review. *Free Radical Biology and Medicine*, 56: 89 – 101.

Sinkalu, V. O. and Ayo, J. O. (2008). Diurnal fluctuations in rectal temperature of Black Harco pullets administered with vitamins A and C during the hot-dry season. *International Journal of Poultry Science*, 7(11): 1065 – 1070.

Sinkalu, V. O., Ayo, J. O., Adelaiye, A. B. and Hambolu, J. O. (2008). Diurnal fluctuations in rectal temperature of Black Harco pullets administered ascorbic acid during the hot-dry season in Nigeria. *The International Journal of Meteorology*, 33(334): 327 – 333.

Sinkalu, V. O., Ayo, J. O., Adelaije, A. B. and Hambolu, J. O. (2009). Influence of co-administration of vitamin A and E on diurnal variation in rectal temperature of Black Harco pullets during the hot-dry season. *Biological Rhythm Research*, 40(3): 257 – 264.

Snedecor, G. W. and Cochran, W. G. (1994). *Statistical Methods*. Oxford and IBH Publishing Corporation, Calcutta, India, 509 pp.

Sohail, M. U., Ijaz, A., Yousaf, M. S. Ashraf, K. Zareb, H., Aleem M. and Rehman, H. (2010). Alleviation of cycle heat stress in broilers by dietary supplementation of mannan-oligosaccharide and *Lactobacillus*-based probiotic: dynamic of cortisol, thyroid hormones, cholesterol, C-reactive protein and humoral immunity. *Poultry Science*, 89(9): 1934 – 1938.

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.

Su, S. Y., Dodson, M. V., Li, X. B., Li, Q. F., Wang, H. W., and Xier Z. (2009). The effects of dietary betaine supplementation on fatty liver performance, serum parameters, histological changes, methylation status, and the mRNA expression level of spot 14α in lands goose fatty liver. *Comparative Biochemistry and Physiology*, 154: 308 – 314.

Sugarmura, K. and Keaney (Jr), J. F. (2011). Reactive oxygen species in cardiovascular disease: a review. *Free Radical Biology and Medicine*, 51: 978 – 992.

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.

Sunil Kumar, B. V., Kumar, A. and Kataria, M. (2011). Effect of heat stress in tropical livestock and different strategies for its amelioration: a review. *Journal of Stress Physiology and Biochemistry*, 7(1): 45 – 54.

Swennen, Q., Geraert, P., Mercier, Y., Everaert, N., Stinckers, A., Willemsen, H., Li, Y., Decuypere, E. and Buyse, J. (2011). Effects of dietary protein content and 2-hydroxy-t-methy/fluidbutanoic acid or DL-methionine supplementation on performance and oxidative status of broiler chickens. *British Journal of Nutrition*, 106(12): 1845 – 1854.

Swu, I. C., Singh, S. P. and Gautam (2012). Constraints perceived by broiler farmers in adoption of scientific poultry production practices. *Veterinary Practitioner*, 13(1): 116 – 120.

Talebi, A., Asri-Rezaei, A., Rozeh-Chai, R. and Sahraei, R. (2005). Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian). *International Journal of Poultry Science*, 4(8): 573 – 579.

Taniguchi, M., Arai, W., Kohno, K., Ushio, S. and Fukuda, S., (2012). Antioxidant and anti-aging activities of 2-0-α glucopyranosyl L-ascorbic acid on on dermal fibroblasts. *European Journal of Pharmacology*, 674: 126 – 131.

Tanizawa, H., Shiraishi, J., Kawakami, S., Tsudzuki, M. and Bungo, T. (2014). Effect of short-term thermal conditioning on physiological and behavioral responses to subsequent acute heat exposure in chicks. *The Journal of Poultry Science*, 51: 80 – 86.

Tao, X. and Xin, H. (2003). Acute synergistic effects of air temperature, humidity and velocity on homeostasis of market-size boilers. *Transactions of the American Society of Agricultural and Biological Engineers*, 46(2): 491 – 497.

Tesseraud, S., Everaest, N., Boussand-onezzine, S., Collin, A., Metayer-Coustard, S. and Berrii, C. (2011). Manipulating tissue metabolism by amino acids: review article. *World's Poultry Science Journal*, 67(2): 243 – 252.

Tijjani, H., Tijjani, B. A., Tijjani, A. N. and Sadiq, M. S. (2012). Economic analysis of poultry egg production in Maiduguri and environs of Borno State in Nigeria. *Scholarly Journal of Agricultural Science*, 2(12): 319 – 324.

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.

Traber, M. G. and Stevens, J. F. (2011). Vitamins C and E: beneficial effects from a mechanistic perspective: review. *Free Radical Biology and Medicine*, 51(5): 1000 – 1013.

Uchendu, C., Ambali, S. F., Yakub, S. L., Lasisi, D. I. and Umosen, A. J. (2011). Comparative effects of vitamin C and acetyl-L-carnithine on subacute chlorpyrifos-induced erythrocyte osmotic fragility in Wister rats. *Advances in Applied Science Research*, 2(5): 297 – 302.

Useni, F., Nakamura, Y., Yamamoto, Y., Bitoh, A., Ono, T. and Kagami, H. (2009). Analysis of developmental changes in avian DNA methylation using a novel method for quantifying genome-wide DNA methylation. *Journal of Poultry Science*, 46(4): 286 – 290.

Venditti, P., Stefand, L. D. and Meo, S. D. (2013). Mitochondrial metabolism of reactive oxygen species: review. *Mitochondrion*, 13: 71 – 82.

Vieira, F. M. C, da Silva, I. J. O., Filho, J. A. D. B., Vieira, A. M .C, Rodrigues-Sarnighausen, V. C. and Danilo de Brito Garcia, D. (2011). Thermal stress related with mortality rates on broilers' preslaughter operations: a lairage time effect study. *Ciencia Rural*, 41(9): 1639 – 1644.

Waldroup, P. W., Mott, M. A., Yan, F. and Frittis, C. A. (2006). Effects of betaine and choline on response to methionine supplementation to broiler diets formulated to industry standards. *Journal of Applied Poultry Research*, 15: 58– 71.

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.

Wang, Y., Jiang, Z., Jin, Z., Tan, H. and Xu, B. (2013a). Risk factors for infectious diseases in backyard poultry farms in the Poyang lake area, China. *Public Library of Science ONE*, 8(6): e67366 – e67376.

Wang, S., Ni, Y., Guo, F., W., Grossmann, R. and Zhao, R. (2013b). Effect of corticosterone on growth and welfare of broiler chickens showing long or short tonic immobility. *Comparative Biochemistry and Physiology*, 164: 537 – 543.

Widjastati, T. and Hernawan, E. (2012). Utilizing of banana peel (*Musa sapientum*) in the ration and its influence on final body weight, percentage of carcass and abdominal fat on broilers under heat stress-condition. *Seria Zootechnic*, 57: 104 – 109.

Wu, B., Peng, X., Fang, J., Zuo, Z., Deng, J. and Huang, J. (2014). Dietary nickel chloride induces oxidative stress, apoptosis and alters bax/bcl-2 and caspase-3 mRNA expression in the cecal tonsil of broilers. *Food and Chemical Toxicology*, 63: 18 – 29.

Xing, J., Kang, L. and Jiang, Y. (2011). Effect of dietary betaine supplementation on lipogenesis gene expression in CpG methylation of lipoprotein lipase gene in broilers. *Molecular Biology*, 38(3): 1975 – 1981.

Yahav, S., Shinder, D., Tanny, J. and Cohen, S. (2005). Sensible heat loss: the broiler's paradox. *World's Poultry Science Journal,* 61: 419 – 434.

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.

Yi, X. J., Kang, L., Hu, Y., QinYing, X., NingBo, Z. and YunLiang, J. (2009). Effect of dietary betaine supplementation on mrna expression and promoter CPG methylation of lipoprotein lipasegene in laying liens. *Journal of Poultry Science*, 46(3): 224 – 228.

Yu, Y., Zhang, H., Tian, F., Zhang, W., Fang, H. and Song, J. (2008). An integrated epigenetic and genetic analysis of DNA methyl transferase genes (dnmts) in tumor resistant and susceptible chicken lines. *Public Library of Science One*, 3(7): e2672 – e2685.

Yu, Y., Zhang, H., Byerly, M. S., Bacon, L. D., Porter T. E., Liu, G. E. and Song, J. (2009). Alternative splicing variants and DNA methylation status of brain-derived neurotropic factor in inbred chicken lines. *Brain Research*, 1269: 1 – 10.

Zhan, X. A., Li, J. X., Xu, Z. R. and Zhao, R. Q. (2006). Effects of methionine and betaine supplementation on growth performance, carcase composition and metabolism of lipids in male broilers. *British Poultry Science*, 47(5): 576 – 580.

Zhang, J., Hou, X., Ahmad, H., Zhang, H., Zhang, L. and Wang, T. 2013. Assessment of free radical- scavenging activity of seven natural pigments and protective effects in AAPH-challenged chicken erythrocytes. *Food Chemistry*, 145: 57 – 65.

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(8): 471 – 476.

APPENDICES

Appendix 1: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Control Broiler Chickens Exposed To Heat Stress (n = 20)

Hour of Day (h)	Mean Cloacal Temperature (°C)	Minimum Cloacal Temperature (°C)	Maximum Cloacal Temperature (°C)	Range Cloacal Temperature (°C)
6:00	41.27 ± 0.04	40.6	42.2	1.6
8:00	41.24 ± 0.04	40.5	41.8	1.3
10:00	41.55 ± 0.06	40.7	42.7	2
12:00	41.98 ± 0.06	40.7	43.2	2.5
14:00	42.13 ± 0.06	41.2	43.5	2.3
16:00	42.16 ± 0.06	41.2	43.4	2.2
18:00	42.23 ± 0.06	41.5	43.7	2.2
Mean ± SEM	41.79 ± 0.16	40.91 ± 0.14	42.93 ± 0.27	2.01 ± 0.16

Appendix 2: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Betaine Administered Broiler Chickens Exposed to Heat Stress (n = 20)

Hour of Day (h)	Mean Cloacal Temperature (°C)	Minimum Cloacal Temperature (°C)	Maximum Cloacal Temperature (°C)	Range Cloacal Temperature (°C)
6:00	41.24 ± 0.04	40.4	42	1.6
8:00	41.27 ± 0.06	40.1	42.6	2.5
10:00	41.59 ± 0.07	41.5	42.8	2.3
12:00	42.00 ± 0.05	41.2	43.1	1.9
14:00	42.34 ± 0.05	41.4	43.5	2.1
16:00	42.19 ± 0.05	41.3	43.1	1.8
18:00	42.19 ± 0.06	41.4	43.3	1.9
Mean ± SEM	41.83 ± 0.17	40.90 ± 0.21	42.91 ± 0.19	2.01 ± 0.12

Appendix 3: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Ascorbic Acid Administered Broiler Chickens Exposed to Heat Stress (n = 20)

Hour of Day (h)	Mean Cloacal Temperature (°C)	Minimum Cloacal Temperature (°C)	Maximum Cloacal Temperature (°C)	Range Cloacal Temperature (°C)
6:00	41.11 ± 0.03	40.4	41.6	1.2
8:00	41.17 ± 0.04	40.6	41.8	1.2
10:00	41.54 ± 0.04	41.4	42.6	2.2
12:00	41.75 ± 0.04	41.2	42.6	1.4
14:00	41.82 ± 0.04	41.1	42.5	1.4
16:00	41.94 ± 0.04	41.2	42.7	1.5
18:00	41.88 ± 0.04	41.3	42.8	1.5
Mean ± SEM	41.60 ± 0.13	40.80 ± 0.15	42.37 ± 0.18	1.49 ± 0.13

Appendix 4: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Broiler Chickens Co-Administered with Betaine and Ascorbic Acid Exposed to Heat Stress (n = 20)

Hour of Day (h)	Mean Cloacal Temperature (°C)	Minimum Cloacal Temperature (°C)	Maximum Cloacal Temperature (°C)	Range Cloacal Temperature (°C)
6:00	41.30 ± 0.05	40.5	42.3	1.8
8:00	41.36 ± 0.04	40.6	42.2	1.6
10:00	41.68 ± 0.04	41.9	42.2	1.3
12:00	42.04 ± 0.04	41.6	43.0	1.4
14:00	42.42 ± 0.06	41.6	43.2	1.6
16:00	42.47 ± 0.06	41.3	43.4	2.1
18:00	42.37 ± 0.07	41.5	43.6	2.1
Mean ± SEM	41.95 ± 0.19	41.14 ± 0.18	42.84 ± 0.23	1.70 ± 0.12

Appendix 5: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Control Broiler Chickens on Day 28 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.5 ± 0.06	40.6	41.6	1
8:00	41.08 ± 0.08	40.5	41.7	1.2
10:00	41.45 ± 0.06	40.8	41.9	1.1
12:00	41.50 ± 0.08	40.7	42.3	1.6
14:00	41.70 ± 0.07	41.2	42.4	1.2
16:00	41.75 ± 0.05	41.2	42.2	1
18:00	41.91 ± 0.05	41.5	42.3	0.8
Mean ± SEM	41.56 ± 0.10	40.93 ± 0.14	42.06 ± 0.12	1.13 ± 0.09

Appendix 6: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Betaine on Day 28 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.05 ± 0.06	40.4	41.6	1.2
8:00	41.25 ± 0.06	40.9	41.9	1
10:00	41.58 ± 0.07	41	42.3	1.3
12:00	41.73 ± 0.05	41.2	42.1	0.9
14:00	42.05 ± 0.07	41.4	42.5	1.1
16:00	41.93 ± 0.09	41.3	42.6	1.3
18:00	41.74 ± 0.05	41.4	42.2	0.8
Mean ± SEM	41.62 ± 0.14	41.09 ± 0.14	42.17 ± 0.13	1.09 ± 0.07

Appendix 7: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Ascorbic Acid on Day 28 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.05 ± 0.05	40.4	41.3	0.9
8:00	41.03 ± 0.06	40.6	41.4	0.8
10:00	41.45 ± 0.08	40.4	42.1	1.7
12:00	41.66 ± 0.05	40.3	42.3	2
14:00	41.95 ± 0.05	41.6	42.3	0.7
16:00	41.95 ± 0.06	41.6	42.6	1
18:00	41.68± 0.0.07	41.3	42.8	1.5
Mean ± SEM	41.54± 0.14	40.89 ± 0.22	42.11 ± 0.22	1.23 ± 0.19

Appendix 8: Mean, Minimum, Maximum and Range Cloacal Temperature Responses Heat-Stressed Broiler Chickens Co-Administered with Betaine and Ascorbic Acid on Day 28 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.11 ± 0.05	40.7	41.5	0.8
8:00	41.22 ± 0.05	40.9	41.7	0.8
10:00	41.57 ± 0.08	40.9	41.9	1
12:00	41.87 ± 0.05	41.6	42.5	0.9
14:00	42.09 ± 0.06	41.8	42.7	0.9
16:00	42.14 ± 0.07	41.6	42.8	1.2
18:00	41.90 ± 0.07	41.5	42.6	1.1
Mean ± SEM	41.70 ± 0.16	41.29 ± 0.17	42.24 ± 0.20	0.96 ± 0.06

Appendix 9: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Control Broiler Chickens Exposed to Heat Stress on Day 35 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.14 ± 0.04	40.8	41.8	1
8:00	41.18 ± 0.04	40.8	41.6	0.8
10:00	41.10 ± 0.05	40.7	41.6	0.9
12:00	42.09 ± 0.08	41.7	42.8	1.1
14:00	42.24 ± 0.08	41.7	42.8	1.1
16:00	42.20 ± 0.08	41.6	42.8	1.2
18:00	42.32 ± 0.08	41.9	43.2	1.3
Mean ± SEM	41.75 ± 0.22	41.31 ± 0.20	42.37 ± 0.26	1.06 ± 0.06

Appendix 10: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Betaine on Day 35 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.14 ± 0.06	40.6	41.6	1
8:00	40.86 ± 0.09	40.1	41.4	1.3
10:00	41.01 ± 0.07	40.5	41.7	1.2
12:00	41.95 ± 0.08	41.3	42.6	1.3
14:00	42.35 ± 0.05	41.9	42.8	0.9
16:00	42.22 ± 0.08	41.7	43	1.3
18:00	42.29 ± 0.09	41.6	43	1.4
Mean ± SEM	41.69 ± 0.25	41.10 ± 0.26	42.30 ± 0.27	1.20 ± 0.07

Appendix 11: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Ascorbic Acid on Day 35 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.01 ± 0.05	40.7	41.5	0.8
8:00	41.09 ± 0.05	40.7	41.6	0.9
10:00	41.40 ± 0.06	40.9	42.1	1.2
12:00	41.75 ± 0.06	41.2	42.2	1
14:00	41.54 ± 0.07	41.1	42.5	1.4
16:00	41.91 ± 0.06	41.2	42.3	1.1
18:00	41.93± 0.06	41.3	42.3	1
Mean ± SEM	41.52± 0.14	41.01 ± 0.09	42.07 ± 0.14	1.06 ± 0.08

Appendix 12: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Betaine on Day 42 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.54 ± 0.06	41	42	1
8:00	41.71 ± 0.08	41.2	42.6	1.4
10:00	42.18 ± 0.08	41.4	42.8	1.4
12:00	42.33 ± 0.08	41.8	43.1	1.3
14:00	42.63 ± 0.09	41.9	43.5	1.6
16:00	42.43 ± 0.07	41.9	43.1	1.2
18:00	42.55 ± 0.08	41.7	43.3	1.6
Mean ± SEM	42.20 ± 0.16	41.56 ± 0.36	42.91 ± 0.19	1.36 ± 0.08

Appendix 13: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Administered with Ascorbic Acid on Day 42 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.29 ± 0.05	40.8	41.6	0.8
8:00	41.39 ± 0.06	40.9	41.8	0.9
10:00	41.76 ± 0.07	41.4	42.6	1.2
12:00	41.85 ± 0.06	41.5	42.6	1.1
14:00	41.99 ± 0.07	41.5	42.5	1
16:00	41.98 ± 0.07	41.5	42.7	1.2
18:00	42.03 ± 0.07	41.6	42.6	1
Mean ± SEM	41.76 ± 0.11	41.31 ± 0.12	42.34 ± 0.17	1.03 ± 0.06

Appendix 14: Mean, Minimum, Maximum and Range Cloacal Temperature Responses of Heat-Stressed Broiler Chickens Co-Administered with Betaine and Ascorbic Acid on Day 42 (n = 20)

Hour of Day (h)	Mean Rectal Temperature (°C)	Minimum Rectal Temperature (°C)	Maximum Rectal Temperature (°C)	Range Rectal Temperature (°C)
6:00	41.69 ± 0.06	41.1	42.3	1.2
8:00	41.61 ± 0.06	41.0	42.2	1.2
10:00	41.92 ± 0.05	41.4	42.2	0.8
12:00	42.33 ± 0.06	41.8	43.0	1.2
14:00	42.62 ± 0.06	42.1	43.0	0.9
16:00	42.68 ± 0.09	41.9	43.4	1.5
18:00	42.48 ± 0.08	41.8	43.2	1.4
Mean ± SEM	42.19 ± 0.17	41.60 ± 0.15	42.76 ± 0.19	1.16 ± 0.09

Appendix 15: Erythrocyte Osmotic Fragility of Broiler Chickens Exposed to Heat Stress (n = 15)

Concentration	Group I	Group II	Group III	Group IV
0.9	11.19 ± 2.22 [a]	4.72 ± 1.48 [b]	3.50 ± 0.54 [b]	3.05 ± 0.68 [b]
0.7	13.65 ± 2.30 [a]	6.66 ± 1.51 [b]	7.17 ± 1.31 [b]	7.00 ± 1.29 [b]
0.5	31.77 ± 3.12	22.76 ± 3.22	25.52 ± 3.36	24.27 ± 3.20
0.3	79.06 ± 2.98	80.00 ± 1.71	76.66 ± 3.12	77.77 ± 2.83
0.1	91.87 ± 1.52	91.95 ± 1.33	90.43 ± 1.74	90.17 ± 1.59
0.0	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

[a,b] = Means with different superscript letters within rows are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 16: Erythrocyte Osmotic Fragility of Broiler Chickens Exposed to Heat Stress on Day 21 (n = 15)

Concentration	Group I	Group II	Group III	Group IV
0.9	14.39 ± 4.04 [a]	7.51 ± 2.65 [ab]	2.26 ± 0.77 [b]	3.87 ± 1.12 [b]
0.7	16.27 ± 4.06	9.35 ± 2.71	6.98 ± 2.47	7.78 ± 1.66
0.5	29.97 ± 4.44	30.38 ± 3.94	24.26 ± 5.77	22.29 ± 4.84
0.3	74.08 ± 5.33	79.72 ± 2.90	76.69 ± 5.20	72.67 ± 4.89
0.1	91.52 ± 2.02	94.02 ± 1.35	88.70 ± 3.03	89.12 ± 2.24
0.0	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

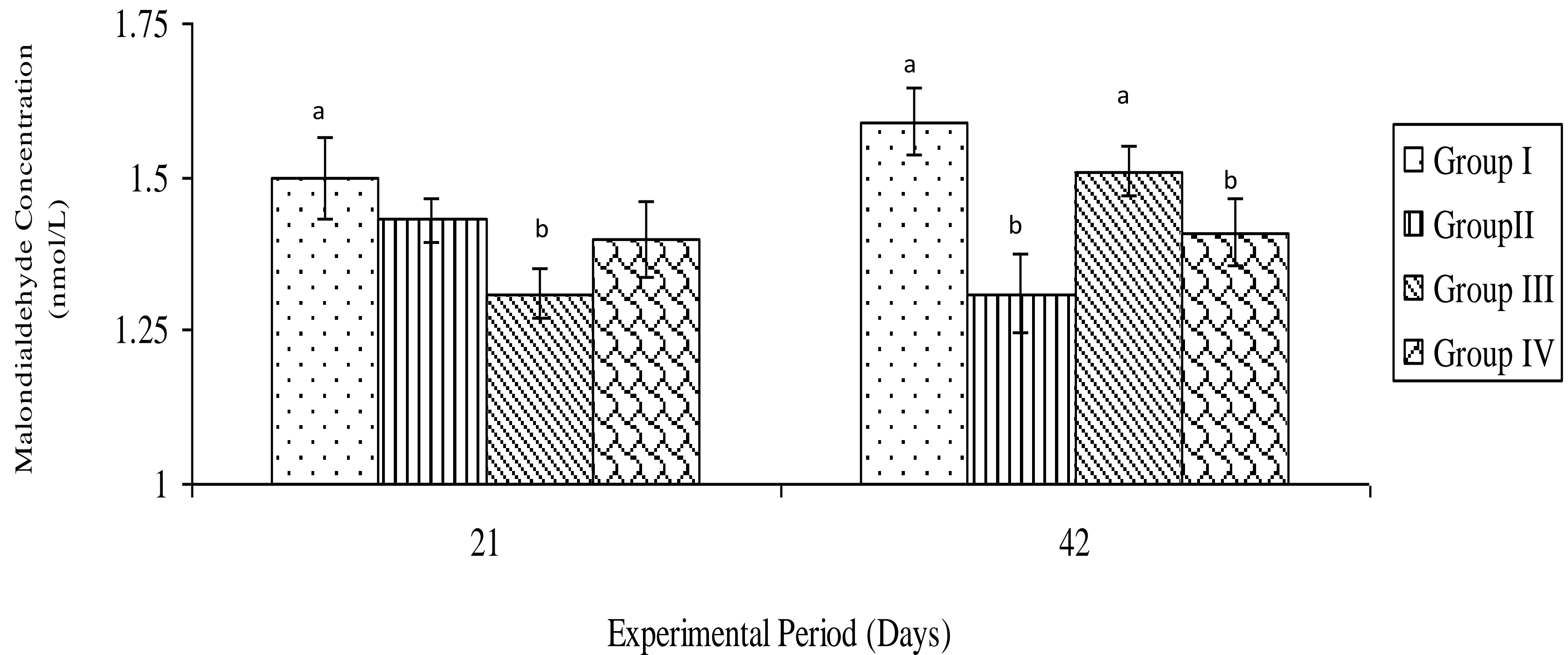
[a,b] = Means with different superscript letters within rows are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 17: Erythrocyte Osmotic Fragility of Broiler Chickens Exposed to Heat Stress on Day 42 (n = 15)

Concentration	Group I	Group II	Group III	Group IV
0.9	7.99 ± 1.65 [a]	1.93 ± 0.98 [b]	4.73 ± 0.61 [ab]	2.22 ± 0.76 [b]
0.7	11.04 ± 2.09 [a]	3.97 ± 1.05 [b]	7.36 ± 0.97	6.23 ± 2.01
0.5	33.58 ± 4.48 [a]	15.14 ± 4.38 [b]	26.77 ± 3.65	26.26 ± 4.30
0.3	84.04 ± 2.23	80.28 ± 1.91	76.63 ± 3.65	82.88 ± 2.37
0.1	92.22 ± 2.34	89.88 ± 2.20	92.16 ± 1.71	91.22 ± 2.29
0.0	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

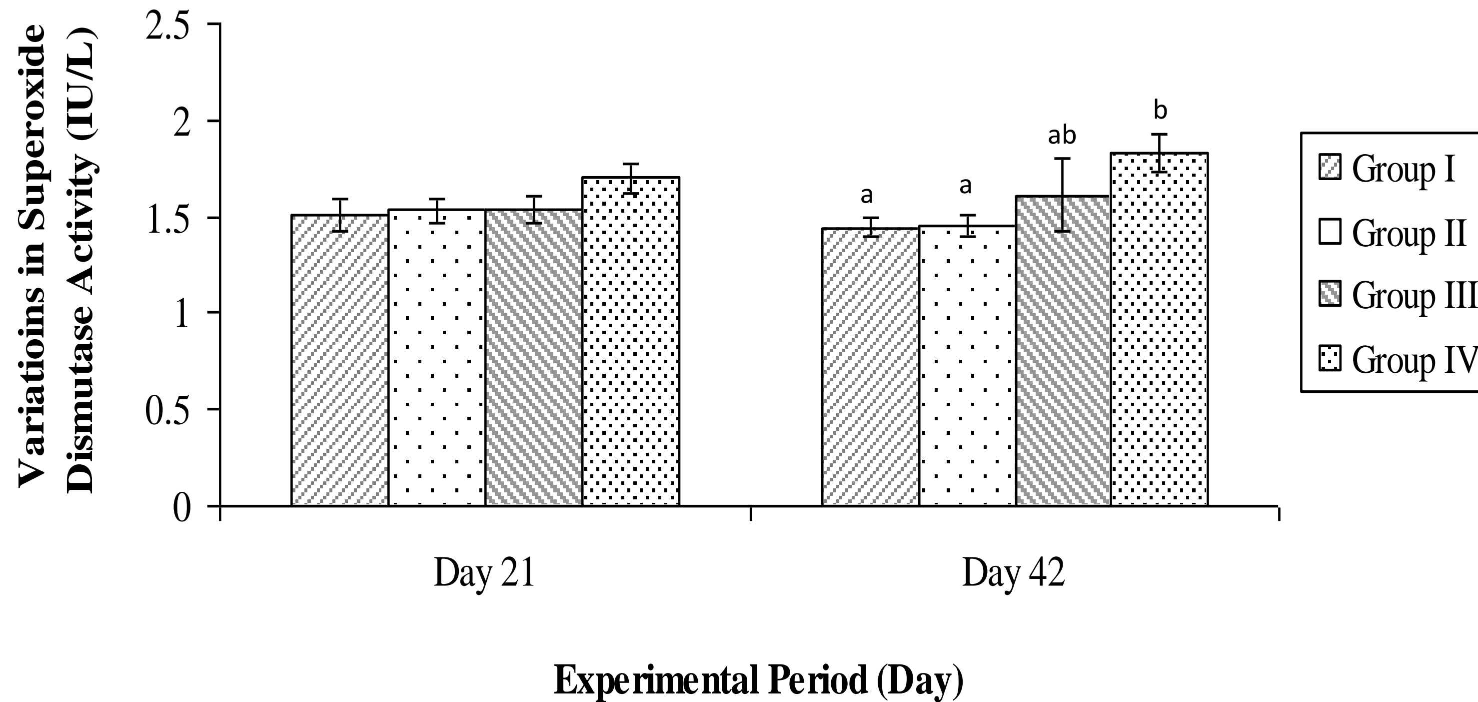
[a,b] = Means with different superscript letters within rows are significantly ($P < 0.05$) different ; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 18: Changes in Malondialdehyde Level of Broiler Chickens on Days 21 and 42 of the Study Period (n = 7)



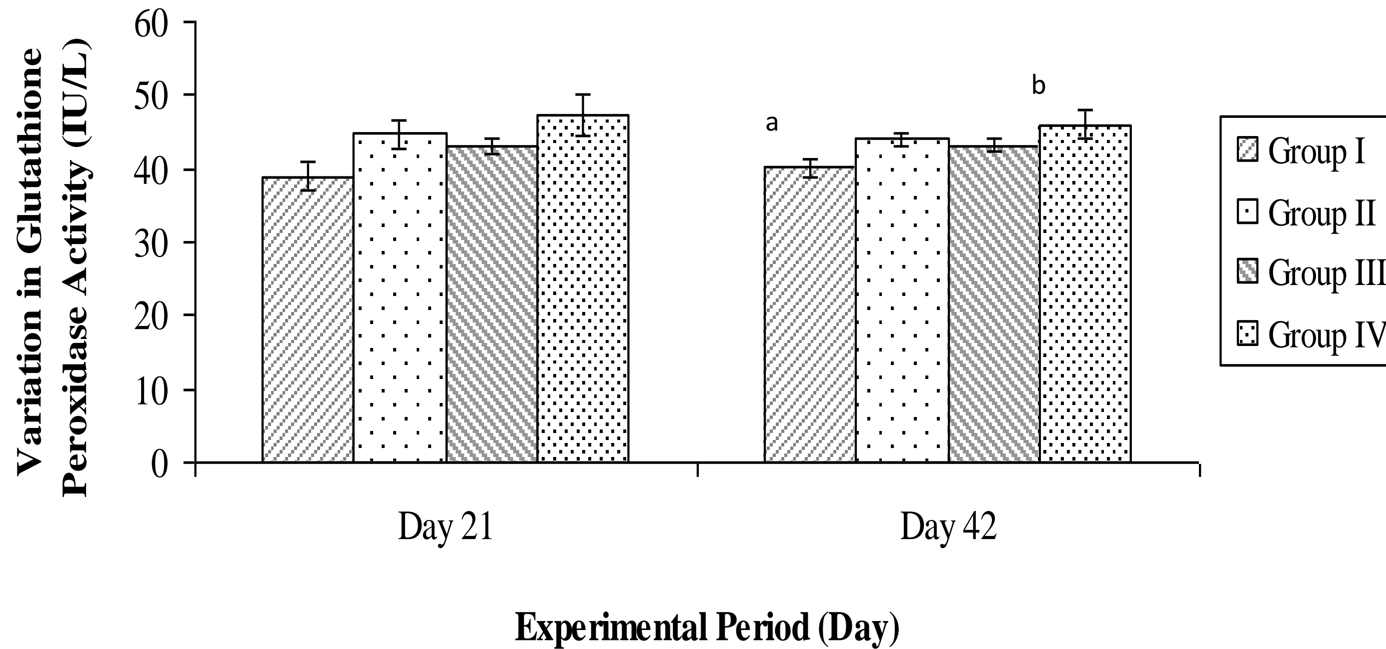
[a,b] = Means with different superscript letters are significantly different ($P < 0.05$); Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 19: Changes in Superoxide Dismutase Activity of Broiler Chickens on Days 21 and 42 of the Study Period (n = 7)



[a,b] = Means with different superscript letters are significantly different ($P < 0.05$); Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 20: Changes in Glutathione Peroxidase Activity of Broiler Chickens on Days 21 and 42 of the Study Period (n = 7)



[a,b] = Means with different superscript letters are significantly ($P < 0.05$) different; Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid

Appendix 21: Proximate Analysis of Feeds Given to Broiler Chickens During the Study Period

Parameters	Pre-starter	Starter	Finisher
Percentage Dry Matter (%)	93.84	93.73	94.14
Metabolisable Energy (M.E.)	3144.34	3406.07	3357.16
Percentage Crude Protein (%)	18.12	16.62	13.75
Percentage Crude Fibre (%)	5.6	3.36	4.38
Percentage Oil (%)	5.45	5.08	5.26
Percentage Ash (%)	11.96	6.95	7.4
Percentage Nitrogen-free Extract (%)	58.07	67.99	69.21

Source: Biochemical Laboratory Unit, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, NIGERIA.

Appendix 22: Economic Analysis for the Administration of Betaine and/or Ascorbic Acid to Broiler Chickens During the Study Period

Economic Parameters	**Group I**	**Group II**	**Group III**	**Group IV**
Cost per Day Old Chicks (₦)	190.00	190.00	190.00	190.00
Total feed intake per Bird (Kg)	3.048	2.81	2.987	3.01
Total feed Consumed (Kg)	60.96	56.20	59.74	60.20
Cost of Feed/ Bag (₦)	2,650.00	2,650.00	2,650.00	2,650.00
Cost of Feed per Kg (₦)	106.00	106.00	106.00	106.00
Cost of Feed Consumed per Bird (₦)	6461.76	5957.20	6332.44	6381.20
Cost Agents Administered (₦)	120.00	1260.00	150.00	1410.00
Cost of Production (per 20 birds) (₦)	6701.76	8477.20	6632.44	9201.20
Cost of Production per Bird (₦)	335.09	423.86	331.62	460.06
Total cost of Production (₦)	525.09	613.86	521.62	650.06
Average Weight of Bird (Kg)	1.723	1.748	1.795	1.858
Selling Price Bird (₦)	1,068.26	1,083.76	1,112.90	1,151.96
Profit per Bird (₦)	549.17	532.90	598.78	572.40

Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid; Note: Assuming that: **i**. All other variable are the same for all groups. **ii**. Selling price per kilogram body weight of broiler chickens is ₦620.00.

Appendix 23: Weekly Weight of Broiler Chickens During the Study Period

Week	Group I	Group II	Group III	Group IV
0	56.88 ± 1.06 [a]	56.38 ± 1.10 [b]	56.38 ± 0.76 [ab]	55.86 ± 1.12 [ab]
1	149.0 ± 3.57 [a]	145.8 ± 3.41 [b]	152.8 ± 2.39 [ab]	153.1 ± 3.48 [ab]
2	272.5 ± 10.56 [a]	252.5 ± 9.23 [a]	305.0 ± 7.16 [b]	263.9 ± 9.74 [a]
3	567.5 ± 17.50 [a]	533.0 ± 16.43 [a]	560.0 ± 12.88 [a]	619.4 ± 18.59 [b]
4	877.5 ± 22.79 [a]	805.0 ± 28.77 [b]	840.0 ± 21.94 [ab]	880.6 ± 30.29 [a]
5	1220.0 ± 29.33	1193.0 ± 35.59 [a]	1220 ± 27.96	1294.0 ± 44.8 [b]
6	1723.0 ± 38.64 [a]	1748.0 ± 57.06	1795.0 ± 37.5	1858.0 ± 62.00 [b]

Group I = Control; Group II = Betaine administration; Group III = Ascorbic acid administration; Group IV = Co-administration with betaine and ascorbic acid