LYCOPENE- AND VITAMIN E-INDUCED MODULATION OF PHYSIOLOGICAL AND REPRODUCTIVE PERFORMANCE INDICES IN LAYING HENS DURING THE DRY SEASON

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MARCH, 2021

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(P17VTPY 9085)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY PHYSIOLOGY

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MARCH, 2021

DECLARATION

I declare that the workin this thesis entitled **"LYCOPENE- AND VITAMIN E-INDUCED MODULATION OF PHYSIOLOGICAL AND REPRODUCTIVE PERFORMANCE INDICES IN LAYING HENS DURING THE DRY SEASON"** has been performed by me in the Department of Veterinary Physiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Joseph AyodeleOBIDI
Name of Student …… ……..
Signature Date

CERTIFICATION

This thesis entitled **"LYCOPENE- AND VITAMIN E-INDUCED MODULATION OF PHYSIOLOGICAL AND REPRODUCTIVE PERFORMANCE INDICES IN LAYING HENS DURING THE DRY SEASON"**by Joseph Ayodele OBIDI meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

To God, in whom we live, in whom we move and in whom we have our being.
To God, who subdued the activities of horsemen in all their mischievous inventions and defeated the crafty enterprises of the wicked, bringing all their treachery and crooked venomous determination to open shameful falsehood;“blessed be the LORD, who has not given us as a prey to their teeth. The snare is broken, and we are escaped”

To the memory of my father, Michael Obidi, who “spored” inquisitive learning for my entire life. Your invaluable legacies echo always to our ears and visibly spotted by our eyes, dearly remembering the sounds of your golden steps in the sand of our history.Rest your soul in the breast of the resident Rock in mount Zion.

ACKNOWLEDGEMENTS

I express my deepest thanks and appreciation to all who may be too numerous to list on pages of this thesis, who have made invaluable contributions to the success of this study. Without their expertise, criticism and cooperation, this thesis would not have been possible. I also wish to express my appreciation to my supervisory team. Professor P. I. Rekwot has been a great mentor with seasoned track record in academic leadership. His excellent virtues have afforded me with invaluable skills and guarded confidence to triumph in practical animal reproduction and doggedness in animal reproduction research endeavour. I am grateful for your leadership role as a mentor. I am also indebted to you for your guidance and criticism to make this work a masterpiece.

I am deeply indebted to Professor J.O. Ayo, who I often find great difficulty in ascribing any definitive characteristic stature because of his uncommon passionate personality. Your initiative in birthing the core theme of this study, your aptitudinal skills and unequalled guidance in anchoring and thrusting the dimension of this research to a level of envy, made indelible landmark that will remain memorable in the sands of my professional career. I express my sincere gratitude to you for finding me worthy, inspite of my shortcoming, to make a part-possessor of your vast dominion in Veterinary Physiology. To say the least, you are rated as a jewel of inestimable value.

I make shouts of gratitude to Professor B. I. Onyeanusi for his supportive arm of maturity during the period of this study. I am indebted to you for as long as this study is read and referred. Thank you for all that you have done for me.

I am grateful to Professor S. B. Oladele for being an oracle in guidance, in counseling and in moral and financial support throughout the period of my study.Thank you for volunteering to be my destiny helper at critical times in the process of my travail.

My gratitude to Professor Lawal Sa'idu, former Director, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. I recall always all that I learned from you in practical poultry production, especially the lessons I received repeatedly on prevention and control of avian diseases. You have built my confidence to take responsibility in "avian culture". Above all, I recall perpetually that you facilitated my interest and initiative in Postgraduate studies in Veterinary Physiology. I remain indebted to you for all that you have done for me, especially the most recent comfort that you have availed me.

My acknowledgement will remain incomplete without reference to the role of late Pastor A. D. Ekah in the successful completion of my degree. It is impossible for me to enumerate his valuable and invaluable contributions to my well-being in life.They are too numerous to remember and such a documentation may never be possible for me to undertake. Your sudden departure to eternity creates a vacuum for all that you loved and raised. I pray that your filial generations be comforted in all that they lay their hands upon to do; never to lack divine messengers in their course in life. Have your rest eternally in the bossom of our Lord Jesus Christ.

Lastly, I thank my wife and children for their love, patience, support and invaluable understanding of my need to devote a great deal of personal time and relatively limited family resources to the success of this study. I am exceedingly grateful.

ABSTRACT

Environmental stress is progressively becoming a globally significant factor of concern to animal agriculture due to climate change.Variations in thermal environmental conditions in the tropical Guinea Savannah, especially when such changes are acute, frequent and abrupt constitute oxidative stress to animal well-being and productivity. The aim of the present study was to determine the ameliorative activity of supplemented lycopene (L) and vitamin E (VE) on thermal stress-induced changes in physiological and performance indices of laying hens during the harmattan and hot-dry seasons. Four hundred, apparently healthy laying hens, aged 41 weeks were divided into four groups each (n = 100): group 1 = L+VE, were coadministered with lycopene (30 mg⁄kg body weight) and vitamin E (250 mg ⁄ kg body weight); group 2 = L, were supplemented with lycopene only ((30 mg ⁄ kg body weight)); group 3 = VE were administered with vitamin E only (250 mg ⁄ kg body weight) and group 4 = CONTROL were not supplemented with any antioxidant. The antioxidants were administered for 5 weeks each, during the harmattan and hot-dry seasons. During the study period physiological and performace parameters were recorded daily and weekly. The results showed that thermal environmental conditions during the harmattan and hot-dry seasons were stressfull to the laying hens, but the hot-dry condition was more stressful to the birds. During the harmattan (cold-dry) season, laying birds were exposed to environmental stress ranging from cold to heat stressor. During the hot-dry season, laying birds were susceptible to severe heat stress almost all the day. The stressful conditions resulted in significant impairment in performance parameters of laying hens. A significant increase in percentage hen-day egg production was recorded in laying hens that were administered antioxidants, lycopene and vitamin E during the two seasons; however, it was evident that coadministration of L+VE to hens resulted in the most significant ($P < 0.05$)

performance parameters. This was followed by laying birds that were administered with lycopene only and vitamin E only, respectively. Hen-day egg production parameter was lowest in control laying hens during the two seasons. Oestrogen and progesterone concentrations were significantly ($P < 0.05$) higher in antioxidant-administered groups of laying hens during the two seasons of the study. Results on thyroid function and thyroid hormone concentrations demonstrated that co-adminstration of lycopene and vitamin E was most significant on thyroid function during the two seasons, indicating that antioxidants surported thyroid function which is known to be dysfunctional during thermally-induced stress condition. Modulatory effect of thyroid hormones activity was more profound during the harmattan season, increasing cloacal temperatures(CTs) during the early hours of the day and decreasing CTs during periods commencing from 12:00 h. Modulatotory roles of antioxidants in the laying hens were in the order: L+VE > L > VE > CONTROL. Significant ($P < 0.05$) increase in CTs was recorded in L+ VE and L groups of laying hens during the study period. Results showed that the effect of VE on CTs was lower than in the former two groups. There was no significant ($P > 0.05$) difference in egg weight in experimental and control groups of laying hens during the seasons. However, internal and external egg parameters were significantly ($P < 0.05$) influenced by the supplementation of antioxidants where L+VE groups of laying hens recorded higher values in eggshell diameter. The group also showed a significant ($P < 0.05$) increase in egg albumen and yolk parameters. During the hot-dry season, ovarian function showed a more resilient aptitude in egg production. Countable white follicles and growing preovulatory yellow ovarian follicles were greater ($P < 0.05$) in number in antioxidant-administered laying hens. There was no transitional shelled egg in-uterus in any of the laying hens supplemented with antioxidants; only in control groups were shelled eggs-in-uteri prominent. It is concluded

that the harmattan and hot-dry seasons were thermally stressful to laying hens. The administration of antioxidants lycopene and/or vitamin E ameliorated the effects of the stress and improved the welfare and productivity of the laying hens.

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LIST OF ABBREVIATIONS

Kg	--	Kilogram
CT	--	Cloacal temperature
ROS	--	Reactive oxygen species
HS	---	Heat stress
DBT	---	Dry-bulb temperature
TNZ	---	Thermoneutral zone
L	---	Lycopene
L+VE	---	Lycopene + vitamin E
VE	---	Vitamin E
CONT	---	Control
T_3	---	Triiodothyronine
T_4	---	Thyroxine
/	---	Per
L	---	Litre

1.0 CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Domestic birds are consistently subjected to diverse and alternating environmental influences as a result of seasonal changes in climatic conditions, which may be favourable to their well-being or inimical to their ultimate productivity. The birds' predisposition to these environmental conditions necessitates their aptitude to undergo profound changes in physiology, morphology and behavioral characteristics across their lifecycles to resist and adapt to environmental diversity to guaranty their survival.For the ultimate capacity to resist extreme changes in environmental conditions which may constitute stress, the neuroendocrinesystem of domestic birds plays a fundamental role in integrating externaland internal signals and orchestrating adequate responsesaimed at maximizing individual fitness (McEwen and Wingfield, 2010; Li *et al*., 2020). Some of thesechanges occur in response to predictable fluctuationsinthe environment like night–day and seasons, which allowyear-round anticipatory organization of major life historystages in cyclic patterns. Super-imposed on predictable components,unpredictable perturbationslike severe weather,loss of social rank, habitat destruction, or human disturbancerequire emergency adjustments in physiology andbehaviour. The adrenocortical response to "stress", whichresults in a rapid elevation of circulating glucocorticoidlevels, provides a major physiologicalmechanism allowing birds to cope with environmentalperturbations. Glucocorticoids, the stress hormones,allow mobilisation of body energy, increase cardiovasculartone, regulate the immune system, and inhibit a varietyof costly anabolic processes including digestion, energystorage, growth, and reproduction in response to "stress"(Sapolsky *et al*., 2000; Sapolsky, 2002). But these hormonesare also fundamental for normal body functions and

normalregulation of energy balance, physiology, morphology,and behaviour under nonstress conditions (Landys *et al*.,2006; Zaglool *et al*., 2019).

Heat stress (HS) is a physiological condition that occurs when animals can no longer regulate their internal euthermic temperature. When livestock are subjected to this inclement thermal environmental stress, it may be detrimental to health, well-being and performance, and in severe extremes, death results (Ayo *et al*., 2011; Mack *et al*., 2013; Ayo *et al*., 2014; Sahin *et al*., 2016). Poultry are particularly susceptible to HS, resulting in high cost of poultry meat and egg production; this ultimately depreciates the quality of poultry products and costing the industry significant monetary losses annually in lost production, losses in animal product quality and losses resulting from death of the animal. One of the most susceptible organs to HS is the reproductive system (Dobson *et al*., 2012; Barrett *et al*., 2019). Heat stress has been shown to depress all levels of the hypothalamic-pituitary-gonadal axis thus, reducing the efficiency of gonadal reproductive function and integrity due to acute or protracted metabolic dysfunction. Together, these deterrents antagonise expected domestic poultry performance and health (Dobson *et al*., 2003; Elnagar *et al*., 2010; Dobson *etal*., 2012; Barrett *et al*., 2019).

The influence of the environment and its interaction with genetic factors is increasingly becoming a focal interest in animal production. It is well appreciated now, that the environment in which farm animals are reared determines to a large extent, their entire lives' responses and their ultimate output. Depending on the range and extent of interaction, meteorological impact on the animal can either be supportive to their well-being or inimical to their targeted productive expectation. Both of these ultimate results are effective through

vital organ-systems of the animal's physiology. During heat and cold stress, the animal reaches a physiological condition where its ability to exert its metabolic machinery to regulate its internal euthermic temperature becomes compromised (Dobson *et al*., 2003; Bouchama *et al*., 2007). Such physiological conditions in livestock are well-known to be detrimental to animal health, well-being and performance. If this condition is severe enough, death becomes imminent (Ayo *et al*., 2011; Dobson *et al*., 2012; Ayo *et al*., 2014).

Domestic chickens are particularly known to be susceptible to HS. They are also known to be susceptible to cold stress (CS). Lien *et al*. (1999) demonstrated that thermal stress conditions are known to result in quantitative and qualitative losses, ranging from deterrent associated with egg and carcass quantity and quality; the animals well-being is also remarkably depreciated in the poultry industry. The health and homeostasis of the animal is affected by multiple disease conditions, environmental and genetic factors. These factors predispose animals to physiological, metabolic or immunological stresses. Collectively, these stresses can lead to significant decrease in animal well-being, work efficiency and physical performance, growth, reproduction and lactation, chronic illnesses, or ultimately death if such deleterious conditions are severe and prolonged. Certain types of stressors such as hypoxia, inflammation and oxidative stress are particularly elicited by environmental conditions, including hyper-and hypothermia. Such environmentally-induced HS has been reported in humans (Basu, 2009; Leon and Helwig, 2010) and domestic animals (Bouchama *et al*., 2007; Bernabucci *et al*., 2010; Pearce *et al*., 2013a; Chauhan *et al*., 2014).

HS has been reported to inflict economic losses to animal agriculture. For instance, it has been estimated that HS in some parts of the United States resulted in losses in the swine

industryamounting to over 300 million dollars per annum (St-Pierre *et al*., 2003). Heat stress may also negatively affect human productivity, especially in outdoor workers in agriculture or industry (Lundgren *et al*., 2013).

Physiologically, HS affects most vital organ-systems of the body, including the nervous, gastrointestinal tract and the reproductive systems. It has been reported that heat-stressed animals adjust their circulatory system to redistribute blood to peripheral circulation in an attempt to maximize radiant heat dissipation, while vasoconstriction occurs in internal organ-systems, including the gastrointestinal tract and the reproductive system; this is to aid in reprioritization of blood flow (Lambert, 2008; Pearce *et al*., 2013a). The resultant effect of this circulatory redistribution compromises the integrity of internal organs, including the integrity of the gastrointestinal tract and gonads (Yan *et al*., 2006).

Vitamin E is the term used for eight naturally-occurring, essential, fat-soluble nutrients, often called tocopherol. Alpha-tocopherol is essentially the most predominant, with the highest biological activity in many species. It has been suggested that the side chain in the 2-position facilitates the incorporation and retention of vitamin E in biological membranes, so that the 6-position is optimal for scavenging reactive oxygen species and terminating lipid peroxidation (Niki *et al*., 1985). Reports have shown the role of vitamin E as a potent antioxidant, the activity of which has been demonstrated to be beneficial in the prevention and management of infertility, and other reproductive anomalies (Masso-Gonzalez and Antonio-Garcıa, 2009; Helmy and Senbel, 2012).

Lycopene is the predominant carotenoid in tomatoes and has been identified as one of the major carotenoids in human serum and tissues (Boileau *et al*., 2002; Palozza *et al*., 2012) and in avian tissues, especially in egg yolk (Olson *et al*., 2008). Although about 90% of the

lycopene in dietary sources is found in the linear, *all-trans* conformation, human tissues contain mainly *cis*-isomers. Several reports have suggested that *cis*-isomers of lycopene are better absorbed than the *all-trans* form because of the shorter length and greater solubility of *cis*-isomers in mixed micelles, and/or as a result of the lower tendency of *cis*-isomers to aggregate (Wu *et al*., 2003). The nutritional significance of lycopene has also been demonstrated in an *in vivo* study of colon cancer, involving rodents (Narisawa *et al*., 1996). Levy *et al.* (1995) have shown that lycopene is more potent than α- and β-carotene in inhibiting the cell growth of various human cancer cell lines. A major hypothesis for the biological activity of carotenoids, including lycopene in disease prevention is based on the antioxidant ability of these compounds. Lycopene can quench singlet oxygen and other ROS, and protect the cells from oxidative damage.

The fact that these carotenoids are also abundant in other fruits and vegetables have been associated with a reduced risk of cancers in epidemiological studies and metabolic pathways for the formation of their oxidation products. It has been concluded that lycopene and other carotenoids can serve as potentially useful chemopreventive agents through their synergistic biological activities. Although lycopene has no vitamin A activity, it has been reported to possess strong antioxidant activity *in vitro* (Boileau *et al*., 2002, 2003).

There is a dearth of information on the ameliorative effects of lycopene and vitamin E on thermally-induced stress in laying hens. The present study was undertaken to assess physiological and reproduction performance changes in laying hens during the harmattan and hot-dry seasons in the Guinea Savannah zone of Nigeria, and the antioxidant roles of

lycopene and vitamin E on the changes associated with thermal stress associated with the seasons.

1.2 Statement of Research Problem

The deleterious effects of thermally-induced stress on the well-being and productivity in domestic animals have been established (Obidi *et al*., 2008a; Ayo *et al*., 2011; Surai *et al*., 2015 Sahin *et al*., 2016). In the Northern Guinea Savannah zone of Nigeria, thermally-induced environmental stress has been associated with the seasonal environmental conditions associated with the zone, especially during the harmattan and hot-dry seasons. The adverse environmental conditions during the seasons have been reported to impair the entire physiological system of animals reared in the zone, including their reproductive efficiency (Ayo *et al*., 2011; Dzenda *et al*., 2013; 2015). The inhibitory effect of environmental stress on animal productive efficiency is further aggravated by the relatively unknown and non-adoption of environmentally controlled housing system for commercial animals reared in the zone, due to the high cost of acquiring and maintaining such advanced livestock housing operating systems. This implies that most of the livestock populations reared in the zone are often exposed to inclement environmental conditions for which they are required to acclimate to survive the harsh thermal environment or death of the animal results from failure of the adaptive process.In both cases, losses on animal agriculture are significantly imminent.

1.3Justification of the Study

Reports have shown some biological significance of nutraceuticals in animal nutrition (Ayo *et al.*, 2014; Surai *et al*., 2015). Several nutraceuticals are now known to possess antioxidant and antistress capacity, through their modulatory roles on animal physiology; and the biological effects are known to mitigate ROS devastating lipoperoxidative activity on biological membranes (Surai *et al*., 2015). Nutritional supplementations of antioxidants such as vitamins, minerals, pre- and probiotics may support animal physiology, including their reproductive performances through their antistress and anti-inflammatory activities. No study on the administration of neutraceuticals for the sustainance, well-being and productivity of chickens, especially during stressful environmental conditions in the harmattan and hot-dry seasons in the Guinea Savannah zone has been conducted.

Carotenoids are the most important pigments for providing attractive colours in fruit and vegetables and may be found in all parts of the plant. They are excellent antioxidants because they quench singlet oxygen and trap peroxyl radicals (Osterlie and Lerfall, 2005). Recent studies have shown that detrimental effects of environmental stress could be partly due to the induction of oxidative stress,diets enriched with antioxidant substances may attenuate the negative effects of the stress (Sahin *et al*., 2016). Tomato and tomato-based products contain some phytochemicals that may have health benefits and are important sources of many established nutrients. Lycopene and vitamin E are potential bioactive compounds found in tomatoes (Agarwal and Rao, 2000). They may alleviate the deleterious effects of thermal stress on the laying hens during the harmattan and hot-dry seasons in the zone.

Lycopene is a major carotenoid present in tomatoes and a highly potent antioxidant that provides protection against cellular damage caused by reactive oxygen species (ROS) (Rao and Agarwal, 1999). Lycopene has also been demonstrated to be a potential cancer chemopreventive agent. Guo *et al*. (2001) reported that there is an inverse relationship between TBA reactive substances value in the thigh meat and egg and dietary antioxidants. The preventive effects of lycopene on oxidative stress have been reported (Sahin *et al*., 2006; 2016).

There has been increasing interest in the investigation of the antioxidant activities of lycopene and vitamin E in poultry production; however, there is a dearth of information in literature on the ameliorative and modulatory effects of lycopene and vitamin E on physiological and reproductive indices of the laying hens in environmental conditions subjecting the birds to thermally-induced stress.in the zone.

1.4 General Aim of the Study

To evaluate the ameliorative effects of lycopene and vitamin E on physiological and performance indices of laying hens during the harmattan and hot-dry seasons.

1.5Specific Objectives of the Study

The study was designed to determine in ISA Brown laying birds during the harmattan and hot-dry seasons the:

i. modulatory and/or ameliorative roles of lycopene and/or vitamin E on cloacal temperature.

ii. effect of lycopene and/or vitamin E supplementation on hen-day egg production and persistence.

iii. effects of lycopene and/or vitamin E supplementation on egg qualitative indices.

iv. effects of lycopene and/or vitamin E on estrogen, progesterone and thyroid hormones of the laying hen.

v. effects of lycopene and/orvitamin E on follicular survival and recruitments in the laying hens.

1.6 Hypotheses

i. Lycopene and vitamin E have no ameliorative effects on physiological and performance indices in ISA Brown laying hens exposed to thermal stress associated with the harmattan and hot-dry seasons.

ii. Harmattan and hot-dry seasons do not induce thermal stress on ISA Brown laying hens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Climatic Classification of the Northern Guinea Savannah Zoneof Nigeria

The Northern Guinea Savannah zone of Nigeria is located between latitude 11˚N, 12′N and longitude 7˚E, 8′E, at an elevation of 650m above the sea level. The zone has average annual maximum and minimum temperatures of 31.8 ± 3.2˚C and 18.0 ± 3.7˚C, respectively. The monthly average rainfall during the rainy season (May–October) is 148 ± 68.4mm (69.2–231.9 mm), while the monthly relative humidity is 71.1 ± 9.7%. The zone is characterized bythree seasons: Harmattan (November–February), hot-dry (March–May), and rainy (June–October) seasons (Ayo *et al*., 2014; Dzenda *et al*., 2015). Each season in the zone has its positive or negative effects on livestock production. For example, the Harmattan season has been described as thermally stressful to goats (Minka and Ayo, 2014), pigs (Adenkola *et al*., 2009), and poultry (Obidi *et al*., 2008a), while the negative impact of environmental stress on poultry is minimal during the rainy season (Obidi *et al*., 2008a, b).

2.1.1 Effects of meteorological elements on animal well-being and production

The meteorological elements constitute a complex and interactive system which acts upon the body of animals, resulting in varied animal responses, depending on the animal species and the intensity of such meteorological condition (Daghir, 2008; Bernabucci *et al*., 2010). Meteorological elements may be jointly expressed as climate, which implies a long term average conditions, or as weather, which makes up the short term fluctuations. Under intensive system of animal production where there is a limited or complete housing-

environment control, meteorological components may still have profound effects on animals in their pens. These effects become more significant on animals where they are produced under extensive system; the impact of meteorological factors on the animal may be either beneficial or detrimental, depending on the magnitude and duration of the variation (Dzenda *et al*., 2011; 2013). It is beneficial to the animal when it is within the range of thermal neutrality, that is, when there is very minimal or even negligible metabolic stress or any physiological burden to the animal's well-being; this implies that within this thermoneutral range, there is no adversity to the animal's productivity (El Halawani, 2010; Liang *etal*., 2016).

On the other hand, meteorological elements impacts could constitute significant detriment where they range beyond the convenience or tolerance of the animal; under this condition the animal's well-being and physiological potential become compromised due to meteorological stress, and most of its productive parameters have been reported to be significantly deterred (Obidi *et al*., 2008a, b; Mack *et al*., 2013, 2015). At this instance, physiological mechanisms of action of meteorological elements on the animal are diverse but are highly interrelated. These mechanisms have been shown to affect the nervous, endocrine, and cardiovascular systems (El Halawani, 2010; Elnagar *et al*., 2010). The impacts of meteorological factors on other systemic function are also evident on the digestive, respiratory and reproductive systems.

2.1.2 Deleterious effects of heat stress on human and livestock

2.1.2.1 Effects on physiology

The deleterious effects of heat stress in human population have been underestimated. It may also appear that mortalities induced by heat stroke and morbidities induced by heat stress in

human populations have been markedly ignored; therefore, any epidemiological report on the subject of environmental stress in humans must be interpreted with caution (EPA, 2014). However, it has been reported that certain proportion of human population are at significant risk of heat stress as a result of their occupation, working condition, age and other predisposing factors (Kovats and Ebi, 2006). The report showed that athletes, soldiers (especially those deployed to combat zones in hot weather conditions), the elderly, firefighters, agricultural workers and people suffering from chronic diseases as in diabetics and children are most susceptible to heat-induced illnesses. For instance, nearly 15,000 people were reported dead in a 2-week heat wave in France alone (Kovats and Ebi, 2006). Other reports showed that a total of 50,000 Europeans died during the heat wave (Kosatsky, 2005; Patz *et al*., 2005). Other reports showed a significant spike in heat-related mortalities in US in year 2006 (EPA, 2015). In more recent years, over 11,000 people were reported dead in Moscow, Russia in a space of 2 months (July and August) in year 2010; this mortality was reported to be higher for the whole country (Barriopedro *et al*., 2011). Although high environmental temperature can be detrimental, the effect of the deterrent can be significantly exacerbated (that is, increasing the thermal burden) by high relative humidity (Leinonen *et al*., 2014).

Heat stress has been markedly correlated to secondary health complications. Such health problems have been reported in cases involving cardiac failure and damages to the central nervous system (Caspani e*t al*., 2004). It must be reiterated that no easy or effective treatment has been discovered for health complications associated with heat-stressed patients, only methods of alleviating this condition are aimed at prevention. Since there are no definitive methods of diagnosing heat-stress-related ailments, heat- related mortalities

are difficult to define (Ostro *et al*., 2009). Therefore, it may not be necessary to suggest that most deaths occurring in domestic animals and even in humans as a result of heat stroke are statistically recorded. This is evident in the recent post-hoc report by EPA (2015) which suggests that reports on heat disaster were often downgraded. This means that in epidemiological studies, such numbers of heat-related casualties are not completely accounted for such that cases of heat stroke may have been misinterpreted for symptoms related to cardiovascular disease conditions and/or for cerebral damage as suggested by Basu (2009).

Generally, it is now well appreciated that our economic animals are markedly threatened by heat stress. Although there are significant advances in technology targeted at thermal comfort in livestock housing, shade and barn management and other heat abatement strategies, the severity of the stress has been reported especially in domestic animals reared under the tropical climatic conditions (Leinonen *et al*., 2014). Although it may not be possible to make accurate universal monetary estimate of losses due to harsh environmental conditions, reported estimates show that such losses amount to multiples of millions of dollars annually. For instance, in the United States alone, monetary losses due to summer-induced decrease in animal production in the swine industry surpass $300 million. A detailed analysis of this sum showed that $113 million was lost on sows and $202 million for growing-finishing swine (St-Pierre *et al*., 2003).

Apart from reported decrease in livestock potential as a result of hot environmental conditions, heat-induced mortalities in farm animals have been documented. For instance, in year 2011, the heat wave of North Carolina was shown to have killed over fifty thousand

broiler chickens in one farm, such casualty was also reported in another turkey farm in Kansas state which showed mortality of over 4,300 turkeys (Hegeman, 2011).

Leinonen *et al*. (2014) reported the aggravating effect of high relative humidity on thermal environmental condition that are already known to be thermally detrimental to animal well-being; the authors showed that complex interaction exists between environmental temperature and relative humidity, noting that high relative humidity with high thermal environment makes the impact of environmental temperature more severe physiological burden for the animal to bear. The combined effects of these environmental factors are now often expressed as temperature humidity index (THI), which is calculated according to animal species (Zulovich and DeShazer, 1990). Generally, the higher the value of this index, the more devastating the environmental condition to the animal. This must be the reason why regions of the world which experience warm and humid climatic conditions at certain periods of the year suffer significant losses on owned livestock enterprises.

2.1.2.2 Effects on animal products

Heat stress has been shown to have several undesirable effects on animal production. Several investigators have reported depreciated standard values for meat, egg and meat and egg products of various animal species, which were traceable to the effect of heat stress (Lu *et al*., 2007; Zhang *et al*., 2012). These changes were demonstrated to have led to the downgrading of these animal products; such changes include off colours in meat, lower carcass and organ weights, lipid peroxidation and changes in fat deposits (Toghyani *et al*., 2012; Barrett *et al*., 2019).

2.1.2.3 Effects on feed consumption and bodyweight gain

Heat stress has been shown to suppress feed consumption, leading to a depression in growth rates of livestock which results in significant losses. Mack *et al*. (2013) and Lara and Rostagno (2013) have reported that heat stress depresses gastrointestinal function in poultry, which leads to digestive abnormalities. It was further demonstrated that thermal stress depressed appetite in poultry, thus resulting in low voluntary feed intake. Losses in live weight, rate of weight gain and parameters associated with reproductive performance have been demonstrated to be depressed in broilers and laying hens during thermal environmental stress.

In growing pigs, it has been reported that heat stress resulted in a 50 % decrease in feed consumption when they were exposed to a 24-h heat stress and body weight in pig during the period was nearly 3 kg less (Pearce *et al*., 2013a). Further studies on pigs reared at 33^{o}C showed that they lost about 1 kg body weight over a 6-day period and feed consumption was reduced by 300 g (Collin *et al*., 2001). In a longer-term study, pigs subjected to heat stress at 32^{o}C for 21 days had a reduction in feed consumption amounting to 771 g which was equated to a decrease of 32% (Renaudeau *et al*., 2012). It has been demonstrated that in a diurnal pattern of heat stress (27-37^{o}C), finishing pigs had a reduction in average daily gain of 870g to 580g and 26% decrease in feed intake over a 28-day period (Song *et al*., 2011). Similar effects of heat stress have been reported in steers. Reports showed that growing steers subjected to a 9-d diurnal heat stress period (29.4-40^{o}C) resulted in a 0.4kg/day dry matter decrease, which was accompanied by a lower average daily gain (1.2kg/day vs. 0.1kg/day) as reported by O'brien *et al*. (2010). In lactating cows, diurnal

heat stress caused about 28% decrease in dry matter intake and a decrease in milk yield (Wheelock *et al*., 2010).

2.1.2.4 Effects on immunity

Public health and food safety concerns are becoming increasingly pronounced in human and animal lives. This concern has recently been associated to heat stress. For instance, in poultry, the food safety concern is based upon egg production (Ferens and Hovde, 2011). Poultry species have been known to be significantly susceptible to colonization in the GIT by *Salmonella* and *Campylobacter* bacteria strains. Such colonization may be increased during heat stress, with mechanisms including increased fecal shedding as well as increased horizontal transmission. Distribution of these pathogens, as well as E. coli is a major public health and economic concern for food safety and food animal production (Ferens and Hovde, 2011).

2.2 Environmental Stress in Domestic Birds

Birds undergo profound changes in physiology, morphology,and behavior across theirlifecycles and the endocrinesystem plays a fundamental role integrating externaland internal signals and orchestrating adequate responsesaimed at maximizing individual fitness (McEwen and Wingfield, 2003a). Some of thesechanges occur in response to predictable fluctuationsinthe environment like night–day and seasons, which allowyear-round anticipatory organization of major life historystages in cyclic patterns. Superimposed on predictable components,unpredictable perturbationslike severe weather,losses of social rank, habitat destruction, or human disturbance require emergency adjustments in physiology andbehavior (McEwen and Wingfield, 2003a; 2003b; 2015). The adrenocortical response to “stress”,whichresults in a rapid elevation of circulating glucocorticoidlevels,

provides a major physiologicalmechanism allowing birds to cope with environmentalperturbations. Glucocorticoids, the stress hormones,allow mobilization of body energy, increase cardiovasculartone, regulate the immune system, and inhibit a varietyof costly anabolic processes including digestion, energystorage, growth, and reproduction in response to “stress”(Sapolsky, 2002; McEwen and Wingfield, 2003b). But thesehormonesare also fundamental for normal body functions and normalregulation of energy balance, physiology, morphology,and behaviour under nonstress conditions (Landys *et al*.,2006;McEwen and Wingfield, 2010, 2015).

The animal’s environment is markedly diversified comprising of factors that are integrative and interactive. The relationship of these complexities in environmental factors may be inimical or benevolent to the animal’s expression of its full genetic characteristics. Environmental stressors can be defined as stressors that are found in the surrounding environment that are not related to genetics. These include drought, cold stress, heat stress, pollutants, noise, health challenges and natural disorders (Lara and Rostagno, 2013; Leinonen *et al*., 2014).The thermoneutral zone of an animal is essentially the range of its thermal tolerance where minimum or no net energy is expended for thermal homeostasis. Within this range of environmental temperature, the animal enjoys its physiological comfort and performs its physical and metabolic functions optimally.

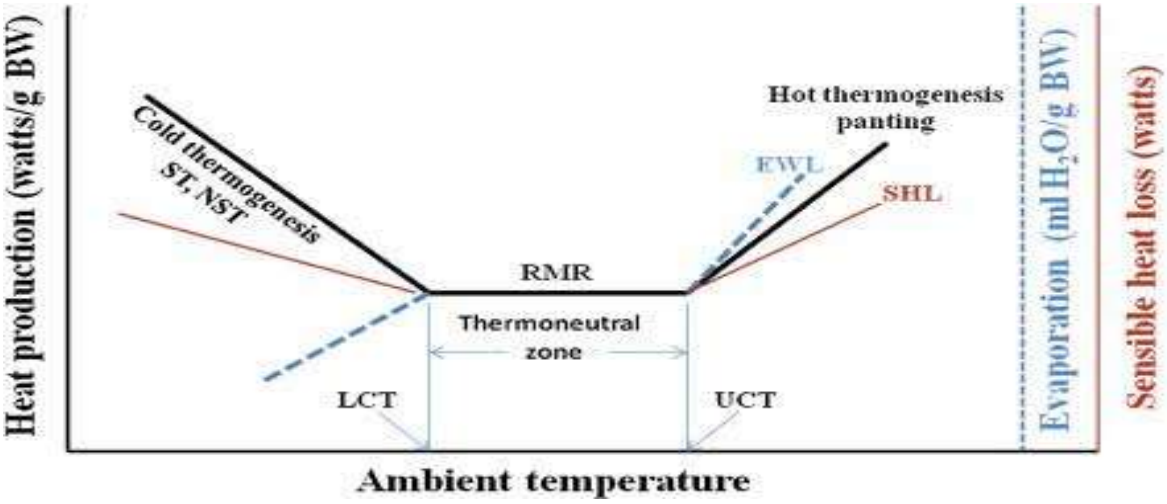

Figure1.1: A General Thermogenetic Curve of Endothermic Organisms.The thermoneutral zone ranged between the lower and upper criticaltemperatures (LCT and UCT, respectively); it represents the organism's resting metabolic rate (RMR). Below the LCT heat generation by shiveringthermogenesis (ST) and nonshivering thermogenesis (NST) increases in response to cold exposure. Above the UCT, thermogenesis increases to permitevaporative water loss (EWL), mainly via panting. Sensible heat loss (SHL) increases above the UCT as a result of increased ventilation, whereas belowthe LCT it increases as a result of the increased differences between the organism surface temperature and the ambient temperature.(Source: Yahav and Giloh, 2012)

Within this zone, basal heat production is at equilibrium with the rate of heat loss to the environment. Below this range, an animal beyond its lower critical temperature (LCT) occurs when ambient temperatures decrease. In most livestock species, this thermal condition is accompanied by increase in voluntary feed intake to avail its body with energy substrate for increased metabolic activity to boost its additional thermal requirement for warmth (Leinonen *et al*., 2014). This metabolic adjustment could be described as an adaptive response to cold stress (Figure 1.1). Failure of these mechanisms to increase an animal's core body temperature results in hypothermia. Conversely, if an animal goes beyond its upper critical temperature (UCT), the animal suffers from hyperthermia which results to heat stress (Daghir, 2008; Felver-Grant *et al*., 2012; Mack *et al*., 2013). If this condition is not adequately and timely ameliorated, the animal descends to heat stroke which results in death. This conudition results from high ambient temperatures or high thermal heat index mediated by increased heat gain and production when the rate of excess heat dissipation does not permit cooling (Basu, 2009; Collier and Collier, 2012; Felver-Grant *et al*., 2012). In view of this, an animal is said to be hyperthermic when it is subjected to, and suffers from an elevated body temperature as a result of its failed thermoregulatory mechanism. This condition is exemplified in the report of Felver-Grant *et al*. (2012), who demonstrated that during hyperthermia, core body temperature increases significantly due to an inability of the animal to dissipate heat. This debilitating hyperthermic condition can result from external factors such as burns and elevated ambient temperature or internal mechanism as in the case of fever or malignant hyperthermia.

During heat stress, there are various ranges of condition and severities of hyperthermia. In mammals, the severity of HS can be described by varying categories of heat-related

illnesses. The most severe form of heat-related illness is heat stroke which is often reported as a terminal heat-induced condition (Collier and Collier, 2012). In man, this situation results when core body temperature rises above 40^{o}C. Under this condition, chances of redemption or survival of the victim tends to be very slim as a result of multi-organ failure. A reported clinical case study of heat stroke by Varghese *et al*. (2005) showed that overall mortality due to heat stroke was 70 %. This figure was reported to be higher, reaching 85 % in cases of those whose conditions were aggravated by multi-organ dysfunction.

2.3 Thermal Tolerance and Heat Transfer

Thermal stress encompasses both cold (hypothermia) and heat (hyperthermia) stress. Under normal physiological conditions, metabolic heat production due to muscle contractions, biochemical reactions, ion pump activity and digestion is offset by the body's ability to dissipate heat by radiation, convection and conduction (Mount, 1978). Heat is transferred to the atmosphere when ambient temperatures are below normal body temperature, implying that there is a temperature gradient. However, when ambient temperatures exceed the body's thermoneutral zone, that is a reverse energy gradient, the only effective heat-loss mechanism is evaporative cooling via sweating or perspiration (Mount, 1978). Figure 1.1 highlights methods of heat gain and heat loss.

Heat can be absorbed from the environment (via conduction, convection and radiation) or generated metabolically due to increased growth, feeding, digestion, lactation and other parameters such as thermogenesis (shivering and energy uncoupling). On the other hand, heat can be removed from the body (via conduction, convection, radiation or evaporation) by means of urination, sweating, panting, defaecation or milk loss. These increments of heat gain/loss are balanced within an animal to maintain euthermia (optimal temperature

range between LCT and UCT) (Krammer and Cohen, 2004; Yahav, 2009). Hypothermia and hyperthermia occur when the body can no longer maintain thermoregulatory homeostasis and this may change with age, health condition, weight and other uncharacterized factors.

2.4 Species Differences in Heat Transfer

There are several species differences in heat transfer mechanisms, especially in terms of heat loss. In sheep, pigs, rodents and dogs, panting is the major mechanism by which heat is dissipated. Panting increases respiration rate and decreases tidal volume, which increases ventilation and evaporation in the oral cavity and esophagus (Yahav *et al*., 2009; Giloh *etal*., 2012). Other mammals such as horses and humans rely predominantly on evaporation via sweating for cooling the core temperature (Fuller *et al*., 2000). Sweating is more effective mechanism of heat dissipation compared to panting because panting requires muscle contraction, which in itself produces heat (Fuller, *et al*., 2000). This has implications for certain species and their susceptibility to heat stress. Specifically, poultry have little or no functional sweat glands, which show that they rely almost entirely on panting for heat dissipation (Zimerman *etal*., 2013; Liang *et al*., 2016; Lu *et al*., 2018). Additionally, poultry have an increased metabolic rate due to genetic selection for rapid lean growth and prolificacy in egg production and possess a substantial layer of subcutaneous adipose tissue which acts as insulator (Yahav and Giloh, 2012). Collectively, poultry have high heat production and greater capacity to store heat, even under thermoneutral conditions. These mechanisms may help partly to explain why poultry may be more susceptible to heat stress compared to other species (Patience *et al*., 2005; McKechnie and Mzihkazi, 2011).

2.5 Pathophysiology of Heat Stress and Stroke

Pathophysiology of heat stroke has been attributed to some important factors. First, heat is toxic to cells and can cause proteins to denature and undergo apoptosis or programmed cell death (Gao *et al*., 2013). Second, in most species, HS is associated with increase in circulating pro-inflammatory cytokines, leading to inflammation (Leon *et al*., 2006; Leon, 2007). Third, heat can injure vascular endothelium (Lugo-Amador *et al*., 2004). Symptoms of environmental hyperthermia include nausea, headaches, low blood pressure, fainting, dizziness, sweating, thirst, hyperventilation, thrombosis (blood clot formation) and endotoxemia (Leon, 2007; Aggarwal *et al*., 2008). These symptoms may range from occurring with a short term, to long-term changes extending to a year. Long-term symptoms of HS can be caused by systemic inflammatory response syndrome (SIRS), which is a whole-body inflammatory state. An increase in core body temperature can decrease splanchnic blood flow to facilitate dissipation of heat while leading to increased gut permeability and an inflammatory response. This, along with the inflammatory effects of the nervous system, can lead to multi-organ failure and death (Leon and Helwig, 2010; Li *et al*., 2020).

Mammals and avian species have a multitude of mechanisms that allow them to acclimate or adapt to conditions such as heat, hypoxia, oxidative stress and inflammation. Many of these mechanisms are common to other environmental stresses such as bacterial infection and metabolic stress. Two main mechanisms are generally thought to be activated to counteract the negative effects of heat exposure: the heat shock response and heat acclimation (Horowitz, 2002; Yahav, 2009). According to the International Commission for

Thermal Physiology, there are multiple terms to describe the various ways mammalian and avian species respond to heat stress as described in (Table 1.1).

Table 1.1: Descriptive Terms Associated With Animal Response to Thermal Stress

Term	Definition
Acclimation	Physiological or behavioural changes occurring within the lifetime of an organism, which reduce the strain or enhance strain endurance.
Acclimatization	Physiological or behavioural changes occurring within the lifetime of an organism in response to changes in its natural climate (e.g. seasonal or geographical)
Adaptation	Changes that reduce the physiological strain produced by stressful components of the total environment. This change may occur within an organism's lifetime (phenotypic) or the result of genetic selection in a specie or subspecies (genotypic)
	Adapted from Bernabucci *et al*. (2010)

Regardless of the environment, acclimation involves physiological changes in hormones, including epinephrine, leptin, prolactin, glucocorticoids, thyroid hormones and somatotropin hormones. These hormonal changes during heat stress are known to occur to maintain homeostasis (Bernabucci, *et al*., 2010). Timing of thermal acclimation can range from days to weeks, whereas homeothemic mechanisms generally take few minutes to hours. Acclimation on the long-term scale has recently been proposed as a homeorhetic mechanism (Collier *et al*., 2008; Yahav *etal*., 2009). Homeorhesis is the orchestrated or coordinated changes in metabolism of body tissues necessary to support a dominant physiological state (Andrewartha *etal*., 2011). It is thought that acclimation may be homeorhetic because it alters set points of certain homeostatic mechanisms. Although acclimation to HS may take as little as a few days to effect, the long-term effects may still negatively alter the animal's health status. These consequences may include nausea, mortality, changes in gut microbiota, reduced feed intake, increased susceptibility to secondary infections and diseases (Bernabucci *et al*., 2010).

2.6 Oxidative Stress and Thermal Stress

Reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are produced during normal cellular metabolism in quantities that promote cellular efficiency. However, it has been demonstrated that during times of excessive ROS production, they can modify lipids, protein and DNA (Figure 1.1). Excessive ROS production in cellular metabolic activities results in oxidative stress and this can contribute to many types of pathological conditions (Ray *et al*., 2012; Li *et al*., 2020). It has been demonstrated that most pathological conditions associated with reproductive failure in farm animals, including avian species occur via mechanisms with high correlation

to oxidative stress. This principle has been extended to substantial cases of reproductive misnomer associated with environmental stress (Mack *et al*., 2013;Sahin *et al*. 2015; 2016). Cell organelle mitochondria serve as an important source of ROS via the leaking or uncoupling of electrons from the electron transport chain.

These free electrons then bind to molecular oxygen (O_2) to form superoxide ($O_2^{\cdot-}$), which is produced by reduction. These ROS bind to lipid, proteins, and DNA and can damage the mitochondria themselves, or other organelles within the cell (Murphy, 2009). Under normal conditions, ROS are likely dismutated by mitochondria superoxide dismutase (Mn-SOD) to form H_2O_2. Mitochondrial electron leakage has been associated with the aging process, as well as several diseases, including neurodegenerative diseases (Liu *et al*., 2002) and conditions associated with reproductive failure in the laying hen (Rozenboim *et al*., 2007; Mack *et al*., 2013; 2015).

In livestock, ROS production from the mitochondria has been shown to increase skeletal muscle protein degradation and reduce feed efficiency (Bottje *et al*., 2009; Grubbs *et al*., 2013). Specifically, in broiler chickens heat-induced ROS has been reported to deter growth rate, muscle development and broiler meat quality (Sahin *et al*., 2016). Pearce *et al.* (2013a, 2013b) have shown that HS decreases gluthathione (GSH), increases 4-hydroxynonenol and 3-methyl histidine in pig intestine and muscle.

Antioxidants serve to combat the effects of ROS and maintain homeostatic redox balance within the body. Some major natural antioxidants produced by the body have been of interest. These include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione transferase (GST) and heme oxygenase-1 (HO-1) (Carocho and

Farrriera, 2013). Some external or non-enzymatic sources of antioxidants have been well reported. They include vitamin A, vitamin C, vitamin E, glutathione, β-carotene and lycopene.

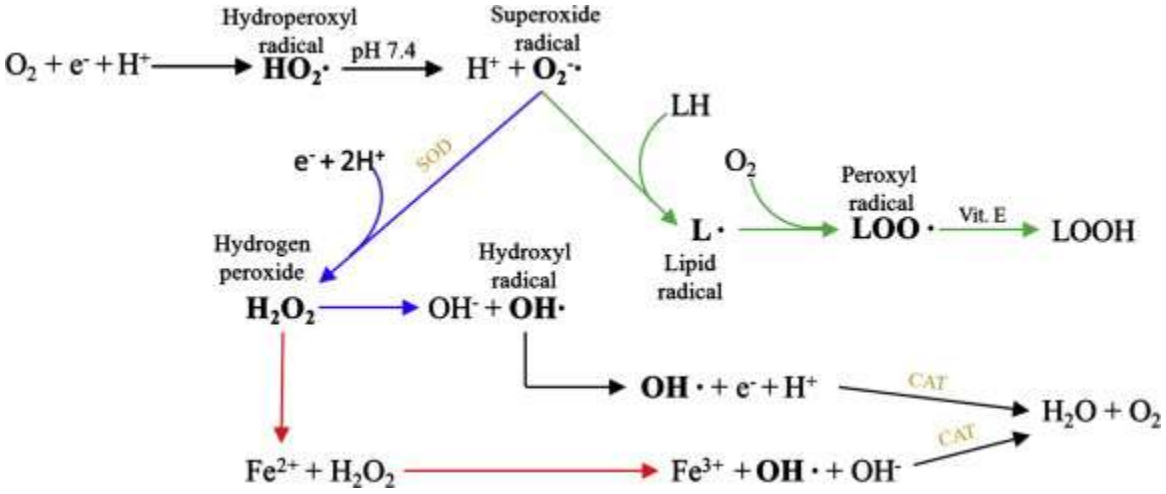

Figure 1.2:Overview of the Reactions Leading to the Formation of ROS (Carocho and Farriera, 2013).

2.6.1 Acute phase response, inflammation and thermal stress

Heat-stressed humans and livestock are prone to an increased acute phase and inflammatory responses (Table 1.2). The acute phase response (APR) is an important part of the innate immune response and serves to initiate the early stages of the acquired/adaptive immune response as well (Cray *et al*., 2009). Acute phase proteins (APP) are found in the blood and are produced by liver hepatocytes in response to the production of pro-inflammatory cytokines (e.g. 16 interleukin (IL) -1β, IL-8, IL-6, and tumor necrosis factor-α (TNF-α). Several cell types such as macrophages, B cells, T cells, mast cells can produce cytokines. There are at least 15 different known cytokines secreted by leukocytes and the three main groups of cytokines can be broken down into pro-inflammatory, anti-inflammatory, and positive/negative growth factors for cells. Adipose tissue has also been shown to be a central player in the inflammatory state as adipocytes secrete cytokines (Fain, 2006). These cytokines, along with nitric oxide (NO) and stress response glucocorticoids can activate the acute phase protein response (Gruys *et al*., 2005).Local inflammation is the body's reaction to tissue injury that is often caused by an infection, but inflammation may also be independent of infection. The innate and adaptive immune systems function to protect against disease by recognizing the presence of a pathogen (virus, bacteria, fungi), containing or eliminating the infection, immune self-regulation, and in the case of adaptive immunity, developing immunological memory to increase resistance during future infections (Dempsey *et al*., 2003).

Pathogens must first pass through physical and chemical barriers before activating the immune response. The innate immune system is activated first and is fast-acting (hours) while the adaptive response takes multiple days but is more effective at clearing

pathogens(Song *et al*., 2011; Pearce *et al*., 2013b). White blood cells, also known as leukocytes, originate in bone marrow and are primarily a part of the immune response. The two main categories of white blood cells are of lymphoid and myeloid lineages. Cells of the adaptive immune response such as B cells, T, cells and innate immune system natural killer (NK) cells arise from lymphoid cells while other cells of the innate response such as dendritic cells, neutrophils, eosinophils, basophils, mast cells, and monocytes/macrophages arise from myeloid lineage. Dendritic cells are responsible for antigen 17 uptake and antigen presentation while macrophages and neutrophils help with phagocytosis. Eosinophils are activated during parasitic infections and mast cells release histamine and active agents (Song *et al*., 2011; Pearce *et al*., 2013b).

Neutrophils are the most numerous and most important cells involved in innate immunity and are considered "first responders" of the immune response. The primary sites of innate immune function in the body are located in lymphoid tissues such as the thymus, lymph nodes, spleen, and mucosal lymphoid system of the gastrointestinal tract. The skin and mucosal lining of the intestine and respiratory systems are the first to encounter pathogens and maintain physical and chemical barriers against invaders. If pathogens overcome these initial obstacles, cells of the innate immune system respond. The APP, serum amyloid A, haptoglobin, α-1 acid glycoprotein are all considered positive acute phase reactants (Eckersall and Bell, 2010). C-reactive protein (CRP) is also a common marker of the positive acute phase reactants. C-reactive proteins act as opsonins by binding residues and polysaccharides on surfaces of bacteria, parasites and fungi to activate complement and phagocytosis. Negative reactants of the APR include transthyretin, retinol binding protein, albumin, transferrin, as well as iron (Fe), zinc (Zn), and calcium (Ca). These negative

APPare all decreased, indicating a temporary increased availability of hormones that are normally bound to these transport proteins (Gruys *et al*., 2005). Heat stress in pigs has been shown to increase haptoglobin concentrations in blood (Song *et al*., 2011; Pearce *et al*., 2013b).

In human heat stroke, concentrations of circulating cytokines are increased. Several pro-andanti-inflammatory cytokines are elevated in heat-stroke patients. The most common of these cytokines are IL-1B, IL-6, interferon-γ, and TNF-α. However, it is interesting to note that mostexamination of human patients occurs at end stages of the disorder or after cooling has occurred,so characterization of the early stages of the cytokine response are poorly understood in humans(Leon, 2007). Circulating lymphocytes are increased during heat stress as well asNK cells and T-cell numbers (Mitchell *et al*., 2002). In poultry, after one week of heat stress T-cell proliferation and B-cell proliferation were decreased in both cyclical and constant HS conditions. Over a four-week period, total white blood cell count (WBC) was increased during cyclical HS but decreased during constant heat stress. The heterophil/lymphocyte ratio wasincreased while antibody titers were decreased (Mashaly *et al*., 2004).

Table 1.2:Effects of Heat Stress on Immune Function in Varying Environmental Conditions

Species	Immunity index	Net effect	Reference
Humans	Plasma IL-6	Increase	Starkie *et al*. (2005) Bouchama *et al*. (1993)
Humans	Plasma TNF-α	Increase	Starkie *et al*. (2005)
Humans	II-1α, II-1β, IFN-γ	Increase	Bouchama*et al*.(1991,1993)
Dairy cows	Serum IL-2	Acute decrease	Liu *et al*. (2013)
Dairy cows	Serum IFN-α	Decrease	Liu *et al*. (2013)
Rats	Plasma IFN-γ, IL-2, IL-4, II-10	Increase	Liu *et al*. (2012)
Rats	SerumTNF-α	Increase	Kluger *et al*. (1997)
Pigs	TissueIL-8	Acute increase	Pearce *et al*. (2013b)
Pigs	Tissue Myeloperoxidase	Increase	Pearce *et al*. (2013b)
Broilers	Macrophage activity	No change	Quinteiro-Filho *et al*. (2012)
Mice	Hypothalamic II-β	Increase	Biedenkapp and Leon(2013)
Baboons	Circulating IL-6	Increase	Bouchama *et al.* (2007)
Baboons	Circulating C3, C4	Decrease	Bouchama *et al.* (2007)

2.6.2 Metabolism and stress

Metabolism is a series of coordinated enzymatic reactions that allow organisms to prioritize energy (ATP) production, grow and reproduce (Baumgard and Rhoads, 2011). Metabolism consists of both catabolism (break down) and anabolism (constructing new molecules). Under stress, basic strategies of catabolic metabolism are involved in the generation of ATP (energy), to generate reducing power, as well as generating building blocks for biosynthesis. The universal currency of energy is ATP, which has high phosphoryl-transfer potential and hydrolysis of ATP drives reactions forward. It is generated from the oxidation of various fuel molecules. In humans the most important metabolic pathways are: glycolysis, citric acid cycle, oxidative phosphorylation, pentose phosphate pathway, urea cycle, fatty acid β-oxidation, and gluconeogenesis. In the 1920's, Otto Warburg discovered that cancer cells metabolize glucose via glycolysis even under normoxic conditions (Upadhyay *et al*., 2013). Various cell types including cancer and dendritic cells have been shown to undergo the Warburg effect, whereby cells shift from oxidative metabolism to glycolytic metabolism. This occurs for a number of reasons; although glycolysis only produces 2 ATP per cycle it is much quicker and more efficient to produce ATP in this manner. The idea is to produce ATP quickly to meet the energy demands of the cell (O'Neill, 2011).

In tumor cells, this shift in metabolism is made possible by induction of HIF-1α, which asa transcription factor promotes transcription of many glycolytic genes. This same response maybe possible in response to lipopolysaccharide (LPS), as LPS has also been shown to stabilizeHIF-1α, and increase gene transcription. Toll like receptor agonists and LPSactivate a metabolic switch to glycolysis in dendritic cells via the PI3K/Akt pathway (Wen *et al*., 2012).Heat stress has been shown to alter metabolism in several different

species. Despite marked reductions in nutrient intake and BW loss, heat-stressed ruminants have increased basaland circulating insulin concentrations (O'Brien *et al*., 2010; Wheelock *et al*., 2010), while failingto mobilize adipose tissue triglycerides despite being in a hypercatabolic state (Bobek *et al*.,1997; O'Brien *et al*., 2010; Rhoads *et al*., 2009; Shwartz *et al*., 2009). This is intriguing because insulin is a potent anabolic signal that is typically down regulated under catabolic conditions.

Heat-stressed animals are thought to be in a catabolic state as epinephrine and cortisol are bothtypically increased during HS (Rhoads *et al.*, 2009; Wheelock *et al*., 2010). Changes in bloodglucose vary depending on species, as well as timing and severity of HS. However, it appears asthough that blood glucose is increased acutely, and then over time as feed intake decreases, as docirculating glucose concentrations. Circulating glucose concentrations have been shown todecrease in heat-stressed pigs (Becker *et al*., 1992) and cows (Shwartz *et al*., 2009). However,other studies in poultry (Bobek et al., 1997), rabbits and pigs (Prunier *etal*., 1997) have shown increases in circulating glucose levels during HS. Heat-stressed pigs also have increased circulating triglycerides and cholesterol (Attia *et al*., 2009; Pearce *et al*., 2013b).

2.7 Dietary Interventions

There is now no controversy whatsoever, about the harmful effects of heat stress on human and animal well-being and productivity, and the tendency for further deterrent must be actively registered in our memory, that is, if no decisive attempt is made to ameliorate or even to counteract heat-induced decimation on the full expression of our animal potential(McCauley *et al*., 1998). It is of interest however, that there are concerted efforts

by several research bodies and institutions to halt and/or recover heat-induced losses in animal production enterprises. One of the efforts of these institutions is targeted towards the application of dietary intervention in stress-mediated pathologies, including heat-induced deterrent to animal production (Kallweit *et al*., 2012; Rhoads *et al*., 2013;Wu *et al*., 2018). In this respect, substantive reports have demonstrated a high potential for nutriceuticals in the prevention and control of heat stress in animal production and animal products. Dietary supplementation is one example of an easily adjustable and affordable option to be utilized in animal agriculture and biomedical studies (Rhoads *et al*., 2013; Gong *et al*., 2020).

In ruminants, glutamine has been shown to enhance the immune response of cattle during heat stress. This is demonstrated by the marked increase in IgG levels and decrease in circulating concentrations of IL-6. It was further shown that cows fed glutamine had a higher cell-mediated immune response than controls (Caroprese *et al*., 2013). Other authors have shown that glutamine improved stress-mediated disruption of intestinal integrity as a result of ethanol and this condition was associated with increased expression of HSP 70 and HSF-1 as reported by Akagi e*t al*. (2013). Glutamine has also been shown to prevent apoptosis in intestinal epithelial cells (Kallweit *et al*., 2012), decrease gut permeability and improve survival following environmental hyperthermia (Singleton and Wischmeyer, 2006). Glutamine has been reported to be a primary biological source of fuel for enterocytes (McCauley *et al*., 1998).

Colostrum has also been demonstrated to be a stimulant for growth of new cells, and has been suggested as a potential protective agent against multiple types of stress (de Moura *et al*., 2013). Bovine colostrum has been shown to prevent intestinal injury (Playford *et al*.,

1999) and permeability in humans and a possible mechanism for this may include antimicrobial and antiviral compounds found in the colostrum (Playford *et al*., 2001). During HS specifically, priorsupplementation of either goat milk powder or bovine colostrum powder have been shown toreduce the impact of HS on intestinal permeability by maintaining transepithelial resistance(Prosser *et al*., 2004).

Zinc has been shown to be essential for normal intestinal barrier function and regeneration of intestinal epithelial cells (Pearse *et al*., 2013a, b). It has been shown to improve intestinal integrity during malnutrition, ethanol-induced intestinal injury (Lambert *et al.,* 2003), inflammatory bowel disease, as well as infectious diarrhoea (Alam *et al*., 1994). During HS in laying Japanese quail, supplementation of zinc sulfate improved feed intake, egg production, egg quality, feed efficiency and nutrient digestibility (Sahin and Kucuk, 2003). In broiler chickens, supplementing zinc sulfate improved weight gain, and feed efficiency while also reducing indices of oxidative stress in the birds (Kucuk *et al.,* 2003; Sahin *etal*., 2003; 2006). It also improves intestinal barrier function during HS in a swine model (Fernandez *et al*., 2013).

Chromium may also be an option during HS and this facilitates insulin action on metabolism and as many studies have demonstrated, insulin is higher in heat-stressed animals compared to pair-fed thermal neutral counterparts (Wheelock *et al*., 2010; Pearce *et al.,* 2013a), which may be part of a survival mechanism. In dairy cattle (Soltan, 2010) and poultry (Lien *et al.,* 1999), chromium supplementation improved growth performance and reduced lipolysis. With that, trivalent chromium increases immune system function by affecting T and B-lymphocytes, macrophages, and cytokines (Shrivastava *et al.,* 2005),

while maintaining integrity of the gastrointestinal tract (Shrivastava *et al.,* 2005). In addition, in quail, supplemental chromium histidinate alleviates oxidative stress through modulation of the hepatic nuclear factor kappa (NFK) β pathway (Orhan *et al.,* 2012).

Overall, high and low thermal environmental conditions may cause detrimental effects to animal production. The negative effects may be correlated significantly to absolute production losses, morbidity rate, as well as high mortality rate. Although HS has been demonstrated to negatively impact several organ systems, gonads and their tracts are highly susceptible to heat and hypoxic injury (Pearce *et al*., 2013a; 2013b). Heat stress, bacteria, and viruses increase intestinal permeability and cause barrier dysfunction, which can lead to endotoxemia. Many of the models utilized to examine the effects of HS have included rodents and cell culture models. However, there is little information examining how laying hens perceive and cope with HS in the Guinea Savannah zone. With that, the mechanisms that lead to this dysfunction have not been characterized in avian model. The present study was aimed at determining physiological responses of the laying hen to thermally-induced stress and the resultant effect on production parameters of the laying hen. The study also examined the ameliorative effects of lycopene and viamin E on physiological and production parameters.

2.8 Oxidative Stress

Reactive oxygen species (ROS) are a necessary evil of aerobic life, being generated continuously during the process of respiration but with the potential to cause oxidative deterioration of protein, lipid, and DNA. ROS generation is elevated by a range of different stress conditions (Pearce *et al*., 2013a; 2013b). The environments of most organisms are

rarely constant, so some resilience to environmental stress is essential in order for organisms to persist. Chemical stressors such as organic and inorganic pollutants can have different modes of action, but one effect common to many of these as well as certain natural stressors, like radiation, is an association with oxidative damage in cells (Avery,2001, 2004 Limon-Pacheco and Gonsebatt,2009). ROS damage is linked to serious degenerative conditions in humans, including amyotrophic lateral sclerosis, Alzheimer's disease, Friedreich's ataxia, and cancer (Roberts *et al*.,2009; Jomova *et al*.,2010). Typical responses by organisms to ROS involve the upregulation of antioxidant proteins, such as the ROS-scavenging peroxidases and superoxide dismutases, or enzymes that reverse oxidative damage, such as methionine sulfoxide reductases.

The oxidative stress responses of a diverse range of organisms are now well characterized (Imlay,2008). Despite this progress, one key unanswered question relates to the principal cellular target(s) of ROS that accounts for their toxicity. It is well known that ROS cause oxidative modification of each of the major cellular macromolecules and that damage to each can be detected during oxidative stress. What is less well known is which putative target first accumulates damage of a severity that precludes cell recovery, i.e., what target accounts for loss of cell viability? There can be more than one such target, the identity of which may depend on the nature of the oxidative stress (Thorpe *et al.*,2004; Imlay, 2008), the organism, its physiological status, and possibly the viability end point in question, e.g., loss of cell integrity or capacity to grow. Nevertheless, identification of such target(s) is now a priority for advancingunderstanding of the critical events during oxidative stress and so, potentially, for devising ways to combat these events in ROS-related disease.

Whereas ROS-mediated damage to cellular constituents is very widely described in the literature, oxidative damage to specific types of target has been linked causally to loss of cell integrity and/or viability. One emphasis is on protein targets, as this area has received the most attention recently. Because of the nature of the question, the evidence discussed is drawn mostly (but not exclusively) from laboratory studies with highly tractable organisms (Thorpe *et al.*, 2004; Imlay 2008). These models lend themselves to the types of manipulation necessary to establish causal associations and tend to be single-celled organisms such as bacteria or yeast. The nature of oxidative stress shares many common key features across prokaryotes and lower and higher eukaryotes (Cabiscol *et al.*, 2000; Imlay, 2008). Moreover, events at the single cell level ultimately may give rise to tissue and organ damage and clinical consequences of oxidative damage in higher organisms. Therefore, insights to ROS targets obtained at the cellular level feed our understanding of the nature of ROS impact on animal health (Genestra, 2007).

As part of normal cellular metabolic processes, aerobic organisms produce endogenousfree radicals, such as reactive oxygen species (ROS), through enzymatic or nonenzymatic chemical processes. Initially, ROS were thought always to be harmful, leading to mutagenesis, aging, and cancer (Thorpe *et al*. 2004;Imlay,2008). The study of enzyme antioxidants, such as superoxide dismutase and glutathione peroxidase, however, began to clarify the mechanisms of ROS metabolism and reveal positive biological effects (Thorpe *et al.*,2004; Imlay 2008). At moderate concentrations, therefore, ROS by-products help to maintain the balance between reduction and oxidation – the redox state – and act as signaling molecules for various physiological functions (Droge,2002; Genestra,2007).

Electron transport chain complexes all possess the potential to add electrons to oxygen, which results in superoxide formation (Imlay,2008). The superoxide anion ($O_2^{\bullet -}$), which is formed when oxygen accepts an electron, is used by the immune system in the defense against invading pathogens. Due to its toxicity, most organisms living in an oxygenated environment utilize an isoform of superoxide dismutase to reduce superoxide levels. Superoxide undergoes enzymatic conversion by superoxide dismutases to hydrogen peroxide, which is then subsequently detoxified by catalases and glutathione peroxidases present in the cytosol. However, while hydrogen peroxide itself is capable of damaging cellular components, it can be a major source of further endogenous radical species such as hydroxyl radicals, by interactions with reduced transitional metal ions such as ferrous or cuprous ions, which are capable of generating further peroxides by interactions with cellular lipids, for example (Droge, 2002).

Further information on how reactive oxygen species interact with cellular biology is well described in the review by Droge (2002). ROS may also be generated by ionizing or ultraviolet radiation (Cooke, 2003; Genestra, 2007). Irrespective of their origin, ROS may interact with and modify cellular biomolecules, such as DNA, which could have potentially serious consequences for the cell, that is, mutation. ROS act directly by damaging DNA, lipids, and proteins or indirectly through the recruitment of inflammatory mediators that trigger a secondary oxidative response. This inflammatory response in turn generates the release of further oxidative species and leads to an overall imbalance in the redox state of the tissue (Genestra, 2007). Extensive DNA damage can also lead to genomic instability. For example, endogenously formed $OH^{\bullet -}$ can lead to alterations in DNA bases (purines and pyrimidines) and cause DNA strand breaks, which in turn affect gene integrity (Droge,

2002; Genestra, 2007). Oxidative stress is, therefore, thought to play an important part in the initiation of various chronic diseases, such as cancer, cardiovascular disease, lung disorders (e.g., chronic obstructive pulmonary disease [COPD]), inflammatory conditions, and metabolic disorders. The mechanisms of oxidative stress are complex. Owing to the presence of many different oxidative species in the cellular environment, specific identification of which these species is directly responsible for a given biological effect can be difficult to determine (Genestra, 2007). Detection of measurable biomarkers associated with oxidative processes and pathways involved in disease development would, therefore, be useful. The complexity also makes it likely that testing multiple biomarkers concurrently will yield the most useful results.

2.9 Effects of Meteorological ElementsonAnimal Physiology

The meteorological elements can influence the animal's body singly, or in combinations.For instance, Collin *et al*. (2005, 2007) and Giloh *et al*. (2012) reported that low ambient temperature plus high air movement (cold) or high ambient temperature and high relative humidity plus solar radiation (heat) exert various effects on animal well-being, which are demonstrated in neuroendocrine, cardiorespiratory, and behavioural responses. Such changes affect the internal environment of birds, including the blood through the nervous and endocrine systems (Zimerman *et al*., 2013;Leinonen *et al*., 2014).

In recent times, the concern and emphasis on the meteorological elements have been orchestrated and has attracted the attention of numerousresearch scholars. This is because meteorological elements are not constant, but are continuously subjected to changes which are now collectively referred to as "climate change" (Leinonen *et al*.,2014). Such changes are known to affect the internal environment, including the blood of the animal.These

effects have been shown to be mediated by the nervous and endocrine systems (Rozenboim *et al*., 2007; Giloh *et al*., 2012; Zimerman *et al*., 2013).

2.10 The Bird's Body Temperature and Regulation

Balance or imbalance of the parameters associated with cumulative heat loss or heat gain in the domestic bird's metabolismwill cause the development of hyper- or hypothermyin birds. Body temperature is the main parameter that representsthe thermal status of an organism; therefore, endothermsaim to maintain a relatively constant temperaturethat will permit efficient functioning of the cells, tissues,and organs (Giloh *et al*., 2012). However, differences are maintained among thetemperatures of different organs as a result of their locations(i.e., visceral or peripheral), activities, and diverse biologicalfunctions (e.g., smooth or striated muscle). With noexception, endotherms will maintain a temperature gradientbetween the core (visceral) and the surface (peripheral) inorder to control heat exchange with the environment (Yahav and Giloh, 2012; Giloh *etal*., 2012; Liang *et al*., 2016).It has been demonstrated that endothermic birds and mammals werecharacterized as homeothermic. However, use of moderntechniques for measuring body temperature established endothermic organismsas heterothermic. Heterothermy involves changes in body temperaturein response to changes in theenvironment, such as alterationsin ambient temperature or in food and water availability.Changingbody temperature from normothermic values in response toenvironmental alterations saves energy and is a key componentof thermoregulation in birds of different sizes exposedto different environments: tropical, subtropical, arid, andmesic (McKechnie and Mzilikazi, 2011; He *et al*., 2018).

It is known, from studies that was conducted duringmany decades and were summarized by Prinzinger*et al*. (1991) and McKechnie and Mzilikazi(2011) that in bird'sbody temperature cycles diurnally and differsamong resting, active, and high-activity phases. In restingbirds, body temperaturemay range from 35.0 to 40.8 °C; in active ones,between 35.6 and 44.6 °C; and high activity increases therange to 40.4-47.7 °C. Despite the fact that endotherms areconsidered to behomeothermic, this is not the case, andtheir body temperature fluctuates diurnally by 2.48 - 2.84 °C,depending on the bird's prevailing metabolic phase (McCafferty *et al*., 2011). Suchextreme development of hyperthermia is in most cases irreversible.Body temperature varies among species also as a resultof body mass, ecological niche (e.g., aquatic), and variedavailability of diet during the year. In geneticallyselected poultry, body temperature in the resting and active phases issignificantly higher than those recorded for undomesticatedbirds; it is related to their higher metabolic rates; and it may be argued that variousenvironmental factors have considerable effects on domesticated birds' body temperature (McCafferty *et al*., 2011; McKechnie and Mzilikazi, 2011).

In general, both invasive and noninvasive techniques areused to measure body temperature in birds. The commonly used ways torecord body temperature in domesticated birds involve invasive techniques,which are time-consuming and may be traumatic as a resultof handling, and may affect body temperature within a few minutes by theinduction of fever that increases body temperature by up to 0.5 °C (Cabanacand Aizawa, 2000; Collin *et al*., 2005; 2007). In both wild and domesticated birds,a telemetry technique is applied, which involves implantationoftemperature-sensitivetransmitters, usually into the abdominal cavity. This enables long-term recording ofundisturbed birds (McCafferty *et al*., 2011).Whereas the telemetry technique involves handling andimplantation, the infra red

thermography technique is an entirelynoninvasive and contact-free method of measuring surfacetemperature, at either short or relatively long distances(McCafferty *et al*., 2011; Giloh *et al.*, 2012; He *et al*., 2018). Thermographicimagingfor body temperature determination can be relevant only if a satisfactorycorrelation exists between the body and surface temperatures;in the case of birds, the surface must be unfeathered.

The use of the temperature of an unfeathered surface is basedon its significant correlation with the vasomotor responsesof the bird; it is well documented that the vasomotor response-vasodilatation or vasoconstriction-is affectedby the bird's body temperature. Studies conducted on broiler chickensdemonstrated that such a correlation could indeed be found;they also demonstrated a high correlation between facialsurface temperature and body temperature. In laboratory studies,where the climatic conditions were relatively similar for allthe chickens, individual body temperatures correlated wellwith individual facial surface temperatures for chickens thatwere exposed to acute environmental heat conditions, bothwith and without ventilation, and to persistent heat withoutventilation (Yahav and Giloh, 2012).It can be concluded that, regarding body temperature, domestic poultry birds are mainlyendothermic, heterothermic organisms.

2.10.1 Responses to environmental temperature

It has been demonstrated that all animal species have a range within which changes in environmental temperature are associated with little or no change in heat production and which is known as the thermoneutral zone for such animal species; within this range, the body temperature is known to be regulated by variations in heat loss. For instance, it has been reported that breeder birds begin to show signs of distress when their environmental

temperature exceeds 26^{o}C, which is widely demonstrated as the upper critical temperature of the avian thermo-neutral zone (Yahav and Giloh, 2012; Zimerman *etal*., 2013).

Unlike mammals, the domestic chicken does not possess sweat glands to aid in heat loss in order to maintain a fairly constant body temperature (Collin *et al*., 2005; 2007). In view of this, domestic birds attempt to dissipate their excess body heat by conduction, convection and radiation. Through conduction, the bird's body heat can be directly transferred to cooler objects (litter materials and slats) with which the bird has a direct contact. In this method of heat loss-convection, the bird transfers heat to the surrounding air.

During a high environmental heat conditions, the bird also attempts to "throw" out its excess heat load by radiation from the skin surface through the air to another object which could be other birds around it. It has been demonstrated that at ambient temperatures between 28^{o}Cand 35^{o}C the methods of heat losses through conduction, convection and radiation are sufficient to maintain the bird's body temperature (McKechie, 2008; Yahav *etal*., 2009; He *et al*., 2018). In this context the bird dilates the blood vessels in its skin, wattles and comb to bring the internal body heat to the skin surface to facilitate heat loss through the aforementioned processes. Floor birds reared on deep litter system will search for cool places in the pen and dig into the litter, aiming to increase the conductive and convective heat losses(Mack *etal*., 2013; Yahav and Giloh. 2012). During high ATs birds droop their feathers; this behavior promotes heat convection through an increase in the surface area of the bird. This may be said to be an advantage over birds in the battery cage which are more susceptible to heat stress because they are unable to seek a cooler place and there is less conductive loss of heat in cages. As the environmental temperature approaches

the body temperature of the bird at about 41^0C, the efficiency of these heat loss mechanisms diminishes.

Andrewartha *etal*. (2011) and Zimerman *etal*.(2013) reported that when the environmental temperature rises above or falls below the thermoneutral zone the heat production increases. The environmental temperatures at these points are called the upper and lower critical temperatures, respectively (Figure 1.1). It is demonstrated that the range of environmental temperature above the upper critical temperature, to which birds can adapt is much narrower than the temperature ranges below the lower critical temperature which is compatible with survival (Giloh *etal*., 2012; Mohammed *et al.*, 2018). The thermoneutral range for any given bird may increase in amplitude with age, if the plane of nutrition is increased or if the bird becomes acclimatized to a lower environmental temperature.

The increase in heat production at the lower critical temperature is due mainly to shivering. The rate of increase in heat production at environmental temperatures below the lower critical temperature is known as the temperature coefficient (McCafferty *et al*., 2011; Yahav *et al*., 2012); this coefficient expresses in percentages the increase in the standard rate of heat production per degree Celsius of reduction in environmental temperature. To a large extent, the value of the temperature coefficient depends on the effectiveness of the bird's insulation; the better the insulation, the lower the temperature coefficient. Increase in heat production at the upper critical temperature is possibly associated with the increase in muscular activity associated with panting, and also to the direct accelerator action of the increase in body temperature on the heat-producing chemical reaction in the body; this phenomenon is called the vant Hoff-Arrhenius effect.

In a cold environment a chicken will reduce its surface area, and thereby its heat loss, by hunching up. It will also fluff out its feathers, thereby increasing its insulation (Yahav *et al*., 2009; Giloh *et al*., 2012). Tucking the head under the wing is an effective way of reducing heat loss, the reduction amounting to about 12 percent in the chicken (Yahav, 2000; Yahav *et al*., 2006). Huddling is another means of heat conservation in the cold. A common method of reducing heat loss from the unfeathered parts of the legs is to "sit" on them. It has been demonstrated that the heat loss in the chicken while standing is from 40 to 50 percent greater than while the bird is sitting (Yahav, 2000; Yahav *etal*., 2006). Increased activity in the cold will increase heat production; food intake is stimulated in a cold environment and depressed under hot conditions.

2.11 Regulatory Mechanism of Endothermy

2.11.1 Neuronal signals to and from thepreoptic anterior hypothalamus

The body temperature of endothermic birds is regulatedthrough a variety of thermoregulatory responses, such asshivering thermogenesis, nonshivering thermogenesis, panting, cutaneous vasomotion, and behavior (Giloh *et al*., 2012; Figure 2.3). All these responses are controlled and orchestrated by the brain to optimize the internal body temperature in orderto facilitate appropriate molecular activities and reactionsinvolving enzymes, ion channels, and several other metabolic factors that may not be fully delineated(Nakamura, 2011).Thethermoregulatory system that enables maintenance ofa relatively constant body temperature consists of a sensory afferent part(involving thermo-, osmo-, and baro-receptors), an integratingpart (the thermoregulatory center), and the commandefferent part (involving neurological and endocrine signals.The *sensory afferent part* comprises thermal informationtransferred by thermoreceptors from the bird's

surface(skin), all internal organs, the spinal cord, and thebrain itself (Patapoutain *et al.*, 2003; Wechselberger *et al*., 2006). Whereas the thermoreceptors in the internalorgans, spinal cord, and brain sense a relatively constantand protected internal environment, those of the skinsense the variable environment outside the body. Changesin the external environment encompass its temperature,relative humidity (RH), and ventilation, all of which affectthe animal's surface temperature, and form the data basetransferred by the peripheral thermoreceptors to the integratingcenter. Skin thermoreceptors are also continuouslyaffected by the body core temperature, as heat istransferredfrom body core to the periphery by blood flow (i.e.the vasomotor response; Nakamura, 2011).

One of the nonthermal information items transferred tothe integrating center refers to another homeostaticparameter, the osmolality, which includes afferentsignals coming from peripheral and central receptors thatare sensitive to osmotic pressure, volume, and sodium concentrationof the extracellular fluid and that can be closelyrepresented by the blood system (Keil *et al*., 1994). Thehomeostasis systems that control thermoregulation andosmolality, respectively, are linked via the evaporative heatlossmechanism: thermogenesis is balanced by energy loss,which depends mainly on evaporative water loss (EWL)which, in turn, depends on the body water and salt equilibria.It is well established that thermal homeostasis is modifiedunder conditions that shift the osmotic balance (Keil *et al*., 1994; Yahav *etal.*, 2012) and vice versa (Landgraf*et al*., 1994), and this interaction was demonstrated in manyendothermic animals. In general, at high ambient temperature, the frequencyof thermal polypnea decreased because of the reduction ofextracellular fluid volume that resulted from the high evaporationrate; this leads to dehydration, hypovolemia, andhyperosmolarity (Yahav *et al*., 2005). In such cases,hyperthermiacontinues to

develop.The integrating partis neuroanatomically located inthe preoptic area, which is located in the rostral pole of thehypothalamus (i.e., the preoptic anterior hypothalamus, PO/AH). The tightcorrelation between thermoregulation and osmoregulationfocuses the maintenance of body temperature and water/salt equilibriumin the PO/AH, so that lesions of this site elicit both severethermoregulatory disturbances and deficiencies in drinkingbehavior and osmoregulation (Kuznetsov and Kazakov, 2000).

This area plays a dual role: it monitors local temperaturechanges and integrates temperature information receivedfrom the periphery (Patapoutain*et al*., 2003; Wechselberger *et al*., 2006). In mammals andbirds, temperature is monitored by temperature-sensitiveneurons, which change their firing rate in accordance withthe hypothalamic temperature (Griffin *et al*., 2001) and canbe described as the hypothalamic setting point (Boulant,2006). These temperature-sensitive neurons form 40% ofthe PO/AH neurons; the rest are temperature-insensitiveneurons. About 75% of the temperature-sensitive neuronsare warmth sensitive (their firing rate increases stronglywhen the hypothalamic temperature increases), whereasabout 25% of them are cold sensitive with a firing rate thatchanges in the opposite sense to the hypothalamus temperature(Tzschentke *et al*., 2004; Boulant, 2006). However, in endothermic mammals the evidence confirms thatthe PO/AH comprises 70% temperature-insensitive neuronsand 20% temperature-sensitive ones (Tzschentke *et al*., 2004).

In various models, thePO/AH serves to define a temperature set point inorder to promote heat production and/or heat loss. When thePO/AH site is cooled below itsthermal set point, efferent neuronal signalswill promote heat production by means that include shivering thermogenesis and nonshivering thermogenesis, peripheral vasoconstriction (in the skin),

and behaviouralresponses that include huddling and seeking a warmenvironment(Boulant, 2006). When PO/AH is warmed above thermal set point, heat lossmechanisms will promote panting, skin vasodilatation, andbehavior that facilitates cooling, such as seeking shade and/or a ventilated environment or exposing nonfeathered skinareas. Each of these responses has its own thermal set point, and the magnitudeof the response depends on the hypothalamic temperature set limit.

However, additional neurons are encompassed bythe concept of hypothalamic set point based on temperature-sensitiveneurons. These are the temperature-guardian neurons(Giloh *et al*., 2012), which were detected in the PO/AH of Muscovy ducks. These neurons reacted exclusivelyto critical brain temperature signals and possibly stimulateda second type of thermoregulatory effector activity. Thistype of neuron falls within Boulant (2006) and Wechselberger *et al*. (2006)two-tier conceptwhich, in this case, operates when body temperature shifts outside the normothermicrange, in cases of hyperthermy orhypothermy.
The efferent neuronscan be characterized according tothemodel, as updated by Boulant (2006).Basically, the warmth- and cold-sensitive and temperature-insensitiveneurons, together with the efferent neurons, controlthe thermoregulatory responses. The efferent neuronsare located within the PO/AH or in sites such as the posteriorhypothalamus and brain stem; they are either spontaneouslyfiring neurons or silent ones that are synapticallyexcited by warmth- and cold-sensitive neurons, or are inhibitedby temperature-insensitive neurons.The endocrine efferent response is mediated mainlyby the hypothalamus–pituitary–thyroid axis. In birds, themetabolic rate is associated with secretion of the hormonethyroxine (T_4), which is secreted by the thyroid gland and isdeiodinated to triiodothyronine (T_3) in all peripheral tissues.

T_3 is the main metabolism-stimulating hormone that was found tobe associated with body temperature regulation (Yahav, 2000; Yahav *etal*., 2009; Yahav *etal*., 2012; Lin*et al.*, 2017).In summary, the mechanisms of both heat productionand heat loss arepermanently activated and are regulated byboth neuronal and hormonal signals.

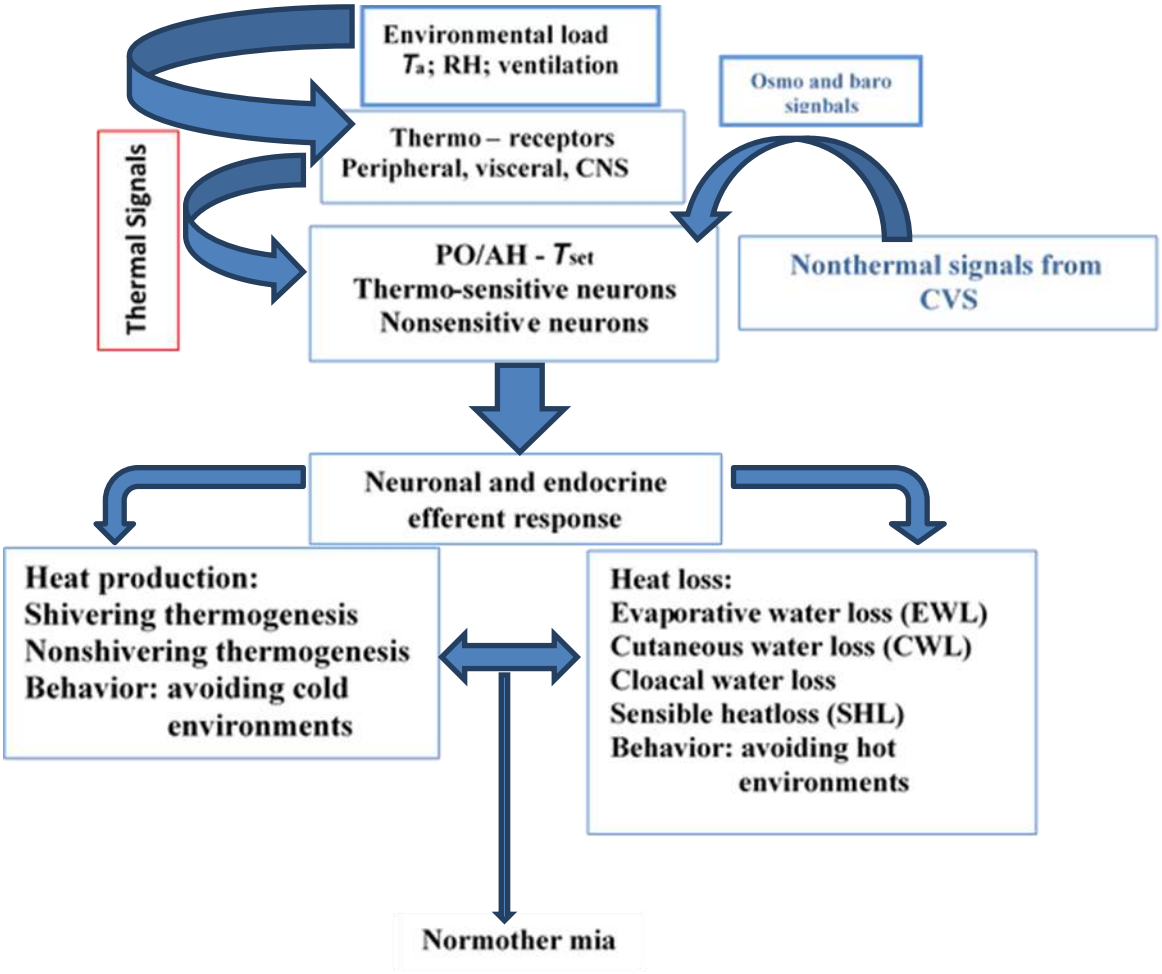

Figure 2.1: Neuronal Regulatory Mechanism of Endorthermy

Schematic flow chart describing how environmental heat load is trnasfered to thermal information by themoreceptors, and transferred to the preoptic anterior hypothalamus (P.O/PA) to elicit themoregulatory responses in the animal (heat production and heat loss efferent response) mediated by the neuronal and endocrine systems. Non-thermal signals, mainly from the blood system can modify the response. ST, shivering them oogenesis; NST, nonshivering themooegesis; CNS, centrsl nervous system; CVS, cardiovascular system; EWL, evaporative water loss; CWL, eutaneous water loss; SHL, sensible heat loss; Tset, set-point temperature (Sources; Nakamura, 2011).

2.12 Physiological Responses to Heat Stress (Hyperthermia)

Multicellular organisms (*eucaryotes*), including poultry have well organized cellular organelles which compose the cell structure. These cells in turn are specialized to make up organs that determine and respond to vital metabolic requirements and dictate physiological responses of the animal for its survival(AndreWartha *et al*., 2011; Zimerman *etal*., 2013; He *et al*., 2018). These specialized structural organizations require optimum range of intrinsic and extrinsic factors, including environmental temperature for the catalogue of their enzyme-controlled metabolic function and, any deviation from this optimum range status threaten the animal's survival as their cellular and even molecular support functions become displaced. If the functional displacement is not promptly rectified to restore homeostasis, then the stability required for all their metabolic responses for survival becomes significantly deterred (Krammer and Cohen, 2004; Katz and Meiri, 2006; Lin*et al.*, 2017).

Various attempts have been made to define stress and to give an indication of the point at which domestic animals are described to be under distress. In this respectAndreWartha *et al*.(2011) and Zimerman *et al*. (2013) demonstrated that stress is the magnitude of forces external to the body which tend to displace its systems from their resting or ground state. Following this definition, it is implied that heat stress in poultry can be characterized and the phenomenon can be defined as a summation of all high temperature-related forces that prompt and induce adjustments occurring from the sub-cellular to the whole animal level to help the chicken avoid physiological dysfunction and for it to better fit its environment. The endeavor by homeotherms to stabilize body temperature within fairly narrow limits is essential to control biochemical reactions and physiological processes associated with

normal metabolism (Krammer and Cohen, 2004; Katz and Meiri, 2006; Zimerman *et al*., 2013). In order to maintain homeothermy, an animal must maintain equilibrium with its environment, which includes radiation, air movement, air temperature and humidity.

Layers prefer ambient temperatures of between 5 and 28^{o}C, the "thermoneutral" zone (TNZ) (Giloh *et al*., 2012; Zimerman *et al*., 2013). At ambient temperatures above 29^{o}C the bird reaches a point where she can no longer cool herself adequately and she enters heat stress. The temperature of the skin underlying the feathered parts of the body is close to the deep body temperature, over a wide range of environmental temperatures. The skin temperatures for the unfeathered extremities however, are much lower than for the feathered areas of skin. When the environmental temperature increases, the skin temperature of the feathered areas does not increase very much, so that little further heat loss from these areas occurs. However, the skin temperatures of the legs, combs and wattles increase considerably and heat loss from them must be increased (AndreWartha *et al*., 2011; Yahav and Giloh, 2012; Lin*et al.*, 2017).

Large increases in the skin temperatures of the extremities also occur when environmental temperature is kept constant and when the body of the bird, excluding the extremities is heated; the increases are largely the result of increases in blood flow to the organs concerned. When environmental temperature is equal to the body temperature, heat cannot be lost from the bird by nonevaporative means; it can be lost however by evaporation of moisture from the respiratory tract. The deep body temperature at which panting is initiated varies from 41^{o}C to 43.5^{o}C in different species of birds (Yahav *et al*., 2004, 2005, 2009; Lin*et al.*, 2017).

Panting or thermal polypnea consists of an increase in respiratory rate and minute volume and decrease in tidal volume (capacity). The reduction in the tidal volume is thought to restrict the hyperventilation to the surfaces of the respiratory tract, which does not participate in the exchange of gases between the blood and the air in the respiratory tract. By this mechanism it is possible that the removal of excessive amounts of carbon dioxide from the blood is lessened (McKechnie and Lovegrove, 2002; Lin*et al.*, 2017). The increased respiratory minute volume results in an increased amount of moisture evaporated from the respiratory tract. Respiratory rate in the chicken may reach a maximum at a deep body temperature of about 44^{o}C. At higher body temperatures the respiratory rate decreases, but the respiratory minute volume remains constant just until the bird dies(Yahav, 2000; 2005; 2009). The decrease in respiratory rate is associated with an increase in tidal volume. Since the minute volume does not increase body temperatures higher than 44^{o}C, and since the amount of heat loss from the respiratory tract is partly dependent on the minute volume, further cooling from the respiratory tract must be the result of the increase in the temperature of the evaporating surface, which in turn will result in an increase in the aqueous vapor pressure at this surface. Since the tidal volume increases at body temperatures exceeding 44^{o}C, the bird probably incurs risk of developing alkalosis (Collin *et al*., 2007; Yahav and Giloh, 2012; Piestun *et al.*, 2017).

During conditions of heat stress in the chicken, there is increased cardiac output and increased blood volume to ensure that the rate of blood flow through the extremities, and also possibly through the evaporating areas of the respiratory tract and through the respiratory muscles involved in panting, is increased (McKechnie and Mzilikazi, 2011; Giloh *et al*., 2012). In the later stages of hyperthermia, when the respiratory rate decreases

and the tidal volume increases, the cardiac output and the blood pressure decrease. Therefore, circulatory failure could be one of the causes of death in the chicken (Collin *et al*., 2005; AndreWartha *etal*., 2011). In the chicken, it has been demonstrated that plasma volume may increase during hyperthermia despite the loss of water by evaporation; however, consumption of water by chickens is higher in hot than in cold environmental conditions; the volume of the feces is also higher (Zhou, 2000; Collin *et al*., 2007; Yahav, 2009; Piestun *et al.*, 2017). This implies that the availability of water is an important factor in the resistance of the avian specie to heat stress. When birds become hyperthermic the increase in body temperature leads to an increase in the temperature gradient between the bird and its environment. However, to be of value, the increased heat loss resulting from this increased gradient must exceed the increased heat production consequent upon the hyperthermia and accelerated contraction of respiratory muscles (Yahav *etal*., 2009; AndreWartha *et al*., 2011; Piestun *et al.*, 2017).

2.12.1 Cloacal evaporation

Hoffman *et al*.(2007) addressed cloacal evaporative water loss; it described its importance for thermoregulation in adult Inca doves. The idea of cloacal evaporation evolved from a study on a desert reptile, the Gila monster; this study showed for the first time in any animal the importance of cloacal evaporation for thermoregulation (DeNardo *et al*., 2004). The study by Hoffman *et al*. (2007)on the Inca dove--a bird that inhabits desert areas and can tolerate high ambient temperatures—showed that at 42 °C these doves can evaporate as much water from the cloaca as from the respiratorytract: 21.2 and 25.4%, respectively, of totalevaporative water loss. These results suggest that Inca doves can employ all the three routesto regulate their thermal status; or, in other words, that all three routes contribute to

the control of body temperature, at least under harsh environmental conditions. Under low ambient temperature, cloacal water loss is minimized and cloacal evaporation is eliminated by constricting the cloacal sphincter in desert inhabitants, the use of respiratory evaporation is limited by respiratory alkalosis and cutaneous evaporation is maximized to its limit, which is determined by the integument structure. In such cases, the cloacal epitheliumis exposed in order to provide further evaporation, which enables these birds to survive the harsh environmental conditions. (Harding *et al.,* 2016; Lin*et al.,* 2017; Piestun *et al.,* 2017)

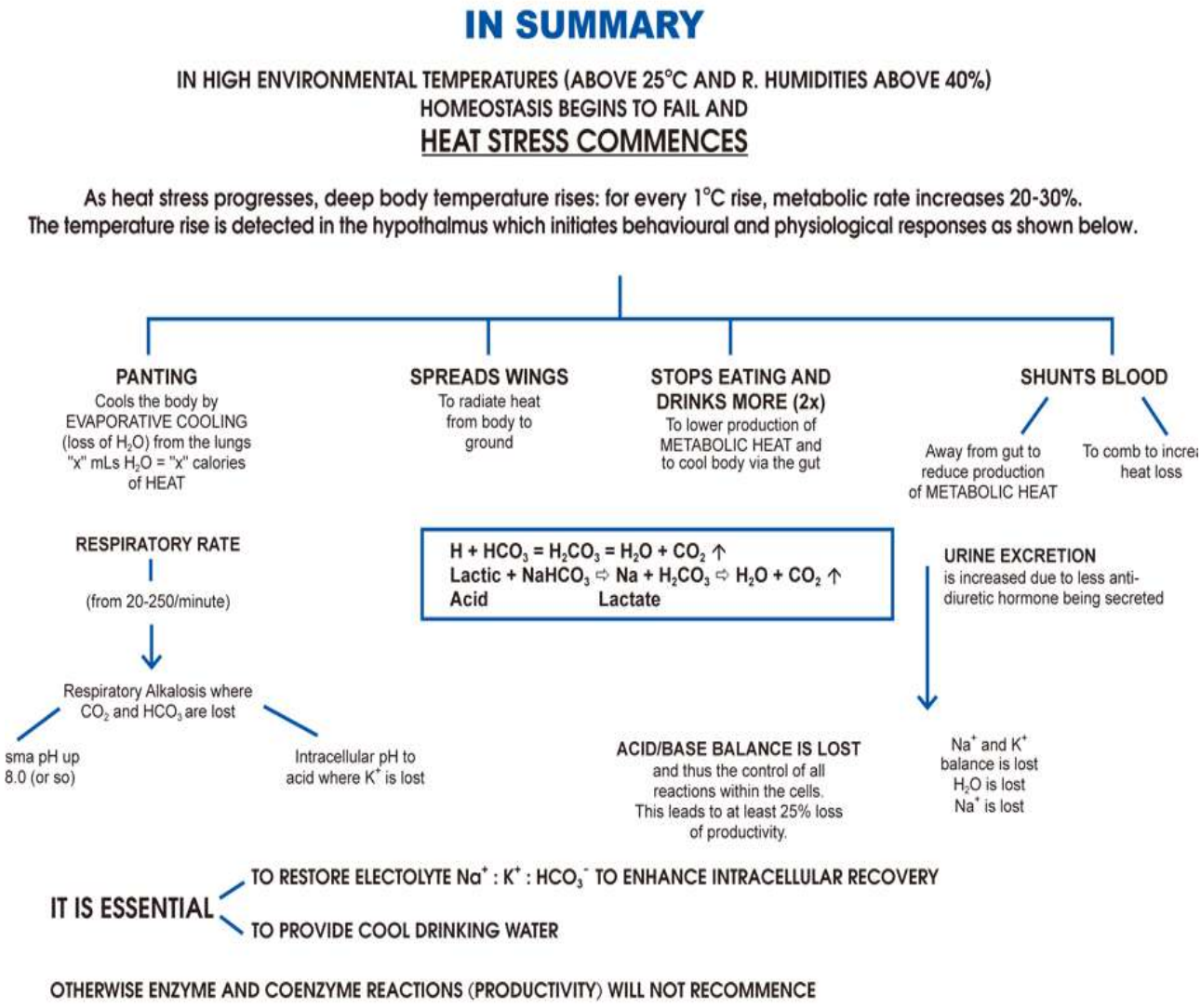

Figure 2.2:Schematic Representation of Physiological Responses to Thermal Stress (Hyperthermia) (Yahav and Giloh 2012; Piestun *et al.,* 2017)

2.13 Physiological Responses to Cold (Hypothermia)

It is evident that the skin temperatures of the uninsulated extremities of the chicken decrease as the environmental temperature decreases. The temperature gradient between the skin of these areas and the air is reduced, and the heat loss from the former is also reduced. Therefore, the maintenance of cold extremities in a cold environment may be regarded as a heat conservation mechanism. At environmental temperatures below 0 ^{o}C., the extremities are prevented from freezing by periodic increases in the flow of warm blood through them, which results in periodic increases in their temperature. This phenomenon is known as "cold vasodilation", which was first shown to occur in the foot of the chicken during immersion in cold water(Piestum *etal*., 2008, 2009; Yahav and Giloh, 2012). It is believed that cold vasodilation was associated with the alternate opening and closing of arteriovenous anastomoses in the foot.

As the environmental temperature decreases below the critical temperature, the rate of heat production increases as a result of increased muscle tone, and of shivering (McKechnie, 2008; Yahav *et al*., 2009; Giloh *etal*., 2012). If the rate of heat loss exceeds the rate of heat production, the body temperature will decrease, implying that the bird will become hypothermic. In this condition, shivering occurs at first, but the heat produced by this means is inadequate to prevent the body temperature from decreasing; this condition increases the respiratory rate slightly, a change that might be associated with a further increase in heat loss, later the respiratory rate and temperature decrease (Zhou, 2000; Yahav *etal*., 2004, 2005; Zimerman *etal*., 2013).

2.14 Effects of Meteorological Elements on Animal Reproduction

Hyperthermia can alter luteinizing hormone (LH) secretion in females, even in the absence of ovarian steroids (Yahav *et al*., 2009), and it is likely that severe heat stress can compromise LH secretion in males as well. However, the major site for disruption of reproductive function appears to be the spermatogenic cell lineage in the testis.Physiologically, the reproductive system is very sensitive to environmental stress in most species. In most animal species excess amounts of cortisol produced under stress, can affect normal functioning of the reproductive system (Yahav *et al*., 2009; Ayo *et al*., 2011). Chronic stress can impair testosterone and sperm production and cause impotence. In women, stress can cause absence or irregular menstrual cycles or more painful periods; stress can also reduce sexual desire.As endotherms, mammals typically function at high core body temperatures that range from approximately 35.8C to 39.8C (Zimerman *et al*., 2013; Yang *et al*., 2010; He *et al*., 2018). These high temperatures, which generally exceed the temperature of the surrounding environment, are achieved through combustion of fuel stuffs to achieve a high metabolic rate (i.e. heat production). Body temperature is closely regulated by matching heat production with heat loss to the environment via conduction, convection, radiation and evaporation. The set-point temperature for regulation of body temperature is not fixed but can vary diurnally or, in some animals, as part of thehibernation response or in response to changes in environmental temperature or other cues (Heldmaier *et al*., 2004). As a group, endotherms can better tolerate low body temperatures than high body temperatures. Indeed, many hibernating species maintain core body temperature at 6-108^{o}F or less (Heldmaier *et al*., 2004). There is less resistance to body temperatures above the set-point temperature: death is likely when temperatures exceed these values by a few degrees because of disruptions in membrane fluidity, protein

structure and, for animals in which sweating or panting exists, electrolyte and fluid loss. In humans, for example, set-point temperature is 37.8^{O}C and potentially lethal effects of hyperthermia are common at body temperatures above 40 - 41.8^{O}C (Jardine, 2007). Not surprisingly then, regulation of core body temperature is a priority over several other physiological functions.

Regulation of body temperature in endotherms can be considered as a homeokinetic control process whereby achievement of equilibrium in body temperature involves dynamic processes that lead to perturbations in other physiological processes. Heat stress in the present study is defined as an environment that acts to drive body temperature above set-point temperature. Heat stress can lead to disruptions in reproductive processes through two general mechanisms. First, the homeokinetic changes to regulate body temperature can compromise reproductive function. One example is redistribution of blood flow from the body core to the periphery to increase sensible heat loss (Yahav, 2000). Another homeokinetic control mechanism for body temperature is reduced feed intake during heat stress (Rozenboim *et al*., 2007; Mack *et al*., 2013). Reducing feed intake reduces metabolic heat production but also can lead to changes in energy balance and nutrient availability that can have large effects on cyclicity, establishment of pregnancy and foetal development. A second mechanism for disruption of reproduction during heat stress is the failure of homeokinetic systems to regulate reproduction (Giloh *et al*., 2012). The rise in body temperature about its regulated set point can compromise function of germ cells, the early developing embryo, and perhaps other cells involved in reproduction. In the present study, the term heat shock will be used to describe the effects of elevated temperature on cellular

function. One objective of the present study is to describe the consequences of heat stress for specific components of the reproductive process in the laying hen.

2.15 The Female Reproductive System

2.15.1 Sexually mature avian ovary

The length of time required to reach sexual maturationdepends upon the species, and it may be as short as severalweeks after hatch (Japanese quail) to as long as 2 to 3 ormore years (e.g., kiwis, penguins, and condors). Sexualmaturation in the domestic hen will occur at about 5months of age when hens are raised under a non-stimulatoryphotoperiod (less than 12 h of light per day), but it can beadvanced significantly by photostimulation beginning atabout 2 months of age (Johnson and Lee, 2016). The functionally mature ovary ofmost birds is arranged with an obvious hierarchy of follicles. As in mammals, both sexual maturation andrecrudescence of the gonads at the onset of seasonal breedingare initiated by changes within the neuroendocrine axisand involve, among other factors, thyroid hormones (Nakao*et al*., 2008; Mancini *et al*., 2016; Garasto *et al*., 2017) and gonadotropin-inhibitory hormone (GnIH) on the hypothalamus. These and additionalhormones and growth factors indirectly (e.g. 3,3′,5-triiodothyronine;T_3) or directly (gonadotropins) impact geneexpression at the level of the ovary. Gene expression canalso be modified by miRNAs acting at a post-transcriptionallevel. For instance, there is evidence that a large numberof miRNAs are differentially expressed in the ovary fromsexually immature versus mature chickens, and in developingovarian follicles relative to the stage of maturation (Kang*et al*., 2013).

The activation and growth of primordial follicles to theprimary follicle stage are associated with the formationof the theca interna layer (from mesenchymal cells). Thedeveloping

theca interna layer is separated from the cuboidal-shapedgranulosa layer by the basal lamina.Growth to the prehierarchal follicle stage (6–8 mm) entailsthe accumulation of lipoprotein-rich white yolk, plus thedifferentiation of the theca into interna and externa layers (Johnson and Lee, 2016).

Vasculature and nervous innervation reaches the folliclethrough the pedicle and radiates through the theca layer.Immediately following selection into the preovulatory hierarchy,preovulatory follicles begin to rapidly grow fromapproximately 9 mm in diameter to 40+ mm over the courseof days. Granulosa cells from preovulatoryfollicles become squamous in shape to facilitate the uptakeof large amounts of vitellogenin and very-low-density lipoprotein(VLDL), except within the germinal disc region.Ovulation of the largest preovulatory follicle eventually occurs at the region of the comparatively avascular stigmaregion (Johnson and Lee, 2016). The mature ovary is supported by a blood supplyfrom the ovarian artery, which usually arises from the leftrenolumbar artery but may branch directly from the dorsalaorta. Within the fully developed ovary,the percentage of blood flow is greatest to the five largestpreovulatory follicles and significantly increases withincreased follicle size and at the time of ovulation (Scanes*et al*., 1982;Johnson, 2011). The ovarian artery divides into many branchesand eventually leads to each follicle stalk (pedicle).

Allveins from the ovary unite into the two main anterior andposterior veins, which subsequently drain into the posteriorvena cava.Both adaptive and innate immune responses have beendescribed within the domestic hen ovary, and these systemsfunction to protect againstcolonization and infection bymicrobial pathogens, as well as to maintain normal

functionsof the ovary. Monocytes, macrophages, and activated B andT cells expressing major histocompatibility complex classII (MHC-II+) molecules have been localized to the thecalayer of healthy cortical follicles plus growing prehierarchaland preovulatory follicles, and more prominently in boththeca and granulosa layers of post-ovulatory and atretic follicles.Cell-mediated immunity within ovarian stromal andtheca tissues is mediated via T cells (CD4+ and CD8+ cells)and represents an effective measure against intracellularpathogens such as*Salmonella* (Barua *et al*., 2001; Baruaand Yoshimura, 2004). Moreover, there is considerable evidencefor a functional innate immune system consistingof β-defensins (small cationic antimicrobial peptides thatexhibit a direct lytic action on microorganisms; (Abdelsalam*et al*., 2012) and Toll-like receptors (pattern recognitionreceptors that activate cytokines, antimicrobial peptides,and immunocompetent cells(Woods *et al*., 2009). A total of14 avian β-defensin genes (AvβD1–14) have been identifiedand these attack a wide range of microorganisms, includingGram-positive and Gram-negative bacteria, fungi, and yeastInnervation to the ovary is provided by both sympathetic(primarily adrenergic) and parasympathetic fibers. Thisinnervation consists of both extrinsic and intrinsic neuralsystems (Madekurozwa, 2008), and provides a number ofneurochemicals (e.g. catecholamines, serotonin, and acetylcholine)and neuropeptides (e.g. neurotropins, vasoactiveintestinal peptide (VIP), and galanin) to the ovary and itsfollicles. Such factors function in diverse roles as in theinitial organization of primordial follicles in the embryonicand early post-hatch ovary, the growth and differentiation offollicles in the adult ovary, the regulation of blood flow, and steroidogenesis.

As puberty approaches and the slow growth of folliclesresumes, the theca layer of primary follicles differentiatesinto discrete theca interna and externa layers with

distinctsteroidogenic profiles. As the follicle grows, the theca layerbecomes increasingly more vascularized except for thestigma (the point of rupture during ovulation), which containsfewer underlying small veins and arteries (Johnson and Lee, 2016). The largestarteries from the follicle stalk are directed toward the fastestgrowing follicles, branch into arterioles, and pass throughthe theca to the basal lamina to form arterial capillaries.There are also an increasingly greater number of neuronspresent within the theca layer as the follicle progressivelymatures. While granulosa cells from early-developing follicles(<2 mm) are closely packed and cuboidal; they eventuallyform a single cell layer and become squamous in shape asthe follicle grows (Johnson, 2011).

The germinal vesicle is localized within the germinaldisc region and includes the oocyte nucleus and the overlyinggranulosa layer. The disc appears as awhite plaque due to a localized,restricted passage of yolkprecursors. This region contains maternal RNA used forprotein synthesis prior to the onset of the transcription fromthe zygotic genome. Additional extra-embryonic RNA islocalized within the cytoplasmic layer directly under theperivitelline membrane, and is proposed to be required foroocyte development (Olszanska and Stepinska, 2008). Thetotal amount of maternal RNA deposited in the avian oocyteis considerably greater than in mammals, but comparableto that in *Xenopus*. In preovulatory follicles, granulosacells within the germinal disc region remain relatively undifferentiated (steroidogenically inactive) and mitoticallyactive, while the outer granulosa layer becomes steroidogenicallyactive but mitotically inactive (Yao and Bahr,2001). Oocytes remain arrested at the first meiotic prophasestage during follicle development, and, in the domestichen, resume meiosis (undergo germinal vesicle breakdown)4–6 h prior to ovulation. One microarray study identifiedno less than five genes

differentially expressed within thegerminal disc region that correlate with enhanced fertility;these genes may eventually be useful as markers of fertilityin the domestic hen (Elis *et al*., 2009).

The ovary of the sexually mature chicken maintainsnumerous primordial follicles smaller than 1 mm diameters that remain embedded within the outer ovarian cortex.These follicles can exist in a quiescent state for months toyears until activated (possibly by the removal from inhibitoryAMH signaling) to begin an extended period of growthand differentiation (Elis *et al*., 2009; Johnson, 2011). As follicles initiate development ("initialrecruitment"), they project from the surface of the cortex andbecome suspended by a stalk (an extension of the ovariancortex) through which blood vasculature and several nervebundles reach each follicle. Typically, there are numerousslow-growing follicles measuring approximately 2–6 mmin diameter that contain small amounts of a protein-richwhite yolk. The selection of a single follicle per day intothe preovulatory hierarchy occurs from a cohort of 6–8 mmprehierarchal follicles (numbering approximately 8–12. The most recently selected follicle (9–12 mm)represents that which has initiated differentiation and rapidgrowth. In the domestic hen, there are typically four to sixlarge, yolk-filled, preovulatory follicles (ranging in sizefrom >12 to 45+ mm in diameter). This final growth phaseprior to ovulation in domestic fowl, ducks, and pigeons generallylasts for 4–6 days (up to 25 days or more in the brownkiwi; Jensen and Durant, 2006). The number of preovulatoryfollicles can range from one or perhaps two for species laying asingle egg (e.g., kiwi and emperor penguin) to eight or morein species that lay 12 to 18 eggs in a clutch (e.g., wild turkeyand ring-neck pheasant).

Importantly, the maintenanceof both prehierarchal follicles in an undifferentiated statetogether with rapidly differentiating preovulatory folliclesrepresents a fundamental distinction between the avian andmammalian reproductive strategies.Specifically, in eutherianmammals the selection of a dominant follicle or follicles ineach estrous or menstrual cycle is rapidly followed by atresiain all growing but subdominant follicles(Stepinska and Bakst, 2006). By contrast, theability to produce a full clutch of eggs requires that a viable,prehierarchical cohort of follicles be maintained such that asingle follicle can be selected for final growth and differentiationon a daily or near-daily basis.Interestingly, the avian perivitelline layer does not preventpolyspermy. Accordingly, at insemination, more thanone male pronuclei may be found in the egg cytoplasm,but only the one located in the center of the germinal discforms the zygote nucleus. It is not yet known how a specificsperm is selected or at which stage between inseminationand fertilization sperm selection takes place. Nevertheless,the remaining sperm degenerate and disappear, presumablydue to the actions of DNases I and II (Stepinska and Bakst,2006). Fusion of the male and female pronuclei (syngamy)initiates embryo development almost immediately, and anumber of cell divisions occur by the time the ovum hasbecome incorporated into the egg and the egg is laid.

2.16 Effects of Thermal Stress on Gonadal Function

2.16.1 Effects of heat stress on semen quality

Thermal stress, especially during the hot season affects all phases of semen production in cocks. Although moderately high temperature stimulates testicular growth in the early phase, and promotes increased semen volume as well as concentration, it subsequently suppresses reproductive capacity, which is manifested in decreased semen quality

andquantity with time (McDaniel *et al*., 1996; Obidi *etal*., 2008a). The frequency of copulation and the quantity and quality of the semen are adversely affected by heat (Ayo *etal*., 2011). On the other hand, the initiation of spermatogenesis occurs later when chicks are reared at 0^{o}C or 10^{o}C than when they are kept at 30^{o}C.

2.16.2 Effects of heat stress on egg quantity and quality

It has been shown for many years that reproductive efficiency in domestic animals including the domestic chicken is significantly decreased by exposure to high temperatures. For instance, (Mack *et al*.(2013) have observed a diminution in egg weight, number of eggs and shell thickness in hens kept at 37.8^{o}C as opposed to birds kept at 21.1 ^{o}C. The diminished egg production in a hot environment is often related to the lower energy intake as it has been demonstrated that heat stress resulting from high AT during the hot season also depresses voluntary feed intake, thereby decreasing the nutrient intake and absorption of birds (Mack*etal*., 2013; Barrett *et al*., 2019).

It has been demonstrated that short daily exposures to heat are sufficient to cause a reduction in egg weight. The inhibitory effect of a hot environment on egg production is intensified if water is withheld from the chickens. The fertility and hatchability of hens are reduced by high environmental temperature; if the environmental temperature is variable the fertility and hatchability of the eggs are also less than when the environmental temperature is kept constant (Obidi *et al*., 2008a). The optimal storage temperature of the egg for maintenance of blastoderm viability is 10-12^{o}C. The fertility of eggs laid in hot summer weather is low (Rozeboim *etal*., 2007).

2.17 Beneficial Roles of Carotenoids in Prevention and Control of Some Diseases

Several reports indicate that carotenoids and tocopherols contribute to the beneficial health effects associated with an increased consumption of fruits and vegetables. It has been suggested that these effects are at least in part related to their antioxidant activity (Key, 2011; Stahl and Sies, 2012). Carotenoids and K-tocopherol trap various types of organic radicals. An interaction between L-carotene and the trichloromethylperoxyl radical was described (Fiedor and Burda, 2014), producing the carotenoid radical cation and a radical adduct (Agarwal *et al*., 2012). The radical adduct is less stable than the radical cation and decays to the cation with a rate constant of 1.8U104 s31. Other free radicals such as nitrogen dioxide, thiyl, and sulfonyl radicals are also rapidly scavenged by L- carotene (Fiedor and and Burda, 2014). Carotenoids react with the radical cation ABTSc. derived from ABTS (2, 2P-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid). This scavenging ability becomes more pronounced with increasing polarity and number of conjugated double bonds (Agarwal *et al*., 2012). Carotenoids efficiently inhibit lipid peroxidation at low oxygen tension, and an interaction of L-carotene with the lipid peroxyl radical leading to the formation of a radical adduct was postulated (Stahl and Sies, 2012). It has been demonstrated that L-carotene incorporated into liposomes prevents them from being oxidized (Key, 2011; Stahl and Sies, 2012]. The reaction of K-tocopherol with a peroxyl radical leads to a relatively stable K-tocopheroxyl radical (Koushan *et al.*, 2013) which is less effective in chain initiation than primary radicals. The K-tocopheroxyl radical can be reduced to K-tocopherol by vitamin C, bile pigments or thiols(Fiedor and Burda, 2014). The protective effect of exogenous K-tocopherol is considerably less than endogenous K-tocopherol in biological membranes due to cooperative interaction with other endogenous antioxidants (Fernandez-Garcia *etal*., 2013; Koushan *etal.,* 2013). Little is known so far

about the antioxidant efficiency and cooperative interactions of different carotenoids and mixtures of carotenoids with vitamin E.

2.18Bioaccessibility and Bioavailability of Functional Dietary Supplements

A fundamental property of any functional ingredient is its chemical structure that determines itsphysicochemical properties as well as the suitability and ease of absorption (Boileau *et al*., 2002; 2003). Functional ingredientscan be absorbed by an active process or through passive diffusion through the membrane. Otherfactors, such as food matrix interactions, also influence the absorption of ingredients becausefunctional ingredients may become bound and form complexes with macromolecules in foodduring food processing or consumption (Holst and Williamson, 2008; Williamson and Clifford 2010). Such complexes have tobe broken as they occur during digestion, before the active ingredients can be absorbed. Theterms bioaccessibility and bioavailability refer to such conditions. The amount of the potentiallyabsorbable form of an active ingredient in the food can be termed as the bioaccessible form ofthe ingredient, whereas bioavailability refers to the amount of active ingredient that is actually absorbed by the body (Boileau *et al*., 2002; 2003).

The absorption of functional ingredients is tremendously influenced by thefood matrix interactions. The uptake of flavonoids was influenced by the chemical structure (e.g., sugar attached to the flavonoid moiety), the presence of hydrophobic molecules, (e.g., lipids inthe diet), and the presence ofemulsifying agents, alcohol, and food matrix components, includingcarbohydrates, proteins, and fiber (Scholz and Williamson, 2007). The uptake of lycopene from thediet is highly favored from a lipid-enriched environment in the product or food. During fruitripening, there are several physiological changes that enhance the

catabolic processes in the cell,and these changes enhance complex formation within the tissue or during the processing of thetissue into juice and pulp (Jacob and Paliyath, 2008; Oke *et al*., 2010;Jacob and Paliyath, 2011). Thus, the bioaccessibilityand bioavailability of active ingredients are influenced by several physicochemical factors.

The molecular characteristics of nutraceuticals have great influence on their absorptionand this may be one reason for the limited uptake and extremely low physiological levels observedin the case of flavonoids. Lipinski's rule of five (Lipinski *et al*., 2001) discusses theinfluence of physicochemical characteristics of small molecular weight functional ingredientsin the diet. Components with relative molecular masses of >500, having more than five hydrogenbond donors and 10 hydrogen bond acceptors, and also showing high hydrophilicityare likely to be absorbed poorly. Flavonoids, especially anthocyanins, are highly hydrophilicmolecules and are usually present as their glycosides. This enables the formation of hydrogenbonds with macromolecular components in food, such as carbohydrates (pectin) and proteins.Digestion of the food matrix can release bound components, which can be absorbed by the body asobserved in carotenoids (Granado-Lorencio *et al.*, 2007). Enzymatic processes, including catabolicbreakdown, methylation, and deglycosylation, that transform the functional ingredient may alsoenhance bioavailability (Yang *et al*., 2008). O-methylated epigallocatechin gallates were observedto be better inhibitors of mast cell–dependent allergic reactions than their non-methylated naturalforms of tea polyphenols (Williamson and Clifford, 2010). Esterification of epigallocatechin gallate, which is highlywater soluble using long chain fatty acids, enhanced its lipophilicity and increased octanol: waterpartition coefficient, suggesting that the esterified form may provide better bioavailability. Therefore, structural modifications

induced during biosynthesis, chemical synthesis,or processing (Oke *et al*., 2010) can greatly influence the bioavailabilityof components that are poorly absorbed by the body.

2.19Biochemistry and Mechanisms of Action of Lycopene

Generally, the activities of carotenoids are known to be measured through their provitamin A activity in biological systems as reported by Clinton (1999). However, it has been well established that lycopene lacks any provitamin A activity as a result of the absence of the beta-ion ring in its biological structure (Stahl and Sies, 1996; Figure 2.3). In view of this, the biological activities of lycopene in humans, and indeed in all biological systems have been attributed to mechanisms of action that are different from that of vitamin A which are exemplified in two major opinions that have been held to explain the anticarcinogenic and antiatherogenic activities of lycopene: a) oxidative and b) non-oxidative mechanisms which were reported by Canadian Medical Association Journal (2000). Furthermore, Heber and Lu (2000) and Wertz *et al*. (2004) documented further details of the mechanisms of action of lycopene which demonstrated the antioxidant properties of lycopene and this characteristic of the carotenoid constitutes the major focus and interest of several studies with regards to its biological activity and perceived mystery. Other mechanisms of lycopene's biological activity have been reported to include the facilitation of gap junction communication, the stimulation of immune system (Chew and Park, 2002; Wertz *et al*., 2004), endocrine-mediated pathways (Wertz *et al*., 2004) and cell cycle regulations (Wertz *et al*., 2004).

Figure 2.3:Chemical Structure of Lycopene(Rao *et al*., 2009)

2.20 Bioavailability, Tissue Distribution and Metabolism of Lycopene

Lycopene, a member of the carotenoid family and mostly found in tomato, is a highly potent antioxidant that provides protection against cellular damage caused by reactive oxygen species (Rao and Agarwal, 1999; Rao and Shen, 2002). Dietary intake of lycopene has been shown to be associated with lower risk for prostate cancer (Kucuk, 2002; Kucuk *et al*., 2002) and tumor incidence. This subject is of major interest because lipid oxidation has been shown to adversely affect sensory properties and utilization of food (Wenk *et al*., 2000). Guo *et al*. (2001) reported that there is a significant inverse relationship between thiobarbituric acid reactive substances (TBARS) value in the thigh meat and egg and the dietary antioxidants.

Lycopene availability from food may depend on several factors. Season, heating, and processing of tomato products may change the amount of bioavailable lycopene. Prolonged heat treatment (>2 h at 100°C) of tomatoes decreases the total carotenoid content and more so in peeled than in unpeeled tomatoes (Grazianie*t al*., 2003; Yang *etal*., 2014). Single-meal studies have shown higher serum levels of lycopene when tomatoes have been consumed cooked rather than raw (Agarwal *etal*., 2001; Floegel *etal*., 2011)and when the tomatoes have been cooked with fat than without (Fielding *etal*., 2001; Ahuja *etal*., 2003), possibly because cooking or heating weakens the bond between lycopene and the tissue matrix and facilitates the passage of lycopene into a lipophilic phase, making it more bioavailable (Rao *etal*., 2005). Cooking or processing has been reported to transform all-*trans*-lycopene to *cis*-lycopene (Grazianie*t al*., 2003; Figures 2.4 and 2.5), which is better absorbed, probably because *cis*-isomers are more soluble in bile acid micelles, although studies have suggested that lycopene is resistant to heat-induced all-*trans* to *cis* conversion under conditions regularly employed in the food industry or during food preparation.

Similarly, 3- and 5-d meal studies have suggested that dietary fat influences plasma levels of lycopene when tomatoes are consumed with olive oil rather than without oil (Ahuja *etal*., 2003; Graziani *etal*., 2003). However, our previous 14-d study of a lycopene rich diet showed that a high monounsaturated fat-enriched sunflower oil diet (36% of energy as total fat and 23.6% of energy as monounsaturated fat) induced similar effects on serum lycopene levels as a high-carbohydrate, low-fat diet (15% of energy as total fat and 4.1% of energy as monounsaturated fat) (Chopra *etal*., 2000; Biesanz *etal*., 2010).

Studies have also compared the effect of carotenoid controlled fruit and vegetable intakes with high monounsaturated fat versus those with high-carbohydrate, low-fat intake on oxidation of isolated low-density lipoprotein (LDL) cholesterol (Ahuja *et al*., 2003; Palozza *etal*., 2010a; Palozza *et al*., 2010b), a risk factor for coronary heart disease (CHD) (Kucuk *et al*., 2002; Graziani *etal*., 2003; Ferrucci*etal*., 2009). In one study the dietary carotenoid intakewas controlled and serum carotenoid levels were measured to confirm similarity. Another study compared the effect of 7-dconsumption of tomato products with extra virgin olive oil versus sunflower oil on antioxidant activity as measured by ferric-reducing ability of plasma and found an increased activity with olive oil (Ahuja *et al*., 2003; Graziani *et al*., 2003; Yang *etal*., 2014).

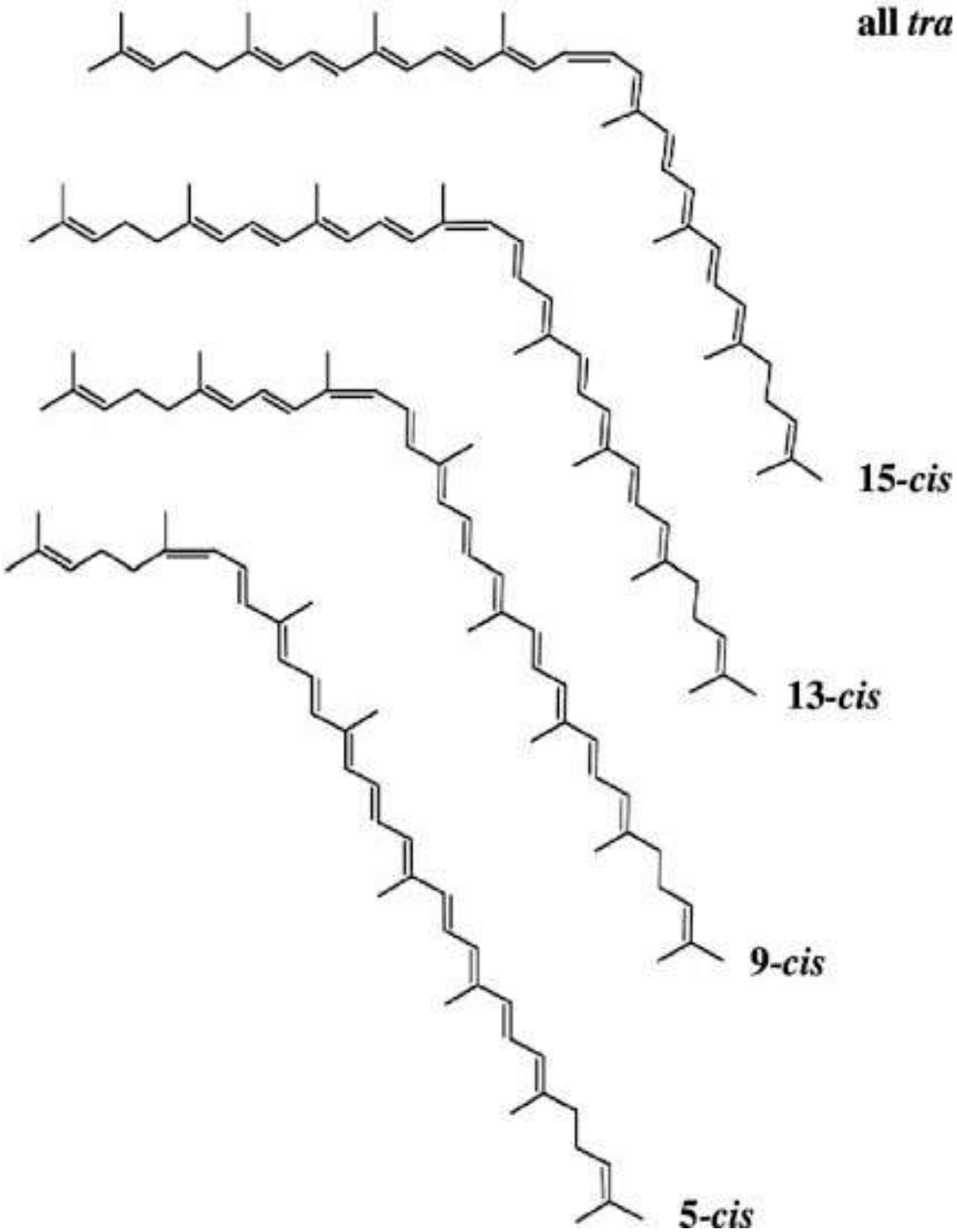

Figure 2.4: The Chemical Structures of Cis-isomers of Lycopene (Rao *et al*., 2009)

Figure 2.5:The Chemical Structure of Trans-isomer of Lycopene

2.21Ameliorative Roles of Lycopene in Stress Physiology

It has been suggested that androgens may stimulate the activities of hepatic enzymes that metabolize or degrade lycopene in the liver or other androgen-responsive tissues. A reduction in circulating androgens would thereby result in decreased lycopene metabolism and clearance, leading to an increase in tissue lycopene concentrations (Boileau *et al*., 2003). However, specific enzymes and/or pathways involved in lycopene metabolism have not been reported. Numerous xenobiotic metabolizing enzymes whose expression is dependent on androgens have been characterized (Costello *et al*., 2000; Boileau *et al*., 2001). One such example is the hepatic lauric acid hydroxylase, cytochrome P450 4A. This enzyme is expressed at much higher levels in male rats than in female rats, and expression is greatly decreased by castration (Costello *et al*., 2000; Yang *etal*., 2014). The possibility that cytochrome P450–type enzyme systems contribute to the catabolism of lycopene or other carotenoids warrants additional investigation as catabolic pathways of carotenoids become better characterized in the future. The non-enzymatic oxidation of lycopene as a result of androgen-induced increases in cellular metabolism could also account for the differences in liver lycopene observed in some studies. Testosterone stimulates the expression of enzymes involved in intermediary metabolism and increases the production of reactive oxygen species in prostate cancer cells (Costello *et al*., 2000; Chang *et al*., 2000; Cook *etal*., 2007).

Lycopene is a potent in vitro antioxidant and has been proposed to protect tissues from activated oxygen byproducts of metabolism in vivo. Food restriction has also been shown to decrease overall oxidative stress (Chang *et al*., 2000; Rao *et al*., 2006), and the increase

in tissue lycopene in energy-restricted rats may also be a result of decreased lycopene oxidation via nonspecific mechanisms.

Lycopene is oxidized via an epoxide to the cyclic oxidation product 2, 6- cyclolycopene-1,5 diol, and the presence of this compound in human serum and breast milk suggests that oxidation of lycopene does occur in vivo (Clinton, 1999; Shanefelt *et al*., 2000). However, details of lycopene oxidation remain obscure, and additional investigation will be needed to address the hypothesis that androgens enhance oxidative consumption of carotenoids. Interestingly, androgens also appear to alter the tissue concentrations of other dietary components thought to modify prostate cancer risk. Boileau *et al*. (2001, 2006) demonstrated in male rats that liver α-tocopherol concentrations are increased in response to castration. Similar to what has been reported with lycopene; the effects of castration on liver α-tocopherol were reversed by testosterone replacement (Boileau *et al*., 2000; 2001). In addition, few studies documented that female rats accumulate significantly more liver vitamin E than do age-matched male rats (Boileau *et al*., 2001, 2006; Rao *et al*., 2006).

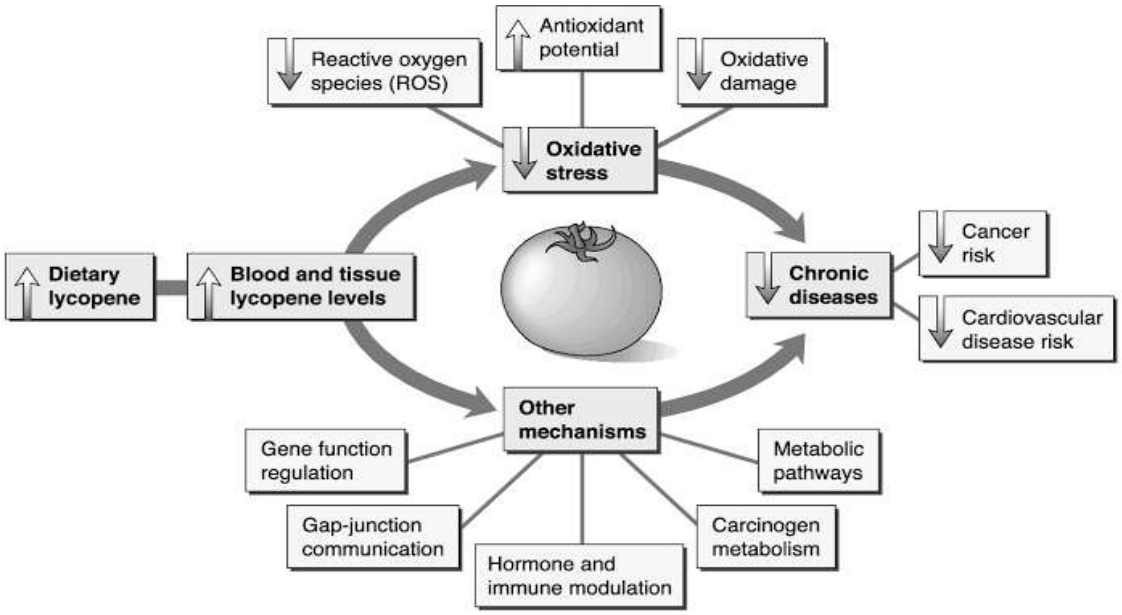

Figure 2.6:Proposed Mechanisms for Lycopene in Chronic Diseases (Canadian Medical Association Journal,2002)

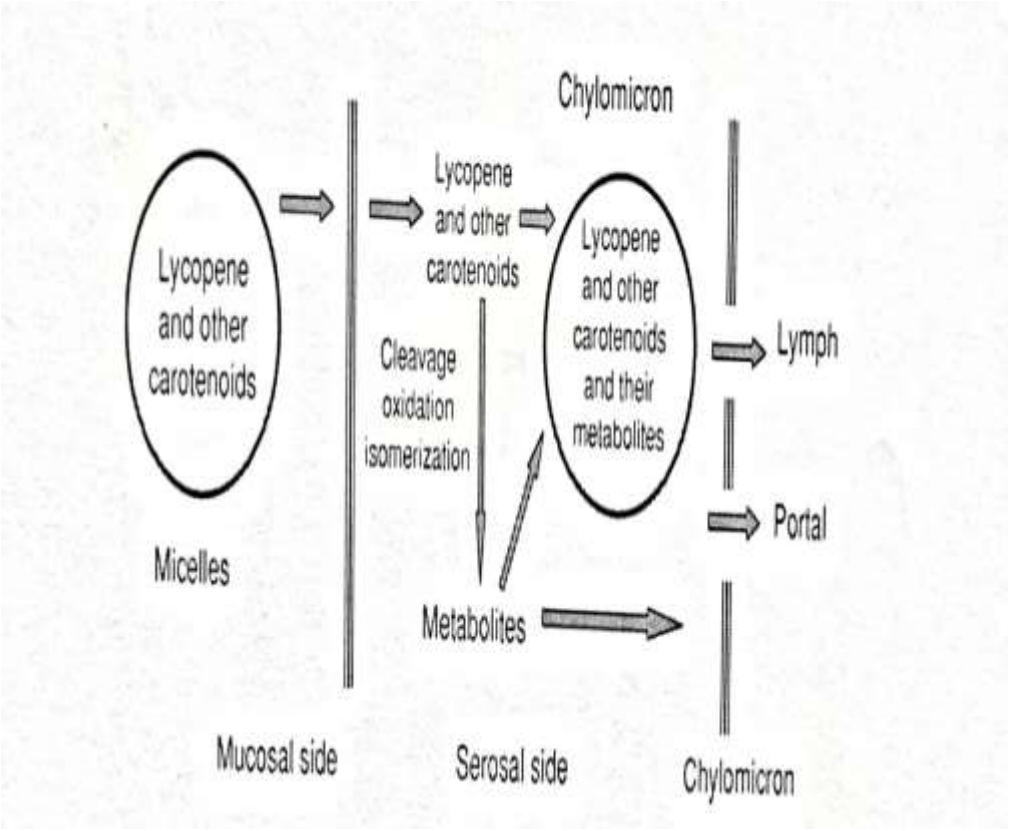

Figure 2.7:Absorbtion and Translocation of Lycopene (Canadian Medical Association Journal, 2002)

2.22 Biochemistry, Kinetics and Mechanisms of Action of Vitamin E

Vitamin E,one of the most powerful antioxidants, has been included into animal feed to improve performance, strengthen immunological status, improve the quality of meat and egg and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of man (Flachowsky, 2000). Poultry cannot synthesize vitamin E; therefore, vitamin E requirements must be met from dietary sources (Sahin *et al*., 2001; Figure 2.6). Vitamin E has been reported to be an excellent biological chainbreaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals. This vitamin is also known to be a lipid component of biological membranes and is considered a major chain-breaking antioxidant (Sahin *et al*., 2001, 2006, 2016). Vitamin E is mainly found in the hydrocarbon part of the membrane lipid bilayer towards the membrane interface and in close proximity to oxidase enzymes which initiate the production of free radicals.Sahin *et al*. (2001, 2002) reported that broilers supplemented with dietary vitamin E had a significant reduction in malondialdehyde (MDA) values, an indicator of lipid peroxidation, in serum and tissue of poultry. However, vitamin E concentration above the physiological requirements does not have any effects. Incorporation of vitamin E into poultry diets has been shown to provide oxidative stability and increase the quality of their eggs and reduce the development of off-flavors while increasing egg production (Cherian *et al*., 1996a). Previous reports have demonstrated that supplemental vitamin E and C significantly alleviated the heat stress-related decrease in the performance of growing Japanese quails suggesting that additional vitamin E

supplementation may be necessary under heat stress conditions (Sahin and Kucuk, 2001a; Sahin *etal*., 2016).

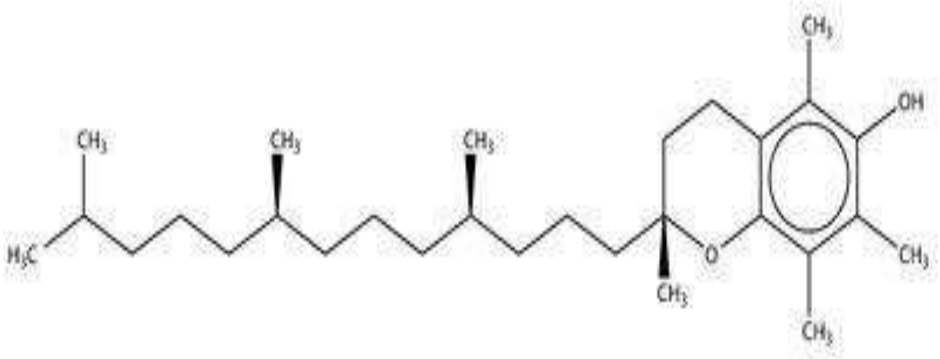

Figure 2.8: Structure of Alpha-Tocopherol form of Vitamin E (Surai, 2009)

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Site and Location of the Study

The experiment was performed in a Poultry Farm in Zaria (latitude 11°N, 12/N and longitude 7°E, 8/E, at an elevation of 650 m above the sea level), located in the Northern Guinea Savannah zone of Nigeria. The zone has an average annual maximum and minimum temperature values of 31.8 ± 3.2°C and 18.0 ± 3.7°C, respectively. The monthly average rainfall during the rainy season (May - October) is 148 ± 68.4 mm (69.2–231.9 mm), while the monthly relative humidity is 71.1 ± 9.7%. The zone is characterized by three seasons: Harmattan (November–February), hot-dry (March–May) and rainy (June–October) seasons (Dzenda *etal*., 2011, 2013).

A dry- and wet-bulb thermometer (Mason's type wet-and dry-bulb hygrometer; GH Zeal limited, London, England) was located in the experimental poultry pen to measure the dry- and wet-bulb temperatures every three hours, beginning from 06:00 h till the end of day at 18:00 h throughout the duration of the study. During the period of the study, other relevant meteorological data were collated from Meteorology Unit, Institute for Agricultural Research, Zaria, located at about 5 km from the experimental site. The present study was conducted during the harmattan season when cold and heat stresses are known to manifest concurrently in poultry and in the hot-dry season, characterised by significant heat stress in the zone.

3.2Season Description and Duration of the Study

The present study was conducted during the harmattan and hot dry seasons, prevailing in the Northern Guinea Savannah zone of Nigeria. The two seasons have been reported to be thermally stressful to animal well-being and productivity.

1) The harmattan season is characterized by low ambient temperature and high air movement (cold) (Ayo *et al*., 1998).

2) The hot-dry season is characterised by high ambient temperature and high relative humidity (Igono *et al*., 1982; Oladele *et al*., 2003; Dzenda *et al*., 2011, 2013). During the season, heat stress is prevalent and the deleterious effect of the stress on animal physiology is reported to be significant (Minka and Ayo, 2009; Ayo *et al*., 2014). Heat stress has been shown to inhibit reproductive efficiency in domestic animals, including poultry (Obidi *et al*., 2008b; Ayo *et al*., 2011).

3.3 Flock Management

The present study was designed with the aim of determining any modulatory and/or ameliorative roles of lycopene and/or vitamin E on physiological and reproductive performance parameters of the domestic hen layer during the stressful harmattan and hot-dry seasons prevailing in the Northern Guinea Savannah zone of Nigeria. To address these objectives, two main studies were conducted during the harmattan and hot-dry seasons. During the peak of the harmattan season, 400 apparently, healthy ISA Brown layers, aged 40 weeks and maintained in deep litter in an open sided poultry house unit were randomly divided into four groups, comprising of one hundred group-penned birds each. The birds were maintained on layer mash (Vital Feed Ltd., Jos, Nigeria) with a nutritive value of 16.50% crude protein, 6.5% crude fibre and 2650.0 kcal/kg metabolisable energy, and were

given access to water *ad libitum.*Routine prophylactic vaccinations and periodic medications were administered to the birds promptly. The same standard flock management routine was extended to the hot-dry season, when a repeated investigation of the objectives of the study was carried out.

3.4 Experimental Design

The present study was designed to have four different penned groups of layers in a laying house, comprising 100 birds per group. Each pen was identified according to its experimental role with "L" representing the pen holding lycopene-administered birds; "L+VE" represented the pen holding a combined lycopene and vitamin E-administered experimental group. The pens with letters "E" and "C" denoted birds that were administered vitamin E only and placebo control birds, respectively. The L-treated group was administered orally with 30 mg lycopene/bird per day, while the control group was given water only. The L+VE-treated birds were also administered orally, with 30 mg lycopene/kg body weight per day combined with 250 mg vitamin E/ kg body weight per day, while the E-penned layers received vitamin E only at a dose of 250 mg/kg body weight per day.

The live weight of 20 randomly selected birds from each group were determined weekly to verify changes in weight gain in the four groups of layers, in order to enable absolute mean weight of each group to be determined.Throughout the period of the study, dry-and wet-bulb ambient temperatures in the poultry house were determined daily at 3-h interval with effect from 06:00 h to 18:00 h using a dry-bulb thermometer (Mason's Type Wet-and Dry-bulb Hygrometer; GH Zeal limited, London, UK).

3.4.1Egg productionand egg weight

During the experimental period, daily hen-day egg production of laying hens was recorded separately according to the identity established for each pen. The hen-day egg production for each group of the experimental period was calculated as total number of eggs produced per group/100 andat the end of each week, a percentage mean hen-day was calculated for each group of laying hens.A sample size of 30 eggs (one tray) was randomly selected from each group of layers and weighed with a digital scale (Mettler Toledo digital precision weighing balance; Model MT- 500) and mean egg weight was determined for each group of layers weekly throughout the experimental period.

3.4.2 Internal egg quality

Samples of 30 eggs collected from each group of laying hens were weighed weekly to determine the average egg weight in each group during the period of study. In order to determine internal egg quality, which is often linked to vital reproductive parameters, chick quality in breeder stock and assessment of merit in commercialpoultry egg-processing, laboratory analyses ofyolk weight, thickness and height, albumen weight, thickness and height were carried out in equal number of randomly selected eggs from the four groups of layers using a vernier calliper (Vernier Caliper, 0-150mm 0.05⁄6” × 1⁄128; G.T. Tools, Japan). Briefly, a sample size of 10 eggs was randomly selected weekly from each group of layers and labeled for proper identification, depending on the administered antioxidant or the control. Egg samples were labeled with an indelible marker as L+VE, L, VE and CONT, respectively for lycopene and vitamin E-, lycopene-, vitamin E-administered and control birds, respectively. Each egg in a sample was further marked indelibly and identified with Figures 1 to 10, subscripted to its parent identity. This implies, for instance,

that each egg in group L+VE was labeled $L+VE_1$-------$L+VE_{10}$ and this order of identification was maintained for all the groups of sampled eggs. The samples were speedily transported to the laboratory for egg quality analysis.Each marked sample was analysed one after the other. Each egg in a sample was carefully weighed with a digital scale (Mettler Toledo digital precision weighing balance; Model MT- 500); the result of the weight was recorded in grammes.

The dimension of each whole egg, that is, the length and breadth of the egg, was taken with a Vennier caliper (Vernier Caliper, 0-150mm 0.05⁄6" × 1⁄128; G.T. Tools, Japan) and the unit was calibrated to the nearest centimeter. This was followed immediately by carefully breaking each egg from the middle ridge of the eggshell to enable easy pouring of the internal liquid content that is, the albumen and yolk into a flat-based white plate without breaking their delicate membranes. As soon as this procedure was accomplished, the dimensions (that is, the breadth and height) of the albumen and the yolk were recorded with a Vennier caliper to the nearest centimeter. This procedure was followed by a careful separation of the albumen from the yolk using a table spoon. The egg components were weighed separately, using a digital scale (Mettler Toledo digital precision weighing balance; Model MT- 500); and the results were recorded to the nearest gramme.

3.4.3 Determination of external egg quality (Egg shell thickness)

Eggshell thickness was determined according to the method of Mack *et al*. (2013). Briefly, the eggshells were prepared by proper identification of individual pairs and in groups. They were exposed to over-night drying, and then having the membrane peeled off from the interior surface.

A micrometer calliper (Mitutoyo, Tokyo, Japan) was used to determine eggshell diameter on three locations on the egg without removing the eggshell membranes. The instrument was attached to an open portion of one-half of the eggshell and carefully screwed to the eggshell until it caught the shell to make a firm grip, without impacting on the eggshell to cracking or breakage. Screws on the micrometer were adjusted to fit the point at which this grip was dimmed to be enough and the reading of the eggshell diameter was carried out with the micrometer calibration corresponding to a calibrated line on the screw.The mean of the three measurements was used as the eggshell thickness for statistical analysis.

3.4.4Eggshell weight

Each pair of eggshell was properly marked for easy identity and eggshells from each group of eggs were packed together and separately from each group and were air-dried in the laboratory for 48 h. At the end of this period, each marked pair of eggshell was weighed with a digital laboratory scale and each weight was recorded in grammes.

3.5Cloacal Temperature

Cloacal temperatures of 20 randomly selected layers from each pen were recorded weekly at 3 hours' interval from 09:00 h using a digital thermometer (Goshen, mode: ECT-1, Israel) as described by Ayo*et al*. (2007) and Makeri et al. (2017). The thermometer was inserted about 2 cm into the cloaca of each layer, and in direct contact with the mucosal wall of the cloaca of each bird. The reading was taken as soon as the thermometer gave an alarm signal after about 1 minute, indicating the end of the reading.

3.6Blood Sampling and Analyses

Blood samples (5 mL) were collected through wing venipuncture from five randomly selected layers from each pen two weeks after the commencement of the study. Subsequent blood sample collection was repeated at the end of the study from each group into five different sample bottles, containing ethylenediaminetetraacetate (EDTA). Samples were speedily transported to the laboratory for haematological and biochemical analyses. Other parameters that were determined in blood samples are activities of antioxidant enzymes, and endocrine profiles.Blood samples were stored on ice until the plasma was separated by centrifugation at 700 x *g* for 20 minutes at 4^{o}C (Sorvall BC 3B Plus, Thermo Fisher Scientific, Waltham, MA, USA). Following centrifugation, the supernatant plasma was collected and stored at -20^{o}C until it was assayed for progesterone, estrogen, and thyroid hormones at the end of the study.

3.6.1 Hormonal concentration analyses

Ten birds were randomly selected from each group of laying hens. Blood was collected via wing venipuncture and plasma was separated by centrifugation at 3,000 x g for 8minutes at 4^{o}C and then stored at – 20^{o}C.The concentrations of estrogen, progesterone and thyroid hormones were determined using micro high performance liquid chromatography as described by van Deventer *et al*. (2011)

3.7Ovarian Follicular and Oviductal Analyses

At the end of the study, the body weight and ovarian weights of each hen weredetermined, using standard procedures.Live layer samples (n = 10) were randomly selected from each pen at the end of the study and humanely decapitated to determine ovarian and oviductal function. The layer's follicular activity was determined as described by Mack *et al*.

(2013).Ovarian follicles were removed from the reproductive tract after the ovarian weight was recorded and the number of mature follicles, distinguished by yellow colour and size approximately ≥2.5 cm in diameter, were countedand countable number of white follicles were determined. Transitional follicles and shelled eggs-in-uteri were noted. The relative ovarian weight was calculated as absolute ovarian weight (g)/body weight (kg). Overall ovarian weights from sacrificed layers were determined and correlated to other afore-mentioned parameters to investigate any antioxidant-induced modulation of follicular growth and follicular survival; this was aimed at determining ovarian resistance to stress-induced regression. Sequence of ovarian recruitment order was investigated also, to determine any supportive influence of antioxidant on this physiological process.

3.8Meteorological Data

A dry- and wet-bulb thermometer located in the experimental poultry pen house was used to record the temperatures every three hours throughout the duration of the study. Daily temperature-humidity index (THI) was determined during the experimental period using the following equation (Zulovich and DeShazer, 1990):

$THI_{layer} = 0.6T_{db} + 0.4T_{wb}$

Where:

THI: temperature-humidity index for the laying hen (°C)

T_{db}: dry-bulb temperature (°C)

T_{wb}: wet-bulb temperature (°C)

3.9 Data Analyses

Data wereanalysed using GraphPad Prism, version 5.03(GraphPad Software, San Diego, California, USA, www.graphpad.com). The data obtained were expressed as mean ±

standard error of the mean (mean ± SEM). Variations in parameters obtained in the brown layers wereanalysed using repeated measures analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test. The relationship between CT of ISA Brown laying hens and ambient temperature and the severity of heat load on hen-day egg production were determined using two-tailed Pearson's correlation analysis. Values of $P < 0.05$ wereconsidered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Harmattan Season

4.1.1 Weekly diurnal fluctuations in dry-bulb temperature during the harmattan season

The meteorological data in the present study showed significant diurnal variation in dry-bulb temperature (DBT) between 6:00 h and 18:00 h daily during the study period (Table 4.1.1). Weekly mean DBT values were significantly ($P < 0.05$) lower during the period between 06:00 h and 09:00 h than between the times of 12:00 h, 15:00 h and 18:00 h; and this pattern of DBT variation and, hence, THI was consistent throughout the experimental period. The lowest DBT of 17.6 ± 1.5 ^{o}C was recorded at 06:00 h during the first week of the study, which was significantly ($P < 0.05$) different from the highest DBT value of 25.0 ± 0.9 ^{o}C, recorded at 09:00 h in the 3rd week of the study (Table 4.1.1). The result of the study showed that DBT varied between these limits between 6:00 h and 9:00 h, although the variations were not significantly ($P > 0.05$) different. The result of the thermal environmental parameter in the present study showed considerable pattern of DBT variation at 12:00 h, 15:00 h and 18:00 h was significantly ($P < 0.05$). It was observed that the lowest value of 18.6 ± 0.9 ^{o}C was recorded at 12:00 h during the first week of the study, and the highest DBT value of 35.3 ± 1.0 ^{o}C was recorded at 18:00 h during the 4th week. There was a significant ($P < 0.05$) variation in DBT values at each hour of the day within this range throughout the experimental period.

During the study period, the laying hens exhibited signs of distress in behavioural responses of birds to varying environmental conditions, including hurdling together, standing and waiting at the direction of the rising sun, in anticipation of a heat source for their warmth in the early hours of the day, especially between 6:00 h and 8:00 h; subsequently, panting behaviour ensued between 15:00 h and 18:00 h of the day; apparently due to increased thermal load (Table 4.1.1).

In summary, there was evidence of diurnal fluctuation in DBT, with increasing trend, beginning from 0:600 h to 18:00 h throughout the period of study. It was noticeable that the variation was accompanied by increased thermal load from time to time as the day progressed.

4.1.2 Temperature-humidity index

The THI varied significantly ($P < 0.05$) between hours of the day and between weeks during the study. It was evident that THI, as an index of heat load, on the laying hen-in-pen ranged between limits that may be regarded as extreme for the laying hens, especially between the times of 12:00 h, and 18:00 h throughout the period of the study. At 12:00 h the lowest THI value of 17.20 ^{o}C was obtained in the 1st week of the study, while the highest THI of 30.60 ^{o}C was recorded during the 3rd week of the study. At 15:00 h, the lowest value of THI of 16.0 ^{o}C was recorded in the 2nd week of the study and the highest THI value of 33.8 ^{o}C was recorded in week 4 of the study. At 18:00 h, during 2nd week, the lowest THI value of 13.4 ^{o}C was obtained while the highest value of 32.6 ^{o}C was recorded at week 4 of the study.

Table4.1.1:Weekly Diurnal Fluctuations in Dry-bulb Temperature (mean ± SEM; ^{o}C) During the Harmattan Season

	Time, h				
Week	6:00	9:00	12:00	15:00	18:00
1.	17.6±1.5[a] (14.0 - 26.0)	18.6 ± 0.9[b] (15.0-21.0)	18.6 ± 0.9[b] (15.0- 21.0)	27.1 ± 0.6[c] (25.0-30.0)	26.7 ± 0.7[c] (25.0-30.0)
2.	17.7 ± 0.7[a] (15.0 -20.0)	18.9 ± 0.5[a] (17.0-21.0)	25.4 ± 0.8[b] (21.0- 27.0)	24.7 ± 2.0[c] (17.0-31.0)	24.0 ± 2.1[c] (15.0-28.0)
3.	23.4 ± 1.0[a] (20.0 -27.0)	25.0 ± 0.9[a] (22.0-28.0)	30.6 ± 1.3[b] (25.0 -34.0)	33.7 ± 0.4[c] (32.0-35.0)	33.5 ± 1.0[c] (30.0-37.0)
4.	21.6 ± 0.6[a] (20.0 -24.0)	24.3 ± 1.2[b] (20.0- 30.0)	32.4 ±0.5[c] (30.0- 34.0)	35.1 ± 0.9[d] (33.0- 39.0)	35.3 ± 1.0[d] (34.0-37.0)
5	18.0 ± 1.1[a] (15.0 -21.0)	20.7 ± 1.6[a] (16.0 -26.0)	25.3 ± 1.3[b] (20.0-30.0)	26.6 ± 1.7[c] (20.0-34.0)	26.1 ± 1.2[c] (22.0-32.0)
6.	22.3 ± 0.9[a] (19.0- 25.0)	22.3 ± 0.9[a] (19.0- 25.0)	28.3 ± 0.7[b] (26.0-31.0)	30.1 ± 0.9[b] (27.0-33.0)	29.4 ± 0.4[b] (28.0-31.0)
Overall mean ± SEM	19.2 ± 1.0 (14-27)	21.6 ± 1.0 (15-30)	27.0 ± 1.0 (15-34)	29.6 ± 1.1 (20-35)	29.2 ± 1.0 (15-37)
Range	13.0	15.0	19.0	15.0	22.0

a, b, c, d = Values with different superscript letters a, b, c and d are significantly ($P < 0.05$) different.

Data in parentheses are minimum and maximum dry-bulb temperature.

Table 4.1.2:Temperature-humidity Index (^{o}C) in Poultry Pen During the Harmattan Season

	Time, h				
Week	06:00	09:00	12:00	15:00	18:00
1	15.70 ± 1.3^{a} (12.40 -21.20)	15.9 ± 0.8^{a} (13.0-18.20)	20.3 ± 0.8^{b} (17.20 -22.80)	22.3 ± 0.7^{b} (19.80-26.0)	22.5 ± 0.7^{b} (19.90 -28.0)
2	15.43 ± 0.6^{a} (13.0 -17.40)	16.5 ± 0.3^{a} (15.0 - 17.40)	21.70 ± 0.8^{b} (17.80 - 24.20)	21.2 ± 1.6^{b} (15.80-26.60)	21.0 ± 0.2^{b} (13.40 -25.20)
3	21.0 ± 0.8^{a} (17.20 - 22.60)	22.3 ± 0.8^{a} (18.40 - 24.0)	27.5 ± 1.3^{b} (22.20 - 30.60)	30.0 ± 0.3^{c} (22.80 - 31.0)	30.0 ± 0.8^{c} (27.0 - 32.20)
4	19.70 ± 0.7^{a} (17.60 -23.20)	21.80 ±1.3^{a} (18.0 - 28.40)	29.0 ± 0.5^{b} (26.40 - 30.0)	31.20 ± 0.6^{b} (29.40-33.80)	30.8 ± 0.4^{b} (29.60 -32.60)
5	16.2 ± 0.9^{a} (13.0 - 18.60)	18.7 ± 1.5^{a} (14.03-23.20)	23.0 ± 1.4^{b} (18.0-27.60)	23.7 ± 1.5^{b} (18.0-30.0)	23.2 ± 1.0^{b} (19.60-28.0)
6	19.1 ± 0.7^{a} (16.60-21.0)	19.3 ± 0.6^{a} (17.40-21.80)	25.5 ± 0.8^{b} (22.80-28.60)	26.5 ± 0.9^{b} (23.40-29.20)	25.2 ± 0.62^{b} (22.80-27.0)
Overall mean ± SEM	18.0 ± 0.7	19.1 ± 0.9	24.5 ± 0.9	26.0 ± 0.9	25.5 ± 0.6

a, b, c = Values with different superscripts are significantly ($P < 0.05$) different
Data in parentheses represent minimum and maximum thermal heat indices

4.1.3 Relationship between temperature and humidity during the harmattan season (Pearson's correlation coefficient)

Pearson's correlation coefficient (r) between THI and hen-day production in the present study assumed no definitive trend. The severity of thermal load on laying hens was more pronounced in control laying birds, with the higher significant correlation co-efficient value of 0.740 at week 3.

Table4.1.3:The RelationshipBetween Thermal Heat Index and Hen-Day During theHarmattan Season

	Layer group			
Week	L+VE	L	VE	CONT.
1	0.264	0.112	0.062	-0.600
2	0.324	-0.628	-0.494	0.031
3	-0.564	0.690**	-0.600	0.740**
4	0.307	0.252	0.253	-0.042
5	0.236	-0.173	0.350	-0.323

**P=< 0.01, L + VE = lycopene + vitamin E, L = lycopene only, E = vitamin E only and CONT = control.

4.1.4 Effects of lycopene and vitamin E on thermal stress-induced changes in hen-day performance indices of ISA Brown layers

Supplemental administration of lycopene and α-tocopherol significantly increased daily egg production, which resulted in significant weekly egg output, when compared with the controls. Data in Table 4.1.4 show the effect of lycopene and vitamin E co-administered to laying hens. Laying hens which received a combination of the antioxidants maintained a significantly high mean laying performance, ranging from the lowest hen-day of 74.1 ± 1.4 % to the highest hen-day of 80.0 ± 1.1 % during the 5^{th} and 3^{rd} week, respectively in peak egg production throughout the experimental period, lasting five weeks. This group of layers had an overall mean of 78.0 ± 1.2 % hen-day at the end of the 5-week study. Layers which were daily supplemented with lycopene alone recorded weekly hen-day egg production, ranging from the lowest hen-day of 72.0 ± 1.3 % to the highest hen-day of 75.7 ± 0.7 %, respectively during 5^{th} and 3^{rd} week. The overall value of the hen-day for this group of layers at the end of the study was 74.20 ± 1.2 %, which was relatively lower than, but not statistically different ($P < 0.05$) from the result of the layers, co-administered with lycopene and vitamin E (Table 4.1.4).

In layers supplemented with vitamin E alone, weekly hen-day egg production ranged from the lowest value of 71.1 ± 1.6 % to the highest value of 76.6 ± 2.2 % hen-day during the 5^{th} and 1^{st} week of the study, respectively. The overall mean of hen-day egg production was 74.20 ± 1.2 %, which was significantly ($P < 0.05$) lower than the value obtained for the layers co-administered with lycopene and vitamin E. The control group of layers was characterised by a consistently low and fluctuating hen-day throughout the experimental period, with their performance ranging from the lowest hen-day of 65.0 ± 1.8 % to the highest value of 69.3 ± 0.7 % hen-day during the 5^{th} and 2^{nd} week, respectively. The overall

hen-day for the control layers was 68.3 ± 1.1 %, which was significantly lower ($P < 0.05$) than that recorded in any of the experimental groups.

Table4.1.4:Effects of Lycopene and Vitamin E on Thermal Stress-induced Changes inHen-Day Performance Indices of ISA Brown Layers (%)During the Harmattan Season (mean ± SEM; n = 100 per group)

	Layer group			
Week	L+VE	L	VE	CONT.
1.	77.7±0.9[a] (73.0 - 80.0)	75.1 ± 1.3[a] (70.0 - 80.0)	76.6 ± 2.2[a] (69.0 - 84.0)	68.6 ± 1.5[b] (65.0 - 77.0)
2.	77.5 ± 1.2[a] (73.0 - 82.0)	74.4±1.2[a] (70.0 - 80.0)	74.6 ± 1.1[a] (70.0 - 80.0)	69.3 ± 0.7[b] (67.0 -72.0)
3.	80.0 ± 1.1[a] (75.0 - 84.0)	75.7 ± 0.7[b] (74.0 -78.0)	73.0 ± 0.7[b] (70.0 -75.0)	69.1 ± 0.5[c] (68.0 -71.0)
4.	79.0 ± 1.4[a] (72.0 - 84.0)	74.0 ± 1.5[b] (68.0 - 81.0)	73.4 ± 1.5[b] (67.0 -78.0)	69.6 ± 1.0[c] (65.0 -72.0)
5.	74.1 ± 1.4[a] (72.0 - 82.0)	72.0 ± 1.3[a] (68.0 - 78.0)	71.1 ± 1.6[a] (66.0 -78.0)	65.0 ± 1.8[b] (60.0 -70.0)
Overall mean ± SEM	78.0 ± 1.2[a]	74.2 ± 1.2[b]	74.0 ± 1.4[b]	68.3 ± 1.1[c]

a, b, c = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum hen-day egg production
L +VE= lycopene + vitamin E, L= lycopene only, E= vitamin E only and CONT= control.

4.1.5 Weekly fluctuations in cloacal temperature (^{o}C) of ISA Brown laying hens during the harmattan season

The present study showed that CT of ISA brown layers varied within and between times of the day (Tables 4.1.5-8). Layers administered with L+VE demonstrated a relatively higher CT, compared to those in other groups, irrespective of the time of day. Thus, a maximum CT of 42.0 ^{o}C was recorded in L+ VE layers at 09:00 h during the 3rd week of the study, while a minimum CT value of 40.30 ^{o}C was recorded at 09:00 h in weeks 1 and 3. Variation within this range was evident, but there was no significant ($P > 0.05$) difference in CT at 09:00 h between weeks in the birds, administered with L+VE throughout the period of investigation. The overall mean value of CT in layers administered with L+VE at 09:00 h was 41.00 ± 0.06 ^{o}C (Table 4.1.5).

At 12:00 h, CT in L+VE group of layers varied between extreme maximum of 42.8 ^{o}C at week 5 and extreme minimum of 40^{o}C at week 1. In some cases, CT varied within this range significantly at noon. The overall mean value of CT at 12:00 h for laying hens in this group was 41.23 ± 0.06 ^{o}C and this value was not significantly different ($P > 0.05$) from that obtained at 9:00 h (Table 4.1.5). At 15:00 h, there was a significant ($P < 0.05$) variation in CT of layers administered with L+VE. It was observed that maximum CT in L+VE layers was 42.8 ^{o}C at week 5, and a minimum CT value of 40.0 ^{o}C was recorded in week 1. Generally, CTs at this time were significantly higher than other times of the day, and this difference was also observed within weeks of the study. The overall value of CT for L+VE layers at 15:00 h period was 41.43± 0.06 ^{o}C, which was higher than the mean value recorded for laying hens at 09:00 h (Table 4.1.5).

Table 4.1.5: Weekly Fluctuations in Cloacal Temperature (^{o}C) of ISA Brown Laying hens Administered with Lycopene and Vitamin E During the Harmattan Season (mean ± SEM; n = 20)

	Time of the day		
Week	09:00 h	12:00 h	15:00 h
1	40.81 ± 007[a] (40.30 - 41.10)	40.90 ± 0.11[a] (40.00 - 41.60)	41.29 ± 0.10[b] (40.00 - 41.70)
2	40.96 ± 0.06[a] (40.40 - 41.30)	41.03 ± 0.05[a] (40.40 - 41.40)	41.20 ± 0.08[a] (40.50 - 41.60)
3	41.02 ± 0.07[a] (40.30 - 42.00)	41.45 ± 0.04[b] (41.00 - 41.80)	41.66 ± 0.03[c] (41.30 - 42.00)
4	41.26 ± 0.05[a] (40.90 - 41.90)	41.54 ± 0.05[b] (41.10 - 41.90)	41.50 ± 0.06[b] (41.10 - 42.10)
5	41.01 ± 0.07[a] (40.50- 41.70)	41.39 ± 0.05[b] (41.10 - 42.00)	41.71 ± 0.09[c] (41.00 - 42.80)
6	40.92 ± 0.06[a] (40.70 - 41.60)	41.08 ± 0.04[a] (40.70 - 41.40)	41.23 ± 0.06[b] (40.80 - 41.70)
Overall mean ±SEM	41.00 ± 0.06	41.23 ± 0.06	41.43 ± 0.06

a, b, c = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperature.

4.1.6 Weekly fluctuations in cloacal temperature (^{o}C) in ISA Brown layers administered lycopene

The pattern of variation in CT for L-administered layers was similar to that recorded in L+VE-administered layers. The extreme maximum CT of 41.80 ^{o}C was recorded in laying hens at 9:00 h in week 3, while the extreme minimum value of 40.0 ^{o}C was recorded in wk 1 (Table 4.6). Although the CT varied between these limits, the variation was not significantly ($P > 0.05$) different from week-to-week at 9:00 h. The overall CT value of 41.0 ± 0.07 ^{o}C was recorded for L-administered birds at 9:00 h, which did not differ from L+VE-administered layers ($P > 0.05$) at the same experimental hour. At 12:00 h, CT in L-administered laying birds showed some variation between weeks; the variation was not significantly ($P > 0.05$) different during the period of the study. At this hour, the extreme maximum CT of 42.7 ^{o}C was recorded at week 5, while the extreme minimum CT of 40.10 ^{o}C was recorded at week 2. The overall value of CT in L-administered layers was 41.21 ± 0.06 ^{o}C, which was not different from that obtained in L+ VE laying birds (41.23 ± 0.06 ^{o}C), at the same hour of the recording (Tables 4.1.5 and 4.1.6).

The CT at 15:00 h in L-administered layers varied less between weeks; except in the 2nd and 3rd weeks, where significant ($P < 0.05$) variation was recorded during the hour of measurement (Table 4.6). The extreme maximum CT of 42.40 ^{o}C was recorded at week 5 of the study, while a minimum CT of 40.10 ^{o}C was obtained at week 4. The overall CT of 41.33 ± 0.06 ^{o}C was recorded for the layers in this group at 15:00 h. This pattern of variation in CT was not obtained in L+VE birds, where CT varied remarkably from week to week.

Table 4.1.6: Weekly Fluctuations in Cloacal Temperature (^{o}C) in ISA Brown Laying Hens Administered with Lycopene During the Harmattan Season (mean ± SEM; n = 20)

	Time of the day		
Week	09:00 h	12:00 h	15:00 h
1	40.59 ± 0.09^{a} (40.00 - 41.20)	41.05 ± 0.06^{b} (40.70 - 41.50)	41.12 ± 0.09^{b} (40.20 - 41.50)
2	40.80 ± 0.09^{a} (40.00 - 41.50)	41.02 ± 0.09^{a} (40.10 - 42.00)	41.06 ± 0.07^{a} (40.40 - 41.50)
3	41.17 ± 0.06^{a} (40.70- 41.80)	41.36 ± 0.04^{b} (40.10 - 41.70)	41.55 ± 0.05^{c} (41.20 - 42.20)
4	40.98 ± 0.07^{a} (40.30 - 41.70)	41.33 ± 0.05^{b} (41.00 - 41.80)	41.42 ± 0.05^{b} (40.30 - 42.10)
5	41.09 ± 0.05^{a} (40.40 - 41.60)	41.45 ± 0.09^{b} (41.00 - 42.70)	41.57 ± 0.07^{b} (41.20 - 42.40)
6	41.02 ± 0.05^{a} (40.60 - 41.60)	41.03 ± 0.06^{a} (40.40 - 41.60)	41.24 ± 0.05^{b} (40.90 - 41.60)
Overall mean ±SEM	40.94 ± 0.07^{a}	41.21 ± 0.06^{b}	41.33 ± 0.05^{b}

a, b, c = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperature.

4.1.7 Weekly fluctuations in cloacal temperature (^{o}C) in ISA Brown laying Hens administered with vitamin E during the harmattan season

Vitamin E-administered laying hens demonstrated a relatively uniform CT between weeks at09:00 h.

The extreme maximum CT of 41.80 ^{o}C was recorded in this group and extreme minimum mean CT value of 40.0 ^{o}C was recorded at week 1 (Table 4.1.7). At the end of the study, an overall mean CT value of 41.0 ± 0.07 ^{o}C recorded at 9:00 h in the layers was not significantly ($P > 0.05$) different from that of L+VE or L-administered layers, measured at the same hour. At 12:00 h, VE-administered birds showed no significant variation in CT except at week 2 (Table 4.7). Extreme maximum CT of 42.10 ^{o}C was recorded at week 4 and extreme minimum CT of 40.6 ^{o}C was obtained at week 6. The overall mean value of CT (41.40 ± 0.07 ^{o}C) recorded at 09:00 h was not different ($P > 0.05$) from those recorded in L+VE and L at the same hour.

Table 4.1.7:Weekly Fluctuations in Cloacal Temperature (^{o}C) in ISA Brown Laying Hens Administered with Vitamin E During the Harmattan Season (mean ± SEM; n=20)

Week	Time of the day		
	09:00 h	12:00 h	15:00 h
1	40.78 ± 0.12^{a} (40.00 - 41.80)	41.16 ± 0.09^{b} (40.50 - 41.80)	40.75 ± 0.07^{a} (40.20 - 41.10)
2	40.94 ± 0.08^{a} (40.20 - 41.60)	41.03 ± 0.06^{a} (40.50 - 41.50)	41.01 ± 0.09^{a} (40.20 - 41.70)
3	41.28 ± 0.04^{a} (41.00 - 41.70)	41.68 ± 0.04^{b} (41.50 - 42.10)	41.62 ± 0.04^{b} (41.62- 41.90)
4	40.94 ± 0.06^{a} (40.50- 41.60)	41.43 ± 0.04^{b} (41.00 - 41.90)	41.23 ± 0.04^{c} (40.90 - 41.50)
5	40.98 ± 0.05^{a} (40.70 - 41.50)	41.63 ± 0.08^{b} (41.10 - 42.80)	41.76 ± 0.07^{b} (41.20 - 42.60)
6	40.88 ± 0.07^{a} (40.10 - 41.40)	41.25 ± 0.07^{b} (40.60 - 42.00)	41.14 ± 0.07^{b} (40.60 - 41.50)
Overall mean ± SEM	41.00 ± 0.07^{a}	41.40 ± 0.07^{b}	41.25 ± 0.07^{b}

a, b, c = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses are minimum and maximum cloacal temperatures.

4. 1.8 Weekly fluctuations in cloacal temperature (^{o}C) in control ISA Brown laying hens during the harmattan season

A close assessment of CT values in control group of layers was consistently lower, compared to the values recorded in experimental groups from week to week. This consistent disparity in CT was more pronounced at 09:00 h and 12:00 h (Table 4.1.8). A maximum CT of 41.30 ^{o}C was obtained at 09:00 h in week 2 of the study and a minimum CT of 39.6 ^{o}C in week 1. There was no significant difference ($P > 0.05$) between week variations in CT in this group at this time. The overall CT of 40.65 ± 0.08 ^{o}C was obtained in the control layers at 09:00 h and this value was lower than those obtained in all the experimental groups. At 12:00 h, CT in control layers did not show significant variation between weeks throughout the study period (Table 4.1.8). The extreme maximum of 41.06 ^{o}C was obtained at week 6 of the study, with the extreme minimum value of 40.00 ^{o}C in week 2. The overall mean CT of 40.95 ± 0.08^{o}C, was obtained in the group at this time, and this value was lower than CT value recorded in any of the experimental groups. The CT at 15:00 h showed a maximum value of 42.10 ^{o}C at weeks 4 and 5. A minimum value of 40.83 ^{o}C was recorded for the birds at week 2. The overall CT value of 41.33 ± 0.06 ^{o}C obtained at the end of the study was not significantly different from those of the experimental groups.

Table 4.1.8:Weekly Fluctuations in Cloacal Temperature (^{o}C) in Control ISA Brown Laying hens During the Harmattan Season (mean±SEM; n=20)

	Time of the day		
Week	9:00 h	12:00 h	15:00 h
1	40.34 ± 0.13^{a} (39.60 - 41.30)	40.95 ± 0.06^{a} (40.60 - 41.20)	41.01 ± 0.06^{b} (40.50 - 41.50)
2	40.66 ± 0.09^{a} (39.90 - 41.30)	40.87 ± 0.07^{a} (40.00 - 41.30)	40.83 ± 0.09^{a} (40.00 - 41.60)
3	40.65 ± 0.07^{a} (40.65- 41.20)	40.83 ± 0.11^{a} (40.83- 41.40)	41.68 ± 0.04^{b} (41.68 - 42.00)
4	40.89 ± 0.09^{a} (40.10 - 41.80)	41.05 ± 0.09^{a} (40.20 - 41.50)	41.56 ± 0.05^{b} (41.30 - 42.10)
5	40.66 ± 0.09^{a} (40.00 - 41.20)	41.06 ± 0.08^{b} (40.00 - 41.50)	41.61 ± 0.05^{c} (41.30 - 42.10)
6	40.75 ± 0.06^{a} (40.20 - 41.20)	40.95 ± 0.08^{a} (40.40 - 42.00)	41.29 ± 0.07^{b} (40.80 - 42.00)
Overall mean ± SEM	40.65 ± 0.08^{a}	40.95 ± 0.08^{a}	41.33 ± 0.06^{b}

a, b, c = Values with different superscript letters are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperatures.

4. 1.9 Modulatory roles of lycopene and vitamin E on concentrations of thyroid hormones during the harmattan season

The concentrations of T_4 varied between experimental and control birds, although the variation was insignificant ($P > 0.05$). Co-administration of lycopene and vitamin E in the layers resulted in the highest mean concentration of T_4 (3.1 ± 0.24 nmol/L). The T_4 values of 2.5 ± 0.9 nmol/L and 1.7 ± 0.7 nmol/L were obtained in lycopene- and vitamin E-administered layers, respectively. The values were greater ($P < 0.05$) than the value of 1.3 ± 0.1 nmol/L, obtained in the control layers. The triiodothyronine concentration of 12.0 ± 1.8 nmol/L, recorded in layers co-administered with lycopene and vitamin E was significantly ($P < 0.05$) higher than the value of 5.70 ± 1.0 nmol/L obtained in control layers. The triiodothyronine concentrations of 7.80 ± 2.5 nmol/L and 7.70 ± 1.5 nmol/L, recorded in lycopene- and vitamin E-administered layers were not significantly ($P > 0.05$) different from the concentration obtained in layers co-administered with lycopene and vitamin E. Thyroid stimulating hormone showed a rather irregular pattern of variation in its concentration, which was not significantly different ($P > 0.05$) between the groups of layers (Table 4.1.9). The highest concentration of thyroid-stimulating hormone of 3.6 ± 0.5 nmol/L was obtained in lycopene-administered birds, followed by that of 2.9 ± 2.0 nmol/L obtained in vitamin E-treated laying hens. The thyroid-stimulating hormone concentration of 2.4 ± 0.5 nmol/L and 1.84 ± 0.6 nmol/L were recorded in the control, and L+ VE co-administered layers, respectively.

Table 4.1.9:Modulatory Roles of Lycopene and Vitamin E on Concentrations of Thyroid Hormones in ISA Brown Layers During the Harmattan Season (mean ± SEM; n = 10 Per Group)

	Layer group			
Hormone ($nmolL^{-1}$)	L+VE	L	VE	CONT.
Thyroxine	3.1 ± 0.24^{a}	2.5 ± 0.9^{a}	1.7 ± 0.7^{b}	1.3 ± 0.1^{b}
Triiodothyronine	12.0 ± 1.8^{a}	7.8 ± 2.5^{b}	7.7 ± 1.5^{b}	5.72 ± 1.0^{c}
Thyroid stimulating hormone	1.84 ± 0.6^{a}	3.6 ± 0.5^{b}	2.9 ± 2.0^{b}	2.4 ± 0.5^{a}

a, b, c = Values with different superscript letters are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

4.1.10 Antioxidant modulation of plasma oestrogen and progesterone (ng/mL) in ISA Brown laying hens

Results in the present study showed a marked depression in the secretion of both oestrogen and progesterone in control laying hens during the harmattan season. Plasma oestrogen concentration was significantly ($P < 0.05$) higher in experimental laying birds, administered with antioxidants, with the values of 123.0 ± 40.0 ng/mL, 105.0 ± 37.0 ng/mL and 97.0 ± 45.0 ng/mL in L+VE, L and VE, respectively, while the control laying hens recorded the value of 72.0 ± 25.0 ng/mL during the study period. Plasma progesterone level decreased ($P < 0.05$) in the control laying hens (0.36 ± 0.10 ng/mL), compared with the values of 1.50 ± 0.35 ng/mL, 1.20 ± 0.07 ng/mL and 0.65 ± 0.08 ng/mL, obtained in L+VE, L and VE groups, respectively.

Table4.1.10:Antioxidant Modulation of Plasma Oestrogen and Progesterone (ng/mL) in ISA Brown Laying Hens During the Harmattan Season (mean ± SEM; n = 10)

	Layer group			
Hormone	L+VE	L	VE	CONT
Oestrogen	123.0 ± 40.0^{a}	105.0 ± 37.0^{a}	97.0 ± 45.0^{a}	72.0 ± 25.0^{b}
Progesterone	1.50 ± 0.35^{a}	1.2 ± 0.07^{a}	0.65 ± 0.08^{b}	0.36 ± 0.10^{b}

a, b = Values with different superscript letters are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4. 1.11Eggshell diameter (mm) and eggshell weight (g) of ISA Brown layers administered with lycopene and vitamin E

External egg parameters: that is, the eggshell diameter and eggshell weight in the present study varied between the groups. This variation was more pronounced in eggshell diameter than eggshell weight (Table 4.1.11). During the 1st week of the study, eggshell diameter showed significant difference ($P < 0.05$) between the experimental and control groups. The values of 0.35 ± 0.02 mm, 0.30 ± 0.02 mm and 0.30 ± 0.02 mm were recorded in L+VE, Land VE, respectively; the values were significantly higher ($P < 0.05$) than 0.20 ± 0.06 mm, recorded in control laying hens. The overall mean values were: 0.30 ± 0.02 mm, 0.27 ± 0.03 mm, 0.25 ± 0.03 mm and 0.18 ± 0.02 mm in L+VE, L, VE and CONT laying hens, respectively (Table 4.1.11).

Table 4.1.11:Eggshell Diameter (mm) and Eggshell Weight (g) of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Harmattan Season (n = 10)

	Week				
Group	1	2	3	4	Overall
L+VE	0.35 ± 0.02^{a} (7.0 ± 0.2)	0.25 ± 0.04^{a} (8.0 ± 0.3)	0.30 ± 0.06^{a} (7.4 ± 0.2)	0.30 ± 0.02^{a} (7.5 ± 0.2^{a})	0.30 ± 0.02 (7.5 ± 0.2)
L	0.30 ± 0.02^{a} (7.0 ± 0.2)	0.20 ± 0.04^{a} (7.0 ± 0.3)	0.25 ± 0.03^{a} (7.5 ± 0.3)	0.32 ± 0.01^{a} (7.1 ± 0.2)	0.27 ± 0.03 (7.2 ± 0.3)
VE	0.30 ± 0.02^{a} (7.0 ± 0.2)	0.25 ± 0.04^{a} (7.3 ± 0.2)	0.20 ± 0.02^{b} (7.2 ± 0.4)	0.25 ± 0.02^{b} (7.7 ± 0.3)	0.25 ± 0.03 (7.3 ± 0.3)
CONT	0.20 ± 0.06^{b} (7.0 ± 0.2)	0.16 ± 0.04^{b} (7.0 ± 0.2)	0.15 ± 0.02^{b} (7.0 ± 0.4)	0.20 ± 0.01^{b} (6.0 ± 0.2^{b})	0.18 ± 0.02 (7.0 ± 0.3)

a, b, c = Values with different superscript between layer groups are significantly ($P < 0.05$) different.

Data in parentheses represent eggshell weight

L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4.1.12 Internal egg parameters of ISA Brown layers administered with lycopene and vitamin E in week 1

The results indicated that the harmattan season exerted influence on internal and external egg parameters throughout the experimental period. During the first week, it was demonstrated that yolk weight in L+VE group of layers was the highest, with the value of 20.0 ±1.0 g, followed by that of L group with the value of 19.0 ±1.2 g. The values were significantly higher ($P < 0.05$) than 17.0 ± 0.5 and 15.0 ± 0.4 g recorded in VE and CONT laying hens, respectively. Albumen weight was higher ($P < 0.05$) in antioxidant-administered layers than in the controls. Values of 38.0 ± 1.5 g, 38.0 ±1.4 g and 36.0 ± 1.0 g recorded in laying hens, compared with the value of 34.0 ± 0.3 g recorded in CONT group of laying hens during the period.

Values of albumen width and albumen height were also higher in experimental birds compared with the controls, following the same trend as albumen weight (Table 4.1.12). There was no significant difference ($P > 0.05$) in percent yolk weight and percent albumen weight within the week; however, percent yolk weight recorded relatively higher values in experimental birds.

Values of egg weight (67.0 ± 2.0 g, 63.0 ± 0.6 g and 62.0 ± 1.6 g, respectively) recorded in L+VE, L and VE groups of laying hens were significantly higher ($P < 0.05$) than 58.0 ± 1.2 g egg weight, recorded in control laying hens (Table 4.1.12).

Table 4.1.12:Internal Egg parameters of ISA Brown Layers Administered with Lycopene andVitamin E During the Harmattan Season (means ± SEM; n = 10 per group)

Week 1	Layer group			
Egg parameter	L+ VE	L	VE	CONT
Yolk weight (g)	20.0 ±1.0[a]	19.0 ± 1.2[a]	17.0 ± 0.5[b]	15.0 ± 0.4[c]
Yolk width (cm)	5.0 ± 0.1	4.3 ± 0.2	4.0 ± 0.1	4.0 ± 0.08
Yolk height (cm)	2.0 ± 0.03[a]	1.7 ± 0.05[a]	1.8 ± 0.03[a]	1.5 ± 0.02[b]
Albumen weight (g)	38.0 ± 1.5[a]	38.0 ± 1.4[a]	36.0 ± 1.0[b]	34.0 ± 0.3[c]
Albumen width (cm)	7.7 ± 0.13[a]	7.5 ± 0.2[a]	6.0 ± 0.10[b]	5.5 ± 0.06[b]
Albumen height (cm)	0.8 ± 0.03[a]	0.7 ± 0.04[a]	0.7 ± 0.03[a]	0.4 ± 0.06[b]
Bloodspot (frequency)	nil	nil	nil	nil
% Yolk weight	30.0	30.0	27.4	26.0
% Albumen weight	57.0	60.0	58.0	58.0
Egg weight (g)	67.0 ± 2.0[a]	63.0 ± 0.6[b]	62.0 ± 1.6[b]	58.0 ± 1.2[c]

a, b, c = Values with different superscripts between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4.1.13Internal egg parameters of ISA Brown layers administered lycopene and vitamin E in week 2

In week 2, yolk weight had the values of 18.0 ± 0.3 g and 17.7 ± 0.4 g in L+ VE and L laying hens respectively, which were significantly higher ($P < 0.05$) than the value of 16.4 ± 0.6 g recorded in VE laying hens. The values were significantly higher ($P < 0.05$) than that obtained in control laying hens (13.1 ± 0.3 g). The trend persisted in the results in albumen weight, which had the values of 38.0 ± 1.5 and 38.0 ± 1.4 g in L+VE and L laying hens, respectively; the value of 36.0 ± 1.0 g was recorded in control laying hens, which was lower than ($P < 0.05$) the corresponding values in L+VE and L. Albumen height were significantly higher ($P < 0.05$) in experimental groups of laying hens than the controls (Table 4.1.13).

Egg weight values of 63.0 ± 1.5 g, 63.0 ± 1.6 g and 62.0 ± 2.2 g were recorded in L+VE, L and VE, respectively; the values were significantly different ($P < 0.05$) from 58.0 ± 1.3 g recorded in control laying hens (Table 4.1.13).

Table 4.1.13:Internal Egg Parameters of ISA Brown Layers Administered Lycopene and Vitamin EDuring the Harmattan Season (means ± SEM; n = 10)

Week 2	Layer group			
Egg parameter	L+VE	L	VE	CONT
Yolk weight (g)	18.0 ± 0.3[a]	17.7 ± 0.4[a]	16.4 ± 0.6[b]	13.1 ± 0.3[c]
Yolk width (cm)	4.5 ± 0.1	4.2 ± 0.5	4.2 ± 0.5	4.0 ± 0.7
Yolk height (cm)	1.9 ± 0.03[a]	1.8 ± 0.02[a]	1.7 ± 0.03[a]	1.5 ± 0.02[b]
Albumen weight (g)	38.0 ± 1.5[a]	38.0 ± 1.4[a]	38.0 ± 1.0[a]	36.0 ± 1.4[b]
Albumen width (cm)	7.2± 0.08	7.2 ± 0.08	7.0 ± 0.10	7.1 ± 00.6
Albumen height (cm)	0.7 ± 0.03[a]	0.7 ± 0.04[a]	0.7 ±0.03[a]	0.5 ± 0.03[b]
Bloodspot (frequency)	nil	nil	Nil	nil
% Yolk weight	28.5[a]	27.6[a]	26.5[b]	22.6[c]
% Albumen weight (g)	59.4	60.3	61.3	62.0
Egg weight (g)	63.0 ± 1.5[a]	63.0 ± 1.6[a]	62.0 ± 2.2[a]	58.0 ± 1.3[b]

a, b, c = Values with different superscript between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4.1.14Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E in week 3

During the 3rd week of the study, yolk weight values in L+VE and L groups of layers were 17.3 ± 0.2 g and 17.2 ± 0.6 g, respectively. The values were higher ($P < 0.05$) than the value of 16.0 ± 0.5 g, recorded in control laying hens (Table 4.1.14). Within the week, there was no significant ($P > 0.05$) difference between groups of layers in yolk width and yolk height; although, the experimental birds recorded relatively higher values. Albumen weight showed a higher trend in experimental birds, the values (38.0 ± 0.8, 37.6 ± 0.8 and 37.6 ± 1.3 g, respectively) were significantly higher ($P < 0.05$) than 36.0 ± 0.9 g, recorded in control laying hens. There was no significant difference ($P > 0.05$) in albumen width and albumen height in all groups within the week. Egg weight showed significant ($P < 0.05$) difference between groups, with L+VE and L groups recording values of 63.4 ±1.6 g and 63.5 ± 1.6 g, respectively. The values were significantly ($P < 0.05$) higher than 60.2 ±1.6 g and 60.0 ±1.0 g recorded in VE and CONT laying hens, respectively (Table 4.1.14)

Table 4.1.14: Internal Egg Parameters of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Harmattan Season (means ± SEM; n = 10)

Week 3	Layer group			
Egg parameter	L+ VE	L	VE	CONT
Yolk weight (g)	17.3 ± 0.2[a]	17.2 ± 0.6[a]	15.3 ± 0.6[b]	16.0 ± 0.5[b]
Yolk width (cm)	4.2 ± 0.05	4.2 ± 0.05	4.2 ± 0.06	4.0 ± 0.08
Yolk height (cm)	1.7 ± 0.03	1.7 ± 0.03	1.7 ± 0.03	1.5 ± 0.03
Albumen weight (g)	38.0 ± 0.8[a]	37.8 ± 0.8[a]	37.6 ± 1.3[a]	36.0 ± 0.9[b]
Albumen width (cm)	7.2 ± 0.08	7.0 ± 0.08	7.1 ± 0.10	7.1 ± 0.06
Albumen height (cm)	0.7 ± 0.05	0.7 ±0.03	0.7 ±0.05	0.7 ± 0.04
Bloodspot (frequency)	nil	nil	Nil	nil
% Yolk weight	27.3	27.3	25.5[b]	27.0
% Albumen weight	60.0[a]	59.5[a]	62.5[b]	60.0[a]
Egg weight (g)	63.4 ± 1.6[a]	63.5 ± 1.6[a]	60.2 ± 1.6[b]	60.0 ± 1.0[b]

a, b = Values with different superscript between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4.1.15Internal egg parameters of ISA Brown laying hens administered with lycopene and vitaminE in week 4

Results of egg parameters in the last week of the study are presented in Table 4.1.15. Results showed a significant difference ($P < 0.05$) in yolk weight between experimental laying hens and the controls. The values of 20.0 ± 0.3 g, 19.6 ± 0.5 g and 19.3 ± 0.5 g were obtained in L+VE, L and VE groups, respectively, which were significantly higher ($P < 0.05$) than that recorded in CONT laying hens (16.3 ± 0.5 g). In this week, yolk height also showed a significantly higher ($P < 0.05$) performance in experimental groups than in the control (L+VE: 1.8 ± 0.02 cm; L: 1.6 ± 0.03 cm; VE: 1.6 ± 0.03cm versus CONT: 1.40 ± 0.03 cm). There was uniformity in albumen weight in L+VE, L and VE groups, with values of 0.8 ± 0.04 g, 0.8 ± 0.04 g and 0.8 ± 0.03 g , respectively which were all significantly higher ($P < 0.05$) than 0.6 ± 0.05 g, recorded in control laying hens. Egg weight in the week varied significantly ($P < 0.05$) between experimental and control birds. The values of 65.0 ± 0.8 g, 65.0 ± 0.8 g and 65.0 ± 0.6 g were recorded in L+VE, L and VE, respectively and were significantly higher ($P < 0.05$) than 59.0 ± 1.0 g, obtained in CONT (Table 4.1.15). During the study, the trend in percent yolk weight and albumen weight did not show any variation; however, the tendency was a higher percentage weight in the values in experimental groups.

Table 4.1.15:Internal Egg Parameters of ISA Brown Laying hens Administered withLycopene and Vitamin E During the Harmattan Season (means ± SEM; n = 10 per group)

Week 4	Layer group			
Egg parameter	L+ VE	L	VE	CONT
Yolk weight (g)	20.0 ± 0.3[a]	19.6 ± 0.5a	19.3 ± 0.5[a]	16.3 ± 0.5[c]
Yolk width (cm)	4.3 ± 0.03	4.2 ± 0.05	4.2 ± 0.07	4.1 ± 0.08
Yolk height (cm)	1.8 ± 0.02[a]	1.6 ± 0.03[b]	1.6 ± 0.03[b]	1.40 ± 0.03[c]
Albumen weight (g)	38.0 ± 0.7[a]	37.4 ± 1.3[a]	37.2 ± 1.0[a]	35.2 ± 0.9[b]
Albumen width (cm)	7.3 ± 0.05	7.0 ± 0.08	7.0 ± 0.10	7.1 ± 0.06
Albumen height (cm)	0.8 ± 0.04[a]	0.8 ± 0.04[a]	0.8 ± 0.03[a]	0.6 ± 0.05[b]
Blood spot (frequency)	nil	nil	Nil	nil
% Yolk weight	30.8[a]	29.2[b]	29.7[b]	27.6[c]
% Albumen weight	58.5	57.5	57.8	59.6
Egg weight (g)	65.0 ± 0.8[a]	65.0 ± 0.8[a]	65.0 ± 0.6[a]	59.0 ± 1.0[b]

a, b, c = Values with different superscript between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT = control layers.

4.2 Hot-Dry Season

4.2. 1 Weekly diurnal fluctuations in dry-bulb temperature (^{o}C) during the hot-dry season

The meteorological data in the present study show significant diurnal variation in dry-bulb temperature (DBT) between 06:00 h and 18:00 h daily during the study period (Table 4.2.1). Comparatively, weekly mean DBT values were significantly ($P < 0.05$) lower during the morning hours between 06:00 h and 09:00 h than 12:00 h, 15:00 h or 18:00 h; and this pattern of DBT variation was consistent throughout the experimental period. The lowest DBT of 24.3 ± 0.5^{o}C was recorded at 06:00 h during the fourth week of the study, which was significantly ($P < 0.05$) different from the corresponding value of 29.43 ± 0.4^{o}C, recorded at 9:00 h in the 3rd week of the study (Table 4.2.1). The result of the study showed that DBT varied between 6:00 h and 9:00 h; although the variations were not significantly ($P > 0.05$) different within time of the day, there was significant difference ($P < 0.05$) between time of the day. The thermal environmental data of DBT varied at 12:00 h, 15:00 h and 18:00 h, and the values were significantly ($P < 0.05$) higher than those of the morning hours. The lowest value of 31.4 ± 0.3^{o}C was recorded at 12:00 h during the 5th week of the study, while the highest DBT value of 36.71 ± 0.6^{o}C was obtained at 18:00 h during the 1st week. There was a significant ($P < 0.05$) variation in DBT values at each hour of the day throughout the experimental period. Cases of heat-induced distress were observed in behavioural responses of birds to the prevailing inclement in thermal environmental conditions, as the laying birds panted frequently, most often from 10:00 h of the day. The intensity of the panting distress (polypnea) rose remarkably between the hours of 12:00 h and 18:00 h of the day.

In summary, the overall weekly DBT ranged between the lowest value of 24.3 ± 0.5^{o}C at 06:00 h and the highest value of 36.7 ± 0.6^{o}C at 18:00 h, showing an increasing trend within the range and time limits.

Table4.2.1: Weekly Diurnal Fluctuations in Dry-bulb Temperature (^{o}C) During the Hot-Dry Season

	Time, h				
Week	06:00	09:00	12:00	15:00	18:00
1	26.0 ± 0.38^{a} (25.0 - 27.0)	27.3 ± 0.4^{a} (25.0 -28.0)	32.6 ± 0.6^{b} (30.0 -35.0)	35.43 ± 0.8^{c} (32.0 -39.0)	36.71 ± 0.6^{c} (35.0 -39.0)
2	25.3 ± 0.4^{a} (24.0 - 27.0)	27.6 ± 0.5^{b} (26 -30)	32.3 ± 0.4^{c} (30.0 -33.0)	34.71 ± 0.5^{d} (33.0 - 36.0)	34.71 ± 0.5^{d} (33.0 -36.0)
3	25.71 ± 0.5^{a} (24.0 - 27.0)	29.43 ± 0.4^{b} (28 -31.0)	33.14 ± 0.5^{c} (32.0 -35.0)	35.0 ± 0.5^{d} (33.0 -37.0)	36.57 ± 0.5^{e} (35.0 -39.0)
4	24.3 ± 0.5^{a} (23.0– 26.0)	28.1 ± 0.7^{b} (25.0 -31.0)	31.6 ± 0.6^{c} (28.0 -33.0)	34.0 ± 0.3^{d} (33.0 -35.0)	34.57 ± 0.3^{d} (33.0 -35.0)
5	26.3 ± 0.5^{a} (24.0 - 27.0)	28.1 ± 0.8^{b} (25.0 -31.0)	31.4 ± 0.3^{c} (30.0 -32.0)	32.6 ± 0.3^{c} (31.0 -33.0)	33.0 ± 0.5^{c} (31 -35.0)
Overall Mean± SEM	25.5 ± 0.5^{a}	28.1 ± 0.6^{b}	32.2 ± 0.5^{c}	34.3 ± 0.5^{d}	35.0 ± 0.5^{d}
Range	(23.0 - 27.0)	(25.0 - 31.0)	(28.0 - 35.0)	(31.0 -39.0)	(31 - 39)

a, b, c, d, e = Values with different superscriptare significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum dry-bulb temperature.

Table 4.2.2:Temperature-humidityindex (THI; ^{o}C) in Poultry Pen During the Hot-DrySeason

	Time, h				
Week	06:00	09:00	12:00	15:00	18:00
1	25.4 ± 0.4^{a} (24.20-26.20)	26.1 ± 0.3^{a} (24.60-26.80)	30.6 ± 0.4^{b} (28.80-31.80)	32.5 ± 0.7^{c} (30.0-35.40)	33.0 ± 0.5^{d} (31.40-35.0)
2	24.5 ± 0.3^{a} (23.20-25.80)	26.4 ± 0.4^{b} (24.80-28.0)	30.1 ± 0.4^{c} (28.40-31.0)	32.0 ± 0.3^{d} (31.0-32.80)	31.8 ± 0.5^{d} (30.20-33.60)
3	24.6 ± 0.8^{a} (22.0-26.60)	28.0 ± 1.7^{b} (27.20-28.40)	31.0 ± 0.4^{c} (30.0-32.20)	33.3 ± 0.4^{c} (30.80-33.40)	33.2 ± 0.6^{d} (31.0-35.40)
4	23.1 ± 0.5^{a} (21.80-24.80)	27.0 ± 0.6^{b} (24.20-29.0)	29.2 ± 0.4^{c} (26.80-30.40)	31.0 ± 0.4^{d} (30.20-32.60)	31.7 ± 0.2^{d} (30.80-32.60)
5	25.3 ± 0.5^{a} (23.20-26.20)	27.0 ± 0.7^{b} (24.20-29.40)	29.5 ± 0.3^{c} (28.40-30.40)	30.3 ± 0.2^{d} (29.40-31.0)	30.5 ± 0.4^{d} (28.60-32.20)
6	24.6 ± 0.3^{a} (23.20-25.20)	26.1 ± 0.3^{b} (24.80-26.80)	28.5 ± 0.3^{c} (26.80-29.40)	29.3 ± 0.4^{c} (27.40-30.20)	29.4 ± 0.3^{d} (28.0-30.60)
Overall mean ± SEM	24.6 ± 0.5^{a}	26.8 ± 0.6^{b}	29.8 ± 0.4^{c}	31.4 ± 0.4^{d}	31.6 ± 0.4^{d}

a, b, c, d = Values of THI values with different superscript letters are significantly ($P < 0.05$) different.

Data in parentheses represent minimum and maximum dry-bulb temperature.

4.2.2 Weekly diurnal fluctuations in temperature-humididy index (^{o}C) during the hot-dry season

Temperature-humidity index in the season showed that thermal load on the laying hens was significant, especially between 09:00 h and 18:00 h. Duringthe study period, the extreme minimum THI (24.20 ^{o}C) was obtainedat 9:00 h in week 4, while the extreme maximum THI (29.40 ^{o}C) was recorded in week 5 at the same time.At 12:00 h extreme minimum THI (26.80 ^{o}C) was recorded at the 4th week of the study; the extreme maximum value (32.2 ^{o}C) was obtained at the 3rd week. Results showed that the extreme minimum THI value of 27.40 ^{o}C was recorded at 15:00 h during the 6th week while the extreme maximum value of 35.40 ^{o}C was obtained in the 1st week. The extreme minimum and extreme maximum values of 28.0 ^{o}C and 35.40 ^{o}C were recorded in the 6th and 3rd week, respectively. The overall THI means (24.6 ± 0.5, 26.8 ± 0.6, 29.8 ± 0.4, 31.4 ± 0.4 and 31.6 ± 0.4) increased significantly ($P < 0.05$) from 06:00 h -18:00 h, respectively (Table 4.2.2)

4.2. 3 Fluctuations in heat index (^{o}C) in the pen during the hot-dry season

Results of thermal index showed heat increments from 06:00 h to 18:00 h throughout the experimental period. Thermal indices at 06:00 h were lowest, with the nadir value of 24.4 ^{o}C obtained at the 4th week of the study; the highest value of 27.3 ^{o}C was obtained at the 5th week. There was no significant ($P > 0.05$) difference in HI between weeks, but there were variations between the limits at this time. At 09:00 h, HI of 27.5 ^{o}C was the minimum during the 1st week. The maximum value of 30.0 ^{o}C was obtained at the 3rd week. Variations within the limits were observed, although between weeks versus time variation was not significant ($P > 0.05$). The overall HI mean was 28.42 ± 0.42 ^{o}C, which was significantly higher ($P < 0.05$) than that obtained at 06:00 h. Extreme minimum and maximum values were 27.5 and 30.0 ^{o}C, respectively.

At 12:00 h, the minimum HI value was 31.0 ^{o}C while the maximum value obtained was 32.2 ^{o}C; there was no significant difference ($P > 0.05$) between weeks in HI, although variations between the limits were evident. The overall HI at 12:00 h was 31.60 ± 0.2 ^{o}C; and the value was significantly ($P < 0.05$) higher than the value obtained at 6:00 h or 09:00 h (Table 3). At 15:00 h, the mean value of 33.4 ± 0.4 ^{o}C was significantly higher ($P < 0.05$) than those obtained at 06:00 h, 09:00 h and 12:00 h (Table 4,2 3). Values of HI at 15:00 h varied between the extreme minimum of 32.0 ^{o}C and the extreme maximum of 34.3 ^{o}C. There was no significant ($P > 0.05$) difference in the overall HI mean value between 15:00 h and 18:00 h. However, the value of 33.78 ± 0.2 ^{o}C, the highest between times of the day obtained at 18:00 h was higher than that recorded at 15:00 h (33.4 ± 0.2 ^{o}C). At 18:00 h, the lowest value of 32 ^{o}C and the highest value of 35 ^{o}C were remarkably higher ($P < 0.05$) than the corresponding values obtained at 06:00 h, 09:00 h and 12:00 h. Between the hours of 15:00 and 18:00, minimum heat index was 32.0 ^{o}C and that of maximum of 35.0 ^{o}C at 18:00 h was higher than 34.3 ^{o}C obtained at 15:00 h.

The overall pattern of severity of ambient temperature in the poultry pen, as depicted from HI, varied significantly ($P < 0.05$) with time of the day in the order: 06:00 < 09:00 h < 12:00 h < 15:00 h =18:00 h (Table 4.2.3). Although overall HI values between 15:00 h and 18:00 h were not significantly ($P > 0.05$) different, the value at 18:00 h was higher than that obtained at 15:00 h.

Table 4.2.3:Fluctuations in Heat Index (^{o}C) in the Pen During the Hot-Dry Season

	Time, h				
Week	06:00 h	09:00 h	12:00 h	15:00 h	18:00 h
1	26.5	27.5	32.2	34.3	34.8
2	26.0	28.0	31.8	33.8	33.7
3	26.0	30.0	31.8	34.1	35.0
4	24.4	28.3	31.0	32.7	33.4
5	27.3	28.3	31.2	32.0	32.0
Overall mean ± SEM	26.04 ± 0.47^{a}	28.42 ± 0.42^{b}	31.60± 0.2^{c}	33.4 ± 0.4^{d}	33.8 ± 0.5^{d}
	(24.4 - 27.3)	(27.5 - 30.0)	(31.0-3 2.2)	(32.0 -34.3)	(32.0-35.0)

a, b, c, d = Values of HI values with different superscript letters are significantly different ($P < 0.05$).

4.2.4 Effects of lycopene and vitamin E on thermal stress-induced changes in hen-day egg production

Data on the influence of daily supplements of antioxidants, lycopene and vitamin E on weekly hen-day egg production of ISA Brown layers are presented in Table 4.2.4. Supplemental administration of lycopene and α–tocopherol significantly ($P < 0.05$) increased daily egg production, which resulted in significant weekly egg output, when compared with the controls.

Data in Table 4.2.4 show the effect of lycopene and vitamin E on hen-day, supplemented alone and/or co-administered to layers. Layers which received a combination of the antioxidants maintained high laying hen-day, ranging from the lowest hen-day of 59.0 ± 1.2 % to the highest hen-day of 66.57 ± 1.2 % during the 4th and 3rd weeks, respectively. A minimum and maximum hen-day egg production value of 56.0 % and 72.0 %, respectively were obtained in laying hens during the period of the study. This group of layers had an overall mean of 62.7 ± 1.0 % hen-day at the end of the 6-week study. Layers which were daily supplemented with lycopene alone recorded weekly mean hen-day, ranging from the lowest hen-day of 59.14 ± 0.8 % to the highest hen-day of 65.1 ± 0.7 %, respectively during 1st and 3rd weeks. A minimum and maximum hen-day egg production value of 54.0 % and 69.0 %, respectively were obtained during the period of the study. The overall result of the hen-day for this group of layers at the end of the study was 62.40 ± 1.1 %, which was not different from the value recorded in the layers, co-administered with lycopene and vitamin E (Table 4.2.4).

In laying hens supplemented with vitamin E alone, weekly hen-day egg production ranged from the lowest value of 59.0 ± 1.9 % and the highest value of 65.3 ± 1.5 % hen-day during

the 1st and 3rd weeks of the study, respectively; a minimum and maximum hen-day egg production values of 52 % and 70 %, respectively were obtained during the period of the study. The overall mean of hen-day for this group of the hens was 62.1 ± 1.2 %, which was not significantly (P > 0.05) lower than that recorded for the laying hens co-administered with lycopene and vitamin E.

The control group of laying hens was characterised by a consistently low but stable hen-day throughout the experimental period, with their performance ranging from the lowest hen-day of 52.8 ± 0.6 % to the highest value of 57.3 ± 0.9 % hen-day during the 5th and 2nd weeks, respectively; a minimum and maximum hen-day egg production values of 50.0 % and 60.0 %, respectively were obtained during the period of the study. The overall hen-day mean for the control hens was 56.0 ± 0.8 %, and this value was lower (P < 0.05) than that recorded in any of the experimental groups.

4.2.5 Correlation co-efficient (Pearsons r) between thermal heat index and cloacal temperature

Pearson's correlation (r) values between CTs and THI demonstrated a significant relationship between both parameters in control laying hens, which were generally high throughout the experimental period. In contrast, except during the second week, L and VE, values in experimental hens were generally low and insignificant (Table 4.2.5).

Table 4.2.4:Effects of Lycopene and Vitamin E on Thermal Stress-induced Changes in Hen-day Egg Production (%) of ISA Brown Layers During the Hot-Dry Season (n=100 Per Group)

	Layer group			
WEEK	L+VE	L	VE	CONTROL
1	60.57 ± 0.7[a] (58.0 - 68.0)	59.14 ± 0.8[a] (56.0 - 61.0)	59.0 ± 1.9[a] (52.0 - 65.0)	57.29 ± 0.9[a] (53.0 - 60.0)
2	65.30 ± 0.6[a] (63.0 – 68.0)	62.86 ± 0.9[a] (59.0 – 66.0)	64.43 ± 1.2[a] (61.0-70.0)	56.70 ± 0.7[b] (54.0 – 59.0)
3	66.7 ± 1.5[a] (62.0 – 72.0)	65.10 ± 1.3[a] (60.0 -69.0)	65.3 ± 1.5[a] (58.0 – 70.0)	55.14 ± 0.5[b] (53.0 -57.0)
4	59.0 ± 1.2[a] (56.0 - 65.0)	62.40 ± 1.0[a] (59.0 – 66.0)	59.43 ± 1.0[a] (56.0 – 62.0)	52.8 ± 0.6[b] (50.0 - 55.0)
5	64.30 ± 0.9[a] (62.0 - 67.0)	63.30 ± 0.7[a] (60.0 - 65.0)	61.71 ± 0.9[a] (60.0 - 65.0)	57.0 ± 1.2[b] (53.0 – 60.0)
6	60.60 ± 0.8[a] (58.0 - 63.0)	61.30 ± 1.6[a] (54.0-65.0)	59.90 ± 0.7[a] (57.0 - 62.0)	56.71 ± 1.1[b] (52.0 - 60.0)
Overall mean ± SEM	62.7 ±1.0[a]	62.4±1.1[a]	62.1 ± 1.2[a]	56.0 ± 0.8[b]
Range	(56 – 72)	(54 - 69)	(52 - 70)	(50-60)

a, b = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum hen-day egg production.
L +VE= lycopene+ vitamin E, L= lycopene only, E=vitamin E only and CONT= control.

Table4.2.5: Correlation Co-efficient (Pearsons r) Between Thermal Heat Index and Cloacal Temperature of ISA Brown Laying Hens During the Hot-Dry Season (n = 20)

	Layer group			
Week	L+VE	L	VE	CONT.
1	0.174^{NS}	0.180^{NS}	0.432**	0.374*
2	0.276^{NS}	0.462**	0.473**	0.450**
3	-0.145^{NS}	0.039^{NS}	0.134^{NS}	0.493**
4	0.340*	0.033^{NS}	0.114^{NS}	0.501**
5	-0.013^{NS}	0.003^{NS}	0.239^{NS}	0.661***

NS = non significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001, n = 20.
L+VE = lycopene + vitamin E-administered laying hens; L = lycopene-administered laying hens; VE = vitamin E-administered laying hens; CONT = control laying hens.

4.2.6 Weekly diurnal fluctuations in cloacal temperature in L+VE-administered ISA Brown laying hens

CT of ISA Brown laying hens had relatively lesser variation within times of the day and between weeks in experimental laying hens, in comparison with the controls (Tables 4.2.3-4.2.6). A close observation at the results showed that laying hens administered L+VE demonstrated a relatively stable CT at 09:00 h and 12:00 h; significantly ($P < 0.05$) higher CTs were recorded at 15:00 h except at week 5 (Table 4.2.6). The lowest CT ($40.88 \pm 0.1^{o}C$) obtained in this group of laying hens at the 1st week at 09:00 h was not significantly different from $41.13 \pm 0.07^{o}C$, reported at 12:00 h during the same week. The values were significantly lower ($P < 0.05$) than the least mean CT, obtained at 15:00 h during the 1st week. Although the highest CTs of $41.59 \pm 0.08\ ^{o}C$ and $41.58 \pm 0.05\ ^{o}C$, recorded at 09:00 h and 12:00 h, respectively were not significantly different, they were lower than the CT value reported at 15:00 h ($41.79 \pm 0.09\ ^{o}C$).

Table 4.2.6: Weekly Diurnal Fluctuations in Cloacal Temperature (^{o}C) in L+VE-Administered ISA Brown Laying Hens During the Hot-Dry Season (mean ± SEM; n = 20)

	Time of the day, h		
Week	09:00	12:00	15:00
1	40.88 ± 0.1[a] (40.0 - 41.80)	41.13 ± 0.07[a] (40.0 - 41.40)	41.50 ± 0.09[b] (40.20 - 42.0)
2	41.23 ± 0.05[a] (40.90 - 41.80)	41.24 ± 0.05[a] (40.60 - 41.60)	41.56 ± 0.1[b] (40.70 - 42.10)
3	41.59 ± 0.08[a] (41.10 - 42.50)	41.58 ± 0.05[a] (41.10 - 41.90)	41.61 ± 0.09[a] (40.90 - 42.20)
4	41.43 ± 0.07[a] (40.70 - 42.10)	41.49 ± 0.04[a] (41.10 - 41.90)	41.70 ± 0.07[b] (41.20 - 42.40)
5	41.42 ± 0.04[a] (41.10 – 41.80)	41.43 ± 0.06[a] (40.90 - 41.80)	41.79 ± 0.09[b] (41.00 - 42.40)
Overall mean ± SEM	41.31 ± 0.07[a]	41.40 ± 0.05[a]	41.63 ± 0.09[b]
Range	(40.0 - 42.50)	(40.0- 41.90)	(40.20-42.40)

a, b = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperature.

4.2.7 Weekly diurnal fluctuations in cloacal temperature in lycopene-administered laying hens

Mean CT in lycopene-administered laying hens showed no significant variation between weeks at 09:00 h throughout the period of study. The lowest value of 41.11± 0.06 ^{o}C was recorded at week 1, while the highest value of 41.76 ± 0.1 ^{o}C was obtained at the 3rd week. At 12:00 h, the values were not different from their corresponding values of 41.22 ± 0.06 ^{o}C and 41.71 ± 0.05 ^{o}C, obtained as the lowest and highest CT at weeks 1 and 3, respectively. Overall, CT recorded at 09:00 h and 12:00 h were not significantly ($P > 0.05$) different. The pattern of CT at 15:00 h showed no remarkable variation between weeks, but the values were relatively higher than those recorded during the other hours of the day (Table 4.2.7).

Table 4.2.7:Weekly Diurnal Fluctuations in Cloacal Temperature (^{o}C) in L-Administered ISA Brown Laying HensDuring the Hot-Dry Season (mean ± SEM; n = 20 Birds Per Time of the Day)

	Time of the day, h		
Week	09:00	12:00	15:00
1	41.11 ± 0.06[a] (40.60 - 41.70)	41.22 ± 0.06[a] (40.80 - 41.90)	41.52 ± 0.08[b] (41.0- 42.30)
2	41.31 ± 0.05[a] (40.90 - 41.80)	41.63 ± 0.08[b] (41.20 - 42.60)	41.70 ± 0.09[b] (40.90- 42.50)
3	41.76 ± 0.1[a] (40.70- 42.70)	41.70 ± 0.06[a] (41.30- 42.30)	41.86 ± 0.08[a] (41.40- 42.40)
4	41.72 ± 0.1[a] (40.90 - 42.80)	41.71 ± 0.05[a] (41.30 - 42.20)	41.83 ± 0.07[a] (41.40- 42.50)
5	41.46 ± 0.06[a] (41.0- 42.10)	41.42 ± 0.09[a] (40.50- 42.10)	41.76 ± 0.08[b] (41.00- 42.50)
Overall mean ± SEM	41.50 ± 0.07[a]	41.50 ± 0.07[a]	41.73 ± 0.08[a]
Range	(40.60-41.72)	(40.50 - 42.60)	(40.90 - 42.50)

a, b = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperature.

4.2. 8 Weekly diurnal fluctuations in cloacal temperature in vitamin E-administered laying hens

In vitamin E-administered laying hens, there was no significant variation in CT at 09:00 h throughout the period of study. At this time, the highest value of 41.90 ± 0.05 ^{o}C was recorded at week 3, while the lowest value of 41.10 ± 0.05 ^{o}C was obtained at the 1st week. Overall CT at 09:00 h was 41.50 ± 0.07 ^{o}C. At 12:00 h CT values also showed no significant variation between weeks, but were relatively higher than those obtained at 09:00 h. Overall, CT at 12:00 h was 41.64 ± 0.07 ^{o}C. Generally, CT values at 15:00 h were relatively higher than other hours of the study. A maximum value (41.89 ± 0.10 ^{o}C) was obtained at week 5, while the minimum value was recorded at the 1st week of the study; overall CT at 15:00 h time was 41.80 ± 0.07 ^{o}C (Table 4.2.8)

Table 4.2.8:Weekly Diurnal Fluctuations in Cloacal Temperature (^{o}C) in VE-Administered ISA Brown Laying HensDuring the Hot-Dry Season (mean ± SEM; n= 20)

	Time of the day, h		
WEEK	09:00 h	12:00 h	15:00 h
1	41.10 ± 0.05[a] (40.80- 41.70)	41.36 ± 0.04[b] (41.10- 41.80)	41.67 ± 0.08[c] (41.0- 42.30)
2	41.28 ± 0.10[a] (41.10- 41.80)	41.52 ± 0.1[a] (41.0 - 42.0)	41.77 ± 0.1[b] (40.80 - 42.50)
3	41.90 ± 0.05[a] (41.50- 42.40)	41.83 ± 0.08[a] (40.90- 42.30)	41.81 ± 0.06[a] (41.40- 42.30)
4	41.60 ± 0.09[a] (40.80- 42.30)	41.76 ± 0.05[a] (41.40-42.50)	41.81 ± 0.09[a] (41.10- 42.50)
5	41.63 ± 0.07[a] (41.00- 42.30)	41.73 ± 0.10[a] (40.40- 42.50)	41.89 ± 0.10[a] (41.0- 42.60)
Overall mean ± SEM Range	41.50 ± 0.07[a] (40.80 – 42.30)	41.64 ± 0.07[a] (40.40-42.50)	41.80 ± 0.09[b] (40.80 - 42.60)

a, b, c = Values with different superscript are significantly ($P < 0.05$) different.
Data in parentheses represent minimum and maximum cloacal temperature.

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4.2. 9Weekly diurnal fluctuations in cloacal temperature in control laying hens

The pattern of CT obtained in control laying hens was different from the experimental groups. There was a significant ($P < 0.05$) CT variation between the hours of the day in the order: 09:00 h < 12:00 h < 15:00 h throughout the period of study. Maximum and minimum values of CT at 09:00 h were 42.10 ^{o}C and 40.50 ^{o}C, respectively. Extreme maximum and minimum mean temperatures of 41.41 ± 0.10 ^{o}C and 41.07 ± 0.05 ^{o}C were obtained at weeks 1 and 5 of the study, respectively; overall CT at 09:00 h was 41.20 ± 0.07 ^{o}C. At 12:00 h, CTs of laying hens were higher ($P < 0.05$) than those of 09:00 h. Extreme maximum and minimum values of CT at 12:00 h were 42.70 ^{o}C and 40.90 ^{o}C, respectively. The highest and lowest mean CTs of 42.06 ± 0.08 ^{o}C and 41.56 ± 0.08 ^{o}C, respectively were obtained at weeks 5 and 2 of the study; overall CT at 12:00 h was 41.84 ± 0.09 ^{o}C. The CT at 15:00 h was the highest in control laying hens ($P < 0.05$) compared to other hours of the day. The lowest and highest values of 41.91 ± 0.07 ^{o}C and 42.21± 0.07 ^{o}C were recorded at the 1st and 3rd weeks, respectively. Extreme maximum and minimum CTs at 15:00 h were 42.60 ^{o}C and 41.40 ^{o}C, respectively (Table 4.2.9). Overall, CT in control laying hens was 42.1 ± 0.07 ^{o}C.

Table 4.2.9:Weekly Diurnal Fluctuations in Cloacal Temperature (^{o}C) in Control ISA Brown Laying HensDuring the Hot-Dry Season (mean ± SEM; n = 20)

	Time of the day, h		
WEEK	09:00 h	12:00 h	15:00 h
1	41.41 ± 0.10^{a} (40.70- 42.10)	41.77 ± 0.11^{b} (41.0- 42.0)	41.91 ± 0.07^{b} (41.40- 42.50)
2	41.11 ± 0.07^{a} (40.70- 41.80)	41.56 ± 0.08^{b} (40.90- 42.0)	42.10 ± 0.05^{c} (41.80- 42.60)
3	41.21 ± 0.05^{a} (40.80- 41.80)	41.91 ± 0.09^{b} (41.30- 42.60)	42.21 ± 0.07^{c} (41.70-42.60)
4	41.18 ± 0.06^{a} (40.50- 41.60)	41.88 ± 0.08^{b} (41.40-42.60)	42.10 ± 0.07^{b} (41.60- 42.60)
5	41.07 ± 0.05^{a} (40.60- 41.40)	42.06 ± 0.08^{b} (41.50- 42.70)	42.06 ± 0.07^{b} (41.50- 42.50)
Overall mean ± SEM	41.20 ± 0.07^{a}	41.84 ± 0.09^{b}	42.1 ± 0.07^{c}
Range	(40.50 - 42.10)	(40.90-42.70)	(41.40 - 42.60)

a, b, c = Values with different superscript letters are significantly (P < 0.05) different.
Data in parentheses represent minimum and maximum cloacal temperature.

4.2. 10 Modulatory roles of lycopene and vitamin E on thyroid hormones concentrations

The thyroid concentration in co-administered lycopene and vitamin E had the highest mean T_3 concentration of 4.8 ± 0.37 nmol/L; and the T_3 concentration of 3.42 ± 0.4 nmol/L was obtained in lycopene-administered laying birds, was not significantly different ($P > 0.05$) from that recorded in L + E-administered laying birds. The VE-administered laying and control hens recorded 1.96 ± 0.2 nmol/L and 1.16 ± 0.1 nmol/L T_3, respectively. The values were not significantly different ($P > 0.05$) between the groups, but were significantly lower ($P < 0.05$) than the corresponding values obtained in L+VE and L groups (Table 4.2.10).

Plasma thyroxine value (15.2 ± 1.7 nmol/L) recorded in birds co-administered with lycopene and vitamin E was significantly ($P < 0.05$) higher than the value (7.6 ±2.5 nmol/L) recorded in L-administered laying hens. The thyroxine concentrations of 6.0 ± 1.5 nmol/L and 6.5 ± 0.9 nmol/ L were recorded in vitamin E-administered birds and control laying hens, respectively. The concentrations did not differ ($P > 0.05$) from that obtained in laying birds administered with lycopene. The concentrations of thyroid-stimulating hormone in L+VE and L laying hens were 4.0 ± 0.5 nmol/L and 3.90 ± 0.5 nmol/L, respectively. The concentrations were significantly lower ($P < 0.05$) than those of 1.22 ± 0.2 nmol/L and 1.3 ± 0.4 nmol/L recorded in VE and control groups of laying hens, respectively (Table 4.2.10).

4.2.11 Modulatory effects of lycopene and vitamin E on plasma oestrogen and progesterone

A marked decrease was obtained in the secretion of both oestrogen and progesterone in control laying hens during the hot-dry season; apparently, due to environmental factors associated with the season, resulting in heat stress. Plasma concentration of oestrogen was

significantly ($P < 0.05$) higher in experimental birds that were administered with antioxidants, lycopene and vitamin E with the values of 203.0 ±33.0 ng/mL, 181.0 ± 27.0 ng/mL and 113.0 ± 31.0 ng/mL in L+VE, L and VE, respectively. In contrast, the control laying hens recorded the value of 87.0 ± 26.0 ng/mL during the period of study. Plasma level of progesterone was also observed to decrease ($P < 0.05$) in the control laying birds; with the value of 0.36 ± 0.37 ng/mL, compared to 1.52 ± 0.33 ng/mL, 0.83 ± 0.07 ng/mL and 0.36 ± 0.37 ng/mL, recorded in L+ VE, L and VE groups, respectively.

Table 4.2.10:Modulatory Roles of Lycopene and Vitamin E on Thyroid Hormones Concentrations in ISA Brown Laying HensDuring the Hot-Dry Season (mean ± SEM; n =10 Per Group; mean ± SEM)

	PEN			
Hormone ($nmol^{-1}$)	L+VE	L	VE	CONT.
Triiodothyronine	4.80 ± 0.37^{a}	3.42 ± 0.4^{a}	1.96 ± 0.2^{b}	1.2 ± 0.1^{b}
Thyroxine	15.2 ± 1.7^{a}	7.6 ± 2.5^{b}	6.0 ± 1.5^{b}	6.5 ± 0.9^{b}
Thyroid-stimulating hormone	4.0 ± 0.5^{a}	3.9 ± 0.5^{a}	1.22 ± 0.2^{b}	1.3 ± 0.4^{b}

a, b = Values with different superscript lettersare significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

Table 4.2.11:Modulatory Effects of Lycopene and Vitamin E on PlasmaOestrogen and Progesterone(ng/mL) Secretion in ISA Brown Laying Hens During the Hot-dry Season (mean ± SEM; n = 10)

	Layer group			
Hormone	L+VE	L	VE	CONT
Oestrogen	203.0 ±33.0[a]	181.0 ± 27.0[a]	113.0 ±31.0[a]	87.0 ±26.0[b]
Progesterone	1.52±0.33[a]	0.83 ± 0.07[b]	0.91± 0.05[b]	0.36 ± 0.37[c]

a, b, c = Values with different superscript lettersare significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

4.2.12 Assessment of ovarian and oviductal activity in lycopene- and vitamin E-administered hens

The ovarian and oviductal activities of the laying hens varied between groups during the hot-dry season (Table 4.2.12). Layers administered with L +VE demonstrated the highest ($P < 0.05$) preovulatory YF (6.0 ± 0.2), compared to other groups. The values of 5.5 ± 0.2, 5.0 ± 0.2 and 4.5 ± 0.3 were recorded in L, VE and control laying hens, respectively. CWF was highest in L+VE laying hens (58.0 ± 1.4) and was significantly higher ($P < 0.05$) than 48.5 ± 0.05, recorded in lycopene-administered laying hens. VE and CONT laying hens recorded 36.5 ± 3.7 and 33.0 ± 2.5 white follicles, respectively; and the values were not significantly ($P > 0.05$) different. The control laying hens had the lowest CWF. There was no significant difference ($P > 0.05$) between the groups in TF, US and POF values; however, only in CONT laying birds were hard-shelled eggs-in-uteri recorded (Table 4.2.12). OW was significantly higher ($P < 0.05$) in L+VE laying hens, with the value of 36.3 ± 1.3 g, than in the control group (32.2 ± 2.0 g). There was no significant difference in the values of 34.0 ± 1.0 g and 33.3 ± 1.7 g recorded in L and VE groups, respectively.

Table 4.2.12: Assessment of Ovarian and Oviductal Activity in Lycopene-and Vitamin E-Administered ISA Brown Laying Hens During the Hot-Dry Season(mean + SEM; n = 10)

	OOA						
Layer group	YF	CWF	TF	US	POF	OW(gm)	Live weight (kg)
L+VE	6.0 ± 0.2	58.0 ± 1.4[a]	0.75 ± 0.1	nil	5.0 ± 0.6	36.3 ± 1.3[a]	1.90 ± 0.6
L	5.5 ± 0.2	48.5 ± 0.5[b]	1.0 ± 0.0	nil	4.0 ± 0.5	34.0 ± 1.0[a]	1.80 ± 0.6
VE	5.0 ± 0.2	36.5 ± 3.7[c]	0.75 ± 0.2	nil	3.0 ± 0.1	33.3 ± 1.7[a]	1.80 ± 0.5
CONT	4.5 ± 0.3	33.0 ± 2.5[c]	1.0 ± 0.0	0.5 ± 0.2	3.0 ± 0.8	32.2 ± 2.0[b]	1.70 ± 0.4

a, b, c = Values with different superscript between layer groups are significantly ($P < 0.05$) different.

OOA = Oviductal and ovarian activity; YF = Yellow follicles; CWF = Countable white follicles; TF = Transitional follicles; US = Uterine status; POF = Post ovulatory follicles; and OW = Ovarian weight.

L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

4.13 Eggshell diameter and eggshell weight of ISA Brown laying hens administered with lycopene and vitamin E

The values of eggshell diameter (thickness) of ISA Brown laying hens during the hot-dry season are presented in Table 4.2.13. In the 1st week of the study, L+VE laying hens recorded the highest ($P < 0.05$) value of 0.33 ± 0.02 mm. The values of 0.24 ± 0.02 mm, 0.22 ± 0.03 mm and 0.23 ± 0.02 mm recorded in L, VE and CONT were not significantly different ($P > 0.05$). No significant difference ($P > 0.05$) was recorded in eggshell weight in all the groups during the first week of the study, although L+VE laying hens recorded a relatively higher value in this parameter.

During the second week, eggshell diameter showed variation between groups (Table 4.2.13). The values of 0.25 ± 0.01 mm, 0.26 ± 0.02 mm and 0.23 ± 0.02 mm were recorded in L+VE, L and VE laying hens, respectively. These values were significantly higher ($P < 0.05$) than 0.19 ± 0.02 mm recorded in CONT layers. Within the week, no significant difference ($P > 0.05$) in eggshell weight was recorded between the groups. In the 3rd week of the study, eggshell diameter was highest in L+VE group (0.30 ± 0.03 mm), which was significantly higher ($P < 0.05$) than the values of 0.23 ± 0.03 mm and 0.20 ± 0.02 recorded in L and VE birds, respectively. These values were significantly higher ($P < 0.05$) than 0.12 ± 0.02 mm, recorded in CONT laying hens. Within the week no remarkable difference ($P > 0.05$) in eggshell weight was evident between groups (Table 4.2.13).

In the 4th week of the study eggshell diameter in L-administered laying hens was highest (0.32 ± 0.02 mm); but was not different from 0.30 ± 0.02 mm, recorded in L+VE laying hens (Table 4.2.14). However, the values were higher ($P < 0.05$) than 0.24 ± 0.02 mm and

0.21 ± 0.01 mm, recorded in VE and CONT birds, respectively. Eggshell weight was the lowest ($P < 0.05$) in CONT laying hens, compared to all other groups.

During the 5th week of the study, eggshell diameter was not different between L+VE, L and VE layers, with the values of 0.24 ± 0.03 mm, 0.23 ± 0.03 mm and 0.20 ± 0.02 mm, respectively. The values were significantly higher ($P < 0.05$) than 0.16 ± 0.02 mm recorded in control laying hens during the hot-dry season. Eggshell weight was significantly lower ($P < 0.05$) in control group (6.5 ± 0.2 g). Overall, eggshell thickness was highest in L+VE group (0.28 ± 0.02 mm), followed by L group (0.23 ± 0.02 mm); laying hens administered with VE had the value of 0.22 ± 0.02 mm, which did not differ from CONT birds (0.18 ± 0.02 mm), but was significantly lower ($P < 0.05$) than the values for the rest of the groups. Similarly, the control laying hens recorded the least eggshell weight (6.7 ± 0.02 g); and was significantly lower ($P < 0.05$) than the values of 7.3 ± 0.24 g, 7.3 ± 0.3 g and 7.2 ± 0.3 g recorded in L+VE, L and VE groups, respectively.

Table 4.2.13: Eggshell Diameter (mm) and Eggshell Weight (g) of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Hot-Dry Season (mean + SEM; n = 10 Per Group)

Group	Week					
	1	2	3	4	5	Overall mean ± SEM
L+VE	0.33 ± 0.02^{a} (7.4 ± 0.3)	0.25 ± 0.01^{a} (7.0 ± 0.3)	0.30 ± 0.03^{a} (7.4 ± 0.2)	0.30 ± 0.02^{a} (7.5 ± 0.2^{a})	0.24 ± 0.03^{a} (7.1 ± 0.2^{a})	0.28 ± 0.02^{a} (7.3 ± 0.24^{a})
L	0.24 ± 0.02^{b} (7.2 ± 0.2)	0.26 ± 0.02^{a} (7.6 ± 0.3)	0.23 ± 0.03^{b} (7.5 ± 0.3)	0.32 ± 0.01^{a} (7.1 ± 0.2)	0.23 ± 0.03^{a} (7.0 ± 0.5^{a})	0.23 ± 0.02^{a} (7.3 ± 0.3^{a})
VE	0.22 ± 0.03^{b} (7.0 ± 0.3)	0.23 ± 0.02^{a} (7.7 ± 0.2)	0.20 ± 0.02^{b} (7.2 ± 0.4)	0.24 ± 0.02^{b} (7.7 ± 0.3)	0.20 ± 0.02^{a} ($6.6 \pm 0.3)^{b}$	0.22 ± 0.02^{a} 7.2 ± 0.3^{a}
CONT	0.23 ± 0.02^{b} (7.0 ± 0.3)	0.19 ± 0.02^{b} (6.3 ± 0.2^{b})	0.12 ± 0.02^{c} (7.1 ± 0.2)	0.21 ± 0.01^{b} (6.5 ± 0.2^{b})	0.16 ± 0.02^{b} (6.5 ± 0.2^{b})	0.18 ± 0.02^{b} (6.7 ± 0.2^{b})

a, b, c = Values with different superscripts between layer groups are significantly ($P < 0.05$) different.

Data in parentheses represent eggshell weight

L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

4.2.14Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E

Internal egg parameters in the laying hens during the hot-dry season are presented in Tables 4.2.14-18. Generally, the parameters in lycopene and vitamin E groups were significantly higher for egg albumen. During the 1st week of the study, L +VE and L birds had significantly higher ($P < 0.05$) albumen heights of 0.63 ± 0.03 cm and 0.62 ± 0.02 cm, respectively, compared with the values of 0.53 ± 0.03 cm and 0.3 ± 0.03 cm recorded in VE and CONT laying hens, respectively (Table 4.2.14). Albumen weight in L+VE laying hens had the highest value of 37.0 ±1.2 g, which was not different ($P > 0.05$) from 36.0 ± 1.5 g and 36.0 ± 0.9 g, recorded in VE and control layers. Within the week, L birds recorded the lowest ($P < 0.05$) albumen weight of 35.0 ± 1.1 g.

4.2.15 Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E

During the 2nd week of the study, no significant difference ($P > 0.05$) was observed in albumen weight in L+VE, L and VE laying hens (Table 4.2.15). However, albumen value of 35.3 ± 1.2 g in control laying hens was lower ($P < 0.05$) than the values obtained in the antioxidant-treated groups. Heights of albumen did not differ between the treated groups of 0.56 ± 0.03 cm, 0.60 ± 0.02 cm and 0.53 ± 0.03 cm in L+VE, L and VE, respectively. The value of 0.42 ± 0.02 cm albumen height in control laying hens was significantly ($P < 0.05$) lower than those recorded in any other group.

Table 4.2.14:Internal Egg Parameters of ISA Brown Laying HensAdministered withLycopene and Vitamin E During the Hot-Dry Season (mean + SEM; n = 10)

Week 1	Layer group			
Egg parameter	L+VE	L	VE	CONT
Yolk weight (g)	15.8 ± 0.4^{a}	16.3 ± 0.4^{b}	15.6 ± 0.5^{a}	14.3 ± 0.5^{c}
Yolk width (cm)	4.3 ± 0.04	4.3 ± 0.06	4.0 ± 0.09	4.2 ± 0.04
Yolk height (cm)	1.6 ± 0.05^{a}	1.7 ± 0.05^{b}	1.6 ± 0.03^{b}	1.4 ± 0.03^{c}
Albumen weight (g)	37.0 ± 1.2^{a}	35.0 ± 1.1^{b}	36.0 ± 1.5^{a}	36.0 ± 0.9^{a}
Albumen width (cm)	7.3 ± 0.12	7.2 ± 0.07	7.1 ± 0.06	7.1 ± 0.02
Albumen height (cm)	0.63 ± 0.03^{a}	0.62 ± 0.02^{a}	0.53 ± 0.03^{b}	0.34 ± 0.03^{c}
Bloodspot (frequency)	nil	nil	nil	nil
% Yolk weight	26.3^{a}	27.6^{b}	26.7^{a}	24.1^{c}
% Albumen weight	61.7	59.3^{b}	62.0	61.0
Egg weight (g)	60.0 ±1.1	59.0 ±1.3	58.5 ±1.9	59.2 ± 1.1

a, b, c = Values with different superscript between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

Table4.2.15: Internal Egg Parameters of ISA Brown Laying Hens Administered withLycopene and Vitamin E During the Hot-Dry Season (mean + SEM; n = 10 Per Group)

Week 2	Layer group			
Egg parameters	L+ VE	L	VE	CONT
Yolk weight (g)	15.0 ± 0.4	15.4 ± 0.1	15.0 ± 0.6	14.7 ± 0.3
Yolk width (cm)	4.3± 0.05	4.2 ± 0.04	4.3 ± 0.04	4.0 ± 0.07
Yolk height (cm)	1.6 ± 0.02	1.6 ± 0.03	1.6 ± 0.04	1.5 ± 0.03
Albumen weight (g)	36.7 ± 1.0[a]	37.0 ± 1.4[a]	37.6 ± 1.6[a]	35.0 ± 1.2[b]
Albumen width (cm)	7.2 ± 0.09	7.2 ± 0.04	7.2 ± 0.01	7.0 ± 0.01
Albumen height (cm)	0.56 ± 0.03[a]	0.60 ± 0.02[a]	0.53 ± 0.03[a]	0.42 ± 0.02[b]
Blood spot (frequency)	nil	nil	1.0	2.0
% Yolk weight	25.4	25.7	25.0	24.0
% Albumen weight	62.2	61.7	62.6	58.3
Egg weight (g)	59.0 ± 1.1	60.0 ± 1.6	60.0 ± 1.7	60.0 ± 1.6

a, b, = Values with different superscriptbetween layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

4.2.16Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E in week 3

The egg albumen weight in laying hens was highest in L group (39.4 ±1.8 g), but was not different ($P > 0.05$) from the value of 38.6 ± 0.7 g recorded in control hens. The values were significantly higher ($P < 0.05$) than those recorded in L+VE (36.4 ±1.0 g) and VE (36.0 ±1.6 g), respectively (Table 4.2.16). The height of egg albumen was significantly higher ($P < 0.05$) in L+VE group (0.70 ± 0.04 cm) than that recorded in L (0.5 ± 0.02 cm), VE (0.5 ± 0.04 cm) and control groups (0.5 ± 0.04 cm) (Table 4.2.16).

4.2.17 Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E in week 4

During the 4th week of the study, egg albumen height was significantly higher in L+ E ($P < 0.05$) than in other groups (Table 4.2.17). In L+VE laying hens the value of 36.4 ±1.1 g, was the highest at week 4 of the study. In control hens, the value recorded (36.2 ± 0.9 g) was higher than that recorded in L (34.2 ± 1.3 g) and VE (33.0 ± 1.0 g) groups of laying hens, respectively. The occurrence of blood spots was more frequent in control laying hens than experimental hens during the period of study (Table 4.2.17).

4.2..18 Internal egg parameters of ISA Brown laying hens administered with lycopene and vitamin E in week 5

During the 5th (final) week, it was observed that albumen weight in L+VE laying hens (36.4 ±1.1 g), was significantly higher ($P < 0.05$) than in VE laying hens (Table 4.2.18). Blood spots were more frequent in control laying hens during the period of study.

Table 4.2.16: Internal Egg Parameters of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Hot-Dry Season (n = 10 per group)

Week 3	Egg parameter			
Layer group	L + VE	L	VE	CONT
Yolk weight (g)	15.0 ± 0.4[a]	15.7 ± 0.8[a]	15.4 ± 0.7[a]	14.0 ± 1.6[b]
Yolk width (cm)	4.5 ± 0.09	4.2 ± 0.05	4.1 ± 0.07	4.1 ± 0.08
Yolk height (cm)	1.6 ± 0.04	1.6 ± 0.02	1.5 ± 0.05	1.5 ± 0.04
Albumen weight (g)	36.4 ± 1.0[b]	39.4 ± 1.8[a]	36.0 ± 1.6[b]	38.6 ± 0.7[a]
Albumen width (cm)	7.2 ± 0.2	7.0 ± 0.1	7.0 ± 0.1	6.8 ± 0.1
Albumen height (cm)	0.7 ± 0.04[a]	0.5 ± 0.02[b]	0.5 ± 0.04[b]	0.5 ± 0.04[b]
Bloodspot (frequency)	nil	nil	nil	3.0
% Yolk weight	25.0	25.0	26.6	22.6
% Albumen weight	60.7	62.5	62.1	62.2
Egg weight (g)	60.0 ± 1.2	63.0 ± 2.7	58.0 ± 2.4[b]	61.0 ± 1.3

a, b = Values with different superscript between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

Table 4.2.17: Internal Egg Parameters of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Hot-Dry Season (n = 10)

Week 4	Egg parameter			
Layer group	L+ VE	L	VE	CONT
Yolk weight (g)	15.2 ± 0.3	15.0 ± 0.1	16.1 ± 0.4	15.3 ± 0.4
Yolk width (cm)	4.4 ± 0.05	4.2 ± 0.04	4.3 ± 0.04	4.4 ± 0.04
Yolk height (cm)	1.6 ± 0.02	1.5 ± 0.04	1.6 ± 0.03	1.5 ± 0.04
Albumen weight (g)	38.4 ± 1.7	38.2 ± 1.5	38.0 ± 1.0	38.5 ± 0.6
Albumen width (cm)	7.4 ± 0.1	7.3 ± 0.1	7.1 ± 0.1	7.0 ± 0.1
Albumen height (cm)	0.7 ± 0.02^{a}	0.5 ± 0.02^{b}	0.5 ± 0.03^{b}	0.5 ± 0.02^{b}
Blood spot (frequency)	nil	nil	1.0	4.0
% Yolk weight	24.5^{a}	24.6^{a}	26.0^{b}	25.0^{a}
% Albumen weight	62.0	63.0	61.0	62.0
Egg weight (g)	62.0 ± 0.8	61.0 ± 1.6	62.0 ± 1.4	62.0 ± 1.1

a, b = Values with different superscript a, b between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

Table 4.2.18: Internal Egg Parameters of ISA Brown Laying Hens Administered with Lycopene and Vitamin E During the Hot-Dry Season (n = 10 Per Group)

Week 5	Layer group			
Egg parameter	L+VE	L	VE	CONT
Yolk weight (g)	15.2 ± 0.3	15.0 ± 0.5	15.3 ± 0.5	15.3 ± 0.5
Yolk width (cm)	4.3 ± 0.05[a]	4.2 ± 0.05	4.2 ± 0.07	4.0 ± 0.08[b]
Yolk height (cm)	1.5 ± 0.03	1.6 ± 0.03	1.6 ± 0.03	1.5 ± 0.03
Albumen weight (g)	36.4 ± 1.1[a]	34.2 ± 1.3[a]	33.0 ± 1.0[b]	36.2 ± 0.9[a]
Albumen width (cm)	7.2 ± 0.05	7.0 ± 0.08	7.0 ± 0.10	7.1 ± 0.06
Albumen height (cm)	0.6 ± 0.03	0.5 ± 0.04	0.5 ± 0.03	0.5 ± 0.06
Blood spot (frequency)	nil	nil	1.0	3.0
% Yolk weight	26.5	26.5	27.3	26.0
% Albumen weight	63.5[a]	61.0[b]	59.0[c]	61.0[b]
Egg weight (g)	57.3 ± 1.5	56.5 ± 1.4	56.0 ± 1.6	59.0 ± 1.0[a]

a, b, c = Values with different superscripta and b between layer groups are significantly ($P < 0.05$) different.
L+VE = lycopene + vitamin E-administered birds; L= lycopene-administered birds; VE = vitamin E-administered layers; CONT= control layers.

CHAPTER FIVE

5.0 DISCUSSION

The present study showed that the mean extreme minimum dry-bulb temperature was 17.6 ±1.5 ^{o}C, which agrees with the reports of Dzenda *et al*. (2013). However, a close observation at the extreme mean maximum DBT of 35.3 ± 1.0 ^{o}C in the present study showed that the value was higher than those reported by Dzenda *et al*. (2015). Thus, the harmattan season in the zone could be thermally stressful to poultry species, in the afternoon hours, as the upper limit of DBT in the present study was significantly higher than the reported upper limit of 24 or 26^{o}C (Holik, 2009; Kingori, 2011) of the thermoneutral (comfort) zone for poultry species in the temperate environment and 18-26^{o}C for poultry reared under tropical environmental conditions (Oluyemi and Roberts, 2000; Ardia, 2013; Fernandes *et al*., 2013).

The phenomenon of thermal stress observed in this study has been reported in the zone for other livestock, including goats (Ayo *et al*., 2007) and pigs (Adenkola *et al*., 2009). In view of the reports, it is suggested that the observed down-turn in hen-day in control layers in the present study could be primarily due to dominant thermal stress, predominantly during the afternoon hours. This report agrees with that of Obidi *et al*. (2008a), who demonstrated that the harmattan season in the Northern Guinea Savannah zone decreased fecundity and fertility in breeder hens and depressed sperm concentration, motility and fertility in breeder cocks. The observed deterrent in the present study may be exacerbated by alternation of cold and hot environmental temperatures, as evident in the fluctuating DBT values, from 17-35 ^{o}C obtained in the present study. Thus, the instantaneous acute and alternating changes in environmental temperature may deter the layers' well-being, including

reproductive performance by inhibitory mechanisms which leave no lag times for the layer's adaptation to short-term fluctuations in environmental temperature as reported by Lu *etal*. (2018). In the course of the study, cases ofdistress were observed in behavioural responses of the laying birds to the varying thermal environmental conditions.The layinghens occasionally hurdled together, "standing and waiting" at the direction of the rising sun in the early hours of the day, especially between 6:00 h and 9:00 h, and panted subsequently between 15:00 h and 18:00 h of the day due to increased thermal load. This was behavioural thermoregulation, indicating that endogeneous corrective mechanisms, resulting in alteration in basal metabolic rate may be frequently activated in the laying hens(Li *et al*., 2020). This was particularly true of the early hours of the day to generate heat, to maintain core body temperature. The behavior was complemented by hurdling, "standing and waiting". In contrast, during 15:00 h and18:00 h, when environmental temperature rose above the comfort zone for the layers, the heat produced was apparently, greater than that required for the maintenance of the body temperature. This excess heat has been shown to be removed from the body by vasomotor-mediated increases in the thermal conductance, which is complemented by panting (thermal polypnea) behaviour (Hulbert andElse, 2004).The results of thermal environment condition and thermal heat index indicated that it was only in the early hours of the day, between 6:00 h and 9:00 h, that the laying hens in the zone had short-lived thermal comfort. It is noteworthy that birds were exposed to heat-induced stress for the rest of their daily activity for a period of over 6 h throughout the harmattan season. Therefore, the harmattan season is further established to be thermally stressful for the laying hen in the zone.

The results of the present study showed that supplemental co-administration or individual inclusion of antioxidants, lycopene and vitamin E significantly supported fecundity in layers during the harmattan season in the zone. Under the thermal environmental conditions in the zone, the performance of the laying hens showed that the antioxidants improved fecundity or daily egg production in the order: lycopene + vitamin E > lycopene alone> vitamin E alone> controls. To our knowledge, the result of the present study, for the first time provided evidence on the beneficial role of lycopene and α-tocopherol in ovarian function in avian reproductive performance; that is, fertility in the zone. Although relatively scanty reports are available for lycopene's supportive role in reproductive function, some studies have demonstrated its significant potential for enhancing immunity and reproductive efficiency in laying hens (Olson *et al*., 2008); lycopene has been shown to support testicular function, resulting in increase in sperm concentration and enhancement of sperm motility (Turk *et al*., 2010).Mehdi *et al*. (2018) and Gong *et al*. (2020) reported the vital roles of carotenoids in ameliorating thermal stress-induced impairment in reproductive performance in laying hens, especially when the inclusion of the carotenoids was combined.

The present study supports the reports, as evident in lycopene's activity as excellent barrier against stress-mediated pathologies to minimize any physiological adversity, which may inflict maximum damage to the layer's well-being and reproductive function during the harmattan season. Absolutely, a salient expression in the result of the present study showed that co-supplementation of lycopene and vitamin E to layers during the harmattan season resulted in the highest hen-day egg production throughout the experimental period. A closer observation of the results showed that the lycopene- and α-tocopherol-treated (either co-

administered or supplemented as individual antioxidants) groups of layers maintained consistently and remarkable hen-day, amounting to over 9 % weekly mean egg output in comparison with the controls. Thus, the relationship in the biological activity of lycopene and vitamin E in sustaining the significant increase in egg output in the present study may be synergistic, suggesting that the antioxidants may exert a modulatory role in amelioration of environmental stress and in the maintenance of metabolic homeostasis in the actively laying hen. This characteristic supportive interaction between carotenoid antioxidants has been demonstrated by Boileau *et al*. (2003).

Persistency is an important genetic characteristic of the laying hen. This desirable characteristic quality in poultry consists in the resolve of laying birds to keep up their performance aptitude to maintain their expected egg production against all odds. It should be noted that this study was conducted on layers, aged 41 weeks at the beginning of the study. Under normal conditions, the rate of egg production and quality in hens and sperm quality in cocks, including other vital indices of fertility in their gametes, would have started to decline due to age-related gonadal dysfunction and metabolic alterations (Avital-Cohen *et al*., 2013), which have been reported to be induced by oxidative stress (Kelso *et al*., 1996; Surai *et al*., 2001; Romero-Sanchez *et al*., 2007; Surai, 2009). Therefore, the avian ovary may be described as anorgan in the front line of oxidative stress-associated pathologies, an organ that is particularly sensitive to redox imbalance resulting in excessive production of reactive oxygen species (ROS). Oxidative stress is viewed as a critical pathogenic factor implicated in the initiation, development and progression of most ovarian dysfunction (Bentov *et al*., 2014), which may be exacerbated during the inclement environmental conditions that are associated with the harmattan season.

It has been shown (Burstein *et al*., 2009; Bentov *et al*., 2014) that such age-related ovarian functional depreciation is significantly associated with stress-mediated compromise in mitochondrial function, as a result of the susceptibility of its structural lipoproteins to oxidative stress. Part of the cumulative effects of the stress is the loss of mitochondrial membrane potential (Liu *et al*., 2000; Lord *et al*., 2013), increased generation of reactive oxygen species by the mitochondrial electron transport chain (Liu *et al*., 2009) and a decline in the efficiency of adenosine triphosphate production (Melov *et al*., 1999; Tarin *et al*., 2002; Lord *et al*., 2013). It is suggested from the present study that the age-related factors may be exacerbated by adverse stress factors in the harmattan season, the combined effects of which deter persistence in the performance of laying hens.

The present study provided evidence for the expression of persistence in antioxidant-administered layers, irrespective of the deterrent associated with age-related factor and the harmattan stress. This finding must be strongly associated with the antioxidant and/or ameliorative effects of combined activities of lycopene and vitamin E. Although combined ameliorative effects on avian reproductive parameters of the two antioxidants may not be preponderant in the literature, an early study has drawn attention to a synergy between lycopene and lutein in their antioxidant property, targeted against oxidative damage (Stahl *et al*., 1998). A study has demonstrated remarkable modulatory anti-inflammatory and antioxidative roles of a dietary mixture of vitamin E and lycopene in obese subjects (Bakker *et al*., 2010). Knowing that most physiological anomalies, including disease conditions and reproductive (gonadal) dysfunction are mediated by inflammatory and oxidative mechanisms, it may be suggested that the findings of the present study

onlycopene and α-tocopherol in alleviating cold-induced stress during the harmattan season may also be due to their characteristic anti-inflammatory and antioxidative properties.

Results on cloacal temperatures from the present study demonstrated in laying hens that during the harmattan season, birds administered with lycopene and vitamin E recorded relatively higher cloacal temperatures, especially during the early hours of the day when thermal environmental conditions tended towards cold stress. With this result, the likelihood for lycopene and vitamin E to increase metabolic activity in laying hens is absolute; this implies that the antioxidants must be associated with a boost in the layer's thyroid function. Therefore, the observed increase in CTsin experimental birds may be attributed to increase in basal metabolism, which apparently activated cellular thermogenic mechanism, to forestall rapid decrease in deep body (core) temperature as a result of heat loss to the bird's cooler environment (Silva, 1995; 2001). This phenomenon may be an active physiological defence mechanism induced by cold in the environment. Supplemental antioxidants, vitamin E and lycopene were supportive of this adaptive response to dry-cold stress in laying hens. Therefore, the results of the present study indicate that lycopene and vitamin E modulated body temperature responses by adaptive mechanisms which alleviated the risk of adverse effects of thermal stress on the health and productivity of layers during the harmattan season.

Harmattan season in the Northern Guinea Savannah zone of Nigeria is characterized predominantly by cold-dry weather condition, especially during the night and the early hours of the day. The severity and intensity of the season may be exacerbated by dusty and windy conditions. However, this characteristic environmental condition is sometimes alternated by a relatively high environmental temperature during the day, at the rising of the

sun. This implies that the dynamics of environmental parameters, typically associated with the season are remarkably interactive(Dzenda *et al*., 2011; Dzenda *et al*., 2013).

The ambient temperature range during the harmattan season in the present study was 17-35°C, which fell outside the critical limits of 20°C and 29.5°C as reported by Dzenda *et al*. (2011) in the zone. The values fell outside the established thermoneutral zone of 12-24°C for poultry species in the temperate region as reported by Holik (2009) and Kingori (2011). The present study showed that the lower critical temperature of 17^{o}C and upper limit of 35^{o}C, respectively fell outside the established range of 18-26°C in the tropics (Oluyemi and Roberts, 2000; Ardia, 2013; Fernandes *et al*., 2013). Therefore, depending on the severity and duration of ambient temperature variations (and its dynamics with other environmental parameters, such as relative humidity), the present study indicates that poultry reared in the zone are prone to remarkable alternating cold and heat stresses as a result of acute environmental temperature changes during the harmattan season. This implies that the complex response and regulatory mechanisms of the laying bird's physiology have to react concordantly to such alternating low and high levels of environmental temperature and, accordingly, cycle-related changes in CT expression. Cyclic environmental temperature fluctuations are, therefore, relevant for modulating physiological responses in laying hens which manifested in CT responses (Barret *et al*., 2019; Li *et al.*, 2020).

This characteristic modulatory effect of environment on the physiology of domestic animal species has been demonstrated (Kendall and Webster, 2009). This observation corroborates the report of Ayo *et al*., (2007) and Ayo *et al*. (2014), who documented that acute meteorological fluctuation in the zone, and the susceptibility of poultry to environmentally-induced stress conditions. This absolute environmentally-induced physiological effect has

also been documented in mammalian species (Keim *et al*., 2002; Vathana *et al*., 2002; Gordon, 2009; Dzenda *et al*., 2015).

Marked diurnal changes in layers' metabolism, which was evident in variations in CTs were observed in the present study. The observation may be dependent on environmental factors; but metabolic parameters at multiple cellular levels, involving circulating nutrient levels, substrate utilization, energy expenditure and thermogenesis have been reported to fluctuate over the course of the day (Yang *et al.,* 2010; Bray *et al.,* 2013; Anwar *et al*., 2018). This implies that the environment and metabolic parameters are concordant in determining body temperature and its pattern of fluctuation in the laying hen.Body temperature and energy expenditure have been reported to vary in the laboratory rodent during the day; these, in association with behavioural factors, including increased physical activity and food intake elicit changes in thermic effect to restore equilibrium (Alberts *et al*., 2006; Yang *et al.,* 2010; Bray *et al.*, 2013; Anwar *et al*., 2018).

With very few exceptions, the extreme maximum CT values obtained in experimental layers in the present study were higher, especially in antioxidant-treated birds than the established normal maximum value of 40^{o}C (Bobek*et al*., 1971) for the poultry species, although the difference was not significant. The maximum CT of 42°C reported for poultry was remarkably ($P < 0.05$) lower than the value of 42.8^{o} C obtained in the present study,evident only in the control layers as reported CT range concurrent with the values documented by Bobek*et al*. (1977). Apparently, this observation implies a metabolic-modulating effect of antioxidants lycopene and vitamin E, by virtue of their antioxidant

activity (Erdman *et al*., 2009; Palozza *et al*., 2010; 2011) on the physiology of the layering hen during the harmattan season.It is likely that part of the resultant biologic effects of lycopene and vitamin E may be associated with thermogenesis, aimed at preventing rapid decline in core body temperature of the layers. Therefore, the antioxidants may have specific vital modulating effects on CTs, aimed at maintaining metabolic homeostasis as reported by Barrett *et al*. (2019).

This phenomenon was evident from the observable increase in CT in the antioxidants-administered layers (Palozza *et al*., 2012). Therefore, it may be suggested that the modulation of cellular responses, which induced the thermogenic effect of lycopene and vitamin E in layers is an adaptive response, targeted at preventing a sudden decline in core body temperature. The diurnal fluctuations observed in the CT of the layers agreed with the findings of Ayo *et al*. (2003), who observed that the CT values of the Shaver Brown pullets varied with the time of the day during the hot-dry season. The diurnalfluctuations recorded in the present studyalso agreed with the findings of Piccione and Caola (2002) that such variations, classical of most animals and birds, are driven by a biological clock located in the suprachiasmatic nuclei of the anterior hypothalamus.

The animal's negative or positive responses to such changes are observed in their well-being and productivity. In avian species as it is in mammals, core body temperature is a vital, measurable index of the thermal comfort. The observed, consistent increase in CT in the experimental birds, especially in L+VE layers was important response in layers' biological reaction to the predominantly cold-dry harmattan season (with alternating high and low ambient temperature). The physiological response to an increase or decrease in body temperature is known to stimulate a compensatory and adaptive mechanism to

restorethe body's homeostasis. This physiological response includes variations in core body temperature (measured mainly as cloacal temperature in *aves*), respiratory rate and heart or pulse rate in mammalian species (Ayo *et al.*, 2007; Ayo *et al*., 2008; McManus *et al*. 2009). High and low ambient temperatures are very important stressors in tropical regions of the world (Altan*et al*., 2003; Li *et al*., 2020), and are known to activate the body's stress mechanisms leading to varying degrees of thermal stress (Gaughan *et al*. 2013; Sharma *et al*. 2013). Results of the present study showed that metabolic (thermogenic) activity, as evident from cloacal temperature responses, was higher in lycopene- and vitamin E-administered layers, wether the antioxidants were administered individually or co-administered.

The experimental laying hens recorded significantly higher circulating triiodothyronine levels and a relatively higher thyroxine activity, compared with the control laying hens; and this corroborates with the results of previous studies (Macini *etal*., 2016; Garasto *etal*., 2017; Zaglool *etal*., 2019). Thyroid hormones have been demonstrated to be significantly associated with increased metabolic activity (Lin *et al*., 2008; Simonides and van Hardeveld, 2008; Welcker *et al*., 2013). The significant variations and relatively higher values of CTs in experimental layers in the present study showed the likelihood of a thermogenic effect of the antioxidants, vitamin E and lycopene, on the metabolic activity of laying hens during the harmattan season.This effect may be mediated by the increase in thyroid function, observable in the results of the present study. Thyroid hormones have been demonstrated to be vital regulator of thermogenesis and basal metabolic rate, thereby effecting alternation in obligatory and facultative thermal homeostasis in cold and hotweather conditions (Silva, 2003, 2011; Zaglool *et al*., 2019).

To our knowledge, before now, no such report on thyroid function in layers has been demonstrated in the zone. Therefore, based on the results of the present study, it may be suggested that the concurrent increase in CT and thyroid activity recorded in the present study was aimed at adaptive thermogenesis in response to cold stress, as a heat saving mechanism in the avian species. The mechanisms have been reported in mammalian species and was associated with increased ATP utilisation (Simonides *et al*., 2001, 2008; Simonides and van Hardeveld, 2008; Zavacki *et al*., 2013). It has been shown that thyroid hormones modulate the physiology of broiler chickens predisposed to stress (Lin *et al*., 2008). Lin *et al*. (2008) showed that neither the acute nor long-term elevation of circulating thyronine levels induce lipid peroxidation in broiler chickens, but rather the hormone enhances enzymatic antioxidant system (SOD in cardiac tissue), suggested to play a vital role in the protection of the bird to increased oxidative challenge. Lin *et al*. (2008) further demonstrated that the responses of redox balance to changed thyroid state may be tissue specific.

The present study showed that domestic layers reared in the harmattan season in the Northern Guinea Savannah zone are susceptible to cold stress, which may result in functional hypothyroidism, by either decreased thyroid hormone production or peripheral deiodination of thyroxine, believed to be associated with stress-related disease conditions (Decuypere *et al*., 2003; Han *et al*., 2005). Results of the present study suggest that the preponderance in plasma triiodothyronine, coupled with the increase in CTs in experimental birds demonstrated defensive physiological mechanism against cold-induced stress and that concurrent environmentally-induced deterrent to the bird's well-being may be associated with the season. It is important to reiterate that the results of the present study on hen-day were supportive of this observation, as an overall 9% mean hen-day above the

control layers was obtained inantioxidant-treated birds during the six-week duration of the study.

The present study showed that the harmattan season in the Northern Guinea Savannah zone is thermally stressful.During the season, supplemental administration of antioxidants L and VE, either individually or their co-administration modulated metabolic activity (apparently through the activation of the hypothalamic-pituitary-thyroid axis) in ISA brown layers. The resultant effect of this modulation was the thermogenic effect recorded as fluxes in the CT of the layers. It is evident that stressors may alter the layer's physiological functions by several potential mechanisms. One of these mechanisms involves alterations of the hypothalamus-pituitary-thyroid axis that in turn modulate any other function, including thermoregulatory and gonadal function. The mechanism of action of thyroid-stimulating hormone and its influence(s) on thyroid hormones in the present study may not be fully delineated.This relationship may only be speculated upon: that there is increased catabolism of thyroid-stimulating hormone in lycopene- and vitamin E-co-administered layers, which may ultimately result in increased secretion of thyroxine and triiodothyronine.Further studies are required to determine these activities during the harmattan season in the zone.

It is agreed that environmental stress constitutes significant adversity to the well-being of domestic chicken. The consequences of adverse effects of environmental stress on avian reproduction have been well demonstrated in previous studies (Mashaly *et al.*, 2004; Obidi *et al*., 2008a; Star *et al*., 2008a; Sharifi *et al*., 2010; Mack *et al*., 2013), but the mechanisms and enormity of such deterrent on avian productivity has not been fully delineated.The present study demonstrated that harmattan season in the Guinea Savannah zone is inimical

to avian reproductive function, apparently due to the alternation between cold- and heat stress which the laying hen may have suffered.The unstable and alternating ambient temperatures recorded during the period in this study predisposed laying hens to thermal stress; environmental conditions beyond the thermoneutral zone (15-25^{o}C) for laying hens were evident, and the prevailing condition which tended to increase ambient temperature during the day may be implicated in the significant reduction in egg weight in control laying hens.The reports of Mashaly *et al*. (2004), Ebeid *et al*. (2012) and Mack *et al*. (2013) showed that egg weight in domestic laying hens was remarkably reduced at high ambient temperatures, which was attributed to decreased feed consumption, reduced feed digestibility and/or reduced efficiency of feed protein and calcium utilisation.

The finding that the harmattan season remarkably depreciated egg shell quality corroborates the reports of Lin *et al*. (2004), Ebeid *et al.* (2012) and Mack *et al*. (2013) that adverse environmental conditions, including ambient temperature are detrimental to eggshell qualitative parameters. Thus, the reports further demonstrated that eggshell breaking strength and eggshell thickness were down-turned; eggshell percentage and eggshell density were deterred when laying hens were exposed to adverse ambient temperatures that degenerated to thermal stress. Again, decreased duodenal epithelial cell calcium uptake and depression in plasma calcium utilisation have been implicated as parts of the mechanisms in the deterrents reported in eggshell parameters (Rozenboim *etal*., 2007; Barret *et al*., 2018; He *et al*., 2018).

The general decrease in internal egg parameters and, hence, in overall internal egg quality in the present study conforms to previous reports (Kirunda *et al.*, 2001; Mack *et al.*, 2013)

that heat-induced stress downgraded internal egg characteristics, which ultimately down-turns the eggs overall qualitative index. The results of the present study demonstrated that the decrease in the overall internal integrity of the avian egg correlated with remarkable decrease in feed utilisation and significant hormonal imbalance in the laying hens; the report of Mashaly *et al.* (2004) who demonstrated an increase in internal egg quality contradicts the findings of the present study. This difference in the findings may be due to variations in heat intensity and duration of the studies.

It is evident in the present study that internal and external egg qualitative parameters finishedin the following decreasing pattern: laying hens administered L+VE was greater than those administered L alone; and these were greater the laying hens which were given vitamin VE alone; VE alone group was greater than the CONT laying hens. The results suggest that the antioxidants administered either singly or in combination to the laying hens mitigated the adversity of thermal stress on internal external egg quality (Kirunda *et al.*, 2001; Ebeid *et al*., 2012; Urso *et al*., 2015, Sahin *et al*., 2016). This implies that the antioxidants prevented the generation and/or mitigated the generation and propagation of outrageous free radicals that may be inimical to biomolecules in avian physiology. Kucuk *et al.* (2003) and Ebeid *et al*. (2012) reported improved egg specific gravity and increased egg weight, eggshell weight, and eggshell strength, when broiler breeders were exposed to cold and heat stress and supplemented with dietary vitamin E. Kirunda *et al*. (2001) reported significant alleviation of stress-related indices in internal egg quality of the hens egg in laying hens exposed to adverse environmental condition, inducing heat stress The results suggest that vitamin E supplementation may improve the performance of hens exposed to adverse thermal environmental conditions and, therefore, predisposed to thermal

stress as may be during the harmattan season. Therefore, vitamin E supplementation to laying hens in adverse environmental conditions prevailing during the seasonmay promote better egg quality.Lycopene is regarded as one of the most effective natural antioxidants. Sahin *et al*. (2008, 2016) reported lycopene's capacity to modulate immune response and activate antioxidant enzymes in domestic chickens during environmental stress. It was demonstrated that the antioxidant improved reproductive parameters, including egg qualitative indices in the laying hens.

Meteorological results obtained in the present study showed that hot season in Northern Guinea Savannah zone of Nigeria was characterized predominantly by intensively hot-dry weather conditions, which may commence in the early hours of the day at the rising of the sun. The intensity of the hot-dry condition becomes progressively severe, with the likelihood of a first wave of heat climaxing at 12:00 h. The intensity of the heat condition may further increase in severity after 12:00 h until 18:00 h. The implication of this pattern of environmental condition is a diurnal variation in prevalent ambient temperature in the zone, which showed that ambient temperature is an important environmental determinant and, therefore, effective in assessing animal well-being. The observation conforms to the reports of Ayo *et al*. (2011) and Dzenda *et al*. (2013; 2015), who demonstrated significant diurnal variations in thermal environmental parameters and concomitant animal physiological responses in the zone.

The findings of the present study showed that minimum and maximum ambient temperatures were 24.3^{o}C and 36.71^{o}C, respectively, with significant variation between the limits. These values contradict the report of Dzenda *et al*. (2013), who recorded minimum

and maximum DBT of 27.5 ^{o}C and 33.5 ^{o}C, respectively in the zone; the difference in ambient temperature limits shows absolutely, that the environmental temperature in the zone is characteristically variable beyond thermoneutral range(18-26 ^{o}C) suggested by Oluyemi and Roberts (2000) and Ardia (2013). It is further evident that THI in the present study also demonstrated significant fluctuation between the extreme minimum and maximum limits of 24.4 ^{o}C and 35.0 ^{o}C, respectively which also contradict the minimum and maximum THI values of 29.0 ^{o}C and 37.0 ^{o}C, respectively reported by Dzenda *et al*. (2013).

The implications of these findings are that the tropical Northern Guinea Savannah may be remarkably prone to acute, unstable and irregular pattern of environmental conditions, especially ambient temperatures during the hot-dry season. The net effect of the extremity of the condition may be intensive and fluctuating thermal stress that may occur in the laying hen in the zone; the inclement condition may result in more deleterious effects, if the adverse environmental condition is prolonged. In the present study,this phenomenon was evident in the result on THI, which is an index of the thermal burden on the laying hens. Thus, the birds were subjected to consistent thermal load which was not easily dissipated as a result of high environmental temperature for a long period of their daily activity during the hot-dry season.

Rozenboim *et al*. (2007) and Tzschentke (2007) reported that such acute variation in environmental conditions induce uncoordinated and immediate non-adaptive biological reactions; the implication of these phenomena is that the condition becomes deleterious to the domestic chicken. Andrewartha *et al*. (2011) and Li *et al*. (2020)have demonstrated that

such environmental pattern of variation deters the entire well-being of the animal. The reports of Ayo *et al.* (2011) and Minka and Ayo (2016) corroborate the findings of the present study that environmental conditions in the zone during the hot season predispose laying hensin the zone to severe thermal stress.

The DBT results in the present study also suggest that during the hot-dry season, significant variation in ambient temperature, with increasing intensity, especially between 06:00 h and 18:00 h was imminent. This finding implies that the fluctuating, inclement ambient temperature leaves no reasonable lag time for labile physiological responses that are amenable for the laying hen's adaptation to heat stress. Again, the report of Tzschentke (2007) and Liang *et al*. (2016) that acute variations in environmental conditions result in non-adaptive reactions is corroborated.This characteristic phenomenon in climatic changes has been shown to be a major mechanism and subject of investigation on the deleterious environmental outrages in poultry production (Mayes *et al*., 2015; Xie *et al*., 2015; Lu *et al*., 2018; Li *et al*., 2020). The reports of Oluyemi and Roberts (2000), Ardia (2013) and Fernandes *et al*. (2013) agree that the zone of thermal comfort for poultry reared in the tropics, including the Guinea Savannah zone is 18-26 ^{o}C. Therefore, it is deduced from the results of ambient temperatures in the present study that the likelihood of the domestic chicken's metabolism within the reported zone of its thermal neutrality was only in the morning at 06:00 h, which may be regarded as a narrow part of its zone of thermal balance.It is of interest, therefore, to conclude that during the hot-dry season, domestic laying hens in the zone were subjected to remarkable heat stress for the rest of the day.

The implication of the report on environmental stress during the hot-dry season in the Northern Guinea Savannah zone is that homeostasis in animals reared in the zone, including laying hens are generally and significantly disturbed beyond the upper tolerant limits of their thermoneutral (comfort) zone.Thus, the bird's regulatory metabolic apparatus may be overworked to near exhaustion in an attempt to re-establish a balance in the metabolism (Altan *et al*., 2003; Dzenda *et al*., 2013). A further implication of this is that there is a down-turn in physiological and performance parameters of the domestic bird, which suggests that the environment, especially the prevailing ambient temperature, is influential on the bird's well-being and productivity (Hansen *et al*., 2004; Ayo *et al*., 2011; Leinonen *et al*., 2014).

The result of thermal stress obtained in this study corroborates the reports of Ayo *et al*. (2007) and Minka and Ayo (2010,2013) who showed the prevalence of thermal stress in the zone during the hot-dry season. Ayo *et al*. (2011; 2014) documented some detrimental effects of heat stress on physiological parameters in poultry. Other reports have shown deleterious effects of heat stress on avian immunity (Marshaly *et al*., 2004; Butts and Sternberg, 2008; Marketon and Glasier 2008; Ghazi *et al*., 2012; Bozkurt *et al*., 2012), hormonal profiles (Rozenboim *et al*., 2007; Willemsen *et al*., 2011), feed consumption and digestive function (Attia *et al*., 2011; Imik *et al*., 2012; Sohail *et al*., 2012). Therefore, it may be concluded that heat stress deters the entire metabolism of the domestic chicken and measures aimed at its mitigation may increase productivity in chickens.

Although, in the Northern Guinea Savannah zone, before now there was no documented evidence in literature relating the detrimental effect of heat stress on fecundity in the laying flock, it is evident in the present study that changes in the thermal environmental

conditions, which resulted in high ambient temperatures are inimical to egg production; thus, result supports the findings of Felver-Grant *et al*. (2012), Sahin *et al*. (2016) and Barrett *et al*. 2019, who demonstrated that adverse thermal environmental condition is a deterrent on fecundity in poultry. The dominant factor that may be adduced to the significant down-turn in hen-day egg production in control layers in the present study could be remarkably related primarily to the dominant thermal stress during the hot-dry season. Furthermore, Obidi *et al*. (2008a) have demonstrated that such deleterious effects of the hot-dry season in the Northern Guinea Savannah zone on fertility and hatchability in breeder hens, and depressed sperm concentration, motility and fertility in breeder cocks.

In the course of the study, cases of heat-induced distress were observed in the behavioral responses of birds to the prevailing environmental condition.Birds began panting (thermal polypnea) shortly after 9:00 h; and in most cases observed, the behavioural response of thermal polypnea lasted remarkably and heightened between 15:00 h and 18:00 h of the day due to increased thermal load or hyperthermia.In some cases, during the study, laying birds were visibly going-off their feeders and concentrating their activities around their drinkers, where they drank more intensively and rested under their feed augers. The results imply that during the hot-dry season, the afternoon and evening hours were especially thermally stressful to the laying hen due to the prevailing inclement environmental condition resulting in preponderant thermal burden. The behavioural expression of panting may be remarkablyrelated to increased thermal load.The observations corroborate the reports of Mack *et al*. (2013), who reported the behavioural responses of laying birds exposed to heat stress.

It has been reported that under thermal environmental conditions, as in the present study, which result in high ambient temperature and increase in thermal load in poultry, panting may be an indication of behavioural thermoregulation in laying hens (Felver-Gant *et al*., 2012; Li *et al*., 2020). Thus, internal corrective mechanisms, resulting in alteration in basal metabolic rate in birds may be frequently activated, especially between 9:00 h to 18:00 h, when environmental temperature rises above the comfort zone for the layers. It has been shown that the heat produced from basal metabolic rate is greater than that required for maintenance of the bird's body temperature, and this excess heat must be removed from the body by vasomotor-mediated increases in the thermal conductance, which is complemented by panting behaviour as reported by Hulbert andElse (2004) and Liang *et al*. (2016).The behaviour may constitute the most significant mechanism of survival from heat-induced stress and stroke in the laying hen.Such behavioural response of domestic birds to heat stress has been reported by Mack *et al*. (2010, 2013), who demonstrated that birds subjected to heat stress spent less time feeding, more time drinking and panting, as well as more time with their wings elevated, less time moving or walking, and more time resting. All the behavioural responses are aimed at finding comfort in the face of life-threatening thermal distress.A long-term effect of these behavioural responses of the laying hen to heat stress may be a negative energy balance, as a result of the rapid increase in cortisol and epinephrine secretion during heat stress; the condition promotes a catabolic metabolism which results in loss of weight (Wheelock *etal*., 2010;Baumgard and Rhoads, 2013; Rhoads *et al*., 2013).

It has been demonstrated that environmental temperatures exceeding the upper critical limit of the thermoneutral zone resulted in heat stress due to a high thermal load, which in turn

triggered significant deterrents, including remarkable increase in the cloacal temperature (Mack *et al*., 2013). Further studies conducted on domestic chickens demonstrated that cloacal temperatures rose significantly when ambient temperatures were increased to 32^{o}C and 33 ^{o}C (Cooper and Washburn, 1998; Xie *et al*., 2015).In the present study, at12:00 h, ambient mean temperatures were about the reported values, and in some cases, between maximum ATs that climaxed at 35 ^{o}C. After 12:00 h, most mean ATs were beyond 34 ^{o}C, and even a maximum of 39 ^{o}C was recorded. It is pertinent to observe that corresponding CT values at these times rose remarkably, especially in the control laying hens. Therefore, it is evident from the findings of the present study, depending on time of the day and duration of the season's thermal intensity that significant correlations between CT and AT exist, and correlations between ambient temperature and CT were very significantin control laying hens. The implication of the result is that during the hot-dry season, the prevalence and intensity of thermal stress increased its inclement impact on the physiology of the bird, and became more inimical than the reported deterrents by Cooper and Washburn (1998), Mack *et al*. (2013) and Xie *et al*. (2015).

It may be emphasized that generally, the values of CTs in experimental birds administered lycopene, vitamin E and a combination of both were relatively less varied at the times of recording, compared with those of the controls where CTs appeared to be more variable within the day during the hot-dry season. This implies that the antioxidants may exhibit modulatory activities, resulting in amelioration on the laying hen's metabolism, the net effect of which is the mitigation of heat-induced stress during the hot-dry season.Sahin *et al*. (2016) reported that lycopene and vitamin E have ameliorative effects on heat stress in domestic birds exposed to extreme of high environmental temperatures.Overall, it was

noticeable that antioxidant administration to laying hens resulted in lower CTs in the afternoon and evening hours, when ATs and, hence, thermal loads were most remarkable. The ameliorative effect was evident in remarkable decrease and relative stability in CTs during the hot-dry season in the zone.

In conclusion, the present study demonstrated that the hot-dry condition of Guinea Savannah is thermally stressful to the laying hens; the administration of vitamin E and lycopene ameliorated the deterrents associated with the inclement season through their adaptive modulatory roles in thyroid function and homeostatic effect on CT of the laying hens.

The importance of thyroid hormones in adaptation to heat stress is related to the central role that the hormones play in the regulation of metabolic rate of birds in different physiological conditions (McNabb, 2000; Johannsen *etal.*, 2012; Zheng *etal.*, 2013). Thyroid hormones are known to regulate and modulate physiological responses and, indeed, attendant biological changes in the animal's interaction with variation in environmental conditions through hormonal signals to regulatory hypothalamic nuclei (Elnagar *et al*., 2010; Xie *et al*., 2015; Grant *et al*., 2018). It has been well documented that acute and chronic heat stress markedly depressed the activity of the thyrotrophic axis in the laying hens; the deterrent results in functional hypothyroidism and the absolute effect are reflected in reduced plasma T_3 concentration (Yahav and McMurtry, 2001; Tao *et al*., 2006; Sohail *et al*., 2010; Mack *et al*., 2013; Xie *et al*., 2015). The reports are corroborated by the results of the present study, which showed considerable decrease in circulating T_3 and T_4concentrationin control

laying hens; this hypothyroid status may be attributed significantly to heat-induced stress (Garasto *et al*., 2017; Grant *et al.*, 2018; Zaglool *et al*., 2019).

To our knowledge, the present study ranks a pioneering investigation in the activity of T_3 and the interaction with L, VE and a combination of both antioxidants in the laying hens during heat-induced stress in the zone. Therefore, the significant increase in serum T_3, which is the more bioactive thyroid hormone in experimental laying hens over the control layers, may only be attributed to the effects of the physiological activity of L, VE and, especially, with L+VE. Lin*et al*. (2008) have reported the effect of increased thyroid hormones in domestic birds, especially during stress-related conditions, when an exogenous thyroid hormone was administered. The authors associated increased output of thyroid hormones to the necessity of the chicken's metabolic apparatus to be ameliorated from the deleterious effect of thermally-induced environmental stress. In effect, the increase in thyroid hormones output in the report was associated with its incidental antioxidant roles, especially in the activation and sustenance of liver enzyme superoxide dismutase activity. Therefore, it may be concluded, from the results of the present study, that the apparent increase in thyroid function and resultant increase in plasma thyroid hormones concentrations played a mediatory role as antistress agent during the hot-dry season, and the function was potentiated by the administered antioxidants.

Contrary to the results obtained in the control laying hens, it is of interest to observe that antioxidant-administered layers recorded remarkable increase in serum T_3concentration. Although serum T_3 concentration in vitamin E-administered layers was not significantly different from the control groups, the values of T_3concentration in L and L+VE groups

werehigher than the control; suggesting that lycopene alone and its metabolic interactions with vitamin E enhanced thyroid function in the hens; especially during the early hours of the day, when environmental temperature may be within the critical limit for the laying hens. Again, Lin*et al*. (2008) reported that increased T_3 concentration in poultry during stress conditions signified a higher incidental antioxidant function of the hormone, meant to reduce deleterious effects of the environmental stress. Therefore, the increase in thyroid function, apparently, induced by the antioxidants in the present study may be attributed to anti-oxidative response, and part of the effects of the response is the ultimate increase in CTs in laying hens as a result of enhanced thermogeny during the early hours of the day. However, the thermogenic effects of antioxidants became depressed at 12:00 h and 15:00 h, when significant increase in environmental temperature was prevalent. The finding implies that lycopene and vitamin E exerted a modulatory role on metabolic activity of the layer, which forestalls outrages in thermogeny of the laying hen.It is also apparent that the modulation follows a circadian rhythm and was climaxed in relative stability of CTs in the experimental birds, aimed at circumventing deleterious effects associated with CT fluctuation.

The result on T_4concentration in the present study must be interpreted with caution, since its response under the present experimental condition may not be clearly understood. Serum concentration of the hormone was significantly higher only in L+VE-treated layers; it even recorded a relatively higher concentration in controls than in vitamin E-treated laying hens, implying that the physiological activity of vitamin E alone may not be sufficient to induce an increase in T_4 concentration during heat stress. Such irregular activity of the hormone has been reported by Elnager *et al*. (2010) and was associated with either a reduced

utilisation of T_3 or reduced deiodinase activity during heat stress. The activity of thyroid stimulating hormone, which is mediated largely through the hypothalamic-pituitary-thyroid axis, has been shown to be modulated by several complex and interrelated external factors, including the influence of environmental factors on its synthesis, secretion, release and action of the hormone. Therefore, thyroid stimulating hormone activity may not also be fully understood in the present study; however, it is possible to speculate that there was an increased TSH release, which in turn enhanced thyroid function and, hence, the significant increase in T_3 and T_4concentrations in L+VE and L experimental layinghens. This result implies a supportive impact of the antioxidants on TSH during the hot-dry season as suggested by McNabb and Darras (2015).

The observed effect of heat stress on reproductive hormones in the present study demonstrated that thermal load is detrimental to endocrine organ functions, which may be interpreted as inhibitory mode on hormonal synthesis and/or release and secretion (Ventura *et al*., 2017). The present study showed that the values of plasma thyroid hormones,oestrogen and progesterone concentration (and probably the optimum balance between them) were significantly lower in control laying hens, which may be attributed to heat-induced stress. This down-turn in thyroid function and reproductive hormones concentration may be implicated in the lowest result on fecundity in control laying hens in the present study, which corroborates the reports of Grant *etal*. (2018) andZaglool *et al*. (2019).

The ultimate deterrent may be associated with inhibition of several hypothalamic and pituitary hormones, which control thyroid,oestrogen and progesterone synthesis, release

and secretion; the effect of the inhibition may be exacerbated by their metabolic dysfuncion which may become magnified in decreased coordination ofovarian functions.The result in the present study supports the findings of Rozenboim *et al.* (2007), Elnagar *et al.* (2010) and Macini *et al*. (2016), who documented significant decrease in plasma oestrogen and progesterone concentrations in laying hens as a result of heat-induced stress. The authors further showed that heat stress specifically deterred plasma concentration and activities ofother hormones, including thyroxine and triiodothyronine, which altogether confounded ovarian functional integrity in laying hens. Thus, based on the results of the present study and reports that have been cited, it may be inferred that thermally-induced stress deteriorates endocrine organ functions and depletes theiradequate hormonal concentrations, including their expected ratio for their concordant andinterdependent activities.

The present study demonstrated a diurnal pattern of variation in ambient temperature, which showed increase from dawn until the end of daylight in the zone. The finding agrees with the reports of Dzenda *et al*. (2013; 2015), who demonstrated a characteristic diurnal variation in ambient temperature, and that ambient temperature is an important determinant of environmental impact on animal physiology and well-being. Results of the present study showed that the tropical Northern Guinea Savannah zone may be remarkably prone to acute and irregular pattern of thermal environmental conditions, especially ambient temperatures during the hot-dry season. The net effect of the extremes of the conditions exerted intensive and fluctuating thermal stress on the laying hens in the zone. Rozenboim *et al*. (2007) and Tzschentke (2007) have reported that such acute variations in thermal environmental conditions induce uncoordinated and immediate non-adaptive biological reactions, implying that the conditions becameinimical to the laying hens. The reports of Ayo *et al.*

(2013) and Minka and Ayo (2016) corroborate the present finding, demonstrating that environmental conditions during the hot season in the zone predispose the laying hens to severe thermal stress.

Depending on the breed, number of developing hierarchical large ovarian yellow follicles has been shown to be between 4 and 6 (Johnson *et al*., 2015).In the present study the large yellow follicles ranged from 4.0 to 6.0, with the lowest mean of 4.0 ± 0.3 and the highest of 6.0 ± 0.2; the result conforms with previous reports (Johnson, 2011; Johnson *et al*., 2015) that developing preovulatory yellow follicles vary between these values and that the number varies from bird to bird. The report of Rozenboim *et al.* (2007)showed that the number of large follicles may be as many as 8. However, it is also known that absolute number of developing yellow follicles is related to pattern of laying, and birds laying in long cycles tend to have more than those laying in short cycles (Johnson, 2011). Therefore, based on number of developingyellow follicles, the present study showed that antioxidant-administered laying hens, especially in the group administered L+VE, was more persistent in lay during the hot-dry season. Again, this finding demonstrated the beneficial effect of lycopene in combination with vitamin E in reducing the risk of adverse effect of thermal stress on laying hens.

The present study showed that number of countable white follicles; that is, pre-hierrachical cohort of follicles was remarkably higher in experimental groups of layers, especially in L+VE laying hens, which had a significant number of countable white follicles above the value documented in the control layers. The result implies that heat-induced atresia, or even

follicular losses due to factors that may not be completely delineated and eventually depopulates ovarian follicles occurred with a relatively lower incidence. It is, therefore, suggestive that the relative increase in hierarchical yellow follicles may be based on greater rate of survival recorded in pre-hierrachical CWFs, which are known to be recruited rapidly from time to time, after a rapid rate of growth, to repopulate ovulated YFs in the ovary (Johnson, 2011; Kim *et al*., 2013). Therefore, the higher number of pre-ovulatory yellow follicles and the increase in number of CWFs may account for the significant increase in ovarian weights recorded in antioxidant-treated birds.Mack *et al*. (2013) and Barrett *et al*. (2019) have demonstrated that stress-induced deterrent on reproduction in laying hens, including heat stress, are due to diverse but interrelated mechanisms.

Alvarez and Hocking (2012) and Barrett *et al*. (2019) proposed that decline in fecundity in laying hens may be due to reduction in rate of recruitment of yellow follicles and an increase in time for the follicles to reach the conditions necessary for ovulation. Another contributing factor to a reduction in egg production could be atresia of large yellow follicles, which increases with stress-induced conditions and contributes to a lower number of successful ovulations. Thus, it is suggested, based on the result of the present study, that heat-induced oxidative stress may decrease the rate of survival of hierarchical yellow follicles, but the deleterious effect may be more pronounced in developing white follicles. The combined effects of heat-induced stress on the ovary may account significantly for mechanisms of the range of reproductive failures reported in laying hens. The present study showed that birds administered with L+VE had the highest limit of 6.0 ± 0.2 yellow follicles, followed by L (5.5 ± 0.2), VE (5.0 ± 0.2) and lastly CONT (4.0 ± 0.3). The result implies that antioxidant-administered birds had the highest laying capacity. Apparently, this

finding suggests that the increase in number of developing yellow follicles and their potential successors or replacements from growing white follicles was due to a combined effects of increase in their selection and a rescue from adverse physiological processes, which would have culminated in atresia (Johnson *et al*., 2015; Johnson and Lee, 2016). The observations attest to the biological function of lycopene and vitamin E as potent antioxidants, which corroborate the reports of Sahin *et al*. (2008; 2016) that the antioxidants are capable of mitigating deleterious effects of oxidative stress during heat-induced stress in laying birds. Thus, lycopene has been reported to be an excellent anti-inflammatory and a guardian of redox signalling which upregulates the antioxidant response elements and modulates the activity ofROS enzymes during stress-related conditions and the ROS radicals (Palozza *et al*., 2010, 2011, 2012; Jiang *et al*., 2020).

Complex, but interactive micro- and macro-biological factors are associated with the development and survival of the avian follicles; the ultimate survival of a follicle implies that these factors have "seasoned" the success of the complex transitional stages of the follicle to reach the target wholesome avian egg (Johnson, 2014; Johnson *et al*., 2015; Johnson and Lee, 2016). It is also implied that a follicle's survival may be threatened by environmental temperature that directly deters its survival factors to function or the response of a follicle to its survival factors.

The present study showed that egg quality in ISA Brown laying hens was down-turned during the hot–dry season, apparently due to heat-induced oxidative stress. Part of the evidence is based on the significant decrease in external and internal egg parameters, especially the decrease recorded in the gel protein albumen integrity; a decrease in albumen

weight and albumen height (albumen viscosity) occurred in control laying hens during the period of the study. The deleterious effect may be associated with rapid deterioration of ovomucin fibres (albumen protein) and albumen dehydration, which decrease the consistency and viscocity, thus, impeding its buffering capacity as reported by Reijrink *et al*. (2009) that inclement thermal environmental conditions such as ambient temperature deter albumen consistency. The deteriorations recorded in internal egg quality in the present study under the prevailing thermal environmental condition in the hot-dry season may be based on these deleterious mechanisms associated with egg-quality deterioration.

Under a normal storage condition, the protein-enriched albumen, is valuable in its function to buffer the avian egg; thus, prolonging the egg's life in storage.In the case of the fertile egg, the albumen sustains the avian embryo through the rich nutrient metabolism. This function is partly determined by the prevailing ambient temperature (Reijrink *et al*., 2009), implying the adverse thermal environmental conditions of heat stress during egg production, storage and incubation result and the associated albumen thinning, that is, and loss of its viscocity.The process has been associated with increase in pH and in excessive generation of carbon dioxide (Reijrink *et al*., 2009).

The incidence of eggs with blood spots obtained in the present study, especially in control laying hens, may be associated with deleterious effect of heat stress, and this may be an index of reduced egg quality. The result agrees with the findings of Jacob *et al*. (2000) who demonstrated that blood spots on egg yolk was due to inclement thermal environmental conditions, including heat stress. Blood spots on yolk have been associated with

haemorrhages of blood vessels in ovary or oviduct or in the infundibulum before albumen was laid down around yolk. The present study showed higher incidences of blood spot in eggs in weeks when mean ambient temperatures were significantly higher.This result demonstrates that high ambient temperature may be associated with haemorrhage, which predisposes the yolk to blood spots.This observation corroborates the report of Jacob *et al*. (2000) that higher ambient temperature predisposes the yolk to incidence of blood spots in eggs.

The impact of lycopene and vitamin E on internal egg parameters in the present study was more evident in the albumen than in the yolk, which shows that antioxidant capacity is a more labile priority in the albumen. Although previous studies have shown the accumulation of dietary carotenoids in the egg yolk, the present study demonstrated that antioxidant effect of lycopene and vitamin E may be more pronounced in the egg albumen. In the present study, the magnitude of the heat-induced decrease in internal egg qualities of the experimental hens was ameliorated by the intervention of the administered antioxidants, lycopene and vitamin E, implying that the antioxidants mitigated the deterioration processes involved in egg albumen (Sahin *et al*., 2016). Although the mechanisms responsible for the effects may not be fully delineated, the findings suggest for the first time in the zone, that the carotenoid, lycopene and vitamin E as antioxidants may co-inhibit pro-oxidant factors and the overproduction of ROS, strongly associated with oxidative stress in the avian egg albumen. In the present study, lycopene and vitamin E mayact synergistically or additively to prevent stress-mediated deterioration in the laying hens' egg.The findings of the present study, apparently, may reinforce the hypothesis of a nutritional modulation of ROS enzymatic producing systems (Sahin *et al*., 2016; Wu *et al*., 2018).

Eggshell quality is an important index of overall egg quality (Hafeez *et al*., 2016), associated with the efficiency of calcium mobilisation and deposition on eggshell in the process of egg formation.This physiological aptitude of the laying hen reduces during unfavorable conditions, including excessive increase in environmental temperatures. Generally, eggshell thickness and eggshell weight in the present study decreased remarkably in control laying hens, implying that there was a decrease in calcium mobilisation and utilisation during heat stress. The results corroborate previous reports (Lin *et al*., 2004; Franco-Jimenez *et al*., 2007; Periera *et al*., 2008; Melesse *et al*., 2010; Mack *et al*., 2013)that heat-induced stress adversely affects qualitative indices of eggshell in laying hens.The deterrent on the birds was associated with reduced calcium intake, consequent upon reduced voluntary feed intake and increased respiratory rate, resulting in respiratory alkalosis, the course of which altered the acid-base balance and blood pH.

The implication of the impairment in eggshell quality is its susceptibility to mechanical damage and progressive deterioration of the internal liquid content of the egg, which ultimately results in egg spoilage (Ventura *et al*., 2017; Wu *et al*., 2018). Again, it was evident that lycopene and vitamin E ameliorated the deleterious effects of heat stress on the laying hens in the zone, which suggests that the antioxidants may improve qualitative indices of the egg in the laying hen during adverse environmental seasons, including the hot-dry period in the zone. This observation supports the reports of Olson *et al*. (2008) and Sahin *et al*. (2008,2016) that lycopene supplementation alleviated heat-induced stress on performance parameters of domestic birds, including egg qualitative indices. The report of Scheideler *et al*. (2010) demonstrated the supportive effect of vitamin E on egg production,

qualitative eggshell indices and integrity in breeder hens, subjected to high ambient temperature.The combined effect of vitamin E and lycopene in alleviation of stress indices in domestic chickens exposed to high oxidant condition has been demonstrated by Lu *et al*. (2014).

In conclusion, the present study indicated that heat stress negatively affected internal and external qualitative indices of the avian egg.Heat stress also impaired ovarian function to the extent that optimum follicular integrity was compromised. However, the administration of lycopene and vitamin E alleviated the adverse effects of heat-stress-induced responses of the laying hens. The results showed that the antioxidants may mitigate the generation of excessive ROS and improve the hen's well-being and reproduction, especially during heat stress.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The present study demonstrated that thermal stress is associated with the harmattan and hot-dry seasons in the tropical Guinea Savannah zone.Dry-bulb temperature and

temperature-humidity index results showed that thermal stress was more prevalent in the zone, during the hot-dry season than the harmattan season. Thedetrimental effects resulting from the environmental adversity impairedthe well-being and reproductive performance of laying hens; the deleterious effects were more evident during the hot-dry season. The present study demonstrated that individual administration of lycopene and vitamin E alleviated thermal stress, and improved laying hens' well-being and reproductive performance. It was more evident that coadministration of the antioxidants yielded more supportive substantive results in most parameters that were considered.

6.2 Conclusions

The results obtained in the present study demonstrated that thermal stress (cold or hot) is associated with the harmattan (cold and heat stress) and hot-dry (predominantly heat stress) seasons and the stress in the seasons adversely affected physiological processes and reproductive performance of laying hens as the seasons depressed the qualitative and quantitative parameters of the avian egg characteristics.

1. Oral supplemental administration of lycopene (30 mg) and vitamin E (250 mg) per bird increased ($P < 0.05$) hen-day egg production by 9% during the harmattan and hot-dry seasons.
2. Coadministration of lycopene and vitamin E resulted in more significant ($P < 0.05$) effects on physiological and performance indices than individual administration of the antioxidants.
3. Antioxidants significantly ($P < 0.05$) mitigated deleterious effects of environmental stress that are associated with internal and external quality of the avian egg.

4. The antioxidants significantly enhanced physiological functions, especially the improvement in thyroid function in laying hens without any adverse effect on metabolism during the harmattan and hot-dry seasons.
5. It was evident that lycopene and vitamin E are nutritional antioxidants and antistress in the laying hen during the harmattan and hot-dry seasons.
6 Lycopene and vitamin E supported survival of yellow and white follicles during cold- and heat-induced stress in the seasons.

6.3 Recommendations

Based on the findings of the study, the following measures against heat-induced oxidative stress are recommended:

1The need to understand the mechanisms of action of lycopene and vitamin E as nutritional antioxidants and antistress agents.

2.The need for regular coadministration of lycopene and vitamin E to laying hens for prevention and control of thermal environmental stress in poultry and poultry products, especially during the dry season, associated with heat-induced oxidative stress.

6.4 Contributions to Knowledge

(1) Thermal stress during the harmattan and hot-dry seasons in the Guinea Savannah zone impaired gonadal function, including ovarian and oviductal activities in laying hens (L+VE: YF = 6.0 ± 0.2 vs CONT: 4.5 ± 0.03); (L+VE: CWF = 58.0 ± 1.4 vs CONT: 33.0 ± 2.5).

(2) The Harmattan season depressed hen-day egg production (L +VE: 78.0 ± 1.2% vs CONT: 68.3 ± 1.1%) during the season; the hot-dry season impaired hen-day egg production from 62.7± 1.0% in L + E group of layers to 56.0 ± 0.8% in CONT laying hens.

(3) The hot-dry season devalued qualitative egg indices; the yolk weight in the hot-dry season for the L+VE group (15.8 g) was significantlyhigher than those of the control group (14.3 g). The yolk weight in the harmattan season for the L+VE group (20.0 g) was significantly higher than the 16.3 g obtained for the control group. Egg shell thickness during the hot-dry season for the L+VE group (0.28 mm) was significantly higher than those of the control group (0.18 mm). Similarly, valuesfor harmattan season for the L+VE group (0.30 mm) was significantly higher than the value obtained (0.18 g) in the control group of laying hens.(L+VE: Yolk weight=) vs (CONT: 14.3 ± 0.5 g) and harmattan season (20.0 ± 0.3 g vs 16.3 ± 0.5g) hot-dry (Eggshell thickness L+VE= 0.28 ± 0.28 mm ± 0.02mm vs CONT = 0.18 ± 0.02mm) and harmattan (L+ VE = 0.30 ± 0.02 mm vs CONT= 0.18 ± 0.02mm).

(4)During the harmattan season, endocrine activities in the laying hens were down-graded by thermal stress in control laying hens. For instance, thyroxine and triiodothyronine concentrations were 3.1± 0.24 $nmolL^{-1}$ and 12.0 ± 1.8 $nmolL^{-1}$ respectively in L + E group of laying hens; compared with 1.3 ± 0.1 $nmolL^{-1}$ and 5.72 ± 10.7 $nmolL^{-1}$ for thyroxine and triiodothyronine, respectively in control laying hens. The values of 123.0 ± 40 ng/mL and 1.50 ± 0.35 ng/mL were recorded for oestrogen and progesterone concentration, respectively in L +VE laying hens which were significantly higher than 72.0 ± 25.0 and 0.36 ± 0.10 for oestrogen and progesterone, respectively in control laying hens.

(5) During the hot dry season, the values of 4.80 ± 0.3 $nmolL^{-1}$ and 15.2 ± 1.7 $nmolL^{-1}$ respectively were recorded for triiodothyronine in L +VE group of laying hens, which were significantly higher than the values 1.2 ± 0.1 $nmolL^{-1}$ and 6.5 ± 9.0 $nmolL^{-1}$, respectively in control laying hens. The values of 203.0 ± 33.0 ng/mL and 1.5 ± 0.33 ng/mL were recorded for oestrogen and progesterone, respectively in L+VE laying hens; these values significantly higher than 87.0 ± 26.0 ng/mL and 0.36 ± 0.37 ng/mL recorded for oetrogen and progesterone, respectively in control laying hens during the hot dry season.

(6) Lycopene and vitamin E served as antioxidants antistress, which alleviated the adverse effects of the stress conditions in the seasons.

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