EFFECTS OF QUERCETIN ON SOME PHYSIOLOGICAL PARAMETERS AND PERFORMANCE OF BROILER CHICKENS RAISED AT DIFFERENT STOCKING DENSITIES

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BY

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

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OCTOBER, 2017

DECLARATION

I declare that the work in this Dissertation, entitled **'Effects of Quercetin on Some Physiological Parameters and Performance of Broiler Chickens Raised at Different Stocking Densities'** has been performed by me in the Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria. The information derived from the literature has been duly acknowledged in the text and in the list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other Institution.

Adelaja Ariyo ABIMBOLA

…… ....

Name of Student Signature Date

CERTIFICATION

This Dissertation, entitled "EFFECTS OF QUERCETIN ON SOME PHYSIOLOGICAL PARAMETERS AND PERFORMANCE OF BROILER CHICKENS RAISED AT DIFFERENT STOCKING DENSITIES" by Adelaja Ariyo ABIMBOLA meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Dissertation is dedicated to the Almighty God in whom all strength is derived.

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ABSTRACT

The study investigated the effects of a potent antioxidant, quercetin, on some physiological parameters, performance, and carcass and meat pH in broiler chickens, raised at different stocking densities. A total of 60 one-day-old Ross 308 broiler chicks were randomly assigned to four treatment groups based on stocking density (12 birds/m^2 or 18 birds/m^2) and 50 mg/kg body weight quercetin treatment (quercetin-treated or untreated). Quercetin was administered *per os* once daily for 28 consecutive days at 18:00 h. The stocking density of 12 birds/m^2 was categorised as low stocking density (LSD) condition while 18 birds/m^2 represented high stocking density (HSD) condition. The circadian cloacal temperature was recorded at 2 h interval, three times, one-week apart on days 22, 29 and 36 while the haematological profile and erythrocyte osmotic fragility (EOF) were recorded on days 28, 35 and 42. Broiler performance was recorded daily. The carcass characteristics and meat pH was determined at the end of the study period. Fluctuations in diurnal cloacal temperature (CT) showed that increasing stocking density induced an elevation ($P < 0.05$) in the overall mean CT of untreated HSD group (40.96 ± 0.02 ºC) while the CT was lower ($P < 0.05$) in the treated HSD group (40.72 ± 0.02 ºC). The CT of the untreated LSD group (40.88 ± 0.02 ºC) was lower ($P < 0.05$) when compared with the untreated HSD group (40.96 ± 0.02 °C). On day 28, the total white blood cell count (TWBC), heterophils and H:L ratio (8.50 ± 0.67 x 10^9/L, 1.35 ± 0.30 x 109/L and 0.19 ± 0.03 respectively) were significantly lower ($P < 0.05$) in the quercetin-treated HSD group when these parameters were compared with those of the untreated HSD group (12.36 ± 1.22 x 10^9/L, 3.91 ± 0.79 x 10^9/L and 0.53 ± 0.13 x10^9/L respectively). The overall mean variation in percentage erythrocyte osmotic fragility (EOF) was significantly higher ($P < 0.05$) at 0.5 %, 0.3 % and 0.1 % NaCl concentrations in the quercetin-treated LSD group with corresponding values of 10.29 ± 6.09 %, 67.41 ± 3.30 % and 88.93 ± 3.47 % respectively, while the EOF was

0.24 ± 0.16 %, 62.21 ± 4.22 % and 85.50 ± 3.56 % in the untreated LSD group. HSD condition caused a higher ($P < 0.05$) erythrocyte osmotic fragility at 0.5 %, 0.3 % and 0.1 % NaCl concentration. The final live body weight (1,344.0 ± 54.22 g) and weight gain (1,120.0 ± 52.48 g/chick) of quercetin-treated HSD was significantly higher ($P < 0.05$) when compared with the final live body weight (1,071.0 ± 60.76 g) and weight gain (842.50 ± 57.07 g/chick) of the quercetin-treated LSD group. The feed conversion ratio was significantly lower in the quercetin-treated HSD group (1.43 ± 0.07) when compared with untreated HSD (1.79 ± 0.10), LSD (2.20 ± 0.10) and quercetin-treated LSD (2.10 ± 0.12) groups. A higher viability ratio was recorded in the quercetin-treated groups. Quercetin administration had a weight-enhancing effect, correlated directly with the increasing age of broiler chicks in quercetin-treated HSD ($r = 0.950$; $P < 0.01$) and quercetin-treated LSD ($r = 0.968$; $P < 0.01$) groups. Low stocking density condition and quercetin administration enhanced meat quality by preventing early rise in pH. This is beneficial to consumers that may wish to refrigerate meat for future consumption. The results suggest that quercetin was most beneficial in conditions of discomfort and, may, be useful as a supplement in broiler chicken production, especially in stress due to HSD. It was concluded that the administration of quercetin at 50 mg/kg body weight enhanced performance through efficient feed utilisation, alleviated stocking density-induced social and physiological stress and prolonged the shelf-life of stored broiler breast meats.

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LIST OF ABBREVIATIONS

%	Per cent
$^{\circ}$C	Degrees Celsius
^{1,2,3}	Numeric superscripts
^{a,b,c}	Superscript letters
CAT	Catalase
CT	Cloacal temperature
d	Day
DBT	Dry bulb temperature
DNA	Deoxyribonucleic acid
e.g.	For example
EDTA	Ethylenediaminetetra-acetate
g	Grams
GSH-Px	Glutathione peroxidase
h	Hour
H:L	Heterophil: Lymphocyte ratio
H_O	Null hypothesis
HSD	High-stocking density
L	Litres
LSD	Low-stocking density
m^2	Square metre
MCHC	Mean corpuscular haemoglobin concentration
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MDA	Malondialdehyde
mL	Millilitres
NaCl	Sodium chloride
N	North
PCV	Packed cell volume

pH	potential of Hydrogen
RBC	Red blood cell count
RH	Relative humidity
ROS	Reactive oxygen species
SEM	Standard error of mean
SOD	Superoxide dismutase
T_{db}	Dry bulb temperature
THI	Temperature-humidity index
TP	Total protein
TWBC	Total white blood cell count
T_{wb}	Wet bulb temperature
UK	United Kingdom

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The influence of stocking density (the number of birds calculated on a weight basis per metre square of different poultry species on growth and productive performance) has generated considerable interest (Skrbic *et al*., 2009a; Tayeb *et al*., 2011). High stocking density (HSD) reduces growth rate (Sekeroglu *et al*., 2011), feed efficiency, livability, carcass quality and body weight in chickens (Puron *et al*., 1995; Feddes *et al*., 2002; Bessei, 2006; Rambau *et al*., 2016). The causes of adverse effects of HSD are: poor air quality as a result of inadequate air exchange, increased microbial activity (Adebiyi *et al*., 2011), leading to increased build-up of ammonia production and reduced access to feed and water in commercial broilers (Feddes *et al*., 2002; Uzum and Toplu, 2013). Despite the clear welfare problems associated with HSD, producers of broiler chickens have derived some economic benefits from it. In the tropics, the total kilogramme produced per unit of space increases with stocking density, profit margins also increase to a point, as birds are raised in increasingly crowded environments (Puron, *et al*., 1995; Utnik-Banas, 2014). However, Kow *et al*. (2015) reported a progressive reduction in feed intake as stocking density increased, but neither season nor stocking density influence feed conversion ratio. Under stressful situations, the behavioural patterns of birds change and, consequently, their energy consumption increases (ZulKifli and Azah, 2004). El-Gogary and Azzam (2014) reported that increased stocking density does not affect plasma total protein.

Studies have shown that some antioxidants (Boots *et al*., 2008; Tan *et al*., 2014), including polyphenols accumulate in tissues to provide antioxidant protection, similar to that offered by vitamin E (Eraslan *et al*., 2007; Sinkalu *et al*., 2008; Durak *et al*., 2010; Eroglu *et al*., 2013;

Surai, 2013) and may, thus, prolong shelf-life as well as preserve keeping quality and taste of meat (Zhang *et al*., 2015).

Quercetin (3, 3^1, 4^1, 5, 7–Pentahydroxyflavone) is one of the ubiquitous flavanol–type flavonoids, predominantly found in edible fruits and vegetables (Anjaneyulu and Chopra, 2004; Zhang, 2005). Quercetin prevents oxidative injury and cell death by scavenging reactive oxygen species (Cox *et al*., 2000), inhibiting xanthine oxidase (Chang *et al*., 1993), lipid peroxidation, and chelating metal ions (Chen *et al*., 1990). Guardia *et al*. (2011) reported non-linear effects of increased stocking density on mortality rate in broiler chickens when raised under experimental conditions. However, under commercial conditions the situation may be very different (Berg and Yngvesson, 2012). Reactive oxygen species (ROS) are increasingly being generated in the body under the influence of stressful conditions and these results in lipid peroxidation (Puttachary *et al*., 2015). Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids. It occurs in biological membranes, inactivates several membrane-bound enzymes, and impairs membrane fluidity (Goel *et al*., 2005) and integrity. Malondialdehyde (MDA) is one of the major oxidation products of peroxidised polyunsaturated fatty acids; therefore, increased MDA content is an important indicator of lipid peroxidation (Kalender *et al*., 2012).

The health status of broiler chickens is linked to their haematological parameters (Kwari *et al*., 2011), which are influenced by diurnal fluctuations (Azeez *et al*., 2009). Leucocytic responses have been used as an indicator of heat or cold stress in poultry (Ben Nathan *et al*., 1976). An increased core body temperature that is due to ambient temperature can influence the changes that are observed in circulating leucocyte components of broiler chickens. In addition, an exposure to heat stress and increased stocking density in broilers causes an increase in both cloacal temperature and heterophil/lymphocyte (H:L) ratio (Altan *et al*., 2000; Altan *et al*., 2003; Sepp *et al*., 2010; Uzum and Toplu, 2013). Erythrocyte osmotic

fragility (EOF) describes the sensitivity to changes in osmotic pressure that an erythrocyte has been exposed to (Oyewale and Durotoye, 1988). It is used as an indicator of oxidative stress in animals (Adenkola and Ayo, 2009; Abdul Wahab *et al*., 2010). Oyewale *et al*. (2011) reported that pH, temperature and storage are among factors influencing EOF.

1.2 Statement of Research Problems

Due to increase in awareness of poultry welfare and concomitant legislation, it has become necessary to determine response of poultry to stress (Wein *et al*., 2017). The stocking densities used in previous studies have been highly variable (Lee *et al*., 2017) but it is universally acknowledged that stocking density affects broiler performance, with poorer performance at high stocking densities (Feddes *et al*., 2002; Velo and Ceular, 2017). It has been reported that lower stocking densities also contributes to farmer's loss (Lee *et al*., 2017). Subjective husbandry systems that are associated with high stocking density may indicate a greater degree of stress, thus, increasing the risk of poor life performance in commercial broilers. This contributes significantly to the loss incurred by producers (EL-Gogary and Azzam, 2014; Ibrahim, 2017). Determination of the optimum stocking density in broiler chickens is still subject of discussion (Tayeb *et al.,* 2011). Many farmers around the world increase stocking density to maximise profitability in production of broiler chickens (Heckert *et al*., 2002; Thaxton *et al*., 2006; Estevez, 2007) and this adversely affects welfare the welfare of broiler chickens (Beloor *et al*., 2010). The intensive management system associated with HSD results in stress and negatively impacts production in broiler chickens; thus, increasing the risk of poor life or welfare and performance in the birds (El-Gogary and Azzam, 2014; Kang *et al*., 2016). Consequently, HSD contributes significantly to the production loss, incurred by farmers (Velo and Ceular, 2017). Some detrimental consequences of increasing the stocking density of broilers include; foot pad lesions (Skrbic

et al., 2015), poor air circulation (Etim *et al*., 2013) and poor litter quality (Taira *et al*., 2014; Bergmann and Schwarzer, 2017; Swiatkiewicz *et al*., 2017).

For many decades, great concerns have been expressed over the continued use of antibiotics as a growth enhancer in animal husbandry and the associated emergence of antibiotic-resistant bacteria strains (Broom *et al.,* 2006; Goliomytis *et al*., 2014; Meek *et al*., 2015). The indiscriminate use of antibiotics in broiler production has enhanced the proliferation of resistant strains of disease-causing microbial organisms. Similarly, farmers are criticised for irrational use of medications, especially antibiotics (WHO, 2014; O'Neill, 2015), and so faced with the problem of how to rear more animals, more efficiently and with higher standards of food safety but without using antibiotics. There is dearth of information on the potential of quercetin to alleviate stressful conditions and preserve the shelf-life of meat in commercial broiler production. Great interest in the development of novel plants and their bioactive metabolites to be used as feed additives in animal nutrition has attracted great research attention. However, scientists are yet to establish a 'balanced antioxidant' that is ubiquitous, effective, affordable, readily available and can be derived from natural source.

1.3 Justification of the Study

There is paucity of information on ameliorative effects of quercetin on physiological responses of broiler chickens to management stressors, including HSD, and meat quality parameters. The objective of this research was, therefore, to evaluate the effect of quercetin administration on some physiologic parameters, including; CT, haematology, erythrocyte osmotic fragility and performance and meat quality characteristics of broiler chickens, raised at different stocking density conditions.

Stocking density is of great significance in broiler chicken production as it influences not only production performance, vitality and health status (Skrbic *et al*., 2009b), but it is also

associated with environmental impact on broiler chickens (Dawkins *et al*., 2004). It eventually reflects on all aspects of broiler production: economic efficiency, quality of products and welfare aspects of broiler chickens (Skrbic *et al*., 2009b).

Scientific research in the area of dietary manipulation of poultry feed is on-going (Bhattacharyya *et al*., 2017) and researchers have reported increased benefits in the use of natural and synthetic antioxidants to promote broiler health and productivity (Makri *et al*., 2017), internal physiological changes and product quality (Lee *et al*., 2017). The health benefits of herbs and botanicals have been demonstrated (Surai, 2002; Wenk, 2003; Surai, 2013) and this has resulted in a growing body of research that has been devoted to natural antioxidants that are currently receiving considerable attention in animal nutrition fields (Lee *et al*., 2017). The study of quercetin has been focused mainly on its therapeutic and antioxidant effects in man (Boots *et al*., 2008). Quercetin has the potential as functional feed additive in poultry production. It improves performance in laying hens (Liu *et al*., 2014). Quercetin administration may compensate for any compromise that is encountered during physiologic stress, especially if due to stocking density in chickens. The study of antioxidant effects and the underlying mechanism of dietary phytochemicals is currently of great research interest (Lee *et al*., 2017). There is, therefore, an urgent need to improve animal welfare and demonstrate the resultant financial benefits to both individual farmers and the society (Dawkins, 2017) using a potent antioxidant. It is, therefore, important to establish the effects of different stocking densities on physiological parameters, performance and meat quality characteristics and to study the modulatory role of quercetin in broiler chickens.

1.4 Research Hypotheses

The following hypotheses were put forward for this research:

H_o:

1. Stocking densities do not have any effect on the cloacal temperature (CT), haematology, meat quality characteristics and performance of broiler chickens.
2. Quercetin administration does not have any significant effect on CT, haematology, performance and meat quality characteristics of broiler chickens, raised at different stocking densities.

1.5 Aim of the Study

The aim of the study was to investigate the effects of quercetin on some physiological parameters and performance of broiler chickens, raised at different stocking-densities.

1.6 Specific Objectives

The specific objectives of the study were:

1. to investigate the effects of different stocking densities on CT, haematology, performance, and meat quality of broiler chickens.
2. to investigate the effects of quercetin on CT, haematology, performance, and meat quality of broiler chickens raised at different stocking densities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Seasons in Northern Guinea Savannah Zone of Nigeria and their Impact on Broiler Production

Seasons in the Northern Guinea Savannah zone of Nigeria have been categorised into three; harmattan, November-February; hot-dry, March-May; and rainy, June-October (Ayo *et al*., 2011). The control of environmental conditions appears to be the key issue, when considering broiler welfare status. Each of the seasons has its beneficial and detrimental effects on livestock production (Bianchi *et al*., 2007; Ayo *et al*., 2011; Dzenda *et al*., 2013). Obidi *et al*. (2008) reported that the negative impact of environmental stress on poultry in this zone was relatively minimal during the rainy season. The hot-dry season in the Northern Guinea Savannah zone of Nigeria may pose the most detrimental threat to poultry production. This is because birds are mainly raised under an extensive management system, which significantly exposes them to the influence of high ambient temperature and high relative humidity, prevailing in the zone (Ayo *et al*., 2011).

Lara and Rostagno (2013) reported that the incidence of bruises is associated with season. In addition, carcass injuries and foot pad dermatitis are more severe during the harmattan season (Meluzzi *et al*., 2008a; Meluzzi *et al*., 2008b). Meteorological elements (climate or weather) are inconsistent and varied. Such variations affect the internal environment of birds, including blood through the nervous and endocrine systems (Simon, 2003). Heat stress depresses growth rate and production, the consequence is down-turn in voluntary intakes in broilers, implying that heat load depresses growth (Sahin *et al*., 2001).

Environmental conditions during transport and holding are known to affect processing yield and meat quality. Traditionally, less consideration has been given to the long-term impact of stress in day-old chicks, considering the fact that in West Africa, day-old chicks are conveyed

over long distances (Bianchi *et al*., 2004). During the hot-dry season, heat stress and excitement prior to slaughter alter meat quality because post-mortem metals in the muscle are modified. Birds reared during this season are associated with poor meat quality (Bianchi *et al*., 2007).

2.2 Pathophysiology of Stress in Broiler Chickens

Increased level of corticosteroid secretion is evident, when the hypothalamo-hypophyseal-adrenocortical axis is stimulated by stress (Ramnath *et al*., 2008). This high secretion has a catabolic effect through increase in ROS generation by altering oxidative metabolism, causing impairment of cellular function and, thus, damage to cell membranes, retarded growth and muscle wasting (Sujatha *et al*., 2010). Animals, in an attempt to overcome and survive adversities, for example environmentally-induced stressors; an adaptive mechanism that tends to acclimatise their physiologic and metabolic activities with their immediate environment is activated. This phenomenon may mask previously observed physiologic or even pathologic changes, thereby leading to the conclusion that the tested physiological indices are within normal ranges.

ROS are free radicals containing oxygen with one or more unpaired electron, which make them highly reactive species. They are continuously generated in the body due to on-going interplay between endogenous metabolic processes and or environmental stress condition (Ayo *et al*., 2011; Sunil *et al*., 2011). The ROS generated during stressful conditions may behave as deleterious and toxic products, involved in tissue and cellular dysfunctions. Over-production of these species may result in protein, lipid and DNA damage (Sies, 2015).

Nevertheless, moderate or low concentrations of ROS are also involved in physiological responses as part of defence mechanisms against infectious agents and signalling process (Cordeiro and Jacinto, 2013). Stress can also impair antioxidant defence mechanisms, which

is manifested as a decrease in the activity of antioxidant enzymes and in the concentration of low-molecular-weight antioxidants in the blood and tissues (Doktor, 2007; Ognik *et al*., 2015). In fact, ROS themselves protect the cell against ROS induced damages by evoking different antioxidant responses in the body, aimed at maintaining or re-establishing homeostasis (Forman *et al*., 2014).

2.3 Stress and its Adverse Effects on Broiler Production

Stress occurring within an animal's body activates the attempt of the animal to cope by evolutionary, neural and endocrine mechanisms (Greenberg *et al*., 2002). Physiological and, especially, immunological and hormonal deficits as well as increased susceptibility of animals to diseases are due to exposure of broiler chickens to different stressors (Quinteiro-Filho *et al*., 2010). Adverse effects of emitted gases such as ammonia, methane, nitrous oxide and vaccination-break, commonly observed in broiler production are often due to negative impact of stress on broilers (Quarles and Kling, 1974; Calvet *et al*., 2011), which increase rate of condemnation and number of under-grade carcasses (Almeida Paz *et al*., 2010). Low stress level (eustress) improves immune function and production parameters indexes. Strong and long-lasting stressors (distress) decrease farm animals' welfare, production parameters and health status (Quinteiro-Filho *et al*., 2010). Reports on the mutual control interactions between the immune system and the central nervous system are involved in stress modulation (Quinteiro-Filho *et al*., 2012).

Dobson and Smith (2000) stated that stress is revealed by the inability of an animal to cope with its environment, a phenomenon that is reflected in failure to achieve genetic potential: growth rate, feed efficiency or disease resistance. Detrimental effects of stress are caused by environmental and management factors or stressors. These include and most especially animal housing when compared with stocking density influence (Dawkins *et al*., 2004), human-animal relationship/animal handling and management, modern production methods,

ambient temperature or changes in photoperiod (day and night cycles). These stressors induce clinical changes in physiological parameters and may impair body functions (Etim *et al*., 2013). The activation of hypothalamic-pituitary-adrenal axis is responsible for the negative effects observed on the chicken performance, immune functions and changes in intestinal mucosal flora (Quintteiro-Filho *et al.*, 2010). Detrimental effects of heat stress on integrity of the intestines, the main niche of *Salmonella* serovars in poultry and farm animals (Andino and Hanning, 2015), account for higher translocations of *Salmonella* enteritidis, resulting in intestinal inflammation and increased *Salmonella* counts in tissues in birds exposed to heat stress (Yadav *et al*., 2016). Heat stress reduces nutrient digestibility and the release of stress hormones. Cortisol is the primary corticosteroid in most mammals, while corticosterone is the corticosteroid in birds (Virden and Kidd, 2009). Corticosterone increases the rate of fat deposition in poultry (Jiang *et al*., 2008) in the abdomen, within muscles and in subcutaneous (He *et al*., 2015).

Stress may be induced in birds by high ambient temperature (Sinkalu *et al*., 2015), increasing stocking density (Zeng *et al*., 2014), road transportation (Leche *et al*., 2013; Minka and Ayo, 2014), alteration in environmental conditions (Dawkins *et al*., 2004; Sohail *et al*., 2012), which activate the hypothalamic-pituitary-adrenal axis to cause cortisol release. Prolonged exposure to stressors impairs immune function and regression of lymphoid tissue, accompanied by increased heterophil-to-lymphocyte ratio (Virden and Kidd, 2009). The negative effects of environmental stressors are common occurrence with worldwide significance.

2.4 Stocking Density: A Factor in Profitable Broiler Production

Major economic losses in broiler production are attributed to physiological stresses, accompanying intensive rearing systems (Zeng *et al*., 2014). The relationship between stocking density and farm profitability is relatively less complex and determined easily than

between stocking density and welfare (Buijs *et al*., 2009). Animal welfare is a multi-factorial concept, based on freedom from disease, ability to perform specific behaviours and environmental and social conditions (Lay *et al*., 2011). Variations exist in the way individual birds respond to stressors. A potential cause of these variations is linked to the fact that stressors do not influence birds in isolation (Lara and Rostagno, 2013) for example, heat stress is accompanied by other stressors, such as limited housing space and inadequate ventilation, social interactions and previous experiences (Boissy *et al*., 2007: Lara and Rostagno, 2013). Different aspects of broiler welfare are influenced at different densities or group sizes, or both (Buijs *et al*., 2009). Factors to consider in determination of stocking density include bird size, feeder space, drinker space, house dimensions, bird welfare, breed, performance (Dozier III *et al*., 2005; The University of Georgia, 2005), nutrition, and economic return (Estevez, 2007).

Stocking density stress is responsible for complexity of adverse effects known today (Skrbic *et al*., 2009a). These effects may be direct and interrelated with other factors of rearing, which eventually reflect on all aspects of broiler production: welfare aspects, quality of products and economic efficiency (Skrbic *et al*., 2009b). Determination of an ideal broiler placement density is an on-going debate (The University of Georgia, 2005). Buijs *et al*. (2009) studied a number of welfare indicators, including physiological (corticosterone concentration), anatomical (bursa weight, leg strength), pathological (dermatitis and mortality) and psychological (fearfulness). The findings indicate that density affect the different indicators of welfare at different levels, and no single critical stocking density could be identified; an implication for determining a specific, acceptable stocking density.

Naturally, providing more space is a pre-requisite for better performance, but the improved environment that the added space may provide is more important. In an experiment that involved 23.8, 17.9, 14.3, 11.9 birds/m^2, Feddes *et al*. (2002) reported that stocking density

did not affect mortality, breast meat yield, carcass grading, incidence of scratches, or carcass quality. It was, therefore, concluded that provided ventilation rate and air circulation were adequate, high yield/unit area was achievable. Thus, birds can be placed at higher stocking density, as long as the correct environment and optimum management practices are provided (Dawkins *et al*., 2004; The University of Georgia, 2005). Some questions of concern with increasing broiler stocking density include the following conditions:

(i) Can stocking density increase even though performance may reduce, yet achieve a satisfactory economic return? and

(ii) Can behavioural and physiological stress be avoided in highly-stocked broilers in compliance to regulation of animal activist groups?

Live body and carcass weight were reported to decrease, when stocking density was reduced; however, bird uniformity was better at high densities (Feddes *et al*., 2002). Increasing the number of chickens in a defined space is a strategy companies practice as an alternative to obtain high production per square meter, but this management practice may create the conditions that are necessary for the proliferation of certain pathogens, mainly those linked with enteric diseases (Fernades *et al*., 2015). It is possible to place broilers at higher densities, but when this is done, broiler environmental management is crucial to optimising broiler performance and welfare (Dawkins, 2008; Feddes *et al*., 2002). Thus, improvement of some rearing condition (optimalisation), primarily stocking density, is possible even in intensive broiler production to satisfy all three aspects of poultry meat production to some magnitude; that is, welfare aspect, quantitative and qualitative aspects.

2.5 Stocking Density and its Adverse Effects on Broiler Production

Overcrowding among other factors such as sudden temperature and, light changes trigger a strong stress response in birds (Ognik *et al*., 2015). Stocking density is a factor that influences the life performance of broilers (Dozier III *et al*., 2005).

High-stocking density has been reported to increase preening, heat stress, locomotion, litter moisture, foot-pad lesions and ammonia production (Cravener *et al*., 1992; Weeks *et al*., 2000; Sanotra *et al*., 2001; Petersen, 2004). The microclimate that exists when birds are placed at high-stocking density was associated with poor air circulation (Etim *et al*., 2013), high moisture and poor litter quality and this predisposes birds to further discomfort (Almeida Paz *et al*., 2010; Bergmann and Schwarzer, 2017; Swiatkiewicz *et al*., 2017). Previous studies have also demonstrated that increasing stocking density adversely affects growth performance, carcass yield and tears, and skin scratches (Feddes *et al*., 2002). High placement density is sex and age dependant (Dozier III *et al*., 2006). The adverse impact of high stocking density is relatively pronounced in male broiler chickens as they consume more feed, gain more weight and have a higher feed conversion ratio. Stocking density was also reported to have more severe effect on the growth of male broiler before 35 day of age (Zouwei *et al*., 2011).

The adverse effect of increasing stocking density in broiler chickens is intensified as the chickens grow (Edriss *et al*., 2003; Skrbic *et al*., 2009a). It is frequently reported that increasing stocking density depresses chicken growth performance, but its mechanisms are not fully understood. The adverse effects of high-stocking density seem to be pronounced at certain age limit in broilers, and may have no adverse effect at all between 10- to 24-day-old broilers. However, high-stocking density increases feed intake in 1 to 10-day-old chicks. There is paucity of information on young broilers (1- to 21-day-old) because the majority of studies deal with the whole rearing period or last period of rearing (Guardia *et al*., 2011). Dozier III *et al.* (2005) reported an advantageous effect of increasing stocking density until broilers are 17 days' old. This was perceived to be due to increased creation of metabolic energy with each added chicken per box, which was beneficial for chicken growth.

Interestingly, increased stocking density of broilers may have a positive impact on the environment. It was found that low stocking density system slightly increased global warming potential of broiler production by 2.0 %. This was attributed to be due to increased heating requirements (Leinonen *et al*., 2014).

2.6 The Role of Antioxidants in Stress Amelioration in Broiler Chicken Production

In the last few years, attention has been drawn to the possibility of exploiting the adaptogenic and antioxidant properties of herbs (Hashemi and Davoodi, 2012). Ognik *et al.* (2015) reported that supplementation of antioxidants in poultry diet alleviates the negative effects of stress. Routine and normal management practices during adverse environmental conditions, if devoid of stress alleviators limit productivity potentials in broiler chickens. Stress factors are initiators of increased levels of corticosterone, a stress hormone in birds (Virden and Kidd, 2009), which increase malondialdehyde (MDA) concentration, and activities of superoxide dismutase (SOD) and catalase (CAT) in the blood plasma of birds (Ognik *et al*., 2015).

Antioxidant research findings demonstrate the ability of exogenous antioxidants to stimulate antioxidant defence mechanisms (Ognik and Czech, 2010; Sinkalu and Ayo, 2016; Zhang and Tsao, 2016). Antioxidants have the ability to mitigate stress by improving the activity of scavengers of ROS, including glutathione peroxidase (GSH-Px) and SOD and decreasing the concentration of MDA (Zienali *et al*., 2011). The concentration of antioxidants in the diet may delay the rate of lipid oxidation in meat and chicken products (Riuz *et al*., 1999). In broilers, damage to bio-molecules, cells and tissues is the resultant effect of oxidative stress (Keshavamurthy, 2013). The ROS decrease immunity and antioxidant defence mechanism of the body, resulting in poor growth rate and production in birds (Ajakaiye *et al*., 2010). Improved growth rate and performance in livestock have been attributed to the vital role antioxidants play in protecting cells from oxidative damage. However, antioxidants have been found to play paradoxical roles *in vivo* (Lei *et al*., 2016); these roles of antioxidant

enzymes in physiology, health, and disease derive from sophisticated molecular mechanisms of redox biology and metabolic homeostasis. Lei *et al*. (2016) further proposed that antioxidant supplementation is not always beneficial as this may be clinically harzadous, especially when no deficiency is established (Ghezzi *et al*., 2016). Antioxidants, including selenium, vitamin E, *Ocimum sanctum* (tulasi) extract increase catalase activity and decrease of MDA concentration in liver tissues by inhibiting lipid peroxidation, but exert no significant effect on glutathione peroxide (GSH-Px) (Keshavamurthy, 2013).

There are complex interactions inside the antioxidant network of the cell/body to ensure an effective maintenance of homeostasis in stress conditions (Surai, 2016). Therefore, supplementation of antioxidants in broiler diet may decrease oxidative reactions during stressful conditions (Ruiz, *et al*., 1999; Hosseini *et al*., 2012). Administration of antioxidants to livestock prior to transport, which is gradually being adopted by poultry farmers in Nigeria, reduces the risk of adverse effects of transportation stress on livestock (Adenkola *et al*., 2009). Polyherbal products (Jadhar *et al.*, 2014) and non-enzymatic antioxidants like vitamin E are extensively being explored as addition in poultry diet. This is because of their antioxidant role in scavenging ROS, generated as a result of heat stress (Ajakaiye *et al*., 2011) during aerobic metabolisim (Lei *et al*., 2016), thus reducing production loss (Jadhar *et al*., 2014).

Ascorbate, α-tocopherol, carotenoid and glutathione are among the several antioxidant molecules, produced endogenously by the body cells. Apart from these molecules, numerous antioxidant enzymes are involved in the removal or quenching of ROS, namely: SOD, CAT, GPx. Thus, the major role in antioxidant defence is accomplished not only by small molecule antioxidant compounds, but also by antioxidant enzymes (Sies, 2015; Lei *et al*., 2016). Endogenous synthesis of these enzymes is only possible when the required co-factor is supplied through the diet; otherwise, there will be deficiency in synthesizing and maintaining

the antioxidant enzyme level; resulting in oxidative stress (Surai, 2002). Keshavamurthy (2013), reported that oxidative stress was a major factor of concern due to the characteristic rapid growth rate in broiler chickens and can be minimized by supplementing broiler diet with antioxidants; either singly or combined, thereby ameliorating stress. Quercetin, one of the most prominent dietary antioxidants, has the special ability of scavenging highly reactive species, such as hydrogen peroxide, superoxide anion, and hydroxyl radicals (Khan *et al*., 2016).

There has been increased interest and research into the process of ROS generation, functional consequences of ROS driven processor and ROS-mediated effects (Espinosa-Diez *et al*., 2015). ROS have long been known to be a component of the killing response of immune cells (that is, weapons of phagocytes) to microbial (that is, pathogen) invasion (Belikov *et al*., 2015). Zeng *et al*. (2014) found that supplementing poultry diet with antioxidants enhanced digestibility and performance in broiler chickens by reducing oxidative stress and immune depression.

Worthy of note is the concept of 'retrograde response' (Sena and Chandel, 2012; Yun and Finkel, 2014). This occurs when mitochondria produce a limited amount of ROS that act as signalling molecules, initiating a molecular stress response that leads to transcriptional change in the nucleus as response to stress, or to an increased energy demand. The transient ROS signal produced generates an endogenous response that scavenges ROS by inducing antioxidant defence enzymes such as SOD and catalase, and other stress defence pathways. Yun and Finkel (2014) further established the concept of mitochondrial hormesis or mitohormesis. This concept, in contrast to ROS playing the role of signalling molecules, shows that sustained high levels of ROS can even cause intracellular damage. The beneficial effects of antioxidants in broiler chickens production may only be observed in birds that are raised in unfavourable conditions (Fernades *et al*., 2015).

2.7 Antioxidants and their Biological Roles

Quercetin has been reported to exert anti-inflammatory (Rezaei-Sadabady *et al*., 2016), anti-fibrotic (Lee *et al*., 2003; Yoon *et al*., 2012), anti-hypertensive, hepatoprotective (Ansar *et al*., 2016; Miltonprabu *et al*., 2016; Tang *et al*., 2016), anti-atherogenic (Perez-Vizcaino *et al*., 2002), anti-diabetic (Mukhopadhay and Prajapati, 2015), insect-repelling (Kubo and Nakanishi, 1977; Adeyemi *et al*., 2010), anti-toxic, immunomodulatory (Mohan *et al*., 2015) and insecticidal (Gressel and Ammann, 2008) properties. Studies have also demonstrated that quercetin exhibits protective neurological activity in the neurones, reproductive system and cardiovascular systems (Joseph and Muralidhara, 2013; Maalik *et al*., 2014; Nathiya *et al*., 2015). Quercetin spares vitamin C and stabilises cell membranes. It also prevents cardiovascular diseases (Sesso *et al*., 2003), and exhibits anticancer, antiviral (Rezaei-Sadabady *et al*., 2016), antibacterial (Waage and Hedin, 1985; Chirumbolo, 2010; Khan *et al*., 2016), antiulcer and antiallergic activities (Stavric, 1994). Most of the pharmacological effects of quercetin are ascribed to its antioxidant activity (Zhang, 2005; Uzun *et al*., 2010; Kalender *et al*., 2012; Liu *et al*., 2012). Quercetin prevents oxidative injury and cell death by scavenging reactive oxygen species (Cox *et al*., 2000), inhibiting xanthine oxidase (Chang *et al*., 1993), lipid peroxidation, and chelating metal ions (Chen *et al*., 1990).

Ognik and Sembratowicz (2011) also reported anti-inflammatory antibacterial, antiviral roles of 5-oxo-1, 2, 4-triazine. *In vitro* analysis of this antioxidant has revealed its capacity as an antifungal agent (Modzelewska-Banachiewicz and Kaminska, 2006).

Extract of *Forsythia suspense* was found to have antifungal, anti-inflammatory (Liu, 2004), antibacterial and antihypertensive (Hao *et al*., 2010) roles. It also enhances nutrient digestibility and performance by reducing oxidative stress and immune depression. In addition, it promotes intestinal colonisation (Zeng *et al*., 2014).

Curcurmin, the active ingredient of turmeric powder, was found to exert the following biologic roles: anti-inflammatory, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoan, antiviral, antivenom, hypotensive and hypocholesteraemic (Chattopodhyay *et al*., 2004; Hosseini *et al*., 2012).

2.8 Quercetin and Its Antioxidant Role in Poultry Production

The International Union of Pure And Applied Chemistry (IUPAC) nomenclature for quercetin is 3,3[1],4[1],5,7–penahydroxyflavone (or its synonym 3,31,41,5,7–pentahydroxy–2–phenylchromer–4–one). Quercetin is regarded as the most diffused and known nature-derived flavonol (Chirumbolo, 2010); belonging to the class of plant secondary metabolites, known as flavonoids (Maalik *et al*., 2014). Quercetin is ubiquitously present in fruits and vegetables (Gomes *et al*., 2015). It is found in apples (Jeanelle and Rui, 2004; Rui, 2004), berries, brassica vegetables, capers, grapes, onions, shallots, tea, tomatoes, many seeds, nuts, flowers, barks and leaves (Kelly, 2011), and some medicinal and aromatic plants (Bhardwaj *et al*., 2010).

Quercetin has been reported for its antiviral and antioxidant applications; hence, its various derivative forms have potential for drug development that may be therapeutically useful in the cure of oxidative stress and lethal virus-induced diseases. The antioxidant activity of quercetin is attributed to its ability to: suppress inflammatory genes (Chrumbolo, 2010; Ravichandran *et al*., 2014), scavenge ROS, donate hydrogen atoms or electrons, chelate metal cations and inhibit chyrosine kinase, suggesting its anti-tumour therapy potential. Pharmacokinetic study reveals that it ameliorates the absorption and metabolism of nutrients, when they enter the physiological system (Sohaib *et al*., 2015). In a bid to emphasize the need to further explore the antibacterial effect of quercetin, currently, the development of resistant bacteria are a challenge; effective and more advanced drugs are required to counter

these bacteria. Quercetin, a bacteriostatic is a good molecule for antibacterial drug research (Maalik *et al*., 2014).

Furthermore, dietary supplementation of quercetin has the potential to improve growth performance, antioxidant capacity, stability of lipids and fatty acid composition in breast meat of birds (Sohaib *et al*., 2015). Quercetin in broiler diets could prolong meat shelf life by reducing the rate of lipid oxidation (Goliomytis *et al*., 2014). In addition, quercetin research in broilers opens up an array of opportunities for scientists desiring to further unveil effects of this agent. Finally, there is dearth of information on potential synergism of quercetin with other chemotherapeutic agents (Chirumbolo, 2010; Kelly, 2011).

2.9 Potential Role of Quercetin in Profitable Broiler Production

Many farmers around the world increase stocking density to maximise profitability in production of broiler chickens (Heckert *et al*., 2002; Thaxton *et al*., 2006; Estevez, 2007; Tayeb *et al*., 2011). The influence of stocking density (number of birds calculated on the basis of weight or bird per meter square) on broiler production has considerable interest among producers in recent years (Abdel-Azeem, 2010). The intensive management system associated with high-stocking density causes a greater degree of stress in production of broiler chickens; thus, increasing the risk of poor life or welfare and performance in the birds (El-Gogary and Azzam, 2014). Consequently, the system contributes significantly to the production loss, incurred by farmers.

The study of quercetin has been focused mainly on its therapeutic and antioxidant effects in man. However, quercetin has the potential as a functional feed additive to boost animal production. Already, it was reported to improve performance in laying hens (Liu *et al*., 2014). Furthermore, the administration of quercetin to broiler chickens should be with caution and this is based on the findings of several workers, that synthetic and naturally sourced

antioxidants like Vitamin C and β-carotene should be with caution as they have dual potentials–as an anti and prooxidant (Schmalhausen *et al*., 2007; Alrawaiq and Abdullah, 2014; Phan-thi *et al*., 2016). However, there is dearth of information on its potential to alleviate stressful conditions in commercial broiler production. From a translational point of view, feeding broiler chickens with diet supplemented with quercetin may influence physiological parameters, biochemical biomarkers and performance indicators in broilers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site and Meteorological Conditions

The experiment was carried out at a poultry house in Ahmadu Bello University (ABU), Zaria (11^{o} $10^{/}$ N, 07^{o} $38^{/}$ E), located in the Northern Guinea Savannah zone of Nigeria. The study was conducted between August 28th and October 9th, 2015, lasting 42 days.

3.2 Experimental Birds and Management

A total of 60 one-day-old, unsexed Ross 308 broiler chicks, purchased from a commercial hatchery at Mayo Belwa, (9° $22^{/}$ $6^{//}$ N and 12° $42^{/}$ $0^{//}$ E) Adamawa State, Nigeria, served as subjects. They were housed in open-sided floor pens. The wall was 1.0 m from the floor and 1.0 m high wire mesh extended from the peak of the wall to the roof. Wood shavings were used as bedding material. The broiler chicks were separated into respective compartments, made of 1 × 1 × 1 m wire mesh with a uniform feeder and drinker space within each compartment. Water and feed was provided *ad libitum* to birds throughout the experimental period. On arrival of the broiler chickens to the pen, they were exposed to 24-h lighting for 6 days. They were fed broiler starter diet from day 1 to 21 and broiler finisher diet from 22 - 42 days respectively. The birds were brood for two weeks and thereafter subjected to prevailing environmental temperature for the remaining thirty six days.

3.3 Experimental Design

Broiler chickens were separated into four stocking density groups as described by Uzum and Toplu (2013). Briefly, 60 one-day-old broiler chicks were identified using tags tied to their legs. Simple random sampling was used to select and assign the broiler chicks into four groups of 18 birds/m^2 in groups I and II, while groups III and IV contained 12 birds/m^2 each.

Broiler chicks in groups II and IV were administered 50 mg/kg body weight of quercetin, while those in groups I and III served as the untreated control broiler chicks. The broiler chicks were given access to feed and water *ad libitum*, brood under continuous lighting until the chicks were 6 days old. Thereafter, all broiler chickens were subjected to 14:00 h light and 10:00 h dark cycle. The flavonoid, quercetin was administered at 06:00 h daily via gavage from day 14 until day 42, when the experiment was terminated (Liu *et al.*, 2014).

3.4 Meteorological Parameters

Bi-hourly circadian dry- and wet-bulb temperature measurements were taken concurrently for each treatment group, using dry- and wet-bulb thermometer (Brannan, UK). From the data, relative humidity (RH) and temperature-humidity index (THI) were calculated. The THI was calculated using the following formula (Tao and Xin, 2003):

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

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

Other meteorological data during the study, including maximum AT, minimum AT, sunshine duration, wind speed and wind direction, were collated from the Institute for Agricultural Research, ABU, Samaru-Zaria, located at a distance of about 0.5 km from the experimental site.

3.5 Cloacal Temperature

Birds were randomly selected and the circadian cloacal temperature of each broiler chicken were recorded weekly using a digital thermometer (Brannan, UK), at 2 h interval, from 07:00 h to 05:00 h as described by Sinkalu *et al*. (2009) on days-22, 29 and 36. The thermometer was inserted, via the cloaca, about 2.0 cm into the rectum and in direct contact with the

mucosal wall of the rectum of each bird. The reading was taken after the thermometer gave an alarm signal, usually after about 1 minute.

3.6 Determination of Haematological Parameters

At 28, 35 and 42 days of age, five broiler chickens were randomly selected from each group and 2.0 mL of blood was aspirated from the wing vein of each broiler chicken into ethylenediaminetetraacetate (EDTA) sample bottles for full blood count in order to estimate H:L ratio. Bleeding procedure was limited to one minute or less to minimise the influence of handling stress. Erythrocyte osmotic fragility was equally determined on days 28, 35 and 42 of the experiment.

3.6.1 Determination of packed cell volume

The packed cell volume (PCV) was determined using standard technique as described by Rehman *et al*. (2003). Non-heparinised capillary tube was filled up to $^{3}/_{4}$ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tube was then centrifuged for 5 minutes at 4,383 × *g* using the Saitexiangyi TG12MX® Micro-haematocrit centrifuge machine. Thereafter, the proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley® Micro-haematocrit Reader.

3.6.2 Haemoglobin determination

Blood haemoglobin concentration was assayed colorimetrically as cyanomethhaemoglobin (Drabkin, 1945). Five millilitres of HICN (Drabkin) solution were measured using a 5-mL syringe into plastic test tubes. Twenty microlitres (20 µL) of blood were measured using a micropipette and added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 × *g* for 15 minutes to separate the empty

RBC from interfering with the reading. The supernatant was separated into a sample bottle. The mixture was absorbed into the haemoglobin meter (XF–C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dL as the haemoglobin concentration.

3.6.3 Red blood cells and total white blood cell count

Red blood cells (RBC) and total white blood cell (TWBC) counts were determined with the Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer.

Blood collected in the EDTA coated sample bottles was slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will cause capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip was properly placed on the haemocytometer. The mixture was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, then left for 5 minutes for cells to settle down.

The light microscope (Olympus-XSZ-107BN), at low power magnification (×40), was used to view the cells and counting was done using the tally counter. For TWBC count, the WBC

in the four outer large squares of the haemocytometer were counted and calculated using the formula below:

$$\frac{\mathrm{N}}{20} = \mathrm{WBC} \times 10^{9}/\mathrm{L}$$

Where *N* = Number of WBC counted in the four outer large squares (or in 64 small squares).

For RBC count, the cells contained in the four corner and central squares in the mid-section of the haemocytometer were counted. Following the "**L**" rule: cells that touched the centre triple lines of the ruling on the left and the bottom sides were counted, but those that touched the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the following formula:

$$\frac{\mathrm{N}}{20} = \mathrm{RBC} \times 10^{12}/\mathrm{L}$$

Where N = Number of RBC counted in the 5 squares in the mid-section of the haemocytometer (or in 160 squares)

Note that both charged sides of the haemocytometer were counted for both the RBC and TWBC, and the average was calculated.

3.6.4 Preparation of smears for differential leucocyte count and thrombocytes estimation

In all the birds, a pair of blood smear was made from each blood sample. A small drop (about 2 µL) of blood was immediately used for the preparation of blood smears each using the standard slide-to-slide technique. The air-dried blood smears were properly labelled using a pencil on the frosted end of the slide and then fixed in a fixing jar containing methanol for 3 minutes and air-dried (Schalm *et al*., 1975).

Staining was done by flooding the blood smears with Wright-Giemsa stain for 3 minutes. An equal amount of Sørensen's buffer (pH of 6.8) was added then mixed gently by blowing using a pipette until green metallic sheen forms on the surface. This was allowed to stand for further 6 minutes. The blood smears were rinsed with the Sørensen's buffer and allowed to stand for a minute for differentiation. The stained slides were then washed copiously with the Sørensen's buffer and the back of the smears were wiped with tissue paper to remove the excess stain and allowed to air dry. These were neatly packed into a slide box until viewing.

Examination of the blood smears was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion (× 1000). One hundred TWBC were counted and classified based on their morphologic features (Campbell and Ellis, 2007). The counting was done using the Marble® Blood Cell Calculator. The differential WBC count was then expressed as a percentage of the individual cell group. The percentage of each cell was then converted into absolute numbers by reference to the total WBC using the formula below:

$$\frac{\text{Percentage of WBC counted } \times \text{TWBC}}{100} = \text{Absolute number } \times 10^9/\text{L}$$

An estimated thrombocyte count was obtained from the stained blood film using the same formula for the indirect estimation of total WBC (Campbell and Ellis, 2007). The absolute number of thrombocytes was estimated by using the formula below:

$$\frac{\text{Number of thrombocytes counted}}{100} \times \text{TWBC} = \text{Absolute thrombocytes } \times 10^9/\text{L}$$

3.6.5 Mean corpuscular values

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using the following standard formulae (Campbell and Ellis, 2007):

$$\text{MCV} = (\text{PCV} \times 10)/\text{RBC} = \text{MCV femto litres (fl)}$$

$$\text{MCH} = (\text{Hb} \times 10)/\text{RBC} = \text{MCH picogram (pg)}$$

$$\text{MCHC} = (\text{Hb} \times 100)/\text{PCV} = \text{MCHC (g/L)}$$

3.6.6 Erythrocyte osmotic fragility

The erythrocyte osmotic fragility (EOF) of birds from each group (n = 5) was determined according to the method described by Oyewale *et al*. (2011) on day 28, 35 and 42. Briefly, 0.02 mL of blood was added to tubes containing increasing concentrations of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0.1, 0.3, 0.5, 0.7 and 0.9% NaCl concentrations). The tubes were gently mixed and incubated at room temperature (24 ^{o}C) for 30 minutes. The content of each tube was then centrifuged at 3,000 *g* for 10 minutes and the supernatant was decanted. The haemoglobin content of the supernatant was determined spectrophotometrically at a wavelength of 540 nm, with distilled water serving as blank. The percentage haemolysis in each concentration of NaCl was evaluated, taking the tube with maximum haemolysis (0 %) as 100 %.

$$\text{Percentage haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 100$$

Graphs of haemolysis in percentage against NaCl concentration were then plotted.

3.7 Performance Parameters

All broiler chickens were weighed individually on arrival; and, thereafter, body weight and feed intake were measured weekly. Body weight gain was recorded weekly. The amount of feed consumed by each broiler chicken was recorded daily in order to determine the feed conversion ratio as described by Uzum and Toplu (2013). Mortality and health status were observed and recorded as they occurred (Zhang *et al*., 2009; Aluwong *et al*., 2013). Moribound and/or dead birds were presented to the Avian Clinic of Ahmadu Bello University, Zaria for the diagnosis of ill-health and/or cause of death.

3.8 Carcass and Meat Quality Measurement

Five broilers from each group, making a total of twenty, were humanely slaughtered after 12 h of feed withdrawal to determine carcass and meat quality at the age of 42 days. Carcass dressing percentages were expressed as percentages of body weight at slaughter. Each carcass was cut into six parts; breast, wing, thigh, drum stick, shank and back as described by Bogosavljevic-Boskovic *et al*. (2006) with slight modification to include shanks. Carcass cut were expressed as percentage of carcass weight. Relative organ weight (gizzard, heart and liver) were determined and expressed as percentage of internal organ weight to final body weight (Uzum and Toplu, 2013). Breast muscle (*Pectoralis major* and *Pectoralis minor*) was used to determine meat pH (Sekeroglu *et al*., 2011). In the study, initial pH of breast meat was determined on day 1 and refrigerated at +4.0 °C. The meat was allowed to cool for 20 min prior to further determination of pH on days 2, 3 and 4 post-slaughter using a digital JENWAY 3505 pH metre equipped, with a penetrating electrode.

3.9 Data Analysis

Data obtained were expressed as mean ± standard error of the mean (± SEM) and analysed using repeated-measures one-way analysis of variance (ANOVA), followed by Tukey's *post-*

hoc test and Pearson's correlation analysis to evaluate significant difference between groups using SPSS statistical package, version 20. Values of $P < 0.05$ were considered significant (Snedecor and Cochran, 1994).

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Quercetin administration and Stocking Density on Overall Circadian Fluctuation in Cloacal Temperature

The effect of quercetin administration and stocking density on the overall circadian fluctuations in CT is shown in Table 4.1. At 7:00 h, the untreated LSD group of 12 birds/m^2 had a significantly higher ($P < 0.05$) mean CT value of 40.64 ± 0.08 °C when compared with the untreated HSD group of 18 birds/m^2, which had a CT of 40.37 ± 0.07 °C. The quercetin LSD group of 12 birds/m^2 had a significantly higher ($P < 0.05$) mean CT value of 40.80 ± 0.05°C, when compared with the quercetin-treated HSD group of 18 birds/m^2 at the same hour. At 17:00 h, the untreated HSD group of 18 birds/m^2 had a significantly higher ($P < 0.05$) mean CT value of 41.44 ± 0.04 °C, when compared with the HSD group of 18 birds/m^2, which had the mean CT value of 41.00 ± 0.05 °C, this trend was again observed at 19:00 h, 21:00 h and 5:00 h. The CT of the untreated HSD group of 18 birds/m^2 was significantly higher ($P < 0.05$) with the value of 41.44 ± 0.04 °C, when compared the untreated LSD group of 12 birds/m^2 which had a mean value of 41.15 ± 0.06 °C at 17:00 h. At 21:00 h, the quercetin-treated LSD group of 12 birds/m^2 had a significantly higher ($P < 0.05$) mean CT value of 40.92 ± 0.08 °C than the quercetin-treated HSD group of 18 birds/m^2 (40.26 ± 0.07 °C); this pattern of significant difference ($P < 0.05$) was equally observed at 1:00 h, 3:00 h and 5:00 h and 7:00 h, when the quercetin-treated LSD group was compared with the quercetin-treated HSD group. At 1:00 h, the mean CT value was significantly higher ($P < 0.05$) in the quercetin-treated LSD group of 12 birds/m^2 (41.04 ± 0.08 °C) when compared with the untreated LSD group (40.79 ± 0.05 °C).

The overall circadian fluctuation in CT as hour of the day increased within corresponding groups of quercetin treatment at HSD and LSD, and is shown in Table 4.4. The CT of broiler

chickens kept at HSD of 18 birds/m^2 rose significantly from value of 40.37 ± 0.07 °C at 7:00 h to 41.44 ± 0.04 °C at 17:00 h ($P < 0.05$). Thereafter, fluctuations in CT did not follow a particular trend until 5:00 h; however, a significant decline ($P < 0.05$) in CT was recorded at 19:00 h with the value of 41.08 ± 0.08 °C ($P < 0.01$). The bi-hourly CT values of broiler chickens within the group that were kept at 18 birds/m^2 + quercetin increased significantly from 7:00 h to 11:00 h with the corresponding value of 40.54 ± 0.06 °C to 40.92 ± 0.08 °C ($P < 0.05$). Furthermore, the CT value within the group rose to 41.10 ± 0.07 °C at 13:00 h, and this value was sustained with minimal variation until 17:00 h. Thereafter, the CT value decreased to 40.55 ± 0.08 °C ($P < 0.05$) and further fluctuations in CT values within the group did not follow a particular trend. The CT values in group of 12 birds/m^2 without quercetin treatment increased significantly as the hour of the day increased from 7:00 h to 11:00 h with the corresponding mean values of 40.64 ± 0.08 °C to 40.99 ± 0.10 °C ($P < 0.05$). Further increase in the mean CT value was recorded at 13:00 h, with the value of 41.14 ± 0.08 °C and CT, and was sustained similarly at this temperature until 17:00 h, after which a significant decrease in the CT value to 40.80 ± 0.07 °C was recorded at 19:00 h ($P < 0.05$). Thereafter, the CT increased to 40.97 ± 0.06 °C at 23:00 h, and gradually decreased to 40.79 ± 0.05 °C at 1:00 h ($P < 0.05$) and increased to 40.86 ± 0.05 °C at 5:00 h. The mean CT value within quercetin-treated group of 12 birds/m^2 was relatively stable, but did not follow a particular trend as the hour of the day increased thereafter. The peak CT value within group of broiler chickens kept at HSD of 18 birds/m^2 without treatment was observed at 17:00 h. The peak CT value in the group of broiler chickens kept at HSD of 18 birds/m^2 and treated with quercetin was obseved at 15:00 h. The acrophase CT within the untreated group of 12 birds/m^2 was 41.15 ± 0.06 °C at 17:00 h. The acrophase CT of 41.25 ± 0.07 in the quercetin-treated group of 12 birds/m^2 was recorded at 11:00 h.

Table 4.1: Effect of quercetin administration and stocking density on overall circadian fluctuation in cloacal temperature of broiler chickens

Time of the day (h)	Stocking density group			
	18 birds/m^2 (n=18)	18 birds/m^2 + Quercetin (n=18)	12 birds/m^2 (n=12)	12 birds/m^2 + Quercetin (n=12)
7:00	$40.37 \pm 0.07^{1,a}$ (38.90 - 41.00)	$40.54 \pm 0.06^{1,2,3,a,b}$ (39.50 - 41.20)	$40.64 \pm 0.08^{1,b,c}$ (39.30-41.40)	$40.80 \pm 0.05^{1,2,c}$ (40.20-41.40)
9:00	$40.74 \pm 0.05^{2,3}$ (39.9-41.50)	$40.61 \pm 0.07^{2,3}$ (39.60-41.70)	$40.65 \pm 0.09^{1,2}$ (39.60-41.80)	$40.82 \pm 0.08^{1,2}$ (39.70-41.50)
11:00	$41.01 \pm 0.08^{4,b}$ (40.00-42.00)	$40.92 \pm 0.08^{4,5,a,b}$ (39.90- 42.60)	$40.99 \pm 0.10^{3,4,a,b}$ (39.90-41.90)	$41.25 \pm 0.07^{3,b}$ (40.30-41.90)
13:00	$41.29 \pm 0.04^{5,6}$ (40.20-41.80)	41.10 ± 0.07^{5} (40.20-42.30)	41.14 ± 0.08^{4} (40.30-42.00)	41.24 ± 0.10^{3} (40.10-42.20)
15:00	$41.32 \pm 0.04^{5,6}$ (40.80-41.80)	41.14 ± 0.05^{5} (40.20-41.90)	41.14 ± 0.05^{4} (40.20-41.70)	$41.18 \pm 0.08^{2,3}$ (40.20-42.00)
17:00	$41.44 \pm 0.04^{6,b}$ (40.90-41.90)	$41.00 \pm 0.05^{4,5,a}$ (40.10-41.70)	$41.15 \pm 0.06^{4,a}$ (40.50-41.90)	$41.19 \pm 0.08^{2,3,a}$ (40.10-41.90)
19:00	$41.08 \pm 0.08^{4,5,b}$ (40.20-41.80)	$40.55 \pm 0.08^{2,3,a}$ (39.20-41.50)	$40.80 \pm 0.07^{1,2,3,a,b}$ (39.80-41.40)	$40.73 \pm 0.15^{1,a}$ (38.90-42.60)
21:00	$40.92 \pm 0.06^{3,4,b}$ (40.00-41.80)	$40.26 \pm 0.07^{1,a}$ (39.00-41.10)	$40.80 \pm 0.06^{1,2,3,b}$ (40.00-41.40)	$40.92 \pm 0.08^{1,2,3,b}$ (40.10-42.20)
23:00	$40.94 \pm 0.05^{3,4}$ (40.00-42.20)	$40.78 \pm 0.05^{3,4}$ (40.00-41.60)	$40.97 \pm 0.06^{2,3,4}$ (40.00-41.90)	$40.79 \pm 0.07^{1,2}$ (40.00-41.80)
1:00	$40.88 \pm 0.06^{3,4,a,b}$ (40.00-41.90)	$40.71 \pm 0.07^{3,4,a}$ (39.70-41.70)	$40.79 \pm 0.05^{1,2,3,a}$ (40.00-41.50)	$41.04 \pm 0.08^{1,2,3,b}$ (40.00-42.20)
3:00	$40.62 \pm 0.06^{1,2,a,b}$ (39.60-41.70)	$40.41 \pm 0.06^{1,2,a}$ (39.20-41.20)	$40.58 \pm 0.08^{1,a,b}$ (39.50-41.70)	$40.79 \pm 0.09^{1,2,b}$ (39.60-41.90)
5:00	$40.95 \pm 0.04^{3,4,b}$ (40.30-41.80)	$40.60 \pm 0.05^{2,3,a}$ (39.70-41.60)	$40.86 \pm 0.05^{1,2,3,4,b}$ (39.90-41.20)	$41.04 \pm 0.06^{1,2,3,b}$ (40.40-41.90)
Overall mean ± SEM	40.96 ± 0.02^{c} (38.90-42.20)	40.72 ± 0.02^{a} (39.00-42.60)	40.88 ± 0.02^{b} (39.30-42.00)	40.98 ± 0.03^{c} (38.90-42.60)

Values in parenthesis represent minimum-maximum. Means with different superscript numbers differ significant within columns. Means with different superscript letters vary significantly ($P < 0.05$) between rows.

4.2 Relationship between Meteorological Parameters and Cloacal Temperature

Table 4.2 shows the relationship between meteorological parameters and CT. There was no significant correlation between the hour of the day and CT ($P > 0.05$) in all the groups. However, there was a negative correlation between both factors in the untreated HSD group of 18 birds/m^2 ($r = 0.485$), when compared with the quercetin-treated HSD group ($r = 0.143$). The relationship was also negative in the untreated HSD group of 18 birds/m^2 ($r = -0.485$), when compared with the untreated LSD group of 12 birds/m^2. The relationship between DBT and the CT was stronger in the untreated HSD group of 18 birds/m^2 ($P < 0.001$; $r = 0.845$) when compared with the quercetin-treated HSD group of 18 birds/m^2 ($P < 0.05$; $r = 0.695$) and untreated LSD group of 12 birds/m^2 ($P < 0.01$; $r = 0.818$). The DBT and CT were positively correlated in both qurcetin-treated HSD of 18 birds/m^2 and quercetin-treated LSD group of 12 birds/m^2 ($r = 0.695$ and $r = 0.674$, respectively). There was a stonger association between DBT and CT in the untreated LSD of 12 birds/m^2 ($P < 0.01$; $r = 0.818$), when compared with the quercetin-treated LSD group of 12 birds ($P < 0.05$). The relationship between RH and CT was significant and positively correlated in the untreated HSD group, when compared with the quercetin-treated HSD group of 18 birds/m^2. A significant negative association was obtained, when untreated ($P < 0.05$; $r = -0.603$) with untreated ($P < 0.05$; $r = -0.615$) LSD groups were compared. There was a significant negative correlation between RH and CT in the quercetin-treated LSD group of 12 birds/m^2 ($P < 0.05$; $r = -0.603$), while the relationship was insignificant in the quercetin-treated HSD group of 18 birds/m^2 ($P > 0.05$; $r = -0.438$). A stronger positive association was found between the THI and CT in the untreated HSD group ($P < 0.001$; $r = 0.848$), when compared with the quercetin-treated HSD group of 18 birds/m^2 ($P < 0.05$; $r = 0.701$). The stronger positive correlation between THI and CT was obtained in the untreated HSD group ($P < 0.001$; $r = 0.848$), when compared with untreated LSD group ($P < 0.01$; $r = 0.821$).

Table 4.2: Relationship between meteorological parameters and cloacal temperature of broiler chickens

Correlated Parameters	Stocking density			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
Hour of the day and CT	-0.485^{NS}	0.143^{NS}	0.481^{NS}	-0.047^{NS}
DBT and CT	0.845***	0.695*	0.818**	0.674*
RH and CT	0.635*	-0.438^{NS}	-0.603*	-0.615*
THI and CT	0.848***	0.701*	0.821**	0.672*

NS = Non-significant correlation ($p > 0.05$), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$;
h = hour of the day, CT = Cloacal temperature, DBT = Dry-bulb-temperature, RH = Relative humidity, THI = Temperature-humidity-index

4.3 Quercetin Administration and Stocking Density on Haematological Values of Broiler Chickens

On day 28 of the study as represented in Table 4.3, the mean value of TWBC was significantly higher ($P < 0.05$) in the untreated HSD group of 18 birds/m^2 ($12.36 \pm 1.22 \times 10^9$/l) when compared with the quercetin-treated HSD group of 18 birds/m^2 ($8.50 \pm 0.67 \times 10^9$/l). A significant difference was not observed at LSD of 12 birds/m^2 ($P > 0.05$) when quercetin was administered ($8.56 \pm 0.44 \times 10^9$/l) and compared with the untreated LSD group ($8.40 \pm 0.93 \times 10^9$/l). The TWBC was significantly higher ($P < 0.05$) in the untreated HSD group of 18 birds/m^2 ($12.36 \pm 1.22 \times 10^9$/l) when compared with the untreated LSD group of 12 birds/m^2 ($8.40 \pm 0.93 \times 10^9$/l). There was no significant change in the mean value of TWBC, when the quercetin-treated HSD group ($8.50 \pm 0.67 \times 10^9$/l) was compared with quercetin-treated LSD group ($8.56 \pm 0.44 \times 10^9$/l). A similar pattern of changes in TWBC between the groups compared was a repeated occurrence in heterophil counts also. The mean value for the H:L ratio was lowest in the quercetin-treated HSD group of 18 birds/m^2 (0.19 ± 0.03), when compared to the other groups of untreated HSD (0.53 ± 0.13), untreated LSD (0.29 ± 0.03), and quercetin-treated (0.29 ± 0.05) groups.

On day 35 as shown in Table 4.4, a significant increase ($P < 0.05$) in red blood cell count was recorded in the untreated HSD group ($2.40 \pm 0.15 \times 10^{12}$/L) when compared with the quercetin-treated HSD ($1.51 \pm 0.24 \times 10^{12}$/L), untreated LSD ($1.73 \pm 0.16 \times 10^{12}$/L) and quercetin-treated LSD ($1.85 \pm 0.16 \times 10^{12}$/L) groups. The variations in other haematological parameters were not significantly different between groups.

On day 42 as represented in Table 4.5, the TP value was higher ($P < 0.05$) in the quercetin-treated HSD group (4.12 ± 0.22 g/dl), when compared with the untreated HSD group (3.00 ± 0.29 g/dl). Although, insignificant, the ratio of TP was higher in the untreated LSD group (3.52 ± 0.17 g/dl), when compared with the untreated HSD group (3.00 ± 0.29 g/dl). The TP

value was not significantly different at LSD when the quercetin-treated 12 birds/m^2 (3.48 ± 0.14 g/dl) was compared with the untreated 12 birds/m^2 (3.52 ± 0.17 g/dl) group. The ratio of TP was higher in the quercetin-treated HSD group (4.12 ± 0.22 g/dl), when compared with the treated LSD group (3.48 ± 0.14 g/dl), although this variation was not significantly different ($P > 0.05$).

Overall, the variations in mean values of haematological parameters were not significantly different ($P > 0.05$), when compared between the different experimental groups (Table 4.6).

4.4 Effect of Quercetin Administration and Stocking Density on *In Vitro* Erythrocyte Osmotic Fragility

There was complete haemolysis (100 %) in the distilled water (control solvent), containing 0.0 % NaCl concentration on each day (Figure 4.1 – 4.3). Similarly, haemolysis was also observed at 0.5 % NaCl on days 35 and 42 of the study but was not observed at 0.7 % and 0.9 % NaCl concentration on day 28. There was a significant change in the percentage haemolysis at 0.5 % NaCl concentration on day 28; with the group of 12 birds/m^2 being the lowest ($P < 0.05$), when compared with the other groups. At LSD of 12 birds/m^2, percentage haemolysis was lower ($P < 0.05$) in the untreated group, when compared with the quercetin-treated group. The percentage haemolysis was also significantly higher ($P < 0.05$) at 0.1 % NaCl in the quercetin-treated LSD when compared with untreated LSD group of 12 birds/m^2. A significant change in the percentage haemolysis occurred at 0.1 % NaCl concentration on day 35 (Figure 4.2), similarly, the group of 12 birds/m^2 was the lowest ($P < 0.05$) when compared with the other groups. There was no significant change ($P > 0.05$) in the percentage haemolysis at the different concentration of NaCl on day 42 (Figure 4.3). Overall, at concentrations of 0.5, 0.3 and 0.1 % NaCl, the untreated group of 12 birds/m^2 had the lowest ($P < 0.05$) percentage erythrocyte osmotic fragility when compared with the other groups. The HSD of 18 birds/m^2 caused a significant increase ($P < 0.05$) in percentage

osmotic fragility in the untreated HSD group at 0.5 %, 0.3 % and 0.1 % NaCl concentration when compared with percentage osmotic fragility in LSD of 12 birds/m^2 (Figure 4.4).

4.5 The Effect of Quercetin Administration and Stocking Density on Growth Performance of Broiler Chickens

The Effect of Quercetin Administration and Stocking Density on Growth Performance of Broiler Chickens is shown in Table 4.7. At low stocking density (LSD) of 12 birds/m^2, the initial body weight (287.90 ± 12.10), final body weight (1,296.00 ± 52.39) and cumulative feed consumption (2,161.00 ± 2.64) were higher ($P < 0.05$) in the untreated LSD group, when compared with LSD group that was administered with quercetin. No significant difference was observed between the means of the initial body weight, final body weight and cumulative feed consumption, when the untreated group of 18 birds/m^2 was compared with the treated group ($P > 0.05$). The feed conversion ratio was markedly lower ($P < 0.05$) in the quercetin-treated HSD group (1.43 ± 0.07), when compared with the untreated 18 birds/m^2 (1.79 ± 0.10), untreated 12 birds/m^2 (2.20 ± 0.10) and quercetin-treated 12 birds/m^2 (2.10 ± 0.12). At HSD, mean values of the initial body weight, final body weight, weight gain and cumulative feed conversion were not significantly different when quercetin-treated HSD was compared with untreated HSD group ($P > 0.05$). The initial body weight (287.90 ± 12.10) and cumulative feed consumption (2,161.00 ± 2.64) was significantly higher ($P < 0.05$) in the untreated LSD group of 12 birds/m^2, when compared with untreated HSD group of 18 birds/m^2. The final live body weight (1,344.00 ± 54.22 g) and weight gain (1,120.00 ± 52.48 g/chick) was significantly higher ($P < 0.05$) in the quercetin-treated HSD group, when compared with quercetin-treated LSD group, with the final live body weight of 1,071.00 ± 60.76 g and weight gain of 842.50 ± 57.07 g/chick (Figure 4.5).

The survivability rate was 94.44 %, 91.70 %, 88.89 % and 83.33 % in the quercetin-treated HSD, quercetin-treated LSD, untreated HSD and untreated LSD groups, respectively. The correlation between the week and body weight was strong in the quercetin-treated HSD ($P <$

0.01; r = 0.9499) group when compared with untreated HSD group ($P < 0.05$; r = 0.8281). The relationship between the week and body weight parameters were also higher in the quercetin-treated LSD group ($P < 0.01$; r = 0.9683) than in the untreated LSD group ($P < 0.05$; r = 0.8783). The relationship between week and body weight was stronger in the quercetin-treated HSD ($P < 0.01$; r = 0.9499) and quercetin-treated LSD ($P < 0.01$; r = 0.9683) groups when compared with the untreated HSD ($P < 0.05$; r = 0.8281) and untreated LSD ($P < 0.05$; r = 0.8783) groups. The strength of this association was similar between the untreated groups while it was similar but stronger between the treated groups.

4.6 Quercetin Administration and Stocking Density on Carcass Characteristics and the Percentages of Internal Organ Weights of Broiler Chickens

The effect of quercetin administration and stocking density on carcass characteristics and the percentage internal organ weight of broiler chickens is shown in Table 4.8. The percentage gizzard (3.38 ± 0.06 %) and breast meat yield (18.75 ± 1.81 %) was significantly higher ($P < 0.05$) in the quercetin-treated LSD when compared with the percentage gizzard (2.83 ± 0.27 %) and breast meat yield (17.38 ± 0.47 %) of the untreated LSD group. The wing yield was significantly higher ($P < 0.05$) in the quercetin-treated HSD group (10.08 ± 0.29 %) when compared with the untreated HSD group (9.44 ± 0.48 %).

4.7 Effect of quercetin and stocking density on the pH of stored breast meat

Table 4.9 represents the effect of quercetin and stocking density on the pH of stored breast meat. The pH of the meat from all the groups increased on a daily basis. The earliest significant rise in pH was first seen on day 3 in the untreated HSD group where the pH value increased from 5.64 ± 0.10 on day 2 to 5.96 ± 0.08 on day 3 ($P < 0.05$). The earliest significant increase in pH of the other groups was recorded on day 4. The pH value of the quercetin-treated HSD was higher in this group when compared with the untreated HSD group; nevertheless, this was not significantly different ($P > 0.05$).

Table 4.3: Effect of quercetin administration and stocking density on haematological values of 28-day-old broiler chicks

Parameters	Stocking density			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
PCV (%)	33.80 ± 0.58	31.20 ± 0.97	32.6 ± 1.33	31.80 ± 1.16
Hb (g/dl)	11.22 ± 0.19	10.38 ± 0.33	10.84 ± 0.45	10.56 ± 0.38
TP (g/dl)	4.12 ± 0.16	3.96 ± 0.26	3.72 ± 0.37	3.56 ± 0.33
RBC × 10^{12}/ l	5.92 ± 0.10	5.58 ± 0.25	5.46 ± 0.25	5.56 ± 0.21
TWBC × 10^9/ l	12.36 ± 1.22^b	8.50 ± 0.67^a	8.40 ± 0.93^a	8.56 ± 0.44^a
Basophils × 10^9/ l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils × 10^9/ l	0.13 ± 0.08	0.06 ± 0.06	0.00 ± 0.00	0.14 ± 0.09
Heterophils × 10^9/ l	3.91 ± 0.79^b	1.35 ± 0.30^a	1.82 ± 0.22^a	1.80 ± 0.30^a
Lymphocytes × 10^9/ l	8.21 ± 1.37	7.05 ± 0.42	6.31 ± 0.70	6.37 ± 0.39
Monocytes × 10^9/ l	0.12 ± 0.07	0.03 ± 0.03	0.07 ± 0.03	0.07 ± 0.04
Heterophil:Lymphocyte	0.53 ± 0.13^b	0.19 ± 0.03^a	0.29 ± 0.03^b	0.29 ± 0.05^b
MCV (fl)	57.13 ± 1.04	56.04 ± 0.78	59.83 ± 1.43	57.22 ± 0.67
MCH (pg)	18.97 ± 0.36	18.64 ± 0.25	19.89 ± 0.46	19.00 ± 0.22
MCHC (g/l)	33.2 ± 0.04	33.27 ± 0.05	33.25 ± 0.04	33.21 ± 0.04

PCV = packed cell volume, Hb = haemoglobin, TP = total protein, RBC = red blood cells, TWBC = Total white blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration
Means with superscripts different letters differ significantly ($P < 0.05$) within rows

Table 4.4: Effect of quercetin administration and stocking density on haematological values of 35-day-old broiler chicks

Parameter	Stocking density			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
PCV (%)	28.00 ± 1.23	19.8 ± 4.07	23.8 ± 4.38	30.80 ± 1.50
Hb (g/dl)	9.33 ± 0.41	6.60 ± 1.36	7.93 ± 1.46	10.27 ± 0.50
TP (g/dl)	3.16 ± 0.29	3.24 ± 0.26	3.92 ± 0.32	3.72 ± 0.23
RBC × 10^{12}/ l	2.40 ± 0.15^b	1.51 ± 0.24^a	1.73 ± 0.16^a	1.85 ± 0.16^a
TWBC × 10^9/ l	5.44 ± 0.66	4.09 ± 0.52	4.03 ± 1.26	4.08 ± 0.95
Basophils × 10^9/ l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils × 10^9/ l	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.02
Heterophils × 10^9/ l	0.58 ± 0.08	0.98 ± 0.57	0.56 ± 0.24	0.66 ± 0.09
Lymphocytes × 10^9/ l	4.78 ± 0.70	3.06 ± 0.23	3.34 ± 1.00	3.21 ± 0.86
Monocytes × 10^9/ l	0.08 ± 0.02	0.03 ± 0.02	0.09 ± 0.05	0.17 ± 0.06
Heterophil:Lymphocyte	0.13 ± 0.03	0.35 ± 0.22	0.15 ± 0.03	0.29 ± 0.07
MCV (fl)	118.00 ± 7.74	131.30 ± 18.53	145.80 ± 30.16	174.20 ± 23.00
MCH (pg)	39.32 ± 2.58	43.77 ± 6.18	48.59 ± 10.05	58.07 ± 7.67
MCHC (g/l)	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00

PCV = packed cell volume, Hb = haemoglobin, TP = total protein, RBC = red blood cells, TWBC = Total white blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration
Means with different superscripts letters vary significantly ($P < 0.05$) within row

Table 4.5: Effect of quercetin administration and stocking density on haematological values of 42-day-old broiler chickens

Parameter	Stocking Density			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
PCV (%)	23.00 ± 1.50	31.00 ± 2.12	24.00 ± 0.63	23.00 ± 3.02
Hb (g/dl)	7.66 ± 0.50	10.33 ± 0.71	8.00 ± 0.21	7.67 ± 1.01
TP (g/dl)	3.00 ± 0.29^{a}	4.12 ± 0.22^{b}	3.52 ± 0.17^{a,b}	3.48 ± 0.14^{a,b}
RBC × 10^{12}/ l	1.59 ± 0.19	2.02 ± 0.27	1.63 ± 0.13	1.46 ± 0.21
TWBC × 10^{9}/ l	3.68 ± 0.28	2.65 ± 0.67	2.53 ± 0.34	4.30 ± 0.87
Basophils × 10^{9}/ l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils × 10^{9}/ l	0.04 ± 0.02	0.07 ± 0.06	0.06 ± 0.03	0.31 ± 0.18
Heterophils × 10^{9}/ l	0.57 ± 0.16	0.61 ± 0.30	0.28 ± 0.07	0.95 ± 0.20
Lymphocytes × 10^{9}/ l	2.96 ± 0.38	1.75 ± 0.36	2.11 ± 0.31	2.92 ± 0.58
Monocytes × 10^{9}/ l	0.11 ± 0.03	0.22 ± 0.06	0.08 ± 0.02	0.10 ± 0.08
Heterophil:Lymphocyte	0.23 ± 0.09	0.28 ± 0.09	0.13 ± 0.03	0.32 ± 0.03
MCV (fl)	150 ± 13.55	161.30 ± 15.25	152.60 ± 17.07	158.50 ± 10.05
MCH (pg)	50.00 ± 4.52	53.77 ± 5.08	50.88 ± 5.69	52.82 ± 3.35
MCHC (g/l)	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00

PCV = packed cell volume, Hb = haemoglobin, TP = total protein, RBC = red blood cells, TWBC = Total white blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration
Means with different superscripts letters differ significantly ($P < 0.05$) within row

Table 4.6: Overall effect of quercetin administration and stocking density on haematological values of broiler chickens

Parameter	Stocking Density			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
PCV (%)	28.27 ± 1.33	27.33 ± 2.03	26.80 ± 1.80	28.53 ± 1.52
Hb (g/dl)	9.41 ± 0.44	9.11 ± 0.68	8.93 ± 0.60	9.50 ± 0.51
TP (g/dl)	3.43 ± 0.19	3.77 ± 0.17	3.72 ± 0.17	3.59 ± 0.14
RBC × 10^{12}/ l	3.30 ± 0.51	3.04 ± 0.50	2.94 ± 0.49	2.96 ± 0.50
TWBC × 10^9/ l	7.16 ± 1.10	5.08 ± 0.75	4.99 ± 0.83	5.65 ± 0.69
Basophils × 10^9/ l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils × 10^9/ l	0.06 ± 0.03	0.05 ± 0.03	0.03 ± 0.01	0.16 ± 0.07
Heterophils × 10^9/ l	1.69 ± 0.49	0.98 ± 0.23	0.89 ± 0.21	1.138 ± 0.17
Lymphocytes × 10^9/ l	5.31 ± 0.76	3.95 ± 0.63	3.92 ± 0.61	4.16 ± 0.54
Monocytes × 10^9/ l	0.10 ± 0.03	0.10 ± 0.03	0.08 ± 0.02	0.11 ± 0.03
Heterophil:Lymphocyte	0.30 ± 0.07	0.27 ± 0.08	0.19 ± 0.03	0.296 ± 0.03
MCV (fl)	108.40 ± 11.38	116.20 ± 13.96	119.40 ± 15.55	130 ± 15.87
MCH (pg)	36.11 ± 3.80	38.73 ± 4.66	39.79 ± 5.19	43.30 ± 5.30
MCHC (g/l)	33.29 ± 0.02	33.31 ± 0.02	33.30 ± 0.02	33.29 ± 0.02

PCV = packed cell volume, Hb = haemoglobin, TP = total protein, RBC = red blood cells, TWBC = Total white blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration

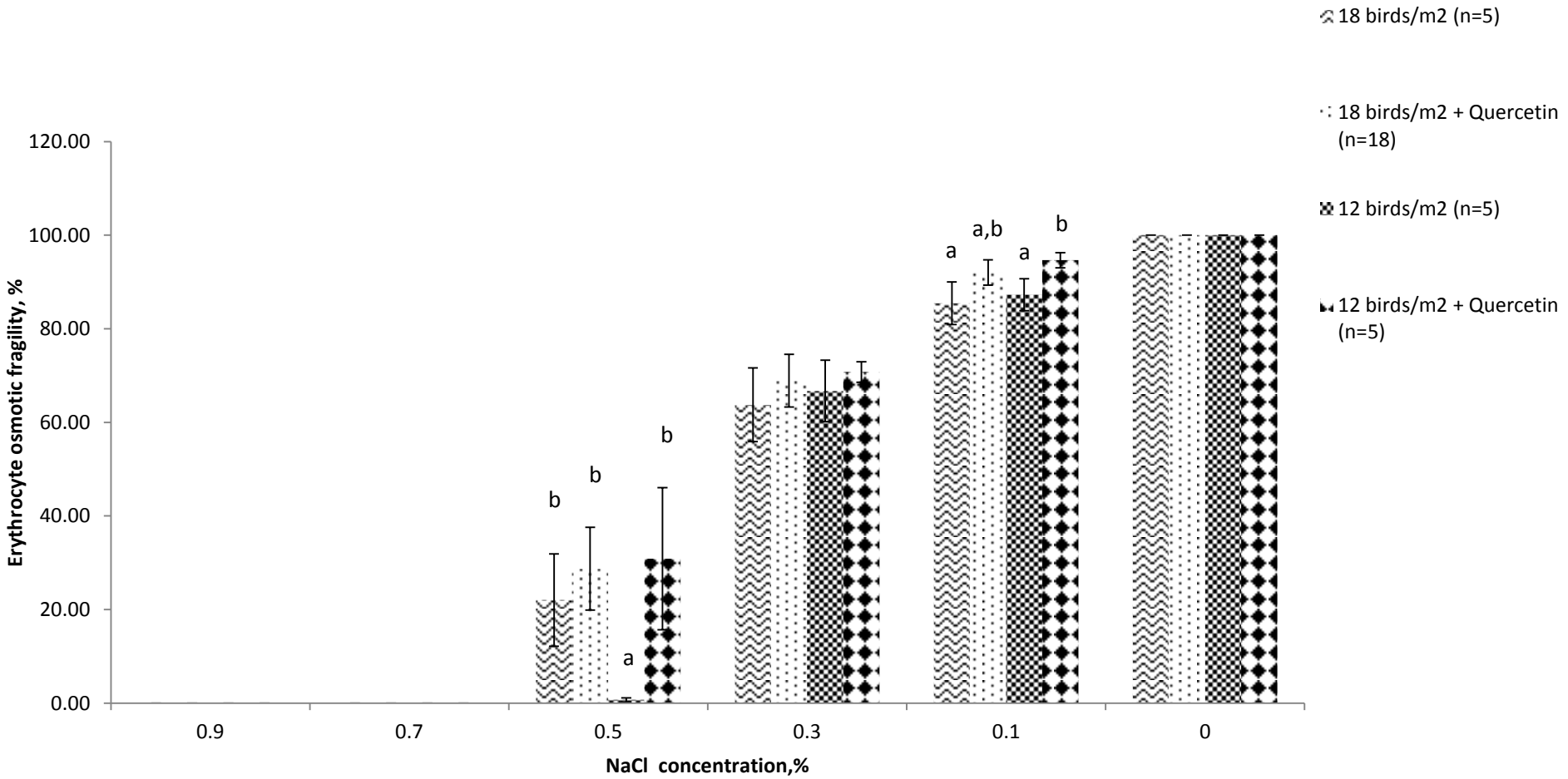

Figure 4.1: Variation in erythrocyte osmotic fragility of 28-day-old ross 308 broiler chickens reared at different stocking densities and administered with quercetin (n=5); [a, b] = Means at the same NaCl concentration having different superscript letters are significantly (P < 0.05) different

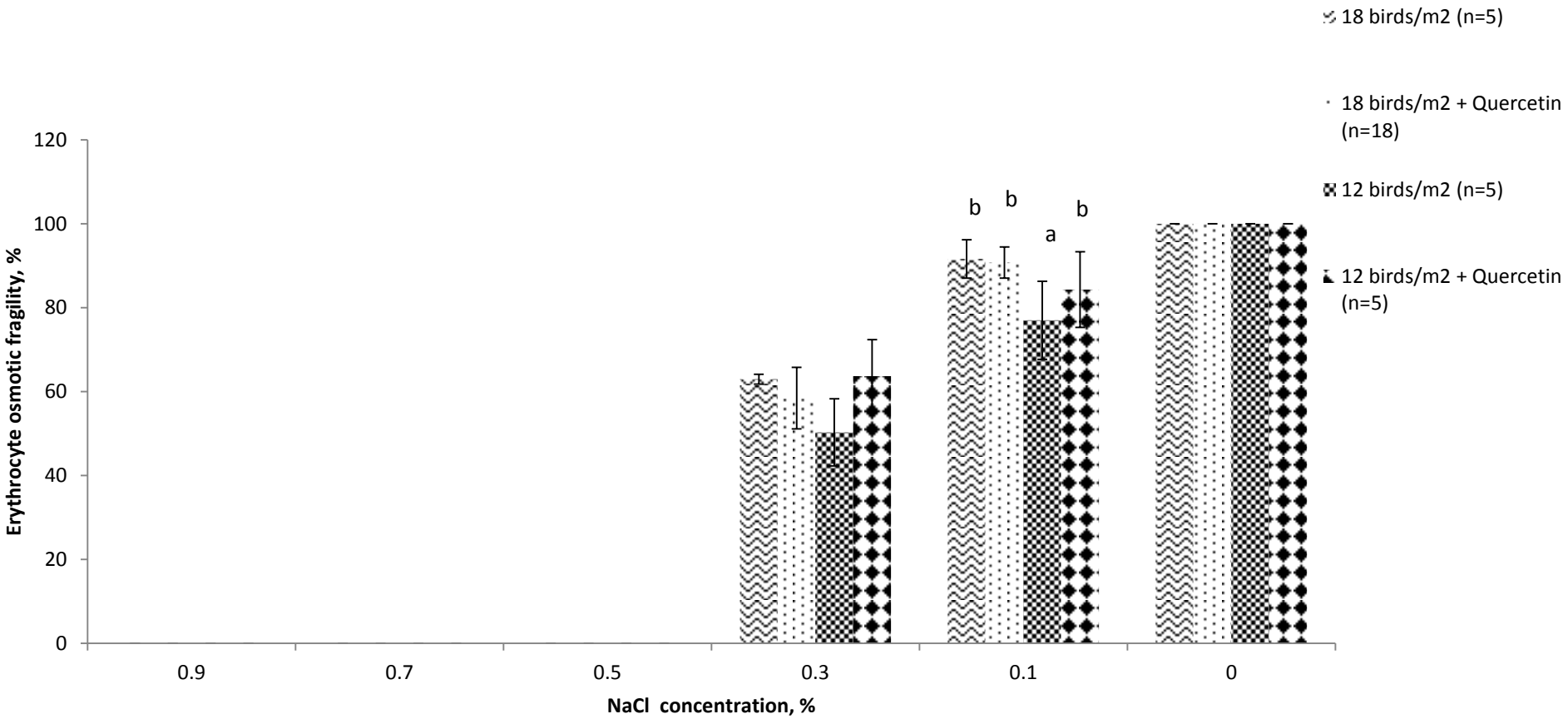

Figure 4.2: Variation in erythrocyte osmotic fragility of 35-day-old ross 308 broiler chickens reared at different stocking densities and administered with quercetin (n=5); [a, b] = Means at the same NaCl concentration having different superscript letters are significantly (P < 0.05) different

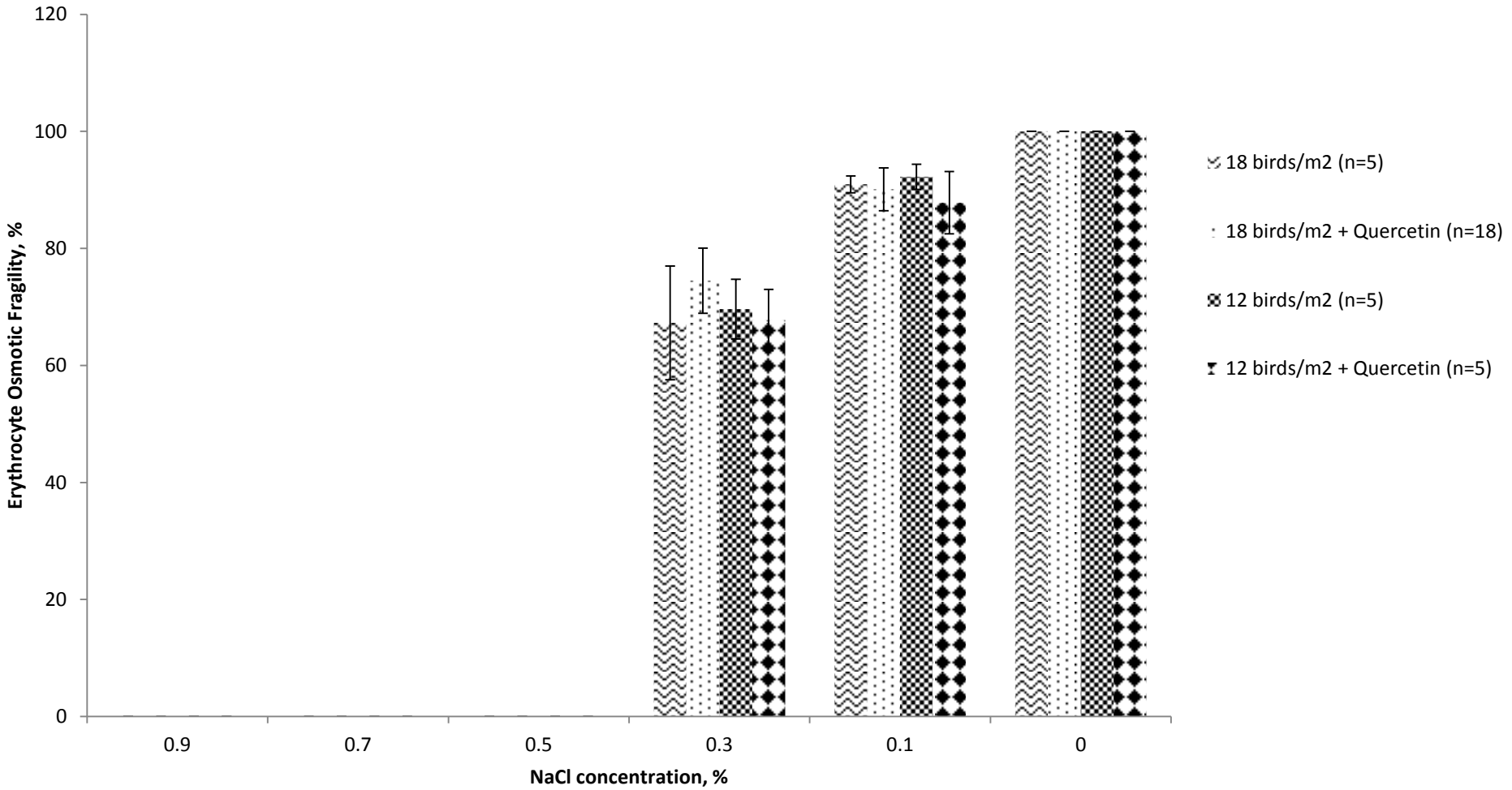

Figure 4.3: Variation in erythrocyte osmotic fragility of 42-day-old ross 308 broiler chickens reared at different stocking densities and administered with quercetin (n=5)

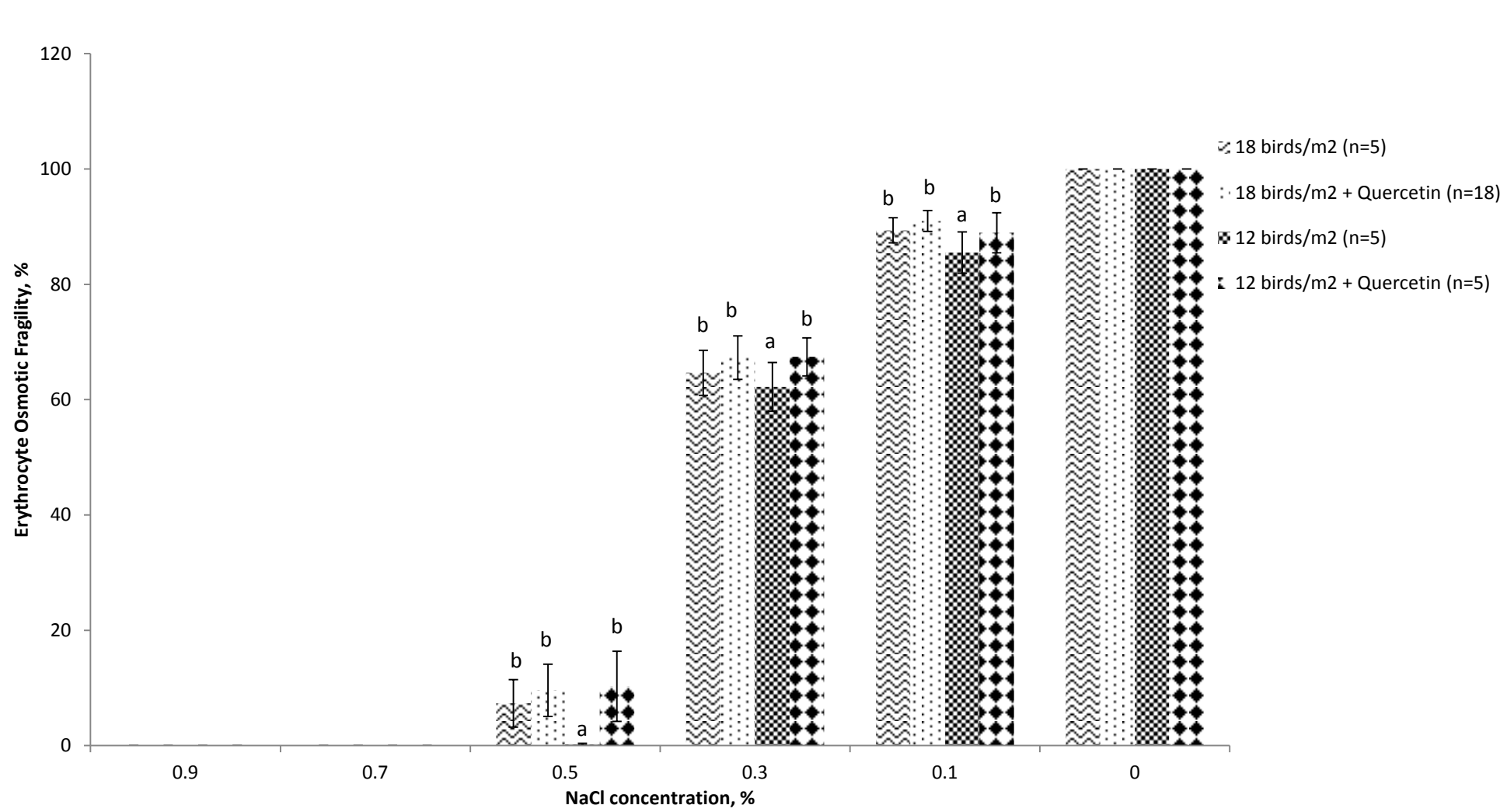

Figure 4.4: Overall mean variation in erythrocyte osmotic fragility of ross 308 broiler chickens reared at different stocking densities and administered with quercetin (n=5); [a, b] = Means at the same NaCl concentration having different superscript letters are significantly (P < 0.05) different

Table 4.7: The effect of quercetin administration and stocking density on performance of broiler chickens

Parameter	18 birds/m^2 (n = 18)	18 birds/m^2 + Quercetin (n = 18)	12 birds/m^2 (n = 12)	12 birds/m^2 + Quercetin (n = 12)
Initial body weight (g) at week 1	245.60 ± 9.60^{a}	224.70 ± 8.61^{a}	287.90 ± 12.10^{b}	228.30 ± 13.47^{a}
Final live body weight (g) at week 6	$1,272.00 \pm 48.21^{a,b}$	$1,344.00 \pm 54.22^{b}$	$1,296.00 \pm 52.39^{b}$	$1,071.00 \pm 60.76^{a}$
Weight gain (g/chick)	$1,027.00 \pm 48.13^{a,b}$	$1,120.00 \pm 52.48^{b}$	$1,008 \pm 47.02^{a,b}$	842.50 ± 57.07^{a}
Cumulative feed consumption (g/chick)	$1,762.0 \pm 0\ 1.67^{a}$	$1,542.00 \pm 2.01^{a}$	$2,161.00 \pm 2.64^{b}$	$1,682.00 \pm 1.60^{a}$
Feed conversion ratio (g feed/gram gain)	1.79 ± 0.10^{b}	1.43 ± 0.07^{a}	2.20 ± 0.10^{c}	$2.10 \pm 0.12^{b,c}$
Viability rate (%)	88.89	94.44	83.33	91.70
Week and Body weight (Correlation)	0.8281*	0.9499**	0.8783*	0.9683**

*= significant correlation ($P < 0.05$), **= highly significant correlation ($P < 0.01$). Superscripts with different letters vary significantly ($P < 0.05$) within rows.

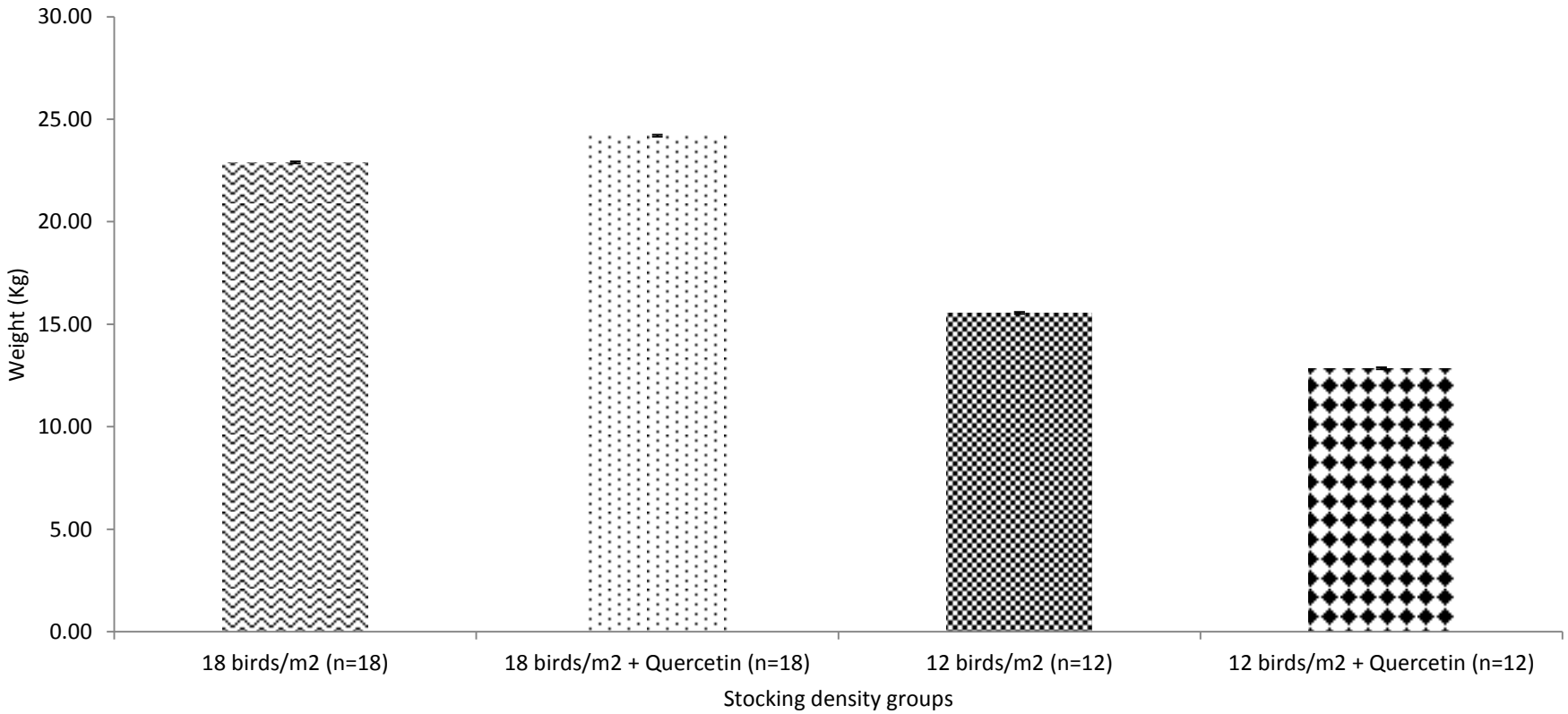

Figure 4.5: Final weights of 42-day-old broiler chickens kept at different stocking densities and administered with quercetin

Table 4.8: Effect of stocking density and quercetin administration on carcass characteristics and the percentages of internal organs of broilers

	Stocking Density Group			
Meat Yield	18 birds/m^2 (n = 5)	18 birds/m^2 + Quercetin (n = 5)	12 birds/m^2 (n = 5)	12 birds/m^2 + Quercetin (n = 5)
Pre-slaughter weight (g)	1400.00 ± 89.44	1470.00 ± 99.50	1390.00 ± 92.74	1220.00 ± 115.80
Eviscerated carcass weight (g)	1082.00 ± 82.34	1084 ± 71.81	1053.00 ± 69.43	921.00 ± 115.8
Carcass weight (%)[1]	77.03 ± 0.96	73.80 ± 0.80	75.84 ± 1.09	75.11 ± 1.28
Shank yield (%)[1]	5.83 ± 0.04	6.13 ± 0.19	5.89 ± 0.17	5.94 ± 0.38
Drum stick yield (%)[1]	11.77 ± 0.12	11.74 ± 0.30	12.2 ± 0.26	11.43 ± 0.12
Thigh yield (%)[1]	12.56 ± 0.39	12.52 ± 0.33	12.94 ± 0.30	11.75 ± 0.45
Wing yield (%)[1]	9.44 ± 0.08^{a}	10.08 ± 0.29^{b}	9.84 ± 0.23^{a}	9.92 ± 0.42^{a}
Breast meat yield (%)[1]	$18.55 \pm 1.27^{a,b}$	15.02 ± 1.37^{a}	17.38 ± 0.47^{b}	18.75 ± 1.81^{b}
Heart (%)[2]	0.60 ± 0.12	0.65 ± 0.06	0.56 ± 0.03	0.59 ± 0.02
Liver (%)[2]	2.91 ± 0.54	3.07 ± 0.34	3.17 ± 0.54	3.50 ± 0.17
Gizzard (%)[2]	2.62 ± 0.34^{a}	2.36 ± 0.19^{a}	2.83 ± 0.27^{a}	3.38 ± 0.06^{b}

[1] = Results are expressed as the percentage of carcass cut weight to eviscerated carcass weight

[2] = Results are expressed as the percentage of internal organ weights to final body (pre-slaughter) weight

Quercetin administration to the LSD group did not exert a significant change in the quercetin-treated LSD group, when compared with the untreated LSD group. HSD resulted in a significantly higher pH in the untreated HSD group (5.96 ± 0.08) when compared with the untreated LSD group (5.68 ± 0.07) on day 3. The interactive effect between quercetin and stocking density did not influence a significant change in pH values, when the quercetin-treated HSD and quercetin-treated LSD were compared.

Table 4.9: Effect of quercetin and stocking density on pH of stored breast meat

Day	Group			
	18 birds/m^2	18 birds/m^2 + Quercetin	12 birds/m^2	12 birds/m^2 + Quercetin
1	5.47 ± 0.06^{1}	5.51 ± 0.05^{1}	5.49 ± 0.10^{1}	5.47 ± 0.08^{1}
2	5.64 ± 0.10^{1}	5.57 ± 0.05^{1}	5.61 ± 0.05^{1}	5.54 ± 0.06^{1}
3	$5.96 \pm 0.08^{2, b}$	$5.73 \pm 0.09^{1, a, b}$	$5.68 \pm 0.07^{1, a}$	$5.71 \pm 0.09^{1, a}$
4	$6.02 \pm 0.04^{2, a}$	$5.98 \pm 0.03^{2, a}$	$5.99 \pm 0.04^{2, a}$	$6.09 \pm 0.04^{2, a}$

Superscripts with different alphabets vary significantly ($P < 0.05$) within rows
Superscripts with different numbers vary significantly ($P < 0.05$) within columns

CHAPTER FIVE

5.0 DISCUSSION

The meteorological data from the study period within and outside the poultry house shows that the AT and THI were optimal for broiler production. This is because the value obtained in the present study for both AT and THI were closed to the thermoneutral zone of 18.0-30.0 °C established for birds in the tropical zones (Pereira and Naas, 2008). The findings disagrees with the report of Karthiayini and Philomina (2014) who reported that rainy season was thermally stressful to broiler chickens but it agrees with the report of Obidi *et al.* (2008). Low AT and THI were recorded during the early hours of 3:00 h to 7:00 h; while higher values were obtained during the hotter hours of 13:00 h to 17:00 h. However, these values recorded during the hotter hours were not sufficient to induce heat stress in the broiler chickens. This may be due to the shorter duration of exposure and partly because of adaptability of the birds to tropical environments. Therefore, the rainy season was not thermally stressful to the broiler chickens.

Lower CT values were recorded during the early hours of the day (1:00 h – 9:00 h) while higher CT values were recorded during the hot hours of the day (13:00 h – 17:00 h). The findings agrees with the results of (Sinkalu *et al*., 2015) that showed similar patterns in birds during the hot-dry season.

At 7:00 h, the group of untreated broiler chickens that was kept at stocking density of 12 birds/m^2 had a lower CT relative to the stocking density group of 18 birds/m^2. Quercetin increased the CT in the treated LSD group, but it decreased CT in the treated HSD group. The effect of stocking density on CT in the untreated LSD was not consistent when compared with the group of untreated HSD group at 7:00 h, perhaps, due to the time. At 17:00 h, increased stocking density caused a higher mean CT value in the untreated HSD group, while

CT was low in untreated LSD group. This implies that high-stocking density of 18 birds/m^2 may contribute to environmental warming, which was a necessary requirement during the brooding stage, but as birds grew; over time, a gradual decrease in ambient temperature was more desirable. The increased CT in the untreated HSD group may also be an indicator that metabolism in this group of chicks was more efficient and this could be linked to the higher final live body weight that was obtained. However, the relationship between variation in CT and final live body weight should be further investigated. At other hours, the CT was not significantly different, when compared between the untreated HSD and untreated LSD groups. Quercetin administration may be responsible for causing the lesser CT value that was obtained at 17:00 h, 19:00 h, 21:00 h and 5:00 h when the treated HSD group was compared with the untreated HSD group. Apparently, quercetin modulated CT at these hours.

The interaction between varying stocking densities and quercetin administration shows that CT was higher in the quercetin-treated LSD group, but lower in the quercetin-treated HSD group at 21:00 h, 1:00 h, 3:00 h, 5:00 h and 7:00 h. The results of circadian rhythmicity were important in order to determine the best time during which the administration of quercetin exerts its modulatory role on thermal homeostasis. This is in line with the findings of Piccione and Caola (2002) who reported that variations of physiological and biochemical functions, and resistance to environmental agents are often quite large, time-dependent and mostly rhythmic. This may offer new insight to animal physiology, pathology, diagnostic possibilities and therapeutic advantages; thus, enhancing scientific understanding of antioxidant effects and the underlying mechanism of quercetin action. At 1:00 h, quercetin significantly increased the CT in the treated LSD group, but the CT was decreased in untreated LSD group, while there was no significant change in CT at other hours on contrasting treated and untreated LSD group. This indicates that quercetin plays a

modulatory role on CT of broiler chickens at different stocking density conditions. Uzum and Toplu (2013) reported that increasing the stocking density to 18 birds/m^2 was responsible for the higher stress status in broiler chickens, and this impinged on broiler welfare. Thus, it is inferred that the administration of quercetin to the group of birds that are kept at 18 birds/m^2 resulted in a significant decrease in CT.

Therefore, the effects of administration of quercetin to broiler chickens may depend on the following: relative metabolic demands of broiler chickens that is age related, stressful factors such as stocking-density and environmental influences. For the first time, this research has elucidated an additional potential of quercetin, that is, a thermodynamic effect.

As the broiler chickens grew and increased in size, they occupied more of the already limited space and these birds eventually became unable to cope with their environment, thus, critical body physiological processes was impaired, especially in the untreated HSD group. This agrees with report of Dobson and Smith (2000), who stated that stress is revealed by the inability of an animal to cope with its environment, a phenomenon that is reflected in failure to achieve genetic potential: growth rate, feed efficiency or disease resistance. The result obtained from the association between RH and CT in the untreated HSD group implies that quercetin administration to the treated HSD group was beneficial. This is because the influence of prevailing high RH on CT fluctuation during the rainy season was not appreciated in the quercetin-treated HSD group. Overcrowded birds are predisposed to stress as a result of inadequate air circulation; the result of the combinative effect of high RH due to the rains and trapped products of metabolism, for example, respired gases, and litter ammonia. Therefore, it is expected that the effects of poor aeration in HSD conditions will markedly affect the untreated HSD the group. At LSD of 12 birds/m^2, the relationship between RH and CT was negatively correlated in both the quercetin-treated and untreated LSD groups. This upholds the earlier statement by Petersen (2004) and Etim *et al*. (2013)

that factors contributing to the increased RH in the micro environment caused discomfort and stress in HSD and overcrowded rearing conditions. It is, therefore, logical that the stocking-density of 12 birds/m^2 was not sufficient to initiate any social discomfort that may be attributed to the prevailing high RH and, therefore, was not stressful.

Based on the stronger correlation between the THI and CT, the impact of HSD was evident in both groups that were untreated with quercetin. Remarkably, the administration of quercetin alleviated the effect of discomfort that attributed to social stress due to high-stocking density. It is inferred that administering quercetin to the LSD group influenced metabolism in a manner that may be more beneficial to the group of 12 birds/m^2 that obtained the treatment, as the relationship between the THI and CT could be described as to be optimally correlated. However, the THI and CT association in the untreated LSD group was more correlated than when compared with the treated LSD group. This clearly means that some other factors (e.g. handling stress and other stressors) other than the characteristic high RH that prevailed in the season were responsible for modulating metabolism and by extension, the CT.

Of interest, was the transition that occurred during the fourth week. The broiler starter feed was replaced with the broiler finisher feed on the 22^{nd} day. Thereafter, it was observed that feed consumption drastically declined and erratic daily feed intake was obseved. This was attributed to the low feed intake by the birds. This finding may be the reason why the final live body weight of the birds at the end of the 42 day experimental study for the different groups were 1.272 kg, 1.344 kg, 1.296 kg and 1.071 kg in the untreated HSD, quercetin treated HSD, untreated LSD and quercetin-treated LSD groups respectively. This agreed with the report of Yo *et al*. (1997), who observed that broiler chickens at 4 weeks of age rejected pelletized feed during the first 24 hour when the form of concentrate (marsh to pellet) was changed. Although, full adaptation to the new size of concentrate required about 3 days (Yo *et al*., 1997), the broiler chickens in the present study did not adapt. Therefore,

the feed was replaced with marshed broiler finisher diet a week later to encourage feed intake by the broiler chickens.

The impact of stocking density on the relationship between THI and CT was also appreciated based on the comparatively greater correlation that was observed in the untreated HSD group when compared with the untreated LSD group. The combined impact of the different stocking-densities and quercetin administration on the association between THI with CT was similar between the quercetin-treated HSD and quercetin-treated LSD groups. This was evident by the similar trend in relationship that was observed between the THI and the CT. This implies that inherent environmental factors or management related factors may have influenced metabolism in all the groups at a uniform intensity. Nevertheless, quercetin administration exerted an appreciable influence on the treated groups, more on the HSD group. Other authors have reported dual roles of quercetin as an antioxidant and a prooxidant (Alrawaiq and Abdullah, 2014). They further stated that the prooxidant may be detrimental to biological systems. Corroborating the report of these authors with the findings from this study, it appears that quercetin may be regarded as a "balanced antioxidant" or even a ''dynamic or auto-regulating antioxidant''. This is attributed to the unique modulatory and selective influence of quercetin, which was observed in the group reared in comfort and the stocking density-induced stressed group. This study has demonstrated that quercetin was selectively potent during stressful condition of overcrowding in broiler chickens. Therefore, stressful environmental conditions in broiler chickens and pullets as reported by some authors (Minka and Ayo, 2008; Sinkalu *et al*., 2015) may be effectively managed with quercetin supplementation also. However, this requires further study.

In this study, the overall influence of the environmental condition and stockmanship, rather than stocking density alone affects the well-being of the birds. The finding is in agreement with that of Dawkins *et al* (2004). Similarly, differences in performance in broiler chickens

that is observed across producers that grow birds under similar densities is due to the variation in the quality of the environment that they supply (Estevez, 2007). Quercetin, when administered to broiler chickens, may be bio-transformed to further health benefitting metabolites such that humans who consume such stock derive some health benefits, but this concept requires further studies. This is of public health significance especially owing to the previously mentioned facts that are inherent in quercetin, for example, anti-inflammatory, antibiotic, antiviral and, anticancer activities, its ability to protect the heart and liver. To corroborate this further, Tan *et al*. (2014) reported that humans who consumed both fruits containing melatonin and other livestock that were supplemented with melatonin derive some health benefits, although this hypothesis requires further investigations. Previous researchers have reported that oral consumption of quercetin was associated with decreased bio-availability and therefore advocated direct inclusion of quercetin in meat products. Although, Kang *et al*. (2012) reported that quercetin was not stored in meat, but it was metabolised in other organs of the digestive tract. Based on these, a formed opinion is that, however the means of administration, consumption of quercetin-treated meat product offer some nutritional benefits.

Kuan *et al*. (1990), reported that increased stocking density produced stress indicated by higher H:L ratio from fourth week onwards. Gross and Siegel (1993), suggested that in birds H:L ratio was a good measure of long-term stress (hours or weeks). They observed that H:L ratio of about 0.2, 0.5, and 0.8 characterized low, optimum and high levels of stress respectively.

On day 28, quercetin caused decreased TWBC, heterophil, and H:L ratio in the treated HSD group. No significant changes was seen in these parameters at LSD. The impact of HSD resulted in an increased TWBC, heterophil, H:L ratio in the untreated HSD group of 18 birds/m^2. This may be linked with the potential of quercetin to affect immunity and

inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins often crucial for a cellular specific function (Chirumbolo, 2010). The combined effect of quercetin administration and varying stocking density did not affect TWBC, heterolphil and H:L ratio. Therefore, quercetin administration at 50 mg/kg stabilised the TWBC, heterolphil and H:L ratio in 28-day-old broiler chickens. The increased stocking density of 18 birds/m^2 resulted in a high TWBC, heterophil count and H:L ratio at this age. This means that at this age, the impact of social stress as a result of increased SD modulated TWBC, heterophil and H:L ratio significantly. This finding is in line with the report of Cotter (2015) and agrees with the report of Karthiayini and Philomina (2014), that the increased serum concentrations of H:L ratio is attributed to the effect of HSD inducing an acute phase response either as a direct physiological response to the stress of high stocking density indirectly or as a result of increased incidence of inflammatory diseases such as foot pad dermatitis. These results suggest that the antistress potency of the quercetin administered was useful in this age group of birds. This age coincided with the period the starter feed was replaced with finisher feed; suggesting that the transition from a particular diet to another may influence physiological changes, which is stressful to broiler chickens. This further warrants supplementation of quercetin during the transition.

The impact of stocking density alone was appreciated by the resultant increase in H:L ratio in both LSD and HSD group; however, the administration of quercetin significantly maintained lower levels of H:L ratio in the HSD group. The administration of quercetin to the LSD group, rather did not affect H:L ratio, even though it was expected that these birds were provided better comfort of space. This may be a clue that quercetin play a pro-oxidant role when birds are raised in comfort of space that is, under LSD condition. This assumption is based on the findings of Eghbaliferiz and Iranshahi (2016) who reported that quercetin has a

dual role, that is, situational antioxidant or pro-oxidant. However, more of antioxidant effect has been linked to quercetin (Leopoldini *et al*., 2006).

On day 35, stocking density had a significant effect on RBC. Stocking density caused an increase in the value of RBC in the untreated HSD group. While quercetin maintained the RBC count at normal levels in the treated HSD group, its effect on RBC of the LSD group was not significantly different when the treated and untreated LSD groups were compared. This may be due to a faster initiation of the adaptogenic response in the quercetin treated LSD and HSD and untreated LSD groups (Gross and Siegel, 1993). This is in line with the findings of Ayo *et al*. (2011), who reported that normal body functions of birds are efficient if the body temperature is kept constant or at least maintained within narrow limits. If the ambient temperature changes gradually, birds adapt to the changes, but if the changes are rapid and especially accompanied with high RH, they induce heat stress (Selye, 1976; Mearns, 1997).

The result from this study however is in agreement with the report of Karthiayini and Philomina (2014) who stated that the continued application of the same stress might cause an adaptive response so that detrimental effects overcrowding stress is not noticed. Furthermore, this adaptability is shown to be age-dependent. In this study, interplay between stress-linked factors (for example, stocking density, environmental and handling) may be considered to have been more pronounced on day 28. This is owing to the fact that the H:L ratio was influenced significantly higher on this day. However, the administration of quercetin might have exerted some positive effect by keeping the H:L ratio at relatively low levels as appreciated in the quercetin-treated HSD group. The 28th day coincided with the week during which the starter feed was replaced with finisher feed for broilers. The ratio of the finisher diet might have influenced *in vivo* metabolic activities in the birds at that age, thereby, causing an increasing basal metabolic activity in order to cope with other interacting

hormonal factors (for example, growth and mineral deposition) that act in concert to promote carcass and meat yield. This finding is in line with the report of (Yo *et al*., 1997), who found that the transition from starter to finisher diet caused a modulation in metabolism by influencing nutrient intake and regulation. The interactive effect between stocking density and quercetin administration did not have a significant effect on RBC. On the 42nd day, quercetin increased the TP in the treated HSD group. The implication of this finding requires further study. Based on this, it is proposed that compensatory mechanisms that support adaptation and survival in broiler chickens may not be constant as birds advance in age. This is in line with the findings of (Karthiayini and Philomina, 2014) who reported that animals' response to adversity is commensurate with the prevailing challenge per time and response varies with age. There is, therefore, the need to further study the social, environmental and nutritional requirements in relation to the age in order to avail with optimum welfare conditions, especially when overcrowding poses risk to poultry health. This study showed that stocking density increased the TP value in the untreated LSD group on the 42nd day, when compared with untreated HSD group. This may be because there was a necessity for a surge in TP at this age, but increasing stocking density to 18 birds/m^2 caused a decline in TP. Similarly, quercetin increased TP in the treated HSD group on day 42. This may be connected with the poor cellular availability of quercetin because of its extensive binding to plasma proteins as reported by Boulton *et al*. (1998). However, the report of Wayner *et al*. (1985) reveals that a large reserve of antioxidant capacity is attributable to plasma proteins when physiological concentrations of natural antioxidants are present in the body. Thus, the production of plasma protein was beneficial and while being age-dependent, may be influenced by stocking density and quercetin administration.

In this study, increasing the stocking density resulted in a higher percentage haemolysis in the untreated HSD group at 5.0 % NaCl on day 28. This assumption was based on the significant

decrease observed in the untreated LSD group. It was also observed that quercetin administration at LSD increased EOF. This is in line with the findings of previous authors (Eghbaliferiz and Iranshahi, 2016; Hadi and Farhan, 2016) who reported that quercetin exhibited both antioxidant and pro-oxidant effects, although further study is necessary to establish this fact using this model. The pro-oxidant effect of quercetin was previously suggested, earlier based on subtle effect of the flavonoid in the quercetin-treated LSD group. This effect in the quercetin-treated LSD group may be attributed to the pro-oxidant effect of quercetin. A higher viability rate was recorded in quercetin-treated groups, when compared with the untreated group. Stocking density did not affect viability rate and this agrees with the findings of Feddes *et al*. (2002) that stocking density did not affect mortality. It is possible to experiment on a dose that establishes 'balanced effect' considering the past reports that quercetin acts both as an antioxidant and pro-oxidant. The result shows that quercetin is a highly potent antioxidant that may be beneficial in adverse welfare conditions.

The Northern Guinea Savannah zone where this experiment was conducted is characterised by a seasonal high AT (Ayo *et al.*, 2011). Therefore, the need for quercetin administration is fully justified under conditions of adverse environmental and social influences, including seasonal change and high-stocking density. Therefore, it is inferred that quercetin incorporation into livestock feeds may be useful in combating the effect of global climatic changes and oxidative stress in uncontrolled environment. It is suggested that indices of stress responses be first established in broiler chickens and only if potential risks exists, such birds should be administered with quercetin. The negative impact of climate change on animal production is more evident in developing countries; previously reported to be ill-prepared to face the challenges of global climate change (Ebenezer, 2015). Supplementation of poultry feed with quercetin is, therefore, inevitable, especially in developing countries like Nigeria.

Quercetin supplementation to the LSD group caused a decrease in the initial body weight, final live body weight and cumulative feed consumption. The stocking-density of 12 birds/m^2 as recommended by Uzum and Toplu (2013) was considered to provide better welfare. Based on the assumption that the stocking density of 12 birds/m^2 is optimum for physiological processes, including metabolism and performance, the benefits derived from administering quercetin to this group of birds require further scientific elucidation. Stressors, if present at all, in this group may not be connected with social stress, emanating from stocking density since the birds were comfortable at this stocking density. Quercetin administration to the LSD group resulted in a lower initial body weight, final body weight, and cumulative feed consumption, but these parameters were higher in untreated LSD group. The reduced value of FCR in the treated LSD group, though not significantly different when compared with the untreated group, may be attributed to quercetin administeration. Quercetin administration was responsible for the reduced cumulative feed consumption and had no significant effect on the initial body weight, final live body weight and cumulative feed consumption in the HSD group, however, the ratio of final live body weight gain and weight gain was higher in the treated group. The lesser FCR that was obtained in the treated HSD group was beneficial and may be attributed to quercetin because least feed was consumed and the final live body weight was highest in this group.

The broiler chickens in the quercetin-treated HSD group, therefore, had a better feed efficiency when compared with the other groups. This could be attributed to the higher metabolic efficiency in the quercetin-treated group during period when this study was conducted. Reducing stocking-density to 12 birds/m^2 resulted in a higher initial live body weight gain and increased cumulative feed consumption while increasing the stocking density to 18 birds/m^2 caused a lower initial body weight gain and lessened the feed that was consumed. Dozier III *et al*. (2006) reported that body weight gain was adversely affected by

increasing stocking density by day 35. Although, the micro-environment in the treated and untreated HSD groups might have impaired respiratory mechanism and other metabolic processes, the result from the present study shows that the survivability in the quercetin-treated groups, irrespective of high or low stocking density was significantly higher than in the untreated groups. The mechanism through quercetin enhanced survivability is not yet fully elucidated in the broiler chickens; however, Chondrogianni *et al*. (2010) reported that quercetin and its derivative (quercetin caprylate) functioned as a proteasome activator and thereby conferring its influence of prolonging cellular lifespan probably via efficient proteolysis. Quercetin also enhanced survival and viability and rejuvenation of HFL-1 primary human fibroblast, thus promoting physiological alterations when applied to cells. This is in line with the report of Saul *et al*. (2008) and Grunz *et al*. (2012) that quercetin conferred longevity to the nematode *Caenorhabditis elegans*. Quercetin administration, to broilers chickens has the potential to boost farmer's income. This is because broiler production per head was higher during the sell-out period. As the birds grew over time, quercetin administration further increased performance in terms of body weight gain. Quercetin, therefore, has the potential to increase live market weight. This implies that the potential for an attractive weight during sell-out period might favour the quercetin-treated groups, should the birds be kept longer than 42 days. The result of carcass characteristics was not in agreement with previous studies of Quercetin increased the breast meat yield in the treated LSD group while the breast meat yield was higher in the untreated HSD group when compared with the untreated LSD group demonstrating that stocking density influenced breast meat yield in the broiler chickens. This disagreed with the carcass characteristics study of Onbasilar *et al*. (2008) and Uzum and Toplu (2013) who reported that there was no significant difference in the meat yield among different stocking density groups. The internal organ weight of the gizzard was higher in the quercetin-treated LSD group but there was no

significant difference in the gizzard yield between untreated HSD and untreated LSD and this disagreed with the finding of Uzum and Toplu, 2013 who reported that increasing stocking density from 12 to 18 birds/m2 caused a significant difference in only heart weight but liver and gizzard weights were not affected. It is recommended that a dose dependent study should be conducted in future study.

The antimicrobial property of quercetin has been well documented (El-Hawary *et al*., 2016; Cetin-Karaca and Newman, 2015; Sannomiya *et al*., 2005). The key factor in meat quality is the pH (Berri *et al*., 2005) and high pH results in shorter shelf life stability (Li *et al*., 2017). Meat having a high pH is indicative of meat deterioration and this has been attributed to the activity of bacterial organisms in meat (Wapi *et al*., 2013; Li *et al*., 2017). Quercetin, due to its antimicrobial property, prevented pH rise possibly by inhibiting the activity of spoilage microbes in the quercetin-treated groups. In this study, the influence of high stocking density resulted in the earliest increase of pH on day 3 in the untreated HSD group. The reduced stocking density of 12 birds/m^2 proved to be beneficial as pH of broiler breast meat was not significantly different when compared with the quercetin-treated HSD and quercetin-treated LSD groups. Thus, increasing stocking density may predispose refrigerated meat of broiler chickens to early deterioration and eventually economic losses due to poor quality and lack of taste appeal to consumers and this agrees with the report of Doktor (2007). However, the administration of quercetin at 50 mg/kg prolonged the shelf-life of the breast fillets. This finding agrees with the report of Goliomytis *et al*. (2014) that quercetin inhibits peroxidation in broiler meats, thus; preserving its keeping quality. This is also in line with the report of Tang and Cronin (2005), that, quercetin, the main antioxidant in onion juice caused a significant reduction in lipid peroxidation in refrigerated cooked turkey breast rolls during storage.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, this study has shown the following:

- ✓ For the first time, the modulatory role of quercetin administration on the CT of broiler chickens maintained at different stocking density conditions.
- ✓ The overall mean CT shows that;

i. the HSD of 18 birds/m^2 caused an increase in CT (40.96 ± 0.02 °C).

ii. the administration of quercetin resulted in a decrease in the CT of HSD group (40.72 ± 0.02 °C).

iii. the interactive effect of quercetin and varied stocking density resulted in higher CT in the quercetin-treated LSD group (40.98 ± 0.03 °C) when compared with the quercetin-treated HSD group (40.72 ± 0.02 °C).

iv. the untreated LSD group had a lower CT (40.88 ± 0.02 °C), but CT increased when quercetin-treated LSD group (40.98 ± 0.03), thus, demonstrating a dynamic thermogenic effect of quercetin.

- ✓ High-stocking density is a physiologic stressor and its detrimental effect on the broiler chickens was alleviated by administering quercetin to broiler chickens.
- ✓ Broiler chickens productivity increased and feed utilisation was more efficient at HSD conditions when quercetin was administered.
- ✓ The shelf-life of stored breast fillets was extended in both groups of quercetin-treated HSD and untreated LSD, thus, improves meat quality.
- ✓ Quercetin alleviated the impact of physiological and social stress at HSD conditions; this is evident by the low levels of H:L ratio in the quercetin-treated HSD group (0.19

± 0.03) when compared with the untreated HSD group (0.53 ± 0.13) in 28-day old broiler chicks.

6.2 Recommendations

From the findings of this study, it is recommended that;

- ✓ further studies should be conducted using similar models in order to scientifically elucidate other roles of quercetin in broiler chickens under different stocking densities.
- ✓ future studies should be conducted to evaluate other physiological stress indicators, viz. plasma corticosterone, glucose, cholesterol and total nitrates and antioxidant enzymes.
- ✓ studies should be carried out to determine the effect of different doses of quercetin on the parameters tested in this study.
- ✓ the presence and levels of quercetin in poultry products, that is, meat and eggs should be determined in future studies.
- ✓ further study should be conducted to compare the effect of quercetin with other antioxidants, for example, vitamins E, C, A and melatonin on the quality of broiler meats.
- ✓ further study should explore use of infrared thermography in order to evaluate surface temperature (and its contribution to sensitive heat loss) as well as to identify radiant temperatures.
- ✓ further study is required to investigate any positive effects of dietary quercetin supplementation on broiler metabolic disorders and to elucidate the respective underlying mechanisms.
- ✓ It is suggested that similar experiment should be carried out during other seasons; especially hot-dry as the present findings demonstrates that quercetin is a selectively

potent antistress and it has a dynamic action on cloacal temperature in broiler chickens.

6.3 Limitations of the Study

- ✓ Automated cell count using automated haemo-analyser machine offers advantage as it is more efficient, gives higher precision and consumes less time. However, there is no standard automated technic for avian blood cell count and differentiation till date. Therefore, blood cell count and differentiation was conducted manually. The development of automated haemo-analyser that is specific for avian species will aid future study.
- ✓ This research was conducted in an experimental setting. It might be better done in a commercial setting so that inferences made can be more generalized for broiler chickens that are kept under different stocking density conditions.
- ✓ Antioxidants are known to reduce the cellular oxidative stress by inhibiting the formation of ROS/reactive nitrogen species through upregulation of cellular defense mechanisms, such as SOD, catalase, or GSH-P*x*. The study of the relationship between quercetin and these antioxidant enzymes, including MDA (an index of lipid peroxidation) would have further elucidated the interplay between antioxidant enzymes, stress induced free radical production and quercetin action in serum.
- ✓ It is important to assay for available quercetin using HPLC analysis in the different body tissues and serum. This will further corroborate the finding that quercetin prevented the early deterioration of stored meat.
- ✓ Power supply was not predictable. Such fluctuations in power supply might influence the absorbance record that was obtained from the spectrophotometer as the machine required warm-up duration. Also, the erratic power outage delayed haematological analysis.

- ✓ The refractive index is determined by the type of cuvette used in the spectrophotometer. The cuvette used in this study was made of glass. However, cuvettes that are made of quartz give a better refractive index relative to glass or plastic cuvettes.
- ✓ Remoteness of the slaughter slab from the Veterinary Physiology Laboratory could affect carcass composition during conveyance. A slaughter slab should be constructed close to the Veterinary Physiology Laboratory.
- ✓ The importation of quercetin from Germany to Nigeria took a long period of time. Considering the beneficial effects of this novel agent in livestock and man, large quantity importation by government agencies may help reduce the cost and increase its availability.

6.4 Contribution to Knowledge

- ✓ The experiment aimed at examining the effects of quercetin on some physiological parameters (cloacal temperature, haematological profile, erythrocyte osmotic fragility), performance, carcass and meat quality characteristics. Result of the study demonstrated a thermogenic potential of quercetin. Thus quercetin may aid a healthy metabolic status in the treated broiler chickens. Increasing stocking density influenced an increase in cloacal temperature (CT). The administration on quercetin caused a lower CT in the treated high stocking density (HSD) group. CT was lower in the low stocking density (LSD) group, further supporting the impact of increased stocking density on the CT of broiler chickens.
- ✓ Quercetin administration caused a reduction in haematological stress indices, i.e. lower total white blood cell count, and lower heterophils and heterophil: lymphocyte ratio. Thus, HSD group, devoid of quercetin administration was associated with

discomfort which may be detrimental to the welfare of untreated broiler chickens at HSD conditions.

- ✓ The EOF was higher ($P < 0.05$) in the untreated HSD group at 0.1, 0.3 and 0.5 % concentration of $NaCl_{(aq)}$.
- ✓ Quercetin prolonged the shelve-life of stored broiler breast meat, also low stocking density of 12 birds/m^2 aided prolonged preservation of stored meat by delaying pH rise.
- ✓ Keeping birds at high stocking density of 18 birds/m^2 with administration of qiercetin increased survival rate and may therefore maximize farmers' profit, yet, without compromising on the welfare of the broiler chickens.
- ✓ Quercetin administration to the HSD group caused a higher final live body weight and weight gain when compared with quercetin treated LSD group. The feed conversion ratio was also lower in the quercetin-treated HSD group when compared with the untreated HSD, untreated LSD and quercetin-treated LSD groups. Quercetin administration improved feed utilization, thus had weight-enhancing effect. This weight-enhancing effect was more correlated with increasing age of the broiler chicks in the quercetin-treated HSD group when compared with quercetin-treated LSD group.

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APPENDICES

Appendix 1: Meteorological data obtained outside the poultry house

Meteorological Parameter	Mean ± SEM	Range
Ambient Temperature, °C: Maximum	31.09 ± 0.46	29.25 - 32.29
: Minimum	22.64 ± 0.11	22.29 - 23.00
Dry-bulb	25.68 ± 0.28	24.55 - 26.34
Relative Humidity, %	77.32 ± 1.25	73.00 - 81.50
Temperature-humidity Index	27.54 ± 0.29	21.95 - 30.38
Sunshine Duration, h/day	6.20 ± 0.58	4.16 - 8.06

Data collated from the Meteorological Unit, Institute for Agricultural Research, Ahmadu Bello University, Zaria.

Appendix 2: Circadian fluctuations of thermal environmental parameters inside the poultry house.

Hour of the Day, h	Dry-bulb temperature, °C	Relative humidity, %	Temperature-humidity Index
07:00	$23.67 \pm 0.58^{a,b}$	93.23 ± 3.55	23.54 ± 0.29^{a}
	(23.00-24.50)	(88.00-100.00)	(22.85-24.28)
09:00	$25.50 \pm 1.04^{a,b,c}$	84.73 ± 3.54	$25.20 \pm 0.69^{a,b}$
	(23.50-27.00)	(81.00-91.80)	(23.35-26.63)
11:00	$29.00 \pm 2.26^{a,b,c}$	80.53 ± 5.76	$28.58 \pm 1.50^{a,b,c}$
	(24.50-31.50)	(73.20-91.90)	(24.35-30.98)
13:00	31.50 ± 0.87^{c}	78.77 ± 3.58	31.03 ± 0.57^{c}
	(30.00-33.00)	(73.40-85.80)	(29.70-32.48)
15:00	31.17 ± 0.93^{c}	80.93 ± 6.07	$30.74 \pm 0.61^{b,c}$
	(30.00-33.00)	(72.90-92.80)	(29.85-32.48)
17:00	$29.83 \pm 0.44^{b,c}$	86.13 ± 6.93	$29.53 \pm 0.21^{b,c}$
	(29.00-30.50)	(79.10-100.00)	(29.00-30.05)
19:00	$28.17 \pm 0.60^{a,b,c}$	86.87 ± 6.65	$27.89 \pm 0.34^{a,b,c}$
	(27.00-29.00)	(78.50-100.00)	(27.00-28.63)
21:00	$28.17 \pm 2.42^{a,b,c}$	80.17 ± 12.45	$27.74 \pm 1.50^{a,b,c}$
	(25.50-33.00)	(57.20-100.00)	(25.28-31.95)
23:00	$25.83 \pm 0.73^{a,b,c}$	88.67 ± 5.78	$25.61 \pm 0.45^{a,b,c}$
	(24.50-27.00)	(81.00-100.00)	(24.50-26.70)
01:00	$26.17 \pm 1.67^{a,b,c}$	89.17 ± 7.18	$25.94 \pm 1.07^{a,b,c}$
	(24.50-29.50)	(75.60-100.00)	(24.35-28.98)
03:00	$23.83 \pm 0.44^{a,b}$	90.70 ± 4.77	23.66 ± 0.25^{a}
	(23.00-24.50)	(84.20-100.00)	(23.00-24.20)
05:00	23.50 ± 0.50^{a}	89.33 ± 5.72	23.30 ± 0.30^{a}
	(23.00-24.50)	(80.40-100.00)	(22.78-24.13)
Mean ± SEM	27.19 ± 0.55	85.77 ± 1.72	26.90 ± 0.52
	(23.00-33.00)	(57.20-100.00)	(22.78-32.48)

Values in parenthesis are minimum - maximum. Superscript letters with different alphabets vary significantly within columns

Appendix 3: Variations in thermal environmental parameters in the poultry house

Day	Dry-Bulb temperature, °C	Relative humidity, %	Temperature-Humidity Index
22	28.79 ± 0.99[b]	78.61 ± 2.33[a]	28.33 ± 0.94
	(24.50-33.00)	(57.20-88.00)	(24.13-32.48)
29	25.67 ± 0.78[a]	96.17 ± 1.45[b]	25.59 ± 0.77
	(23.00-30.00)	(85.80-100.00)	(22.85-29.85)
36	27.13 ± 0.91[a,b]	82.53 ± 2.37[a]	26.77 ± 0.86
	(23.00-31.30)	(72.90-100.00)	(22.78-30.90)
Overall	27.19 ± 0.55	85.77 ± 1.72	26.90 ± 0.52
	(23.00-33.00)	(57.20-100.00)	(22.78-32.48)

Values in parenthesis represent minimum-maximum. Means with different superscript letters vary significantly ($P < 0.05$) within rows

Appendix 4: Between-Group Variation in Erythrocyte Osmotic Fragility of 28-Day-Old Broiler Chickens at different Stocking Densities

Density Group	NaCl Concentration (%)					
	0.9	0.7	0.5	0.3	0.1	0
18 birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	22.03 ± 9.86^{b}	63.76 ± 7.88	85.47 ± 4.55^{a}	100.00 ± 0.00
18 birds/m^2 + Quecertin (n=5)	0.00 ± 0.00	0.00 ± 0.00	28.72 ± 8.83^{b}	68.91 ± 5.64	92.02 ± 2.69^{a,b}	100.00 ± 0.00
12 birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.71 ± 0.44^{a}	66.71 ± 6.55	87.27 ± 3.40^{a}	100.00 ± 0.00
12 birds/m^2 + Quercetin (n=5)	0.00 ± 0.00	0.00 ± 0.00	30.88 ± 15.17^{b}	70.77 ± 2.20	94.65 ± 1.62^{b}	100.00 ± 0.00

Superscripts with different letters vary significantly within columns

Appendix 5: Between-Group Variation in Erythrocyte Osmotic Fragility of 35-Day-Old Broiler Chickens at different Stocking Densities

Density Group	NaCl Concentration					
	0.9	0.7	0.5	0.3	0.1	0
18birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	62.97 ± 1.16	91.65 ± 4.57^b	100.00 ± 0.00
18birds/m^2+ Quecertin (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	58.44 ± 7.35	90.79 ± 3.71^b	100.00 ± 0.00
12birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	50.30 ± 8.00	77.01 ± 9.30^a	100.00 ± 0.00
12birds/m^2+ Quecertin (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	63.69 ± 8.69	84.34 ± 9.02^b	100.00 ± 0.00

Superscripts with different alphabets vary significantly within columns

Appendix 6: Between-Group Variation in Erythrocyte Osmotic Fragility of 42-day-Old Broiler Chickens at different Stocking Densities

Density Group	NaCl Concentration					
	0.9	0.7	0.5	0.3	0.1	0
18birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	67.25 ± 9.72	90.94 ± 1.43	100.00 ± 0.00
18birds/m^2 + Quecertin (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	74.48 ± 5.55	90.10 ± 3.67	100.00 ± 0.00
12birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	69.63 ± 5.10	92.23 ± 2.16	100.00 ± 0.00
12birds/m^2 + Quercetin (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	67.76 ± 5.23	87.81 ± 5.32	100.00 ± 0.00

Appendix 7: Table: Between-Group Variation in Overall Erythrocyte Osmotic Fragility broiler chickens at different stocking densities

Density Group	NaCl Concentration					
	0.9	0.7	0.5	0.3	0.1	0
18 birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	7.34 ± 4.12^b	64.66 ± 3.91^b	89.35 ± 2.17^b	100.00 ± 0.00
18 birds/m^2 + Quecertin (n=5)	0.00 ± 0.00	0.00 ± 0.00	9.57 ± 4.53^b	67.28 ± 3.78^b	90.97 ± 1.82^b	100.00 ± 0.00
12 birds/m^2 (n=5)	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.16^a	62.21 ± 4.22^a	85.5 ± 3.56^a	100.00 ± 0.00
12 birds/m^2 + Quercetin (n=5)	0.00 ± 0.00	0.00 ± 0.00	10.29 ± 6.09^b	67.41 ± 3.30^b	88.93 ± 3.47^b	100.00 ± 0.00

Superscripts with different letters vary significantly within columns

Appendix 8: Daily Feed Consumption of Broiler Chickens

Day	Untreated HSD (n=18)	Quercetin-treated HSD (n=18)	Untreated LSD (n=12)	Quercetin-treated LSD (n=12)
1	16.42	11.14	17.75	15.94
2	19.86	14.00	21.66	19.44
3	23.31	16.86	25.56	22.94
4	26.75	19.72	29.56	26.44
5	29.64	22.58	33.47	29.94
6	31.14	25.44	35.31	31.53
7	31.94	29.08	37.19	33.13
8	33.22	29.39	38.50	33.75
9	34.39	29.69	39.81	34.38
10	35.56	30.00	41.13	35.00
11	36.72	30.31	42.44	35.63
12	37.89	30.61	43.75	36.25
13	41.06	31.31	46.50	37.38
14	41.92	32.22	47.44	38.06
15	42.78	33.14	48.38	38.75
16	43.64	34.06	49.31	39.44
17	44.50	34.97	50.25	40.13
18	45.36	35.89	51.19	40.81
19	47.22	37.64	53.13	42.19
20	41.67	20.83	35.94	28.13
21	36.81	31.25	40.41	33.28
22	41.67	41.67	44.88	38.47
23	38.17	33.11	48.22	32.91
24	34.67	24.58	51.56	27.34
25	39.58	30.56	57.91	38.22
26	44.50	36.53	64.25	49.09
27	44.83	37.58	67.97	50.44
28	45.17	38.64	71.69	51.78
29	45.50	39.69	75.41	53.13
30	45.83	45.75	79.13	54.47
31	46.17	41.81	82.84	55.81
32	46.50	42.86	86.56	57.16
33	42.06	43.92	38.22	39.66
34	44.86	46.44	43.22	41.59
35	47.75	48.97	48.31	43.53
36	50.64	51.50	53.41	45.50
37	53.53	54.03	58.50	47.47
38	56.42	56.56	63.59	49.44
39	59.31	59.08	68.69	51.41
40	62.78	61.67	73.75	53.28
41	64.50	62.94	76.28	54.22
42	65.36	63.58	77.53	54.69
Mean ± SEM	41.94 ± 1.67	36.70 ± 2.01	51.44 ± 2.64	40.05 ± 1.62

Appendix 9: Weekly Weight Record of the Untreated HSD Group of 18 birds/m^2

n = 18	Week					
	1	2	3	4	5	6
1	240	500	700	800	1200	1500
2	190	400	600	950	1200	1500
3	290	600	900	625	650	900
4	190	400	600	750	1000	1300
5	290	600	900	1050	1150	1700
6	190	400	600	725	900	1200
7	240	500	650	1150	1500	1100
8	290	600	900	1150	1350	1300
9	315	650	1000	750	900	1200
10	265	550	850	925	1050	1300
11	265	550	650	775	850	1300
12	290	600	700	800	1000	1300
13	240	500	600	900	1050	1300
14	265	550	800	1000	1100	1450
15	215	450	600	700	550	850
16	215	450	500	600	950	1300
17	240	500	600	725	1000	1300
18	190	400	550	600	750	1100
Mean ± SEM	245.60 ± 9.60	511.10 ± 19.20	705.60 ± 34.75	831.90 ± 41.01	1008.00 ± 54.87	1272.00 ± 48.21

Appendix 10: Weekly Weight Record of the Quercetin-treated HSD Group of 18 birds/m^2

n =18	Week					
	1	2	3	4	5	6
1	190	400	800	850	1000	1700
2	290	600	900	1150	1250	1550
3	240	500	700	950	1100	1450
4	215	450	700	830	1080	1350
5	190	400	650	750	850	1200
6	240	500	700	750	850	1150
7	265	550	900	1150	1050	1200
8	240	500	800	950	1100	1400
9	240	500	650	750	1000	1200
10	165	350	550	700	900	1200
11	190	400	650	950	950	1200
12	265	550	800	1150	1350	1850
13	215	450	800	1000	1200	1500
14	240	500	700	880	1000	1300
15	190	400	550	850	900	1200
16	165	350	500	650	750	1100
17	265	550	550	700	700	1000
18	240	500	800	1000	1150	1650
Mean ± SEM	224.70 ± 8.61	469.40 ± 17.22	705.60 ± 28.26	889.40 ± 37.63	1010.00 ± 40.20	1344.00 ± 54.22

Appendix 11: Weekly Weight Record of Untreated LSD Group of 12 birds/m^2

n = 12	Week					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	340	700	850	1075	1100	1400
2	265	550	650	975	1200	1400
3	240	500	600	650	800	1250
4	265	550	800	800	900	1250
5	265	550	600	750	950	1300
6	240	500	600	650	800	1150
7	315	650	800	900	1000	1400
8	365	750	950	1200	1250	1500
9	290	600	750	850	920	1050
10	315	650	750	900	1050	1100
11	315	650	800	1050	1250	1650
12	240	500	500	650	750	1100
Mean ± SEM	287.90 ± 12.10	595.80 ± 24.20	720.80 ± 37.67	870.80 ± 52.30	997.50 ± 50.66	1296.00 ± 52.39

Appendix 12: Weekly Weight Record of Quercetin-treated LSD Group of 12 birds/m^2

n = 12	Week					
	1	2	3	4	5	6
1	290	600	900	1050	1050	1000
2	215	450	700	800	700	900
3	240	500	800	980	1150	1500
4	290	600	1000	1180	1200	1500
5	190	400	650	780	800	1100
6	265	550	900	1200	750	950
7	165	350	650	730	800	1000
8	190	400	600	600	750	950
9	240	500	850	900	1050	1000
10	265	550	800	900	900	1100
11	150	330	600	630	750	950
12	240	500	700	780	800	900
Mean ± SEM	228.30 ± 13.47	477.50 ± 26.52	762.50 ± 38.00	877.50 ± 56.53	891.70 ± 50.31	1071.00 ± 60.76