EVALUATION OF CHANGES IN CLOACAL TEMPERATURE, TONIC IMMOBILITY, VIGILANCE AND PERFORMANCE OF BROILER CHICKENS ADMINISTERED WITH PROBIOTIC AND FISETIN DURING THE EARLY RAINY SEASON

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BY

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

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ZARIA, NIGERIA

OCTOBER, 2018

DECLARATION

I declare that the work reported in this dissertation, titled **'Evaluation of Changes in Cloacal Temperature, Tonic Immobility, Vigilance and Performance of Broiler Chickens Administered with Probiotic and Fisetin During the Early Rainy Season'** was carried out by me in the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The information derived from literature has been duly acknowledged in the text and in the list of references provided. No part of this dissertation has been presented for another degree or diploma at any university.

Victory Osirimade SUMANU

……	.....	.....
Name of Student	Signature	Date

CERTIFICATION

This dissertation, entitled **'EVALUATION OF CHANGES IN CLOACAL TEMPERATURE, TONIC IMMOBILITY, VIGILANCE AND PERFORMANCE OF BROILER CHICKENS ADMINISTERED WITH PROBIOTIC AND FISETIN DURING THE EARLY RAINY SEASON'** carried out by Victory Osirimade SUMANU 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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Ahmadu Bello University, Zaria

DEDICATION

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

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ABSTRACT

High ambient temperature and high relative humidity occurring during the early rainy season cause oxidative stress, adversely affecting broiler chicken production. The aim of the study was to evaluate the changes in cloacal temperature, tonic immobility and performance indices of broiler chickens, administered with probiotic and fisetin during the early rainy season. Sixty Arbo Acre breed of broiler chickens at day-old, allotted into four groups of 15 birds each, were used. Group I (Control) was given only distilled water; Group II, fisetin (5 mg/kg); Group III, probiotic (4.125×10^6 cfu/100 mL); and Group IV, fisetin and probiotic (5 mg/kg and 4.125×10^6 cfu/100 mL, respectively), for the first seven days of life. All administrations were done orally through a gavage. Cloacal temperature of the broiler chickens, dry-bulb temperature, relative humidity and temperature-humidity index in the pen were obtained bi-hourly, from 07:00 – 07:00 h, at days 21, 28 and 35 of the study period. Tonic immobility and vigilance were evaluated at 7:00, 13:00 and 18:00 h at days 21, 28 and 35 of the study period. Body weight of each broiler chicken was recorded at days 7, 14, 21, 28, 35 and 42. Feed and water intake were measured on daily basis beginning from day 1-42 of the experiment. Absolute feed and water intakes were calculated. The dry-bulb temperature, relative humidity and temperature-humidity index (30.57 ± 0.36 °C, 79.22 ± 1.26% and 30.10 ± 0.34, respectively) recorded were predominantly outside the thermoneutral zone, indicating that the broiler chickens were subjected to heat stress. At day 21, the cloacal temperature recorded in probiotic (40.32 ± 1.90 °C) group was significantly lower ($P < 0.05$) than in the control group (41.39 ± 0.03 °C). At day 28, the cloacal temperature in broiler chickens administered with fisetin +

probiotic (40.40 ± 0.03 °C) was significantly lower ($P < 0.05$), when compared with that of the control group (41.58 ± 0.03 °C). Tonic immobility duration in broiler chickens administered with fisetin + probiotic (99.90 ± 9.43 s) was lower than that of the controls (139.70 ± 11.69 s). The vigilance behavioural ranking was highest (1.64 ± 0.06) in the control group, while the lowest value (1.45 ± 0.06) was obtained in fisetin + probiotic group. Broiler chickens administered with probiotic alone, and fisetin + probiotic had higher ($P < 0.05$) feed intake at days 35 and 42, when compared respectively with those of the control and fisetin groups. Water intake was higher ($P < 0.05$) in the probiotic administered group than in any other group only at day 42. The feed conversion ratio was lower ($P < 0.05$) in the probiotic supplemented group (0.42 ± 0.13) than that of the control group (1.20 ± 0.08). The live weight gain of the broiler chickens was highest ($P < 0.05$) in the probiotic group. It was concluded that the administration of probiotic and/or fisetin ameliorated the adverse effects of heat stress on broiler chickens during the thermally stressful early rainy season, and the best performance was obtained in broiler chickens administered with probiotic alone.

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ABBREVIATIONS

ANOVA	Analysis of Variance
AT	Ambient Temperature
BBB	Blood-Brain Barrier
CD36	Cluster of Differentiation
cfu	Colony forming unit
cm	Centimeter
CT	Cloacal temperature
ºC	Degree Celsius
DBT	Dry-bulb Temperature
E	East
FCR	Feed Conversion Ratio
g	Gram
GSH	Reduced Glutathione
h	Hour
IL-1	Interleukin-1
LDL	Low-Density Lipoprotein
mg	Milligram
mL	Milliliters
MOS	Mannan Oligosaccharide
MT	Mettler Toledo®
N	North
$OH^{\cdot}$	Hydroxyl radical
RH	Relative Humidity

ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
s	Seconds
T_3	Triiodothyronine
T_4	Tetraiodothyronine
TEAC	Trolo -Equivalent Activity Concentration
THI	Temperature-Humidity Index
TI	Tonic immobility
TNF-α	Tissue Necrotic Factor-alpha
USA	United States of America
V	Vigilance
WBT	Wet-bulb Temperature
µg	Microgram

CHAPTER ONE

1.1 Background of the Study

Broiler production in Nigeria involves the keeping of chickens of heavy meat breeds for the purpose of obtaining good quality meat (Johnson *et al*., 2018), which are usually sold live or processed at six to eight weeks of age (Balami *et al*., 2014). Broiler production is carried out in all parts of Nigeria without any known religious, social or cultural inhibitions associated with the consumption of broiler meat (Stadig *et al*., 2016). The key to a successful broiler production depends on systematic and efficient management programme adopted by the farmer (Sahil *et al*., 2017). Specifically, broiler chickens have short production cycles (Addisu *et al*., 2013). The high demand for poultry products, the success of exotic breeds and the ease of mastering the techniques has made poultry production to develop to the status of agribusiness in Nigeria (Sanni, 2015). Though broiler chickens, to some extent, may acclimatize to some levels of oxidative stress, resulting from heat, they experience some organ damage due to lipid peroxidation, caused by hyperthermia (Rajkumar *et al*., 2018). Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules (Tian *et al*., 2018). Oxidation is a chemical reaction that transfers electron from a substance to an oxidising agent. Although oxidation reaction is important during heat production in animal body, it may also damage the tissue (Puttachary *et al*., 2015). Antioxidants are widely used as ingredient, in dietary supplements, and their supplementation provides beneficial effects against stress-induced tissue damage (Sun *et al*., 2015); for example, probiotic and fisetin.

Probiotics are living microorganisms (Aluwong *et al*., 2017), which when administered in adequate amount confer health benefits on the host (Gatrell *et al*., 2018). Probiotic micro-organisms have been shown to have beneficial effects during *in-vivo* trials, accompanied by much promising new potentials as developed by *in-vitro* experiments (Jahromi *et al*., 2016). In general, probiotics improve intestinal microbial balance, provide protection against gut pathogens and modulate immune system (Song *et al*., 2013). Emergence of new probiotic products has spurred increasing number of research groups exploring new probiotic strains and potential novel health functions of probiotics (Aluwong *et al*., 2013). Probiotics are supplemented into animal feed such as that for ducks, broiler chickens, cattle and in aquaculture for fishes and prawns (Sugiharto *et al*., 2017). The Genera of probiotic microorganisms commonly used for animals include *Bifidobacterium, Lactococcus, Lactobacillus, Bacillus, Streptococcus* and those of yeast are *Saccharomyces* and *Candida* (Sugiharto *et al.,* 2017). Feeding of probiotics has beneficial impacts on commercial animals by enhancing weight gain (Jhony *et al*., 2018), increasing feed conversion efficiency, increasing egg production, lowering the incidence of disease and mortality rate (Aluwong *et al*., 2013).

Flavonoids are commonly found in most plants (Sandeep *et al*., 2018). They exert a significant range of biological activities such as antioxidant, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating and antiviral effects (Khan *et al*., 2013). Flavonoids and their polymers comprise one of the largest groups of phytonutrients that has beneficial health effects. These important polyphenolic compounds under the class of plant secondary metabolites exert significant effects in the biological system (Youns *et al*., 2017). Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a dietary flavonoid in fruits and vegetables,

including strawberries, grapes, cucumbers and onions (Chen *et al*., 2015). Fisetin exert its antioxidant effect by scavenging for free radicals and preventing or slowing the oxidation of free radicals (Sandeep *et al*., 2018). It has extensive physiological and pharmacological activities, including antioxidant, anti-inflammatory, anti-neoplastic and neuroprotective effects (Prakash *et al*., 2013). It enhances behavioural performance and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity (Chuang *et al*., 2014). Furthermore, fisetin suppresses oxidative and neuroinflammatory responses in microglial cells (Yuan *et al*., 2015). Average daily intake of fisetin was computed as 0.4 mg (Sundarraj *et al*., 2018). The highest concentration of fisetin is found in strawberries (160 μg/g) (Chen *et al*., 2015). Recently, fisetin has become a subject of research because it is found in various human foods and its antiproliferative effect (Su *et al*., 2017). It is a chemopreventive/chemotherapeutic agent in cancer and a neuroprotective agent. Youns *et al*. (2017) indicate that fisetin is a promising novel antioxidant.

The combined effects of high ambient temperature (AT) and high relative humidity (RH) have been shown to cause heat stress in chicken (Egbuniwe *et al*., 2018). Fluctuations in the environmental conditions will enable the animal to adjust and cope with its environment (Vielma *et al*., 2014). High AT significantly retards the growth of animals (Mohammed *et al*., 2018). Reduced growth has been considered a consequence of reduced feed intake (Rhoads *et al*., 2013). Feed intake is related to environmental temperature in chickens (Sohail *et al*., 2013).

Tonic immobility (TI) and vigilance in birds are anti-predator behaviours of birds (Suzuki *et al*., 2013), used to determine the level of fear or stress (Campo *et al*., 2014). The adverse relationships between underlying fearfulness and performance may be linked with

occurrence of the adrenal and hypothalamus axis response. Although fear and stress are not the same, they are similar, fear elicitation involves adrenergic, dopaminergic, and cholinergic systems, which play a very important role in the physiological stress response in poultry, especially in predator or prey-like interactions (Wilson *et al*., 2015). They are also important in the measurement of adaptability of birds to environmental stress factors, including climate variability (Wang *et al*., 2014). Fear reactions are produced in stressful situations, including those related to predator defence (Sinkalu *et al*., 2016).

1.2 Statement of the Research Problem

Thermal environmental parameters exert significant adverse effects on farm animals reared in the tropics (Ravikumar *et al*., 2016). Direct thermal environmental conditions acting on animals include high AT and high RH, especially their combination (Schuller *et al*., 2013) which have been shown to cause stress in chickens, particularly during the various seasons (Dunning *et al*., 2016). Of all stress factors adversely affecting productivity in the tropical environment, AT and RH and, especially, their fluctuations are the most crucial changes in the thermal environment because they cause a variety of physiological responses in birds and animals (Schuller *et al.,* 2013; Minka and Ayo*,* 2016). The combined effect of high AT and RH induce heat stress in broiler chickens (Wang *et al*., 2014).

1.3 Justification of the Study

There are several reasons why people engage in poultry production and these include the provision of income, meat, ornamental, animal products and by-products and manure (Addisu *et al*., 2013). Gross margin analyses for poultry production have shown that it holds great potential for profitable economic returns. The structure of Nigerian poultry farming is such that 80-90% of the nation's poultry production lies in the hands of small

holders or other traditional groups (Maduka *et al*., 2016). It has been established that cloacal temperature (CT) is an important index in determining the response of poultry to changes in thermal environmental conditions. The cloacal temperature of broilers is known to be affected by seasonal rhythms. Stress is aggravated in birds by excessive high AT and relative humidity (RH) and also by wide fluctuations in the AT between the day and night time (Minka and Ayo, 2016). Heat stress and its impact on broiler chickens are manifested in physical, mental and emotional activities. Neurobehavioural changes are often the first signs of disease and an indicator of welfare status (Song *et al*., 2014).

There are three seasons in the Northern Guinea Savannah zone of Nigeria: the rainy (early and late), cold-dry (harmattan), and the hot-dry seasons (Dunning *et al*., 2016). Therefore in order to adequately interprete in clinical or research setting, the accurate normal values or range of values recorded in healthy animals during the dry and rainy seasons and at different hours of the day are necessary. There is a need to understand in full the pattern of changes in CT values of broiler chickens subjected to heat stress during the early rainy season in the zone (Minka and Ayo, 2014). Therefore, the information obtained from the present study may enhance the current understanding of effects of probiotic and/or fisetin on the CT, tonic immobility, vigilance and performance of broiler chickens. Such data may improve performance in broiler chickens reared during the thermally stressful early rainy season in the zone.

1.4 Aim of the study

To investigate the effect of probiotic and fisetin on CT, tonic immobility, vigilance and performance of broiler chickens.

1.5 Objectives

The objectives of the study were to investigate in broiler chickens, reared during the early rainy season in the Northern Guinea Savanna zone, the effects of probiotic and fisetin on:

i. Circadian variation in cloacal temperature.

ii. Tonic immobility and vigilance.

iii. Performance indices: feed intake, water intake, feed conversion ratio and live weight gain.

1.6 Statement of the Research Hypotheses

i. Probiotic and fisetin do not exert significant effect on cloacal temperature in broiler chickens.

ii. Probiotic and fisetin do not modulate significantly tonic immobility and vigilance of broiler chickens.

iii. Probiotic and fisetin do not exert significant effect on performance indices of broiler chickens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Poultry

Poultry are domesticated birds kept by humans for their meat, eggs or their feathers (Sahil *et al*., 2017; Qi *et al*., 2018). These birds are members of the super order *Galloanserae* (fowl), especially the order *Galliformes* (which includes chickens, quails and turkeys) (Stadig *et al*., 2016). Poultry also include other birds that are slaughtered for their meat, such as the young of pigeons (known as squabs) but does not include similar wild birds hunted for sport or food and known as game (Rajkumar *et al*., 2018). The word ”poultry” comes from a French/Norman word *poule,* itself derived from a Latin word *pullus,* which means small animal (Sonawane *et al*., 2017).

The domestication of poultry took place several thousand years ago (Igwe *et al*., 2015). This may have originally been as a result of people hatching and rearing young birds from eggs collected from the wild, but later involved keeping the birds permanently in captivity (Samour *et al*., 2016). Selective breeding for fast growth, egg laying ability, conformation, plumage and docility took place over the centuries and modern breeds often look very different from their wild ancestors (Okpala *et al*., 2016). Although some birds are still kept in small flocks in extensive systems, most birds available in the market today are reared in intensive commercial enterprises (Egbuniwe *et al*., 2015). Globally, poultry is the second most widely eaten type of meat and along with eggs, provides nutritionally beneficial food containing high-quality protein accompanied by a low proportion of fat (Hasan *et al*.,

2015). All poultry meat should be properly handled and sufficiently cooked in order to reduce the risk of food poisoning (Gattani *et al*., 2016).

2.2 Poultry Production

Production of chickens (*Gallus gallus*) for human consumption dates back as far as 7,000 years ago (Abdel-Hafeez *et al*., 2018), and there has been a continuous selection for specific desired traits through selective breeding of parent stock to achieve the desired results (Hasan *et al*., 2015). *Gallus gallus* is the major ancestor species, but *Gallus sonneratii* has also contributed to the genetic make-up of the domestic chicken (Archer, 2016). Furthermore, the knowledge of gene sequencing has accelerated the identification of causal mutations determining major morphological differences between wild *Gallus* and domestic breeds (Gattani *et al*., 2016). Evidence has shown that the critical issues of low production and inefficiency in resource allocation and utilisation in poultry production have adversely affected farmers in Nigeria (Okpala *et al*., 2016). Some of these constraints could be overcomed by forming cooperative groups to obtain credit facilities from the government and financial institutions (Egbuniwe *et al*., 2015). Poultry industry is an important subsector of livestock production and plays an important role in economic growth. In some poultry-producing countries, high environmental temperature is one of the most important inhibiting factors to poultry production (Aluwong *et al*., 2017). Besides improving environmental management, nutritional strategies have been developed to partially alleviate the negative impacts of heat stress in birds (Egbuniwe *et al*., 2015), including feeding diet with increased energy density, addition of salts, antioxidant vitamins and minerals in heat-stressed poultry diets (Sinkalu *et al*., 2015). Recently, dietary supplementation of probiotics, prebiotics and synbiotics has also been implemented in

poultry to counteract the negative effects of heat stress (Sonawane *et al*., 2017). Poultry enterprise is becoming complex by the day, given the rapid strides in technology, changing market dynamics and growing scale of production. Due to some factors, starting from the procurement of chicks to their final disposal, entrepreneurs are faced with numerous constraints (Sahil *et al*., 2017). Factors such as acute heat stress at marketing age, especially in broiler chickens raised in open houses with poor ventilation, and impaired heat exchange, result in economic losses (Stadig *et al*., 2016). Some considerations should therefore be given to the microclimate within the broiler houses as birds experience heat stress (Igwe *et al*., 2015).

2.3 Management Systems in Poultry Industry

Domestic chickens are prone to infectious and zoonotic diseases which have negative impact on poultry production and poses a serious threat to human health (Johnson *et al*., 2018). In Nigeria, good management practices are being advocated to meet the desires of customers (Balami *et al*., 2014). Despite the popularity of backyard poultry system, diseases easily spread in this setting (Kim *et al*., 2014). The most crucial limitations affecting village chicken production are diseases, predators, shortage of supplementary feeds, problems with the housing and lack of veterinary services (Wondmeneh *et al*., 2016). Outbreaks of diseases in poultry mostly result in areas where the locations of farms or trade overlap with habitats for wild birds (Suryanti *et al*., 2014). An integrated system of production is now advocated in today's poultry industry which takes into consideration animal health and product safety in a sustainable way. It puts together factors such as an improved diet, biosecurity and innovative processing and packaging to improve the safety of poultry products. Intensive system of production and some practices such as antibiotic

usage, stocking density and movement of animals which result in affordable products, have created serious health concerns for humans (Asaduzzaman *et al*., 2017). Industry-driven researches into poultry welfare should be emphasized due to factors bordering on human health, environmental impact and cost (Stadig *et al*., 2016).

Managing the environment is most important especially for broiler chicks in order to conserve heat and reduce energy lost (Zhang *et al*., 2015). Also the feeding management and stocking density is very essential for broiler to enhance efficient growth rate and to reduce injuries and mortality (Johnson *et al*., 2018). Litter management is also a crucial aspect of environmental management and is fundamental to bird health and performance and to final carcass quality (Samour *et al*., 2016).

2.3.1 Extensive (free-range) system

This system of poultry management is the most common in the rural areas of Nigeria (Maduka *et al*., 2016). In this management system, the birds are not confined and are thus free to feed on their own, with the low cost of management however the free-range management system cannot be practiced on an intensive commercial seal (Wondmeneh *et al*., 2016).

2.3.2 Semi-intensive system

This system requires a permanent housing with attached fence. This involve the process by which the birds are confined and also allowed to move out and search for feed, this system is suitable where land is limited and also in small-holder farms (Brian, 2016). Under this system, disease conditions may be common and therefore requires close monitoring and control (Kamel *et al*., 2017).

2.3.3 Intensive system

This is used mainly for commercial and small scale poultry production; it involves confining birds indoors either in battery cages or on deep litter within a large controlled environment (Pichova *et al*., 2016). The food and water requirements of birds are made available all the time (Cheng *et al*., 2018). The system affords effective insulation of the birds from outside atmospheric condition, while perches are provided for them as from the fourth week of age (Nobis *et al*., 2016). This system is recommended for large-scale commercial broiler production because it allows high-stocking density, efficient management of resources and labour, resulting in high production output (Archer and Mench, 2017).

2.4 Definition of Broiler Chickens

Broiler chickens are a gallinaceous domesticated fowl, bred and raised specially for meat production (Bai *et al*., 2016). They are hybrid of the egg-laying chicken, both being a subspecies of the red jungle fowl (*Gallus gallus*) (Ahmed *et al*., 2018). Typical broilers have white feathers and yellowish skin (Wang *et al*., 2016). Most commercial broilers reach slaughter weight between five to seven weeks of age, although slower growing breeds reach slaughter-weight at approximately fourteen weeks of age (Zheng *et al*., 2016).

2.5 Broiler Chicken Production

Broiler production in Nigeria involves the keeping of chickens of heavy meat breeds for the purpose of getting good-quality meat products, usually sold live or processed at six weeks of age (Aluwong *et al*., 2017). Broiler production is carried out in all parts of the country with no known religious, social or cultural inhibitions associated with their consumption

(Egbuniwe *et al.*, 2017). Global broiler meat production rose to 84.6 million tones in 2013, the largest producers were the United States (20%), China (16.6%), Brazil (15.1%) and the European Union (11.3%) (Maduka *et al*., 2016). Specifically, investment in broiler enterprise is attractive because the production cost per unit is low relative to other types of livestock. Poultry meat is very tender and broiler enterprises have short production cycles (Wondmeneh *et al*., 2016). The high demand for poultry products, the success of exotic breeds and the ease of mastering the techniques of poultry production among other factors has made it developed to the status of agribusiness in Nigeria as distinct from subsistence production (Sanni, 2015). The key to successful broiler production such as Arbo acre breed of broilers depends on a systematic and efficient management programme the farmer has adopted (Muhammad *et al*., 2018). In addition, it is advisable to do proper planning and preparation well on time for the arrival of chicks on site (Nobis *et al*., 2016).

The production cycle of broilers comprises their purchase from hatcheries and rearing for six weeks, after which the chicken house is cleaned, disinfected and allowed to rest for two weeks (Youngstedt *et al*., 2016). At six weeks, the broilers attain an average weight of 2 kg and are selected, slaughtered, packaged and sold to different market outlets (Carter *et al*., 2016). A complete cycle is therefore 8 weeks long, making it a total of 6 to 7 complete cycles annually. The common diseases of poultry can be prevented under good management practice and biosecurity measure which reduces mortality of 5-10% per year (Kamel *et al*., 2017). Due to artificial selection for rapid growth and husbandry used in poultry production, broilers are susceptible to several welfare concerns, particularly skeletal malformation and dysfunction, skin and eye lesions and congestive heart conditions (Pourabedin *et al*., 2014). The broiler breeding stock grow to maturity and beyond but also

have welfare issues related to frustration of high feeding motivation and beak trimming (Jahromi *et al*., 2016). Broiler chickens are usually grown as mixed-sex flocks in large sheds under intensive condition, but some breeds can be grown as free-range flocks (Bozkurt *et al.*, 2014). Chickens are often the most common and wide spread domestic animals and with a population of 19 billion in 2011, there are more chickens in the world than any other species of birds (Okpala *et al*., 2016). Modern commercial broiler chickens are artificially selected and bred for large scale, efficient meat production and grow much faster than egg laying hens or traditional dual purpose breeds (Mookiah *et al.*, 2014).

2.5.1 Economic importance of broiler chickens

The advantages of broiler chickens production are: 1), ease of management 2), high turnover, 3), fast returns on investment and 4), wide acceptance for consumption (Sinkalu *et al*., 2015). Domestic chickens are also considered important biological model for researches in biomedical science (Aluwong *et al*., 2017). Broiler chickens provides nutritionally beneficial food containing protein of high quality, this is accompanied by low levels of fat which have a favourable mix of fatty acid. There is a shift in emphasis from broiler chickens for poultry meat, to spent layers in developing countries (Zhang *et al*., 2014). There have also been significant improvements in poultry meat production in Nigeria due to efforts made in the use of improved breeds for production and the intensification of management systems of poultry (Samour *et al*., 2016). Poultry meat is relatively cheap for purchase by consumers and broiler chicken production is profitable because it has a positive net return on investment (Guo *et al*., 2018). Broiler meat has gained wide acceptance because it is a healthier alternative to red meat (Zhang *et al*., 2015). The human population in Nigeria, which is on the increase, has children between the ages

of 0 - 14 years, constituting 37% of the population (National Bureau Statistics, 2016). This has resulted in increased demand for protein intake, which poultry meat reliably supplies (Jang *et al*., 2014). Owing to rising world population and demand for animal-based protein, there is increased pressure on animal production, such as broiler chickens, maximizing the yield permitted by the genetic make-up to meet the high demand (Liu *et al*., 2015).

2.6 Thermoneutral Zone of Broiler Chickens

The thermoneutral zone can be defined as the range of ambient temperature during which regulatory changes in metabolic heat production or evaporative heat loss in birds is not induced (Egbuniwe *et al*., 2015). In the tropics, the diurnal ambient temperature fluctuations usually exceed the thermoneutral zone of chickens resulting in heat stress (Aluwong *et al*., 2017). Ambient temperatures outside the thermo-neutral zone of birds, irrespective of age, may negatively affect their energy balance and fitness (de Vogel *et al*., 2014). Elevated temperature negatively affects production, reproductive potentials, immune responses and health status of livestock including broilers (Singh *et al*., 2015). It has been reported that the thermoneutral zone for poultry in the tropics is between 18 – 24 °C and between 12 – 26 °C in temperate regions. It was also reported that the most favourable temperature range for poultry is between 12 – 26 °C (Ravikumar *et al*., 2016). The cardinal factor in understanding thermodynamic responses of homeotherms to their environments is by evaluating the energy involved in biological processes (Alaeldein *et al*., 2016).

2.7 Environmental Factors and the Welfare of Broiler Chickens

The Northern Guinean Savannah zone of Nigeria (11° 10/ N, 07° 38/ E), has annual ambient temperature ranging between 18.0 ± 3.7 °C and 31.8 ± 3.2 °C. Its seasons are mainly

harmattan (November-February), hot-dry (March-May) and rainy (June-October) seasons (Dzenda *et al*., 2013). Production of broiler chickens is directly influenced by meteorological factors, such as AT and RH (particularly during the hot-dry months), and their physiology (Egbuniwe *et al*., 2015). During hot conditions, characterised by high AT, high RH and radiant energy, there is decreased ability of animals to dissipate heat (Chuen-Yu *et al*., 2018). This initiates compensatory and adaptive mechanisms to return the body to homeostasis (Egbuniwe *et al*., 2017). When this persists, the difference between AT and body temperature of broiler chickens decreases, causing reduced rate of sensible heat loss (Hassan *et al*., 2018), which further results in mortality due to hyperthermia (Bughdadi, 2014). It is necessary to assess the environmental parameters of rearing broilers because they affect performances of the birds (Okpala *et al*., 2016). For instance, heat stress, resulting from high AT and RH negatively affects poultry performance in the tropical and subtropical regions (Song *et al*., 2014). The temperature-humidity index, an index of thermal comfort integrating the effects of AT and RH, is used to evaluate the degree of thermal stress in livestock (Kumar *et al*., 2017).

2.8 Stress Factors and Their Adverse Effects on Poultry Production

Stress is any factor that threatens the health of the body or has an adverse effect on its functioning, such as injury, disease or anxiety (Egbuniwe *et al*., 2017). The existence of one form of stress tends to diminish the body's resistance to other forms (Sinkalu and Ayo, 2018). Constant stress brings about changes in the balance of hormones in the body (Aluwong *et al*., 2013). Stress has been described as a consequence of adverse effects of the environment or management system, which forces changes in the physiology or behaviour of an animal to avoid malfunctioning (Haiyun *et al*., 2017). Thus, stress assists the animal

to cope with its environment (Bolisa *et al.*, 2017). The concept of stress, as a general adaptation syndrome, is a non-specific response of the body to various extraneous factors called stress factors or stressors (Brian, 2016). Any factor which disrupts physiological and/or psychological stability is a stressor and reaction to a stressor is termed stress (Aluwong *et al*., 2017). Behavioural changes are often the first and primary signs of distress. Stress is important to livestock production because it reduces the ability of animals to combat diseases and gain live weight (Zheng *et al.,* 2016).

2.9 Tonic Immobility and Vigilance Behavioural Responses of Broiler Chickens

One component of stress in poultry is fear, and the duration of tonic immobility (TI) and vigilance is the physiological indicator of fear (Archer and Mench, 2017). Both anxiety and fear are emotional status which increases with age in broiler chickens (Alm *et al*., 2016). They are damaging stressors resulting in impaired animal welfare (Qi *et al*., 2018), poor production and predispose birds to depressive-like behaviour (Sinkalu *et al*., 2016). TI and vigilance is a fear response because it is attenuated by procedures that reduce fear and enhanced by those that increase it (Egbuniwe *et al*., 2016). Some characteristics of fear described by Pichova *et al*. (2016) include temporary suppression of the righting response, reduced vocalisation, intermittent eye closure, rigidity, Parkinsonian-like muscle tremors in the extremities, altered electroencephalographic patterns and changes in heart rate, respiration and core body temperature (Melleu *et al*., 2016). The TI is the last in a series of defensive behaviours displayed in response to attack by a predator (Archer, 2016), thought to function by reducing stimuli leading to further attack (Stadig *et al*., 2016).

The TI is less affected by external influences under the action of compulsory fixation (Alm *et al*., 2015). TI duration is also related to aggressive behaviour (Zebunke *et al*., 2015),

pecking, and cannibalism among hens in commercial poultry industry hence, it is considered as an indirect diagnostic tool to assess stress, in poultry (Favreau-peigne *et al*., 2016). It is also an adaptive psycho-physiological response (Favati *et al*., 2016). Studies have shown that genetic predispositions of poultry influence responses of birds to heat stress in terms of behaviour and production (Garcia-Longoria *et al*., 2015). Birds confronted with threats exhibit fear-induced freezing called TI, mostly observed in prey species as a defense mechanism (Herrington *et al*., 2015). Fear as an adaptive behaviour which protects the animal from psycho-chemical damage, eliciting a reaction to the perception of actual danger (Huth and Archer, 2015). It is important to understand the behaviour of animals because it affects their production and welfare (Kim and Velando, 2015). It is practically impossible to completely eliminate fear-inducing (stressful) situations during the rearing of birds (Mamo *et al*., 2015). Duan *et al*. (2014) described broiler chickens showing short or long TI responses as low-fear or high-fear responders, respectively.

2.10 Cloacal Temperature Responses in Broiler Chickens

The hot-dry season in the Northern Guinea Savannah zone of Nigeria extends from March to May (Dzenda *et al*., 2013) and has been demonstrated to be thermally stressful to poultry (Egbuniwe *et al*., 2018). The thermal environmental conditions during the hot-dry season directly influence the health and welfare of the birds (Makeri *et al*., 2017). Cloacal temperature (CT) is one of the indices of heat stress, reflecting the core body temperature (Aluwong *et al*., 2017). It indicates the balance between heat loss and heat gain in broiler chickens (Iyasere *et al*., 2017). Thus, changes in CT during stress may be of value in determining the resistance of broiler chickens to stressful conditions (Sinkalu *et al*., 2015). It was reported that the normal upper limit of body temperature in poultry does not exceed

42.2 °C (Aluwong *et al*., 2017). Prevention of hyperthermia is vital for maintaining proper pituitary-gonadal axis to improve reproductive performance and efficiency of poultry production in the tropics (Bughdadi, 2014).

Stress responses are integrally associated with changes in acid-base balance, performance, immunocompetence, body temperature, behavioural and haematological parameters (Sinkalu *et al*., 2016; Egbuniwe *et al*., 2017). Heat stress in broiler chicken is evaluated by measuring the cloacal temperature (Ahmed *et al*., 2018), which is a true reflection of internal body temperature and a reliable index of thermal balance (Lindholm *et al*., 2016). Core body temperature is often the marker rhythm of choice due to ease of measurement, particularly in field conditions (Makeri *et al*., 2017). Relatively constant body temperature are maintained by birds under normal body conditions, but when the internal heat production and heat gain from the environment are greater than the rate of heat dissipation, the body temperature increases (Aljuobori *et al*., 2016). Heat stress induces oxidative stress which impairs the normal body function, leading to increased morbidity, mortality, poor meat quality, and decreased productivity (Nobis *et al*., 2016). The management of broiler chickens under continuous lighting programmes may increase the ambient temperature (if high-energy bulbs are used) during hot conditions, reduce duration or depth of sleep, and induce chronic stress (Youngstedt *et al*., 2016). Sleep deprivation may further increase physiological stress, associated with production of broiler chickens (Carter *et al*., 2016).

The Arbo Acre breed of broiler chickens is ultra-converters and has shown best performance efficiencies across multiple metrics, including feed conversion, live ability, and growth rate (Aluwong *et al*., 2017). In order to alleviate the detrimental effects of high environmental temperature on oxidative stress and performance of poultry, dietary

manipulations are preferable to other methods because of their practicability and lower cost. Other methods of mitigating the deleterious effects of heat stress on broiler chickens in tropical zones of the world, reported to be inefficient, include adjustment of ventilation rates and the use of cooling systems (Hamrita and Conway, 2017).

2.11 Antioxidants as Anti-stress Agents in the Amelioration of Thermal Stress in Broiler Chickens

It has been demonstrated that during stress, reactive oxygen species (ROS) are generated in the body (Sonawane *et al*., 2017). ROS have been shown to induce lipoperoxidation of cytomembranes (Michiko *et al*., 2017), resulting in cell damage and destruction (Egbuniwe *et al*., 2017). Antioxidants protect lipids from peroxidation by radicals (Lee *et al*., 2018), they are effective because they readily give up their own electron to free radicals (Egbuniwe *et al*., 2016). When a free radical gains electrons from an antioxidant, it no longer attacks the cell and the chain reaction of oxidation is broken (Makeri *et al*., 2017). Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules (Muhan *et al*., 2018). Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent (Vesco *et al*., 2017).

Although oxidation reaction is important during heat production in the animal's body, it may also damage the tissue (Wang *et al*., 2015). Antioxidants are widely used as ingredient, in dietary supplements, and their supplementation provides beneficial effects against stress-induced tissue damage (Aluwong *et al*., 2017). Previous studies have shown that diets enriched with antioxidant substances, such as probiotic, fisetin, vitamins A, C, and E and zinc and chromium, may be used to attenuate the negative effects of environmental stress (Yogev *et al*., 2017). This fact suggests that adverse effects of environmental stress are largely due to induction of oxidative stress (Al-Rukibat *et al*., 2017).

2.12 Definition of Probiotics

Probiotics are live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance (Adjei-Fremah *et al*., 2018). They are viable single or mixed cultures of microorganisms that when given to animals or humans beneficially affect the host by improving the properties of the indigenous microflora (Aluwong *et al*., 2013).

2.13 Probiotics and their Antioxidant Effects

The Genera of probiotics microorganisms commonly used for animals include *Bifidobacterium, Lactococcus, Lactobacillus, Bacillus, Streptococcus* and yeast such as *Sacromyces* and *Candida* (Sugiharto *et al.,* 2017). The probiotic *Saccharomyces cerevisiae,* is one of the live microorganisms that when administered through the digestive tract, has a positive impact on the host health through its direct nutritional effect (Aluwong *et al*., 2017). Probiotics act by reducing feed conversion, resulting in an increase in daily weight gain (Bai *et al*., 2016). For several decades, yeasts have been used to improve feed utilisation by farm animals, and they have played an important role since the ban on using antibiotics as growth promoters in animal feed in the European Union in 2006 (Zheng *et al*., 2016). Yeast is a rich source of protein, fat, vitamin B and enzymes such as cellulase and phytase (Haque *et al*., 2017). The yeast cell walls contain 29-64% betaglucan, 31% mannan, 1-2% chitin and mannan oligosaccharide (MOS), and these components are considered immune stimuli (Nie *et al*., 2015). Yeast also has an important role in improving animal productivity, maintaining favourable gut ecology and improving gut health. In addition, yeast cell walls contain antioxidant enzymes (peroxidases), such as superoxide

dismutase, catalase and glutathione (Aluwong *et al*., 2013). Moreover, yeast can be used as a practical means to improve the utilisation of agricultural by-products in animal nutrition and to reduce environmental pollution and the cost of feeding (Swiatkiewicz *et al*., 2014). However, the effect of yeast depends on the dietary composition and nutrient profiles of animal feeds (Sandeep *et al*., 2018).

Several works have indicated the potential benefits of probiotics in ameliorating the impaired physiological conditions in poultry due to stress (Lei *et al*., 2013; Sohail *et al*., 2013). Probiotic-enhanced water acidifier (combination of sorbic acid, citric acid, sodium chloride, sodium citrate, potassium chloride, zinc sulphate, ferrous sulphate, magnesium sulphate, cellulase) helps in restoring serum sodium and potassium levels of broilers following exposure to any form of stress (Wang *et al*., 2016). Probiotic treatment has been demonstrated to increase serum concentration of triiodothyronine (T3) and tetraiodothyronine (T4) in broiler chickens subjected to heat stress (Aluwong *et al*., 2013). Considering that thyroid hormones play a vital role in stimulating the synthesis of many structural proteins, enzymes and hormones (Barbakadze *et al*., 2014), the increased levels of thyroid hormones following probiotic supplementation are reasonably expected to improve digestion and metabolism in heat-stressed chickens (Pourabedin *et al*., 2014). One possible factor that might be responsible for enhancing the concentrations of T3 and T4 in probiotics-supplemented, heat-stressed birds was the reduced circulating level of corticosterone as elevated concentration of corticosterone may result in hypothyroid activity (Aluwong *et al*., 2013). It has been reported that probiotic treatment increases uric acid level in the serum of heat-stressed birds (Hasan *et al*., 2015).

2.14 Probiotics and its Beneficial Effects in Poultry Production

As part of the nutritional strategies, inclusion of feed additives in the diet has been conducted for ameliorating the negative effects of stress in poultry (Adjei-Fremah *et al*., 2018). Among feed additives, probiotics have gained more attention from poultry nutritionists as this additive is capable of improving the physiological conditions, intestinal morphology and structure (Jahromi *et al*., 2016), immune system and, thus, performance and well-being of poultry (Al-Fataftah and Abdelqader, 2014). A number of studies have reported the potential benefits of probiotics on the intestinal microbial diversity and population in poultry subjected to high ambient temperature (Sohail *et al*., 2013). The mechanisms through which probiotics elicit beneficial impacts and/or re-establish the balanced intestinal microbial diversity and populations in birds have been elucidated (Sugiharto, 2014). In modern broiler production, excessive fat deposition, especially of abdominal fat, is a major concern in the poultry industry (Bozkurt *et al.*, 2014). Fat is a main form of energy deposition in the body therefore, excessive fat deposition demands high energy supply and results in energy loss (Wang *et al*., 2016). This, in turn, causes an increase in overall feed cost as well as in reduced feed efficiency. Furthermore, the more body fat that a chicken has, the lower the possible lean meat yield of that chicken, which is highly problematic because lean meat has the high nutritional value and it is preferred by consumers (Mookiah *et al.*, 2014). Accordingly, because low lean meat yield is undesirable for consumers, it also causes processing losses (Adjei-Fremah *et al*., 2018). It is evident that excessive fat in broiler chickens is detrimental for the broiler industry (Bai *et al*., 2016). Naturally, based on the afore-mentioned information, reducing excessive fat deposition is a priority for broiler producers and consumers (Wang *et al*., 2015).

Attempts have been made to mitigate the problem of fat deposition using nutritional strategies, such as the dietary supplementation of probiotics and plant extracts, and some researchers have demonstrated that this is an efficient means of preventing excessive fat deposition. Aluwong *et al*. (2013) showed that the supplementation of a yeast probiotic in broiler feeds significantly decreased abdominal fat weight by 16.67% in the group fed 1.5% and by 28.626% in the group fed 2.0% compared with the controls. Therefore, these results indicate the potential of specific diets and dietary supplements to prevent excessive fat deposition in broiler carcasses (Walid *et al*., 2017). Probiotics have been used to improve broiler chickens growth performance, apparent nutrient digestibility, immune function, digestive enzyme activity, and intestinal tract bacteria and morphology (Sun *et al.*, 2015). Considering that uric acid is an important antioxidative agent, the increased level of uric acid may be a mechanism by which probiotic helps in alleviating the oxidative damage following stress in birds (Sugiharto *et al*., 2017).

The increased level of total serum glucose in response to stress has been reported to be alleviated by provision of probiotic-enhanced water acidifier. In this case, probiotics might decrease the concentration of corticosterone that in turn decreases gluconeogenesis in stressed birds (Wang *et al*., 2016). Stress is associated with the decreased concentration of some haematological variables such as haemoglobin (Haque *et al*., 2017). Hasan *et al*. (2015) showed that probiotics (Protexin® Boost) increased haemoglobin concentration in birds subjected to a stressor. The different type of probiotics used in the diets of stressed birds seemed to be the reason for the discrepancy (Zheng *et al*., 2016). It should be noted that probiotics have been useful in improving the immune system of birds subjected to stress (Beski and Al-Sardary, 2015).

Probiotics may reduce the circulating level of corticosterone leading to lowered heterophil to lymphocyte (H/L) ratio and improved immune responses (Mingmongkolchai and Panbangred, 2018). Yang *et al*. (2015) suggested that corticosterone possess immunosuppressive effects in poultry, and therefore the reduced corticosterone level may be beneficial in restoring the normal function and development of immune system. The lowered antibody response to infectious agents is usually believed to increase the mortality of stressed chickens (Sohail *et al*., 2013). Probiotic administration has been found to enhance antibody responses as well as leucocyte count in birds reared under hot temperature (Yang *et al*., 2015). In most cases, the relative weight of immune organs may represent the capability and functionality of the immune system in poultry (Song *et al*., 2014). Lymphoid organ involution due to heat stress in poultry may be prevented by probiotic administration (Griggs and Jacob, 2015).

Probiotics may reduce the level of corticosterone responsible for the involution of lymphoid organs under heat stress (Sohail *et al*., 2013). It is well known that intestinal microbial diversity and population affect the development of immune system of poultry. Dietary probiotic administration has been able to improve the intestinal microbial diversity and population, and thus immune system of poultry under heat stress condition (Al-Fataftah and Abdelqader, 2014). The mechanisms through which intestinal microbial ecosystem affects the immune system of birds have extensively been reviewed elsewhere (Sugiharto, 2014). Recently, dyslipidaemia (elevation of plasma cholesterol, triglycerides, or both) has been believed to be a marker of inflammation in human studies (Aulinas *et al*., 2015). Concomitant with the enhanced levels of the conventional inflammatory marker such as tissue necrotic factor-alpha (TNF-α) and interleukin (IL-1), stress in poultry was also

associated with the dyslipidaemia (Habibian *et al*., 2014). There is growing evidence that probiotics can control dyslipidaemia in birds subjected to a stressor. As part of the nutritional strategies, inclusion of feed additives in the diet has been conducted in ameliorating the negative effects of stress in poultry (Sugiharto, 2014). Among feed additives, probiotics have gained more attention from poultry nutritionists as this additive is reportedly capable of improving the physiological conditions, intestinal morphology and structure, immune system and thus performance and well-being of stressed poultry (Jahromi *et al*., 2016).

2.15 Effect of Probiotic on Immunity of Broiler Chickens

Probiotics are live microbial feed additive that maintain microbial balance in digesta in the gastro-intestinal tract of the host animal (Wang *et al*., 2016). Administration of probiotics in chicken feed has been reported to benefit chickens by lowering *Escherichia coli* and *Salmonella* populations in their intestines (Sugiharto *et al*., 2017), with subsequent improvements of feed conversion and body weight gain (Bai *et al*., 2016). Dietary supplementation with probiotic exerts positive effects on productivity, stimulating the immune system, improving the meat quality of broiler chickens and improving the antioxidant capacity in poultry raising (Park and Kim, 2014). Probiotics reduce the level of cholesterol in broiler chickens (Zheng *et al*., 2016). Probiotic supplementation may also depress the concentration of cholesterol in blood and yolk of chickens, and it may benefit broiler chickens by improving innate immunity and body growth (Parr *et al*., 2015). These considerations are particularly relevant in tropical and sub-tropical environments or during summer in temperate areas where high environmental temperatures are inevitable (Xie *et al*., 2015). In poultry production, heat stress manifested as reduction in weight gain and

elevation in feed conversion ratio can be ameliorated by the use of probiotics as feed supplements (Aljuobori *et al*., 2016).

2.16 Sources and Structure of Fisetin

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a bioactive flavonol molecule (Fig. 2.1) (Sundarraj *et al*., 2018), found in vegetables, nuts beans and fruits, such as strawberry, apple, persimmon, grape, onion and cucumber at concentrations in the range of 2-160 g/g (Mahmoud *et al*., 2017). Flavonoids and their polymers comprise one of the largest groups of phytonutrients that afford beneficial health effects (Nan *et al*., 2017). These important polyphenolic compounds under the class of plant secondary metabolites exert significant effects ($P < 0.05$) in the biological system (Jui-Hung *et al*., 2017). It was reported that the average total intake of flavonoids in the United States was 1 g/day (Kang *et al*., 2016). No reports have been documented in Nigeria.

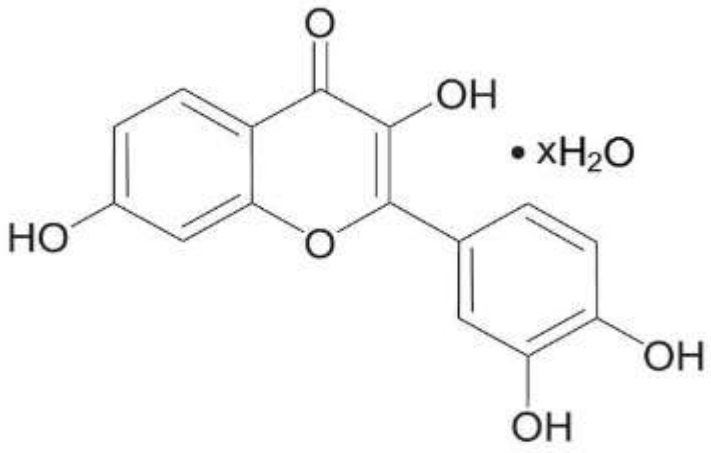

Figure 2.1: Molecular structure of fisetin (Source: Su *et al*., 2017).

2.17 Fisetin and Its Antioxidant Effects

Flavonoids and their metabolites can be transported across the blood-brain barrier (BBB), making them ideal targets in the therapeutic utility for neurodegenerative disorders (Chen *et al*., 2015). The intake of flavonoids and isoflavonoids was estimated by calculations from the database and based upon a preliminary examination of 40 food items, covering at least 80% of total food consumption (Chiang *et al*., 2015). Average daily intake of fisetin per capita of flavonoids was computed as 0.4 mg (Ferreira *et al*., 2018). The highest concentration of fisetin was found in strawberries (160 lg/g) followed by apple (26.9 1g/g) and persimmon (10.5 lg/g) (Chen *et al*., 2014). In the past years, fisetin was a subject of research because of its presence in various human foods and its antiproliferative, apoptotic, and antioxidant activities (Currais *et al*., 2014). Fisetin has been reported as a chemopreventive/chemotherapeutic agent in several types of cancer and also as a neuroprotective agent. Several studies indicate that fisetin is a promising novel antioxidant with many potentials (Su *et al*., 2017).

Recently, the use of natural dietary substances found in fruits, vegetables and herbs has received considerable attention as chemopreventive and chemotherapeutic agent worldwide (Kang *et al*., 2016). The approach of cancer prevention using non-toxic novel plant-derived agents has been encouraged (Syed *et al*., 2014). Flavonoids are commonly found in most plants and exert a significant range of biological activities such as antioxidant, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, and antiviral effects (Chen *et al*., 2015). The ability of flavonoids to scavenge free radicals contributes to their marked antioxidant activity and significant biological effects (Mansuri *et al*., 2014). When an imbalance occurs between antioxidants and reactive oxygen species (ROS), it

results in oxidative stress (Pawar *et al*., 2018). It is a consequence of a mismatch between the production of the ROS and the ability to defend against them (Kim *et al*., 2015). It has been implicated in the development of many diseases, including diabetes mellitus, retinal degeneration, neurodegenerative diseases, mutagenesis, carcinogenesis, and ageing (Wu *et al*., 2015).

Antioxidative properties of fisetin have been examined by both cyclic voltammetry- and quantum-chemical-based calculations (Hsien-Yu *et al*., 2015). The trolox-equivalent activity concentration (TEAC) value of fisetin has been reported to be 2.80 – 0.06 (Kang *et al*., 2015). Fisetin was found to be a planar molecule exerting a cross-conjugation effect. The hydroxyl bond (OH) dissociation energy and dipole moment specified that fisetin had high antioxidant capacity (Li *et al*., 2014). It has been reported that fisetin strongly binds between the polar head and hydrophobic tail of the phospholipids around the interfacial region of the egg phosphatidylcholine liposomes (Khan *et al*., 2014). This region is easily available to the free radicals and serves as the reaction site for the antioxidant activity of the fisetin molecule and inhibits lipid peroxidation (Seo and Jeong, 2015). It was found that fisetin partitioned well into the membrane in solid gel and liquid crystalline phases, and the fisetin molecules were generally present near the head group region of the lipid bilayer membrane (Smith *et al*., 2016).

Fisetin has been reported to inhibit human low-density lipoprotein (LDL) oxidation *in vitro* (Zhuo *et al*., 2015). Fisetin along with quercetin and myricetin had the lowest oxidation potential, more active than trolox, and seemed to be the most active compound in ferric reducing antioxidant power assay, which determines the reducing capacity of a compound

directly (Kang *et al*., 2014). The effects of fisetin, morin, and myricetin were investigated on the susceptibility of LDL to oxidative modification. Fisetin had stronger inhibitory activity than morin and myricetin (Sundarraj *et al*., 2018). It was found that fisetin, morin, and myricetin prevented LDL from oxidation, in part, through reducing CD36 gene expression in macrophages, a possible effect in ameliorating atherosclerosis (Lee *et al*., 2018). Fisetin has been shown to increase intracellular glutathione (GSH) levels in the mouse hippocampal HT-22 cells both in the presence and absence of glutamate (Su *et al*., 2017). The increase in GSH metabolism provides protection from glutamate, as glutamate decreases the level of GSH by inhibiting the uptake of cystine necessary for the production of GSH (Ravichandran *et al*., 2014). Treatment with fisetin caused increased nuclear translocation and activity (Sundarraj *et al*., 2018).

2.18 Impact of Stress on the Performances of Broiler Chickens

The deleterious effects of stress on the growth performance of broiler chickens cannot be overemphasized (Park and Kim, 2014). In laying hens, stress was also found to decrease egg production (Alms *et al*., 2015). The impaired performances of poultry subjected to stress have been associated with a number of factors including poor appetite and reduced feed intake (Baxter *et al*., 2018). Impaired digestion due to damage of intestinal morphology and lowered digestive enzyme activity and metabolism (due to lowered activity of thyroid hormones), altered endocrine status as well as reduced blood triglyceride and total cholesterol levels in broiler chickens (Song *et al*., 2014). In addition, various studies have also shown that fermented products have a regulatory effect on lipid metabolism (Chen *et al*., 2014). In broiler chickens, fatty acid synthesis occurs mainly in

the liver, and the adipose tissue is the primary site of fat storage as triglycerides, especially the abdominal fat tissue (Sugiharto *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site and Thermal Environmental Conditions

The experiment was conducted at the Department of Veterinary Physiology, Ahmadu Bello University, Zaria (11° 10/ N, 07° 38/ E), located in the Northern Guinea Savannah zone of Nigeria. The birds were kept under natural conditions, without artificial control of the environment. As a result, they were subjected to the naturally prevailing conditions of fluctuating ambient temperature (AT) and (RH). The study was carried out during the early rainy season from June to July, 2017 (Dzenda *et al*., 2013).

3.2 Experimental Animals and Management

A total of 60, apparently healthy day-old broiler chicks (Arbor Acres), comprising both sexes were purchased from a reputable hatchery in Nigeria, they served as the subjects. They were kept in a poultry pen under an intensive management system. The floor was littered with wood shavings and the broiler chicks were allowed access to water and feed *ad libitum.* The broiler chicks were fed with broiler starter (day 1 - 28) and broiler finisher (day 29 - 42) produced by a Commercial Feed Mill, Kaduna, Nigeria. The proximate analysis of the feeds (Nutrition Laboratory, Mark Farms, Osara, Nigeria) is shown in Table 4.1. The poultry pen was made of concrete floor, cement block with aluminum roofing and cardboard ceiling. The dimension of the pen was 8.4 m × 5.6 m × 1.91 m and the birds were stocked at the density of 15 birds/m^2. Biosecurity measures were ensured by providing foot-wears and protective clothing for all the assistants, and the pen was not accessible to any non-essential person, animals or other birds.

3.3 Meteorological data from the study area

The AT at the experimental site was measured using a wet- and dry-bulb thermometer (Brannan® Sapphire Instruments, New Delhi, India) thrice per day at 07:00 h, 13:00 h and 18:00 h, and the RH was calculated using Osmon's hygrometric table (Narinda Scientific Industries, Haryana, India). The dry-bulb temperature (DBT) and wet-bulb temperature (WBT) were recorded every two hours for three days, one week apart, on days 21, 28 and 35 of the experiment. The temperature-humidity index (THI) was determined using the following formula (Tao and Xin, 2003):

THI = 0.85(Tdb) + 0.15Twb (for broilers), where THI = temperature-humidity index for broiler chickens, Tdb = dry-bulb temperature and Twb = wet-bulb temperature. The parameters were recorded inside the poultry house on each day of the experiment.

3.4 Ethical Approval

The conduct of this research was approved by the Ethical Committee on Animal Use and Care of the Ahmadu Bello University, Zaria with reference number ABUCAUC/2018/021.

3.5 Experimental Design

The 60 birds were weighed individually at weekly interval using a Mettler Toledo® digital precision weighing balance with a sensitivity of 0.01 g (Model MT-500D). Group I was administered with distilled water *ad libitum*; Group II, fisetin at a dose of 5 mg/kg; Group III, probiotic (*Saccharomyces cerevisiae*) at a dose of 4.125×10^6 cfu/100 mL using the competitive exclusion method; and Group IV, administered with probiotic (*Saccharomyces cerevisiae*) and fisetin (5 mg/ml) and probiotic (4.125×10^6 cfu/100 mL), respectively. All

administration were done orally for the first seven days of life (Aluwong *et al*., 2017). The broiler chickens were divided by simple randomisation into four groups of 15 each, as follows: group I, control; group II, fisetin; group III, probiotic; and group IV, combination of probiotic (*Saccharomyces cerevisiae*) and fisetin. Probiotic (*Saccharomyces cerevisiae*) (Montajat Pharmaceuticals, Bioscience Division, Dammam 31491, Saudi Arabia) and fisetin (Sigma Inc., New Orleans, Louisiana, USA), were administered for 7 days orally via a 1 mL-tuberculin syringe, starting from 1-day old. Each bird was identified on the leg by using a masking tape for proper recordings.

3.6 Measurements of Cloacal Temperature

The cloacal temperature (CT) values were recorded as an indicator of the body temperature (Sinkalu *et al*., 2014), using a digital clinical thermometer (Krusser Thermometer® Amazon, Berlin, Germany). Measurements of CT were taken using standard procedures (Minka and Ayo, 2013) over a 24-h period. After restraining each broiler chicken, the CT was taken by inserting the thermometer at about 3 cm into the cloaca and was tilted to make contact with the wall of the cloaca. The dry- and wet-bulb temperatures were recorded concurrently with the CT values. The readings were taken from 07:00 to 07:00 h of the next day, on days 21, 28 and 35 of the study. Manufacturer's instructions were followed to ensure accurate readings. The CT was measured on three days only in order to reduce the adverse effects of stress due to handling of the birds, which increases body temperature (Choi *et al.,* 2015).

3.7 Measurement of Tonic Immobility Responses

Induction was performed on days 21, 28 and 35 as described by Sinkalu *et al*. (2016) in 60 broiler chickens (15 in each groups) at 07:00 h, 13:00 h, and 18:00 h by placing a broiler chicken at each hour of measurement on its back on an improvised cradle and gently pressing its breast for 15 seconds. Each cradle was constructed from Dunlop foams, measuring 50 × 40 × 25 cm and covered with cloth, similar to those used by Wang *et al*. (2014). The centre of the cradle was scooped to accommodate each broiler chicken on dorsal recumbency. Any broiler chicken that righted itself within 2 seconds as gently caught again and the procedure was repeated. If TI was not induced after three attempts, the duration of TI was then recorded as 0 second. If the broiler chicken did not resume a standing position after 600 seconds, which was the maximum duration of TI allowed, the induction process was interrupted (Egbuniwe *et al*., 2016).

3.8 Ranking of Vigilance Behaviour

The vigilance behaviour at self-righting of each TI test was observed in broiler chickens and were ranked as described by Sinkalu *et al*. (2016). Briefly the ranking was performed with a slight modification on a scale of 1 to 3; with 1 representing fearlessness, and 3 representing fearfulness. Vigilance behaviour of each broiler chicken at self-righting was ranked as follows:

i: if the process of self-righting was quick and spontaneous (≤ 180 seconds with no vocalisation)

ii: if the process was slow with a short vigilance period (> 180 seconds/or < 600 seconds with peeping and vocalisation)

iii: if the process was very slow and with a prolonged period of vigilance (600 seconds).

3.9 Performance Parameters

3.9.1 Measurement of feed intake

Daily feed intake of the broiler chickens was measured once a day at 07:00 hour during the period of the experiment. The mean values for each, 1 week experiment was used as the daily feed intake values for each week during the study period. The weight of the feed before placement was measured using a Mettler Toledo® Digital Precision weighing balance (Model MT-500D, Columbus, Ohio, USA). The remaining feed was measured again 24 h later. Absolute feed intake was calculated as the difference between the amount of feed supplied to the broiler chickens and the amount that remained at the end of each consumption period. The feed intake and feed conversion ratio (FCR) were calculated using the formula: (Aluwong *et al*., 2013),

FCR = Total feed consumed by birds/total weight gain.

3.9.2 Measurement of water intake

Daily water intake of broiler chickens was measured once a day at 07:00 h during the experimental period. The mean values for each week of the experiment was used as the daily water intake values for each week of the study period. A graduated cylinder was used to measure the volume of water before placement. The remaining water was measured again 24 h later the next day (Aluwong *et al*., 2013).

3.9.3 Measurement of live weight gain

All the broiler chickens were weighed using A Mettler Toledo® Digital Precision weighing balance with a sensitivity of 0.01 g (Model MT-500D, Columbus, Ohio, USA) before and after each 7-day feeding and watering, the average of each week was considered as the live weight gain for that particular study period (Aluwong *et al*., 2013).

3.10 Data Analyses

The data obtained were expressed as mean ± standard error of the mean (Mean ± SEM). Values were subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test to compare differences between the means obtained from the control and treatment groups. Pearson's correlation and regression analysis were used to evaluate the relationships between thermal environment and cloacal temperature parameters. The behavioural parameters (TI and vigilance) were analysed using Kruskal-Wallis analysis of variance. GraphPad Prism 5.03 for windows (GraphPad Software, San Diego, California, USA) was used for the analyses. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

Table 4.1: Composition and Proximate Analysis of Broiler Chicken Diets

Feed Composition	Starter	Finisher
Ingredients (%)		
Crude protein	22.00	19.5
Fat	5.10	3.80
Crude fibre	4.30	3.00
Calcium	1.20	1.20
Available phosphorus	0.45	0.44
Methionine	0.56	0.50
Lysine	1.30	1.20
Metabolisable energy (Kcal/kg)	3000.00	3100.00
Proximate analysis		
Crude protein (%)	22.00	21.00
Fat (%)	7.90	6.80
Crude fibre (%)	4.30	3.00
Calcium (%)	2.00	2.00
Available phosphorus (%)	0.80	0.70
Methionine (%)	0.56	0.50
Lysine (%)	1.20	1.20
Metabolisable energy (Kcal/kg)	2900.00	2980.00

4.1 Weekly Mean, Minimum and Maximum Variations in Thermal Environmental Parameters during the Study Period

During the study period, week 3 had the highest mean DBT (30.86 ± 0.67 °C) and week 5 (30.43 ± 0.56 °C) had the least mean DBT (Table 4.2). There was no significant ($P > 0.05$) difference in DBT between the weeks of the study period. The overall DBT for the study period ranged from 26.00 - 36.00 °C, with a mean of 30.57 ± 0.36 °C. The RH during the study period ranged from 49.00 - 93.00%, with a mean of 79.22 ± 1.26%. The RH for Week 5 (81.57 ± 1.64%) was the highest during the study period, week 3 had the least mean RH (74.67 ± 2.15%) and was significantly ($P < 0.05$) lower than those for weeks 4, and 5 (Table 4.2). The mean THI for the study period was 30.10 ± 0.34 and it ranged from 25.60 - 35.30. There was no significant ($P > 0.05$) difference in THI between the weeks of the study period. The THI for week 5 (30.01 ± 0.54) was the least during the study period and it ranged from 25.70 - 33.40. Week 4 (30.20 ± 0.62) had the highest THI value, with a range of 25.70 - 35.30 (Table 4.2).

Table 4.2: Weekly Mean, Minimum and Maximum Variations in Thermal Environmental Parameters during the Study Period

Weeks	DBT (°C)	RH (%)	THI
3	30.67 ± 0.67 (26.00 - 36.00)	74.67 ± 2.15 (49.00 - 93.00)	30.09 ± 0.64 (25.55 - 35.10)
4	30.62 ± 0.66 (26.00 - 36.00)	81.43 ± 2.41 (61.00 - 93.00)	30.20 ± 0.62 (25.70 - 35.30)
5	30.43 ± 0.56 (26.00 - 34.00)	81.57 ± 1.64 (67.00 - 93.00)	30.01 ± 0.54 (25.70 - 33.40)
Overall Mean ± SEM	30.57 ± 0.36 (26.00 - 36.00)	79.22 ± 1.26 (49.00 - 93.00)	30.10 ± 0.34 (25.60 - 35.30)

Where RH = Relative humidity, THI = Temperature-humidity index, DBT = Dry-bulb temperature, n = 15

4.2 Variations in Thermal Environmental Parameters on Selected Days of the Study Period

The mean DBT at Day 28 (28.85 ± 0.45 °C) of the study was higher compared to those of days 21 (26.85 ± 0.25 °C) and 35 (28.54 ± 0.39 °C). There was no significant ($P > 0.05$) difference in DBT between days 21, 28 and 35 of the study (Table 4.3). Day 21 (77.92 ± 2.56%) had the least RH compared to days 28 (81.46 ± 3.11%) and 35 (79.00 ± 2.62%). The mean THI was highest on day 28 (28.47 ± 0.38) as compared to days 21 (26.40 ± 0.23) and 35 (28.05 ± 0.33) (Table 4.3). There were no significant ($P > 0.05$) differences in RH and THI between days 21, 28 and 35 of the study period.

Table 4.3: Variations in Thermal Environmental Parameters on Selected Days of the Study Period (mean ± S.E.M)

Parameters	Day 21	Day 28	Day 35
DBT (°C)	26.85 ± 0.25 (26.00 - 28.00)	28.85 ± 0.45 (27.00 - 32.00)	28.54 ± 0.39 (27.00 - 31.00)
RH (%)	77.92 ± 2.56 (58.00 - 92.00)	81.46 ± 3.11 (63.00 - 93.00)	79.00 ± 2.62 (62.0 - 93.00)
THI	26.40 ± 0.23 (25.10 - 27.55)	28.47 ± 0.38 (26.85 - 31.10)	28.05 ± 0.33 (26.55 - 30.10)

RH = Relative humidity, THI = Temperature-humidity index, DBT= Dry bulb temperature, n = 15

4.3 Circadian Variation in Cloacal Temperature Responses of Broiler Chickens during the Study Period

At day 21 of the study period, the CT values in the probiotic (40.32 ± 1.90 °C) and probiotic + fisetin (40.49 ± 0.03 °C) were significantly lower ($P < 0.05$) when compared with the control group (41.39 ± 0.03 °C). At the same day of the study period, the mean CT value of the fisetin group did not differ significantly ($P > 0.05$) from that of the control group (Table 4.4). At day 28 of the study period, CT values did not differ significantly ($P > 0.05$) in the broiler chickens supplemented with probiotic (41.26 ± 0.06 °C), when compared with the corresponding value in the control group (41.58 ± 0.03 °C) (Table 4.5). At day 28 of the study period, the CT value of the fisetin-group did not differ (41.58 ± 0.03 °C); while the co-administered group showed a highly significant (40.40 ± 0.03 °C, $P < 0.001$) decrease when compared with the value obtained from the control group (Table 4.6). The CT in the co-administered group did not differ on day 35 of the study period, when compared with that of the controls (41.78 ± 0.03 °C). The overall CT value in the fisetin and co-administered groups increased (41.68 ± 0.03; 41.41 ± 0.03 °C), but there was no significant ($P > 0.05$) difference in the values obtained from the groups. In the group administered with probiotic, the CT value differed significantly (40.10 ± 0.11 °C; $P < 0.05$), when compared to the value (41.78 ± 0.03 °C), recorded in the control group. There was no significant ($P > 0.05$) difference in both overall minimum CT values on days 21 and 35 of the study period, in the probiotic and/or fisetin groups, when compared respectively with those of the control group (Table 4.6).

In the present study, on day 21 the probiotic administered group showed a diurnal fluctuation in CT from 40.37 ± 0.08 °C (07:00 h) to 41.06 ± 0.08 °C (07:00 h). However,

the groups administered with probiotic and the co-administered group exhibited decline in CT during the hot periods of the day (13:00 h – 17:00 h) (Table 4.4). At day 28 and 35 of the study, probiotic and the co-administered group showed a gradual decrease in CT during the hot hours of the day (13:00 h -17:00 h), compared to the control group (Table 4.5 and 4.6, respectively).

Table 4.4: Circadian Variation in Cloacal Temperature of Broiler Chickens during Day 21 of Study

Hours	Control (n= 15)	Fisetin (n= 15)	Probiotic (n= 15)	P+F (n=15)
7:00	41.48 ± 0.06 [a] (41.10 - 41.90)	41.43 ± 0.06 [a] (41.10 - 41.80)	40.37 ± 0.08 [b] (40.70 - 41.90)	40.54 ± 0.10 [a] (40.90 - 42.20)
9:00	41.53 ± 0.05 [a] (41.20 - 41.90)	41.46 ± 0.06 [a] (41.10 - 41.80)	40.37 ± 0.08 [b] (40.70 - 41.90)	40.38 ± 0.10 [b] (40.70 - 42.00)
11:00	40.62 ± 0.11 (40.00 - 41.50)	41.23 ± 0.10 (40.40 - 41.70)	41.43 ± 0.06 (41.10 - 41.80)	41.07 ± 0.13 (40.20 - 42.10)
13:00	41.55 ± 0.09 [a] (40.40 - 41.70)	40.26 ± 0.11[b] (39.50 - 41.20)	41.02 ± 0.09 [a] (40.60 - 42.10)	41.39 ± 0.08[a] (40.50 - 41.90)
15:00	41.57 ± 0.10 [a] (41.10 - 42.40)	41.49 ± 0.07 [a] (41.00 - 41.90)	40.99 ± 0.10 [c] (40.50 - 41.40)	41.07 ± 0.05[b] (40.60 - 41.30)
17:00	41.83 ± 0.11 [a] (40.00 - 41.70)	41.33 ± 0.11 [a] (40.00 - 41.60)	40.93 ± 0.08 [b] (40.10 - 41.30)	40.88 ± 0.14 [b] (39.80 - 41.80)
19:00	41.32 ± 0.11 [a] (40.70 - 42.10)	41.27 ± 0.08 [a] (40.70 - 41.80)	40.30 ± 0.06 [a] (40.80 - 41.80)	40.19 ± 0.06 [a] (40.80 - 41.60)
21:00	41.04 ± 0.07 (40.50 - 41.60)	41.09 ± 0.09 (40.20 - 41.50)	40.95 ± 24.67 (40.80 - 41.30)	41.32 ± 0.08 (40.60 - 41.80)
23:00	41.18 ± 0.09 (40.70 - 42.20)	41.15 ± 0.06 (40.50 - 41.40)	41.10 ± 0.06 (41.10 - 42.00)	40.34 ± 0.08 (40.80 - 41.70)
1:00	41.29 ± 0.10 (40.60 - 42.20)	41.08 ± 0.08 (40.50 - 41.70)	41.07 ± 0.10 (40.80 - 42.10)	41.48 ± 0.10 (40.90 - 42.60)
3:00	41.19 ± 0.11 (40.10 - 41.90)	41.15 ± 0.08 (40.50 - 41.70)	41.35 ± 0.10 (40.80 - 42.10)	41.48 ± 0.10 (40.90 - 42.60)
5:00	41.33 ± 0.06 (41.00 - 41.90)	41.27 ± 0.08 (40.90 - 42.00)	40.15 ± 0.06 (41.00 - 41.90)	41.30 ± 0.14 (40.80 - 42.50)
7:00	41.33 ± 0.10 (40.40 - 41.80)	41.28 ± 0.10 (40.60 - 42.00)	41.06 ± 0.08 (40.60 - 41.70)	41.45 ± 0.09 (40.70 - 42.00)
Overall mean ± SEM	41.39 ± 0.03 [a] (40.00 - 42.40)	41.20 ± 0.03 [a] (39.50 - 42.00)	40.32 ± 1.90 [b] (40.10 - 41.30)	40.49 ± 0.03 [a] (39.80 - 42.60)

Values in parenthesis are minimum–maximum. [a,b,c] = Means with different superscript letters within rows are significantly different ($P < 0.05$) P+F = Probiotic + Fisetin

Table 4.5: Circadian Variation in Cloacal Temperature of Broiler Chickens during Day 28 of Study

Hours	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	P+F (n=15)
7:00	41.35 ± 0.14 (40.00 - 42.20)	41.29 ± 0.16 (40.10 - 42.00)	41.08 ± 0.19 (39.00 - 41.90)	41.25 ± 0.21 (39.60 - 42.70)
9:00	41.33 ± 0.14 (40.00 - 42.20)	41.34 ± 0.14 (40.10 - 42.00)	41.10 ± 0.13 (40.00 - 41.70)	41.17 ± 0.18 (39.60 - 42.70)
11:00	41.68 ± 0.12 (40.10 - 42.00)	41.61 ± 0.13 (40.70 - 42.50)	41.28 ± 0.06 (41.10 - 41.90)	41.50 ± 0.09 (41.10 - 42.20)
13:00	41.83 ± 0.16 (40.00 - 41.80)	41.56 ± 0.15 (40.50 - 42.80)	41.38 ± 0.11 (40.50 - 42.40)	41.65 ± 0.10 (41.00 - 42.40)
15:00	41.82 ± 0.11[a] (40.70 - 41.90)	41.77 ± 0.12[a] (40.90 - 42.70)	41.32 ± 0.08[a] (41.30 - 42.30)	41.44 ± 0.16[a] (40.20 - 42.60)
17:00	41.83 ± 0.12[a] (40.00 - 41.60)	41.49 ± 0.14[a] (40.90 - 42.60)	41.27 ± 0.06[a] (41.00 - 41.90)	41.43 ± 0.10[a] (40.80 - 42.30)
19:00	41.79 ± 0.16 (40.50 - 42.00)	41.06 ± 0.08 (41.00 - 42.90)	41.13 ± 0.10 (40.10 - 41.80)	41.28 ± 0.08 (40.80 - 42.00)
21:00	41.29 ± 0.11 (40.50 - 42.00)	41.43 ± 0.14 (40.60 - 42.40)	41.07 ± 0.06 (40.80 - 41.60)	41.19 ± 0.08 (40.80 - 42.00)
23:00	41.69 ± 0.08 (40.80 - 42.00)	41.65 ± 0.14 (41.00 - 42.60)	41.39 ± 0.11 (39.00 - 41.90)	41.14 ± 0.12 (41.00 - 42.00)
1:00	41.59 ± 0.15 (40.30 - 42.60)	41.55 ± 0.16 (40.70 - 42.60)	41.35 ± 0.14 (40.60 - 42.50)	41.09 ± 0.11 (40.40 - 41.70)
3:00	41.48 ± 0.13 (40.00 - 42.00)	41.35 ± 0.21 (40.20 - 42.70)	41.09 ± 0.11 (40.90 - 42.70)	41.27 ± 0.09 (40.70 - 41.80)
5:00	41.75 ± 0.09 (40.90 - 42.00)	41.69 ± 0.16 (41.00 - 42.80)	40.45 ± 0.67 (30.90 - 41.70)	41.25 ± 0.08 (40.80 - 41.80)
7:00	41.50 ± 0.06 (41.10 - 41.90)	41.45 ± 0.06 (41.10 - 41.80)	41.37 ± 0.08 (40.70 - 41.90)	41.54 ± 0.10 (40.90 - 42.20)
Overall mean ± SEM	41.58 ± 0.03[a] (40.00 - 42.60)	41.36 ± 0.04[a] (40.10 - 42.90)	41.26 ± 0.06[a] (39.00 - 42.70)	40.40 ± 0.03[b] (39.60 - 42.70)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters within rows are significantly different ($P < 0.05$) P+F= Probiotic + Fisetin

Table 4.6: Circadian Variation in Cloacal Temperature of Broiler Chickens during Day 35 of Study

Hours	Control(n=15)	Fisetin (n=15)	Probiotic (n=15)	P+F (n=15)
7:00	41.67 ± 0.17 (40.10 - 42.70)	41.51 ± 0.08 (41.00 - 42.10)	41.48 ± 0.05 (41.20 - 42.00)	41.53 ± 0.07 (41.20 - 42.20)
9:00	41.53 ± 0.05 (41.20 - 41.90)	41.51 ± 0.05 (41.10 - 41.80)	40.37 ± 0.11 (40.20 - 41.90)	41.54 ± 0.07 (41.20 - 42.20)
11:00	41.60 ± 0.15 (40.20 - 42.00)	41.47 ± 0.09 (41.00 - 42.00)	41.36 ± 0.15 (40.00 - 42.40)	41.49 ± 0.20 (40.40 - 42.60)
13:00	41.63 ± 0.09[a] (40.70 - 42.00)	41.58 ± 0.11[a] (40.80 - 42.60)	40.43 ± 0.08[b] (41.30 - 42.50)	41.57 ± 0.10[a] (41.10 - 42.30)
15:00	41.83 ± 0.12 [a] (40.80 - 42.30)	41.72 ± 0.10 [a] (41.00 - 42.50)	41.33 ± 0.11[a] (40.80 - 42.50)	41.54 ± 0.18 [a] (41.20 - 42.40)
17:00	41.82 ± 0.15 [a] (40.30 - 42.40)	41.66 ± 0.08 [a] (41.20 - 42.30)	41.32 ± 0.12 [a] (40.50 - 42.10)	41.44 ± 0.10 [a] (40.80 - 42.30)
19:00	41.74 ± 0.11 [a] (40.80 - 42.10)	41.60 ± 0.12 [a] (40.30 - 42.30)	41.24 ± 0.09 [b] (40.70 - 42.10)	41.41 ± 0.10 [a] (40.09-42.00)
21:00	41.66 ± 0.15 (40.10 - 42.20)	41.60 ± 0.10 (40.80 - 42.00)	41.26 ± 0.08 (41.00 - 42.00)	41.35 ± 0.12 (40.80 - 42.70)
23:00	41.74 ± 0.99 (40.70 - 42.10)	41.52 ± 0.07 (41.30 - 42.10)	40.31 ± 0.06 (41.10-41.90)	41.39 ± 0.07 (41.00-42.00)
1:00	41.63 ± 0.09 (41.00 - 42.00)	41.65 ± 0.08 (41.00 - 42.00)	41.43 ± 0.08 (41.00 - 41.90)	41.53 ± 0.11 (40.90 - 42.20)
3:00	41.54 ± 0.12 (40.60 - 42.00)	41.55 ± 0.08 (41.00 - 42.30)	41.43 ± 0.08 (40.90 - 42.00)	41.45 ± 0.06 (41.10 - 41.80)
5:00	41.65 ± 0.10 (41.10 - 42.60)	41.62 ± 0.06 (41.00 - 41.90)	41.56 ± 0.09 (41.10 - 42.30)	41.41 ± 0.08 (41.00 - 42.00)
7:00	41.48 ± 0.06 (41.10 - 41.90)	41.46 ± 0.06 (41.10 - 41.80)	40.37 ± 0.08 (40.70 - 41.90)	41.54 ± 0.10 (40.90 - 42.20)
Overall mean ± SEM	41.78 ± 0.03 [a] (40.10 - 42.70)	41.68 ± 0.03 [a] (40.30 - 42.60)	40.10 ± 0.11[b] (40.00 - 42.50)	41.41 ± 0.03 [a] (40.40 - 42.70)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters within rows are significantly different ($P < 0.05$) P+F= Probiotic + Fisetin

4.4 Relationships (r) between Environmental and Cloacal Temperature Parameters in Broiler Chickens Administered with Probiotic and/or Fisetin during the Early Rainy Season.

There was a positive correlation r = 0.895*** and 0.925*** ($P < 0.05$) between the DBT and the cloacal temperature of the broiler chickens in the probiotic group and probiotic + fisetin group at day 21 and also in the fisetin, probiotic and probiotic + fisetin groups, respectively at day 28. The correlation was highly significant r = 0.700**, 0.775** and 0.690** respectively ($P < 0.05$) on day 21 and day 35 in the control, fisetin group and the control group, respectively. The correlation was non significant ($P > 0.05$) in the fisetin, probiotic and probiotic + fisetin groups at day 35 of the study.

There was a negative correlation which was non significant r = -0.375^{NS}, -0.403^{NS}, -0.427^{NS} and -0.420^{NS} respectively ($P > 0.05$) between the RH and the cloacal temperature of the broiler chicken in the control, fisetin, probiotic and probiotic + fisetin groups at day 21. Control, fisetin and probiotic groups at day 28 and the fisetin group at day 35. The negative correlation was significant r = -0.674*, -0.600* and -0.527* respectively ($P < 0.05$) at day 28 in the fisetin + probiotic group and in the probiotic and probiotic + fisetin groups at day 35. The negative correlation was highly significant ($P < 0.05$) at day 35 of the study in the control group.

There was a positive correlation r = 0.895*** and 0.878*** ($P < 0.05$) between the THI and the cloacal temperature of the broiler chickens in the probiotic and probiotic + fisetin groups at day 21. The fisetin, probiotic and probiotic + fisetin groups at day 28 had a positive correlation. The correlation was significant r = 0.597*, 0.595* and 0.679* ($P < 0.05$) at day 21 in the fisetin group and the control group at day 28 and day 35, respectively.

The correlation was non significant ($P > 0.05$) in the control group at day 21, the fisetin, probiotic and the probiotic + fisetin groups at day 35 of the study.

Table 4.7: Relationships between Thermal Environmental and Cloacal Temperature Parameters in Broiler Chickens Administered with Probiotic and/or Fisetin during the Early Rainy Season

Attributes	Correlated parameters	Control	Fisetin	Probiotic	Probiotic+Fisetin
Day 21	DBT	0.700**	0.775**	0.895***	0.925***
	RH	- 0.375^{NS}	- 0.403^{NS}	- 0.427^{NS}	- 0.420^{NS}
	THI	0.457^{NS}	0.597*	0.840***	0.878***
Day 28	DBT	0.575*	0.833***	0.809***	0.872***
	RH	- 0.525^{NS}	- 0.459^{NS}	- 0.527^{NS}	- 0.674*
	THI	0.595*	0.853***	0.823***	0.877***
Day 35	DBT	0.690**	0.529^{NS}	0.474^{NS}	0.534^{NS}
	RH	- 0.707**	- 0.546^{NS}	- 0.600*	- 0.587*
	THI	0.679*	0.526^{NS}	0.453^{NS}	0.527^{NS}

^{NS}= Non-significant ($P > 0.05$) correlation. *= Significant ($P < 0.05$) correlation.

= Highly significant ($P < 0.01$) correlation. *= Very highly significant ($P < 0.001$) correlation. DB= Dry bulb temperature; RH= Relative humidity; THI= Temperature-humidity index

4.5 Diurnal Fluctuations in Tonic Immobility Duration of Broiler Chickens during the Study Period

The duration of TI at 7:00 hours in the probiotic (123.10 ± 18.43 s) or fisetin supplemented (130.20 ± 17.40 s) groups of broiler chickens were shorter, when compared with that of the control group (150.20 ± 22.43 s) (Table 4.8). The TI duration in the co-administered (131.60 ± 14.66 s) group of broiler chickens was also shorter ($P < 0.05$) than that recorded in the control group (150.20 ± 22.43 s) (Table 4.8). At 13:00 hours, the durations of TI in the probiotic and the co-administered groups (119.20 ± 19.84 s and 124.40 ± 18.53 s, respectively) were lower than that of the control group (146.20 ± 21.16 s). At 13:00 hours, the shortest TI duration was recorded in the probiotic group with the value of 119.20 ± 19.84 s, which was closely followed by the duration obtained in the co-administered group (124.40 ± 18.53 s). Similarly at 18:00 hours, the shortest duration of TI was obtained in the probiotic supplemented group of broiler chickens, with the value of 98.16 ± 14.59 s, and this was also closely followed by that of the co-administered group (112.70 ± 15.84 s). At 07:00 and 18:00 hours, the longest durations of TI recorded were in the control broiler chickens, with values of 150.20 ± 22.43 s and 159.00 ± 19.92 s, respectively (Table 4.8).

Table 4.8: Diurnal Fluctuations in Tonic Immobility Duration of Broiler Chickens during the Study Period

Hours	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	P + F (n=15)
7:00	150.20 ± 22.43 (0.00 - 565.00)	130.20 ± 17.40 (0.00 - 480.00)	123.10 ± 18.43 (0.00 - 497.00)	131.60 ± 14.66 (0.00 - 410.00)
13:00	146.20 ± 21.16 (0.00 - 520.00)	144.00 ± 21.73 (0.00 - 582.00)	119.20 ± 19.84 (0.00 - 546.00)	124.40 ± 18.53 (0.00 - 510.00)
18:00	159.00 ± 19.92 [a] (0.00 - 540.00)	124.60 ± 16.30 [a] (0.00 - 425.00)	98.16 ± 14.59 [b] (0.00 - 359.00)	112.70 ± 15.84 [a] (0.00 - 510.00)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters within rows are significantly different ($P < 0.05$) P + F= Probiotic + Fisetin

4.6 Effect of Age on Fluctuations in Tonic Immobility Duration of Broiler Chickens during the Study Period

The duration of TI in control group rose as the age of the birds increased, and at day 35, TI was 155.90 ± 22.69 s. However, the shortest duration was recorded in the co-administered group with the value of 128.80 ± 14.12 s. At day 28 the shortest TI was recorded in the probiotic supplemented group with the value of 92.73 ± 14.80 s. While the longest TI was recorded in the control group with the value of 195.70 ± 22.71 s (Table 4.9). Day 21 has the longest duration of TI in the control group with the value of 147.20 ± 20.60 s, however the co-administered group has the shortest duration of TI with the value of 109.90 ± 13.59 s.

The overall mean duration of TI was shortest ($P < 0.05$) in the co-administered group with the value of 122.90 ± 9.43 s followed by the probiotic administered group with the value of 124.80 ± 10.41 s. The longest duration of TI was recorded in the control group with the value of 179.70 ± 11.69 s (Table 4.9).

Table 4.9: Effect of Age on Fluctuations in Tonic Immobility Duration of Broiler Chickens during the Study Period

Days	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
21	147.20 ± 20.60 (0.00 - 520.00)	132.90 ± 21.28 (0.00 - 582.00)	128.90 ± 16.48 (0.00 - 497.00)	109.90 ± 13.59 (0.00 - 437.00)
28	195.70 ± 22.71[a] (0.00 - 530.00)	130.90 ± 20.58[a] (0.00 - 490.00)	92.73 ± 14.80[b] (0.00 - 420.00)	116.50 ± 18.68[a] (0.00 - 510.00)
35	155.90 ± 22.69 (0.00 - 565.00)	129.80 ± 16.46 (0.00 - 480.00)	134.60 ± 17.60 (0.00 - 546.00)	128.80 ± 14.12 (0.00 - 326.00)
Overall mean ± SEM	179.70 ± 11.69[a] (0.00 - 565.00)	133.50 ± 11.28 [a] (0.00 - 582.00)	124.80 ± 10.41[a] (0.00 - 546.00)	99.90 ± 9.43[b] (0.00 - 510.00)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters within rows are significantly different ($P < 0.05$)

4.7 Diurnal Fluctuations in Vigilance of Broiler Chickens during the Study Period

At 7:00 hours the vigilance behavioural ranking of 1.40 ± 0.10 was the lowest recorded in the co-administered group of broiler chickens as compared to the value of 1.52 ± 0.11 and 1.53 ± 0.10 obtained from the probiotic and fisetin supplemented groups respectively, the value of 1.56 ± 0.11 was recorded in the control group which was shown to be the highest value at the said time (Table 4.10).

At 13:00 hours the highest vigilance behavioural ranking of 1.64 ± 0.12 was obtained from the control group while the lowest value of 1.51 ± 0.10 was recorded in the co-administered group (Table 4.10). At 18:00 hours the lowest vigilance behavioural ranking of 1.44 ± 0.10 was obtained from the co-administered group with the highest value of 1.62 ± 0.11 recorded in the control group (Table 4.10).

Table 4.10: Diurnal Fluctuations in Vigilance of Broiler Chickens during the Study Period

Hours	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
7:00	1.56 ± 0.11 (1.00 - 3.00)	1.53 ± 0.10 (1.00 - 3.00)	1.52 ± 0.11 (1.00 - 3.00)	1.40 ± 0.10 (1.00 - 3.00)
13:00	1.64 ± 0.12 (1.00 - 3.00)	1.58 ± 0.11 (1.00 - 3.00)	1.56 ± 0.11 (1.00 - 3.00)	1.51 ± 0.10 (1.00 - 3.00)
18:00	1.62 ± 0.11 (1.00 - 3.00)	1.62 ± 0.12 (1.00 - 3.00)	1.51 ± 0.12 (1.00 - 3.00)	1.44 ± 0.10 (1.00 - 3.00)

Values in parenthesis are minimum–maximum

4.8 Effect of Age on Fluctuations in Vigilance of Broiler Chickens during the Study Period

At 21 day of the experimental study, the vigilance behavioural ranking of (1.36 ± 0.09) which was the lowest ($P < 0.05$) was recorded in the co-administered group of broiler chickens as compared to the value of 1.56 ± 0.11 and 1.62 ± 0.12 obtained from the probiotic and fisetin supplemented groups, respectively, the value of 1.67 ± 0.11 was recorded in the control group which was shown to be the highest value (Table 4.11).

At day 28 of the experimental study, the highest ($P > 0.05$) vigilance behavioural ranking of 1.67 ± 0.13 was obtained from the control group while the lowest ($P > 0.05$) value of 1.45 ± 0.11 was recorded in the co-administered group (Table 4.11). At day 35 of the experimental study, the lowest ($P > 0.05$) vigilance behavioural ranking of 1.40 ± 0.10 was obtained from the probiotic supplemented group with the highest value of 1.56 ± 0.11 recorded in the control group (Table 4.11).

The overall mean value of vigilance behavioural ranking had the highest value of 1.64 ± 0.06 in the control group, while the lowest value of 1.45 ± 0.06 was obtained in the co-administered group (Table 4.11).

Table 4.11: Effect of Age on Fluctuations in Vigilance of Broiler Chickens during the Study Period

Days	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
21	1.67 ± 0.11 [a] (1.00-3.00)	1.62 ± 0.12 [a] (1.00-3.00)	1.56 ± 0.11 [a] (1.00-3.00)	1.36 ± 0.09[b] (1.00-3.00)
28	1.67 ± 0.13 (1.00-3.00)	1.56 ± 0.11 (1.00-3.00)	1.62 ± 0.12 (1.00-3.00)	1.45 ± 0.11 (1.00-3.00)
35	1.56 ± 0.11 (1.00-3.00)	1.49 ± 0.10 (1.00-3.00)	1.40 ± 0.10 (1.00-3.00)	1.51 ± 0.11 (1.00-3.00)
Overall mean ± SEM	1.64 ± 0.06 (1.00-3.00)	1.58 ± 0.06 (1.00-3.00)	1.57 ± 0.07 (1.00-3.00)	1.45 ± 0.06 (1.00-3.00)

Values in parenthesis are minimum–maximum. [a ,b]= Means along the same row with different superscript letters are significantly different

4.9 Effects of Probiotic and/or Fisetin on Feed Intake of Broiler Chickens during the Early Rainy Season

There were no significant ($P > 0.05$) differences in feed intake among the experimental groups, when compared with the values obtained in the control group from days 7 to 28 of the experimental period (Table 4.12). Feed intake in the probiotic and co-administered group was significantly ($P < 0.05$) higher (1741.00 ± 103.60 g/bird and 1360.00 ± 38.09 g/bird) on day 35, and in the probiotic administered group (2705.00 ± 49.53 g/bird) on day 42, when compared with those of the control and any of the treatment groups (Table 4.12). The overall mean feed intake was higher ($P < 0.05$) in the probiotic group (1268.00 ± 121.30 g/bird) than in the control group (905.60 ± 57.81 g/bird) (Table 4.12).

Table 4.12: Effects of Probiotic and/or Fisetin on Feed Intake of Broiler Chickens during the Early Rainy Season

Days	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
7	278.60 ± 50.63 (80.00 - 500.00)	264.70 ± 43.81 (115.00 - 459.00)	284.10 ± 49.29 (85.00 - 500.00)	273.00 ± 40.80 (108 - 438)
14	755.40 ± 64.14 (550.00 - 955.00)	731.30 ± 76.04 (500.00 - 960.00)	768.30 ± 70.65 (550.00 - 1000.00)	731.00 ± 75.90 (500.00 - 961.00)
21	1067.00 ± 56.04 (790.00 - 1200.00)	1059.00 ± 64.68 (722.00 - 1200)	1078.00 ± 55.58 (810.00 - 1200.00)	1062.00 ± 56.50 (780.00 - 1200.00)
28	1156.00 ± 37.02 (1000.00 - 1300.00)	1130.00 ± 71.09 (844.00 - 1400)	1239.00 ± 101.00 (969.00 - 1800.00)	1122.00 ± 34.41 (960.00 - 1200.00)
35	935.00 ± 62.31[a] (730.00 - 1132.00)	1068.00 ± 56.84[a] (910.00 - 1300.00)	1741.00 ± 103.60[b] (1400.00 - 2246.00)	1360.00 ± 38.09[b] (1200.00 - 1500.00)
42	1298.00 ± 114.10[a] (800.00 - 1600.00)	1413.00 ± 75.23[a] (1070.00 - 1600.00)	2705.00 ± 49.53[b] (2550.00 - 2850.00)	1467.00 ± 61.67[a] (1220.00 - 1650.00)
Overall mean ± SEM	905.60 ± 57.81[a] (80.00 - 1600.00)	932.70 ± 62.06[a] (115.00 - 1600.00)	1268.00 ± 121.30[b] (85.00 - 2850.00)	991.00 ± 66.33[a] (108.00 - 1650.00)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters within columns are significantly different ($P < 0.05$)

4.10 Effects of Probiotic and/or Fisetin on Water Intake of Broiler Chickens during the Early Rainy Season

There were no significant ($P > 0.05$) differences in water intake among the experimental groups when compared with the control group from days 7 to 35 of the experimental period (Table 4.13). Water intake in the probiotic group was significantly ($P < 0.05$) higher (4003.00 ± 110.60 ml) when compared with the control and any other group at day 42 of the experimental period. There was no significant ($P > 0.05$) difference in the overall mean water intake among the experimental groups when compared with the control group (Table 4.13).

Table 4.13: Effects of Probiotic and/or Fisetin on Water Intake of Broiler Chickens during the Early Rainy Season

Days	Control (n=15)	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
7	702.90 ± 139.40 (120.00 - 1200.00)	670.00 ± 132.90 (60.00 - 1030.00)	707.10 ± 136.50 (150.00 - 1200.00)	650.70 ± 126.60 (50.00 - 955.00)
14	1471.00 ± 56.55 (1240.00 - 1700.00)	1418.00 ± 65.57 (1200.00 - 1700.00)	1479.00 ± 53.65 (1260.00 - 1700.00)	1400.00 ± 72.28 (1150.00 - 1700.00)
21	1819.00 ± 157.40 (1280.00 - 2500.00)	1803.00 ± 165.90 (1220.00 - 2500.00)	1874.00 ± 120.60 (1390.00 - 2425.00)	1745.00 ± 174.40 (1000.00 - 2345.00)
28	2414.00 ± 110.20 (1950.00 - 2880.00)	2297.00 ± 259.30 (900.00 - 2960.00)	2303.00 ± 133.40 (1800.00 - 2880.00)	2264.00 ± 114.10 (1900.00 - 2800.00)
35	2364.00 ± 191.10 (1500.00 - 3000.00)	2225.00 ± 203.40 (1800.00 - 3000.00)	2890.00 ± 152.90 (2300.00 - 3290.00)	2502.00 ± 188.10 (1785.00 - 3160.00)
42	3272.00 ± 116.10^{a} (2900.00 - 3600.00)	3473.00 ± 121.60^{a} (3200.00 - 4000.00)	4003.00 ± 110.60^{b} (3320.00 - 4500.00)	3552.00 ± 117.10^{a} (3250.00 - 4000.00)
Overall mean ± SEM	1976.00 ± 135.70 (120.00 - 3600.00)	1945.00 ± 148.10 (60.00 - 4000.00)	2165.00 ± 169.00 (150.00 - 4350.00)	1982.00 ± 150.20 (50.00 - 4000.00)

Values in parenthesis are minimum–maximum. ^{a,b} = Means with different superscript letters within columns are significantly different ($P < 0.05$)

4.11 Effects of Probiotic and/or Fisetin on the Live Weight Gain of Broiler Chickens during the Early Rainy Season

The live weight gain of broiler chickens in the treated groups from days 7 to 28 of the study period showed no significant ($P > 0.05$) differences when compared with the control group (Table 4.14). At days 35 and 42 of the study period, body weight differed significantly ($P < 0.05$) in the probiotic supplemented group (1001.00 ± 51.39 g and 1242.00 ± 63.39 g, respectively) when compared with the values obtained in the control group (810.70 ± 42.02 g and 983.90 ± 59.18 g, respectively). There was no significant ($P > 0.05$) difference in the overall mean body weight among the experimental group when compared with the control group (Table 4.14).

Table 4.14: Effects of Probiotic and/or Fisetin on the Live Weight Gain of Broiler Chickens during the Early Rainy Season

Days	Control	Fisetin (n=15)	Probiotic (n=15)	Probiotic+Fisetin (n=15)
7	100.40 ± 3.13[a] (81.00 - 114.00)	99.40 ± 1.97[a] (81.00 - 112.00)	99.53 ± 2.30[a] (84.00 - 117.00)	99.93 ± 2.96[a] (70.00 - 117.00)
14	262.70 ± 7.35[a] (224.00 - 307.00)	258.80 ± 4.72 [a] (218.00 - 291.00)	260.70 ± 10.10[a] (198.00 - 336.00)	259.90 ± 9.90[a] (170.00 - 313.00)
21	535.20 ± 12.20[a] (466.00 - 606.00)	540.30 ± 10.80[a] (477.00 - 623.00)	521.20 ± 25.40[a] (321.00 - 669.00)	523.90 ± 21.70[a] (377.00 - 663.00)
28	832.20 ± 37.74[a] (558.00 - 940.00)	746.40 ± 22.95[a] (575.00 - 928.00)	736.50 ± 40.17[a] (441.00 - 999.00)	712.30 ± 38.13[a] (425.00 - 912.00)
35	856.50 ± 54.80[a] (476.00 - 1132.00	840.20 ± 30.43[a] (683.00 - 1083.00)	930.40 ± 31.48[b] (780.00 - 1202.00)	872.60 ± 49.79[a] (548.00 - 1109.00)
42	983.90 ± 59.18[a] (476.00 - 1362.00)	989.70 ± 29.34[a] (780.00 - 1129.00)	1106.00 ± 41.49[b] (888.00 - 1438.00)	1020.00 ± 58.19[a] (611.00 - 1358.00)
Overall mean ± SEM	579.40 ± 35.62[a] (81.00 - 1362.00)	579.10 ± 34.44[a] (81.00 - 1129.00)	609.00 ± 39.33[b] (84.00 - 1438.00)	581.40 ± 37.31[a] (70.00 - 1358.00)

Values in parenthesis are minimum–maximum. [a,b] = Means with different superscript letters across rows are significantly different ($P < 0.05$)

4.12 Values of Water and Feed Intake and Feed Conversion Ratio of Broiler Chickens Supplemented with Probiotic and/or Fisetin

There was no significant ($P < 0.05$) difference in the average water intake value and average live weight gain among the experimental groups when compared with the control group (Table 4.15). The average feed intake value was significantly ($P < 0.05$) higher in the probiotic supplemented group (1268.00 ± 121.30 g/bird) when compared with the values obtained in the control group (Table 4.15). The feed conversion ratio was significantly ($P < 0.05$) lower in the probiotic supplemented group 0.42 ± 0.13 than in the control group (1.20 ± 0.08) (Table 4.15).

Table 4.15: Values of Water and Feed Intake and Feed Conversion Ratio of Broiler Chickens Supplemented with Probiotic and/or Fisetin

Parameters	Control	Fisetin	Probiotic	P + F
Average water intake (ml)	1976.00 ± 135.70 (120.00 – 3600.00)	1945.00 ± 148.10 (60.00 – 4000.00)	2251.00 ± 169.9.00 (150.00 – 4350.00)	1982.00 ± 150.20 (50.00 – 4000.00)
Average feed intake (g/bird)	905.60 ± 57.81[a] (80.00 – 1600.00)	952.70 ± 62.06[a] (115.00 – 1600.00)	1288.00 ± 121.30[b] (85.00 – 2850.00)	991.00 ± 66.33[a] (108.00 – 1650.00)
Average body weight (g/bird)	579.40 ± 35.62 [a] (81.00 – 1362.00)	584.80 ± 34.84 [a] (81.00 – 1208.00)	888.40 ± 44.62 [b] (84.00 – 1849.00)	598.20 ± 38.51 [a] (70.00 – 1453.00)
Feed conversion ratio	1.20 ± 0.08[a] (0.73 – 1.38)	0.91 ± 0.13[b] (0.56 – 1.31)	0.42 ± 0.13[c] (0.34 – 1.02)	0.72 ± 0.14[b] (0.53 – 1.32)

[a,b,c] = Means with different superscript letters across rows are significantly different ($P < 0.05$). P + F = Probiotic + Fisetin, n = 15

CHAPTER FIVE

DISCUSSION

The result of the present study showed that the DBT values (26.00 – 36.00 °C) obtained was outside the thermoneutral zone for broiler chickens above 3 weeks old, which is 18 – 24 °C (Aluwong *et al*., 2017). The RH obtained (49.00 – 93.00%) in the present study was also predominantly outside the optimal range 65 – 70% for broiler chickens between the ages of 3 – 6 week old (Egbuniwe *et al*., 2015). This finding agrees with that of de Oliveria *et al*. (2013), who reported that the optimum AT and RH values for broiler chickens are 21 °C and 74%, respectively. Thus, the early rainy season (June – July) in the Northern Guinea Savannah zone of Nigeria, during which the study was carried out, had high ambient temperature and high RH, which was thermally stressful to broiler chickens.

The THI revealed the impact of dry- and wet-bulb temperatures on animals (Tao and Xin, 2003). In the present study, the overall mean value of THI recorded was 30.10 ± 0.34, which was stressful to the broiler chickens (Egbuniwe *et al*., 2015). Sinkalu *et al.* (2015) showed that THI values greater than 20.8 induce heat stress in broiler chickens. Thermal stress, prevailing during the early rainy season may adversely affect energy balance and the fitness of birds (Ardia, 2013). This may further result in poor performance, immune suppression and high mortality; hence, the administration of antioxidants, such as probiotic and fisetin could be useful during the early rainy season. The administration may ameliorate the risk of the adverse effect of heat stress on the health and performance of

broiler chickens, reared during the early rainy season. Since heat stress induces oxidative stress and, consequently, excessive production of ROS (Lara and Rostagno, 2013; Egbuniwe *et al*., 2018), this finding becomes the rationale for modulating the adverse effects of heat stress by the administration of probiotic and/or fisetin.

In the present study, circadian fluctuation in mean CT values was recorded in both the treated and control groups. This result is in agreement with the finding of Egbuniwe *et al*. (2015) that the CT fluctuates with the hour of day. The observation that CT values significantly decreased at 13:00 – 17:00 h in the probiotic group on days 21 and 35 showed that probiotic administration exerted a significant hypothermic effect on the broiler chickens, especially during the hot period of the day (13:00 – 17:00 h). This result is in agreement with the findings of Hasan *et al*. (2015) and Aluwong *et al.* (2017), who reported that CT decreases with increase in hour of the day in broiler chickens, administered with probiotic. The finding indicated that probiotic administration to broiler chickens, subjected to heat stress, may have shown some improvement in the central thermoregulatory system (Tanizawa *et al.*, 2014). The result of this study showed that probiotic exerted a significant ($P < 0.05$) decrease in CT at 13:00 h. At the later hour of 17:00 h, the co-administered group showed a significant ($P < 0.05$) decrease in CT. This finding indicated that probiotic, and its co-administration with fisetin, may decrease the metabolic rate, as evidenced by the lower CT values in treated groups, when compared with the control group at 13:00 h and 17:00 h.

Sugiharto *et al*. (2017) demonstrated that increased metabolic processes that accompanies increase in body weight generates more heat in the body. This results in quick attainment of

market weight in antioxidant treated broiler chickens, despite the adverse effects of high AT and RH. The result of the present study also showed, for the first time, that probiotic and its co-administration with fisetin may exert some hyperthermic effect during the early phase of growth; but at maturity, the broiler chickens may adapt to heat exposure. The result demonstrated that probiotic administration, both singly and in combination with fisetin, increased the CT values in broiler chickens during the cool hours of the day, 21.00 h – 7.00 h. This finding may be because probiotic decreased the thermoregulatory set-point in the hypothalamus. This may facilitate the ability of the birds to adjust to the prevailing high AT during the early rainy season that elicited some level of heat stress, especially starting from the early age of the birds. Consequently, it increased the resilience of the broiler chickens to the adverse effects of heat stress at maturity. This finding agrees with that of Vesco *et al*. (2017), who demonstrated that antioxidant administration to chicks resulted in hyperthermia at an early age of 0 – 4-day-old, but adaptation to heat stress may occur after this age. The study of Vesco *et al*. (2017) was carried out in Brazil during the thermally stressful season, while the present study was performed during the early rainy season in the Northern Guinea Savannah zone of Nigeria. The decline in CT during the hot hours of the day (13:00 h – 17:00 h) may further serve as evidence that probiotic promotes resilience of broiler chickens to heat stress at maturity.

Probiotic has been shown to enhance growth in broiler chickens (Aluwong *et al.,* 2013; Zhang *et al.,* 2014). Further investigations are required to elucidate its effects on heat stress of broiler chickens. The fluctuations in the CT were obtained at various hours for all the experimental groups. Body temperature fluctuations reflect the stressful nature of AT, RH, and the thermoregulatory mechanism required to maintain homeothermy (Makeri *et al*.,

2017). Therefore, broiler chickens are apparently, stressed, whenever a high range of CT occurs. The wider the fluctuation, the more marked is the heat stress, and the higher the risk of adverse effects upon optimal growth and health (Vesco *et al*., 2017). At 7:00 h, probiotic, fisetin and probiotic + fisetin modulated the CT restoring it to similar level as that of the control group, thereby ensuring homeothermy in the broiler chickens. It is very challenging for the chickens to maintain core body temperature during high AT, as they lack sweat glands, relying on evaporative cooling (panting) to keep them cool (Aluwong *et al*., 2017). Several workers have reported increased CT in adult chickens during heat stress (Robinson *et al*., 2016). The present study demonstrated that CT was significantly increased by the thermal environmental parameters, indicating that thermoregulatory mechanisms to increase sensible heat loss in the birds were not able to cope with the thermal environmental parameters.

During the study period, the broiler chickens were reared under natural, thermally stressful environmental conditions of the early rainy season in the zone. The DBT (26.00 -36.00 °C), RH (49% - 93%), and THI (25.60 - 35.30) values recorded during the study period fell predominantly outside the thermoneutral zone for broiler chickens. The normal ranges for DBT (18 - 21 °C), RH (65 - 70%) and THI values of 20.8 (Egbuniwe *et al*., 2015; Aluwong *et al*., 2017) are optimal in raising broiler chickens.

The results of the present study showed that the broiler chickens reared under heat stressed conditions and administered with probiotic and/or fisetin recorded the shortest duration of TI, when compared with the control group, whereas the longest TI duration was observed in the control broiler chickens. The result is in agreement with the finding of Sinkalu *et al*.

(2016), who showed that broiler chickens subjected to heat stress exhibited reduced welfare, evidenced by longer TI duration. It further implies that heat stress heightened fear responses in broiler chickens, demonstrated by increase in TI duration. The decreased fear responses in broiler chickens supplemented with probiotic and/or fisetin may be attributed to the fact that the antioxidants ameliorated heat stress by scavenging for ROS. The result of the present study agreed with the findings of Ghareeb *et al.* (2014) that TI demonstrated the beneficial effect of the antioxidants in the alleviation of behavioural response induced by heat stress. The stress is an index of fearfulness and its duration indicates the level of stress in birds (Ghareeb *et al*., 2014). The finding of significantly ($P < 0.05$) longer duration of TI at day 28 than that of day 21 in the control broiler chickens suggested that the fear response increased with age in the group without antioxidant administration, and that the rearing of broiler chickens during the thermally stressful conditions in the zone may adversely affect their welfare and health.

The negative impact of the season on the birds was very pronounced at day 35 of age, when they were expected to attain market weight and be slaughtered for meat or transported to the market for sale. The metabolic rate and, consequently, heat generation has been shown to increase as the broiler chickens grow older, which further aggravates the thermal load on the birds (Surgiharto *et al*., 2017). The administration of antioxidants of probiotic and fisetin ameliorated the adverse effects of excess heat load, imposed on the broiler chickens by decreasing their fear responses. The findings demonstrated, for the first time, that probiotic administration to stressed broiler chickens, especially in combination with fisetin reduced the duration of TI, and suggested that both agents exerted antioxidant effects by scavenging ROS, generated in excess due to the heat stress.

The results of the study showed that probiotic and the co-administered (probiotic + fisetin) groups of broiler chickens exhibited high vigilance behaviour as evidenced by a short ranking of vigilance, when compared to the values obtained from the control and fisetin-administered broiler chickens. The findings demonstrated, for the first time, that probiotic markedly reduced fear, which is a damaging stressor in broiler chickens. Furthermore, the results agreed with the finding of Sinkalu *et al*. (2016) that TI is an inherently stressful and fearful experience, which magnifies the degree of perturbation. The administration of probiotic and/or fisetin to broiler chickens elicited boldness and confidence by inducing alertness and suppressing fear, which agreed with the findings of Wang *et al*. (2014) that antioxidant administration reduces fear in broiler chickens.

Melleu *et al*. (2016) carried out similar studies in pigeons and reported that high ambient temperature and age may affect the defensive mechanism of pigeons, when exposed to heat stress. The findings may be responsible for the high values recorded during the hot hours of the day (13:00 h) and as the birds aged during the experimental days 28 and 35, respectively. Thus, probiotic and/or fisetin administration induced calming effect, and increased the alertness of the broiler chickens, which may enhance performance and productivity. This finding agreed with the report of Sinkalu *et al*. (2016) that melatonin, a potent antioxidant, exerts a calming effect and increases the performances of broiler chickens. The decrease in vigilance of broiler chickens was an evidence of stressful conditions, shown to drastically decrease feed intake, feed conversion ratio (FCR) and, consequently, the overall weight gain of the broiler chickens (Baxter *et al*., 2018; Mohammed *et al*., 2018). Therefore, the administration of probiotic and/or fisetin may alleviate behavioural stress responses in broiler chickens.

The improvement in feed intake by dietary probiotic supplementation often resulted in improved growth performance (Mohammed *et al*., 2018). In the present study, though there was no significant difference in the first 4 weeks of life in feed intake between the treatment and control groups. However, the probiotic supplemented group had the highest feed intake and body weight on the 5th and 6th weeks of life. The improvement in live weight is attributed to the improved digestion and absorption of nutrients in the digestive tract due to the presences of yeast cells of *Saccharomyces cerevisiae.* The results of the present study are in agreement with that of Wang *et al*. (2016), who reported that dietary additions of probiotics and prebiotics, increases feed intake in broiler chickens. The results of this study disagreed with that of Bai *et al*. (2016), who reported a decrease in feed intake in the group administered probiotics during his experimental period. The results of this study agreed with that of Aluwong *et al*. (2013), who reported an increase feed intake in the probiotic administered group during his study, he also reported that feed intake of broiler chickens increases with a high dose of probiotic administration. Increase feed intake helps to increase the live weight gain of broiler chickens (Mahmoud *et al*., 2017).

The results showed that water intake was increased by dietary probiotic supplementation in the broiler chickens, which often resulted in improved growth performance. In the present study, though there was no significant difference ($P > 0.05$) in the first 5 weeks of life in water intake between the treatment groups. However, the probiotic supplemented group had the highest water intake, feed intake and body weight on the 5th and 6th weeks of life. The improvement in live weight is attributable to the improved digestion and absorption of nutrients in the digestive tract due to the presences of yeast cells of *Saccharomyces cerevisiae.*

The results of the present study are in agreement with that of Wang *et al*. (2016), who reported that dietary additions of probiotics and prebiotics, increases feed intake which may as well increase water intake in broiler chickens. The results of this study agreed with that of Aluwong *et al*. (2013), who reported an increase feed intake which may concurrently increase water intake in the probiotic administered group during his study, he also reported that feed intake of broiler chickens increases with a high dose of probiotic administration. Increase feed intake may have a positive effect on water intake to aid digestion which therefore causes an increase in the live weight gain of broiler chickens.

Probiotic acts by reducing feed conversion ratio resulting in an increase daily live weight gain (Wang *et al*., 2016). This is achieved through the physiological improvement of digestion by balancing the resident gut microflora. Essentially, they help the animal to fulfil its genetic potential. The significant ($P < 0.05$) decrease in feed conversion ratio obtained in the present study agrees with the findings of Aluwong *et al*. (2013) and Bai *et al*. (2016), who reported that probiotic supplementation improved the performance of broiler chickens. Probiotic as growth promoters can be used as an alternative non antibiotic feed additives because they do not have side effects on consumers, but they improve growth indices of broiler chickens. Therefore the co-administration of probiotic and fisetin in broiler chicken did not reduce the FCR as compared to the values obtained in the administration of probiotic singly to the broiler chickens in this study.

The results of this study showed that probiotic supplementation had no significant effect on the live weight gain of broiler chickens from the 1st to the 4th week of life. This may be due to the time it took for the probiotic to reestablish the conditions of eubiosis in the digestive system of the broiler chickens (Zheng *et al*., 2016). However, from the 5th to the 6th weeks of life, body weight differed significantly between the control and probiotic group, but not within the control and fisetin group. The overall mean result showed that the highest body weight was recorded in the probiotic administered group. This was made possible by the reduction in the FCR, enhanced intestinal absorptive area through increased villus length and added gut mucosa antigenic response. This finding is in agreement with previous studies conducted by Bai *et al.* (2016), in which increases in body weights were reported in broilers fed diets supplemented with probiotics. Also this result demonstrated that probiotic supplementation has a more positive effect on live weight gain when administered to broiler chickens from the first day of life. Aluwong *et al*. (2013), reported that cell walls of *Saccharomyces cerevisiae* improves the nutrient absorption from the intestinal mucosa and suggested that this factor may be responsible for the improvement in performance of broiler chickens, supplemented with *Saccharomyces cerevisiae.*

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

Based on the findings of this study, it was concluded that:

i. The thermal environmental conditions in the early rainy season induced heat stress, evident by high DBT (26.00 - 36.00 °C), which was outside the thermoneutral zone (18 - 24 °C) for broiler chickens in the tropics.

ii. The CT values in broiler chickens administered with probiotic (40.10 ± 0.11 °C), fisetin (41.68 ± 0.03 °C) and fisetin + probiotic (41.41 ± 0.03 °C) decreased ($P < 0.05$), compared to the value obtained in controls (41.78 ± 0.03 °C). Broiler chickens treated with probiotic alone had the lowest CT (40.10 ± 0.11 °C, $P < 0.01$), compared to all other groups.

iii. The duration of TI and vigilance in probiotic-treated broiler chickens (99.90 ± 9.43 s and 1.45 ± 0.06, respectively) were significantly shorter ($P < 0.05$) than in the controls (179.70 ± 11.69 s and 1.64 ± 0.06 s, respectively).

iv. Feed intake (1268.00 ± 121.30 g), water intake (2165.00 ± 169.00 ml) and body weight (609.00 ± 39.33 g) were significantly higher ($P < 0.05$) in probiotic-administered broiler chickens than in the controls (905.60 ± 57.81 g, 1976.00 ± 135.70 g, and 579.40 ± 35.62 g, respectively).

v. Feed conversion ratio was significantly lowest ($P < 0.01$) in the broiler chickens (0.42 ± 0.13) treated with probiotic, compared to the ratios in fisetin (0.91 ± 0.13), probiotic + fisetin (0.72 ± 0.14) group, and especially the controls (1.20 ± 0.08, $P < 0.001$).

6.2 Recommendations

It is recommended that:

i. Probiotic and/or fisetin should be administered to broiler chickens exposed to heat stress in order to improve their health and, consequently, productivity.

ii. In addition to CT, other indices such as behavioural responses and performance may be useful diagnostic tools for evaluating the adverse effects of stress on broiler chickens during the early rainy season.

iii. Further studies are required to elucidate the molecular mechanism by which fisetin administration, both singly and in combination with probiotic, modulates CT responses in heat-stressed broiler chickens.

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