Dietary inclusion of virginiamycin to ameliorate the effect of heat stress on broilers

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DECLARATION

I, Luhandri van der Walt declare that this dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Livestock Production at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Luhandri van der Walt

SIGNATURE: …….

DATE: ……..

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ABSTRACT

Heat stress is a cause of great economic loss in poultry production throughout the world. Many methods have been researched to find a solution to alleviate the negative effects of heat stress. Methods include but are not limited to: adequate ventilation and facility orientation, the acclimation of birds to elevated temperatures, electrolyte and acid-base balance to maintain body fluid homeostasis, water management to aid in evaporative cooling, feed management by means of fasting and nutrition to ensure a balance diet during reduced intake and non-nutritive feed additives to improve performance. Previous research has shown that the inclusion of virginiamycin (VM) in poultry diets during periods of heat stress may improve growth rate, feed intake, feed conversion ratio, survivability and carcass weight. This was tested by conducting an experiment on 1408 male broilers. A randomised block design was used with 32 pens in each side of the house. Chicks were randomly assigned to four treatments with 16 replications, each containing 22 broilers. Data was statistically analysed as a randomised block design with the GLM model and mortality data by Chi-square. The broilers were subjected to a temperature profile which simulated a cyclic heat wave with high temperatures during the day and lower temperatures at night. From day 16 to 32, house temperature was gradually increased from 8:00-11:00 until the set maximum temperature of the profile was achieved and decreased at 18:00 to 24 °C. The control group received a diet without antibiotic growth promoters. The second group which was a positive control group received a diet with zinc bacitracin 15% (334 g/ton). The third group received a diet with VM (20 g/ton) in all feeding phases whilst the fourth group received VM (20 g/ton) in all feeding phases with exception of the post-finisher phase. Broiler performance, intestinal morphology and carcass and blood composition of birds were measured. The addition of VM to the feed of broilers subjected to heat stress had no significant ($P<0.05$) effect on body weight or carcass and blood composition. Mortality within the third and fourth group was significantly lower, both before and after the onset of high cyclic temperatures. Supplementation of VM could be beneficial to lower the mortality rate of birds subjected to heat stress.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
cFCR	Cumulative feed conversion ratio
AVT	Arginine vasotocin
BMD	Bacitracin methylene disalicylate
Ca	Calcium
CF	Crude fiber
Cl	Chloride
CP	Crude protein
CRF	Corticotropin-releasing factor
CS	Corticosterone
DM	Dry matter
E	Epinephrine
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
FCR	Feed conversion ratio
HSP	Heat shock protein
HSP70	Heat shock protein 70
KCl	Potassium chloride
LH	Luteinizing hormone
mEq	Milliequivalants
MT	Mesotocin
Na	Sodium
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NE	Norepinephrine
NC	Negative control
NH_4Cl	Ammonium chloride
NRC	National research council
PEF	Performance efficiency factor
RH	Relative humidity
SEM	Standard error of mean
T_3	Triiodothyronine

T_4	Thyroxine
VM	Virginiamycin
VM1	Virginiamycin, treatment 3
VM2	Virginiamycin, treatment 4
WSC	Water soluble carbohydrates
ZLTE	Zone of thermoregulation effort
ZMM	Zone of minimal metabolism
ZnB	Zinc bacitracin

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Chapter 1
Introduction

Poultry science research aims to improve both the breed and the quality of feed for high productivity (Chaiyabutr, 2004). Since the commercial broiler of today is a fast growing and efficient bird with extraordinary production potential, these birds have become more susceptible to stress. The rapid growth rate of modern broiler genotypes increase metabolic heat production whereas the capacity to dissipate heat does not (Teeter, 1994). A combination of high ambient temperature and relative humidity (RH) often challenge the thermal balance of poultry. During the summer months, these environmental factors may adversely affect poultry production, leading to great economic loss.

High environmental temperatures stimulate physiological, behavioural, neuroendocrine and molecular responses in poultry to maintain body temperature within the normal range. When the ambient temperature exceeds the birds' thermal neutral zone, birds will adjust metabolic heat to maintain their physiological status (Song & King, 2015). Yousef (1985) concluded that temperatures near 18-21 °C will provide the optimal temperature for maximum growth rate of broilers from three weeks of age. At temperatures of 30 °C and above (Yousef, 1985), undesirable effects on feed intake, feed efficiency, growth rate, carcass quality and survivability will be evident (Teeter & Belay, 1996; Wiernusz, 1998). Studies conducted by Mack *et al.* (2013), Mashaly *et al.* (2004) and Quinteiro-Filho *et al.* (2010) further found that not only does heat stress adversely affect production efficiency but also innate immunity, health and endocrine functions.

The majority of heat stress research that has been conducted either applied a constant heat stress exposure or simulate commercial conditions with cyclic temperatures. Constant heat exposure does not allow for a recovery period and could be more detrimental (Mashaly *et al.*, 2004). Acute heat stress is typically experienced for a short duration, nonetheless, negative effects might occur but is of minimal concern (Virden & Kidd, 2009). Panting results in a reduction of carbon dioxide in the blood of broilers when exposed to heat stress of short duration. The unaffected blood pH level might indicate that the carbon dioxide could be maintained for a short period (Lin *et al.*, 2006). However, chronic heat stress for an extended duration results in impaired immune function and production performance (Virden & Kidd, 2009).

Many management strategies have been researched to assist in alleviating the detrimental effects of heat stress. Nutrition and management may have a pronounced impact on optimal production during the period birds are exposed to heat distress (Wiernusz, 1998). Environmental changes such as facility orientation, roof insulation and ventilation could be used to counteract the effects of heat stress.

Furthermore, nutritional changes and the addition of additives in water or feed could also be useful either alone or in combination with the environmental changes. Virginiamycin (VM), an antibiotic growth promoter, has been found to improve the growth and feed efficiency of broilers (Buresh *et al.*, 1985). It has also been found to improve protein utilisation (Miles *et al.*, 1984), mortality rate (Teeter, 1994), intestinal digestion and absorption of carbohydrates and fat in broilers (Eyssen & de Somer, 1963).

The aim of this trial was to determine whether virginiamycin will alleviate the detrimental effects of heat stress on broiler performance. The null hypothesis was that VM will not improve broiler production, gastro intestinal health or carcass composition. The alternative hypothesis was that VM will improve broiler performance, gastro intestinal health or carcass composition.

Chapter 2
Literature review

2.1 Introduction

High ambient temperatures are among the important environmental stressors in poultry production (Lin *et al.*, 2004). There is a upward trend in the global environmental temperatures, which demands effective means to economically improve the thermo tolerance of broilers in hot climates without affecting productivity (Ahmad & Sarwar, 2006). Heat stress has an undesirable effect on poultry production and when coupled with high humidity even more harmful effects may occur (Butcher & Miles, 2012). The detrimental effects of heat stress on poultry range from physiological, immunological and microbial challenges which results in abnormal or impaired performance (Sugiharto *et al.*, 2016). High temperatures lead to behavioural, physiological, neuroendocrine and molecular responses in broilers, in order to prevent death from heat exhaustion (Butcher & Miles, 2012). The development of effective interventions to reduce the negative effects heat stress has on the welfare and productivity of poultry has been widely investigated (Lara & Rostagno, 2013). Lara & Rostagno (2013) found that birds subjected to heat stress have a reduced feed intake, an increased water intake, and showed increased panting and a higher frequency of resting behaviour. Furthermore, exposure to heat stress also presented impaired growth rate, meat yield and enhanced fat deposition, therefore, not allowing broilers to reach their full genetic potential (Lu *et al.*, 2007). Many responses will be initiated by a variety of stressors that allows the bird to regulate homeostasis in the body. Understanding these responses will assist in making decisions regarding the rearing conditions of birds.

2.2 Thermal balance and thermoregulation in broilers

Poultry, being homeothermic like other warm-blooded animals, have the ability to maintain a constant internal environment within an external comfort zone (thermal neutral zone) of around 18-36°C (Chaiyabutr, 2004). The thermal neutral zone is a temperature range that allows the animal not to actively regulate body temperature. As the bird age and matures, thermal neutral zone decreases, at hatching the comfort zone range from 32-35 °C to approximately 18-24 °C at four weeks of age (Teeter & Belay, 1996), since the body weight of the bird increases more rapidly than the surface area (Wiernusz, 1998). Figure 2.1 illustrates that broilers need to be provided with an optimal environment in order to maintain their thermal balance for continued optimal performance. Stress is minimised when the rearing ambient

temperature of poultry is within the thermal neutral zone thereby supporting optimal feed efficiency, health, growth rate and productivity (Deaton *et al.*, 1978). The body temperature of poultry remain relatively constant with fluctuating temperatures and varies with size, sex and breed (Chaiyabutr, 2004). Body temperature will remain at a constant of 40.5-41.5 °C if heat is neither gained from the environment nor dissipated. When the body temperature falls below 23 °C death occurs, however, the upper critical margin is much less flexible with mortalities occurring at temperatures above 45-47.2 °C (DEFRA, 2005).

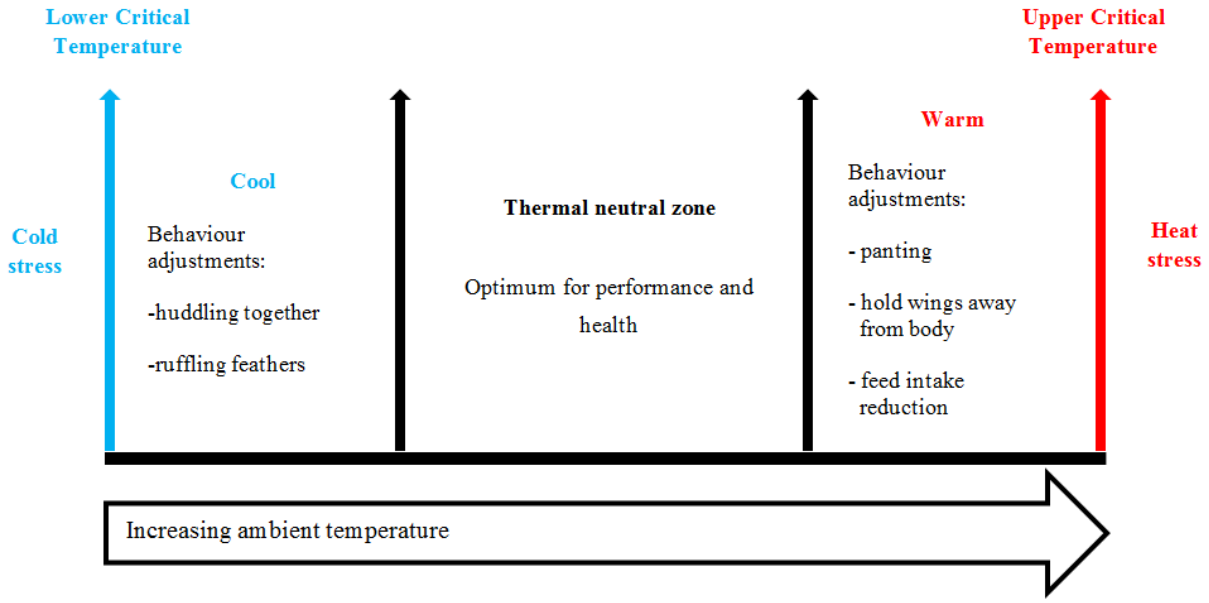

Figure 2.1 Effect of environmental temperature on the behaviour of poultry (DEFRA, 2005)

Exposure of poultry to a hot environment and/or performing vigorous physical activity might increase body temperature by 1 °C or 2 °C as heat is stored (Etches *et al.*, 2008), physiological processes will therefore be stimulated to avoid a build-up of excessive heat in the body (Teeter & Belay, 1996). Lara & Rostagno (2013) suggested that birds undergoing heat stress spend less time on feeding, restrict their movement and increase their water intake as well as panting (hyperventilation). Exposure to periods of high environmental temperatures increases hyperventilation of birds to dissipate heat through evaporative cooling. As a result, muscle activity is increased, which leads to the increased energy requirement associated with heat stress (Butcher & Miles, 2012).

Figure 2.2 shows the effect that environmental temperature has on homeothermic animals and their responses (Hillman *et al.*, 1985). The z*one of normothermia* indicates body temperature as a constant that is sustained over a wide range of environmental temperatures. The lower critical temperature [a] is the minimum environmental temperature at which life could be sustained regardless of whether it is over a period of days. A decline of environmental temperature below that of the lower critical temperature (*zone of hypothermia*) would result in a reduction of body temperature and consequently death. Thermoregulatory processes are initiated between the lower critical temperature [a] and critical thermal maximum [g] to allow for the management of fluctuating ambient temperatures until incipient hyperthermia [h] is reached. The *zone of least thermoregulatory effort* (ZLTE) is the comfort zone in which minimal effort is used to regulate body temperature. The *zone of minimal metabolism* (ZMM) includes higher temperatures that could be managed by an increase in evaporative and sensible heat loss (radiation, conduction and convection heat transfer). Sensible heat loss only alleviates a rise in body temperature until the body has reached the same temperature as the environment and usually an increase in body temperature will follow [f]. Increases in metabolic heat production occur both below ZMM and above ZLTE, to supply energy towards maintaining body temperature and panting, respectively.

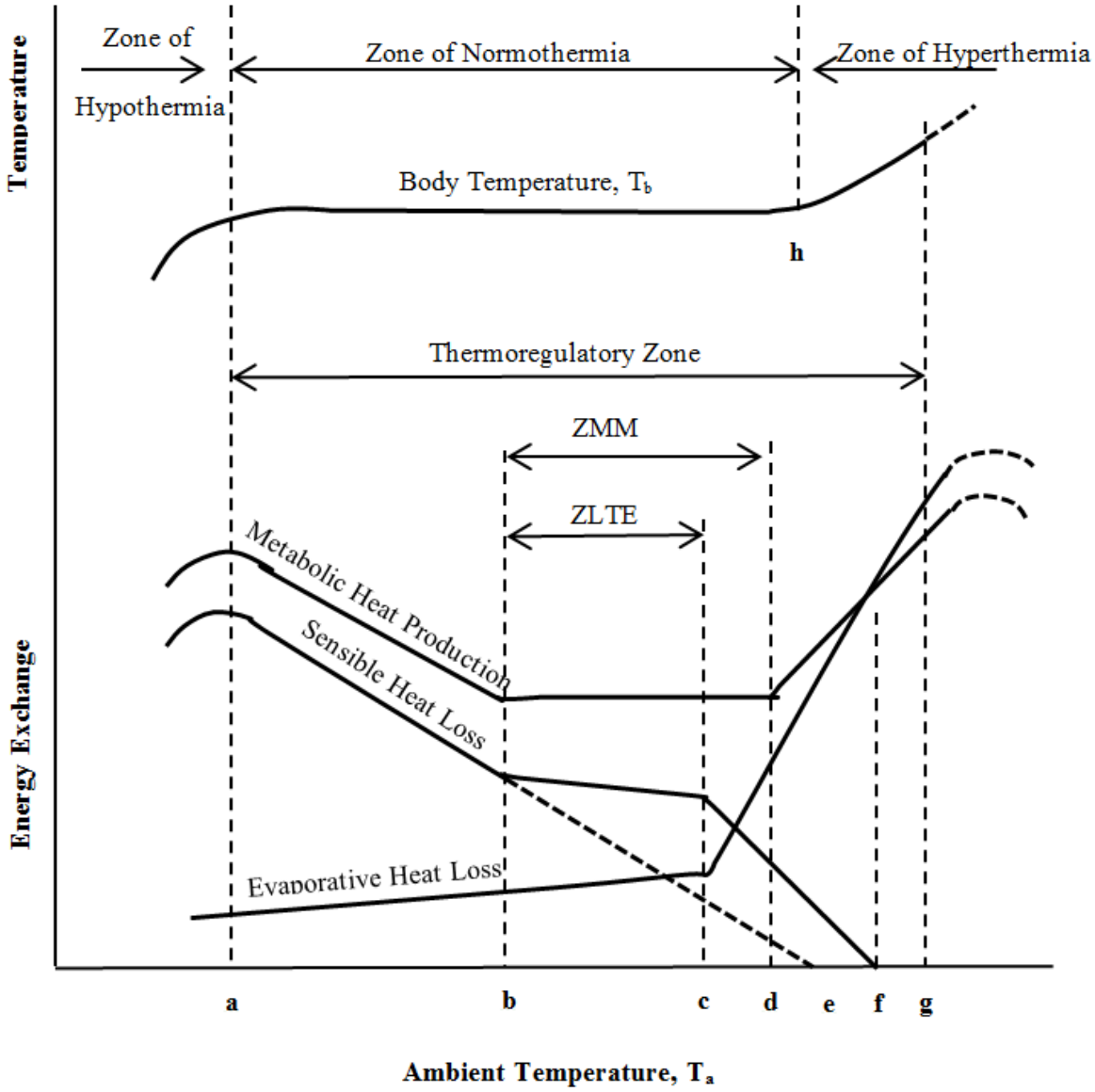

Figure 2.2 The effect of ambient temperature (T_a) on the body temperature of homeothermic animals (T_b) and the initiation of different responses (Hillman *et al.*, 1985)

Relative humidity provides an indication to what level the air is saturated by water at a given temperature (Wiernusz & Teeter, 1993). The interaction between temperature and relative humidity (RH) is demonstrated in Table 2.1. Temperatures experienced by birds (apparent) depend on both dry bulb temperature and RH. Heat loss to the environment by the bird through evaporative moisture loss from the respiratory tract will become less effective with an increase in RH, which will increase the apparent temperature experience by chicks. Lower RH requires an increase in temperature, as the apparent temperature will be reduced. Temperature could be adjusted accordingly if chick behaviour is

frequently observed and monitored to provide an indication of whether the temperature is too low or too high (Aviagen, 2014).

Table 2.1 Dry bulb temperature required to achieve equivalent temperatures at varying RH; dry bulb temperatures, at the ideal RH at an age, are light grey (Aviagen, 2014)

Age (Days)	Dry Bulb Temperature (°C) at RH%				
	40	**50**	**60**	**70**	**80**
Day old	36.0	33.2	30.8	29.2	27.0
3	33.7	31.2	28.9	27.3	26.0
6	32.5	29.9	27.7	26.0	24.0
9	31.3	28.6	26.7	25.0	23.0
12	30.2	27.8	25.7	24.0	23.0
15	29.0	26.7	24.8	23.0	22.0
18	27.7	25.5	23.6	21.9	21.0
21	26.9	24.7	22.7	21.3	20.0
24	25.7	23.5	21.7	20.2	19.0
27	24.8	22.7	20.7	19.3	18.0

2.3 Behavioural responses of poultry to heat stress

Stress is defined by Selye (1976) as a nonspecific response of the body to any demand, whereas a stressor is an agent that produces stress at any time. Therefore, when there is an adverse stimulus that disrupts the homeostasis of an animal, the response will be stress (Lara & Rostagno, 2013). Heat stress is a multiple adaptive response during which an animal is exposed to high-prolonged environmental temperatures and the heat load exceeds the animal's capacity for heat dissipation (Chaiyabutr, 2004).

The difference between energy intake and energy loss is equal to the net energy stored in the body tissue of a bird. A potential source of energy is the metabolism of feed and high ambient temperatures while potential utilisation of energy is low ambient temperatures and maintenance of normal body temperature. Birds use various adaptive responses that will allow for the increase or decrease of energy both from and to the environment; if these responses fail it could be fatal (Etches *et al.*, 2008). Many tropical and subtropical parts of the world with high environmental temperatures require poultry to respond with complicated physical, physiological and behavioural changes. These responses facilitate domestic birds to manage heat stress. Behavioural adjustments occur during heat distress to assist in

maintaining body temperatures within thermal homeostasis. These adjustments are rapid, and precise and are highly flexible when the thermal homeostasis is challenged. The behavioural adjustments are less costly compared to physiological or anatomical responses (Lustick, 1983).

Non-evaporative cooling is the primary and most energy efficient heat dissipation method when the ambient temperature is low to intermediate (Wiernusz & Teeter, 1993). Areas of the skin covered by feathers remain close to the inner body temperature throughout a wide range of environmental temperatures with minimal heat loss, whereas temperatures of non-feather surfaces fluctuate to a great extent (Chaiyabutr, 2004). Behavioural patterns of birds during high ambient temperature include less standing-lying frequency and overall standing or crouching time (Tanizawa *et al.*, 2014). Birds also stand with their wings away from the body to increases body surface area that assist in reducing body insulation and increasing the blood flow to the body surface (Bottje & Harrison, 1984). Heat distressed birds tend to minimise social behaviour and when caged, distancing themselves from one another (Etches *et al.*, 2008). This creates a larger interface for heat transfer between the bird and the environment to maximise sensible heat loss.

Thermally stressed birds may have an increase in water consumption and reduction of feed intake to compensate for an increase in evaporative cooling. Evaporative cooling becomes the primary cooling mechanism for poultry when the environmental temperature equals or exceeds the birds' body temperature (Wiernusz, 1998). Heat transfer to the body surface increase by reducing the peripheral resistance to blood flow and increasing resistance to the viscera (Bottje & Harrison, 1984). Appetite is suppressed only a few hours after the occurrence of high temperatures, whilst water intake increases immediately after the onset of high ambient temperatures (Lott, 1991), which is critical for survival. The immediate increase in water intake facilitates the immediate demand for evaporative cooling (Etches *et al.*, 2008) and Belay & Teeter (1993) reported heat distressed broilers adjust water consumption and renal handeling of water therefore affecting evaporative cooling. Feed metabolism and heat production are restored during the post heat stress recovery period (Teeter, 1994; Teeter & Belay, 1996).

2.4 Acclimation of broilers to high ambient temperature

Under high temperature conditions birds alter their physiological responses, which involve the functional integration of several organs (Etches *et al.*, 2008) to allow them to meet the metabolic needs, trying to reduce body temperature and maintain homeostasis (Lara & Rostagno, 2013).

Acclimation is a physiological adjustment of an organism to change in a certain environmental condition even though it could decrease performance (Lin *et al.*, 2004). The theory of heat stress acclimation could be defined as the attainment of new energy equilibrium and physiological homeostasis

during exposure to recurrent high temperatures (Lin *et al.*, 2004). Exposure to high ambient temperatures results in an initial temperature increase of the peripheral tissue of a bird and subsequently the core body temperature (Boone & Hughes, 1971a). Acclimation to high environmental temperature might be associated with lower basal metabolic rate (Smith & Oliver, 1971). Boone (1968) suggested that the body temperature of birds started to increase at an environmental temperature of 30 °C when the increase was rapid but when the ambient temperature increase was gradual the body temperature only started to rise at 33 °C (Boone & Hughes, 1971b).

2.4.1 Sensible heat loss in poultry

Sensible heat loss, also known as non-evaporative heat loss, in birds occurs by means of radiation, conduction and convection. High ambient temperature increases capillary vasodilation and adjust the physical behaviour of birds (Belay & Teeter, 1993). In birds, naked or poorly feathered areas, also known as the thermal windows, such as the beak, eyes, feet, under the wings and comb will, together with the respiratory tract, contribute to heat loss (Schmidt-Nielsen, 1990). A greater surface area are created by birds for sensible heat loss via convection or radiation when standing (Zhou *et al.*, 1997). Birds have arteriovenous heat exchange systems where the warm arterial blood from the body core is exchanged with cooler venous blood that dissipates heat to the environment through these thermal windows (Midtgård, 1989). Furthermore, an arteriovenous heat exchange system also exists between the optic cavity and the brain (Midtgård, 1989), where heat could also be dissipated through the eye, buccal cavity, beak and nasal passage, however it has not yet been established to what extent these mechanisms are used (Etches *et al.*, 2008).

Plumage of a bird fulfils an important function in thermoregulation due to the insulation capacity it beholds. Housed hens tend to have feather loss on the neck, back and breast regions caused by cages or crowding (Hill & Hunt, 1978). Poor feathering, regardless of the reason, improves sensible heat loss considerably (Richards, 2009). Major genes in birds could contribute to their tolerance to high ambient temperatures. Firstly, the naked neck gene (*Na*) reduces the feather mass relatively to body weight; up to 20% heterozygous (*Na/na*) and 40% homozygous (*Na/Na*). The advantage of this gene has been recognised since the 1980's (Lin *et al.*, 2006; Richards, 2009). Broilers containing the gene (*Na*) could have an improved growth rate, feed efficiency and body temperature, all of which could be an advantage during high temperatures allowing dissipation of heat more efficiently compared to normal broilers (Patra *et al.*, 2002). Secondly, the frizzle gene (*F*) has an effect on the size and curling of feathers which may reduce heat insulation. The *F* gene has proven to be less effective than the *Na* allele in broilers exposed

to elevated temperatures. However, in slow growing lines the effect is significant (Lin *et al.*, 2006). Both these genes could be beneficial to broiler production in regions with high ambient temperatures.

2.4.2 Respiration rate

During heat exposure panting in poultry allows for heat to be dissipated by means of evaporative cooling through the surface of the mouth and respiratory pathways (Chaiyabutr, 2004; Etches *et al.*, 2008), increasing both the respiration rate and saliva secretion (Teeter & Belay, 1996; Chaiyabutr *et al.*, 1997). Up to 80% of the heat production of stressed birds may be dissipated through evaporative cooling, which is greatly impacted by the RH of the external environment (Wiernusz & Teeter, 1993; Teeter & Belay, 1996). When the narrow limit of core temperature is exceeded, the respiration rate will increase. During the course of acute heat stress, birds are able to alter their respiration rate from 25 breaths per minute to 250 breaths per minute (Linsley & Burger, 1963). Lee *et al.* (1945) found that when the temperature was adjusted from 29 °C to 35 °C with a RH of 50-60%, hens responded with an increase in water evaporation from 5-18 g/h. The increased rate of respiration involves energy expenditure and results in the addition of calories to the head load of the bird, which furthermore enhances the dissipation requirements and risk for respiratory alkalosis. However, to increase heat dissipation, evaporative cooling is the only solution and should be assisted by producers (Teeter, 1994; Teeter & Belay, 1996).

The higher respiration rate increase water loss from the body that may likely increase blood viscosity resulting in elevated resistance to blood flow and possibly reducing blood flow to heat exchanged surface (Zhou *et al.*, 1997). Lungs, kidneys and various other buffer systems controls the blood pH levels to avoid rapid pH changes (Suganya *et al.*, 2015). Throughout heat stress, the respiratory rate escalates to increase evaporative heat loss, which is crucial for maintaining body temperature (Bottje & Harrison, 1984). Therefore, an increased alveolar ventilation rate will reduce the partial pressure of carbon dioxide (PCO_2) due to excessive loss of carbon dioxide (CO_2). The bicarbonate buffer system will furthermore reduce hydrogen ions and increase the pH of the blood, resulting in respiratory alkalosis (Linsley & Burger, 1963). Inconsistencies of respiratory alkalosis in studies have been observed in heat-stressed poultry. No significant variation in the blood pH was found in turkey hens (Kohne & Jones, 1975b) or broilers (Siegel, 1980) when exposed to high cycling temperatures. In contrast, Kohne & Jones (1975a) presented respiratory alkalosis in hyperthermic turkey hens. Blood pH in broilers that were chronically heat stressed has shown to be higher than those birds that were not heat stressed (Kohne & Jones, 1975a). According to Teeter *et al.* (1985), the effect of hyperventilation on blood pH may be affected by the degree and duration of thermal stress, as well as the degree of acclimation to high temperatures.

2.4.3 Cardiovascular response to heat stress

Birds with acute hyperthermia typically presented with decreased heart rate and peripheral vasodilation, resulting in an increased cardiac output, a decreased peripheral resistance and a decline in blood pressure as the body temperature rise (Whittow *et al.*, 1964; Darre & Harrison, 1987). An increased cardiac output seems to alleviate heat distress in birds as birds with normal cardiac output were in a condition of cardiac and respiratory failure (Whittow *et al.*, 1964). Peripheral vasodilation assists heat transfer from the body core to the environment through use of the comb, wattles and shanks (Nolan *et al.*, 1978), dissipating heat to the environment therefore lowering the deep body temperature to normal (Darre & Harrison, 1987). Research has indicated that acclimation of birds to high environmental temperatures caused a reduction in cardiac output, an increase in blood pressure and normalisation of peripheral resistance (Whittow *et al.*, 1964; Darre & Harrison, 1987; Dawson & Whittow, 2000).

2.4.4 Heat shock proteins

When living organisms are thermally challenged, a set of proteins called heat shock proteins (HSP) are synthesised (Ganter *et al.*, 2006; Staib *et al.*, 2007). Heat shock proteins play an important part in the survival of stressed cells (Etches *et al.*, 2008) through the protection and repair of damage cells (Gu *et al.*, 2012) and the stabilisation of the internal environment (Gabai *et al.*, 1997). These evolutionary conserved stress proteins are classified according to molecular size (10-150kDa) (Benjamin & McMillan, 1998). Three major molecular groups of HSPs that are found in chickens are 20-30, 65-80 and 80-90 kDa (Atkinson *et al.*, 1983).

Heat stress result in oxidative stress which generates a redox imbalance favouring pro-oxidants, therefore inactivating cellular antioxidant defenses and inducing oxidative changes in cells (Mahmoud *et al.*, 2003). Heat shock proteins have been found to bind to heat sensitive proteins thereby preventing degradation, protect and repair cells and tissues during heat exposure (Ganter *et al.*, 2006). Broilers that were exposed to heat distress showed an increase in both HSP70 mRNA and protein in broiler liver within 3 hours (Gabriel *et al.*, 1996). The role of HSP70 in the gastrointestinal tract has been studied extensively. Under acute heat stress the activity of broilers' digestive enzymes were significantly improved with overexpression of HSP70 but no change in the morphology of the intestines were found (Hao *et al.*, 2012). Gu *et al.* (2012) suggested that HSP70 significantly elevates antioxidant enzyme activity and inhibited lipid peroxidation, therefore, HSP70 presented to protect the intestinal mucosa in broilers. Al-Aqil & Zulkifli (2009) suggested that birds acclimated to high environmental temperatures possess improved expression of HSP70, which could improve the ability of birds to cope with stressors

such as transport in hot humid climates in comparison to birds reared in environmental controlled houses. Heat shock proteins have therefore been the subject of intense studies (Gabriel *et al.*, 1996; Al-Aqil & Zulkifli, 2009; Gu *et al.*, 2012; Hao *et al.*, 2012) ascribed to the extensive functions they possess in both stressed and normal cells.

2.4.5 Adaptation of the endocrine system

Birds possess both peripheral temperature receptors and deep body temperature sensitive elements. The responsiveness of birds may involve temperature-dependent synaptic transmission and intrinsically thermal response within the central nervous system (Dawson & Whittow, 2000). The endocrine system consists of glands that produce and secrete hormones into the circulatory system. These chemical substances produced by the body regulate activities of tissue or organs. Hormones initiate and regulate metabolism, growth, development and reproductive processes. When birds are exposed to high temperatures, hormonal systems are initiated to facilitate physiological and behavioural responses in an attempt to remain in homeostasis. Major adaptation of the endocrine system in response to heat stress will be discussed in the following sections.

2.4.5.1 Neurohypophysis (posterior pituitary gland)

Arginine vasotocin (AVT) and mesotocin (MT) are hormones derived from the neurohypophysis in birds, homologues to mammalian vasopressin and oxytocin, respectively. In non-mammal, AVT is an antidiuretic hormone released when dehydration occurs, in turn stimulating the kidneys to reabsorb water. However, it is believed that AVT plays a role in heat dissipation and plasma osmolality but is independent from each other in chickens (Robinzon *et al.*, 1988; Wang *et al.*, 1989). Wang *et al.* (1989) found that the plasma concentration of AVT changed after 90 minutes of constant exposure to 32 °C or 60 minutes at a temperature of 37 °C, without a significant change of plasma osmolality, Azahan & Sykes (1980) supported these findings. However, Arad *et al.* (1985) observed that only after 48 hours of dehydration AVT plasma levels increased. Furthermore, AVT injections administered in birds have proven to reduce the temperatures of shank and comb (Robinzon *et al.*, 1988). Wang *et al.* (1989) concluded that AVT secretion in non-acclimated birds was due to an elevated environmental temperature while AVT's key stimulant in acclimated birds was an increased osmolality.

Koike *et al.* (1986) suggested that mesotocin is a diuretic hormone, due to the facilitated release of MTs when birds were hypotonicaly infused by saline and positively correlated with renal blood flow. In birds, both heat stress and high levels of AVT suppressed MT, but it is still unclear what role MT fulfills

in thermoregulation. Furthermore, it has been proposed that thermoregulation is facilitated by either the central nervous system or peripheral mechanisms. Both suppression of MT and increase in plasma AVT may assist in the conservation of body fluids when birds are thermally stressed (Wang *et al.*, 1989).

2.4.5.2 Adenohypophysis (anterior pituitary gland)

It is well established that growth hormone (GH) plays a role in lipid metabolism. Research indicated that the administration of GH on several mammalian species depleted fat depots in the body, improved lipid transport to the liver, increased ketogenesis, depressed respiratory quotient and accelerated oxidation of lipids (John *et al.*, 1973). John *et al.* (1975) found that GH plasma level increased significantly when pigeons were dehydrated and exposed to high environmental temperatures for three consecutive days at 28 °C, 31 °C and 36.5 °C, respectively. It is believed that GH diverts high energy substrates towards muscle metabolism for the purpose of panting while birds are enduring heat distress (Etches *et al.*, 2008).

The regulation of the avian reproductive system is a function of the hypothalamic pituitary adrenal axis. The hypothalamus produces gonadotropin-releasing hormones (GnRH) that stimulates the anterior pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone, successively regulating the ovarian and testicular function (Ottinger & Bakst, 1995).

The hypothalamus could be the primary focus for heat stress as it receives both neural and endocrine inputs that might result in a general inhibition of the reproductive system. Diminished egg production of hens is a consequence of thermal stress on the reproductive performance and suspected to be partially influenced by the ovulatory hormones as well. Heat stress reduces the levels of GnRH, LH and preovulatory surges of LH and progesterone. Furthermore, corticosterone, which is also present at high concentrations during heat stress, is known to suppress circulating LH in birds (Donoghue *et al.*, 1989).

2.4.5.3 Pineal gland

Melatonin is a hormone secreted by the pineal gland of animals, including birds, when in a dark environment. Melatonin contributes to many biological functions such as thermoregulation, behaviour and circadian rhythm. It also plays a part within the reproductive and cardiopulmonary systems (Apeldoorn *et al.*, 1999; Daghir, 2008; Sinkalu *et al.*, 2015). During periods of darkness melatonin levels in both the plasma and pineal gland were high with body temperatures relatively low, conversely melatonin levels during daylight periods were low and body temperatures higher (John *et al.*, 1978).

Vasodilation and blood flow of peripheral tissue were enhanced in the presence of elevated levels of melatonin resulting in radiating excess heat through the legs, therefore, lowering the body temperature (Jones & Johansen, 1972). Cogburn *et al.* (1976) observed that the pineal gland has an important function in the regulation of body temperature since there was a delay in the restoration of normal body temperature in pinealectomised birds.

2.4.5.4 Adrenal glands

The location of the paired adrenal glands is at the anterior and medial to the cephalic lobe of the kidney. Adrenal glands are composed of the cortical and chromaffin tissues that are intermingled. Adrenal glands are vital in the regulation of homeostasis in birds. Corticosterone (CS) is one of the predominant glucocorticoids secreted from the adrenal cortex. The central nervous system receives impulses from both neural and endocrine origin to stimulate production of corticotropin-releasing factors (CRF) by the neurons (Siegel, 1980). Transportation of CRF to the pituitary gland is facilitated by the hypothalamic portal vascular system, where adrenocorticotropic hormone (ACTH) synthesis is stimulated and released into the general circulatory system (Freeman, 2007). The major target tissue of ACTH is the adrenal cortex, which increases synthesis of all adrenocortical hormones with CS and aldosterone in the majority. Primary stress response mediators are ACTH, aldosterone and CS. These mediators have an extensive effect throughout the body on multiple target tissues (Edens & Siegel, 1976). All these responses have an impact on the physiological responses of the birds' ability to manage high environmental temperatures (Etches *et al.*, 2008). Plasma CS is elevated with response to thermal stress; maintaining high levels of CS is only possible for a short duration as the concentration of CS declines after the initial surge. Therefore, other behavioural or physiological responses could be initiated to alleviate heat stress after the initial surge of CS (Etches *et al.*, 2008). Exposure of chicks to a temperature of 43 °C increased plasma CS within 30 minutes but pre-exposure concentration was reached within 120 minutes (Edens, 1978). An experiment done by Edens & Siegel (1976) indicated significant increase of glucose, CS and catecholamines when birds were exposed to a high temperature of 45 °C for 2 hours. After onset of heat, glucose and CS peaked at 45 minutes and 70 minutes, respectively, thereafter a rapid decline occurred. Moreover, in support of above-mentioned studies, several others found that CS concentrations increased as birds were thermally challenged (El-Halawani *et al.*, 1973; Pilo *et al.*, 1985; Tanizawa *et al.*, 2014). Research has thus proven that adrenal cortical tissue respond to environmental stressors.

Catecholamines, epinephrine (E) and norepinephrine (NE) are synthesised and secreted by the chromaffin tissue. Stress stimulates the release of these substrates, which is similar to that of the adrenal

cortical response; the latter and ACTH both stimulate E and NE release (Carsia & Harvey, 2000). Rapid energy expenditure during heat stress is supported by catecholamines that increase the blood glucose levels. According to Thurston *et al.* (1993), turkeys tend to have a greater glycemic response to catecholamines compared to chickens that could indicate that catecholamines may be species specific. El-Halawani *et al.* (1973) found an increased release of CS and catecholamines initiated by the initial response to temperature stress, therefore could be correlated to adaptive changes leading to temperature acclimatisation. Stressors result in elevated levels of catecholamines in the blood that lead to peripheral vasoconstriction, increased heart rate and increased plasma glucose in poultry (Freeman, 2007). Catecholamines may also have an effect on body temperature of chicks. It has been suggested that the hypothalamus infused with catecholamines lowers the body temperature (Marley & Stephenson, 1970). Initially, the turnover rate of NE in the brain was increased and E decreased when turkeys were exposed to acute thermal stress at 32 °C. A reduction in NE turnover rate was noted and E left unaffected when birds were exposed to prolonged elevated temperatures (El-Halawani *et al.*, 1973). Furthermore, while the NE turnover rate did not increase by prolonged heat exposure in the brain, the NE turnover rate in the heart was increased.

2.4.5.5 Thyroid gland

As with vertebrates, birds synthesised triiodothyronine (T_3) and thyroxine (T_4) from iodination of tyrosine in the thyroid gland. Regulation of body temperature, development (growth and maturation of birds) (McNabb, 2000), metabolic rate (Danforth Jr & Burger, 1984), as well as hatching, moulting and reproduction (Decuypere & Eduard, 1988) are processes which involve thyroid hormones. Regulation of body temperature and metabolic rate is an important role of thyroid hormones when birds are exposed to heat stress. Williamson *et al.* (1985) found that when birds were exposed to high environmental temperatures the feed consumption of birds decreased, which caused a decline in T_3, levels. The metabolic rate of birds declined following a thyroidectomy but after T_4 was administered the metabolic heat increased (Mellen & Wentworth, 1962) as well as the ability of birds to regulate body temperature (Davison *et al.*, 1980). Chicks exposed to heat stress were able to prolong survival time after a radiothyroidectomy procedure that reduced the thyroid function. Therefore, a suppression of thyroid function may alleviate the effects of heat stress in birds (Bowen *et al.*, 1984).

Discrepancies have been found regarding the function of T_3; it is assumed that T_3 stimulates metabolic rate and heat stress depresses both thyroid hormones. T_3 and T_4 serum levels significantly decreased in pigeons when exposed to high ambient temperatures (Bowen & Washburn, 1985). However, Pilo *et al.* (1985) found no change in serum T_4 when birds were exposed to heat stress, but T_3 serum

levels increased with exposure to cold. Furthermore, May *et al.* (1986) found no differences in the concentration of thyroid hormones between high temperature acclimated and non-acclimated broilers.

2.5 Effect of heat stress on broiler performance and health

Several studies documented that broilers subjected to high ambient temperature have poor performance. The most important factor affecting poor performance is a reduction in feed intake, but only 67% of the reduced broiler growth was found to be due to a reduced feed intake (Daghir, 2009). Heat stress increases body temperature of a bird that may result in a decrease of feed consumption, growth rate, feed efficiency (Teeter & Belay, 1996; Zulkifli *et al.*, 2006; Quinteiro-Filho *et al.*, 2010) and survivability (May *et al.*, 1987; Teeter & Belay, 1996; Quinteiro-Filho *et al.*, 2010). The reduction in feed consumption could result in an alteration of energy and protein intake (Al-Batshan & Hussein, 1999). A decrease in body protein gain and protein efficiency as a result of heat stress in birds was reported by Cahaner & Leenstra (1992) and Temim *et al.* (2000). Deterioration of meat quality traits could be attributed to high rates of lipid peroxidation (Ain Baziz *et al.*, 1996; Howlider & Rose, 2007) and altered electrolyte balance when birds were exposed to heat stress (Babinszky *et al.*, 2011).

Environmental stressors, such as thermal stress, may cause changes in the intestinal epithelial structures (Burkholder *et al.*, 2008), resulting in a decrease of villi height and crypt depth, therefore, adversely affecting the absorption of epithelium in the gut (Sandikci *et al.*, 2004; Yamauchi *et al.*, 2006; Parsaie *et al.*, 2007). It has been suggested that greater absorption of available nutrients is related to the villi length and the increase in surface area. Conversely, the reductions of absorptive function are related to shorter villi accompanied by a reduced surface area (Yamauchi *et al.*, 2006; Parsaie *et al.*, 2007).

Heat stress has shown to increase the corticosterone serum levels of broilers according to Quinteiro-Filho *et al.* (2010) and Tanizawa *et al.* (2014). Zulkifli & Siegel (2007) indicated that stress might lead to a reduction in lymphocytes and an increase in heterophils in chickens. Borges *et al.* (2004) confirmed these effects in broilers under heat stress. Total white blood cell count was significantly reduced in birds subjected to heat stress (Trout & Mashaly, 1994; Mashaly *et al.*, 2004). The activity of both T- and B-lymphocytes was not significantly impacted but lymphocytes from the heat stressed birds had the lowest activity. These findings indicated that heat stress does not only have an adverse effect on production performance but also the immune function (May *et al.*, 1986; Mashaly *et al.*, 2004).

2.6 Management strategies to minimise the effect of heat stress in broilers

Many methods have been researched to reduce the adverse effects of heat stress. Some are either aimed at enhancing growth rate or enhancing bird survival. Physical functions of the birds will be affected by heat dissipation mechanisms during high ambient temperatures. Various management aspects could be considered to reduce detrimental effects of heat stress such as water management, acclimation of poultry to high ambient temperatures and supplementing water or diet with minerals to correct the acid-base imbalance (Chaiyabutr, 2004).

2.6.1 Housing

Adequate ventilation in broiler houses are essential to minimise heat stress during periods of high ambient temperature. Optimal ventilation will also aid in removing ammonia, carbon dioxide and moisture from the housing facility (Butcher & Miles, 2012). Ventilation fans should be strategically placed throughout the house to facilitate ventilation (Teeter & Belay, 1996), as the movement of air over birds will increase and promote convective cooling (Pawar *et al.*, 2016). The ventilation system can be particular efficient during the evening to assist in rapid removal of excessive heat, which will allow the birds to maintain homeothermy and provides an opportunity for growth compensation (Teeter, 1994; Wiernusz, 1998).

Poultry housing should always have an east-west orientation, minimising the direct sunlight entering the naturally ventilated house. Direct sunlight on the bird could create conditions resulting in heat stress, which might not be normal at a certain given temperature. Birds would move away from side walls to escape the effect of direct sunlight, causing the birds to flock together, thereby increasing the effective density and birds with high body temperature will be in direct contact with each other. The increased stocking density will not allow for sufficient air movement between and over the birds. Houses orientated in an east-west direction may not fully benefit from wind blowing in the same direction, however, this is not a significant concern for narrow houses of less than 12 metres (Daghir, 2008).

The roof overhang should be adequate to keep direct sunlight out (Daghir, 2008). According to Daghir (2008), the ideal length of the overhang is a function of the side wall height as well as the opening to the ground, with a minimum of 0.6 metres in most occasions. Taller side walls and larger side wall openings should have use an overhang of 1.25 metres or more. Longer overhangs may also assist to keep rain away from the walls.

Obstructions like trees and shrubs around housing should be placed where it does not affect prevailing winds, whereas the vegetation should be kept trimmed (Pawar *et al.*, 2016). Trimmed

vegetation also discourages both rodents and wild bird nesting. Tall trees with canopies above the sidewalls could be beneficial during high ambient temperatures. Grass covered surroundings compared to bare ground could be helpful by reducing the reflection of sunlight into the house, as the temperature of vegetation could be 30 °C cooler compare to the bare ground (Daghir, 2008).

Both the condition of the roof and insulation will affect the heat gain of a house. A shiny roof reflects much more radiation than a rusted or dark roof, therefore roofs should be kept clean and rust free. Roof insulation eliminates heat by acting as a thermal barrier, reducing the amount of heat entering the house (Daghir, 2008).

2.6.2 Acclimation

Heat stress acclimation or thermal conditioning at an early age could be beneficial for poultry re-exposed to heat stress at a later stage. This process allows poultry to survive short periods of acute heat distress while having a lower body temperature which previously resulted in a notable rise in bird body temperature (Chaiyabutr, 2004). Mortality rates in acclimated birds were noticeably lower than that of unacclimated birds. Arjona *et al.* (1988) suggested that exposing birds to high temperatures at 5 days of age could have a markable lower mortality rate when birds were again exposed to heat distress at 36 days of age. Body temperatures of acclimated broilers were significantly lower than unacclimated broilers when exposed to a temperature of 41 °C for 3.5 h (May *et al.*, 1987). Lott (1991) proposed that acclimated and unacclimated birds given access to feed for 1 hour prior to heat exposure consumed 60 mL and 50 mL of water per broiler, respectively, during heat exposure. Broilers' water consumption that received no feed prior to heat stress, were 58 mL and 30 mL per broiler, respectively.

Studies done by May *et al.* (1987) and Zhou *et al.* (1997) suggested that acclimated broilers might have the ability to alter the thermoregulation physiological responses such as lower heat production, heart rate and body temperature and higher panting rate. May *et al.* (1987) found that chicks with a initial exposure of 40 °C for 3 hours at 6 days of age showed a delay in behavioural responses when exposed to 40 °C for 15 minutes at 10 days of age, compared to the control group. Rectal temperature and plasma corticosterone (CS) levels measured at the end of the heat exposure were lower in acclimated chicks than in the control group, but no difference was found in the respiratory rate (Tanizawa *et al.*, 2014). Overall, acclimation may be beneficial to improve heat tolerance in poultry exposed to heat stress.

2.6.3 Water management

Water consumption is a crucial component during heat stress and is influenced by the ambient and water temperature and pH, addition of salts to water or feed, dietary protein levels, age and physiological state of the birds (Yousef, 1985). As mentioned previously, approximately 80% of the heat produced by the birds is dissipated via evaporative cooling (Van Kampen, 1981; Ahmad & Sarwar, 2006). As stated by Belay & Teeter (1993) and Smith & Teeter (1988) water acts as a heat receptor; for that purpose an increase in water consumption could benefit the birds by increasing water dissipation per breath. An experiment conducted by Belay & Teeter (1993) suggested that heat loss per breath would increase by 30% when basal water consumption increased by 20%. Addition of salts or reducing the temperature of water may increase water consumption, subsequently elevating evaporative cooling (Belay & Teeter, 1993).

The optimum water pH as recommended by Socha *et al.* (2003) ranges between 6 to 8.5, whereas optimal water temperature according to Beker & Teeter (1994) is between 20 °C to 24 °C or below 26.7 °C (Ahmad & Sarwar, 2006). However, there are significant interactions between the addition of salts to drinking water and temperature (Wiernusz, 1998). Teeter *et al.* (1987) found that supplementing water with potassium chloride (KCl) will only have a positive effect on feed consumption and growth rate when the temperature of water consumed is below that of the bird's body temperature. Lowering the water temperature without salts also proved to be beneficial to stimulate water intake. Consequently, an increase in water intake may produce an increase in urine production resulting in wet litter (Van Kampen, 1981); producers should consider both the advantages and disadvantages of increased water intake.

2.6.4 Electrolytes and acid-base balance

Under high temperatures the maintenance of body fluid homeostasis is vital to ensure adequate performance in broilers. Normal tissue function depends on maintaining body fluid homeostasis keeping the intracellular and extracellular fluids stable. Electrolytes can be divided into cations and anions (Naseem *et al.*, 2005). Major ions such as sodium (Na), potassium, chloride (Cl), phosphate , calcium (Ca), magnesium and sulphate are involved in body fluid homeostasis of birds (McDonald *et al.*, 2011). In many animal species including poultry, it is known that potassium, the most abundant intercellular cation, is required for normal metabolic maintenance processes (Chaiyabutr, 2004).

Huston (1978) concluded that the concentration of plasma potassium was reduced by heat stress, whereas potassium retention in the body decreased and enhanced urinary potassium excretion (Smith & Teeter, 1987). Concerns following potassium depletion may be the modifications of physiological

functions such as cell volume regulation, metabolic processes, nerve conduction and the excitation-contraction in muscle cells. However, studies have been conducted on dietary alterations that could counteract these responses but with contradictive results (Chaiyabutr, 2004). Ahmad & Sarwar (2006) concluded that dietary electrolyte balance of birds depend on ambient temperature, age and the duration of high environmental temperatures. Milliequivalents (mEq) is used to express the ability of potassium and Na to neutralise hydroxyl groups and Cl ability to neutralise hydrogen ions. These ions have the largest effect on the acid-base balance of a bird. Numerous studies suggested that the requirements of electrolytes were around 250 mEq/kg of feed for optimal growth and feed utilisation. Survivability of birds experiencing heat distress depended largely on water intake, which relied on electrolyte balance of the diet and age of the bird. The ability of birds to regulate body temperature improved with higher water intake that will affect rectal temperatures and caused an increase in litter moisture due to increased water excretion (Ahmad & Sarwar, 2006).

Strategies have been researched to combat respiratory alkalosis that may cause a suppression in growth rate of birds by means of supplementing feed or water with electrolytes such as sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), KCl, calcium chloride and ammonium chloride (NH_4Cl) (Teeter *et al.*, 1985; Ahmad & Sarwar, 2006). Electrolytes are necessary in various concentrations and combinations to maintain physiological functions of poultry during hot weather (Brake *et al.*, 1998). Deyhim & Teeter (1991) observed that broilers subjected to a temperature of 35 °C and supplemented water with KCl and NaCl had an increased water consumption of 68% and 35%, respectively, as compared to the control group. Survivability improved by 10% (Deyhim & Teeter, 1991) in birds supplemented with KCl and male birds gained 10.5% weight when supplemented with NaCl (Smith, 1994) compared to those that received no supplementation. Smith (1994) and Whiting *et al.* (1991) found that no carcass qualities were affected by electrolyte supplementation. Teeter *et al.* (1985) found that broiler growth may be improved by supplementing 1% NH_4Cl or 0.5% $NaHCO_3$ under heat stress. Studies have shown that the addition of electrolytes such as NH_4Cl, KCl and/or $NaHCO_3$ to drinking water (Ahmad & Sarwar, 2006) as well as carbonated water (Bottje & Harrison, 1984) improved feed and water intake of broilers. Similarly, Balnave & Oliva (1991) found an improved feed intake, weight gain and feed efficiency by supplementing $NaHCO_3$ to the diet or water. Smith & Teeter (1988) suggested that the survivability of birds increased the total marked weight by 3% when KCl is supplemented, 14% when birds were fasted and 8% for a combined treatment. Research further demonstrated that $NaHCO_3$ elevated the deficiency of bicarbonate that resulted in respiratory alkalosis, even though it had an unfavourable effect on the blood pH, NH_4Cl could be used to reduce the blood pH (Benton *et al.*, 1998). Bottje & Harrison (1984) found that heat stressed birds had less of Ca available for eggshell formation due to a reduced ionic Ca in the blood; this is apart from the lower dietary Ca ingestion due to a reduction

of feed intake (Richards, 1970). High ambient temperature exposure might alter the birds' electrolyte balance, causing the plasma Na^+ to decrease and Cl^- to increase, influencing amino acid (glutamic acid) metabolism, which plays an important part in metabolic regulation in acid-base balance of birds (Austic & Calvert, 1981, cited by Chaiyabutr, 2004).

2.6.5 Feed management and nutrition

Environmental temperatures greatly affects feed intake and ultimately the growth rate of the broilers (Daghir, 2009). The behavioural response of birds to high environmental temperature will cause a reduction in feed intake, while reducing the total nutrient intake. Many dietary alterations have been researched and implemented with varying degrees of success (Chaiyabutr, 2004).

2.6.5.1 Fasting

Feed consumption increases the heat production in the body by the processes of digestion, absorption and metabolism of nutrients, which may result in an increased mortality rate during the cource of acute heat stress. Feed withdrawal could reduce heat production, effectively lowering the body temperature and mortality of broilers (Yalçin *et al.*, 2001). Fasting, on the other hand, may result in a reduction in growth rate and lead to a later marketing age. However, producers should decide whether it will be more beneficial to have a fast growth rate compared to high mortality rates (Daghir, 2009), as a heat stressed bird's growth rate is already reduced. Thompson & Applegate (2006) suggested that feed withdrawal may also affect the integrity of intestines due to an alteration in the morphology and reduction in the intestinal mucus. Research showed that removal of feed once heat stress has begun is of little value; time should allow for the bird's digestive tract to be cleared of digesta and substrate availability to reduce metabolic heat (Wiernusz, 1998). Teeter *et al.* (1987) established that removal of feed as little as 3 h prior to heat distress may increase survival rates of broilers.

2.6.5.2 Energy density

An increase in the energy content of feed can partially overcome the depressed growth during heat stress (Daghir, 2009). Replacing carbohydrates with fat as source of energy can reduce the heat increment of feed. This enables the birds to reduce the dynamic effect of the feed and assists in managing the heat stress (Daghir, 2009). Diets containing high fat (5%) at ambient temperatures of 29-36 °C could reduce the unfavourable effect of heat stress (Ghazalah *et al.*, 2008). Dietary fat may also reduce the

passage rate of feed in the gastro intestinal tract of poultry allowing for greater nutrient utilisation (Mateos *et al.*, 1982), therefore increasing the energy value of other dietary constituents (Mateos & Sell, 1981). Teeter & Belay (1996) have consistently found that increased dietary energy and/or lowering caloric-protein ratio increase both mortality and weight gain.

2.6.5.3 Protein quality

Broiler performance may be affected by high environmental temperature particularly during the finishing phase, which occurs at four to six weeks of age. Lower growth rate and a reduction in the protein retention efficiency may be a possible result of an alteration in protein metabolism in heat stressed birds (Temim *et al.*, 2000). Broiler performance could be improved by reducing the dietary protein levels and supplementing poor quality or unbalanced protein diets with essential amino acids further reducing the heat increments (Daghir, 2009; Pawar *et al.*, 2016) and alleviating heat distress consequences (Daghir, 2009). Results obtained by Cahaner *et al.* (1995) indicated that nutritional requirements of broilers were affected by both ambient temperature and by the genotype. Growth rate and meat yield were considerably decreased in fast growing commercial broilers when they were exposed to high ambient temperature and fed a high protein diet. Protein digestion resulted in an increase in heat production compared with carbohydrates and fat. The decreased bird performance may be due the greater heat increment of protein. Therefore, the decreased dietary protein may lower the heat increment (Daghir, 2008). A study conducted by Temim *et al.* (2000) concluded that chronic heat exposure at a temperature of 32 °C caused a reduction in muscle protein deposition in broilers due to a decrease in protein synthesis. Although there was a 5 % increase in protein intake no restoration of protein synthesis was noted. No significant differences were detected by Rahman *et al.* (2002) in the performance of heat exposed broilers when receiving either a 23 % or 21 % protein diet.

Specific amino acid requirements of broilers under high temperatures have been studied and the responses vary (Daghir, 2009). Low protein diet with 16 % crude protein supplemented with lysine, methionine, threonine, arginine and valine compared to a 20% crude protein diet, did not elevate the negative effects of heat stress (Temim *et al.*, 2000). Corzo *et al.* (2003) established that increased lysine dietary levels appeared necessary to accommodate depressed feed intake and improve feed efficiency. Brake *et al.* (1998) and Chamruspollert *et al.* (2004) found that ideal amino acid balance may be different for broilers during normal thermal neutral conditions and high environmental temperatures. Broilers' ideal amino acid balance vary with different dietary conditions such as dietary electrolytes (Daghir, 2009; Pawar *et al.*, 2016). Brake *et al.* (1998) established that at high temperatures an increase in the Arg:Lys ratio improved food conversion without affecting growth. According to Balnave & Brake (2001), high

Arg:Lys ratio with sodium bicarbonate improved broiler performance. Amino acid levels could be increased as a percentage of the diet up to 30 °C, but beyond an environmental temperature of 30 °C no additional increase in amino acid levels could be justified as growth will be depressed (Daghir, 2009). Additionally, the length of heat exposure may affect protein utilisation in hyperthermic birds. Therefore, it is important to distinguish between long- and short-term heat exposures when interpreting research on heat stress (Gonzalez-Esquerra & Leeson, 2005). The imbalance of dietary amino acids increases the dietary nitrogen excretion that produces ammonia build up with an unfavourable effect on broiler performance and welfare (Miles *et al.*, 2004).

2.6.5.4 Vitamins

High environmental temperatures lower nutrient intake and utilisation thereof (Sahin *et al.*, 2009), it may lead to a reduction in oxidative damages and lowering plasma concentration of vitamins (eg. A, C and E) and minerals (eg, Zn). Mobilisation and excretion of vitamins and minerals within tissue increase during heat stress, resulting in a marginal vitamin and mineral deficiency (Sahin *et al.*, 2009). Supplementation of vitamins and minerals to heat-stressed broilers demonstrated a reduction in mortality and improved growth (Pawar *et al.*, 2016). Daghir (2009) observed that supplementation of a vitamin A, D, E and B complex to the drinking water of heat distressed broilers could be beneficial for both performance and immune function. Whereas, vitamin A, D, E and B complex could improve laying performance and immune function, serving as a prophylactic to a stressful environment (Ferket & Qureshi, 1992).

Lin *et al.* (2002) showed that supplementation of vitamin A at 8000 IU/kg diet alleviated the detrimental effects that heat stress had on egg production. The same authors found that higher levels of vitamin A were needed to enhance antibody production of hens when exposed to heat after the vaccination against Newcastle disease. Supplementation of zinc (30 mg/kg) and vitamin A (15,000 IU retinol/kg) in combination or separately, has shown to enhance weight gain, feed efficiency, carcass traits and reduced serum malondialdehyde in broilers (Kucuk *et al.*, 2003). Broilers supplemented with both vitamin A (15,000 IU retinol/kg) and zinc (30 mg/kg) had shown the potential to prevent performance depression related to heat stress (Kucuk *et al.*, 2003).

Vitamin C (ascorbic acid) can be synthesised by poultry and is not a required supplement under normal conditions. However, it is common practice to supplement ascorbic acid to either feed or water in hot regions (Pawar *et al.*, 2016). The general recommendation which to include ascorbic acid during periods of higher ambient temperature, is 1 g/L drinking water (Daghir, 2009). In the biological system ascorbic acid is an important antioxidant that could assist in preventing oxidative injuries caused by heat

stress in broilers (Lin *et al.*, 2000). Supplemental ascorbic acid improved carcass traits and crude protein content and reduced crude fat in broilers subjected to high ambient temperature (Kutlu, 2001). A study by McKee & Harrison (1995) concluded that supplemental ascorbic acid not only improved feed intake but also reduced plasma CS and the heterophil: lymphocyte ratio.

Supplementation of a diet with 60 IU of vitamin E/kg feed led to an improvement in feed intake, egg production, vitelline membrane strength and yolk and albumen solids (Kirunda *et al.*, 2001). Dietary supplemental levels of 250 mg/kg vitamin E proved to alleviate the adverse effect of high temperatures on egg production (Bollengier-Lee *et al.*, 1998; 1999). Puthpongsiriporn *et al.* (2001) found that vitamin E supplemental levels of 65 IU/kg feed improved egg production and immune function of birds during chronic heat stress. It is recommended that vitamin E should be administered before, during and after heat stress in order to partially alleviate the adverse effect of chronic heat stress in layers (Bollengier-Lee *et al.*, 1999). The primary concern for the incorporation of vitamins in hot regions are factors such as transportation delays, temperature, moisture, oxidation, peroxides and trace minerals that affect vitamin stability. These problems could be limited by means of antioxidants, gelatine-encapsulated vitamins, correct storage conditions, and to delay the addition of fats until feed is ready to be fed (Daghir, 2009).

2.6.6 Non-nutritive feed additives

2.6.6.1 Nicarbazin

Nicarbazin is an effective anticoccidial that has repeatedly been documented to increase mortality in heat distressed birds (Teeter & Belay, 1996; Wiernusz, 1998). Maxiban™ (Elanco Animal Health, Greenfield, Illinois, USA) a combination of narasin (50 mg/kg) and nicarbazin (50 mg/kg) was researched with the objective to reduce the recommended levels of nicarbazin from 125 mg/kg to 50 mg/kg and determine whether the lower level of nicarbazin would reduce the toxicity during high ambient temperatures. Data collected in the study mentioned indicated that Maxiban™ increased the mortality rate of male broilers, therefore, demonstrating nicarbazin toxicity in heat stressed broilers (Wiernusz & Teeter, 1991). Conversely, during a study conducted by Teeter & Belay (1996) it was suggested that nicarbazin toxicity could be alleviated by both supplementing salt to the drinking water and fasting. The inclusion of nicarbazin should therefore be limited to the temperate periods of the year, since nicarbazin toxicity has been suggested to be related to heat production (Wiernusz & Teeter, 1995).

2.6.6.2 Antibiotic growth promoters

Antibiotics are supplemented to prevent or control diseases. Supplementation of growth promoters at sub therapeutic levels modifies microbiota or their products in the direct environment of the host and inside the gastrointestinal lumen (Visek, 1978). It has been reported that antimicrobial agents used as feed additives, decreased microbial production of toxins that suppresses growth (Sandikci *et al.*, 2004). Antibiotic growth promoters do not sterilize the gastrointestinal tract but control specific microbial populations (Ferket, 2004). According to Visek (1978) animals supplemented with antibiotics at sub therapeutic levels have a lower metabolic rate and contain less tissue in intestinal cell walls and associated lymphoid structures. As a result slower cells rejuvenation leads to less tissue in the intestinal walls and rapid nutrient absorption. The beneficial effects of antibiotic supplementation could potentially assist in alleviating heat stress with regards to lower heat production, reduce immune challenge and improve nutrient absorption (Belay & Teeter, 1994).

Zinc bacitracin

The first discovery of bacitracin was made in 1943 when *bacillus* was isolated from the wounds of a seven year old girl (Brander *et al.*, 1991). Bacitracin is a metallopeptide antibiotic that is widely used against Gram positive bacteria. However, for the biological activity of the antibiotic it requires a divalent metal such as Zn^{+2} (Ming & Epperson, 2002).

Zinc bacitracin (ZnB) fed at sub therapeutic levels has enhanced performance of different species such as pigs, horses and fowl (Elsaeed, 2015). Zinc bacitracin is a growth promoter which is given orally and which is not absorbed through the intestinal tract, indicating that the mode of action is primarily restricted to intestinal Gram-positive bacteria (Eyssen & de Somer, 1963). Research by Eyssen & de Somer (1963) has shown that feed efficiency increased with antibiotic treatments. The intestinal walls in animals that were supplemented with antibiotics were thinner than that of with the control group and similar to germ-free animals. These investigations support the hypothesis that some bacteria interfere with absorption of nutrients by thickening the intestinal walls.

Results regarding the effect of antibiotics on carcass parameters are inconsistent. A reduction of abdominal fat was noted by Wojick & Plaur (1983) when ZnB were fed at 100 mg/kg feed and Bartov (1992) found that the weight of liver, small intestines and abdominal fat reduced when chicks were fed 25 mg/kg ZnB. Similarly, broiler chicks fed ZnB showed significant reduction in liver and heart weight compared to control groups, with no effect of the drug on the weight of the proventiculus (Franti *et al.*, 1972). Izat *et al.* (1990a) determined that ZnB at 50 mg/kg feed did not have a significant effect on the

overall dressing percentage and the weight of different carcass portions. Layers fed a diet that contained 55 mg/kg ZnB had a significant improvement in egg production, fertility and hatchability (Damron *et al.*, 1991; Männer & Wang, 1991). The results indicated that the effectiveness of ZnB on layers were more pronounced when kept under stressed conditions (Männer & Wang, 1991).

Virginiamycin

Virginiamycin (VM) is an antibiotic that was first discovered in 1955 and was isolated from a mutant of *Streptomyces virginiae* (De Somer & Van Dijck, 1955). Virginiamycin is known to be effective against Gram-positive microorganisms in the gastrointestinal tract. The development of resistance is negligible and therefore no risk of transferable drug resistance exists. In practice, transferable drug resistance is limited to Gram-negative *Enterobacteriaceac.* The activity of VM is mainly limited to the gut, therefore making tissue residue uncommon (Miles *et al.*, 1984).

Since the discovery of virginiamycin the effects thereof have been researched on various animal species with varying results. Under thermal neutral conditions, supplementation of VM improved growth and protein utilisation (Miles *et al.*, 1984). Cervantes *et al.* (2008) conducted a study to demonstrate the nutrient sparing effect of VM and the cost effectiveness. An increase in breast meat weights were found in those birds that were fed a low nutrient density diet containing 15 mg/kg VM from day 30 to day 49, compared to a standard withdrawal diet and a low nutrient density diet without VM. The intestinal mass and passage rate of digesta were reduced in broilers that were supplemented with VM and had a sparing effect of amino acids and minerals. A sparing of at least 0.231 MJ/kg and 2% protein were calculated (Cervantes *et al.*, 2008).

Virginiamycin supplementation has shown to improve body growth rate, feed efficiency (Leeson, 1984; Miles *et al.*, 1984; Salmon & Stevens, 1990a; Cervantes *et al.*, 2011) and carcass weight (Leeson, 1984; Miles *et al.*, 1984) but mortality was unaffected according to Miles *et al.* (1984), Waibel *et al.* (1991) and Salmon & Stevens (1990a). In contrast, Proudfoot *et al.* (1990) reported that VM added to the feed did not improve body weight at 21 days of age. Belay & Teeter (1994) found that supplementation of VM at 15 mg/kg for broilers during high ambient temperatures increased weight gain (2.1%) and lower mortality (5.1%) at 20 mg/kg. Saleable carcass weight was linearly increased with VM supplementation as survivability were improved (Belay & Teeter, 1994). Broilers supplemented with VM at 0, 15 and 20 mg/kg were subjected to either thermal neutral (24 °C) or heat stress conditions (24-35 °C) (Teeter, 1994). It was established that birds exposed to the thermal neutral environment and supplemented VM at 15 and 20 mg/kg compared to the negative control had improved survivability by 1.5 % and 2.1 %, respectively, gain/feed ratio by 2 % and 6.1 %, respectively, and weight gain by 1.3 % and 2.2 %,

respectively. Within the heat stressed condition, VM supplemented at 15 and 20 mg/kg compared to the negative control, enhanced weight gain by 3.1 % and 1.7 %, respectively, as well as gain/feed ratio by 7.5 % and 10 %, respectively, and survivability by 3.1 % and 6.2 %, respectively. No carcass parameters were affected. The improved survivability could be the result of either a reduced immune challenge and/or heat production (Teeter, 1994).

Virginiamycin lowers bacterial count in the gut, resulting in less gut cell destruction. Therefore, less new cells with shorter villi would be necessary to replace older gut cells. Other factors such as food passage may also cause destruction of gut cells, therefore, alterations in the gut morphology could not be related to a mere factor (Khodambashi Emami *et al.*, 2012). Under thermal neutral environments, Parsaie *et al.* (2007) reported that the duodenal villi were greater when compare to jejunal villi, which remained unaffected, whilst ileal villi decreased in birds supplemented with VM (200 mg/kg). Baurhoo *et al.* (2007) observed a decrease in the height of jejunum villi when fed VM (11 mg/kg). In a study conducted by Khodambashi Emami *et al.* (2012), duodenal villi height were increased and crypt depth decreased; these results might be attributed to the antimicrobial properties of VM (Baurhoo *et al.*, 2007). Quinteiro-Filho *et al.* (2010) presented that broilers subjected to heat stress (either 31 or 36 °C) exhibited no change in the villi height or crypt depth of the duodenum, jejunum or ileum. Butcher & Miles (2012) presented data showing that broiler chicks fed VM have a lower total villus area, shorter villus height and crypt depth in the ileum compared to chicks fed the control diet or bacitracin methylene disalicylate (BMD). Supplementing either VM or BMD decreased both weight and length of the intestines, as well as a thinner muscularis mucosa in the ileum in birds fed VM compared to control. Henry *et al.* (1986) reported a reduction in relative intestinal weight and mucosal membrane thickness, with the improved carcass yield a result of a lower weight of the intestines (Henry *et al.*, 1986; Izat *et al.*, 1990b; Salmon & Stevens, 1990b).

Limited research is available on the effect VM has on heterophil and lymphocytes in birds exposed to heat distress. Zulkifli *et al.* (2006) determined that birds subjected to high ambient temperatures had a significantly higher lymphocyte count and lower heterophils count when being fed VM (20 mg/kg), compared to the control group.

Additional benefits of supplementing VM to broiler diets included improved phosphorus utilisation and bone ash, so that the amount of phosphorus required for one gram of body weight was reduced (Buresh *et al.*, 1985). Research done by Cox *et al.* (2003) demonstrated that antimicrobial feed additives such as VM significantly reduced *Salmonella* populations, however, natural *Campylobacter* populations were unaffected.

2.7 Conclusion

Heat stress in broilers can occur when ambient temperatures increase above the thermal neutral zone of the bird, resulting in a rise of body temperature. In order to reduce body temperature the bird initiates physiological, behavioural, neuroendocrine and molecular responses to maintain body temperature. Heat stress could result in a reduction in feed intake, growth rate, carcass quality and an increase in mortality. Furthermore, heat stress might impair intestinal morphology, immune function, health and endocrine functions.

Management strategies aimed to reduce the negative effects of heat stress can be achieved through the manipulation of the environment or supplementation of the diet with non-nutritive feed additives, electrolytes, vitamins and minerals. Virginiamycin has proven to alleviate the detrimental effects that heat stress has on broilers. Broilers supplemented with VM when exposed to heat stress have been reported to improve growth rate, feed efficiency and carcass weight. Studies have further reported a reduction in immune challenge and gastrointestinal mass that could be related to a decrease in body heat production in broilers. Supplementing VM could therefore be beneficial in alleviating the detrimental effects of heat stress on broiler performance and health.

Chapter 3
Materials and Methods

3.1 Housing and husbandry

The trial was conducted in an environmentally controlled broiler house on the Hillcrest Experimental Farm, University of Pretoria, South Africa. Animal ethical approval was obtained by the University of Pretoria's Animal Ethical Committee (EC077-14) before commencement of the trial. The duration of the trial was 32 days in total. The facility contained 96 identical pens, each with a surface area of 2.25m^2. The broiler facility was divided into two sides, house 1 and house 2, each side containing 48 pens. Only 32 pens per side were used in this experiment with each side divided into 4 blocks. One thousand four hundred and eight (1408) day-old male Ross 308 chicks from the same breeder flock were purchased from National Chicks (Boshkop, Pretoria). Chicks were feather sexed for a second time upon arrival to ensure only healthy male chicks were selected for the trial. During sexing, all chicks received a numbered neck tag for easy identification throughout the trial and after slaughter. Twenty two birds were placed in each pen at a stocking density of 9.78 birds/m^2.

The broiler house was cleaned and disinfected two weeks prior to onset of the trial. Nipple drinkers were flushed and inspected individually before and during the trial to ensure all were in working order. The concrete floor of all pens was covered with pine shavings 10 cm deep to assist in thermal insulation and moisture absorption. Approximately forty eight hours prior to placement of chicks the environmental controlled house was preheated to an ambient temperature of 36 °C and a litter temperature of 34 °C. Floor temperatures were monitored by means of a manual infrared thermometer. Feed and water were provided *ad libitum* and the tube feeders were shaken and refilled in the mornings and evenings to ensure consistent feed availability. Fountain drinkers, tray feeders and paper were utilised additionally to tube feeders and nipple drinkers for the first 7 days to ensure easy access to feed and water. The tray feeders, papers and fountain drinkers were cleaned twice daily.

House temperatures were automatically recorded every hour by a DOL 53X Climate Computer (Skov) which were connected to one temperature sensor at chick level in each of the house. Four additional loggers were installed per side of the house to measure temperature in 15 minute intervals. The position of each logger in the house was noted so that readings could be correlated to the adjacent trial groups at the end of the trial. From placement to 15 days of age standard temperature cycles were followed, day 16 and onwards temperatures were set above the birds' comfort zone. In Table 3.1 the intended temperature cycles are given. The temperature profile simulates a cyclic heat stressed

environment with temperatures gradually increased from 8:00 to 11:00 until the maximum temperature was achieved of the set profile. Temperature was then decreased at 18:00 to 24°C from day 16 to 32. Due to low ambient environmental temperatures the desired maximum temperatures were difficult to obtain. Table 3.2 illustrates the average house temperature as recorded by the loggers.

Table 3.1 Temperature profile for induced cyclic heat stress

	Temperature cycles		
Days	**Morning (8:00-11:00)**	**Afternoon (11:00-18:00)**	**Evening (18:00-08:00)**
Day old - 15 days	Standard*	Standard*	Standard*
16-19	24-36	36	36-24
20-24	24-32	32	32-24
25-28	24-39	39	39-24
28-32	24-32	32	32-24

***** The Ross broiler management manual was used as reference for the standard brooding temperatures. Initial temperature was slowly reduced to keep birds at an optimum temperature. Day 0-6 was reduced from 36 to 30 °C , days 6-12 from 30 to 28 °C, days 12-15 from 28 to 25 °C.

A standard Ross lighting programme as recommended by Aviagen (2014) was implemented with 23 hours of light and 1 hour of darkness until a body weight of 160 g was obtained, thereafter the light was reduced gradually to 16 hours and 8 hours darkness per day for the remainder of the trial.

Phibro Animal Health (Pty) Ltd. supplied all vaccines that were used during the trial. On the day of hatching the chicks were sprayed with Infectious Bronchitis and Newcastle Disease vaccines. Vaccinations were again administered orally through the drinking water on day 15 (Infectious Bursal Disease) and 21 (Newcastle Disease) using fountain drinkers.

Table 3.2 Actual temperatures as measured by loggers in both sides of the house during the course of the trial

	Actual Temperature: House 1				Actual Temperature: House 2			
	Temperatures (°C)				**Temperatures (°C)**			
Day	**Average**	**Median**	**Minimum**	**Maximum**	**Average**	**Median**	**Minimum**	**Maximum**
0	34.3	34.3	32.8	35.5	34.8	35.1	32.8	36.5
1	33.0	33.1	31.7	34.4	33.3	33.6	31.7	35.4
2	31.8	31.6	30.9	33.4	32.0	32.0	30.9	33.6
3	30.5	30.2	28.8	32.6	30.7	30.5	28.8	32.5
4	30.1	30.2	28.6	31.0	30.4	30.4	29.0	32.0
5	29.5	29.5	28.0	30.5	29.5	29.5	27.6	31.2
6	28.6	28.7	27.7	29.5	29.1	29.0	27.7	30.4
7	29.0	29.0	28.2	29.6	29.2	29.1	27.8	30.5
8	27.9	27.7	27.3	29.0	27.8	27.8	27.0	29.1
9	27.2	27.2	26.4	28.3	26.8	26.8	26.1	27.9
10	26.6	26.6	25.4	28.0	26.5	26.5	25.1	27.8
11	26.6	26.6	24.2	27.8	26.7	26.7	25.0	28.2
12	27.4	27.5	26.0	29.1	27.7	27.7	25.5	29.4
13	26.4	26.3	25.1	27.7	26.3	26.2	24.5	27.5
14	26.3	26.2	24.9	28.4	26.5	26.5	23.8	28.4
15	25.0	24.8	23.2	27.3	24.9	24.8	22.7	27.3
16	32.3	33.5	24.2	35.1	32.4	34.0	23.9	35.1
17	32.5	33.7	23.7	35.5	32.7	34.1	23.7	35.7
18	32.6	33.7	26.1	35.7	32.6	33.8	25.4	35.3
19	32.1	33.0	24.7	35.3	32.2	33.2	24.7	35.1
20	29.8	30.7	24.1	31.8	29.9	30.9	23.2	32.1
21	30.0	31.1	24.9	32.1	30.2	31.2	24.7	32.1
22	30.1	31.0	25.2	32.2	30.3	31.3	25.1	32.3
23	29.8	30.8	24.2	32.0	30.0	31.1	24.1	32.0
24	30.1	30.9	25.2	32.1	30.3	31.2	24.2	32.2
25	32.2	32.9	25.2	34.8	32.4	33.0	25.6	35.3
26	32.7	33.6	25.3	35.4	33.1	34.0	25.7	35.8
27	33.2	34.2	25.7	36.1	33.7	34.6	26.1	36.7
28	32.4	33.3	26.0	35.3	32.5	33.4	25.8	35.3
29	30.3	31.3	25.1	32.4	30.2	31.2	24.3	31.9
30	30.3	30.9	24.0	32.4	30.2	31.0	24.0	32.0
31	30.3	31.0	24.6	32.1	30.3	31.0	24.6	31.9
32	29.1	30.2	23.0	32.2	29.6	30.5	22.3	32.5

3.2 Diet and diet formulation

Feed was formulated to meet or exceed nutrient requirements for broilers (NRC, 1994) to achieve optimum performance by the broiler birds. A four-phase feed program (Table 3.3) was used. A local feed mill, Penville (Pty) Ltd. (Pretoria), manufacturing of the experimental diets by blending with a fountain mixer. Starter crumbles were fed from day 0-7 followed by grower crumbles from day 7-21, finisher pellets (4 mm diameter) from day 21-28 and post-finisher pellets from day 28-32. A coccidiostat (salinomycin) and phytase enzyme (Axtra Phy, Danisco; 1000 P) were included in all the feed according to standard commercial practise in South Africa. The only difference between experimental feeds was the inclusion of antibiotics in the feed. The following dietary treatments were included (Table 3.5):

1. Negative control group with no antibiotic growth promoters in the basal feed.
2. Positive control group with Albac 15 % (zinc bacitracin) added to the basal feed (334 g/ton Albac) in all feed phases except for the post-finisher phase.
3. Treatment group with Stafac 500 (virginiamycin) added to the basal feed (40 g/ton Stafac 500) in all feed phases (including post-finisher).
4. Treatment group with Stafac 500 (virginiamycin) added to the basal feed (40 g/ton Stafac 500) in all feed phases except for the post-finisher phase.

Table 3.3 Feed ingredient (%) and calculated nutrient (g/kg) composition for starter, grower, finisher and post-finisher (on *as is* basis)

Dietary ingredient	Starter	Grower	Finisher	Post-finisher
Yellow maize	53.747	57.487	62.642	64.944
Soya oilcake meal	21.076	13.045	8.871	4.992
Full fat soya	7.508	10.015	12.512	14.976
Sunflower oilcake	5.005	7.511	5.035	6.723
Gluten 60	8.009	7.414	6.853	4.431
Lysine	0.419	0.381	0.359	0.351
Methionine - DL	0.166	0.097	0.099	0.139
Threonine	0.049	0.024	0.022	0.047
Tryptophan	-	-	-	0.002
Wheat bran	0.209	0.749	-	-
Crude soya oil	-	-	0.973	1.296
Limestone	1.720	1.571	1.338	1.172
Monocalcium Phosphate	1.227	0.973	0.608	0.331
Salt	0.218	0.224	0.234	0.231
Choline chloride	0.000	0.009	0.009	0.019
Sodium bicarbonate	0.285	0.188	0.135	0.137
Coxistac 12% (Salinomycin)	0.050	0.050	0.050	-
Phytase feed enzyme (Axtra Phy 1000 P)	0.010	0.010	0.010	0.010
Broiler Starter Premix	0.300	0.250	0.250	0.200

Table 3.4 Nutrient composition (%) of starter, grower and finisher diets, as formulated and analysed (on *as is* basis)

	Starter		Grower		Finisher		Post-finisher	
	Formulated	**Analysed**	**Formulated**	**Analysed**	**Formulated**	**Analysed**	**Formulated**	**Analysed**
Moisture	10.902	12.300	10.904	12.300	10.946	12.400	10.934	12.500
Dry matter	89.039	87.700	89.038	87.700	88.996	87.600	89.008	87.500
Metabolisable energy (MJ/kg)	12.039	-	12.285	-	13.038	-	13.203	-
Crude protein	24.000	25.700	22.000	24.000	20.000	20.400	18.408	18.500
Crude fat	3.864	3.830	4.396	4.05	5.780	5.570	6.552	6.250
Crude fibre	3.892	4.250	4.400	5.44	3.837	5.020	4.230	5.200
Ash	4.851	5.05	4.446	4.430	3.916	3.510	3.676	3.320
Calcium	1.100	0.940	0.990	0.930	0.825	0.820	0.711	0.730
Total phosphorus	0.689	0.660	0.633	0.660	0.515	0.530	0.454	0.440
Sodium	0.220	0.290	0.180	0.320	0.160	0.300	0.159	0.330
Water-soluble carbohydrates	-	1.11	-	1.060	-	0.900	-	0.720
Starch	-	27.100	-	40.400	-	43.400	-	45.400

Table 3.5 Dietary treatment inclusion levels used during this study

		Treatment inclusion levels	
		Zinc bacitracin (mg/kg)	**Virginamycin (mg/kg)**
Treatment 1	Starter	-	-
	Grower	-	-
	Finisher	-	-
	Post-finisher	-	-
Treatment 2	Starter	50.1	-
	Grower	50.1	-
	Finisher	50.1	-
	Post-finisher	-	-
Treatment 3	Starter	-	20
	Grower	-	20
	Finisher	-	20
	Post-finisher	-	20
Treatment 4	Starter	-	20
	Grower	-	20
	Finisher	-	20
	Post-finisher	-	-

3.3 Feed sampling and analysis

One sample (1kg) of the basal feed from each of the four phases was taken during feed manufacturing, before remixing with the additive. Proximate analysis of feed was conducted, which included dry matter (DM), ash, crude protein (CP), crude fibre (CF) and crude fat. Additionally, feed was analysed for Ca, P, Na, starch and water-soluble carbohydrates (WSC) at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria (Table 3.4). Salinomycin in the basal diets were analysed at CAL labs, Stormill, Johannesburg. Once mixed with the additive, five cores (200 g each) were taken randomly throughout each feed treatment for all four phases. The five subsamples per feed were pooled, mixed and stored for analysis. Levels of the virginiamycin and zinc bacitracin were analysed on the 16 feed samples (4 treatments x 4 phases) at FDA Laboratories, Brooklyn, Pretoria. The concentrations of antibiotics in the feed are shown in Table 3.6.

Table 3.6 Analysed concentrations (mg/kg) of zinc bacitracin and virginiamycin in the feed on an *as is* basis

		Starter	Grower	Finisher	Post-finisher
Treatment 2	**Zinc bacitracin**	48.9	49.5	50.0	0
Treatment 3	**Virginiamycin**	18.0	22.0	21.0	21.2
Treatment 4	**Virginiamycin**	18.0	22.0	21.0	0
All treatment groups	**Salinomycin**	58.0	59.5	57.0	0

Dry matter was measured by drying a crucible in the oven at 105 °C for one hour. After removing the crucible from the oven, it placed in a desiccator to allow cooling down for an half an hour. Empty weight of crucible was recorded and 2 g of the sample was added and again the weight was recorded. Crucibles were then placed in an oven for 24 hours at 105 °C, thereafter placed in a desiccator to cool down and weighed. The DM was measured according to the AOAC's official method of analysis (AOAC., 2000b, Official method of analysis 934.01). The formula used to determine the DM percentage:

$$\% \text{ DM} = \frac{\text{mass of crucible and dried sample}}{\text{mass of crucible and sample}} \text{ x } 100$$

Ash content was obtained according to AOAC's official method of analysis (AOAC., 2000c, Official method of analysis 942.05). After the weight of the dried sample was obtained, the crucibles were then placed in a muffle furnace for 2 hours at 250 °C after which the temperature was increased to 600 °C for another 4 hours. Crucibles were then left in the furnace for at least 2 hours to cool down and then placed in a desiccator for 30 minutes before weights were recorded. The following formula was used to calculate the ash percentage:

$$\% \text{ Ash} = \frac{\text{mass of crucible and ash sample}}{\text{mass of crucible and dried sample}} \text{ x } 100$$

Crude fibre was analysed according to AOAC's official method of analysis (AOAC., 2000d, official method of analysis 962.09). Sintered glass crucibles were used to weigh in 1 g of sample and recorded. The crucibles were then placed on the hot extraction unit, with the addition of 150 mL boiling sulphuric acid solution and three drops of n-octanol to each tube. The solution was then boiled for 30 minutes then rinsed three times each with 30 mL of hot distilled water. A sodium hydroxide solution of 150 mL and three drops of n-octanol were then added to each tube and boiled for another 30 minutes and once again rinsed with hot distilled water. Crucibles were then placed in a drying oven overnight, allowed to cool down for 30 minutes in a desiccator and weighed. A furnace oven was used to ash crucible samples at

550 °C for 3 hours afterwards, allowed to cool slowly to 250 °C and then placed in a desiccator for 30 minutes and weighed. The following formula was used to calculate the percentage of CF in the samples:

$$\% \text{ CF} = \frac{\text{mass of crucible and boiled sample} - \text{mass of crucibel and ash}}{\text{mass of crucible and sample}} \text{ x } 100$$

The Leco Trumac Nitrogen determinator uses the Dumas Method to determine the nitrogen content of the samples. According to the AOAC's official method of analysis (AOAC., 2000f, offical method of analysis 968.06). The CP percentage was calculated by multiplying the nitrogen content by 6.25.

The Foss Soxtec system was used to determine the crude fat percentage with ether extraction according to the approved procedure (AOAC., 2000a, offical method of analysis 920.39). Filter paper was used to weigh 2 g of sample, folded and then placed into an extraction thimble. Thereafter, thimbles were placed into the extractor tubes, weight of beakers were recorded and placed under the extractor tubes. Approximately 40 mL of 60-80 °C petroleum ether were added and allowed to boil and condense for 2.5 hours. Collection of petroleum ether followed for a further 30 minutes. Beakers were then placed into a drying oven for at least 2 hours prior to weighing. Determination of crude fat was calculated by the following formula:

$$\% \text{ Crude fat} = \frac{\text{mass of crude fat}}{\text{mass of sample}} \text{ x } 100$$

Water-soluble carbohydrates (WSC) were analysed according to the AOAC's official method (AOAC., 2000g, offical method of analysis 974.06). Standard curves with absorbance (Spectrophotometer, Analytic Jena Specord 200 plus) against glucose concentration were used to calculate the WSC.

Starch analysis was determined according to the method described by MacRae & Armstrong (1968) and Faichney & White (1983) (AOAC., 1984, offical method of analysis 996.11). Firstly, sugars were removed and secondly gelatinisation by autoclaving and enzymatic hydrolysis took place. Lastly, glucose readings were determined by a spectrophotometer (Analytic Jena Specord 200 plus). A glucose standard curve was drawn to calculate the starch percentage.

A Perkin Elmer 5100PC Atomic Spectrophotometer was utilised to analyse Ca concentrations following the method of Giron (1973). Phosphorus content was analysed by procedures described by the AOAC's official method of analysis (AOAC., 2000e, offical method of analysis 965.17) using a spectrophotometer (Analytic Jena Specord 200 plus). The Na content was determined following the

manufactures' instruction for the Varian SpectrAA 400 Atomic Absorption Spectrometer Instruction Protocol (2004).

3.4 Water analysis

Tshwane Municipal water was used for the duration of the trial. A water sample was collected in a sterilised plastic bottle at the water tank supplying the broiler facility and tested by Nvirotek laboratories, Ifafi, Hartbeespoort. Results given in Table 3.7 indicate that the water used in the broiler facilities was suitable for both human and animal consumption.

Table 3.7 Chemical and microbial analysis of water used at broiler facilities

Chemical analysis	
Calcium (mg/L)	26.59
Magnesium (mg/L)	14.38
Potassium (mg/L)	6.93
Sodium (mg/L)	29.9
Iron (µg/L)	1.9
Manganese (µg/L)	1.8
Copper (µg/L)	4.3
Zinc (µg/L)	32.6
Boron (µg/L)	21.6
Bi-carbonate (mg/L)	134.2
Carbonate (mg/L)	0
Sulphate (mg/L)	33.21
Ammonia (mg/L)	0
Nitrate (mg/L)	1.45
pH	8.29
Conductivity (mS/m)	38.3
Dissolved Solids (mg/L)	245.12
Chloride (mg/L)	27.82
Microbial analysis	
Escherichia coli (Count/mL)	<1
Total coliform bacteria (Count/mL)	<1
Heterotrophic plate count (Count/mL)	>73.8

3.5 Experimental design

A randomised block design was used in this trial. One thousand four hundred and eight male birds were randomly placed in 64 pens with 22 chicks per pen. There were four treatments with 16 pen replicates per treatment. Each side of the house was divided into two blocks, four blocks in total, with 16 pens per block. Each of the four treatments was randomly replicated four times per block. The broilers were exposed to cycling high temperatures to simulate typical heat wave conditions. Standard temperatures as recommended by the breeder company (Ross 308 manual) were applied up to and including day 15. Higher than normal temperatures was introduced from day 16 onwards, with the house being heated during the day and then allowed to cool down naturally during the night.

3.6 Data recording

3.6.1 Performance measurements

Pen weight was recorded on day of placement and days 7, 14, 21, 28 and 32. The average body weight of a bird was calculated by dividing the total pen weight by the number of birds present. On day 14 and 28 the average body weight per bird per pen was used to select birds for slaughtering purposes. Feed intake per bird was calculated by dividing the total feed intake by the number of birds in the pen. Feed intake was measured weekly on the same days that the birds were weighed (days 0, 7, 14, 21, 28 and 32). Each pen had its own feed bin with a known quantity of feed at the beginning of each week. At the end of the feed phase surplus feed (including feed left over in feeders) was subtracted and feed consumed calculated.

Weekly feed conversion ratio (FCR) and cumulative feed conversion ratio (cFCR) were measured weekly and corrected for all mortalities by adding the gained weight of mortalities to the total pen weight gain within a given period. The performance efficiency factor (PEF) for the 32-day grow-out period was also calculated by using formula:

$$\text{FCR} = \frac{\text{total feed intake per pen}}{(\text{total live pen weight at end of week period} + \text{motality weight} - \text{total pen weight at start of week})}$$

$$\text{cFCR} = \frac{\text{total feed intake per pen}}{(\text{total live pen weight at end of given period} + \text{motality weight} - \text{total pen weight at start of trial})}$$

$$\text{PEF} = \frac{\text{livability x live weight (kg)}}{\text{age in days x FCR}} \text{ x } 100$$

3.6.2 Gut morphology

A one-centimeter segment of the duodenum, jejunum and ileum were excised for evaluation of villi height and crypt depth. A bird close to average pen weight was selected on days 14 and 32. The total pen weight was divided by the number of birds present in the pen to calculate the average body weight per pen. Birds were then individually weighed to select a bird closest to the average body weight. The birds were culled by means of cervical dislocation and eviscerated immediately to minimise deterioration of the intestinal wall. The sample of the duodenum was taken 5 cm from the ostium to the beginning of the mesentery, the jejunum sample was taken at the end of the mesentery 8 cm towards Mercel's diverticulum and the ileum sample was taken 6 cm from Mercel's diverticulum towards the ileocaecal junction. All intestinal segments were rinsed and stored in a ten percent formalin buffer solution. Paraffin was used to embed intestinal tissue and a 5 µm section was prepared and placed onto microscope slides, then stained with haematoxylin-eosin for light microscope examination. Bancorft's Theory and practices of histological techniques were used as the procedure staining (Suvarna *et al.*, 2013). Average villi height and crypt depth were measured for each sample using a program ImageTool (alpha 3). Measured villus heights were taken from the villus/crypt junction to the top of the villus and the crypt depth was measured from lamina propria to the villus/crypt junction.

3.6.3 Carcass composition

One bird per replicate were weighed and slaughtered on day 33 in order to measure carcass composition. Slaughtering was done through electrical stunning and bleeding out from a single laceration to the neck. The carcasses were then scalded in a 60 °C water bath for approximately 1 minute, whereafter it was defeathered in a rotary drum mechanical picker. Shanks and heads were removed from the carcasses before evisceration and sample collection proceeded. Digesta of the intestinal tract were removed to measure and record the empty weights of the proventriculus, gizzard, duodenum, jejunum, ileum and caeca and weights were expressed as percentage of body weight. Complete eviscerated carcasses were weighed and recorded, followed by weighing the excised half a breast muscle and abdominal fat pad. Carcass dressing percentages were calculated by the following formula:

$$\text{Dressing \%} = \frac{\text{carcass weight}}{\text{live body weight}} \text{ x } 100$$

3.6.4 Blood analyses

Blood samples were obtained from one bird per replicate, selected close to average pen weight on days 14, 15, 32 and 33. Blood was collected from the same birds that were sacrificed for sampling of intestines and measurement of carcass composition. Birds were rendered unconscious by electrical stunning and blood collected in one vacutainer without any additves and one heparine vacutainer from the jugular vein after laceration and stored at 2-8 °C directly after collection. Red cell counts were obtained with a ADVIA 2120 Haematology system (Department of Clinical Pathology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort).

The prepared blood smear was stained with Wright-Giemsa and then followed by a blood smear evaluation and manual leukocyte differential count. The leukocytes were counted over 10 fields in the monolayer area using a 40x objective, and divided the total by 10 to get an average per 40x field (Campbell & Ellis, 2007; Clark *et al.*, 2009; Weiss *et al.*, 2010). Blood samples intended for CS analysis was centrifuge at 3000 rpm for 15 minutes and then stored between 2-8 °C. The analyses were conducted at the Department of Reproduction, Faculty of Veterinary Science, University of Pretoria, Onderstepoort. The concentration of CS was measured using an assay kit for the quantitative determination of endogenic chicken corticosterone in serum (CORT Elisa kit: chicken CS, Cusabio Technologies, Houston, USA). The detection range was 0.5-20 ng/mL. This assay has high sensitivity and excellent specificity for detection of CS, no significant cross-reactivity or interference between CS and analogues was observed.

3.7 Statistical Analysis

Data were statistically analysed as a randomised block design with the GLM model (Statistical Analysis System, 2016) for the average effects over the trial. Repeated Measures Analysis of Variance with the GLM models were used for repeated weekly or periodic measurements. Means and standard error of mean (SEM) were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuals, 1989). Mortality data was analysed using Chi-square (Statistical Analysis System, 2016). The variation due to block effect was accounted for by including block as fixed effect in the model. The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + H_j + BH_k + TH_l + e_{ijkl}$$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_I = effect of the i^{th} treatment

H_j = effect of the j^{th} house

BH_k = effect of the k^{th} block in house

TH_l = effect of the l^{th} treatment house interaction

e_{ijkl} = error associated with each Y

Chapter 4

Results

4.1 Broiler performance

Body weight

Table 4.1 presents broiler body weights for the duration of 32 days. At the start if the trial (day 0) body weight between treatments did not differ. After the first week birds from the Negative Control (NC) were significantly heavier than the Zinc bacitracin (ZnB) treatment and one of the treatments that received virginiamycin. Birds within the Virginiamycin 2 (VM2) treatment were significantly lighter ($P < 0.05$) than the NC group on day 14. No further significant differences ($P > 0.05$) were noted between the body weights of birds from the different treatments on days 21, 28 and 32.

Table 4.1 The effect of virginamycin on average weekly body weight (g) of broilers subjected to high cyclic temperatures from day 16[1]

	Days of age					
Treatment[2]	**0**	**7**	**14**	**21**	**28**	**32**
Negative control	44.55	205.51[a]	560.29[a]	1085.7	1710.9	2075.8
Zinc bacitracin	44.66	202.13[b]	550.42[ab]	1073.7	1695.9	2057.2
Virginiamycin 1	44.23	202.55[ab]	551.48[ab]	1085.6	1689.7	2061.1
Virginiamycin 2	44.18	199.83[b]	549.73[b]	1075.4	1682.0	2044.5
SEM	0.197	1.177	3.519	6.557	10.316	12.517

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Feed intake

Weekly feed intakes are shown in Table 4.2 the end of the second week feed intake of birds in the VM1 treatment group was significantly ($P < 0.05$) higher than the treatment group ZnB. The remainder of the weeks indicated no significant differences ($P > 0.05$) between the feed intake from the different treatment groups.

Table 4.2 Effects of virginiamycin on the average weekly feed intake (g/bird) of broilers subjected to high cyclic temperatures from day 16[1]

	Days				
Treatment[2]	**0- 7**	**7-14**	**14-21**	**21-28**	**28- 32**
Negative control	168.24	414.49[ab]	765.59	973.28	633.37
Zinc bacitracin	166.71	407.93[b]	764.52	967.69	631.17
Virginiamycin 1	167.93	434.11[a]	746.93	950.05	625.58
Virginiamycin 2	167.13	413.66[ab]	755.79	947.59	624.89
SEM	1.254	7.548	12.131	11.401	6.721

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Average cumulative feed intake

Table 4.3 denotes the average cumulative feed intake with no substantial differences ($P > 0.05$) on 7, 21, 28 and 32 days of age. The group of broilers in the VM1 treatment group showed a significant ($P < 0.05$) higher feed intake compared to the birds in ZnB group during the first two weeks of the trial.

Table 4.3 Effects of virginiamycin on the average cumulative feed intake (g/bird) of broilers subjected to high cyclic temperatures from day 16[1]

	Days				
Treatment[2]	**0-7**	**0-14**	**0-21**	**0-28**	**0-32**
Negative control	168.24	582.73[ab]	1348.32	2321.60	2954.97
Zinc bacitracin	166.71	574.65[b]	1339.17	2306.86	2938.03
Virginiamycin 1	167.96	596.85[a]	1350.24	2328.39	2964.76
Virginiamycin 2	166.59	581.63[ab]	1332.97	2276.16	2899.43
SEM	1.180	7.596	10.657	18.834	23.288

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Feed conversion ratio

After the first week, birds from the NC group has a significantly lower ($P < 0.05$) feed conversion ratio (FCR) than birds from the VM2 group, whilst birds from groups NC and ZnB had the lowest FCR during the second week of the trial compare to that of the VM1 group (Table 4.4). No significant differences ($P > 0.05$) were noted in the weekly FCR after the second week.

Table 4.4 Effects of virginiamycin on the feed conversion ratio of broilers subjected to high cyclic temperatures from day 16[1]

	Days				
Treatment[2]	**0-7**	**7-14**	**14-21**	**21-28**	**28-32**
Negative control	1.045[b]	1.167[b]	1.645	1.602	1.780
Zinc bacitracin	1.059[ab]	1.168[b]	1.663	1.602	1.746
Virginiamycin 1	1.056[ab]	1.236[a]	1.616	1.607	1.761
Virginiamycin 2	1.065[a]	1.192[ab]	1.603	1.561	1.765
SEM	0.007	0.020	0.024	0.018	0.033

[a,b] Means within a column with no common letters differ significantly (P < 0.05).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Cumulative feed conversion ratio

Significant differences (P <0.05) were observed in Table 4.5 during the course of the second week of the trial. Cumulative FCR of treatment groups NC and ZnB were substantially (P <0.05) lower in comparison to birds fed VM1.

Table 4.5 Effects of virginiamycin on the average cumulative feed conversion ratio corrected for mortalities of broilers subjected to high cyclic temperatures from day 16[1]

	Days				
Treatment[2]	**0-7**	**0-14**	**0-21**	**0-28**	**0-32**
Negative control	1.045	1.129[b]	1.359	1.462	1.531
Zinc bacitracin	1.058	1.132[b]	1.368	1.459	1.516
Virginiamycin 1	1.050	1.171[a]	1.380	1.478	1.540
Virginiamycin 2	1.063	1.154[ab]	1.369	1.450	1.507
SEM	0.007	0.014	0.007	0.011	0.011

[a,b] Means within a column with no common letters differ significantly (P < 0.05).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Performance efficiency factor

No significant differences (P >0.05) were noted in Table 4.6 between the performance efficiency factor (PEF) values of the different treatments groups (Table 4.6).

Table 4.6 Effects of virginiamycin on the performance efficiency factor of broilers subjected to high cyclic temperatures from day 16[1]

Treatment[2]	
Negative control	371.52
Zinc bacitracin	376.23
Virginiamycin 1	360.43
Virginiamycin 2	378.06
SEM	8.481

[a,b] Means within a column with no common letters differ significantly (P < 0.05).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

4.2 Carcass measurements

Carcass composition

Results of the carcass composition in Table 4.7 indicated no significant (P >0.05) variation in body, carcass, half breast, abdominal fat weight or carcass dressing percentage.

Table 4.7 The effect of virginiamycin on carcass composition (g) of broilers subjected to high cyclic temperatures from day 16[1]

Treatment[2]	Body weight	Carcass weight	Carcass dressing %	Half breast	Abdominal fat
Negative control	2102.5	1529.1	72.75	194.11	27.19
Zinc bacitracin	2061.3	1496.6	72.63	191.87	26.98
Virginiamycin 1	2086.3	1521.3	72.94	195.40	26.96
Virginiamycin 2	2061.3	1500.6	72.81	194.22	26.14
SEM	18.243	14.234	0.229	2.444	1.088

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Intestinal weights

This research did not reveal meaningful differences ($P > 0.05$) of intestinal weights expressed as the percentage of body weight between treatment groups throughout high cyclic temperatures (Table 4.8).

Table 4.8 The effect of virginiamycin on intestinal weight is expressed as % of body weight of broilers subjected to high cyclic temperatures from day 16[1]

Treatment[2]	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Cecum
Negative control	0.345	1.38	0.484	0.848	0.698	0.238
Zinc bacitracin	0.326	1.42	0.496	0.896	0.731	0.245
Virginiamycin 1	0.323	1.36	0.467	0.834	0.688	0.238
Virginiamycin 2	0.320	1.34	0.491	0.879	0.741	0.228
SEM	0.0102	0.0441	0.4845	0.0247	0.7144	0.0621

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

4.3 Intestinal morphology

Day 14 & 32

Results of intestinal measurements are indicated in Table 4.9 of day 14 and Table 4.10 of day 32. On day 14 ileum villi heights of the NC group were significantly ($P < 0.05$) shorter compared to treatment groups ZnB and VM1. No differences ($P > 0.05$) were noted in the intestinal measurements on the last day of the trial between treatments groups.

Table 4.9 The effect of virginiamycin on intestinal measurements (nm) of broilers subjected to thermal neutral conditions (day 14)[1]

	Duodenum		Jejenum		Ileum	
Treatment[2]	**Villi height**	**Crypt depth**	**Villi height**	**Crypt depth**	**Villi height**	**Crypt depth**
Negative control	2966.2	278.56	2105.9	221.35	952.48[b]	248.89
Zinc bacitracin	3387.1	256.54	2077.1	234.81	1182.3[a]	251.33
Virginiamycin 1	3267.9	251.10	1974.2	242.90	1181.6[a]	259.66
Virginiamycin 2	3247.6	260.62	2075.9	212.28	1011.9[ab]	264.93
SEM	141.987	16.438	119.007	20.791	73.367	21.494

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Table 4.10 The effect of virginiamycin on intestinal measurements (nm) of broilers subjected to high cyclic temperatures from day 16 (day 32)[2]

	Duodenum		Jejenum		Ileum	
Treatment[1]	**Villi height**	**Crypt depth**	**Villi height**	**Crypt depth**	**Villi height**	**Crypt depth**
Negative control	3775.4	405.98	2764.7	304.04	1946.7	235.42
Zinc bacitracin	3726.0	407.13	2843.9	345.84	1860.3	248.32
Virginiamycin 1	3866.7	379.52	2515.4	309.17	1896.0	202.55
Virginiamycin 2	3685.4	340.88	2823.8	364.63	1883.6	229.86
SEM	140.766	39.067	151.249	33.805	105.808	18.279

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

4.4 Blood composition

Complete blood count

Day 14 & 32

Blood collected from the broilers on day 14 before commencement of high cycling temperatures are shown in Table 4.11 Negative control group had a significant variation ($P < 0.05$) in segmented heterophils in comparison with VM1. No significant differences ($P > 0.05$) were noted in the complete blood count of broilers during the high cyclic temperature phase of the trial (Table 4.12).

Table 4.11 The effect of virginiamycin on the complete blood count of broilers (day 14) subjected to high cyclic temperatures from day 16[1]

	Treatment [2]				
	Negative control	**Zinc bacitracin**	**Virginiamycin 1**	**Virginiamycin 2**	**SEM**
Red cell count ($x10^{12}$ /L)	2.43	2.43	2.49	2.49	0.051
White cell count ($x10^{9}$ /L)	20.16	20.4	15.19	19.66	1.838
Segmented Heterophil ($x10^{9}$ /L)	7.91[a]	6.53[ab]	4.01[b]	6.25[ab]	1.210
Lymphocyte ($x10^{9}$ /L)	9.82	9.16	9.34	10.23	0.991
Monocytes ($x10^{9}$ /L)	1.02	1.02	0.77	0.72	0.216
Eosinophil ($x10^{9}$ /L)	0.57	2.21	0.49	1.46	0.633
Basophil ($x10^{9}$ /L)	0.8	1.11	0.59	1.00	0.188
Packed cell volume (%)	34.44	33.63	34.91	34.53	0.794

[a,b] Means within a row with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Table 4.12 The effect of virginiamycin on the **c**omplete blood count of broilers (day 32) subjected to high cyclic temperatures from day 16[2]

	Treatment[2]				
	Negative control	**Zinc bacitracin**	**Virginiamycin 1**	**Virginiamycin 2**	**SEM**
Red cell count ($x10^{12}$ /L)	2.61	2.62	2.59	2.63	0.063
White cell count ($x10^{9}$ /L)	21.60	23.51	23.23	22.98	1.909
Segmented Heterophil ($x10^{9}$ /L)	5.00	6.87	7.23	6.08	1.045
Lymphocyte ($x10^{9}$ /L)	13.26	13.03	12.29	12.63	0.880
Monocytes ($x10^{9}$ /L)	1.00	0.92	0.98	0.99	0.246
Eosinophil ($x10^{9}$ /L)	0.89	1.12	1.04	1.42	0.252
Basophil ($x10^{9}$ /L)	1.06	1.58	1.57	1.86	0.387
Packed cell volume (%)	31.88	31.67	30.91	31.44	0.722

[a,b] Means within a row with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

Corticosterone

Corticosterone serum levels collected from birds on days 14 and 31 presented no differences (P >0.05) between the treatment groups (Table 4.13).

Table 4.13 The effect of virginiamycin on corticosterone serum levels of broilers before (day 14) and during (day 31) high cyclic temperatures[1]

Treatment[2]	Day 14 (ng/mL)	Day 31 (ng/mL)
Negative control	1.17	1.40
Zinc bacitracin	1.60	1.55
Virginiamycin 1	1.04	1.59
Virginiamycin 2	1.72	1.83
SEM	0.266	0.199

[a,b] Means within a column with no common letters differ significantly (P < 0.05).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

SEM: Standard error of the mean

4.5 Mortality

No significance was found (p > 0.05) between moralities comparing treatments, periods and within weeks during the trial (Table 4.14). The only significance obtained (Table 4.15) was within treatment 1 and 2 and periods. Within period 1 there was a significance (p < 0.05) between moralities in period 1 (27.27%) versus period 2 (72.73%), as well as in treatment 2, period 1 is 30.77% and period 2 is 69.23%.

Table 4.14 The effect of virginiamycin on weekly mortality of broilers (number of mortality) for a total period of 32 days subjected to high cyclic temperatures[1]

	Days					
Treatment	**0-7**	**8-14**	**15-21**	**22-28**	**29-32**	**Total**
Negative control	4	5	12	8	4	33
Zinc bacitracin	2	6	12	3	3	26
Virginiamycin 1	5	12	6	6	1	30
Virginiamycin 2	3	7	6	5	1	22

[a,b] Means within a column with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

Table 4.15 The effect of virginiamycin on the mortality of broilers (number of mortality) before (period 1) and after (period 2) the onset of high cyclic temperatures between periods and within treatments[1]

	Period 1	**Period 2**
Treatment	**0 -15**	**16-32**
Negative control	9 [a]	24 [b]
Zinc bacitracin	9 [a]	17 [b]
Virginiamycin 1	17	13
Virginiamycin 2	10	12

[a,b] Means within a row with no common letters differ significantly ($P < 0.05$).

[1] High cyclic temperature was introduced at 32 °C and increased to 39 °C from day 16 to day 32. Temperature was gradually increased from 08:00 to 11:00 and returned to thermal neutral conditions at 18:00.

[2] The negative control group contained no antibiotic growth promoters. Zinc bacitracin served as the positive control group and contained 50 mg/kg zinc bacitracin with the exception of the post-finisher phase. Both Virginiamycin 1 and Virginiamycin 2 contained 20 mg/kg virginiamycin in all feed phases with the exception of the post-finisher phase for Virginiamycin 2.

Chapter 5
Discussion

5.1 Broiler performance

Temperature is one of the most important variables affecting feed intake and weight gain. High ambient temperatures result in a reduction of feed intake and weight gain, therefore affecting broiler performance (Berrong & Washburn, 1998; Daghir, 2009). Virginiamycin supplementation has been known to improve growth rates (Leeson, 1984; Miles & Harms, 1984; Henry *et al.*, 1986), feed efficiency (Leeson, 1984; Miles *et al.*, 1984; Henry *et al.*, 1986; Leeson *et al.*, 2005) and carcass yields (Leeson, 1984; Miles & Harms, 1984) under thermal neutral environments. Increased feed intake and greater body weight gains were observed during the starter (1-21 days) and grower (22-42 days) periods in birds supplemented with VM under thermal neutral conditions compared with the control (Rasouli & Jahanian, 2019).

In this study, body weight gain, feed intake, weekly feed conversion and PEF of broilers did not differ significantly between the treatments before or during high cycling temperatures. These results contradict numerous studies where growth rate, feed conversion ratio (Belay & Teeter, 1994; Rahimi & Khaksefidi, 2006; Zulkifli *et al.*, 2006) and feed intake (Belay & Teeter, 1994; Zulkifli *et al.*, 2006) were improved with the addition of VM in the feed of broilers exposed to heat stress. Results of this trial corroborate the findings of Belay & Teeter (1994) and Rahimi & Khaksefidi (2006) that no improvement were found in the carcass weight, feed intake (Rahimi & Khaksefidi, 2006) or body weight gain (Proudfoot *et al.*, 1990) of broilers supplemented with VM during heat stress. Little evidence in the current experiment support the notion of Zulkifli *et al.* (2000) that antibiotics such as VM ameliorates the response of broilers to heat stress. It should be noted that the feed analysis in this study indicated that the Na levels range 0.29-0.33 g/100g were much higher compared to the formulated levels 0.159-0.22 g/100g. This might have increased the water intake and the response of birds to heat stress and potentially to VM.

5.2 Carcass measurements

Thermally stressed broilers exhibit depressed protein retention due to decreased muscle protein synthesis and deposition (Temim *et al.*, 2000), an increase in total carcass fat and abdominal fat were observed by Howlider & Rose (2007); Al-Batshan & Hussein (1999) found that abdominal fat were not

affected by heat stress. Carcass composition measured by Belay & Teeter (1994) and Teeter & Belay (1996) was unaffected in broilers that were fed VM during both thermal neutral and high cycling temperatures. Furthermore, within the control group (no feed additives) that was exposed to high ambient temperatures, a decrease in dressing percentage and an increase in carcass fat was found. Breast weight was unaffected by VM fed to broilers under thermal neutral conditions (Leeson *et al.*, 2005). In contradiction of the above mentioned studies improved carcass weight (Henry *et al.*, 1986; Izat *et al.*, 1990b; Salmon & Stevens, 1990b; Rasouli & Jahanian, 2019) and breast weight (Rasouli & Jahanian, 2019) were found in birds fed VM under thermal neutral conditions, this could possibly be subscribed to the lower intestinal weight. The results of this study are therefore in agreement with Belay & Teeter (1994) where no significant differences were found in carcass composition of birds fed VM.

5.3 Intestinal measurements and morphology

Antibiotics control the growth and proliferation of microorganisms. Both VM and ZnB control lactic-acid producing bacteria as well as other gram-positive microorganisms but they do not have the same mode of action (Miles *et al.*, 2006). Zinc bacitracin suppresses cell wall formation by preventing peptidoglycan strands to form and thereby inhibiting protein synthesis (Kahn *et al.*, 2005). Virginiamycin blocks the ribosomal activity which inhibits the protein synthesis (Cocito, 1979). Ferket (2004) reported that antibiotic growth promoters control certain microorganism populations rather than sterilising the GIT. Miles & Harms (1984), Henry *et al.* (1986) and Izat *et al.* (1989) reported VM supplementation reduced intestinal weight under thermal conditions that may be the result of the thinning of intestinal mucosal membrane due to antibiotics (Coates *et al.*, 1955). The current research study is not in congruence with the above-mentioned studies, as results concluded that there were no statistical significant differences between intestinal weights on day 32 of treatment groups exposed to high ambient temperature.

Intestinal measurements that were taken on day 14 presented significance in the villi height of the ileum with the longest in both treatments groups ZnB and VM in comparison with the NC group. Under thermal neutral environments, Parsaie *et al.* (2007) reported that the duodenal villi were greater, jejunal villi remained unaffected whilst ileal villi decreased in birds supplemented with VM. Similarly, Khodambashi Emami *et al.* (2012) found duodenal villi height were increased and crypt depth decreased. The jejunal villi height were greater with a decreased crypt depth in birds given VM (Rasouli & Jahanian, 2019). The changes in intestinal morphology might be attributed to the antimicrobial properties of antibiotics such as VM and ZnB (Baurhoo *et al.*, 2007). The intestinal measurements taken on day 32, presented no significance in the morphology of the intestinal tract of the broilers. These

findings are in agreement with Quinteiro-Filho *et al.* (2010) that found broilers subjected to heat stress (either 31 or 36 °C) exhibited no change in the villi height or crypt depth of the duodenum, jejunum or ileum. In contradiction with the present study, Henry *et al.* (1986) suggested that the villi height of both the ileum and duodenum were shorter in those birds supplemented with VM, under thermal neutral conditions.

5.4 Blood composition

Heat stress has shown to have an immunosuppressing effect on broilers, although using different measurements such as lower levels of circulating antibodies and weights of lymphoid organs (Lara & Rostagno, 2013). Aengwanich (2008) found a reduction of bursa weight in thermally stressed broilers and decreased lymphocyte numbers in the cortex and medulla areas. The blood composition in this study presented that the segmented heterophils were significantly lower in the VM1 group in comparison with the NC group, before the onset of high thermal conditions. No further significant variation were found in the blood composition of either of the treatment groups during exposure of high cyclic temperatures during this study. These results are in line with Rahimi & Khaksefidi (2006) that found no significant differences in the white blood cell count, heterophils and lymphocytes when birds were under heat distress. According to Chancellor & Glick (1960) and Zulkifli & Siegel (2007) heterophils decrease and lymphocytes increase initially at the onset of high cycling ambient temperatures. Whereas, Prieto & Campo (2010) found increased levels of heterophils and reduced number of circulating lymphocytes. Mashaly *et al.* (2004) found a lower white blood cell count when birds were exposed to chronic heat stress.

Heat stress induces the release of corticosterone from the adrenal glands (Edens, 1978) and increased the plasma CS levels in poultry (Edens & Siegel, 1975; Garriga *et al.*, 2006). In this study, high ambient temperature did not affect the plasma concentration levels of CS. Similarly, Mack *et al.* (2013) reported that temperature did not affect the plasma CS concentrations of laying hens. This is in contrast with previous studies that have shown increased plasma CS concentrations in layers (Tanizawa *et al.*, 2014), broilers (Quinteiro-Filho *et al.*, 2010) and turkeys (El-Halawani *et al.*, 1973) both in acute (Quinteiro-Filho *et al.*, 2010) and chronic (Soleimani *et al.*, 2011) heat stressed broilers. According to Etches *et al.* (2008), from the initial response to thermal stress, increased plasma CS concentration could only possibly be maintained for a short period of time, thereafter CS starts to decline. The current study suggests that birds supplemented with VM had no differences in plasma CS concentration compared to other treatments, before or after exposure to high cyclic temperatures. These results could have been

affected by the stress birds felt during transportation to the abattoir and electrical stunning, therefore, possibly preventing the treatments to ameliorate the effect of heat stress.

5.5 Mortality

Mortality and poor performance of broilers is of concern when the ambient temperature is near or exceeds the body temperature (40.5-41.5 °C) of the bird (Ryder *et al.*, 2004). Berrong & Washburn (1998) found an increased mortality rate when broilers were exposed to 32 °C and 38 °C environments. Teeter & Belay (1996) found that there was an increase in survivability of birds fed VM 20 mg/kg as to the control, during both thermal neutral and heat distressed environments. Presumably survivability and better performance may be due to reduced immune challenge, heat production (Teeter, 1994) and improved nutrient absorption efficiency (March *et al.*, 1978) as a result of VM supplementation. Results obtained from Miles *et al.* (1984), Salmon & Stevens (1990a) and Waibel *et al.* (1991) contradict that VM had no effect on improving the mortality rate, under thermal neutral environments. No significance between mortality was observed when comparing treatments, period and within weeks during this trial. However, a significant difference was obtained within treatment NC and ZnB and periods. Within both NC and ZnB treatments the significance were between the mortality in period 1 versus period 2; this significance can be ascribed to the exposure of broilers to high ambient temperature during period 2. Mortality remained unaffected for both VM treatment groups therefore implying that during heat stress VM prevented an increase in mortality rate. These results corroborate the findings of Zulkifli *et al.* (2006) that survivability in broilers subjected to heat stress remained unaffected when fed VM.

Chapter 6
Conclusion

One of the most important factors that affect poultry production efficiency is high cycling temperatures throughout the world, even though the problem could be seasonal and variable in duration. Good management is the beginning of optimal broiler production, since commercial broilers are fast growing and efficient birds that are highly susceptible to heat stress. Management considerations should therefore include the environment and/or diet of broilers to alleviate the detrimental effects of heat stress. High ambient temperature affects broiler performance, carcass weights and composition, immunity, and mortality, all of which could lead to a great economic loss. Many studies have established that thermally stressed broilers has lower feed intake which affects the feed efficiency, weight gain and carcass weight. Carcass quality could be affected due to a possible increase in total carcass and abdominal fat in heat stressed birds. Research conducted has found that the immunity of heat stressed broilers may be suppressed as the total white blood cells and lymphocytes activity were negatively affected, and mortality rate increased.

Experiments designed to assess the efficiency of dietary antibiotics, such as VM, in alleviating heat stress related problems in broiler chickens are few and reports are often contradictory. Nevertheless, VM has proven to alleviate the detrimental effects of thermally stressed broilers. It has been reported that VM improved broiler performance and reduced immune challenge, gastrointestinal mass and mortality. In the current research, mortality remained unaffected for treatment VM1 and VM2 whereas the NC and ZnB had a significant increase in mortality during high ambient temperature. The unaffected mortality of broilers before and during heat stress might be due to lower heat production. The lowered heat production would be expected to be a result of both a reduction in immune challenge and reduced gastrointestinal tract mass. No significance was further found in the measured parameters of this trial between the treatment groups when broilers were exposed to the high cyclic temperatures. It has been established that the use of VM as an additive to alleviate heat stress did not improve broiler performance, carcass measurements or blood composition in comparison with the NC or ZnB treatment groups. Therefore, the benefit of supplementing VM to reduce mortalities may outweigh the costs of the inclusion of VM in the ration.

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