RESPONSES OF BROILER CHICKENS FED BETAINE HYDROCHLORIDE SUPPLEMENTATION UNDER DEXAMETHASONE INDUCED STRESS CONDITION

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE, DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE AHMADU BELLO UNIVERSITY, ZARIA

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DECLARATION

I hereby declare that this thesis entitled "**RESPONSE OF BROILER CHICKENS FED BETAINE HYDROCHLORIDE SUPPLEMENTED DIETS UNDER DEXAMETHASONE INDUCED STRESS CONDITIONS**" has been written by me under the supervision of Professor (Mrs) G.T. Iyeghe-Erakpotobor, Professor P.P. Barje and Dr. (Mrs) O.M. Daudu. It is the result of my investigation and has not been part of presentation for any other qualification in this or any other institution. All literature has been duly acknowledged and a list of references provided.

_____ _____

Lawrence Anebi ADEMU Date

CERTIFICATION

This thesis entitled "**RESPONSES OF BROILER CHICKENS FED BETAINE HYDROCHLORIDE SUPPLEMENTED DIETS UNDER DEXAMETHASONE INDUCED STRESS CONDITIONS**" by Lawrence Anebi ADEMU meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

To Adrian Ademu: gone but not forgotten

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ABSTRACT

Three studies were carried out to investigate the response of broiler chickens fed betaine hydrochloride supplemented diets under dexamethasone induced stress conditions. The first experiment was conducted using 240 day-old *Arbor acre* broiler chickens. There were four treatments with three replicates (n = 20 birds/rep) to which concentrations of dexamethasone at 0, 1, 2 and 3 mg/L of water were administered daily. Results indicated a decrease ($P > 0.05$) in rectal temperature and increase ($P > 0.05$) in respiratory rate with increasing dose of dexamethasone. Birds receiving 0 mg dexamethasone had higher ($P < 0.05$) final body weight, daily weight gain, daily feed intake and feed conversion ratio. Corticosterone and thyroxine levels were significantly ($P < 0.05$) increased with an increase in dose of dexamethasone. Carcass cut weight of thigh and drumstick were significant ($P < 0.05$) with increasing dose of dexamethasone. Liver weights were higher ($P < 0.05$) in the dexamethasone groups. Dexamethasone increased tibia weight, length, and weight/length index and robusticity index significantly ($P < 0.05$). Villus height was higher ($P < 0.05$) in the groups containing dexamethasone compared with the control. Feed intake, rectal temperature and respiratory rate were good predictors of final weight. The second experiment was conducted using 240 day-old *Arbor acre* broiler chickens. There were four treatments with three replicates (n= 20 birds/rep) to which concentrations of dexamethasone at 0, 1, 2 and 3 mg/L of water were administered daily. All dexamethasone treated birds were fed 0.15% betaine HCl in their diets. Results indicated a decrease ($P > 0.05$) in rectal temperature with increasing dose of dexamethasone. Birds receiving 0 mg dexamethasone had the highest ($P < 0.05$) final body weight, average daily weight gain, average daily feed intake and feed conversion ratio. Thigh and drumstick weights rose significantly ($P < 0.05$) with increasing dose of dexamethasone. Betaine HCl effect on tibia weight, length, and weight/length index were significant ($P < 0.05$) with the control group

performing better than the betaine treated groups. Feed intake, rectal temperature and respiratory rate were good predictors of final weight. The third experiment was conducted using 300 day-old *Arbor acre* broiler chickens. There were five treatments with three replicates (n= 20 birds/rep) made up of a treatment containing no dexamethasone and no betaine serving as the control. Another treatment was fed 0.30% betaine only supplemented diets, with the other three treatments given daily dexamethasone concentrations at 1, 2 and 3 mg/L of water, respectively. All dexamethasone treated birds were fed 0.30% betaine HCl in their diets. Results indicated a decrease ($P > 0.05$) in rectal temperature and respiratory rate with increasing dose of dexamethasone, especially with the betaine only group. Birds in the control and betaine only diets had significantly ($P < 0.05$) higher final body weight, daily weight gain and feed conversion ratio. Haematological indices were significantly ($P < 0.05$) increased for packed cell volume, heterophil, lymphocyte counts and hetrophil-lymphocyte ratio. Heart weight was significantly ($P < 0.05$) higher and the betaine only group had the least weight. Betaine increased tibia weight, length and weight/length index and ash was significantly ($P < 0.05$). Bursa weight was significantly ($P < 0.05$) higher with the 3 mg treated group having the least weight. Crypt depth was significant ($P < 0.05$) with the betaine-containing groups having higher crypth depth. The results showed that feed intake, rectal temperature and respiratory rate were good predictors of final weight. It was concluded that dexamethasone-induced stress had negative effects on broiler performance, organs, and tibia and blood compositions. Betaine HCl had positive effects on broiler performance, serum parameters, carcass cuts, viscera and immune organs. Thermoregulatory parameters with growth performance indices were good predictors of final weight of broiler chickens.

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CHAPTER ONE

1.0 INTRODUCTION

It is estimated that world food consumption will double by 2050 as more developing nations improve their economic status and per capital meat consumption increase (Godfray *et al*., 2010). This places an increasing pressure on animal producers especially poultry farmers to maximize output levels of their animals to meet this growing demand. Physiological stress is one of many concerns facing the modern broiler producer. The term stress is very familiar to most researchers, but there is no universal definition for stress. When a stressor is actually causing a negative impact on the well-being of an animal, this can be defined as distress (Moberg, 2000). Another broader definition states that stress is any biological response elicited when an animal perceives a threat to its homeostasis (Moberg, 2000). Extremes of ambient temperature is an important stressor that confronts poultry in many regions of the world and large economic losses occur because of mortality and decreased production (Altan *et al*., 2000).

The thermoneutral zone for poultry is 18°C–24°C in the tropics and 12°C–26°C in the temperate zones, but this often gets exceeded in the tropics, resulting in heat stress (Holik, 2009; Dei and Bumbie, 2011). When the hypothalamic-pituitary-adrenocortical axis is activated, the hypothalamus produces corticotrophin-releasing factor, which in turn stimulates the pituitary to release adrenocorticotropic hormone (ACTH) (Mormède *et al.,* 2007). Secretion of ACTH causes the cells of the adrenal cortical tissue to proliferate and to secrete corticosteroids. The main active hormone of the axis is cortisol in cattle, sheep, pig, mink, fox and fish, and corticosterone (CS) in birds and rodents (Mormède *et al.,* 2007). These are cholesterol-derived steroids synthesized in the fascicular zone of the adrenal cortex under the control of the pituitary hormone. ACTH is synthesized by specialized cells (corticotrophs) of the anterior pituitary gland

(Mormède *et al.*, 2007).The production of glucocorticoids is increased by stress; therefore, corticosterone can be used as a biomarker of stress in poultry.

In chickens, adrenal corticosteroids are secreted shortly after exposure to stress and elevated levels of plasma glucocorticoids have been used as an index of the response to stress in poultry (Siegel, 1995). By elevating circulatory corticosteroids and decreasing thyroid activity, heat stress impairs broiler performance, especially adult birds, because the ability to dissipate heat decreases with age (Mahmoud *et al*., 2014). Drastic decline in feed intake occurring in heat-stressed birds is a physiological response to minimize intrinsic heat production. It is aimed at maintaining thermal homeostasis, thus decreasing feed efficiency, live weight gain, and survival rates (Faria-Filho *et al*., 2007). Lower breast-meat yield and higher carcass-fat deposition are the other deleterious effects of heat stress that lower the economic value of broiler carcasses (Geraert *et al*., 1996; Ain-Baziz *et al*., 1996). Corticosterone has an indirect role in lipid metabolism by causing the rate of fat deposition to increase in poultry (Jiang *et al*., 2008; Yuan *et al*., 2008). Glucocorticoid hormone secretion also has implications for mineral metabolism, thus corticosteroids have been directly implicated in the development of osteoporosis in stressed animals (Siegel and Latimer, 1970). It has also been shown that glucocorticoids support the action of catecholamines, which have been shown to increase urinary calcium and sodium excretion (Fink and Brody, 1978).

Corticosteroids have been shown to inhibit several immune system functions in various species, as demonstrated in depressed number of circulating lymphocytes, which results in an increase in the ratio of circulating heterophils to lymphocytes, the most recognizable symptom of stress in poultry (Siegel, 1995). Regression of the thymus, bursa, and spleen has also been demonstrated in chickens after corticosterone or ACTH administration *(*Puvadolpirod and Thaxton, 2000a).

Similarly, synthetic glucocorticoid dexamethasone administration mimics the adverse effects of increased corticosterone. Dexamethasone (doses ranging from 0.2 to 4.0 mg/kg) has been used as an immune suppressive agent (Fowles *et al*., 1993), mediator of prenatal stress (Maccari *et al*., 2003) and to induce oxidative stress in laying hens (El-Habbak *et al*. 2005) and cockerels (Eid *et al*., 2006). Aengwanich (2007) demonstrated that synthetic glucocorticoid; dexamethasone in doses of up to 6 mg/kg in their diets had many effects on broilers like internal glucocorticoid.

Certain feed additives (selenium, vitamins C and E, α-lipoic acid, α-tocopherol, prebiotics and probiotics) enhance performance in heat-stressed broilers (Ghazi Harsini *et al*., 2012; Hamano, 2012; Imik *et al*., 2012; Khan *et al*., 2012; Sandhu *et al*., 2012; Sohail *et al*., 2012). Betaine, a methyl group donor, functions in lipid metabolism by stimulating oxidative catabolism of fatty acids through carnitine synthesis. Betaine is also an organic osmolyte and does not interfere with enzyme function or upset metabolism (Simon, 1999). As an osmolyte, betaine may have a stabilizing function on cells subjected to osmotic stressors, such as in the case of coccidiosis infection (Klasing *et al*., 2002) by regulating the water balance, resulting in the stability of tissue metabolism especially in the gastrointestinal tract (Lipinski *et al.*, 2012). Dietary supplementation of betaine presumably reduces the requirement for other methyl-group donors, such as methionine and choline (Siljander-Rasi *et al*., 2003). Florou-Paneri *et al*. (1997) showed that between 30 and 80 percent of supplemental methionine can be substituted by betaine without negative effects on performance. As a feed additive, betaine is most commonly added to animal diets as anhydrous betaine; betaine monohydrate, and betaine hydrochloride (Kidd *et al*., 1997; Eklund *et al*., 2005). Within the body, betaine is synthesized from choline (Sakomura *et al*., 2013).

1.1 Justification

A variety of stressors have been used to study stress responses in poultry species. These stressors include mediation of the adrenal glands directly by exogenous administration of adrenocorticotropin (ACTH) and exogenous administration of steroid moieties, including corticosterone, cortisone, cortisol, deoxycorticosterone, and dexamethasone. In addition, various environmental conditions, including hot and cold regimes, have been employed. Other stressors that have been evaluated include injections with various pharmacological preparations, such as reserpine, propranolol, norepinephrine, serotonin, and l -dopa (Puvadolpirod and Thaxton, 2000c). Although all of these methods have produced signs of physiological stress (Puvadolpirod, 1997), there seems to be more similarity in some of the responses that are typically demonstrated when stress is induced at the adrenal level.

The administration of corticosterone or analogues of corticosterone is a promising tool in the research on adaptation to stress in broiler chickens (Post *et al.,* 2003). Majority of studies on corticosteroids have focused only on the immunosuppressive actions of the stress hormones (Strickland *et al.*1986; Wiegers and Reul, 1998; Post *et al.*, 2003; Lopez *et al*., 2007), and not on their effect on performance indices, lipid metabolism and mineral retention.

Stress is a great problem for the broiler industry; however, nutritional improvements may be able to lessen its effects. Betaine content is low in animal feedstuffs; hence, supplementation is essential for improving performance and stress resistance in poultry (Wang *et al.* 2004). Singh *et al*. (2015) reported that synthetic betaine HCl (2 g/kg diet) may be used as a tool to improve performance of broilers under thermal stress conditions. Attia *et al*. (2009) also showed that the impact of severe heat stress could partially be overcome by adding betaine (1g/kg of diet) to the diet in slow-growing broilers. Supplemental dietary betaine improved weight gain and feed

conversion in some poultry studies (Mathews and Southern, 2000; Hassan *et al*., 2005), whereas other studies showed minimal or no effect of betaine on animal performance (Zulkifi *et al*., 2004; Feng *et al*., 2006).

Literature indicates clearly that a reliable model to study stress in poultry is lacking. One possible strategy for improvement in this area is to conduct nutritional research by using a model that induces physiological stress in broilers using a specific stressor. If researchers had knowledge of a nutrient potential to ameliorate the detrimental effects of physiological stress, then future research involving nutrition and more specific stressors could be conducted more efficiently. The two major criteria of an acceptable stress model are a treatment that is exact and highly repeatable and a predictable set of stress responses that occur in a known temporal pattern. The purpose of this study was to define a stress model in chickens. This model employed continuous delivery of a defined dosage level of dexamethasone for a defined time period via drinking water.

1.2 Objectives of the Study

The objectives of the study are:

1. To determine of the effect of varying doses of dexamethasone on:
    - Thermoregulatory responses of broiler chickens
    - Growth performance and carcass traits of broiler chickens
    - Immune organ weight and tibia composition in broiler chickens
    - Blood chemistry, corticosterone and thyroxine concentrations of broiler chickens
2. To determine the effect of betaine hydrochloride supplemented diets in dexamethasone-treated broiler chickens on:

- Thermoregulatory responses
- Growth performance and carcass traits.
- Immune organ weight and tibia composition.
- Blood chemistry.

Research Hypotheses

The research hypotheses are:

H_{01}: Treatment with dexamethasone has no effect on thermoregulatory responses, production performance, carcass traits, immune traits and blood parameters of dexamethasone stressed birds,

H_{A1}: Treatment with dexamethasone has effect on thermoregulatory responses, production performance, carcass traits, immune traits and blood parameters of dexamethasone stressed birds

H_{02}: Treatment with betaine hydrochloride has no effect on thermoregulatory responses, production performance, carcass traits, immune traits and blood parameters of dexamethasone stressed birds,

H_{A2}: Treatment with betaine hydrochloride has effect on thermoregulatory responses, production performance, carcass traits, immune traits and blood parameters of dexamethasone stressed birds.

CHAPTER TWO

2.0 LITERATURE REVIEW

The broiler industry has achieved great efficiency through improved genetics, improved nutrition, and the advent of the confined animal feeding operation. Due to confinement and rapid growth of today's broilers, heat stress is a major problem. Some problems associated with extreme heat stress in broilers include respiratory alkalosis, decreased performance (Lara & Rostagno, 2013); decreased breast meat yield and slower growth rate (Aksit *et al*., 2006).

One of the challenges the producer must overcome in the pursuit of his production goals is potential stressors that the broiler may experience during life. Undoubtedly, the bird experiences various stressors each day in production. Short-term stress can be expected, and with the exception of situations such as acute heat stress, is typically of minimal concern. However, long-term stress can have far-reaching detrimental effects on poultry production. Climate change, human disturbance and poor housing quality are some factors that can lead to chronic stress in birds (Scheuerlein *et al*., 2001; Schoech *et al*., 2009). Chronic stress that lasts for days or even weeks can have deleterious effects, such as inhibiting reproductive functions and reducing body weight (Breuner *et al*., 2008). One way to study the effects of stress on the performance of the birds is treating birds with exogenous glucocorticoids to stimulate its rise in plasma which occurs during stress (Tasker and Herman, 2011). Glucocorticoid concentrations can then be related to physiological or behavioural changes that occur to restore homeostasis (Sapolsky *et al*., 2000). The main outcome of this pathway is elevated blood corticosterone, the primary glucocorticoid in birds (Romero and Reed, 2008). Research needs to be conducted to aid in producers' ability to combat the detrimental effects of stress in poultry through different nutritional regimens. A possible strategy for improvement in this area is to conduct research by using a model that

induces physiological stress in broilers using a specific stressor (hypo or hyper-thermic temperatures, elevated sound levels, over-illumination, overcrowding). If researchers had knowledge of a nutrient or combination of nutrients that offers the potential to ameliorate the detrimental effects of physiological stress, then future research involving nutrition and more specific stressors could be conducted more efficiently. Hence, research with more practical applications could be conducted more easily if a better foundation were available.

The world's population is constantly increasing, as well as the number of developed nations, meaning that the amount of animal protein produced will need to increase in order to meet demand in the future. To achieve this all production systems, even broiler production as efficient as it is, will need to improve efficiency. Improved efficiency will not be allowed to come at the expense of animal welfare, so improvements will have to be made carefully.

2.1 Use of Dexamethasone in Inducing Physiological Stress

Dexamethasone is an immunosuppressant drug that is widely used to study some stressor effects in disease development and course in poultry (Huff *et al.*, 2001, 2013; Shini *et al.*, 2010). Given its similarity to endogenous corticosteroids, dexamethasone treatment is thought to reproduce the effects of high levels of corticosterone, thus mimicking stress associated signaling pathways (Devenport *et al*., 1989). Glucocorticoid has been designated as the stress hormone because its levels in circulation rise sharply in response to stress (Hardy *et al*., 2005). As one of the analogs to glucocorticoid, dexamethasone has been used in many studies to simulate the effects of glucocorticoid (Eid *et al*., 2006).

Dexamethasone is a synthetic glucocorticoid that has been widely used for in vitro and in vivo studies of the glucocorticoid effects on a number of different cellular and physiological responses (Bertolo *et al,* 2011). In biological psychiatry, dexamethasone has been extensively

used to probe Hypothalamus-Pituitary-Adrenal (HPA) axis negative feedback sensitivity to glucocorticoids (Ribeiro *et al.,* 1993). In literature, dexamethasone has been employed as an experimental model of stress effects on various diseases, such as clostridial dermatitis (Huff *et al*., 2013). These studies have inferred that the immunomodulatory effects of dexamethasone some how mimic those commonly induced by stressors, i.e., they may similarly trigger the development or modify the course of poultry diseases (Huff *et al*., 2013; Shini *et al.*, 2010). Sabeur *et al* (1993) found that when chickens received dexamethasone, it caused muscular dystrophy and reduced growth. Dexamethasone has been found to negatively affect feed intake in studies involving poultry (Sapolsky *et al.,* 2000; Malheiros *et al*., 2003; Lin *et al.,* 2004; Hanafy and Khalil, 2015).

2.2 Betaine and its Applications in Animal Nutrition

Although the normal broiler body temperature is 41°C, optimum growing temperature is 21 to 24°C, and heat stress can be elicited by conditions such as high temperature and high humidity depending on age (Teeter and Belay, 1996). The detrimental effects of heat stress have been shown to be alleviated by dietary means (Khan *et al*., 2012; Sandhu *et al*., 2012; Sohail *et al*., 2012), prior exposure to heat treatment (Arjona *et al*., 1988; De Basilio *et al*., 2001), chilled drinking water (Smith and Teeter, 1987), fasting (Ait-Boulahsen *et al*., 1989), and house cooling measures (Teeter and Belay, 1996). Thus, heat stress exerts adverse effects on the broiler industry, and nutritional improvements may lessen its effects.

Betaine, also known as trimethylglycine, is a zwitterionic quaternary ammonium compound. Betaine is found in many foods e.g sugar beets, wheat bran, spinach (Zeisel *et al*., 2003), and since it can be manufactured in the mitochondria, it is not considered essential (Craig, 2004). Betaine supplementation to diets for livestock has increased during the last decade (Feng *et al*.,

2006; Fernandez-Figares *et al*., 2008). Betaine, the trimethyl derivative of the amino acid glycine, is a naturally occurring compound, which is widely distributed in many plants and animal tissues. It is present in large quantities in aquatic invertebrates and sugar beets, but also in wheat, wheat products and lucerne meal (Kidd *et al*., 1997; Chendrimada *et al*., 2002). Common sources of betaine are sugar beets and their by-products such as molasses and condensed molasses solubles (Eklund *et al*., 2005). As a feed additive, betaine is also available in purified form and most commonly added to animal diets in the form of anhydrous betaine, betaine monohydrate and betaine hydrochloride (Kidd *et al*., 1997; Eklund *et al*., 2005). Betaine is stable and non-toxic (Yu *et al*., 2004).

Due to its chemical structure, betaine has a number of different functions both at the gastrointestinal and metabolic level (Eklund *et al*., 2005). Betaine donates its labile methyl group which can be used in transmethylation reactions for synthesis of substances like carnitine and creatine (Kidd *et al*., 1997). Therefore, the dietary supplementation of betaine may reduce the requirement for other methyl group donors such as methionine and choline (Siljander-Rasi *et al*., 2003). Betaine is an essential osmoprotectant, primarily in the kidneys, liver, and brain, and large amounts of betaine can accumulate in cells without disrupting cell function; importantly, this role of betaine protects cells, proteins, and enzymes under osmotic stress (Kempson *et al*., 2013). Betaine is considered the most effective organic osmolyte and its function as an osmolyte is based on the fact that it is a zwitterion, which is, carrying both a positive and a negative charge on the same molecule at the same time (Zheng *et al*., 2005). Due to its osmotic properties, betaine may have the potential to improve the digestibility of specific nutrients (Eklund *et al*., 2006). It accumulates in gastrointestinal cells, regulating water flux across the intestinal epithelium. Betaine is also shown to inhibit cellular apoptosis and to reduce energy expenditure

of gastro-intestinal cells. Schrama *et al*. (2003) found a 5% reduction in energy requirements for maintenance of the gastrointestinal cells in pigs fed betaine. Furthermore, betaine is involved in protein and energy metabolism due to its methyl group donor function (Eklund *et al*., 2005).

Betaine is absorbed in the duodenum. Human studies have shown rapid absorption and distribution with a peak increase in the serum one to two hours following food intake. Weigand and Kirchgessner (1981) reported that betaine is absorbed in the gastrointestinal tract (GIT), whereas up to three-fourths of it could remain at the intracellular level. Intracellular accumulation takes place via active and passive transport systems. Specifically, betaine can be freely filtered in the kidney and reabsorbed into the circulation, so it is primarily excreted in sweat instead of urine (Craig *et al*., 2010).

Following betaine supplementation to diets for poultry, several authors reported a reduction in abdominal fat weight, whereas breast meat yield was increased in broiler chicken (Zhan *et al*., 2006), turkeys (Noll *et al*., 2002) and meat ducks (Wang *et al*., 2004). Under heat stress conditions, supplementation of betaine enhanced egg production and egg shell quality in laying hens (Ryu *et al*., 2002), and to improve weight gain of broiler chicks (Farooqi *et al*., 2005). Attia *et al.* (2009) showed that the effect of severe heat stress could partially be overcome by adding betaine to the diet in slow-growing broilers. Adding 1 kg of betaine to the diet improved weight gain and feed conversion compared to a negative control treatment. More importantly, rectal temperatures decreased from 43.2°C to 41.9°C compared to the negative control. Panting, a mechanism of heavily breathing to lose heat via evaporation was also reduced from 78.3 breaths per minute to 63.9 breaths per minute. These results on performance, rectal temperature and panting were confirmed by Hassan *et al*. (2011), who showed a clear dose-response effect when betaine was added in 250 g, 500 g, 750 g or 1,000 g per metric ton of feed to the diet of rabbits

kept under severe heat stress conditions. In contrast, Zulkifli *et al*. (2004) could not show any effects of betaine on weight gain and feed conversion in broilers reared under heat stress conditions. Waldroup and Fritts (2005) also did not show any effect of betaine supplementation on carcass characteristics in poultry. The involvement of betaine in lipid metabolism offers an interesting perspective in meat production to satisfy consumer's needs for lean meat. Due to the reduction of carcass fat content and increase in carcass lean, betaine is often referred to as 'carcass modifier' (Jacela, 2011).

The mode of action of betaine as 'carcass modifier' may be related to its methyl group donor properties (Eklund *et al*., 2005). The improvement in carcass lean percentage may be attributed to a higher availability of methionine and cystine for protein deposition (McDevitt *et al*., 2000). An enhanced utilization of dietary amino acids for protein synthesis may result in fewer amino acids available for deamination and eventual synthesis of adipose tissue (Wallis, 1999). Accordingly, changes in hormone levels and growth factors involved in the regulation of fat synthesis and degradation, as well as lower activities of lipogenic enzymes have been observed following dietary betaine supplementation (Huang *et al*., 2006).

2.3 The Concept of Stress

The term stress is very familiar to most researchers. However, there is no universal definition for stress. One definition states that stress is any situation that elicits the biological stress mechanisms of an animal (Selye, 1936). Another broader definition states that stress is any biological response elicited when an animal perceives a threat to its homeostasis (Moberg, 2000). When a stressor is actually causing a negative impact on the well-being of an animal, this can be defined as distress. The stress response is sometimes quantified in order to specify and determine

the stressors'severity. It can be classified as mild, moderate and severe stress, and the outcome differs (Moberg, 2000).

Stressors stimulate the Sympathetic-Adrenal-Medullary (SAM) axis and the HPA axis, which enhances the production of glucose needed for survival during stress (Shini *et al*., 2008). Ognik and Sembratowicz (2012) claimed that the main distinguishing factor between a stressor and non-stressor is the activation of the HPA axis.

Stress can be classified as either acute or chronic. Acute stress can be defined as a single exposure to a stressor (Schoenfeld and Gould, 2012), whereas chronic stress is induced if either the stressor itself or the after-effect of the stressor lasts for a long term (Wiepkema and Koolhaas, 1993). No specific duration has been reported as to when chronic stress is experienced, however studies have used a range of duration from 7 to 49 days (Post *et al*., 2003; Olanrewaju *et al*., 2006; Shini *et al*., 2008; Vahdatpour *et al*., 2009). It has been argued that acute stress is beneficial to the animal (Dickens *et al*., 2010) because the initiation of the stress response is associated with an increase in blood glucose, increased behaviour directed at fleeing or freezing, increased vigilance and increased cognition (Romero *et al*., 2009). However, studies show that prolonged secretion of corticosterone during chronic stress can have damaging effects on the animal, such as suppression of the immune system, breakdown of muscle due to gluconeogenesis, cardiovascular problems, fear, suppressed cognition and induced depression (Romero *et al*., 2009). To perceive how long-term stress can eventually cause a negative impact on the well-being of an animal, it is necessary to understand the physiological processes an animal undergoes when it is confronted with a stressor.

Stress responses can be categorized as specific or non-specific (Siegel, 1980). Specific stressors are typically short-term, such as a sudden increase in environmental temperature and animals

typically react to specific stressors by trying to combat the stressor (Siegel, 1980). Long-term or non-specific stress, however, results in the animal taking measures to adapt the stressor, rather than dealing with it directly (Siegel, 1980). When an animal first encounters a stressor, the neurogenic system is activated. The neurogenic system is composed of the sympathetic postganglionic neurons and adrenal medullary tissue. This response was previously referred to as the "fight or flight" response. Activation of the neurogenic system leads to marked increases in blood pressure, muscle tone, nerve sensitivity, blood sugar, and respiration (Siegel, 1980). This is brought about by secretion of the neurogenic amines epinephrine and norepinephrine (Siegel, 1971).

2.4 Hypothalamic-Pituitary-Adrenal (HPA) Cortical System

The HPA axis has the classical architecture of the major neuro-endocrine systems. The main active hormone of the axis is cortisol in cattle, sheep, pig, mink, fox and fish, and corticosterone in birds and laboratory rodents.Once secreted; corticosterone is transported through the blood to the target organs which have receptors for binding with the hormone (Nelson, 2005). Corticosterone receptors are present in the brain, liver, kidney, lung, intestines, thymus, bursa of fabricius and the nasal gland (Hess, 2006). Corticosteroids are cholesterol-derived steroids synthesized in the fascicular zone of the adrenal cortex under the control of the pituitary hormone, specifically adrenocorticotropic hormone (ACTH). The ACTH is synthesized by specialized cells of the anterior pituitary gland (corticotrophs) and its release is triggered by the coordinated action of two neuropeptides, the corticotrophin-releasing hormone (CRH) and argininine vasopressin (AVP) that are synthesized in specialized neurons of the paraventricular nucleus of the hypothalamus and released in the capillary bed of the median eminence from

where they reach the pituitary directly via the hypothalamic–pituitary portal circulation (Fink, 2010).

The hypothalamus receives numerous inputs from other hypothalamic nuclei (these inputs relay metabolic and nycthemeral signals), from the brain stem (in relation with neural inputs from the periphery), from the subfornical organ (that monitors blood plasma composition) and from the limbic system (that generates signals related to the emotional state). This multiplicity of signals converging at the hypothalamus explains the sensitivity of the HPA axis to a wide range of stimuli from both internal and external origin. Furthermore, cortisol/corticosterone exerts a negative feedback on the axis by acting on the pituitary corticotrophs, the hypothalamus and higher levels in the central nervous system. This feedback action of cortisol/corticosterone participates in the return of the HPA axis activity to basal levels after stimulation (Manteuffel, 2002).

Stressful situations cause an increase in the activity of the hypothalamo–pituitary–adrenal (HPA) axis, and this has been studied in great detail in mammals (Vazquez, 1998; Makino *et al*., 2002; Tsigos and Chrousos, 2002) as well as in birds (Harvey and Hall, 1990). Being the end-product of the action of the HPA axis, glucocorticoids act at several loci to exert a negative feedback, which results in an inhibition of adrenocorticotropin (ACTH) secretion. Failed attempts to combat or flee from the stressor immediately results in the activation of the HPA system (Siegel, 1980). When this system is activated, the hypothalamus produces corticotrophin-releasing factor, which in turn stimulates the pituitary to release ACTH (Holmes and Philips, 1976). Secretion of ACTH causes the cells of the adrenal cortical tissue to proliferate and to secrete corticosteroids (Holmes and Philips, 1976). If corticosteroids remain at elevated levels in circulation, there are many possible effects, including, but not limited to, changes in glucose and mineral metabolism,

cardiovascular diseases, hypercholesterolaemia, gastro-intestinal lesions, and alterations in immune system function (Siegel, 1995). The distribution and delivery of corticosteroids to the tissues are controlled, at least partially, by corticosteroid-binding globulins (Siegel, 1995).

In mammals, corticosteroid secretion appears to be mediated largely by the hypothalamus and pituitary (Siegel, 1980). This is evident because in mammals, when the anterior pituitary is transplanted to other areas of the body or when the neuro-humoral link from the hypothalamus to the pituitary is severed, circulating corticosteroid decline to levels similar to those occurring following surgical extirpation of anterior pituitary (Siegel, 1980).

2.5 Core Body Temperature (CBT) in Poultry

The total heat produced in the course of digestion, excretion and metabolism of nutrients is called heat increment. Within a certain range of ambient temperature and besides unvarying feed and nutrient intake the total heat production of the animal remains constant (Babinszky *et al*., 2011). This temperature range is called the thermoneutral zone. In a thermoneutral environment, the heat production of the animal is at the minimum, and thus the dietary energy can be used for production (growth, egg and milk production) efficiently. Unfavorable temperatures (too cold or too hot environments) lead to an increased heat production by the animal, that is, there is more loss of energy, and inconsequence less energy remains for production at the same level of energy intake, and the efficiency of energy utilization deteriorates.

The upper and lower critical temperatures vary for different animal species and age groups. Those for day old and finishing broiler chickens are 25-32ºC and 16-26ºC, respectively (FASS, 2010). Like all other avian species, birds are homeothermic in nature, meaning that they have the ability to maintain a stable internal (that is, core body temperature, CBT) environment under

extreme environmental conditions (Schwab and Schafer, 1972), which gives birds the opportunity to adapt through changes in their physiology or behaviour under environmental conditions that are outside the normal conditions (Iyasere, 2014). However, this may depend on the severity and duration of the environmental conditions. One of the homeostatic processes involves the regulation of CBT (Sherwood *et al*., 2005) since the normal CBT of broilers is between 40.6-41.7^{o}C (Olanrewaju *et al*., 2010).

The body of an animal can be divided into core and periphery. The body core consists of the central nervous system (brain and spinal cord), visceral organs and parts of the skeletal musculature, while the periphery includes the other parts of the skeletal musculature, skin, feathers, un-insulated extremities and subcutaneous fat layer (Schwab and Schafer, 1972). The main thermoregulatory centre is the hypothalamus which depends on signals sent from temperature receptors (thermoreceptors) present on body surfaces (periphery) and in the core (central). The anterior hypothalamus controls heat loss, while the posterior controls heat gain (Rastogi, 2007).

Broiler chickens exposed to high temperature and relative humidity (RH) find it difficult to maintain their core body temperature (Borges *et al*., 2007) because hot-humid environments reduce the opportunity for heat loss through sensible and evaporative means (Widowski, 2010). Hence, an animal can be said to be under heat stress if the prevailing environmental conditions (high ambient temperature and RH) impair heat loss from the body, thus resulting in an increase in core body temperature (CBT) (Jensen and Toates, 1997). The standard means of measuring CBT is from a digital thermometer inserted into the rectum of the animal (Quimby *et al*., 2009). However, the procedure of handling and restraining the bird could interfere with core body

measurement (Lowe *et al*., 2007). In addition, this method is laborious to use on a large population of animals.

2.6 Mechanisms for Combating Stress in Poultry

The secretion of corticosterone is initiated by a wide range of stressors (Cockrem, 2007) which could either be from an internal or an external source (Borell, 2001). External stressors listed for broiler breeders are extreme heat and cold or high humidity, bright light, wet litter, poor ventilation, rapid growth, catching, transport and overcrowding (Rosales, 1994). Chickens have cooling mechanisms that allow them to survive at above optimal temperatures. The two main methods that chickens use are evaporative and non-evaporative cooling. Non-evaporative cooling occurs via convection and is enhanced by air movement in the house, while evaporative cooling occurs through the evaporation of water from the lungs. This method of heat dissipation is enhanced by low humidity and increased respiratory rates. It has been shown that non-evaporative cooling is more efficient than evaporative cooling (Teeter and Belay, 1996) and is therefore the first line of defense, but as the ambient temperature rises to near body temperature, this method becomes ineffective (Teeter and Belay, 1996) and evaporative cooling becomes necessary. As the ambient temperature gets closer to broiler body temperature, respiration rate will increase (Linsley and Burger, 1964) thereby initiating a higher rate of evaporative cooling (Teeter and Belay, 1996). High humidity will also compound the problem, since evaporative cooling rate is inversely related to humidity (Teeter and Belay, 1996). It is understood that chickens have means to cool themselves, but due to its ineffectiveness under certain climatic conditions, proper management is paramount (Summers, 2013).

When temperatures exceed the effective range for non-evaporative cooling, the way that chickens cool themselves causes certain physiological changes that further complicate the issue.

Evaporative cooling is accomplished by increasing the respiratory rate (Toyomizu *et al*., 2005; Renaudeau *et al*., 2011), also known as panting, and this can lead to respiratory alkalosis (Sandercock *et al*., 2001; Toyomizu *et al*., 2005; Steiss and Wright, 2008; Imik *et al*., 2013). Acid-base imbalance has been shown to cause electrolyte imbalance (Borges *et al*., 2004) and increased electrolyte excretion (Belay *et al*., 1992). Acid-base imbalance and the resulting electrolyte imbalance, caused by heat-induced panting, may play a major role in the decreased performance (Bartlett and Smith, 2003; Smith *et al*., 2003; Ahmad and Sarwar, 2006; Quinteiro-Filho *et al*., 2010; Willemsen *et al*., 2011; Ghazi Harsini *et al*., 2012; Quinteiro-Filho *et al*., 2012; Sohail *et al*., 2012), altered meat quality (Aksit *et al*., 2006; Imik *et al*., 2012; Zhang *et al*., 2012), and increased mortality (Arjona *et al*., 1988) recorded in heat-stressed broilers. To increase evaporative cooling from body surfaces, birds can splash water on their combs and wattles (Dawson and Whittow, 2000).

2.7 Physiological Consequences of Long-term Stress

Predominantly, the effects of long-term physiological stress can be categorized as immunological or metabolic. Extremes of ambient temperature are important stressors that confront poultry in many regions of the world and large economic losses can occur because of mortality and decreased production (Altan *et al*., 2000). Broiler chickens subjected to heat stress show elevated corticosterone levels and lower thyroid hormones (Mahmoud *et al*., 2014). Corticosteroids have been shown to inhibit several immune system functions in various species, including lymphocyte proliferation, immunoglobulin production, cytokine production, cytotoxicity, and anti-inflammatory agents (Munck *et al*., 1984). Poultry treated with CS or ACTH has demonstrated depressed number of circulating lymphocytes (Siegel and Latimer, 1970). The effect of this reduction in lymphocyte numbers is an increase in the ratio of circulating heterophils to

lymphocytes, which is probably the most recognizable symptom of stress in poultry (Siegel, 1995). The cause of this decrease in lymphocyte numbers is probably due to the regression of lymphoid tissue caused by the presence of circulating corticosterone for prolonged periods (Siegel, 1971). Regression of the thymus, bursa, and spleen has been demonstrated in chickens after CS or ACTH administration (Puvadolpirod and Thaxton, 2000 a, b, c). Glick (1967) observed that lymphocytes were depleted at germinal centers after ACTH or corticosterone injections, indicating that lymphocyte production is inhibited by lymphoid tissue atrophy.

The most pronounced consequence of chronic stress is probably the alteration of metabolic function. Primarily, stress-induced metabolic alterations seem to be focused on the mobilization or production of glucose for energy needed to maintain homeostasis in the presence of the stressor. During stress, animals adapt by creating a tissue priority hierarchy by which nutrients are devoted to certain tissues based on their order of importance (Touchberry, 1984). For example, stressed animals devote nutrients according to the following tissues, in order from greatest to least: neural, visceral, bone, muscle, and adipose (Touchberry, 1984).

One of the chief metabolic functions of corticosterone is to promote gluconeogenesis by causing the liberation of substrates from body tissues necessary for endogenous glucose production (Exton, 1979). From a meat production standpoint, the most detrimental effect of this action is the catabolism of structural protein to free amino acids for use as gluconeogenic substrates (Puvadolpirod and Thaxton, 2000d). There is evidence that this glucose production occurs at the expense of structural protein in poultry treated with corticosterone. Glucocorticoids have a particular predilection for inducing the atrophy of skeletal muscle, with cardiac muscle being largely protected (Sandri *et al*., 2006; Shimizu *et al*., 2011). Glucocorticoids have an indirect role

in lipid metabolism. Corticosterone exerts a permissive effect on catecholamines to initiate lipolysis (Fain, 1979). Glucocorticoid hormone secretion also has implications for mineral metabolism. Corticosteroids have been directly implicated in the development of osteoporosis in stressed animals (Siegel and Latimer, 1970). This is probably caused by interference with intestinal absorption of calcium through inhibition of the synthesis of carrier proteins (Feher and Wassermen, 1979). Additionally, glucocorticoids support the action of catecholamines, which have been shown to increase urinary calcium (Morey and Kenney, 1964) and sodium (Fink and Brody, 1978) excretion.

Studies reveal that corticosterone administration decreased feed intake and duodenal and jejunal epithelial cell proliferation of young broilers (Hu and Guo, 2008), which in turn results in lower duodenal and jejunal villus height and crypt depth (Hu and Guo, 2008). Villus height is postively related to villus surface area (Mitchell and Carlisle, 1992); reduction of villus height during villus growth would decrease the expansion of small intestinal surface area and subsequently decrease the absorptive capacity (Moran, 1985). The effect of stress on the absorption of nutrients in the intestine of animals may occur in two ways. One is nonspecific, in which the absorption of nutrients is affected by changes in the intestinal mucosa morphology, the activities of digestive enzymes, and intestinal motility (Tsukada *et al*., 2002); the other is specific, in which the absorption of nutrients is affected by changing the expression of transporter that is particular to nutrients (Shepherd *et al.*, 2004).

Glucose is the main nutrient that is released through the hydrolysis of carbohydrate. Many types of carbohydrates can be utilized only when they are hydrolyzed to glucose, so glucose plays an important role in carbohydrate nutrition. However, it has been shown that corticosterone administration increased glucose and calcium absorption (Nasir *et al*., 1999).

2.8 Methods for Inducing Physiological Stress

To understand the stress responses and their effect on animals under a controlled environment, researchers have simulated conditions to mimic stress through exogenous administration of corticosterone in different forms, namely injecting the hormone directly into the animal (Gao *et al*., 2008), adding the hormone to the animal's feed or water (Post *et al*., 2003 and Shini *et al*., 2008) or implanting the hormone into the body (Puvaldopirod and Thaxton, 2000a; Olanrewaju *et al*., 2006). One limitation of the administration of corticosterone through drinking water is the inability to regulate the dosage consumed by the birds, since this depends on the amount of water consumed. However, a regulated dose can be administered by injecting the animal directly or offering birds mealworms which had been previously injected with a known dose of corticosterone.

The indirect feeding of corticosterone to the bird remains the only non-invasive method of administering corticosterone (Müller *et al*., 2009) since it does not involve piercing through the skin (Dawkins, 2004) as is the case with injection or implanting, therefore preserving the body integument and minimizing pain and discomfort to the animal. In their work with White-crowned Sparrows, Breuner *et al.*, (1998) used a non-invasive method of inducing acute stress by injecting corticosterone into mealworms before offering them to the sparrows. Their results showed a peak in plasma corticosterone levels seven minutes after the birds ingested the corticosterone-injected mealworms, which returned to baseline levels an hour later.

In a study where chronic stress was mimicked, Olanrewaju *et al*. (2006) used exactly the method of Puvadolpirod and Thaxton (2000a) to induce physiological stress in broiler chickens. Their aim was to extend the work of Puvadolpirod and Thaxton (2000a) by investigating the effect of chronic stress on acid-base balance. They showed that corticosterone level of birds implanted

with the ACTH mini-osmotic pump was elevated on the 4th and 7th day post implant. This was accompanied with a significant increase in PCO_2, HCO_3^-, haematocrit, haemoglobin and glucose levels whereas PO_2, Na^+, K^+ and Cl^- levels were suppressed. Thus, apart from the elevated corticosterone causing an increased energy supply (glucose) through gluconeogenesis, there was the loss of electrolytes through the urine. While physiological stress caused increased PCO_2 and decreased PO_2 levels in the blood, the reverse was observed under heat stress where there was an increase in CO_2 loss during panting (Hocking *et al*., 1994).

For most commercial broiler producers, attainment of high body weight within a specific period is an important objective to maximise the output of the housing system. However, rapid weight gain may not be achieved if the flock is exposed to conditions that could result in chronic stress. In a study designed to simulate chronic stress conditions in broilers, results showed that the greater the concentration of corticosterone intake by the birds, the lower their final body weight indicating that chronic stress can have a direct effect on growth performance as nutrients are diverted away from growth (Vahdatpour *et al.*, 2009).

2.9 Target Parameters for Measuring Stress

2.9.1 Growth Parameters

The most recognizable effect of corticosterone or ACTH treatment on live performance is a sharp reduction in body weight (BW) gain (Siegel *et al*., 1989; Puvadolpirod and Thaxton, 2000a, b; Aengwanich, 2007; Li *et al*., 2009; Vahdatpour *et al.*, 2009). This reduction in BW gain often occurs despite significant increases in feed intake (Siegel and Van Kampen, 1984; Puvadolpirod and Thaxton, 2000d). Heat stress adversely affects voluntary feed intake, body weight gain, carcass characteristics and mortality (Mashaly *et al*., 2004 and Khan *et al*., 2012). In fact, a significantly negative correlation exists between body temperature and weight gain (R^2 = -

0.4), feed intake (R^2 = -0.31) and feed conversion ratio (R^2 = 0.24) in birds exposed to heat stress at 32^{o}C (Cooper and Washburn, 1998). The reduction in feed intake during heat stress could be related to decreased blood flow to the digestive system (Wolfenson *et al*., 1981), thus heat production associated with digestion, absorption and utilization of nutrients is suppressed (Syafwan *et al*., 2011). Elevations in feed conversion have been observed in broilers given injections of ACTH (Puvadolpirod and Thaxton, 2000d). Stressed broilers also typically display increases in abdominal fat deposition (Bartov *et al*. 1980). A reduction in muscle accretion, as well as an increase in abdominal fat deposition, occurs in poultry treated with corticosterone or ACTH (Siegel and Van Kampen, 1984). These effects most likely occur because of the action of glucocorticoids on glucose metabolism.

2.9.2 Blood Parameters

One common measurement of stress in poultry is to measure plasma corticosterone concentration in birds after treatment with CS or ACTH (Siegel, 1995). Metabolism of corticosterone takes place in the liver and also in the gut through bacterial deconjugation (Möstl and Palme, 2002). The clearance of corticosterone from the body system decreases as the bird ages (Hess, 2006); hence adult birds may be more likely to have higher circulating corticosterone concentration than younger birds presumably because of decreased activity of the liver. Corticosterone is mostly measured from blood plasma or serum (Mormède *et al*., 2007). However, there are several limitations to this method, including high variability of corticosterone levels, procedures such as catching and restraining the bird elevates corticosterone levels if blood is not sampled within 2-3 minutes of the bird being caught (Mormède *et al.*, 2007). Another short-coming of blood corticosterone is that under chronic (prolonged) stress, corticosterone levels in the blood can be reduced to base-line levels by the negative feedback mechanism, which assists in the regulation

of the concentration of corticosterone in circulation (Pariante and Lightman, 2008) such that it becomes difficult to differentiate between a chronically stressed animal and a control.

Evidence is available that the immune status of chickens, as indicated by differential leucocyte counts or H/L ratios, can also be affected by stressors. These, like environmental stressors, cause an elevation in plasma corticosterone concentrations, and consequently increase H/L ratios due to leukopenia (lymphopenia) and heterophilia (Gehad *et al*., 2002; Wang *et al*., 2003; Shini *et al*., 2004, 2005). Elevations in circulating levels of corticosterone in poultry after treatment with ACTH have been demonstrated repeatedly (Puvadolpirod and Thaxton, 2000 a, b, d). Nagra and Meyer (1963) measured effluent blood from the adrenal vein after intravenous (IV) injections of ACTH in several avian species and found that plasma corticosterone level increased by as much as 250% post injection. ACTH, whether produced endogenously or administered exogenously, causes avian plasma corticosterone levels to increase. It can, therefore, be surmised that stress responses are fairly similar when treatment with either ACTH or corticosterone is used.

Several other stress validation signals exist, ranging from increases in blood glucose (Puvadolpirod and Thaxton, 2000a, c) to cholesterol (Puvadolpirod and Thaxton, 2000 a, b; Gross and Siegel, 1983). Blood glucose levels are increased under heat stress due to an increase in secretion of adrenalin, noradrenalin and glucocorticoids (Borges *et al*., 2007) to increase energy available to survive the stressful condition (Ognik and Sembratowicz, 2012). Apart from heat stress, simulation of stress through exogenous administration of corticosterone has also been shown to increase blood glucose levels (Puvaldopirod and Thaxton, 2000d; Post *et al*., 2003 and Olanrewaju *et al*., 2006). Gluconeogenesis is the process whereby non-carbohydrate substrates like proteins and fats are converted into glucose to avail birds with the required energy to survive during stress (Ognik and Sembratowicz, 2012) because of the suppression of the digestive

processes. In a study by Iyasere (2014) who fed broiler corticosterone-injected mealworms, there was a tendency for corticosterone treated birds to have a greater level of blood glucose. Olanrewaju *et al*. (2006), Vahdatpour *et al*. (2009) and Lin *et al*. (2004) also found that corticosterone-treated birds had increased blood glucose levels. According to Aengwanich (2007), heterophil, heterophil/lymphocyte ratio, total white blood cell count, body temperature, respiratory rate, packed cell volume and feed intake were significantly increased, while lymphocyte, haemoglobin, average daily gain and body weight of broilers were significantly decreased in dexamethasone-treated broiler chickens. Reports from studies on cholesterol have varied, with some reporting an increase (Staels *et al*., 1991; Shini *et al*., 2008), decrease (Giudetti and Gnoni, 1998) or no change (Wang *et al*., 2012b). Siegel (1995) noted that hypercholesteremia is one of many symptoms associated with long term stress.Triglyceride reports have also been varied, with some reporting a decrease (De La Cruz *et al.*, 1987) and others an increase (Lin *et al.*, 2006; Shini *et al.*, 2008). Sahin and Kucuk (2001) reported that stress increases serum glucose, triglyceride and cholesterol.

2.9.3 Organ Weight Indices

Liver weight has been consistently shown to increase in broilers treated with CS or ACTH (Puvadolpirod and Thaxton, 2000 a, b, c; Malheiros *et al*., 2003). This is probably due to an increase in liver fat because liver lipids have been shown to increase significantly in broilers treated with ACTH during the process of fat breakdown for the production of glucose (Puvadolpirod and Thaxton, 2000 a, b). Corticosteroids have also been shown to stimulate protein synthesis in the liver leading to liver muscle enlargement (Baxter and Rousseau, 1979). Other common validation procedures for the occurrence of physiological stress after corticosterone or ACTH treatment involve the measurement of immune system parameters. One

such example is to measure the lymphoid organs (that is, bursa, thymus, and spleen) because regression of these organs has been shown repeatedly to occur as a result of corticosterone or ACTH treatment (Post *et al*., 2003). It has been shown that heat stress had immunosuppressant effects on birds and resulted in decreasing weights of lymphoid organs, total circulating antibodies, and phagocytic ability of macrophages (Quinteiro-Filho *et al*., 2012).

2.9.4 Gastro-intestinal Tract

The gastrointestinal tract of broiler chickens is about 1.5% of BW; however, approximately 6% to 8% of the energy derived from the diets is consumed by it (Spratt *et al*., 1990). The small intestinal epithelium is a compound multiple cell system, which determines the growth potential of broiler after it hatches (Uni *et al.*, 1998). The development of intestinal morphology and function resulted in the development of chickens (Yamauchi and Tarachai, 2000). Many studies have shown that stress could affect the intestinal function of animals (Cera *et al.*, 1988; Olsen *et al*., 2005) and further disturb the absorption of nutrients (Thiesen *et al*., 2003; Shepherd *et al.*, 2004; Garriga *et al*., 2006; Albin *et al*., 2007). The effects of stress on the absorption of nutrients in the intestine of animals may occur in two ways. One is non-specific, in which the absorption of nutrients is affected by changes in the intestinal mucosa morphology, the activities of digestive enzymes, and intestinal motility (Tsukada *et al*., 2002); the other is specific, in which the absorption of nutrients is affected by changing the expression of transporter that is particular to nutrients (Shepherd *et al*., 2004).

2.9.5 Bone Characteristics

There is a need for better understanding of bone strength in poultry because bone breakage and associated infections contribute to mortality, low productivity, and carcass condemnations. Market age poultry often suffer from lameness and bone deformities, which can cause bone

breakage during catching and transportation and which create problems during processing (Gregory and Wilkins, 1992; Julian, 1998; Knowles and Wilkins, 1998). Bone weakness and other bone problems also constitute significant animal welfare issues because of lameness and mortalities stemming from leg weakness and osteoporosis in laying hens (Aziz-Abdul, 1998). Bone is a dynamic tissue influenced by physiological, nutritional, and physical factors such as mechanical stress and physical activities. Like mammalian bones, the avian long bones are made up of compact cortical bone, which is the outer shell surrounding the cancellous or trabecular bone and marrow space. The trabecular bones are organized as a lattice and provide larger surface areas and show high turnover rates (Albright, 1987). The architectural organization of the lattice structure can also be important for the strength of a bone. Based on the structural organization, bone is either lamellar or woven. Percentage of tibia ash is commonly and routinely used as a measure of bone mineralization.

Corticosteroid hormones have strongly been implicated in mammalian osteoporosis (Reid, 1998). These steroids have multitudes of effects on cells such as slowing cell division and differentiation. In mature animals, corticosteroids can affect remodeling, perhaps by preventing the recruitment of osteoblasts and causing bone to weaken by preventing normal bone formation. Synthetic corticosteroid, (dexamethasone) decreases bone strength of turkeys, although the severity of the effect was age dependent (Rath *et al*., 2000). The severity of the effects of dexamethasone was much reduced when the drug was administered at an age when bone development had nearly reached completion (Rath *et al*., 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted at the Teaching and Research farm of the Department of Animal Science, Ahmadu Bello University, Samaru, Zaria. The farm is located on Latitude 11º 9' 45'' N and Longitude 7º 38' 8'' E, at an altitude of 610 m above sea level (Ovimaps, 2012). Zaria is located in the Northern Guinea Savannah zone of Nigeria. The area has three distinct seasons; namely the hot dry season from March to May, the warm rainy season from June to September, and a cool dry season from November to February with a mean annual rainfall of about 1011±16.1 mm (Oluwasemire and Alabi, 2004). The area has an average relative humidity of 36.0% during the dry season and 78.5% for the wet season. The average minimum and maximum temperatures recorded in the area are 15.6 and 38.5°C respectively (NCAT, 2008).

3.2 Experiment 1: EFFECT OF VARYING LEVELS OF DEXAMETHASONE ON PERFORMANCE AND THERMOREGULATORY RESPONSES OF BROILER CHICKENS

3.2.1 Experimental Design, Diets and Management of Birds

Two hundred and forty day old *Arbor acre* broiler chicks were used in this experiment. They were randomly allotted to four experimental treatments namely 0, 1, 2 and 3 mg dexamethasone. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design. A maize/soybean meal based broiler starter and finisher diet was formulated according to NRC (1994) nutrient requirement for broiler chickens (Tables 3.1 and 3.2) and fed to all birds. Daily doses of dexamethasone (1, 2 and 3 mg) was administered by dissolving in a litre of water and supplied for twenty-eight days beginning from when the birds attained 14 days of age. Dexamethasone administration was terminated 14 days prior to termination of the

experiment at day 56 to allow a two week readjustment phase. Birds receiving no dexamethasone (0 mg) in their drinking water served as the control.

The birds were raised on deep litter and housed in 2.5 m x 1.96 m bird pens for each replicate with feed and water provided *ad libitum*. All routine and management practices were strictly adhered to. Parameters measured include initial weight, feed intake and final weight while weight gain and feed conversion ratio were calculated as follows:

$$\text{Weight gain} = \text{Final weight} - \text{Initial weight}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

Initial weight was taken at the start of the experiment at day 1 while feed intake and weight gain were taken weekly. Mortality records were taken as they occurred. The experiment lasted 56 days from the month of May to June.

3.2.2 Thermoregulatory Measurements

Indoor temperature and relative humidity readings were recorded daily using an electronic digital thermo-hygrometer (HTC-1). Both readings were taken in the morning (8.00 am) and afternoon (3.00 pm) throughout the experimental period and were used to calculate the morning and afternoon THIs. Rectal temperatures and respiratory rates were measured by placing a digital thermometer in the rectum and counting of respiration (breath/minute) with the aid of a stopwatch, respectively. Temperature-humidity index (THI) was calculated using the formular of Tao and Xin (2003).

$$THI = 0.85\,T_{db} + 0.15\,T_{wb}$$

Where,

THI = temperature-humidity index in °C

T_{db} = dry-bulb or ambient temperature in °C

T_{wb} = wet-bulb temperature in °C

Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression functions by Stull (2011). Heat stress was classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

3.2.3 Haematological and Serum Analyses

Brachial vein blood samples (4 ml) were collected from two birds per replicate on days 28, 42 and 56 for haematological, metabolite and hormone assay. Blood samples collected into collection tubes containing EDTA (Ethylenediamminetetraacetic acid) were analyzed for packed cell volume, haemoglobin, leucocyte, erythrocyte, heterophils and lymphocytes using an auto haematology analyser (HA-17600). Whole blood was collected in tubes containing no EDTA for serum metabolites and hormonal assay. All samples were run in duplicate and kit calibrators and controls were included in each analysis. Absorbance was measured at 450 nanometers (nm), with a reference wavelength of 650 nm, in an ELISA microplate reader.The haematological and serum analyses were carried out at the Haematology Laboratory of the Faculty of Medicine, Ahmadu Bello University, Zaria. The immunoassays for corticosterone and thyroxine were carried out at the Ahmadu Bello University Teaching Hospital Haematology Laboratory, Zaria.

3.2.4 Organ Collection and Examination

On day 56 of the experiment, three birds per replicate were slaughtered and eviscerated. Body weights, as well as weight of organs including liver, kidneys, bursa of fabricius, spleen, and the

thymus were measured using a sensitive digital scale. Relative weights of each organ expressed as percentage of live weight were determined. Tissue samples were obtained from the breast region of the birds and analysed for drug residue. For small intestine histomorphometric analysis, samples of the jejenum were harvested and fixed in 10% formal saline. Fixed tissues were histologically processed according to the method of Bancroft and Stevens (2008).

3.2.5 Determination of Tibia Geometric Properties

During carcass analysis at day 56, the left tibia of three birds per replicate was removed. Tibia were then labeled and immersed in boiling water (100 °C) for 15 minutes according to the procedure described by Applegate and Lilburn (2002) for complete tissue removal. Length of each bone was measured using a meter rule. The distance from proximal to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing balance (Satorius ENTRIS). The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al*., 1991). The Robusticity index was determined using the formula described by Reisenfeld (1972).

$$Robusticity\ Index\ =\ \frac{Bone\ length}{Cube\ root of bone\ weight}$$

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to the procedure described by A.O.A.C. (1990). The percentage ash was then determined relative to dry weight of the tibia.

Table 3.1: Ingredient Composition and Calculated Analysis of the Diet for Starter Broiler Chickens

Ingredients	
Maize	55.00
Soyabean cake	24.00
Groundnut cake	14.00
Maize offal	2.00
Limestone	1.00
Bone meal	3.00
Common salt	0.30
Vitamin premix	0.30
Lysine	0.20
Methionine	0.20
Total	100.00
Calculated analysis	
ME KCal/kg	2860
Crude protein	23.06
Ether extract	4.62
Crude fibre	4.25
Calcium	1.22
Available phosphorus	0.52
Lysine	1.24
Methionine	0.60

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg.

Table 3.2: Ingredient Composition and Calculated Analysis of the Diet for Finisher Broiler Chickens

Ingredients	
Maize	62.5
Soya cake	18.0
Groundnut cake	15.0
Limestone	0.50
Bone meal	3.00
Common salt	0.30
Vitamin premix	0.30
Lysine	0.20
Methionine	0.20
Total	100.00
Calculated analysis	
ME KCal/kg	2950
Crude protein	21.05
Ether extract	4.48
Crude fibre	3.77
Calcium	1.03
Av. Phosphorus	0.51
Lysine	1.09
Methionine	0.58

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg.

3.3 Experiment 2: EFFECT OF 0.15% BETAINE HYDROCHLORIDE ON PERFORMANCE AND THERMOREGULATORY RESPONSES OF BROILER CHICKENS UNDER DEXAMETHASONE INDUCED STRESS

3.3.1 Experimental Design, Diets and Management of Birds

Two hundred and forty day old *Arbor acre* broiler chicks were used in this experiment. They were randomly allotted to four experimental treatments namely 0, 1, 2 and 3 mg dexamethasone. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design. A maize/soybean meal based broiler starter and finisher diet was formulated according to NRC (1994) nutrient requirement for broiler chickens (Tables 3.3 and 3.4). Birds administered 1, 2 and 3 mg dexamethasone in drinking water were given diets supplemented with Betaine HCl at 0.15%. Daily doses of dexamethasone (1, 2 and 3 mg) was administered by dissolving in a litre of water and supplied for twenty-eight days beginning from when the birds attained 14 days of age. Dexamethasone administration was terminated 14 days prior to termination of the experiment at day 49 to allow a two week readjustment phase. Birds receiving no dexamethasone (0 mg) in their drinking water served as the control.

The birds were raised on deep litter and housed in 2.5 m x 1.96 m bird pens for each replicate with feed and water provided *ad libitum*. All routine and management practices were strictly adhered to. Parameters measured include initial weight, feed intake and final weight while weight gain and feed conversion ratio were calculated as follows:

$$\text{Weight gain} = \text{Final weight} - \text{Initial weight}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

Initial weight was taken at the start of the experiment at day 1 while feed intake and weight gain were taken weekly. Mortality records were taken as they occurred. The experiment lasted 49 days from the month of August to September.

3.3.2 Thermoregulatory Measurements

Indoor temperature and relative humidity readings were recorded daily using an electronic digital thermo-hygrometer. Both readings were taken in the morning (8.00 am) and afternoon (3.00 pm) throughout the experimental period. Rectal temperatures and respiratory rates were measured by placing a digital thermometer in the rectum and counting of respiration (breath/minute) with the aid of a stopwatch respectively. Temperature-humidity index (THI) was calculated using the formular of Tao and Xin (2003).

$$THI = 0.85\,T_{db} + 0.15\,T_{wb}$$

Where,

THI = temperature-humidity index in °C

T_{db} = dry-bulb or ambient temperature in °C

T_{wb} = wet-bulb temperature in °C

Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression functions by Stull (2011). Heat stress was classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

3.3.3 Haematological and Serum Analyses

Brachial vein blood samples (4 ml) were collected from two birds per replicate on days 21, 35 and 49 for haematological and metabolite determination. Blood samples collected into collection tubes containing EDTA (Ethylenediamminetetraacetic acid) were analyzed for packed cell

volume, haemoglobin, leucocyte, erythrocyte, heterophils and lymphocytes using an auto haematology analyser (HA-17600). Whole blood was collected in tubes containing no EDTA for serum metabolite determination. All samples were run in duplicate and kit calibrators and controls were included in each analysis.The haematological and serum analyses were carried out at the Haematology Laboratory of the Faculty of Medicine, Ahmadu Bello University, Zaria.

3.3.4 Organ Collection and Examination

On day 49 of the experiment, three birds per replicate were slaughtered and eviscerated. Body weights, as well as weight of organs including liver, kidneys, bursa of fabricius, spleen, and the thymus were measured. Relative weights of each organ expressed as percentage of live weight was determined.

3.3.5 Determination of Tibia Geometric Properties

During carcass analysis, the left tibia of three birds per replicate was removed. Tibiae were then labeled and immersed in boiling water (100 °C) for 15 minutes according to the procedure described by Applegate and Lilburn (2002) for complete tissue removal. Length of each bone was measured using a meter rule. The distance from proximal to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing balance. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al.*, 1991). The Robusticity index was determined using the formula described by Reisenfeld (1972).

$$Robusticity\ Index\ =\ \frac{Bonelength}{Cube\ rootofbone\ weight}$$

Table 3.3: Ingredient Composition and Calculated Analysis of the Diet Supplemented with 0.15% Betaine HCl for Starter Broiler Chickens

Ingredients	Diet	Diet + 0.15% Betaine
Maize	55.00	54.85
Soyabean cake	24.00	24.00
Groundnut cake	14.00	14.00
Maize offal	2.00	2.00
Limestone	1.00	1.00
Bone meal	3.00	3.00
Common salt	0.30	0.30
Vitamin premix	0.30	0.30
Lysine	0.20	0.20
Methionine	0.20	0.20
Betaine HCl	-	0.15
Total	100.00	100.00
Calculated analysis		
ME KCal/kg	2860	2860
Crude protein	23.06	23.05
Ether extract	4.62	4.61
Crude fibre	4.25	4.25
Calcium	1.22	1.22
Available phosphorus	0.52	0.52
Lysine	1.24	1.24
Methionine	0.60	0.60

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg.

Table 3.4: Ingredient Composition and Calculated Analysis of the Diet Supplemented with 0.15% Betaine HCl for Finisher Broiler Chickens

Ingredients	Diet	Diet + 0.15% Betaine
Maize	62.50	62.50
Soyabean cake	18.00	17.85
Groundnut cake	15.00	15.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Common salt	0.30	0.30
Vitamin premix	0.30	0.30
Lysine	0.20	0.20
Methionine	0.20	0.20
Betaine HCl	-	0.15
Total	100.00	100.00
Calculated analysis		
ME KCal/kg	2950	2940
Crude protein	21.05	20.98
Ether extract	4.48	4.47
Crude fibre	3.77	3.76
Calcium	1.03	1.03
Available phosphorus	0.51	0.51
Lysine	1.09	1.09
Methionine	0.58	0.58

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg.

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to the procedure described by A.O.A.C. (1990). The percentage ash was then determined relative to dry weight of the tibia.

3.4 Experiment 3: EFFECT OF 0.30% BETAINE HYDROCHLORIDE ON PERFORMANCE AND THERMOREGULATORY RESPONSES OF BROILER CHICKENS UNDER DEXAMETHASONE INDUCED STRESS

3.4.1 Experimental Design, Diets and Management of Birds

Three hundred day old *Arbor acre* broiler chicks were used in this experiment. They were randomly allotted to five experimental treatments. Each treatment was replicated three times with twenty birds per replicate in a completely randomized design.A maize/soybean meal based broiler starter and finisher diet was formulated according to NRC (1994) nutrient requirement for broiler chickens (Table 3.5 and Table 3.6). Birds receiving 0 mg dexamethasone served as the control without betaine supplementation. Birds administered 1, 2 and 3 mg dexamethasone in drinking water along with a betaine only group were given diets supplemented with Betaine HCl at 0.30%. Daily doses of dexamethasone (1, 2 and 3 mg) was administered by dissolving in a litre of water and supplied for twenty-eight days beginning from when the birds attained 14 days of age. Dexamethasone administration was terminated 14 days prior to termination of the experiment at day 49 to allow a two week readjustment phase. Birds receiving no dexamethasone (0 mg) in their drinking water served as the control.

The birds were raised on deep litter and housed in 2.5 m x 1.96 m bird pens for each replicate with feed and water provided *ad libitum*. All routine and management practices were strictly adhered to. Parameters measured include initial weight, feed intake and final weight while weight gain and feed conversion ratio were calculated as follows:

$$\text{Weight gain} = \text{Final weight} - \text{Initial weight}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

Mortality records were taken as they occurred. The experiment lasted 49 days from the month of November to December.

3.4.2 Thermoregulatory Measurements

Indoor temperature and relative humidity readings were recorded daily using an electronic digital thermo-hygrometer. Both readings were taken in the morning (8.00 am) and afternoon (3.00 pm) throughout the experimental period. Rectal temperatures and respiratory rates were measured by placing a digital thermometer in the rectum and counting of respiration (breath/minute) with the aid of a stopwatch respectively. Temperature-humidity index (THI) was calculated using the formular of Tao and Xin (2003).

$$THI = 0.85\, T_{db} + 0.15\, T_{wb}$$

Where,

THI = temperature-humidity index in °C

T_{db} = dry-bulb or ambient temperature in °C

T_{wb} = wet-bulb temperature in °C

Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression functions by Stull (2011). Heat stress was classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Table 3.5: Ingredient Composition and Calculated Analysis of the Diet Supplemented with 0.3% Betaine HCl for Starter Broiler Chickens

Ingredients	Diet	Diet + 0.30% Betaine
Maize	55.00	54.70
Soyabean cake	24.00	24.00
Groundnut cake	14.00	14.00
Maize offal	2.00	2.00
Limestone	1.00	1.00
Bone meal	3.00	3.00
Common salt	0.30	0.30
Vitamin premix	0.30	0.30
Lysine	0.20	0.20
Methionine	0.20	0.20
Betaine HCl	-	0.30
Total	100.00	100.00
Calculated analysis		
ME KCal/kg	2860	2860
Crude protein	23.06	23.05
Ether extract	4.62	4.61
Crude fibre	4.25	4.25
Calcium	1.22	1.22
Available phosphorus	0.52	0.52
Lysine	1.24	1.24
Methionine	0.60	0.60

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg

Table 3.6: Ingredient Composition and Calculated Analysis of the Diet Supplemented with 0.30% Betaine HCl for Finisher Broiler Chickens

Ingredients	Diet	Diet + 0.30% Betaine
Maize	62.50	62.50
Soyabean cake	18.00	17.70
Groundnut cake	15.00	15.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Common salt	0.30	0.30
Vitamin premix	0.30	0.30
Lysine	0.20	0.20
Methionine	0.20	0.20
Betaine HCl	-	0.30
Total	100.00	100.00
Calculated analysis		
ME Kcal/kg	2950	2940
Crude protein	21.05	20.98
Ether extract	4.48	4.47
Crude fibre	3.77	3.76
Calcium	1.03	1.03
Available phosphorus	0.51	0.51
Lysine	1.09	1.09
Methionine	0.58	0.58

Nutrivitas broiler premix provided per 1 kg of diet Vitamin A, 4,000,000 I.U; Vitamin D_3, 800,000 I.U; Vitamin E 16,000 mg; Vitamin K_3, 800 mg; Vitamin B_1, 600 mg; Vitamin B_2, 2,000 mg; Vitamin B_6, 1,600 mg; VitaminB_{12}, 8 mg; Niacin, 16,000; Calpan, 4,000; Folic acid, 400 mg; Biotin, 40 mg; Choline chloride, 120,000 mg; Manganese, 32,000 mg; Iron, 16,000 mg; Zinc, 24,000 mg; Copper, 3,200 mg; Iodine, 320 mg; Cobalt, 120 mg; Selenium, 80 mg

3.4.3 Haematological and Serum Analyses

Brachial vein blood samples (4 ml) were collected from two birds per replicate on days 21, 35 and 49 for haematological and metabolite determination. Blood samples collected into collection tubes containing EDTA (Ethylenediamminetetraacetic acid) were analyzed for packed cell volume, haemoglobin, leucocyte, erythrocyte, heterophils and lymphocytes using an auto haematology analyser (HA-17600). Whole blood was collected in tubes containing no EDTA for serum metabolite determination. All samples were run in duplicate and kit calibrators and controls were included in each analysis. The haematological and serum analyses were carried out at the Haematology Laboratory of the Faculty of Medicine, Ahmadu Bello University, Zaria.

3.4.4 Organ Collection and Examination

On day 49 of the experiment, three birds per replicate were slaughtered and eviscerated. Body weights, as well as weight of organs including liver, kidneys, bursa of fabricius, spleen, and the thymus were measured. Relative weights of each organ expressed as percentage of live weight was determined. Tissue samples were obtained from the breast region of the birds and analysed for drug residue. For intestine histomorphometric analysis, samples of the jejenum portion of the small intestine were harvested and fixed in 10% formal saline. Fixed tissues were histologically processed according to the method of Bancroft and Stevens (2008).

3.4.5 Determination of Tibia Geometric Properties

During carcass analysis, the left tibia of three birds per replicate was removed. Tibiae were then labeled and immersed in boiling water (100 °C) for 15 minutes according to the procedure described by Applegate and Lilburn (2002) for complete tissue removal. Length of each bone was measured using a meter rule. The distance from proximal to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing

balance. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al*., 1991). The Robusticity index was determined using the formula described by Reisenfeld (1972).

$$Robusticity\ Index\ =\ \frac{Bonelength}{Cube\ rootofbone\ weight}$$

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then ashed in a muffle furnace at 600°C for 6 hours according to the procedure described by A.O.A.C. (1990). The percentage ash was then determined relative to dry weight of the tibia.

3.5 Data Analysis

All data collected from the experiments were subjected to analysis of variance (ANOVA) using the General Linear Model Procedure of JMP SAS (2012). The model used is as follows:

$$Yi = \mu + \delta i + \varepsilon i$$

Where

Y_i = individual observation;

μ = overall mean of the population;

δ_i = effect of the ith dexamethasone level and

ε_i = random error.

Growth performance data for the 14- 28, 28-56 and 28-49 day periods were analyzed separately. Initial weights at the finisher stages were expressed as co-variates of other performance indices. Where the result of ANOVA was statistically significant, Tukey's (1949) post-hoc test for multiple comparisons was performed to compare means of all groups.

Multiple Regression Models were used to determine the relationship between environmental parameters and growth performance indices as shown below:

$$Y = \beta o + \beta iX + \mathcal{E}$$

Where,

Y = dependant variable;

X = independent variable (growth performance indices);

$\beta o, \beta i$ = regression coefficient or regression parameters

$\mathcal{E}$ = random error.

The haematological data collected over several periods were analyzed using repeated measures analysis according to JMP® SAS (2012) using the model:

$$Y_{ijk} = \mu + \alpha_i + \delta_{ij} + t_k + (\alpha * t)_{ik} + \mathcal{E}_{ijk}$$

Where:

Y_{ijk} = observation *ijk*

μ = the overall mean

α_i =the effect of treatment i

t_k =the effect of period k

$(\alpha * t)_{ik}$ = the effect of interaction between treatment i and period k

δ_{ij} = random error with mean 0 and variance between animals within treatment and it is equal to the covariance between repeatedmeasurements within animals

$\mathcal{E}_{ijk}$ = random error with the mean and variance, the variance between measurements within animals.

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment 1: EFFECT OF VARYING LEVELS OF DEXAMETHASONE ON PERFORMANCE AND THERMOREGULATORY RESPONSES OF BROILER CHICKENS

4.1.1Thermoregulatory Responses

The temperature-humidity index (THI) inside the poultry house during the experimental period for mornings and afternoons is shown in Figure 4.1. THI in the mornings averaged 26.4 and 32.8 in the afternoon. In general, THI in the afternoons were 19.5% higher than in the morning. The effect of varying doses of dexamethasone on rectal temperature of broiler chickens is shown in Figure 4.2. The average rectal temperature across the treatment groups ranged between 41.6ºC to 42.28ºC and was not significant ($P > 0.05$). A decrease in rectal temperature with increasing doses of dexamethasone was observed with birds on 3 mg dexamethasone having the lowest rectal temperature.

The effect of varying doses of dexamethasone on respiratory rate of broiler chickens is shown in Figure 4.3. The average respiratory rate across the treatment groups ranged between 201.33 and 215.00 breaths per minute. A non-significant increase ($P > 0.05$) in respiratory rate with increasing doses of dexamethasone was observed with birds on 3 mg dexamethasone having the highest respiratory rate.

4.1.2 Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 14- 28)

Growth performance of broiler chickens fed varying doses of dexamethasone is presented in Table 4.1. Differences among the dietary treatments in final weight ranged from 591.30 to

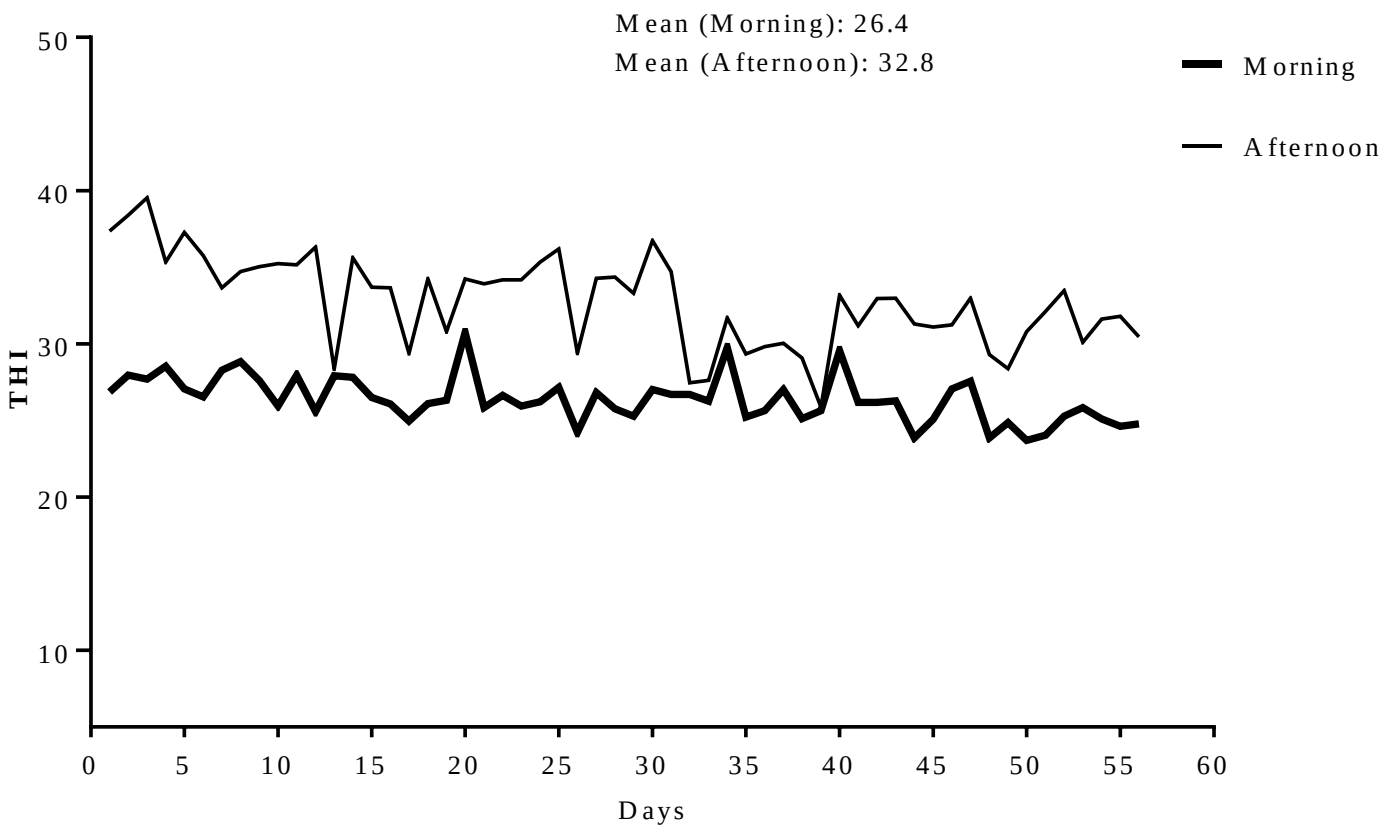

Figure 4.1: Daily Temperature-humidity index inside the poultry house during the experimental period

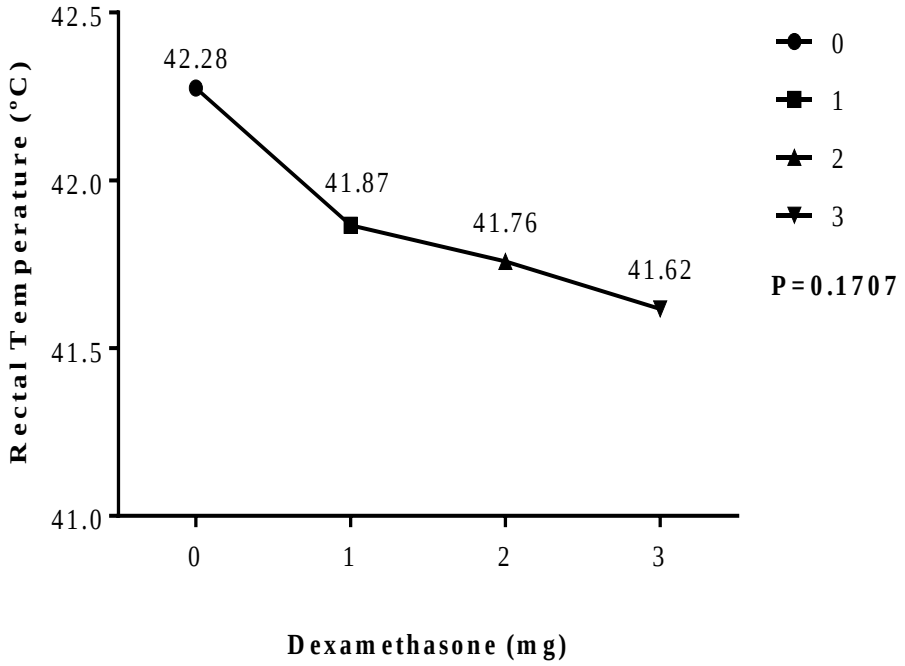

Figure 4.2: Effect of Varying Doses of Dexamethasone on Rectal Temperature of Broiler Chickens

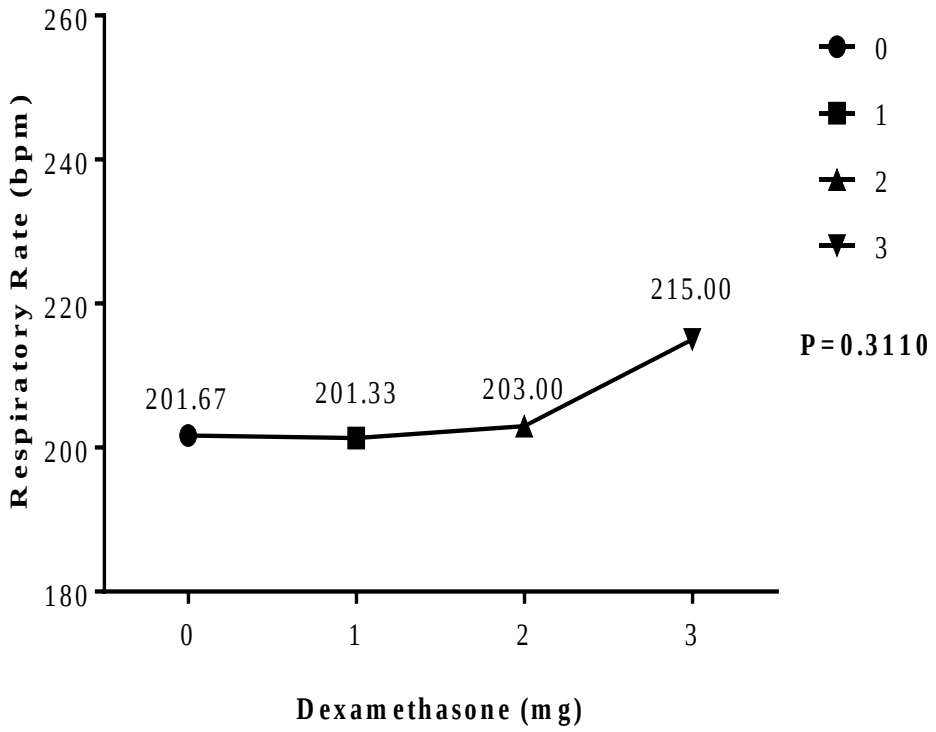

Figure 4.3: Effect of Varying Doses of Dexamethasone on Respiratory Rate of Broiler Chickens

803.33 g. Chickens fed 0 mg dexamethasone had higher ($P < 0.05$) final weight than those fed dexamethasone.

Final weight decreased ($P < 0.05$) with increasing doses of dexamethasone with birds administered 2 and 3 mg dexamethasone having the least final live weight and being similar ($P > 0.05$). The average daily feed intake ranged from 52.15 to 63.02 g/bird/day across the dietary treatments. Feed intake of birds receiving 0 and 1 mg dexamethasone were higher ($P < 0.05$) compared to other treatment groups. The average daily weight gain ranged from 23.24 to 33.41 g. Birds in the control (0 mg dexamethasone) had higher ($P < 0.05$) daily weight gain compared to those offered dexamethasone. Among the birds offered dexamethasone, those that received 2 and 3 mg dexamethasone had the least weight gains.

Feed conversion ratio (FCR) ranged from 1.89 to 2.39. Birds in the control and 1 mg dexamethasone groups had better ($P < 0.05$) FCR values than those offered 2 and 3 mg dexamethasone. Birds on 2 mg dexamethsaone had higher ($P < 0.05$) mortality but were similar ($P > 0.05$) with other dexamethasone groups.

4.1.3 Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 28-56)

Growth performance of broiler chickens fed varying doses of dexamethasone during day 28-56 is presented in Table 4.2. Final weight ranged from 2058.62 to 2483.62 g and was higher ($P < 0.05$) in chickens fed 0 mg dexamethasone. The average daily feed intake ranged from 120.51 to 158.23 g/bird/day with birds on 0 mg dexamethasone having higher ($P < 0.05$) feed intake compared to other dietary treatments. Feed intake decreased ($P < 0.05$) with the introduction of dexamethasone. Average daily weight gain ranged from 45.86 to 61.34 g with birds in the control (0 mg dexamethasone) having higher ($P < 0.05$) weight gain compared to other

Table 4.1: Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 14-28)

Parameters	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Initial weight (g/bird)	101.67	102.50	102.50	103.33	0.93	0.6722
Daily Feed intake (g/b/d)	63.02^{a}	58.65^{ab}	57.39^{b}	52.15^{c}	1.03	<.0005
Final weight (g/bird)	803.33^{a}	673.42^{b}	607.02^{bc}	591.30^{c}	16.09	<.0001
Daily weight gain (g/b/d)	33.41^{a}	27.19^{b}	24.02^{bc}	23.24^{c}	0.74	<.0001
Feed conversion ratio	1.89^{a}	2.16^{ab}	2.39^{b}	2.25^{b}	0.08	0.0093
Mortality (%)	0.30^{b}	0.20^{ab}	0.36^{a}	0.10^{ab}	0.14	0.0502

$^{a, b, c}$ Means with different superscript on the same row differ significantly ($P < 0.05$), g/b/d = gram/bird/day, SEM: Standard error of the mean

Table 4.2: Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 28-56)

Parameters	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Initial weight (g/bird)	803.33[a]	673.42[b]	607.02[bc]	591.30[c]	16.09	<.0001
Daily Feed intake (g/b/d)	158.23[a]	135.26[b]	137.92[b]	120.51[c]	2.91	<.0001
Final weight (g/b)	2483.63[a]	2202.97[c]	2307.49[b]	2058.62[d]	41.79	<.0001
Daily weight gain (g/b/d)	61.34[a]	51.14[c]	54.79[b]	45.86[d]	1.48	<.0001
Feed conversion ratio	2.64[b]	2.65[b]	2.51[a]	2.66[b]	0.05	.0052
Mortality (%)	0.10	0.20	0.06	0.10	0.14	0.4563

[a, b, c , d] Means with different superscript on the same row differ significantly ($P < 0.05$), g/b/d = gram/bird/day, SEM: Standard error of the mean

treatment groups. Feed conversion ratio ranged from 2.51 to 2.66 with birds on 2 mg dexamethasone performing best. FCR of the other treatment groups including the control were similar ($P > 0.05$). Mortality among the treatment groups was not significant ($P > 0.05$).

4.1.4 Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 14-56)

Growth performance of broiler chickens fed varying doses of dexamethasone is presented in Table 4.3. The average final weight ranged from 1896.28 to 2765.59 g with chickens fed 0 mg dexamethasone having higher ($P < 0.05$) final weights compared to other dietary treatments. Birds administered 3 mg dexamethasone had the least ($P < 0.05$) final weight. The average daily feed intake ranged from 90.76 to 118.21 g/bird/day. Birds on 0 mg dexamethasone had higher ($P < 0.05$) feed intake compared to other dietary treatments. Birds on 3 mg had the least ($P < 0.05$) feed intake with birds on the other dexamethasone levels (1 and 2 mg) being similar ($P > 0.05$).

The effect of dexamethasone on the average daily weight gain ranged from 36.59 to 54.37 g with birds in the control (0 mg dexamethasone) having higher ($P < 0.05$) daily weight gain compared to the birds on dexamethasone. Birds receiving 3 mg dexamethasone had the least ($P < 0.05$) weight gain. Feed conversion ratio ranged from 2.18 to 2.48 with birds in the control performing best ($P < 0.05$). FCR increased ($P > 0.05$) with with increasing levels of dexamethasone. Birds on 2 mg dexamethasone had the highest ($P < 0.05$) mortality.

4.1.5 Effect of Period and Varying Doses of Dexamethasone on Haematological Indices of Broiler Chickens

The effect of varying doses of dexamethasone on haematological indices of broiler birds is presented in Table 4.4. All haematological indices except erythrocyte were not significantly ($P > 0.05$) different across treatment groups. Erythrocyte values ranged from 4.97 to 4.39 x 10^{12}/l.

Table 4.3: Effect of Varying Doses of Dexamethasone on Performance of Broiler Chickens (day 14-56)

Parameters	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Initial weight (g/b)	101.67	102.50	102.50	103.33	0.93	.6722
Daily Feed intake (g/b/d)	118.21[a]	102.45[b]	103.05[b]	90.76[c]	1.49	<.0001
Final weight (g/b)	2765.59[a]	2212.72[b]	2178.10[b]	1896.28[c]	51.63	<.0001
Daily weight gain (g/b/d)	54.37[a]	43.07[b]	42.36[b]	36.59[c]	1.04	<.0001
Feed Conversion Ratio	2.18[a]	2.38[b]	2.43[b]	2.48[b]	0.04	.0046
Mortality (%)	0.10[ab]	0.20[b]	0.60[a]	0.06[ab]	0.14	0.0209

[a, b, c] Means with different superscript on the same row differ significantly ($P < 0.05$), g/b/d = gram/bird/day, SEM: Standard error of the mean

Birds fed 2 mg dexamethasone had significantly higher ($P < 0.05$) erythrocyte values compared to those on 1 mg dexamethasone.

The PCV and haemoglobin (Figure 4.4) showed response among the levels of dexamethasone from days 28 to 56. Birds on 1 and 3 mg dexamethasone showed an increase ($P > 0.05$) in PCV and hemoglobin concentration on days 28 through to 56, those on 2 mg and the control peaked at day 42 and decreased ($P > 0.05$) thereafter. Figure 4.4 showed that the highest ($P > 0.05$) values of leucocyte were observed on day 28 for the control. Birds fed 1 mg dexamethasone showed progressive, though not significant ($P > 0.05$), increase in leucocyte count with time, peaking at day 56. Erythrocyte counts (Figure 4.4) showed an increasing ($P > 0.05$) trend for birds in the 1 mg dexamethasone group with the lowest ($P > 0.05$) values observed on day 28. Birds in the control group showed an initial decline in erythrocyte count before rising at day 42. Birds receiving 2 mg dexamethasone had higher ($P > 0.05$) erythrocyte count compared with other treatment groups on day 42.

Figure 4.5 shows a decreasing trend for heterophils for the the treatment groups with the exception of the 1 mg dexamethasone group where an increase was observed at day 56. Higher hetrophil counts ($P > 0.05$) was observed for birds in the 2 mg dexamethasone group compared with other treatment groups on day 28. The lowest heterophil counts were observed on day 56 for birds in the control and 3 mg dexamethasone group. Birds on the control, 1, 2 and 3 mg dexamethasone showed an increase ($P > 0.05$) in lymphocyte counts from day 28 to 42. Birds in the 3 mg group showed a decrease in lymphocyte within the same period. A decline in heterophil-lymphocyte ratio among the control and dexamethasone containing groups (2 and 3 mg), with the exception of birds receiving 1 mg, where a decline on day 42 and a subsequent rise on day 56 thereafter were observed.

Table 4.4: Effect of Varying Doses of Dexamethasone on Haematological Indices of Broiler Chickens

Parameters	Dexamethasone levels (mg/l)				SEM	P value	Ref*
	0	1	2	3			
Packed cell volume (%)	27.22	25.72	29.33	29.00	1.17	0.0702	22-35
Haemoglobin (g/dl)	9.02	8.53	9.76	9.62	0.39	0.0739	7-13
Leucocyte ($x10^9$/l)	12.63	13.29	13.16	10.43	1.37	0.5378	1.2-3.0
Erythrocyte ($x10^{12}$/l)	4.62^{ab}	4.39^{b}	4.97^{a}	4.89^{ab}	0.18	0.0250	2.5-3.5
Heterophil (%)	14.61	15.06	16.50	17.44	2.37	0.4385	15-40
Lymphocyte (%)	84.67	82.89	82.78	80.06	2.22	0.1911	45-70
H:L	0.18	0.18	0.21	0.22	0.03	0.3714	-

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$); H:L= Heterophil-Lymphocyte ratio; *Reference values of Jain (1993)

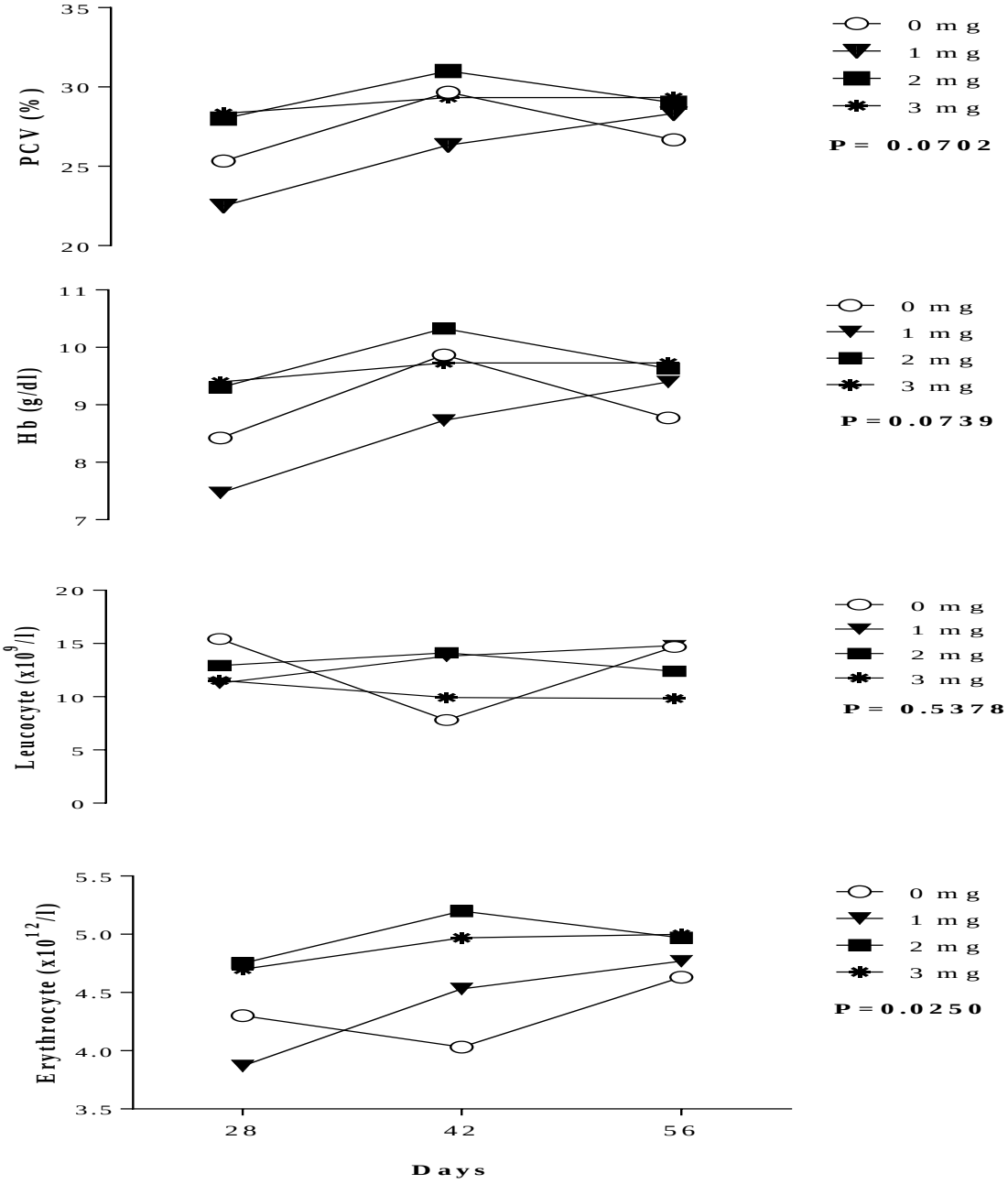

Figure 4.4: Effect of Period on Packed Cell Volume, Haemoglobin, Leucocyte and Erythrocyte counts in Dexamethasone-stressed Broiler Chickens

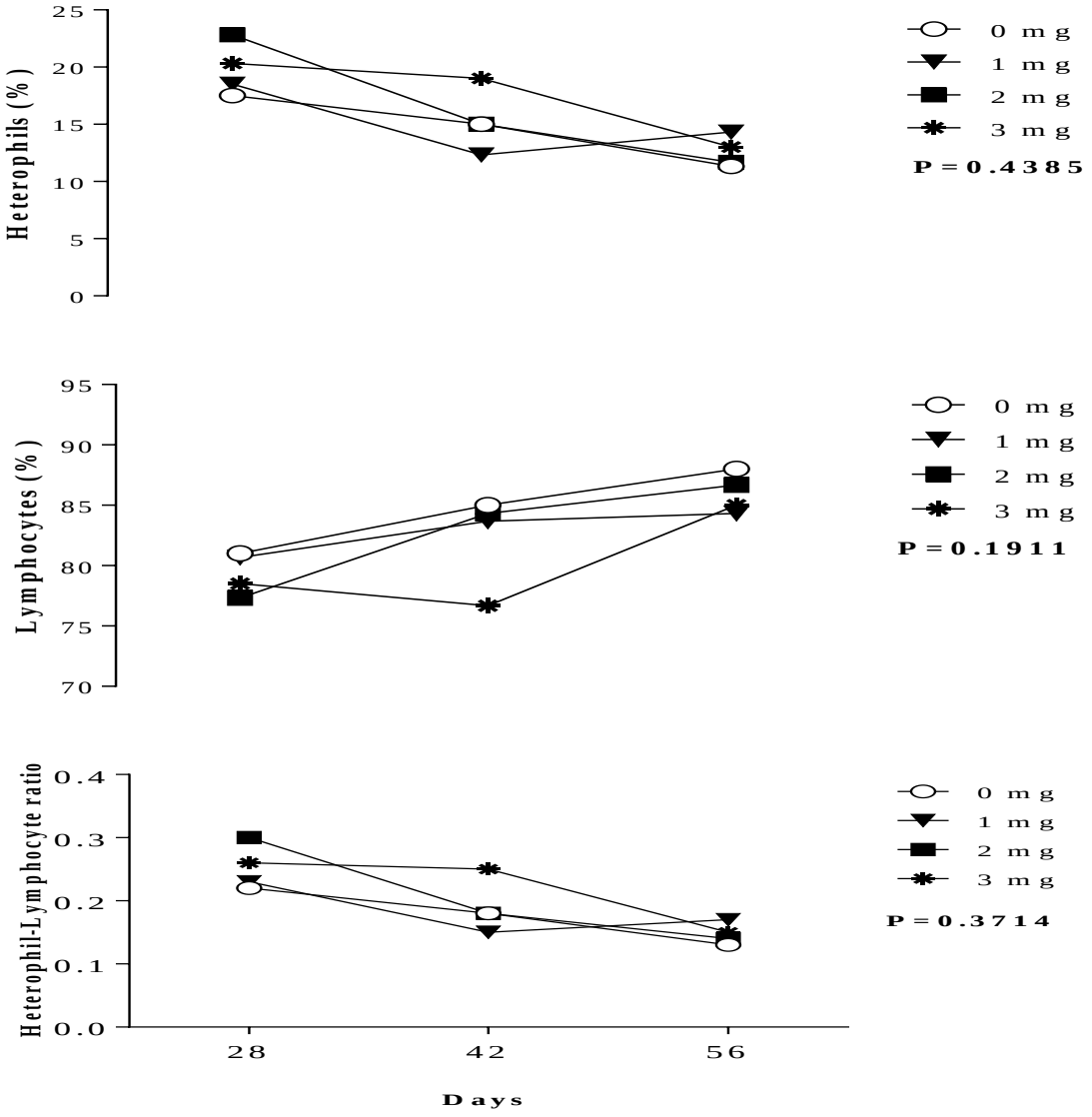

Figure 4.5: Effect of Period on Heterophil, Lymphocytes and Heterophil-Lymphocyte Ratio in Dexamethasone-stressed Broiler Chickens

4.1.6 Effect of Period and Varying Doses of Dexamethasone on Serum Chemistry of Broiler Chickens

The effect of varying dexamethasone doses on serum chemistry in stress induced broiler chickens was not significant ($P > 0.05$) (Table 4.5). Both glucose and cholesterol were within normal reference ranges. On days 28, 42 and 56, glucose levels decreased among birds in treatment groups 2 and 3 mg from day 28 to 42 (Fig. 4.6). The lowest glucose level was observed on day 56 for birds in the control. Birds in the 1 mg dexamethasone group showed a steady increase ($P < 0.05$) in glucose levels, while control birds showed a sharp decline ($P < 0.05$) in glucose levels on day 56. Similarly, cholesterol levels decreased with the lowest levels ($P > 0.05$) observed on day 56 across treatment groups. Birds in the control group had highest ($P > 0.05$) cholesterol levels compared to other treatment groups for days 28, 42 and 56. There was a decreasing ($P > 0.05$) trend observed among the dexamethasone groups for triglycerides from day 42 after a gradual rise on day 28.

4.1.7 Effect of Period and Varying Doses of Dexamethasone on Corticosterone and Thyroxine levels of Broiler Chickens

The effect of varying doses of dexamethasone on corticosterone and thyroxine concentration in broiler chickens is presented in Table 4.6. On day 28, corticosterone levels ranged between 47.67 – 68.67 µg/l. Birds on 1 mg dexamethasone had the highest ($P < 0.05$) corticosterone levels compared to birds in other treatment groups. Thyroxine levels, ranging from 66.67 – 81.00 ng/ml, were not significantly ($P > 0.05$) different across treatments. On day 42, corticosterone levels ranged between 47.33 – 75.00 ng/ml. Birds offered 2 and 3 mg dexamethasone had higher ($P < 0.05$) corticosterone levels than those in the 0 and 1 mg group. Thyroxine levels ranged from 65.33 – 82.00 ng/ml. Birds in the control, 2 and 3 mg dexamethasone groups had higher

Table 4.5: Effect of Varying Doses of Dexamethasone on Serum Chemistry of Broiler Chickens

Parameters	Dexamethasone levels (mg/l)				SEM	P value	Ref*
	0	1	2	3			
Glucose (mg/dl)	243.22	226.06	256.39	232.00	18.95	0.6973	197-299
Cholesterol (mg/dl)	200.33	141.22	184.44	149.56	24.00	0.1912	129-297
Triglycerides (mg/dl)	93.67	86.67	72.44	54.61	24.99	0.7101	-

*Reference values of Clinical Diagnostic Division (1990), SEM: Standard error of the mean

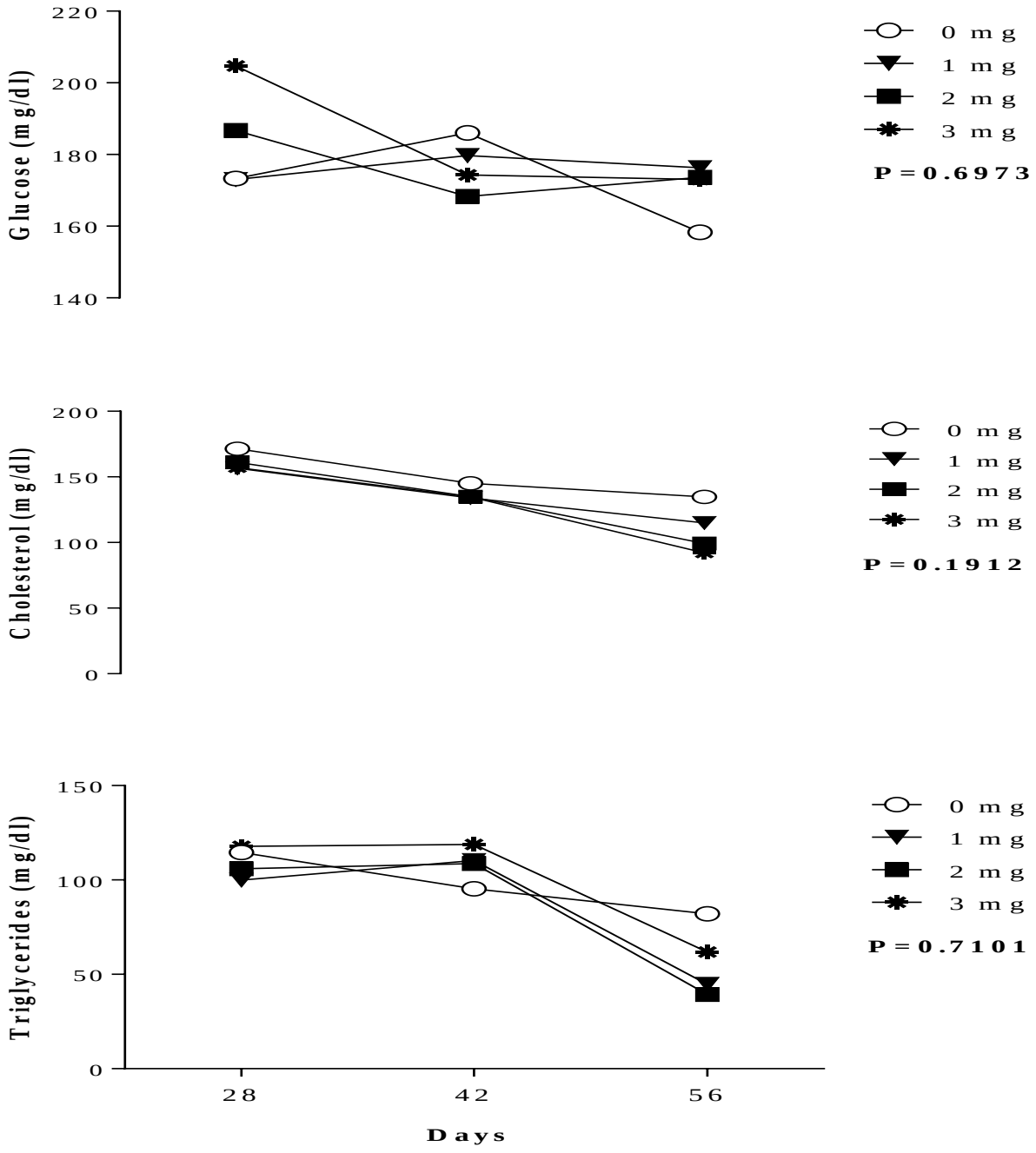

Figure 4.6: Effect of Period on Glucose, Cholesterol and Triglycerides in Dexamethasone-stressed Broiler Chickens

Table 4.6: Effect of Period and Varying Doses of Dexamethasone on Corticosterone and Thyroxine levels of Broiler Chickens

Parameters	Dexamethasone levels (mg/l) 0	1	2	3	SEM	P value
Day 28						
Corticosterone (µg/l)	54.67[b]	68.67[a]	47.67[b]	54.00[b]	2.18	0.0009
Thyroxine (ng/ml)	77.67	80.00	81.00	66.67	5.08	0.2467
Day 42						
Corticosterone (µg/l)	47.33[b]	53.33[b]	68.33[a]	75.00[a]	2.90	0.0005
Thyroxine (ng/ml)	84.67[a]	65.33[b]	76.33[ab]	82.00[a]	3.43	0.0172

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

($P < 0.05$) thyroxine levels, while those in 1 and 2 mg dexamethasone groups had similar ($P > 0.05$) levels.

4.1.8 Effect of Varying Doses of Dexamethasone on Carcass Cut Parts and Organs Weight of Broiler Chickens

The effect of varying doses of dexamethasone on carcass cut parts and organs weight is shown on Table 4.7. Result on breast weight was not significant ($P > 0.05$). The effect of dexamethasone on thigh weights (9.77 to 10.84) was significant ($P < 0.05$). Birds on 0 and 1 mg had significantly higher ($P < 0.05$) thigh and drumstick weights compared to those on 2 and 3 mg.

Liver weights ranged from 1.72 to 2.27% of live weight and were significant ($P < 0.05$). Birds in the 1, 2 and 3 mg dexamethasone group had higher ($P < 0.05$) liver weights. Liver weights were observed to increase with increasing levels of dexamethasone. Gizzard, heart, kidney and intestine weights were not significant ($P > 0.05$).

4.1.9 Effect of Varying Doses of Dexamethasone on Tibia Geometry of Broiler Chickens

The effect of varying doses of dexamethasone on broiler chicken tibia geometry is presented in Table 4.8. The effect of dexamethasone on tibia weight was significant ($P < 0.05$) ranging from 8.63 to 12.21g. Birds in the control and 1 mg dexamethasone had the highest ($P < 0.05$) tibia weight, while birds on 3 mg dexamethasone had the lowest ($P < 0.05$) tibia weight. Dexamethasone effect on tibia length was also significant ($P < 0.05$) ranging from 9.27 to 10.98 cm, with tibia length decreasing with increasing levels of dexamethasone. Birds on the control had the highest ($P < 0.05$) tibia length, while those on 3 mg dexamethasone had the lowest tibia length. Dexamethasone effect on tibia weight/length index ranged from 0.93 to 1.11. However, birds that received 0, 1 and 2 mg dexamethasone had higher tibia weight/length index ($P < 0.05$). Birds treated with 3 mg dexamethasone had the lowest ($P < 0.05$) index.The robusticity index

Table 4.7: Effect of Varying Doses of Dexamethasone on Carcass Cut Parts and Organs Weight of Broiler Chickens

Parameters (%LW)	Dexamethasone levels (mg/l) 0	1	2	3	SEM	P value
Breast	22.71	20.32	22.24	21.25	0.86	0.2393
Thigh	10.84[a]	10.47[ab]	9.77[b]	9.79[b]	0.22	0.0044
Drumstick	5.15[a]	5.12[a]	4.59[b]	4.77[b]	0.10	0.0019
Organs						
Liver	1.72[b]	2.07[ab]	2.13[ab]	2.27[a]	0.13	0.0389
Gizzard	1.94	2.08	2.12	2.09	0.12	0.7232
Heart	0.39	0.46	0.50	0.52	0.04	0.1007
Kidney	0.41	0.45	0.50	0.47	0.04	0.4630
Intestine	4.68	4.60	5.39	5.91	0.39	0.0838

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.8: Effect of Varying Doses of Dexamethasone on Tibia Geometry of Broiler Chickens

Tibia Compositions	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Tibia weight (g)	12.21^{a}	11.03^{ab}	9.73^{bc}	8.63^{c}	0.35	<0.0001
Tibia length (cm)	10.98^{a}	10.20^{b}	9.68^{c}	9.27^{d}	0.09	<0.0001
Tibia weight/length index (g/cm)	1.11^{a}	1.08^{a}	1.00^{ab}	0.93^{b}	0.03	0.0049
Robusticity index (cm/g^3)	4.77^{a}	4.59^{ab}	4.54^{b}	4.52^{b}	0.05	0.0125
Ash (%)	38.25	36.62	37.56	38.79	3.42	0.9729

$^{a, b, c, d}$ Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

ranged from 4.52 to 4.77, decreasing with increasing level of dexamethasone. However, there was no difference ($P > 0.05$) among birds fed dexamethasone. Effect of varying doses of dexamethasone on ash composition ranged from 36.62 to 38.79 and was not significant ($P > 0.05$).

4.1.10 Effect of Varying Doses of Dexamethasone on Immune Organs Weight of Broiler Chickens

Result showing the effect of varying doses of dexamethasone on immune organs weight of broiler chickens is presented in Table 4.9. Dexamethasone effect on spleen, thymus and bursa was not significant ($P > 0.05$).

4.1.11 Effect of Varying Doses of Dexamethasone on Jejunum Mucosal Morphology of Broiler Chickens

Result showing the effect of varying dexamethasone doses on jejunum mucosal morphology of broilers chickens is presented in Table 4.10. Villus height was significant ($P < 0.05$), ranging from 19.10 to 30.42 μm with birds receiving 2 mg dexamethasone having higher ($P < 0.05$) villus height compared to other treatment groups. Villus height increased with increasing levels of dexamethasone.Villus width, crypt depth and absorption area were not significantly ($P > 0.05$) different for all treatment groups.

4.1.12 Regression of Final Weight with Feed Intake and Thermoregulatory Parameters of Dexamethasone Stress-induced Broiler Chickens

Result showing the multiple regression of final weight with feed intake and thermoregulatory parameters (respiratory rate and rectal temperature) of dexamethasone stress-induced broiler chickens is shown in Table 4.11. An R^2 value of 0.97 was obtained when feed intake, rectal temperature and respiratory rate were combined in a multiple regression equation and when only feed intake and respiratory rate were used. An R^2 value of 0.95 was recorded when feed intake and rectal temperature were used and when only feed intake was used as a sole predictor.

Table 4.9: Effect of Varying Doses of Dexamethasone on Immune Organs Weight of Broiler Chickens

Parameters (% LW)	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Spleen	0.07	0.07	0.07	0.06	0.01	0.9590
Thymus	0.09	0.19	0.20	0.20	0.03	0.0674
Bursa of Fabricius	0.07	0.09	0.07	0.06	0.01	0.4586

SEM: Standard error of the mean

Table 4.10: Effect of Varying Dexamethasone Doses on Jejunum Mucosal Morphology iof Broiler Chickens

Parameters	Dexamethasone levels (mg/l)				SEM	P value
	0	1	2	3		
Villus height (μm)	19.10^{b}	24.13^{b}	30.42^{a}	24.01^{b}	1.34	0.0024
Villus width (μm)	3.10	2.69	2.67	2.90	0.66	0.9624
Crypt depth (μm)	14.23	16.29	12.09	14.33	1.93	0.5302
Absorption area (μm^2)	348.79	409.03	511.50	437.58	70.40	0.4734

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.11: Regression of Final Weight with Feed Intake and Thermoregulatory Parameters of Broiler Chickens under Dexamethasone-induced Stress

Parameters	Model	N	R^2	Adjusted R^2	P value
FI	FW = -1019.50 + 31.68FI	12	0.95	0.95	<0.0001
FI, RT	FW = -1395.33 + 31.45FI + 9.56RT	12	0.95	0.94	<0.0001
FI, RR	FW = -1396.37 + 32.09FI + 1.70RR	12	0.97	0.97	<0.0001
FI, RT, RR	FW = 309.29 + 33.21FI – 44.15RT + 1.83RR	12	0.97	0.97	<0.0001

FI-feed intake, RT-rectal temperature, RR- respiratory rate, FW-final weight, R^2-co-efficient of determination

4.2 Experiment 2: EFFECT OF 0.15% BETAINE HYDROCHLORIDE ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BROILER CHICKENS UNDER DEXAMETHASONE-INDUCED STRESS

4.2.1 Thermoregulatory Parameters

The average temperature-humidity index (THI) inside the poultry house during the experimental period in the mornings and afternoons is shown in Figure 4.7. In the mornings, THI averaged 27.1 and 32.3 in the afternoon. In general, THI in the afternoons were 16.1% higher than in the mornings. The effect of betaine HCl (0.15%) on rectal temperature of broiler chickens under dexamethasone induced stress is shown in Figure 4.8. The average rectal temperature across the treatment groups ranged between 41.58 to 41.77 ºC and was not significantly ($P > 0.05$) different. The trend, however, showed a decrease in rectal temperature with increased doses of dexamethasone treated with 0.15% betaine HCl.

The effect of 0.15% betaine HCl on respiratory rate of broiler chickens under dexamethasone induced stress is shown in Figure 4.9. The respiratory rate across the treatment groups ranged between 99.00 and 101.33 breaths per minute and was not significantly ($P > 0.05$) different. However, a decrease in respiratory rate with increased doses of dexamethasone was observed.

4.2.2 Effect of 0.15% Betaine Hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 14-28)

Growth performance of starter broiler chickens fed 0.15% betaine HCl with varying doses of dexamethasone is presented in Table 4.12. Average final weight ranged from 623.33 to 926.05 g, with chickens fed 0 mg dexamethasone having higher ($P < 0.05$) final weights compared to those in dietary treatments receiving betaine HCl and dexamethasone.

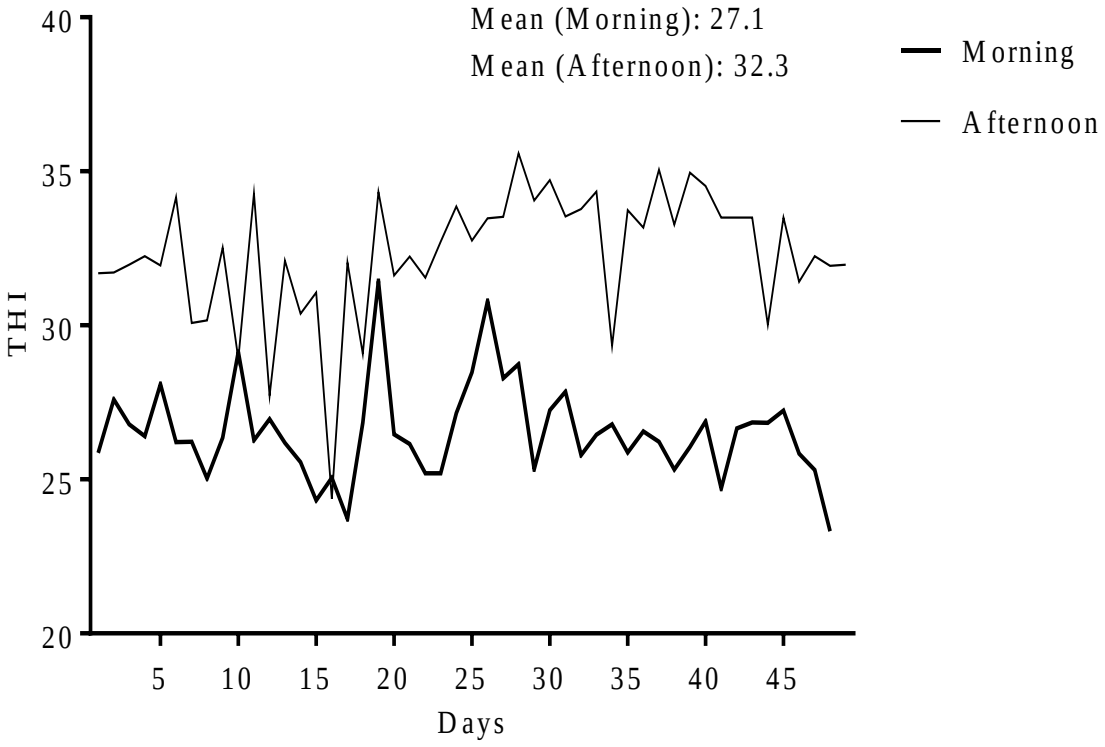

Figure 4.7: Daily Temperature-humidity index inside the poultry house during the experimental period

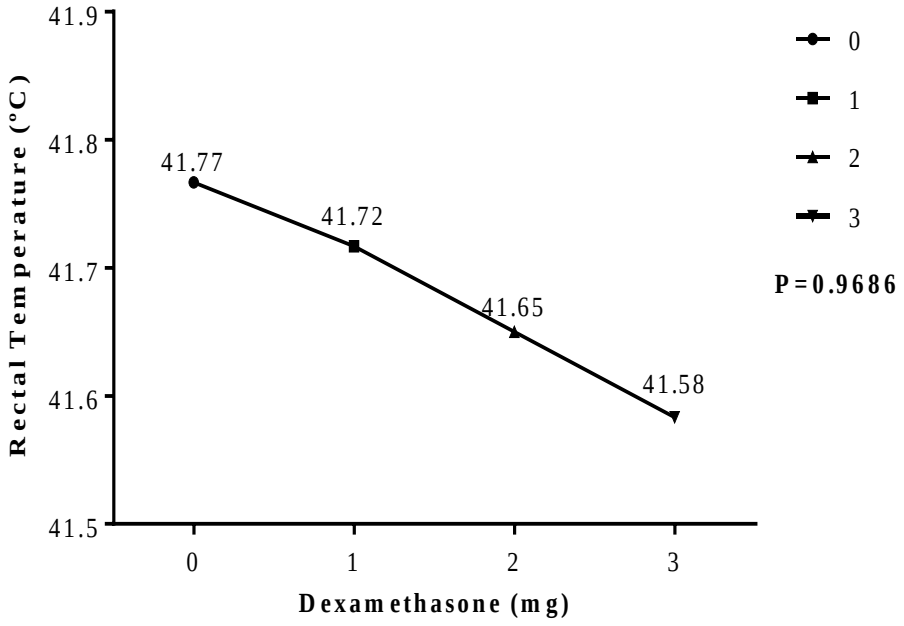

Figure 4.8: Effect of 0.15% Betaine HCl on Rectal Temperature of Dexamethasone stress-induced Broiler Chickens

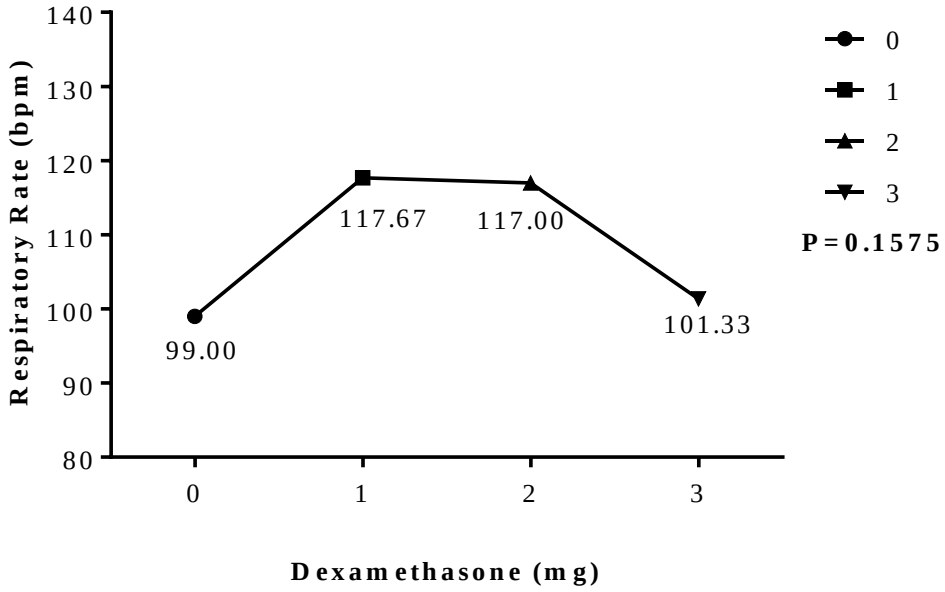

Figure 4.9: Effect of 0.15% Betaine HCl on Respiratory Rate of Dexamethasone stress-induced Broiler Chickens

Table 4.12: Effect of 0.15% Betaine Hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 14-28)

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters	0	0.15	0.15	0.15	SEM	P value
Initial weight (g/b)	115.83	115.83	115.83	115.00	0.72	0.8018
Daily Feed intake (g/b/d)	69.91^{a}	62.39^{b}	59.48^{c}	60.67^{bc}	0.89	<.0001
Final weight (g/b)	926.05^{a}	709.83^{b}	636.67^{c}	623.33^{c}	24.62	<.0001
Daily Weight gain (g/b/d)	38.58^{a}	28.29^{b}	24.80^{c}	24.21^{c}	1.17	<.0001
Feed Conversion Ratio	1.81^{a}	2.23^{ab}	2.40^{bc}	2.51^{c}	0.09	.0032
Mortality (%)	0.10	0.20	0.30	0.30	0.07	0.2192

$^{a, b,c}$Means with different superscript on the same row differ significantly ($P < 0.05$); g/b/d= gram/bird/day, SEM: Standard error of the mean

Birds administered 2 and 3 mg dexamethasone had similar ($P > 0.05$) final live weight. Feed intake ranged from 59.48 to 69.91 g/bird/day across the dietary treatments. Animals on dexamethasone treated with 0.15% beataine HCl had lower ($P < 0.05$) feed intake compared to those on the control (0 mg). Feed intake of birds on dexamethasone was similar ($P > 0.05$). The effect of dexamethasone on the average weight gain ranged from 24.21 to 38.58 g. Birds in the control (0 mg dexamethasone) had higher ($P < 0.05$) daily weight gain compared to the other treatment groups. A downward trend in weight gain was observed with increasing doses of dexamethasone inspite of betaine treatment.

Feed conversion ratio ranged from 1.81 to 2.51 with birds on the control and1 mg dexamethasone groups performing best ($P < 0.05$). Differences among the dexamethasone groups were significant ($P < 0.05$) showing an increasing trend with increasing levels of dexamethasone. Mortality among the treatments was not significant ($P > 0.05$), showing an increasing trend inspite of betaine HCl addition.

4.2.3 Effect of 0.15% Betaine hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 28-49)

Growth performance of finisher broiler chickens fed 0.15% betaine HCl with varying doses of dexamethasone is presented in Table 4.13. Differences among the dietary treatments for final weight ranged from 1522.33 to 2133.55 g with chickens fed 0 mg dexamethasone having higher ($P < 0.05$) final weights compared to other dietary treatments which were similar ($P > 0.05$).The daily feed intake of the birds varied from 114.15 to 127.53 g/bird/day across the dietary treatments. Animals in the control had higher ($P < 0.05$) feed intake compared to those fed betaine HCl. The average weight gain ranged from 38.02 to 67.12 g with birds in the control having

Table 4.13: Effect of 0.15% Betaine Hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 28-49)

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters	0	0.15	0.15	0.15	SEM	P value
Initial weight (g/b)	926.05[a]	709.83[b]	636.67[c]	623.33[c]	24.62	<.0001
Final weight (g/b)	2133.55[a]	1687.22[b]	1522.33[b]	1635.23[b]	32.02	<.0001
Daily Weight gain (g/b/d)	67.12[a]	45.87[b]	38.02[b]	43.39[b]	1.52	<.0001
Daily Feed intake (g/b/d)	127.53[a]	116.89[bc]	114.15[c]	119.93[b]	2.41	.0003
Feed Conversion Ratio	1.66[a]	2.57[b]	2.91[c]	2.57[bc]	0.04	.0005
Mortality (%)	0.10	0.10	0.30	0.16	0.14	0.2112

[a, b,c]Means with different superscript on the same row differ significantly (P < 0.05); g/b/d= gram/bird/day

higher ($P < 0.05$) daily weight gain compared to the betaine HCl and dexamethasone containing treatments. Feed conversion ratio ranged from 1.66 to 2.91 with birds in the controlperforming the best ($P < 0.05$). Birds on dexamethasone had similar ($P > 0.05$) feed conversion. Mortality results were not significant ($P > 0.05$).

4.2.4 Effect of 0.15% Betaine Hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 14-49)

Growth performance of broiler chickens fed 0.15% betaine HCl with varying doses of dexamethasone is presented in Table 4.14. Final weight ranged from 1541.67 to 2088.79 g, with birds in the control having higher ($P < 0.05$) final weight compared to dietary treatments receiving betaine HCl. The feed intake ranged from 84.19 to 104.81 g/bird/day across the dietary treatments with birds in the control having higher ($P < 0.05$) feed intake compared to those fed betaine HCl which were similar ($P > 0.05$).

The effect of dexamethasone on weight gain ranged from 33.95 to 46.97 g. Birds in the control had higher ($P < 0.05$) daily weight gain thanbetaine containing treatments. Feed conversion ratio differed ranged from 2.23 to 2.48 with birds on the control, 1 and 3 mg dexamethasone performingthe best ($P < 0.05$). Mortality result was not significant ($P > 0.05$) for all treatments

4.2.5 Effect of 0.15% Betaine Hydrochloride on Haematological Indices of Dexamethasone Stress Induced Broiler Chickens

Result showing the effect of 0.15% betaine HCl on haematological indices of dexamethasone stress-induced broiler chickens is presented in Table 4.15. All haematological indices did not differ significantly ($P > 0.05$); they exceeded reference values. Across the days of study, PCV and haemoglobin (Figure 4.10), gave a similar response where birds on the control, 1 and 2 mg dexamethasone showed a decline ($P > 0.05$) from day 21 followed by an increase on day 35,

Table 4.14: Effect of 0.15% Betaine HCl on Performance of Dexamethasone Stress Induced Broiler Chickens (day 14-49)

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters	0	0.15	0.15	0.15	SEM	P value
Initial weight (g/b)	115.83	115.83	115.83	115.00	0.72	0.8018
Final weight (g/b)	2088.79^{a}	1690.35^{b}	1541.67^{c}	1657.52^{bc}	30.45	<.0001
Daily Weight gain (g/b/d)	46.97^{a}	37.49^{b}	33.95^{c}	36.73^{bc}	0.73	<.0001
Daily Feed intake (g/b/d)	104.81^{a}	89.21^{b}	84.19^{b}	87.27^{b}	1.58	<.0001
Feed Conversion Ratio	2.23^{a}	2.38^{ab}	2.48^{b}	2.38^{ab}	0.03	.0041
Mortality (%)	0.00	0.10	0.30	0.16	0.15	0.2754

$^{a, b,c}$Means with different superscript on the same row differ significantly ($P < 0.05$); g/b/d= gram/bird/day, SEM: Standard error of the mean

Table 4.15: Effect of 0.15% Betaine HCl on Haematological Indices of Dexamethasone Stress Induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	1	2	3			
	Betaine HCl (%)						
Parameters	0	0.15	0.15	0.15	SEM	P value	Ref*
Packed cell volume (%)	37.74	40.83	39.57	41.89	1.61	0.2593	22-35
Haemoglobin (g/dl)	11.60	12.57	12.50	13.06	0.47	0.1912	7-13
Leucocyte ($x10^9$/l)	79.54	82.89	85.89	86.31	3.74	0.4360	1.2-3.0
Erythrocyte ($x10^{12}$/l)	2.58	2.71	2.67	2.85	0.14	0.3098	2.5-3.5
Heterophils (%)	1.93	2.28	1.79	2.19	0.55	0.9012	15-40
Lymphocytes (%)	91.46	90.43	90.89	90.78	1.07	0.8883	45-70
H:L	0.02	0.03	0.02	0.02	0.01	0.9017	-

H:L= Heterophil-Lymphocyte ratio; *Reference values of Jain (1993), SEM: Standard error of the mean

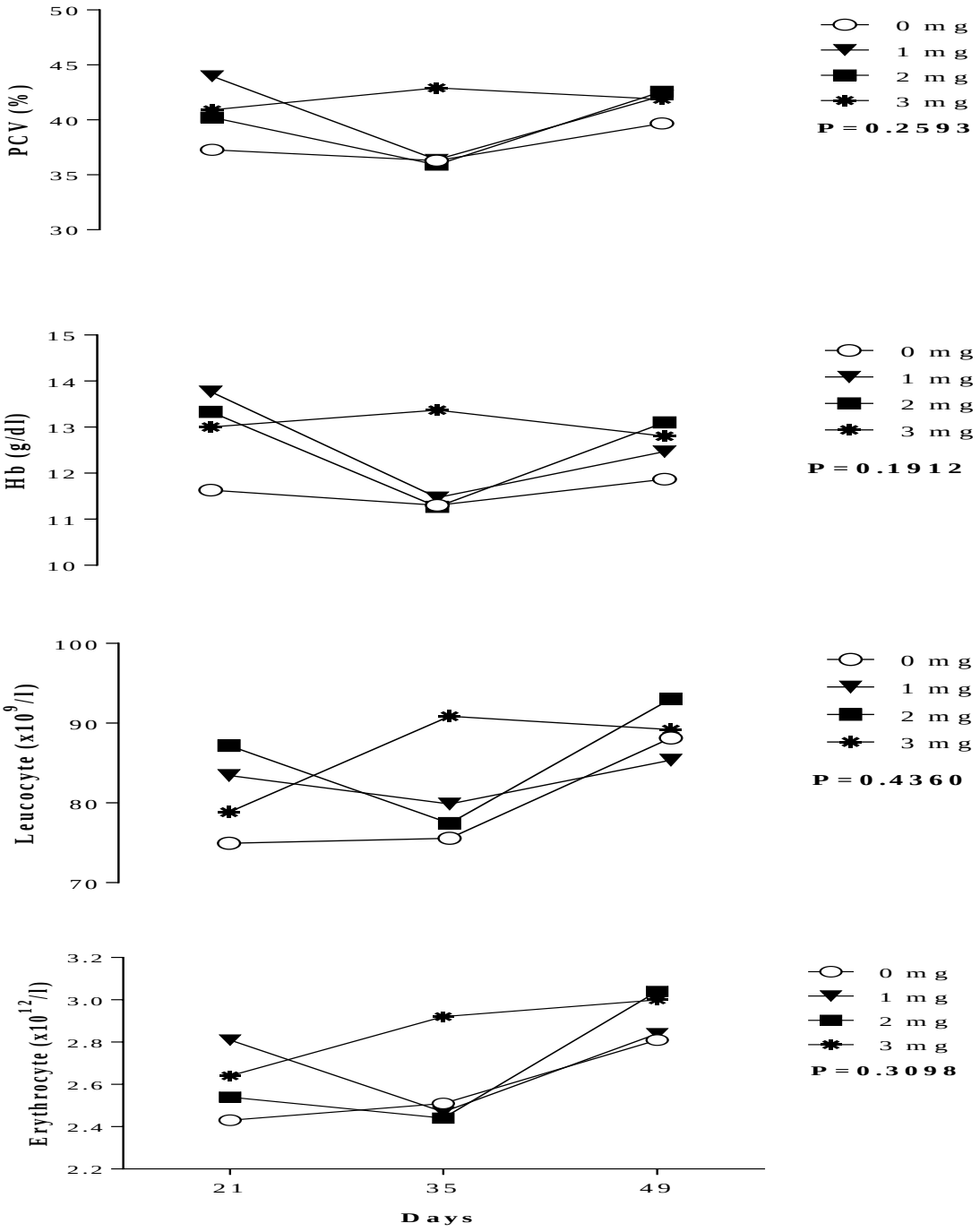

Figure 4.10: Effect of Period on Packed cell volume, Haemoglobin, Leucocyte and Erythrocyte counts in Dexamethasone Stress-induced Broiler Chickens fed 0.15% Betaine HCl

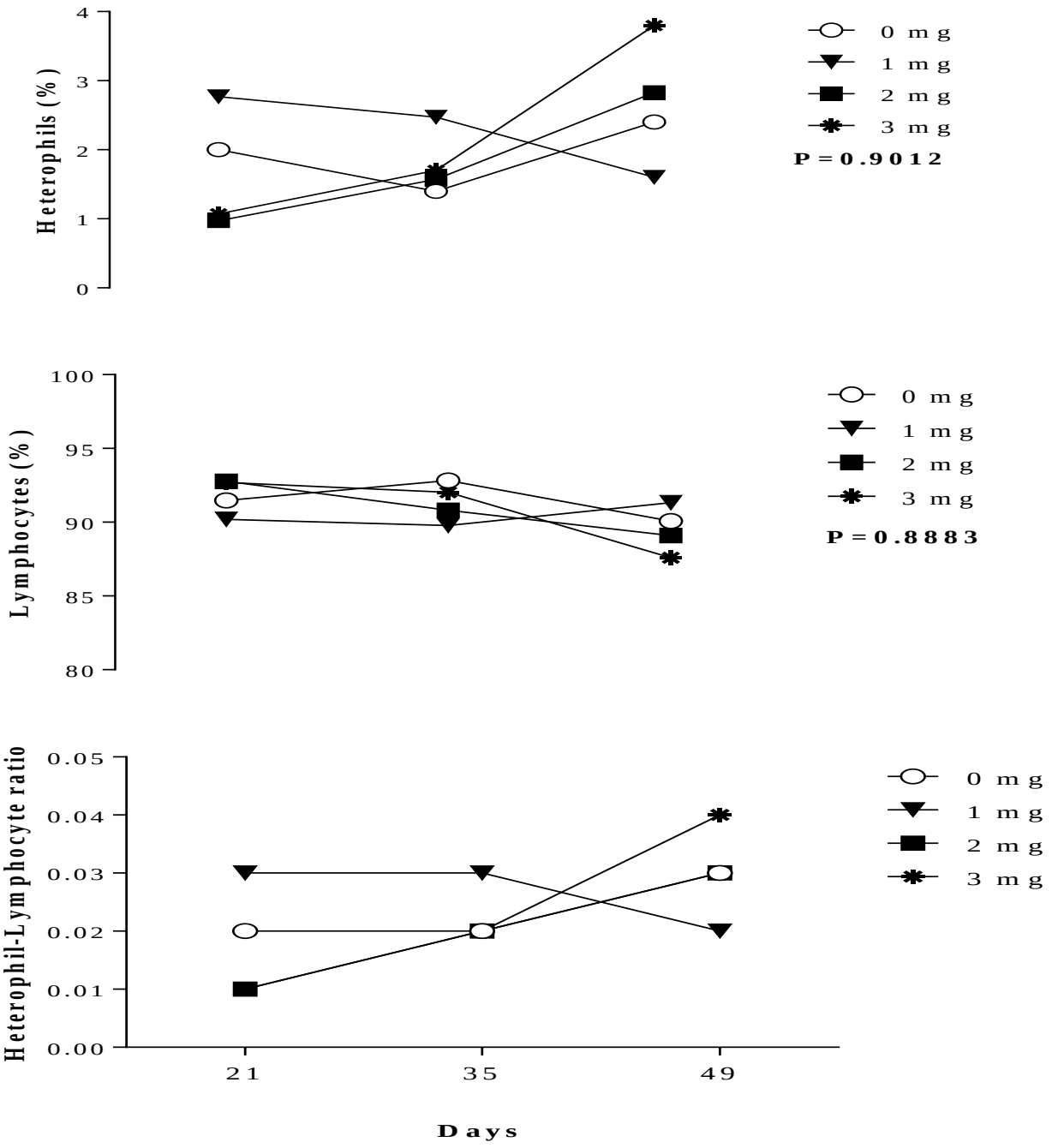

Figure 4.11: Effect of Period on Heterophils, Lymphocytes and Hereophil-Lymphocyte Ratio in Dexamethasone Stress-induced Broiler Chickens fed 0.15% Betaine HCl

with the exception of birds on 3 mg which increased on day 35 and decreased ($P > 0.05$) thereafter. Figure 4.10 showed that the highest ($P > 0.05$) values of leucocytes were reached on day 49 for the birds receiving 2 mg dexamethasone. Birds in the control group showed an increasing trend across the days; streadily increasing before sharply rising on day 49. Birds on the 1 and 2 mg groups showed a decline on day 35 before rising and peaking on day 49. Erythrocyte values showed an increasing trend for birds in the control and 3 mg dexamethasone group with the 1 and 2 mg group showing a decrease ($P > 0.05$) on day 35 before increasing on day 49.

Heterophil values (Figure 4.11) for the 1 mg group showed a downward trend with the lowest ($P > 0.05$) heterophil levels observed on day 49 while an increasing trend for birds in the 2 and 3 mg group was observed. Birds in the 1mg group showed a steady increase ($P > 0.05$) in lymphocyte across the days of study while birds on the 2 and 3 mg dexamethasone showed a decrease ($P > 0.05$). Birds receiving 1 mg showed a decline on day 35 after remaining unchanged from day 21 to 35 for heterophil-lymphocyte ratio while an increase among the control, 2 and 3 mg dexamethasone groups was observed. The highest ($P > 0.05$) heterophil-lymphocyte ratio was observed on day 49 for the 2 and 3 mg group.

4.2.6 Effect of 0.15% Betaine HCl on Serum Chemistry of Dexamethasone Stress-induced Broiler Chickens

Results showing the effect of 0.15% betaine HCl on serum chemistry of dexamethasone stress-induced broiler chickens are presented in Table 4.16. All serum parameters were not significant ($P > 0.05$) and were outside the reference values. For glucose values (Figure 4.12), birds on the control and 1 mg showed an increasing trend from day 21 to day 35 before decreasing with lower

Table 4.16: Effect of 0.15% Betaine HCl on Serum Chemistry of Dexamethasone Stress Induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	1	2	3			
	Betaine HCl (%)						
Parameters	0	0.15	0.15	0.15	SEM	P value	Ref*
Glucose (mg/dl)	172.56	176.33	176.22	184.00	7.15	0.6939	197-299
Cholesterol (mg/dl)	150.40	135.19	131.73	127.94	15.41	0.0668	129-297
Triglycerides (mg/dl)	97.30	84.99	84.67	99.49	18.53	0.4254	-

*Reference values of Clinical Diagnostic Division (1990), SEM: Standard error of the mean

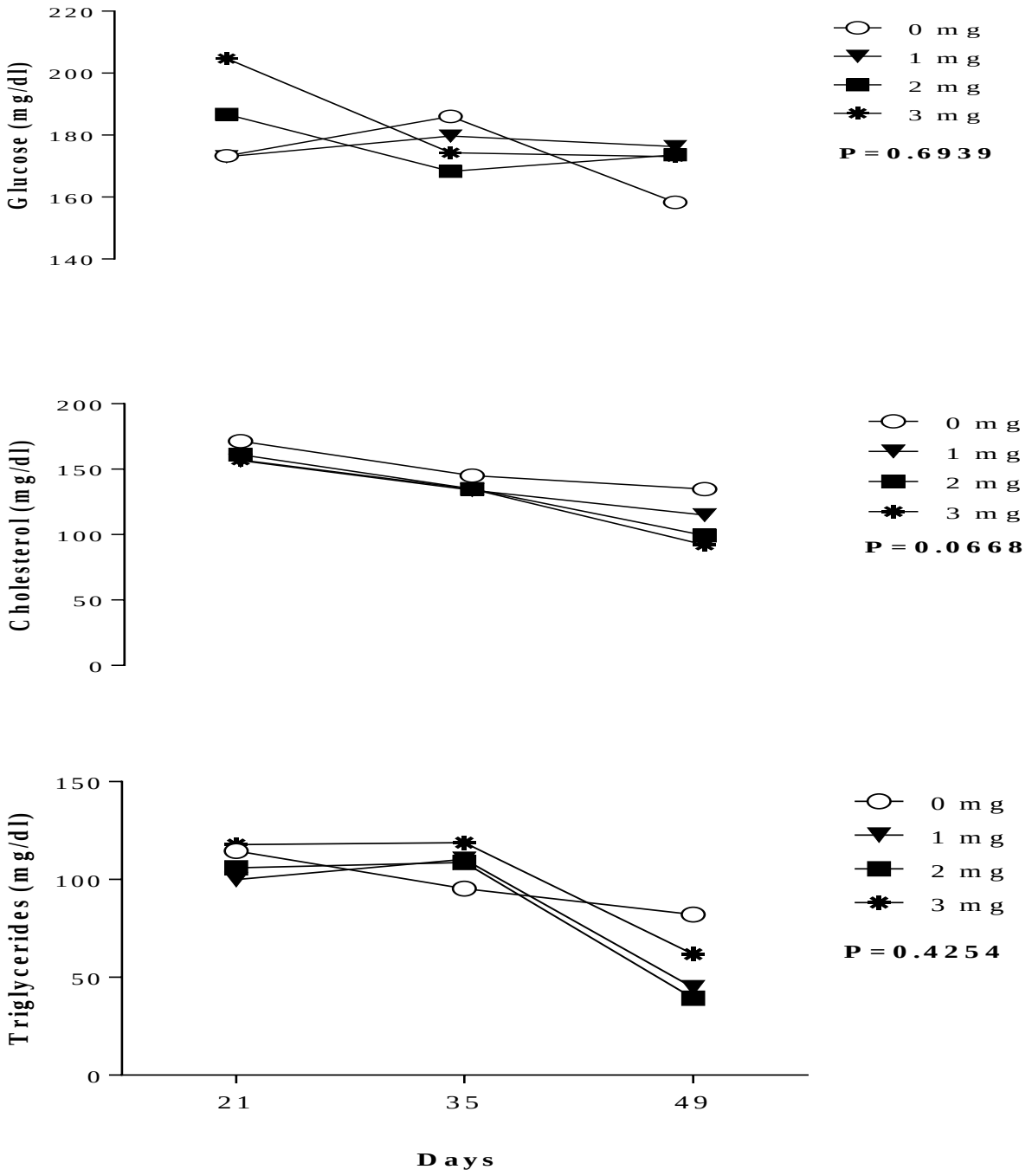

Figure 4.12: Effect of Period on Glucose, Cholesterol and Triglycerides in Dexamethasone Stress Induced Broiler Chickens fed 0.15% Betaine HCl

values on day 49. Birds on the 2 and 3 mg group showed an opposite trend, decreasing from day 21 to day 35. Cholesterol showed a decreasing trend for all the treatment groups including the control with lowest levels observed on day 49. Dexamethasone groups receiving betaine HCl had lower levels of cholesterol compared to the control. There was also a downward trend observed for the treatment groups for triglycerides with the lowest triglyceride levels reported on day 49 after an initial increase on day 35.

4.2.7 Effect of 0.15% Betaine Hydrochloride on Carcass Cut parts and Organs Weight of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.15% betaine HCl on carcass cut parts and organs weight of dexamethasone stress-induced broiler chickens is shown in Table 4.17. Breast weights were not significant ($P>0.05$). The effect of betaine HCl on thigh weights ranged from 9.63 to 10.82 with birds on the control and 2 mg dexamethasone having higher ($P<0.05$) thigh weights, although those on 2 mg were similar ($P>0.05$) with those on 1 and 3 mg. For drumstick weights, birds in the control had higher ($P<0.05$) weights compared with the rest of the betaine HCl treatments which were similar ($P>0.05$). Liver, gizzard, heart and intestine weights were not significant ($P>0.05$). Kidney weights ranged from 0.41 to 0.61 with the dexamethasone groups having higher ($P<0.05$) weights.

4.2.8 Effect of 0.15% Betaine Hydrochloride on Tibia Geometry of Dexamethasone Stress-induced Broiler Chickens

Results showing the effect of 0.15% betaine HCl on tibia composition of dexamethasone stress-induced broiler chickens are presented in Table 4.18. Tibia weight ranged from 4.49 to 6.36 g with birds on the control having higher ($P<0.05$) tibia weights while all betaine treated treatments were similar ($P>0.05$). The effect of betaine HCl on tibia length ranged from 8.20 to 9.50 cm, with birds in the control having the highest ($P<0.05$) tibia length.

Table 4.17: Effect of 0.15% Betaine HCl on Carcass Cut parts and Organs Weight of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters (% LW)	0	0.15	0.15	0.15	SEM	P value
Breast	21.30	21.84	21.39	21.15	0.66	0.8879
Thigh	10.82[a]	9.63[b]	10.06[ab]	9.88[b]	0.23	0.0109
Drumstick	5.27[a]	4.70[b]	4.72[b]	4.63[b]	0.11	0.0025
Organs						
Liver	2.03	2.16	1.95	2.21	0.14	0.5561
Gizzard	2.14	2.51	2.44	2.39	0.10	0.1078
Heart	0.42	0.46	0.41	0.40	0.02	0.2806
Kidney	0.41[b]	0.53[ab]	0.45[ab]	0.61[a]	0.04	0.0201
Intestine	5.31	5.59	5.05	5.86	0.35	0.4124

[a, b]Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.18: Effect of 0.15% Betaine HCl on Tibia Geometry of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Tibia Compositions	0	0.15	0.15	0.15	SEM	P value
Tibia weight (g)	6.36[a]	5.03[b]	4.58[b]	4.49[b]	0.28	0.0004
Tibia length (cm)	9.50[a]	8.32[b]	8.20[b]	8.32[b]	0.11	0.0001
Tibia weight/length index (g/cm)	0.67[a]	0.60[ab]	0.56[b]	0.54[b]	0.03	0.0158
Robusticity index (cm/g^3)	5.15	4.86	4.94	5.04	0.07	0.0576
Ash (%)	35.74	40.92	43.52	35.32	3.61	0.3587

[a, b]Means with different superscript on the same row differ significantly ($P < 0.05$)

All birds fed betaine HCl had similar ($P > 0.05$) tibia lengths. Tibia weight/length index ranged from 0.54 to 0.67 g/cm. The birds in the control and 1 mg group gave higher ($P < 0.05$) index and it decreased with increasing doses of dexamethasone. Birds receiving 2 and 3 mg dexamethasone were similar ($P > 0.05$) with those on 1 mg dexamethasone. Effect of betaine HCl on robusticity index was not significant ($P > 0.05$). Effect of betaine HCl on ash composition ranged from 35.32 to 43.52 and was also not significant ($P > 0.05$).

4.2.9 Effect of 0.15% Betaine Hydrochloride on Immune Organs Weight of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.15% betaine hydrochloride on immune organs weight of dexamethasone stress-induced broiler chickens is presented in Table 4.19. Results on all immune organs were not significant ($P > 0.05$).

4.2.10 Prediction of Final Weight with Feed Intake and Thermoregulatory Parameters of Dexamethasone Stress-induced Broiler Chickens fed 0.15% Betaine HCl

Result showing the multiple regression of final weight with feed intake and thermoregulatory parameters (respiratory rate and rectal temperature) of dexamethasone stress-induced broilersfed 0.15 % betaine HCl is shown in Table 4.20. An R^2 value of 0.96 was obtained when feed intake, rectal temperature and respiratory rate were combined in a multiple regression equation. An R^2 value of 0.95 was recorded when feed intake and rectal temperature were used and also when only feed intake was used as a sole predictor.

Table 4.19: Effect of 0.15% Betaine HCl on Immune Organs Weight of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)					
	0	1	2	3		
	Betaine HCl (%)					
Parameters (%LW)	0	0.15	0.15	0.15	SEM	P value
Spleen	0.07	0.08	0.09	0.09	0.02	0.8053
Thymus	0.29	0.22	0.27	0.24	0.04	0.6175
Bursa of Fabricius	0.06	0.07	0.07	0.06	0.01	0.4384

SEM: Standard error of the mean

Table 4.20: Regression of Final Weight with Feed Intake and Thermoregulatory Parameters of Dexamethasone Stress-induced Broilers fed 0.15% Betaine HCl

Parameters	Model	N	R^2	Adjusted R^2	P value
FI	FW = -537.31+ 24.97FI	12	0.96	0.96	<.001
FI, RT	FW = -2034.35 + 24.93FI – 35.99RT	12	0.96	0.95	<.001
FI, RR	FW = -381.24 + 24.85FI – 0.70RR	12	0.96	0.95	<.001
FI, RT, RR	FW = -2193.41 + 24.75FI +44.84RT – 0.94RR	12	0.96	0.95	<.001

FI-feed intake, RT-rectal temperature, RR- respiratory rate, FW-final weight, R^2-co-efficient of determination

4.3 Experiment 3: EFFECT OF 0.30% BETAINE HYDROCHLORIDE ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BROILER CHICKENS UNDER DEXAMETHASONE-INDUCED STRESS

4.3.1 Thermoregulatory parameters

The average temperature-humidity index (THI) inside the poultry house during the experimental period for mornings and afternoons is shown in Figure 4.13. In the mornings, THI averaged 23.5 and 31.5 in the afternoon. In general, THI in the afternoons were 25.4% higher than in the mornings.

The effect of 0.3% betaine HCl on rectal temperature of broiler chickens under dexamethasone induced stress is shown in Figure 4.14. The rectal temperature across the treatment groups ranged between 41.29 to 41.47 ºC and was not significant ($P > 0.05$). Numerically however, a relative decrease in rectal temperature with increasing doses of dexamethasone was observed. Birds in the betaine only group had the lowest rectal temperature compared to other treatment groups. The effect of 0.3% betaine HCl on respiratory rate of broiler chickens under dexamethasone induced stress is shown in Figure 4.15. The respiratory rate across the treatment groups ranged between 98.44 and 121.00 breaths per minute and was not significant ($P > 0.05$). However, a decrease in respiratory rate with increasing dose of dexamethasone was observed. Birds in the betaine only group had the lowest respiratory rate.

4.3.2 Effect of 0.30% Betaine Hydrochloride on Performance of Dexamethasone Stress Induced Broiler Chickens (day 14-28)

Growth performances of broiler chickens fed 0.30% betaine HCl under dexamethasone-induced stress (day 14-28) is presented in Table 4.21. The daily feed intake varied from 50.09 to 60.93 g/bird/day across the dietary treatments. Feed intake of birds in the control was higher ($P < 0.05$) than other treatment groups with animals in the dexamethasone group having similar ($P > 0.05$)

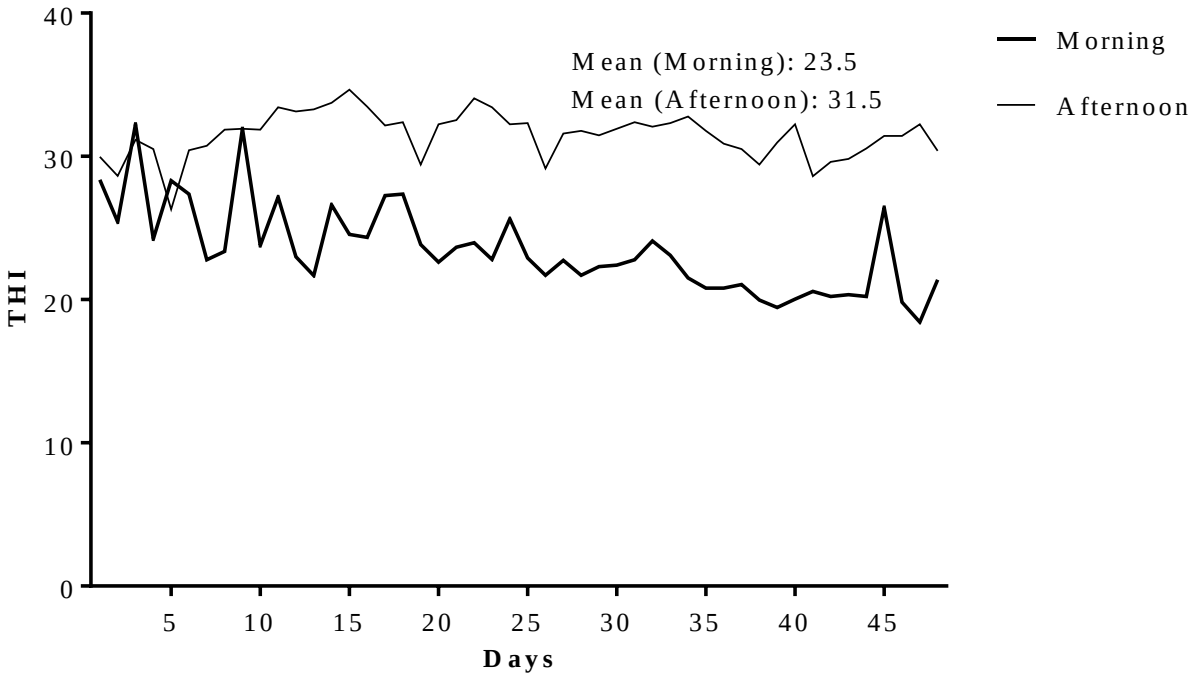

Figure 4.13: Daily Temperature-humidity index inside the poultry house during the experimental period

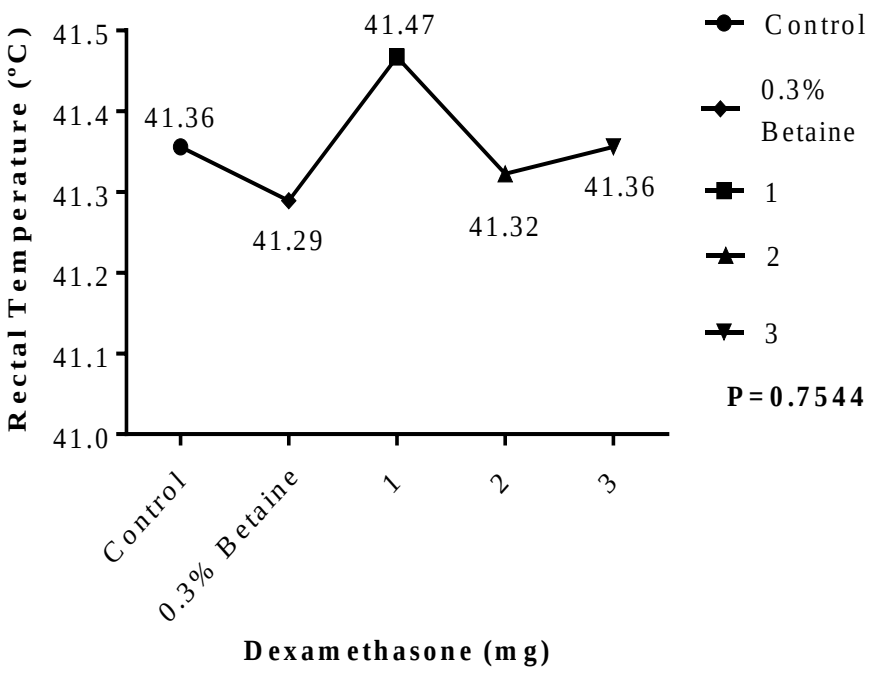

Figure 4.14: Effect of 0.30% Betaine HCl on Rectal Temperature of Dexamethasone Stress-induced Broiler Chickens

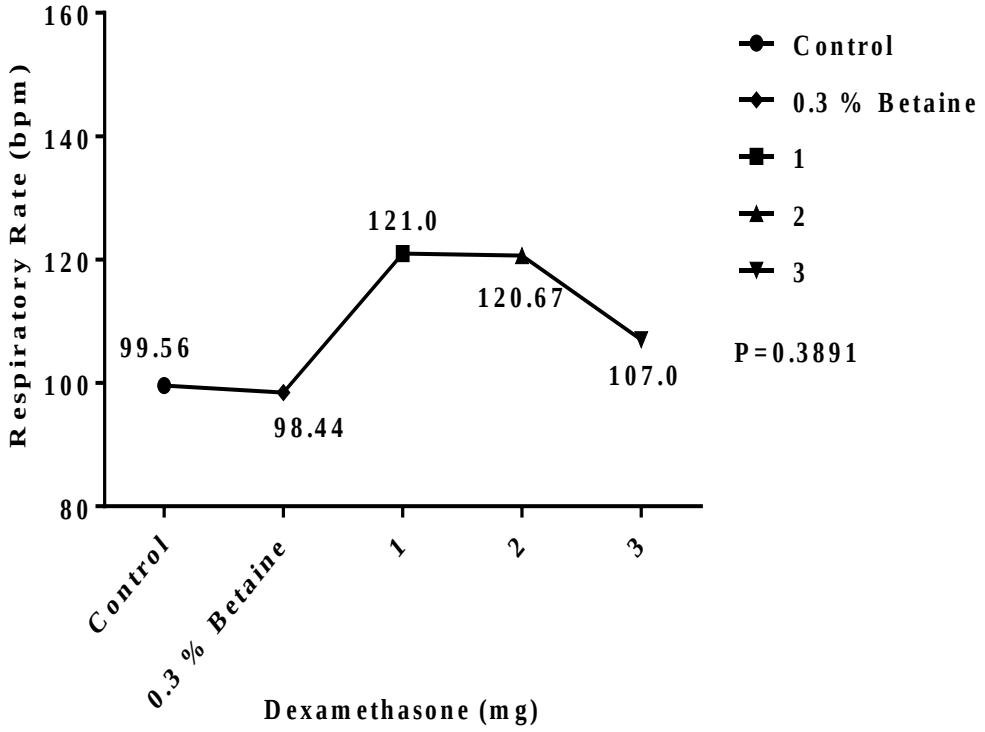

Figure 4.15: Effect of 0.30% Betaine HCl on Respiratory Rate of Dexamethasone Stress-induced Broiler Chickens

Table 4.21: Effect of 0.30% Betaine HCl on Performance of Dexamethasone Stress-induced Broiler Chickens (day 14-28)

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value
Initial weight (g/b/)	113.33	112.5	113.33	113.33	114.17	0.75	0.6554
Daily Feed intake (g/b/d)	60.93[a]	56.38[b]	51.37[c]	50.09[c]	50.64[c]	0.88	<0.0001
Final weight (g/b)	1100.00[a]	1025.65[a]	858.33[b]	848.91[b]	808.33[b]	18.67	<0.0001
Daily Weight gain (g/b/d)	35.24[a]	32.61[a]	26.61[b]	26.27[b]	24.79[b]	0.66	<0.0001
Feed conversion ratio	1.73[a]	1.73[a]	1.93[ab]	1.91[ab]	2.05[b]	0.05	0.0067
Mortality (%)	0.30	0.20	0.30	0.20	0.10	0.13	0.2742

[a, b,c, d] Means with different superscript on the same row differ significantly ($P < 0.05$), g/b/d= gram/bird/day, SEM: Standard error of the mean

feed intake. Differences among the dietary treatments for final weight ranged from 808.33 g to 1100.00 g with birds in the control and the betaine only supplemented diet having higher ($P > 0.05$) final weights compared to the dexamethasone groups. The effect of betaine HCl on the daily weight gain ranged from 24.79 to 35.24 g. Birds in the control and the betaine only supplemented diet groups had higher ($P < 0.05$) daily weight gain. Birds in the dexamethasone groups had similar ($P > 0.05$) weight gain. Feed conversion ratio ranged from 1.73 to 2.05. Birds in the control, the betaine only supplemented diet, 1 and 2 mg dexamethasone had significantly ($P < 0.05$) better FCR values.Mortality among the treatments were not significant ($P > 0.05$).

4.3.3 Effect of 0.30% Betaine Hydrochloride on Performance of Dexamethasone Stress-induced Broiler Chickens (day 28-49)

Growth performances of broiler chickens fed 0.30% betaine HCl under dexamethasone-induced stress (day 28-49) is presented in Table 4.22. The daily feed intake ranged from 149.17 to 162.37 g/bird/day across the dietary treatments with birds in the control having higher ($P < 0.05$) feed intake compared to the other treatments with animals on 3 mg having the least.

Final weight ranged from 2178.39 to 2591.80 g with birds in the control and betaine only supplemented diet groups having higher ($P < 0.05$) final weights compared to the dexamethsone treatments. The daily weight gain ranged from 59.53 to 79.22 g. Birds fed betaine only supplemented diets and the control had higher ($P < 0.05$) weight gain compared to dexamethsone treatments. Birds administered 2 and 3 mg dexamethasone had the lowest ($P < 0.05$) weight gain.

Feed conversion ratio ranged from 1.97 to 2.62 with birds fed betaine only supplemented diet and the control giving the best ($P < 0.05$) FCR. Birds on 1 and 3 mg dexamethasone were similar ($P > 0.05$) with the poorest FCR. Mortality records were also significant ($P < 0.05$) with those in the control and 1 mg groups having the highest mortality.

Table 4.22: Effect of 0.30% Betaine HCl on Performance of Dexamethasone Stress-induced Broiler Chickens (day 28-49)

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value
Initial weight (g/b)	1100.00[a]	1025.65[a]	858.33[b]	848.91[b]	808.33[b]	18.67	<0.0001
Daily Feed intake (g/b/d)	162.37[a]	157.71[b]	156.76[b]	157.03[b]	149.17[c]	1.70	0.0001
Final weight (g/b)	2576.41[a]	2591.80[a]	2288.47[b]	2183.01[c]	2178.39[c]	48.15	<.0001
Daily Weight gain (g/b/d)	78.48[a]	79.22[a]	64.77[b]	59.75[c]	59.53[c]	2.29	0.0016
Feed conversion ratio	2.03[a]	1.97[a]	2.42[c]	2.62[d]	2.49[c]	0.06	0.0052
Mortality (%)	0.30[a]	0.20[b]	0.30[a]	0.00[c]	0.00[c]	0.04	0.0009

[a, b,c, d] Means with different superscript on the same row differ significantly ($P < 0.05$), g/b/d= gram/bird/day, SEM: Standard error of the mean

4.3.4 Effect of 0.30% Betaine Hydrochloride on Performance of Dexamethasone Stress-induced Broiler Chickens (day 14-49)

Growth performances of broiler chickens fed 0.30% betaine under dexamethasone-induced stress is presented in Table 4.23. The daily feed intake varied from 90.77 to 107.40 g/bird/day across the dietary treatments. Feed intake of birds in the control was higher ($P < 0.05$) compared to other treatment groups with animals in the 2 and 3 mg dexamethasone group having similar ($P > 0.05$) feed intake. The final weight ranged from 2092.11 to 2700.00 g, with birds fed betaine only supplemented dietand the control having higher ($P < 0.05$) final weight compared to the dexamethsone treatments. Birds in the dexamethasone groups had similar ($P < 0.05$) final weights. The daily weight gain ranged from 40.37 to 52.79 g. Birds on the control and birds fed betaine only supplemented diets had higher ($P < 0.05$) weight gain compared to the birds in the dexamethasone groups which had similar ($P > 0.05$) weight gain.

Feed conversion ratio ranged from 1.95 to 2.30 with birds in the control and those fed betaine only diet having better ($P < 0.05$) FCR compared to birds receiving dexamethasone which were similar ($P > 0.05$). Mortality results were not significant ($P > 0.05$).

4.3.5 Effect of 0.30% Betaine Hydrochloride on Haematological indices of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.30% betaine hydrochloride on haematological indices of dexamethasone stress induced broiler chickens is shown in Table 4.24. Haemoglobin, red and leucocyte counts were not significant ($P > 0.05$) for all treatments. Pack cell volume ranged between 37.48 and 43.32%. PCV among the dexamethasone groups decreased ($P < 0.05$) with increasing doses of dexamethasone. Birds in the control, betaine only, 1 and 2 mg dexamethasone groups had similar ($P > 0.05$) PCV values.

Table 4.23: Effect of 0.30% Betaine HCl on Performance of Dexamethasone Stress-induced Broiler Chickens (day 14-49)

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value
Initial weight (g/b)	113.33	112.50	113.33	113.33	114.17	0.75	0.6554
Daily Feed intake (g/b/d)	107.40^{a}	101.51^{b}	95.32^{c}	94.54^{cd}	90.77^{d}	0.97	<.0001
Final weight (g/b)	2700.00^{a}	2661.89^{a}	2238.16^{b}	2125.93^{b}	2092.11^{b}	47.62	0.0001
Daily Weight gain (g/b/d)	52.79^{a}	52.03^{a}	43.36^{b}	41.07^{b}	40.37^{b}	0.97	<.0001
Feed conversion ratio	2.03^{a}	1.95^{a}	2.20^{b}	2.30^{b}	2.25^{b}	0.03	<.0001
Mortality (%)	0.30	0.00	0.20	0.20	0.23	0.14	0.0937

$^{a, b,c, d}$ Means with different superscript on the same row differ significantly (P<0.05), g/b/d= gram/bird/day, SEM: Standard error of the mean

Table 4.24: Effect of 0.30% Betaine HCl on Haematological Indices of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)							
	0	0	1	2	3			
	Betaine HCl (%)							
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value	Ref*
Packed cell volume (%)	41.50^{ab}	39.13^{ab}	43.32^{a}	40.23^{ab}	37.48^{b}	1.04	0.0076	22-35
Haemoglobin (g/dl)	27.05	25.96	27.83	26.50	24.88	8.04	0.0909	7-13
Leucocyte (x10^{9}/l)	100.70	90.41	100.67	93.35	86.97	3.90	0.1113	1.2-3.0
Erythrocyte (x10^{12}/l)	2.79	2.67	2.76	2.73	2.77	0.09	0.9133	2.5-3.5
Heterophils (%)	1.02^{b}	1.20^{b}	2.07^{a}	2.17^{a}	1.15^{b}	0.15	0.0027	15-40
Lymphocytes (%)	95.02^{a}	95.10^{a}	87.38^{b}	91.75^{ab}	93.97^{a}	1.13	0.0037	45-70
H:L	0.01^{b}	0.01^{b}	0.02^{a}	0.02^{a}	0.01^{b}	0.001	0.0001	-

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$), H:L= Heterophil-Lymphocyte ratio; *Reference values of Jain (1993), SEM: Standard error of the mean

Heterophil levels ranged between 1.02 and 2.17%, with birds receiving 1 and 2 mg dexamethasone having higher ($P < 0.05$) heterophil counts than the other treatment groups which were similar ($P > 0.05$). Lymphocyte levels were also higher ($P < 0.05$) for the control, betaine only diet, 2 and 3 mg dexamethasone groups. Heterophil-Lymphocyte ratio ranged between 0.01 and 0.02. Birds in the 1 and 2 mg dexamethasone groups had higher ($P < 0.05$) ratios than the other treatment groups which were similar ($P > 0.05$). All haematological indices exceeded the reference range values.

The PCV and haemogblobin (Figure 4.16) were significant ($P < 0.05$) on day 21. Birds in the 1 mg dexamthasone group gave the highest PCV and haemoglobin levels on day 21 showing a decreasing trend with increasing doses of dexamethasone. A steady decline for PCV was also observed on day 35 for the dexamethasone containing groups with birds in the 3 mg group giving the lowest ($P < 0.05$) PCV levels on day 35. The highest ($P > 0.05$) values of leucocyte were reached on day 35 for the control group, showing a decreasing trend from the control to the dexamethasone containing treatments. Birds in the dexamethasone groups also gave a downward trend on day 21 with the betaine only and the 3 mg dexamethasone group having the lowest ($P < 0.05$) leucocyte count. Erythrocyte cell counts showed an irregular trend across the treatments and were not significant ($P > 0.05$) for days 21 and 35.

Heterophil values (Figure 4.17) showed an increasing ($P < 0.05$) trend for birds on day 21, with the highest hetrophil obtained with birds in the 2 mg group. A decreasing ($P < 0.05$) trend was observed at day 35 for the dexamethasone containing groups with the 1 mg dexamethasone group having the highest ($P > 0.05$) heterophil, while the control group remained the same at both days 21 and 35. Birds on 1 mg dexamethasone showed the lowest lymphocyte count on day 21

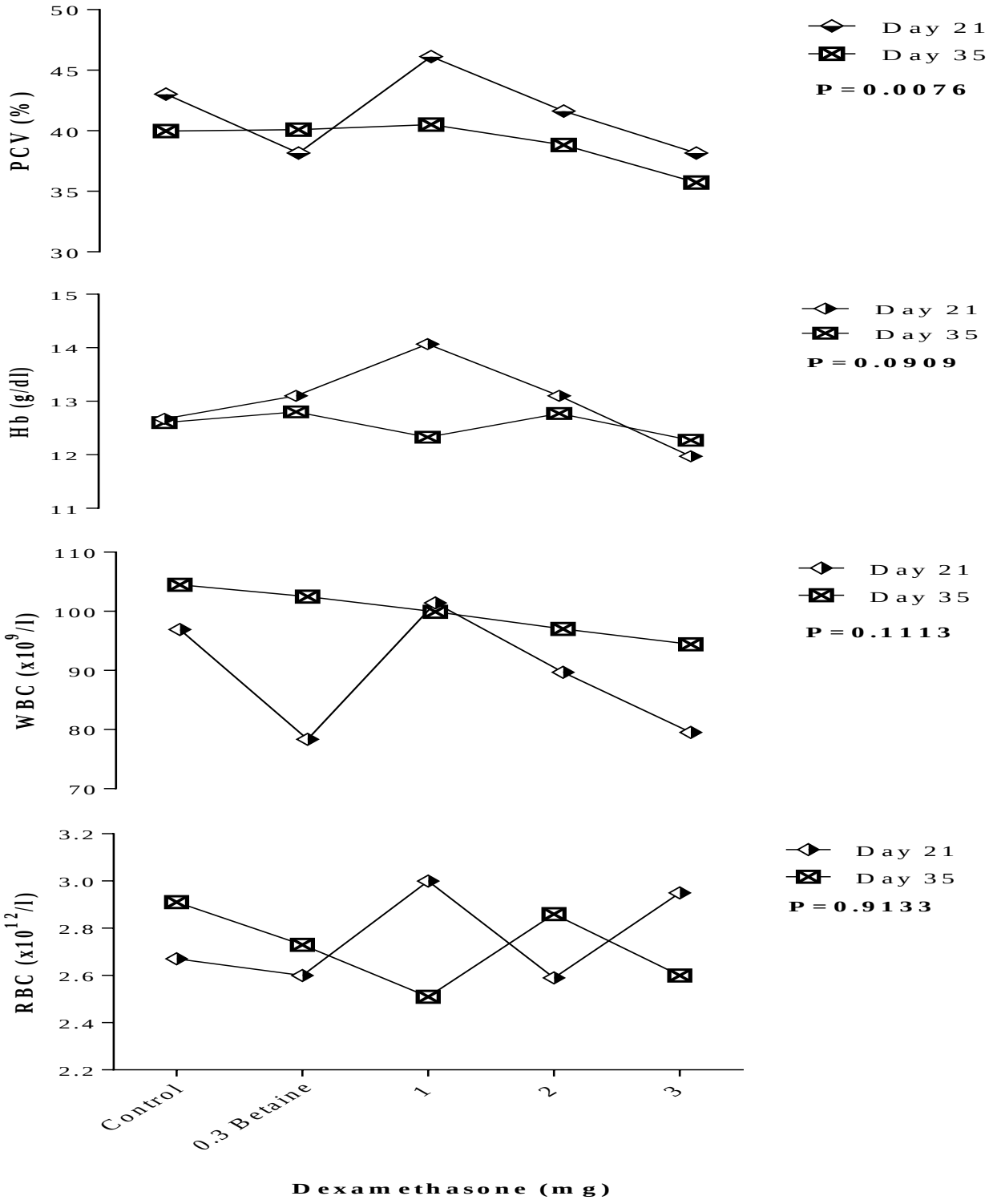

Figure 4.16: Effect of 0.30% Betaine HCl on Packed Cell Volume, Haemoglobin, Leucocyte and Erythrocyte counts in Dexamethasone Stress-induced Broiler Chickens

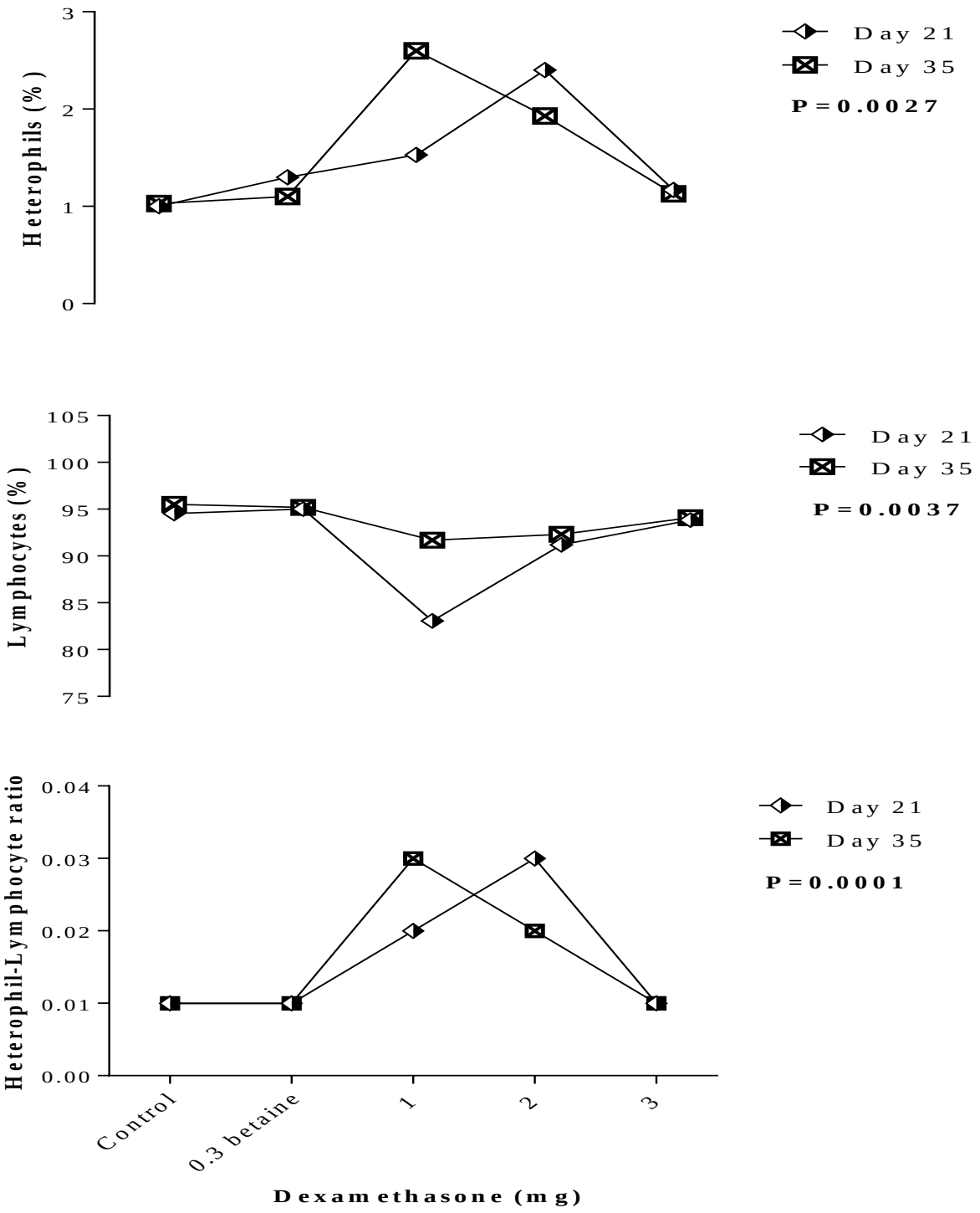

Figure 4.17: Effect of 0.30% Betaine HCl on Heterophils, Lymphocytes and Hereophil-Lymphocyte Ratio in Dexamethasone Stress-induced Broiler Chickens

with little or no change between days 21 and 35 for other treatment groups.There was no difference ($P > 0.05$) between the control, the betaine only and the 3 mg dexamethasone group on days 21 and 35 for heterophil-lymphocyte ratio where it remained unchanged. The 1 and 2 mg dexamethasone groups gave the highest ($P < 0.05$) ratios on days 35 and 21, respectively with a decreasing ($P < 0.05$) trend observed among the dexamethasone group with increasing doses of dexamethasone on day 35.

4.3.6 Effect of 0.30% Betaine HCl on Serum Chemistry of Dexamethasone Stress-induced Broiler Chickens

Results showing the effect of 0.30% betaine HCl on serum chemistry of dexamethasone stress-induced broiler chickens are presented in Table 4.25. Glucose and cholesterol levels were not significant ($P > 0.05$). Triglyceride values ranged between 283.38 and 316.03 mg/dl. Birds in the control, betaine only, 2 and 3 mg dexamethasone had higher ($P < 0.05$) values. Glucose and cholesterol values were not within the reference values given.

Glucose (Figure 4.18) levels were higher on day 21 with birds on 2 mg dexamethasone having higher ($P > 0.05$) levels at day 21. On day 35, birds in the control group had higher ($P > 0.05$) glucose levels compared to other groups. Glucose levels declined with increased levels of dexamethasone. Cholesterol was similar across all treatments on day 21. Birds in betaine only group gave the lowest ($P > 0.05$) cholesterol level on day 35 with dexamethasone groups slightly above levels observed on day 21. Results on the triglycerides were significant ($P < 0.05$) with levels on day 35 above day 21 levels. A rising trend was observed among the dexamethasone containing groups on day 21. The betaine only group was similar ($P > 0.05$) with the 2 and 3 mg dexamethasone groups.

Table 4.25: Effect of 0.30% Betaine HCl on Serum Chemistry of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)							
	0	0	1	2	3			
	Betaine HCl (%)							
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value	Ref*
Glucose (mg/dl)	74.28	66.05	70.38	75.87	67.00	4.64	0.2306	197-299
Cholesterol (mg/dl)	370.20	308.70	386.05	390.53	382.82	25.90	0.2719	129-297
Triglycerides (mg/dl)	293.80^{ab}	312.68^{ab}	283.38^{b}	315.15^{ab}	316.03^{a}	12.69	0.0239	-

[a, b]Means with different superscript on the same row differ significantly ($P < 0.05$); *Reference values of Clinical Diagnostic Division (1990), SEM: Standard error of the mean

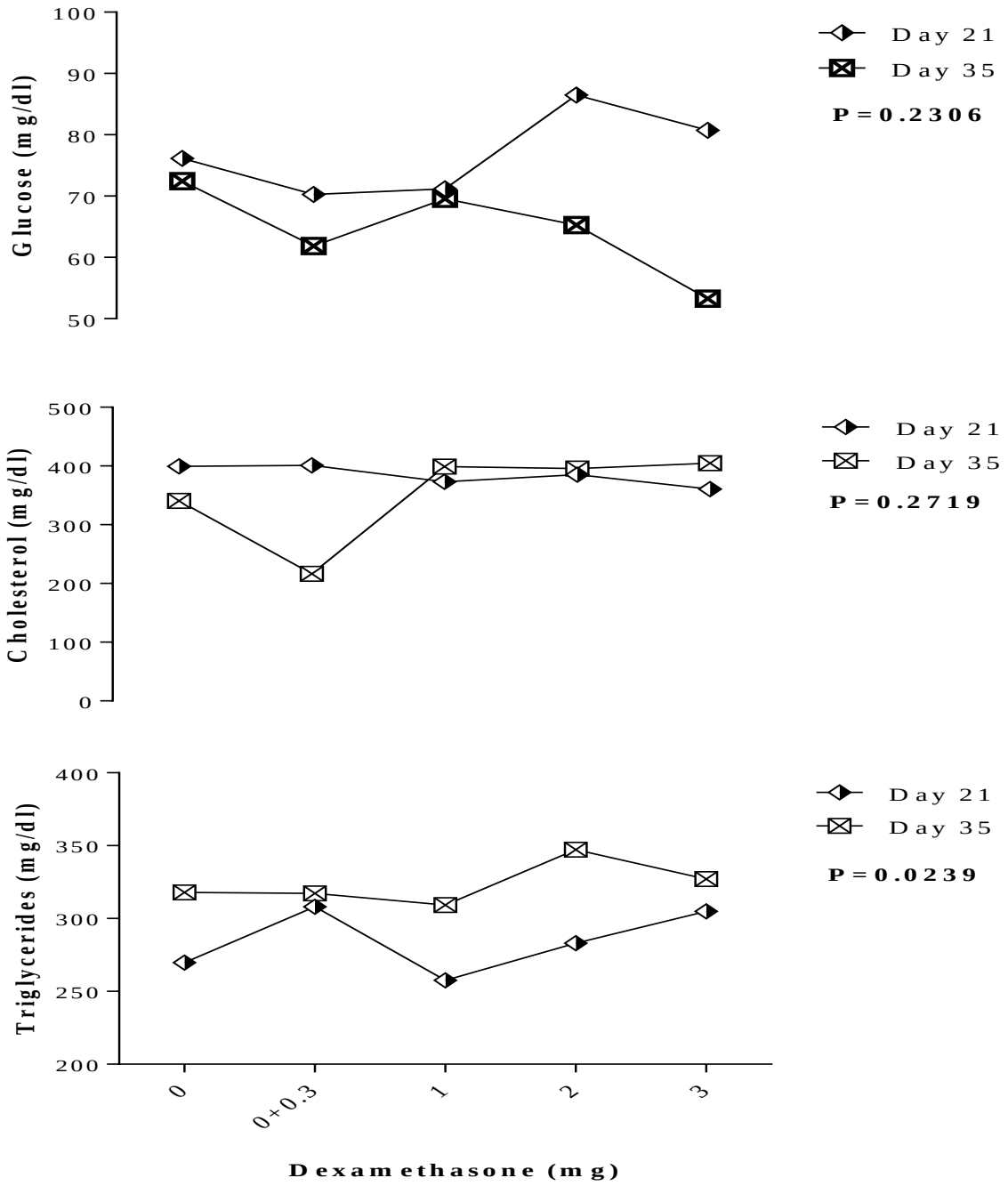

Figure 4.18: Effect of 0.30% Betaine HCl on Glucose, Cholesterol and Triglycerides in Dexamethasone Stress-induced Broiler Chickens

4.3.7 Effect of 0.30% Betaine Hydrochloride on Carcass Cut Parts and Organs Weight of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.30% betaine HCl on carcass cut parts and organs weight of dexamethasone stress-induced broiler chickens is shown on Table 4.26. All carcass cut weights were not significant ($P > 0.05$). Liver, gizzard and kidney weights were also not significant ($P > 0.05$). The effect of 0.3% betaine HCl on heart weight ranged from 0.48 to 0.62, with birds fed betaine only supplemented diets ($P > 0.05$) having the least weight.

4.3.8 Effect of 0.30% Betaine Hydrochloride on Tibia Geometry of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.30% betaine HCl on tibia geometry of dexamethasone stress-induced broiler chickens is presented in Table 4.27. Tibia weight ranged from 4.98 to 6.63 g, with the birds on control, betaine only, 1 and 2 mg groups being higher ($P < 0.05$). Tibia weight decreased with increasing levels of dexamethasone. The percentage decrease relative to the control group for tibia weight was 1.05 % (betaine only diet), 9.2 % (1 mg), 18.6 % (2 mg) and 24.9 % (3 mg), representing an increase in tibia weight reduction with increasing dose of dexamethasone. The effect of dexamethasone on tibia length ranged from 8.93 to 10.15 g with the control and birds fed 0.3% betaine only supplemented diets having higher ($P < 0.05$) tibia length compared to those in the dexamethasone groups. The percentage decrease in tibia length relative to the control group was -3.4 % (betaine only supplemented diet), 4.07 % (1 mg), 6.6 % (2 mg) and 9.1 % (3 mg). All dexamethasone containing treatments were similar ($P > 0.05$). Tibia weight/length index ranged from 0.54 to 0.67 with birds in the control, betaine only diets and 1 mg group having a higher ($P < 0.05$) weight/length index compared to those in the 2 and 3 mg dexamethasone groups. Robusticity index was not significant ($P > 0.05$).

Table 4.26: Effect of 0.30% Betaine HCl on Carcass Cut Parts and Organs Weight of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters (%LW)	0	0.3	0.3	0.3	0.3	SEM	P value
Breast	26.23	26.43	23.76	23.78	25.87	0.88	0.0896
Thigh	11.03	10.97	11.19	10.60	10.76	0.22	0.3669
Drumstick	4.87	4.96	5.20	4.97	4.74	0.17	0.4436
Organs							
Liver	2.22	2.21	2.25	2.15	2.16	0.06	0.7890
Gizzard	1.70	1.68	1.87	2.00	1.90	0.11	0.2329
Heart	0.53^{ab}	0.48^{b}	0.62^{a}	0.58^{ab}	0.57^{ab}	0.03	0.0215
Kidney	0.59	0.60	0.69	0.60	0.69	0.03	0.0640

[a,b] Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.27: Effect of 0.3% Betaine HCl on Tibia Geometry of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Tibia Compositions	0	0.3	0.3	0.3	0.3	SEM	P value
Tibia weight (g)	6.63[a]	6.56[a]	6.02[ab]	5.40[ab]	4.98[b]	0.29	0.0009
Tibia length (cm)	9.82[a]	10.15[a]	9.12[b]	9.17[b]	8.93[b]	0.14	<.0001
TWLI (g/cm)	0.67[a]	0.65[a]	0.67[a]	0.58[b]	0.54[b]	0.03	0.0380
Robusticity index (cm/g^3)	5.24	5.38	4.96	5.28	5.48	0.14	0.1293
Ash (%)	42.06[ab]	40.43[ab]	42.38[ab]	46.09[a]	28.79[b]	3.45	0.0447

[a,b]Means with different superscript on the same row differ significantly ($P < 0.05$), TWLI= Tibia weight/Length index, SEM: Standard error of the mean

Ash values ranged from 28.79 to 46.09 with the birds fed 3 mg dexamethasone having the lowest ($P < 0.05$) ash percentage. All other treatment groups were similar ($P > 0.05$).

4.3.9 Effect of 0.30% Betaine Hydrochloride on Immune Organs Weight of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of 0.30% betaine HCl with varying doses of dexamethasone on immune organ weights of broiler chickens is presented in Table 4.28. Results on spleen and thymus were not significant ($P > 0.05$). Bursa weights ranged from 0.00 to 0.05, with birds in the control, betaine only, 1 and 2 mg dexamethasone groups having higher ($P < 0.05$) bursa weights.

4.3.10 Effect of 0.30% Betaine HCl on Jejunum Mucosal Morphology of Dexamethasone Stress-induced Broiler Chickens

Result showing the effect of varying dexamethasone doses on jejunum mucosal morphology in stress induced broilers is presented in Table 4.29. Result on villus height, villus width and absorption area were not significant ($P > 0.05$). Crypt depth ranged from 11.70 to 22.87. Birds receiving betaine only supplemented diet, 2 and 3 mg dexamethasone had higher ($P < 0.05$) crypt depth.

4.3.11 Regression of Final Weight with Feed Intake and Thermoregulatory Parameters of Dexamethasone Stress-induced Broiler Chickens fed 0.3% Betaine HCl

Result showing the multiple regression of final weight with feed intake and thermoregulatory parameters (respiratory rate and rectal temperature) of dexamethasone stress-induced broilers treated with betaine HCl is shown in Table 4.30. An R^2 value of 0.92 was obtained when feed intake, respiratory rate and rectal temperature were combined in a multiple regression equation. An R^2 value of 0.88) was recorded when feed intake and rectal temperature were used and when only feed intake was used as a sole predictor.

Table 4.28: Effect of 0.30% Betaine HCl on Immune Organs Weight of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters (% LW)	0	0.3	0.3	0.3	0.3	SEM	P value
Spleen	0.12	0.12	0.13	0.17	0.13	0.02	0.4627
Thymus	0.52	0.45	0.71	0.49	0.60	0.07	0.0784
Bursa of Fabricius	0.04^{ab}	0.02^{ab}	0.05^{a}	0.02^{ab}	0.00^{b}	0.01	0.0341

[a,b]Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.29: Effect of 0.30% Betaine HCl on Jejunum Mucosal Morphology of Dexamethasone Stress-induced Broiler Chickens

	Dexamethasone levels (mg/l)						
	0	0	1	2	3		
	Betaine HCl (%)						
Parameters	0	0.3	0.3	0.3	0.3	SEM	P value
Villus height (μm)	38.63	36.85	37.00	38.33	31.28	3.84	0.6707
Villus width (μm)	2.24	2.31	3.24	3.26	2.43	0.54	0.5053
Crypt depth (μm)	11.70^{b}	17.17^{ab}	12.12^{b}	22.87^{a}	15.26^{ab}	1.82	0.0089
Absorption area ($\mu m^{2)}$	540.66	534.06	734.79	817.02	492.51	160.64	0.5558

[a, b]Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of the mean

Table 4.30: Regression of Final Weight with Feed Intake and Thermoregulatory Parameters of Dexamethasone Stress-induced Broiler Chickens fed 0.3% Betaine HCl

Parameters	Model	N	R^2	Adjusted R^2	P value
FI	FW = -1788.67 + 42.41FI	12	0.88	0.87	<.001
FI, RT	FW = 2606.66 + 42.08FI – 105.50RT	12	0.88	0.86	<.001
FI, RR	FW = -1250.62 + 39.91FI – 2.68.00RR	12	0.91	0.89	<.001
FI, RT, RR	FW = 6059.84 + 39.07FI – 173.94RT – 3.00RR	12	0.92	0.89	<.001

FI-feed intake, RT-rectal temperature, RR- respiratory rate, FW-final weight, R^2-co-efficient of determination

CHAPTER FIVE

5.0 DISCUSSION

5.1 Thermoregulatory Parameters

Extremes of ambient temperature is an important stressor that confronts poultry in many regions of the world and large economic losses can occur because of mortality and decreased production (Altan *et al*., 2000). The results obtained in the dexamethasone study indicated that THI in the afternoons was higher by 19.5% than THI in the morning indicating the absence of heat stress in the morning and the presence of very severe heat stress in the afternoon. During the 0.15% betaine study, THI in the afternoons was higher by 16.1% than THI in the morning, indicating the absence of heat stress in the morning and the presence of very severe heat stress in the afternoon. During the 0.30% betaine study, THI in the afternoons was higher by 25.4% than THI in the morning indicating the absence of heat stress in the morning and the presence of very severe heat stress in the afternoon. Thermal sensitivity to high temperature increases with body weight (Lin *et al*., 2004). Tao and Zin (2003) also observed that the body temperature of male broilers (BW = 2.8 kg) tends to rise at THI 30.0.

Rectal temperature may be considered one of the indicators of metabolic rate in broilers (Moberg, 2000), and increases when birds are exposed to high ambient temperature. Rectal temperature of the broilers reached temperatures of up to 42.28ºC for the control, revealing that the condition of the experimental room as evident in the THI exerted thermal stress on them. Under the condition of induced stress in the dexamethasone study as compared with the control, the decrease in rectal temperature may be a successful consequence of the birds combating the effect of dexamethasone and attempting a return to homeostasis where a decrease of up -1.56%

relative to the control was observed in the 3 mg group. Reports by Aengwanich (2007) showing a decrease in rectal temperature in broilers receiving between 1-5 mg/kg dexamethasone agrees with the results of this study. Results on rectal temperature in both betaine studies where a decrease in rectal temperature of up to -0.45% (0.15% betaine) and -0.10% (0.30% betaine) for the 3 mg groups especially at 0.30% betaine supplementation for the betaine only group (-0.17%) agrees with the study of Singh *et al.* (2015), who fed betaine to methionine and choline deficient diets. These results are also supported by Hassan *et al*. (2011) and Nofal *et al*. (2015) who concluded that betaine supplementation in diets significantly reduced rectal temperature. As an osmolyte, betaine may have a stabilizing function on cells subjected to osmotic stressors, such as coccidiosis (Klasing *et al*., 2002) by regulating the water balance, resulting in the stability of tissue metabolism especially in the gastro-intestinal tract (Lipinski *et al.*, 2012) protecting them from heat shock. Intestinal cells use betaine as an osmolyte to prevent dehydration due to a high solute concentration of intestinal contents. This is important to maintain the metabolic activities of intestinal cells. Despite the stress imposed by dexamethasone on the other groups, betaine HCl helped these birds cope with this stress as evident in lower rectal temperatures. Zhan *et al*. (2006) also reported that betaine feeding decreased rectal temperature from 43.2ºC to 41.9ºC.

Results from both betaine studies however disagree with those obtained by Gudev *et al*. (2011), who observed that betaine supplementation or enviromental temperature fluctuation did not change rectal temperature. Even though rectal temperature decreased among birds subjected to dexamethasone induced stress irrespective of betaine HCl addition, whether at 0.15% or 0.3%, the efficacy of betaine HCl in decreasing rectal temperature is evident in the third study where lower rectal temperatures were achieved in birds fed 0.30% betaine HCl only supplemented diet

even in the presence of THI averaging 29.09. This was below that observed in the control and dexamethasone groups.

One of the visible symptoms of a bird under heat stress is panting or increased respiratory rate (Etches *et al*., 2008). The dexamethasone study showed a rising trend in respiratory rate with increasing dose of dexamethasone. Increased respiratory rate of up to 6.61% with increasing doses of dexamethasone was observed in the dexamethasone study. Signals from the peripheral thermoreceptors in the skin or changes in blood temperature are sent to the anterior hypothalamus to initiate heat loss by triggering vasodilation and panting (Rastogi, 2007). This behavior is similar to birds under stress and this helps birds reduce body temperature through evaporative heat loss to return to homeostasis. When the environmental temperature exceeds the comfort limit, chickens suffer heat related stress resulting in physiological and metabolic changes (Borges *et al.,* 2007). This study also agrees with Aengwanich (2007) who administered dexamethasone at 1-5 mg/kg to broiler chickens.

The trend of increased repiratory rate was less evident in the betaine studies. Both betaine HCl studies showed a decreasing trend for respiratory rate relative to the dexamethasone study, indicating the ameliorative effect of betaine. Respiratory rate was found to be reduced even below that of the control group for the betaine group (-1.12%) when betaine was supplemented at 0.30%. When the environmental temperature exceeds the thermoneutral zone, birds reduce their feed intake in order to decrease the heat production associated with feed consumption and metabolism, as well as increase non evaporative heat loss followed by evaporative cooling (Teeter and Belay, 1996).

5.2 Effect of Dexamethasone and Betaine Hydrochloride on Performance of Broiler Chickens

The decreased feed intake in the dexamethasone study agrees with Lin *et al.* (2004), Malheiros *et al*. (2003) and Hanafy and Khalil (2015) who administered dexamethasone to Japanese quails. The reduction in feed intake may be a direct effect of high temperature (Ferket and Gernat, 2006) or an indirect effect caused by the elevated body temperature which in turn regulates the pattern of feed intake (Lin *et al*., 2004) so that under high body temperature birds eat less in order to avoid excess heat load. The effect of dexamethasone on food intake in birds may be dose-dependent and this appears to be more severe at higher doses. It is also in agreement with the report of Sapolsky *et al.* (2000) who reported that dexamethasone caused a decrease in intake and appetite. The reduction in feed intake during stress could also be related to decreased blood flow to the digestive system (Wolfenson *et al*., 1981), thus suppressing heat production associated with digestion, absorption and utilization of nutrients (Syafwan *et al*., 2011). Decreased feed intake is a physiological response to minimize intrinsic heat production and to maintain the thermal homeostasis, thus bringing down feed efficiency (Faria Filho *et al*., 2007). In both betaine studies dexamethasone still caused a reduction in feed intake compared to the control. Under heat stress condition, reduction in feed intake may be due to a lower heat preservation energy requirement (Freeman, 1988).

In the 0.30% betaine study, a decrease in feed intake was also observed in the betaine only supplemented diet group. This disagrees with Awad *et al*. (2014) who reported that feeding of betaine at the rate of 1.5 g/kg in the diet resulted in significantly higher feed intake as compared to the control group. Similarly, Sakomura *et al*. (2013) also reported that betaine supplemented to broilers significantly increased feed intake as compared to the control group.

The decreasing trend observed in the dexamethasone study for final weight could be as a result of the attempts by the birds to combat the additional stress caused by dexamethasone resulting in reduced feed intake and weight loss. This is in agreement with a study by Vahdatpour *et al.* (2009) who simulated chronic stress conditions in broilers, from day 1 to 49 of age. Results had shown that the greater the concentration of corticosterone intake by the birds, the lower their final body weight. Aengwanich (2007) and Li *et al*. (2009) also reported that the body weight of broilers receiving up to 6 mg kg^{-1} dexamethasone in diets or by injection into the abdomen was significantly lower than control group and this decrease was consistent with increasing levels of dexamethasone.

The decreasing trend in final weight in the betaine studies at 0.15 and 0.30% supplementation disagrees with with those obtained by Attia *et al*. (2005) and Dunshea *et al*. (2007) who found that addition of betaine to poultry diet improved body weight significantly. However, there was no heat stress neither was dexamethasone administered in the studies cited. Decreased body weight observed in the dexamethasone containing treatments can be attributed to reduced feed intake, in which birds divert metabolizable energy required for production to performance in the maintenance of homeothermy (Mckee *et al*., 1997).

The effect of dexamethsone on weight gain in the dexamethasone study clearly showed a decrease in weight gain with increasing dose of dexamethasone. This decrease in weight gain was maintained at both starter and finisher phases. This is in agreement with the report of Sabeur *et al.* (1993), who observed that when chickens received dexamethasone, it caused muscular dystrophy and reduced growth. Irrespective of betaine supplementation at 0.15 and 0.30%, this decrease in weight gain was maintained in the dexamethasone treated groups. The betaine only

supplemented group however remained similar (P>0.05) with the control group. This trend disagrees with studies by Augustine *et al*., (1997) who reported positive effect on weight gain when administering betaine at 0.15% inclusion to broilers infected with *Eimeria acervulina*. Birds respond to being stressed by decreasing feed intake which results in decreased weight gain and feed efficiency (Sakomura, *et al*., 2013). It, however, agrees with Waldroup and Fritts (2005) who observed no positive effects for weight gain, efficiency, or mortality. Supplemental dietary betaine improved weight gain and feed conversion in some poultry studies (Mathews and Southern, 2000; Hassan *et al*., 2005). Under heat stress conditions, supplementation of broiler diets with 0.1% betaine improved weight gain, compared with control birds (Farooqi, *et al*., 2005).

High FCR observed in the dexamethasone study for the dexamethasone groups is contrary to the report by Aengwanich (2007) who reported no significant (P>0.05) difference when broiler chickens were treated with dexamethasone. The increase in feed conversion ratio indicates that the administration of dexamethasone induced physiological stress where glucose metabolism is favoured over protein synthesis which had a negative impact on feed to tissue conversion and (Eid *et al*., 2003; Malheiros *et al*., 2003; Lin *et al*., 2004; Virden *et al*., 2007). In the 0.15% betaine study feed conversion ration did not improve. Result on FCR agrees with studies by Teeter *et al*. (1999) where a decrease in FCR was observed from 0-14 days at 0.15% betaine inclusion. These results obtained also agree with reports by Attia *et al*., (2009) who showed that the impact of severe heat stress could partially be overcome by adding betaine to the diet in slow growing broilers. In his study, adding betaine at 0.10% to the diet improved weight gain and feed conversion, compared to negative control treatment.

At 0.3% betaine inclusion, FCR in the betaine only supplemented group agrees with Waldroup *et al.* (2006) who showed improved feed conversion in broilers fed additional choline or betaine. Haldar *et al*. (2011) showed feed conversion improved from 1.67 in positive control to 1.63 (1.3 kg betaine HCl) and 1.62 (2.0 kg betaine HCl). Waldroup *et al.* (2006) also reported that with 500 mg/kg or 1000 mg/kg betaine supplementation there was improved feed conversion ratio at 35 and 42 days. Improvement in FCR was more evident among the dexamethasone groups at the starter phase with the 1 and 2 mg dexamethasone groups being similar ($P>0.05$) with the control. This finding agrees with the reports of El-Husseiny et al. (2007) and Honarbakhsh *et al*. (2007), who reported improved FCR at betaine levels of up to 0.10 and 0.23 % inclusion respectively.

5.3 Effect of Dexamethasone and Betaine Hydrochloride on Haematological Indices of Broiler Chickens

Blood is an important and reliable medium for assessing the physiological and health status of individual animals (Egbe-Nwiyi *et al*., 2000). Result on heterophil, lymphocyte and H/L ratio during the dexamethasone study is in agreement with reports by Jain (1993) and Aengwanich (2007), who both administered dexamethasone to chickens and reported increased heterophil, and H/L ratio with decreased lymphocytes. The dexamethasone study observed increased heterophil and H/L trends at days 28 and 42 and 56 with decreasing lymphocyte trends, though not significant. Jain (1993) explained that dexamethasone induced lymphopenia as a result of lympholysis in blood, DNA damage, lymphoid tissue atrophy and increased shift of lymphocytes from the blood to other body compartments which leads to a decrease in circulating blood lymphocytes. Poultry treated with corticosterone or ACTH demonstrated depressed numbers of circulating lymphocytes (Siegel and Latimer, 1970). Evidence is available that the immune status of chickens, as indicated by differential leucocyte counts or H/L ratios, can also be affected by

stressors. These, like environmental stressors, cause an elevation in H/L ratios due to leucopenia (lymphopenia) and heterophilia (Gehad *et al*., 2002; Wang *et al*., 2003; Shini *et al*., 2004, 2005). With the exception of leucocytes and lymphocytes, other haematological indices were within the reference ranges.

At betaine HCl inclusion of 0.15%, the dexamethasone groups had generally higher heterophil percentages and significantly lower lymphocyte percentages than the control group. These findings are in disagreement with Nofal *et al*. (2015) who showed that supplementation of betaine in diet significantly decreased heterophil percentage but lymphocyte percentage was significantly increased,whereas, H/L ratio was significantly reduced. Awad *et al*. (2014) reported that adding betaine in diets significantly increased the lymphocyte percentage where as heterophil and H/L ratio was significantly decreased compared to the control group. Gudev *et al*. (2011) reported that supplementing betaine at the level of 1.5g/kg in feed significantly increased the lymphocyte and significantly decreased heterophil percentage. Mashaly *et al*. (2004) reported that reduction of lymphocyte during heat stress is due to the increase in inflammatory cytokines which stimulate the hypothalamic production of corticotrophin releasing hormone under heat stress.

Packed cell volume, haemoglobin and leucocyte count in the dexamethasone study did not increase with increasing doses of dexamethasone. This is in agreement with reports by Aengwanich (2007) where broilers received dexamethasone at 0, 1, 2, 3, 4, 5 and 6 mg kg^{-1} in their diet and reported significant ($P<0.05$) increases in these indices with increasing doses of dexamethasone. Dexamethasone caused increases in erythrocyte count above the control. This

result is contrary with the work of Siegel (1968) that neither cortisol nor ACTH influenced hematocrit of young chicks.

When betaine HCl was included at 0.3%, results agree with the studies of Gudev *et al*. (2011) where PCV levels tended to be lower in dexamethasone groups relative to the control. The observed decline in hematocrit especially in the betaine only group could be associated with the reported regulatory effect of betaine on erythrocyte membrane ATP-ases, via conformational changes which results in cell volume control (Craig, 2004). The decline in leucocyte count observed on day 21 is contrary to reports by Gross and Siegel (1983) where treatment with dexamethasone resulted in increases in leucocyte count.

5.4 Effect of Dexamethasone and Betaine Hydrochloride on Serum Chemistry of Broiler Chickens

Glucose level was similar during the days of study during the dexamethasone study. Results from this study agrees with the work of Li *et al.* (2009) who reported that no significant ($P>0.05$) difference was found in broilers injected with 1 and 5 mg of dexamethasone/kg of body weight. At 0.15% level of inclusion, betaine produced an increasing trend in glucose levels on day 21 to 35 before no significant effect was observed on day 56. Previous studies have shown that administration of corticosterone increased glucose and calcium absorption (Nasir *et al.*, 1999). Primarily, stress-induced metabolic alterations seem to be focused on the mobilization or production of glucose for energy needed to maintain homeostasis in the presence of the stressor. This was clearly demonstrated on day 21 where dexamethasone containing treatments had higher glucose levels compared to those in the control. No difference was seen on day 56 where all treatment groups were similar. This may indicate an ameliorative effect of betaine on

dexamethasone treated birds. Also at 0.30% inclusion, no changes in glucose on days 21, 35 and 56 was observed. This indicates that betaine HCl helped mitigate the effect of the induced stress, hence glucose absorption remained unchanged. Under conditions of simulated chronic stress, physiological stress induces a higher glucose level in blood, which is second only to corticosterone (Puvadolpirod and Thaxton, 2000a, Odihambo *et al.,* 2006; Olanrewaju *et al*., 2006; Lin *et al*.,2007).

Cholesterol result during the dexamethasone study showed a variable trend during the various study periods. Reports from previous studies on cholesterol have varied, with some reporting an increase (Staels *et al*., 1991), decrease (Giudetti and Gnoni, 1998) or no change (Wang *et al*., 2012b). Result on cholesterol from day 35 to 56 disagrees with studies by Shini *et al.* (2008) who reported significantly ($P<0.05$) increased effect of corticosterone administration on plasma cholesterol. This decline coincides with the two-week period when dexamethasone administration was terminated. The 0.15% betaine HCl study showed similar cholesterol values among the treatment groups on days 21 and 35. A decrease however occurred on day 49 with a decreasing trend observed with increasing dexamethasone doses. This is contrary to reports by Puvadolpirod and Thaxton (2000a) where they reported increased cholesterol levels under continuous delivery of ACTH by mini-osmotic pumps. Siegel (1995) noted that hypercholesteremia is one of many symptoms associated with long term stress. This phenomenom was clearly demonstrated on day 35 for the third study (0.30% betaine inclusion) where the control and the dexamethasone groups displayed high cholesterol levels with the exception of the betaine only group, indicating the efficacy of betaine HCl in combating long term stress under heat stress conditions.

Similar triglyceride values observed in the dexamethasone study disagrees with Shini *et al.* (2009). Triglyceride results in other studies have varied, with decrease (De La Cruz *et al.,* 1987) and an increase (Lin *et al.,* 2006; Shini *et al.,* 2008) in concentration. Triglyceride values increased at day 21 through to 35 before a decrease was observed on day 56. With the addition of 0.15% betaine HCl, triglyceride levels remained similar for all treatment groups on days 21 and 35 with a sharp decline at day 56 for lower levels of dexamethasone which may indicate the ability of betaine to alleviate the induced stress. Sahin and Kucuk (2001) reported that stress increased serum glucose, triglyceride and cholesterol.

5.5 Effect of Dexamethasone and Betaine Hydrochloride on Corticosterone and Thyroxine levels of Broiler Chickens

Corticosterone level is expected to increase with increasing doses of dexamethasone and dexamethasone and corticosterone levels seem to be time-dependent. Corticosterone levels have been shown to initially rise and reach the peak within 2 to 4 days, followed by a gradual decrease to control levels within 8 to 12 days after corticosterone treatment (Puvadolpirod and Thaxton, 2000b; Post *et al*., 2003). A similar occurrence was observed on day 28 when dexamethasone treatments exhibited levels even lower than that of the control indicating a successful return to homeostasis.

However, sustained elevated levels of plasma corticosterone above the control were observed on day 42 which may indicate a form of chronic stress. Elevations in circulating levels of corticosterone in poultry after treatment with ACTH have been demonstrated (Puvadolpirod and Thaxton, 2000 a, b, d). The results are in agreement with reports by Shini *et al.* (2008) who reported that administration of corticosterone in drinking water increased circulating

corticosterone above the baseline and induced effects similar to responses to stressors. The increase in corticosterone levels is the result of the activation of HPA axis in avian species (Quinteiro-Filho *et al*., 2010) and is a response of the body to a stressor (Selye, 1976). The stressor is detected by the cortex in the brain which activates HPA by sending a signal to the hypothalamus; because ACTH, whether produced endogenously or administered exogenously, causes avian plasma corticosterone levels to increase, it can be concluded that stress responses are fairly similar when treatment with ACTH, corticosterone or dexamethasone is used. It is important to note that this increase is to a certain extent because high corticosterone levels inhibit secretion of ACTH by means of standard, negative feedback loop.

Glucocorticoids have been reported to influence thyroid activity and thyroid hormones in birds (Darras *et al*., 1996) by stimulating the secretion of thyroid stimulating hormone (TSH), by making the thyrotrophs in the adenohypophysis more sensitive to thyroxine releasing factor (TRF). Thyroxine increases heat production. Bowen and Washburn (1984) reported suppressed secretion of thyroid gland hormones, that is, T_3 (triiodothyronine) and T_4 (thyroxine), under stress conditions of increased temperature. This was not observed on days 28 and 42, where thyroxine levels remained similar with the control. In general, results of heat-mediated alterations on thyroxine concentrations are inconsistent with studies reporting decrease (Bobek *et al.* 1980), increase (Cogburn and Freeman, 1987; Elnagar *et al.*, 2010), or no alteration (Mitchell and Carlisle, 1992; Mack *et al.* 2013).

5.6 Effect of Dexamethasone and Betaine Hydrochloride on Carcass Cut Parts and Organs Weight of Broiler Chickens

Dexamethasone in the first study showed no negative effect on breast weight. Wang *et al.* (2012a) studied dexamethasone-induced intramuscular lipid accumulation in chickens and

reported no significant (P>0.05) effect of dexamethasone on breast muscle mass. Both betaine inclusion levels showed no improvements in breast weights. Waldroup and Fritts (2005) reported no improvements in breast meat yield of broilers fed diet containing 0.1% betaine. The authors suggested that the response of the bird to betaine supplementation may be age dependent.
This disagrees with the reports of improved breast yield by McDevitt *et al*. (2000) and Waldroup *et al*. (2006) when 0.5% betaine was supplemented in broiler diets. Other studies also reported increased breast yield in broiler chicken (Zhan *et al*., 2006), turkeys (Noll *et al*., 2002) and meat ducks (Wang *et al*., 2004).

Dexamethasone in the first study reduced thigh weights. Dong *et al*. (2007) and Virden *et al.* (2007) reported that thigh cuts were significantly suppressed by corticosterone treatments. From a meat production standpoint, this points to the detrimental effect of dexamethasone in catabolising structural protein to free amino acids such as glutamine and alanine for use as gluconeogenic substrates (Puvadolpirod and Thaxton, 2000d). A similar trend was observed at 0.15% inclusion of betaine, showing that dexamethasone had a negative effect on thigh weights. The result on thigh cuts in the 0.3% betaine study clearly show betaine had a positive effect among treatment groups pointing to the possibility of thigh cut weight improvements with betaine especially among the dexamethasone containing treatments. Nofal *et al*. (2015) observed that carcass weight, dressing, thigh, breast and giblets percentages were significantly ($P \leq 0.01$) improved by betaine supplementation at 0.1 or 0.2% compared to the control group. Also, Noll *et al*. (2002) and Hassan *et al.* (2005) reported that dietary supplementation of betaine improved carcass yield and breast muscle yield by approximately 3 -15% in poultry. Under the condition of induced stress with dexamethasone, carcass cut weights were comparable to the control; hence

betaine proved beneficial in improving carcass cut weights. Several authors (Esteve-Garcia and Mack, 2000; Attia *et al*., 2005; Pirompud *et al*., 2005; Zhan *et al*. 2006) reported improved carcass slaughter characteristics with inclusion of betaine.

Increased liver weight is always regarded as one index of stress condition (Puvadolpirod and Thaxton, 2000a). Liver result from the dexamethsaone study agrees with previous studies where liver weight has been consistently shown to increase in broilers treated with corticosterone or ACTH (Puvadolpirod and Thaxton, 2000 a, b, c; Malheiros *et al*., 2003). In this study an increase in liver weight of up to 32% was observed relative to the control. This is probably due to an increase in liver lipid due to the process of gluconeogenesis, because liver lipids have been shown to increase significantly in broilers treated with ACTH (Puvadolpirod and Thaxton, 2000 a, b). This may also be partially attributable to liver tissue enlargement because corticosteroids have been shown to stimulate protein synthesis in the liver (Baxter and Rousseau, 1979). Liver weight did not increase for the two betaine studies, and this, apparently, indicates no accumulation of lipid. Betaine, a methyl group donor, functions in lipid metabolism by stimulating oxidative catabolism of fatty acids through carnitine synthesis (Simon, 1999). This may indicate the ability of betaine to mitigate the effects of dexamethasone. Neto *et al*. (2000) reported that liver weight was not affected by betaine. Konca *et al*. (2008) also reported no positive benefits of betaine supplementation on liver weight.

The heart assists in heat dissipation by increasing blood flow (Yahav *et al*., 1997) to organs that are actively involved in heat dissipation such as comb, wattles and respiratory tracts (Wolfenson *et al*., 1981). With the need to cope with increased cardiac output under stress, the heart enlarges due to blood accumulation. No significant changes in heart weight were observed across the

dexamethasone and 0.15% betaine studies. However, at 0.3% betaine inclusion, birds had significantly lower heart weights compared to the control, indicating that birds were not under any form of stress requiring increased cardiac output. The non-significant differences in visceral organ (gizzard, kidney and intestine) weights across all three studies are in agreement with Vahdatpour *et al.* (2009) who observed no significant ($P<0.05$) effects on visceral organ weights with elevated corticosterone intake.

5.7 Effect of Dexamethasone and Betaine Hydrochloride on Tibia Geometry of Broiler Chickens

The decrease in tibia length and weight in the dexamethasone study is in agreement with Rooman *et al*. (1999) who reported a decrease in tibia length and weight with increasing doses of dexamethasone in mice. In a study by Rath *et al.* (2000), the bones from younger turkeys were more susceptible to corticosteroid-induced stunting of growth. This may be as a result of the reduction in growth rate observed among doses of dexamethasone in relation to the control which was more severe with higher doses of dexamethasone.

Tibia weight was improved by betaine at 0.30% at dexamethasone levels of up to 2 mg even though the betaine only group was similar to the control. Maddahian *et al.* (2017) stated that betaine supplementation increased tibia length of birds under heat stress. However, Konca *et al*. (2008) reported tibia length and weight were not affected by dietary betaine.

Tibia weight length index in all the studies showed a similar trend where a decrease was observed with higher levels of dexamethasone irrespective of betaine inclusion level. The higher the index is, the denser the bone (Monteagudo *et al*., 1997). This may be attributed to the shorter bone length among the dexamethasone groups. Decreasing robusticity index in the dexamethasone study disagrees with Rath *et al.* (2000) who found that dexamethasone decreased

bone strength of turkeys. Robusticity index is a measure of bone strength, and a low robusticity index indicates a strong bone structure. Robusticity index was not significant in both betaine studies, indicating betaine HCl was able to prevent the weakening effect of dexamethasone at both levels of betaine inclusion.

Ash result in the first study indicates dexamethasone did not decrease bone mineralization. Ash results also proved to be similar with the addition of betaine at 0.15%, but increased with the addition of betaine at 0.30%. Bone mineralization provides compressional strength to bone, the bone ash content or bone mineral densities have been used as indices of bone strength (Rath *et al.*, 2000).

5.8 Effect of Dexamethasone and Betaine Hydrochloride on Immune Organs Weight of Broiler Chickens

Regression of the thymus, bursa, and spleen has been demonstrated in chickens after corticosterone or ACTH administration (Post *et al*. 2003). This, however, was not observed in this study where dexamethasone was used. Inclusion of betaine in the second and third studies did not negatively affect immune organ weights. Spleen, thymus and bursa weights were contrary to reports by Yang *et al.* (2005), who reported that the relative weight of the spleen was reduced in broiler chickens treated with corticosterone in comparison with corn oil-exposed counterparts. This may point to the efficacy of betaine HCl at both inclusion levels to reverse the negative effects such as reduction in immune organ weights. The results matched the findings of Hamidi *et al*. (2010) and Klasing *et al*. (2002) who reported that betaine promoted immune function of broiler chickens. Quinteiro-Filho *et al*. (2012) also found no negative impact on immune organ (spleen, thymus and bursa) weights.

5.9 Effect of Dexamethasone and Betaine Hydrochloride on Jejunum Mucosal Morphology of Broiler Chickens

Many studies have shown that stress could affect the intestinal function of animals (Cera *et al*., 1988; Saunders *et al*., 1994; Olsen *et al*., 2005) and further disturb the absorption of nutrients (Thiesen *et al*., 2003; Shepherd *et al.,* 2004; Garriga *et al*., 2006; Albin *et al*., 2007). Results on villus height, absorption area and crypt depth which was similar among treatments for the dexamethasone study disagrees with Li *et al.* (2009) who reported that dexamethasone decreased villus height and absorption area but increased the crypt depth significantly. Villus height and the mucosa area are apparent indicators of the ability of the intestine to absorb nutrients and crypt depth is an indicator of the maturation of intestinal epithelium: a deeper crypt indicates a more mature intestinal epithelium (Li *et al.*, 2009). The results indicate that dexamethasone did not negatively affect nutrient absorption.

Results of the betaine study is contrary to studies by Hu and Guo (2008), which revealed that corticosterone administration decreased jejunal epithelial cell proliferation of young broilers which in turn lowered jejunal villus height and crypt depth. This indicates the ability of betaine to improve the intestinal morphology of dexamethasone induced stress broiler chickens. Yoo *et al.* (2016) showed supplementation of antioxidants increased villus height but decreased crypt depth in broilers reared under chronic heat stress. This is contrary to the results of the betaine studies where villus height remained unchanged and crypt depth improved among the betaine containing groups and especially the betaine only group where it had higher crypt depth compared to the control. Crypt depth is an indicator of the maturation of intestinal epithelium: a deeper crypt indicates a more mature intestinal epithelium (Li *et al.*, 2009). In intestinal cells subjected to osmotic disorder and dehydration, betaine is taken up and may have a stabilizing

function (Kettunen *et al*., 2001). It also stimulates intestinal epithelial cell proliferation, feed digestibility and absorption of nutrients (Augustine and Danforth, 1999).

5.10 Regression of Final Weight from Feed Intake and Thermoregulatory Parameters of Broiler Chickens fed Dexamethasone and Betaine Hydrochloride

It will be observed that the regression analysis were highly significant ($P<0.005$) in all the equations with the R^2 values being more than 90% in some groups across all three studies. In predicting the final weight of broiler chickens under dexamethasone induced stress conditions, making use of not only performance indices like feed intake but also body response parameters such as respiratory rate and rectal temperature is vital as evident in the R^2 value being further improved when body response parameters were included in the model. With up to 97 % of the variance of the dependent variable (final weight) being explained by the independent variables (feed intake, rectal temperature and respiratory rate) both performance indices and body response parameters are very good predictors for body weight in broilers.

Respiratory rate and rectal temperature are inversely related to feed intake which directly affects the final weight. Predicting final weight from thermoregulatory parameters is highly reliable, hence the high R^2 in all three studies.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Three studies were carried out to investigate the response of broiler chickens fed betaine hydrochloride supplemented diets under dexamethasone induced stress conditions.The first experiment to determine the effect of varying doses of dexamethasone on performance and thermoregulatory responses of broiler chickens was conducted using 240 day-old *Arbor acre* broiler chickens. There were four treatments each having three replicates with twenty birds per replicate. Concentrations of dexamethasone at 0, 1, 2 and 3 mg/ L of water were supplied daily. Results showed that dexamethasone had a negative effect on production performance and carcass traits. It elevated corticosterone and thyroxine levels of broiler chickens and had a negative effect on tibia geometric properties of broiler chickens. Dexamethasone had no significant effect on immune organs and haematological indices of broiler chickens. Dexamethasone also had a negative effect on the villus height of the jejunum. The results of multiple regression analysis showed that it was possible to predict body weight of broilers using performance indices and body response parameters under induced stress conditions.

The second experiment to determine the effect of betaine hydrochloride supplementation on the performance and thermoregulatory responses of dexamethasone stress induced broiler chickens was conducted using 240 day-old *Arbor acre* broiler chickens. There were four treatments each having three replicates with twenty birds per replicate. Concentrations of dexamethasone at 0, 1, 2 and 3 mg/L of water were supplied daily. All dexamethasone treated birds were supplemented with 0.15% betaine HCl in the diet. Results showed that 0.15% betaine HCl did not alleviate the

negative effect of dexamethasone on production performance of broiler chickens. The addition of 0.15% betaine HCl had non significant effect on hematological indices of broiler chickens. Betaine HCl at 0.15% did not alleviate the negative effect of dexamethasone on tibia geometry of broiler chickens but had a positive effect on immune organs, breast, liver and heart weights of broiler chickens. Multiple regression analysis showed that it was possible to predict body weight using performance indices and body response parameters of betaine treated broilers under induced stress conditions.

The third experiment to determine the effect of betaine hydrochloride on the performance and thermoregulatory responses of dexamethasone stress induced broiler chickens was conducted using 300 day-old *Arbor acre* broiler chickens. There were five treatments each having three replicates with twenty birds per replicate. A treatment containing no dexamethasone and no betaine served as the control, a treatment containing no dexamethasone and fed 0.30% betaine HCl served as another treatment. The other three treatments were given concentrations of dexamethasone at 1, 2 and 3 mg/L of water, respectively and supplied daily. All dexamethasone-treated birds were fed 0.30% betaine HCl in their diets. Results showed that 0.30% betaine HCl decreased both rectal temperature and respiratory rate of birds, especially in the betaine only group. It had no positive effect on production performance of broiler chickens under dexamethasone induced stress. The addition of betaine HCl at 0.30% had a positive effect on FCR especially in the betaine only group. The addition of 0.3% betaine HCl had a significant effect on hematological indices of broiler chickens particularly with pack cell volume index. Betaine HCl also had a positive effect on tibia geometry especially in tibia weight and ash. It also had a positive effect on immune organs (spleen and thymus), carcass traits and liver weights of

broiler chickens. Betaine HCl also showed a positive effect of jejunum morphometric indices such as villus height, villus width and absorption area. Multiple regression analysis showed that it is was possible to predict bodyweight using performance indices and body response parameters of betaine treated broilers under induced stress conditions.

6.2 CONCLUSION

From the dexamethasone and betaine studies, it can be concluded that:

- Dexamethasone had a negative effect on production performance and carcass traits.
- It elevated corticosterone and thyroxine levels of broiler chickens and had a negative effect on tibia geometric properties of broiler chickens.
- Dexamethasone also had a negative effect on the villus height of the jejunum
- Betaine HCl at 0.15% did not alleviate the negative effect of dexamethasone on production performance of broiler chickens.
- The addition of betaine HCl at 0.30% had a positive effect on FCR especially in the betaine only group.
- The addition of 0.3% betaine HCl had a significant effect on hematological indices of broiler chickens particularly with pack cell volume index
- It had a positive effect on immune organs (spleen and thymus), carcass traits and liver weights of broiler chickens.
- It had a positive effect on tibia geometry especially in tibia weight and ash.
- Betaine HCl also showed a positive effect of jejunum morphometric indices such as villus height, villus width and absorption area.

6.3 RECOMMENDATION

The administration of dexamethasone via drinking water is an effective model to study the response of broiler chickens under induced stress. Administation of 1 mg dexamethasone was enough to elicit a stress response in broiler chickens. Stress related responses such as increased respiratory rate, reduced growth performance, increased circulating corticosterone levels, immune organ, carcass and regression and its negative effect on carcass and organ traits has been demonstrated. Future studies studying stress responses in poultry should employ the use of dexamethasone to simulate natural stress conditions and its application at lower doses should be investigated to see if stress response can be achieved due to its efficacy at 1 mg/L.

The addition of betaine HCl had a beneficial effect on thermoregulatory parameters, performance, especially FCR and carcass traits of dexamethaone treated/untreated broiler chickens, particularly immune organs, and carcass and organ traits. Management strategies for combating stress in broiler chickens should employ the use of betaine HCl, especially in its potential in helping broilers cope with heat stress conditions under commercial broiler production.

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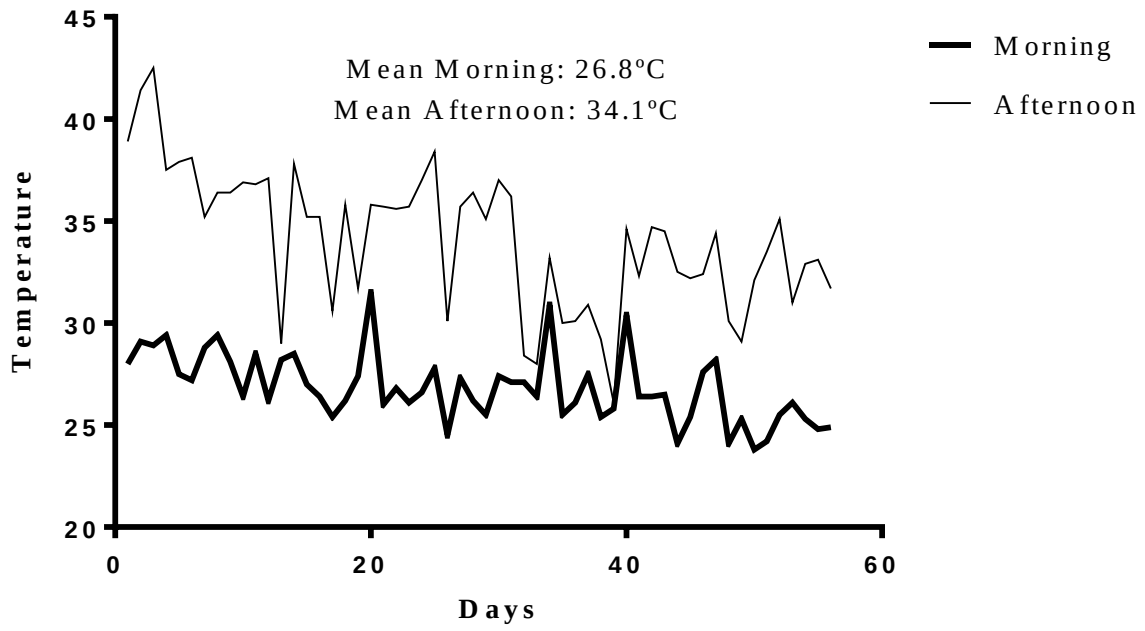
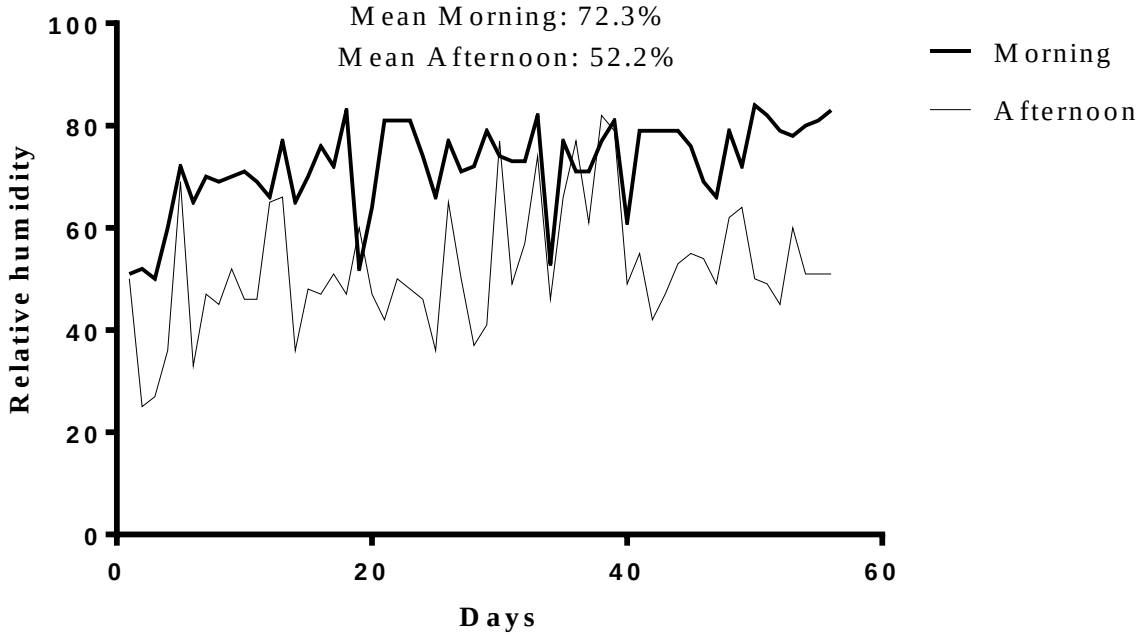
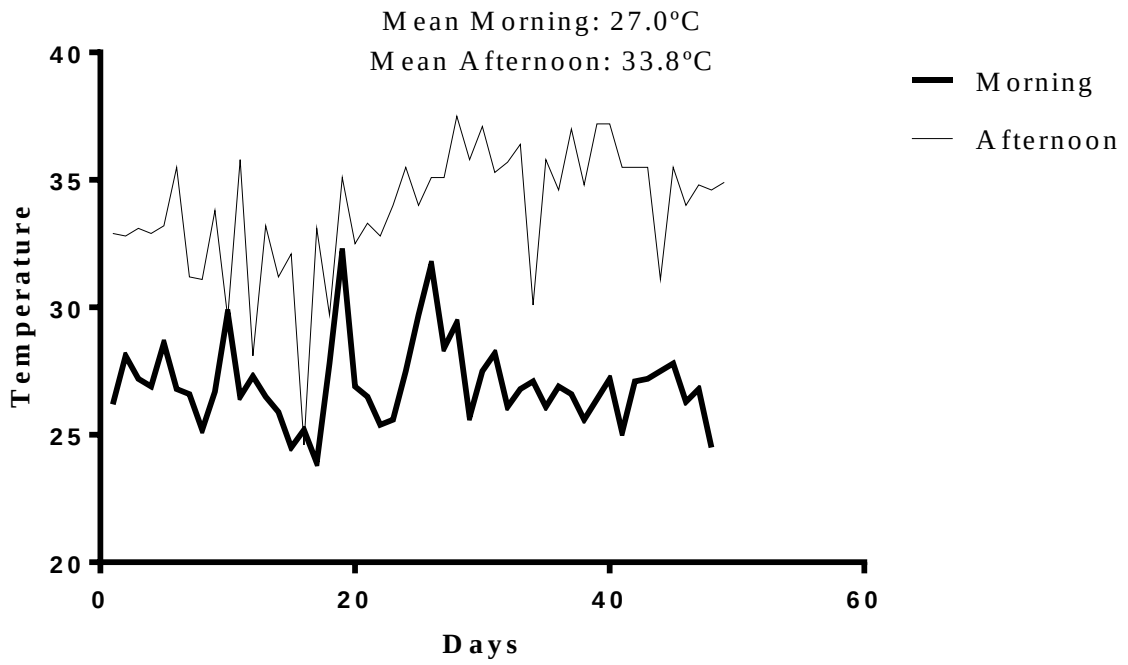


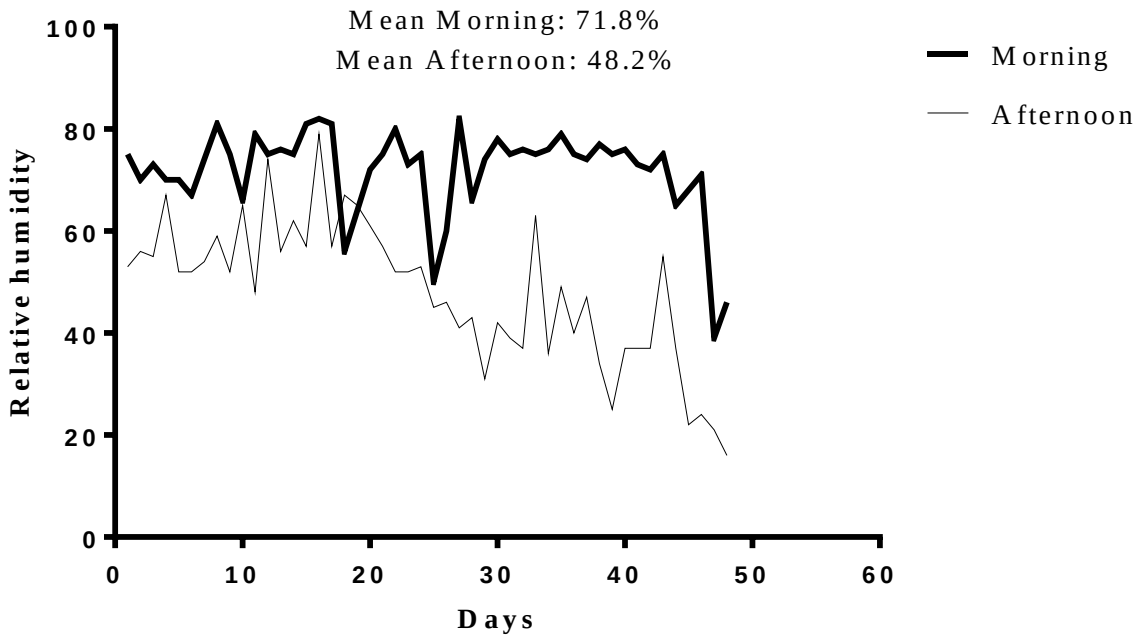
Appendix I: Daily Temperatures during the Dexamethasone study (May-June)



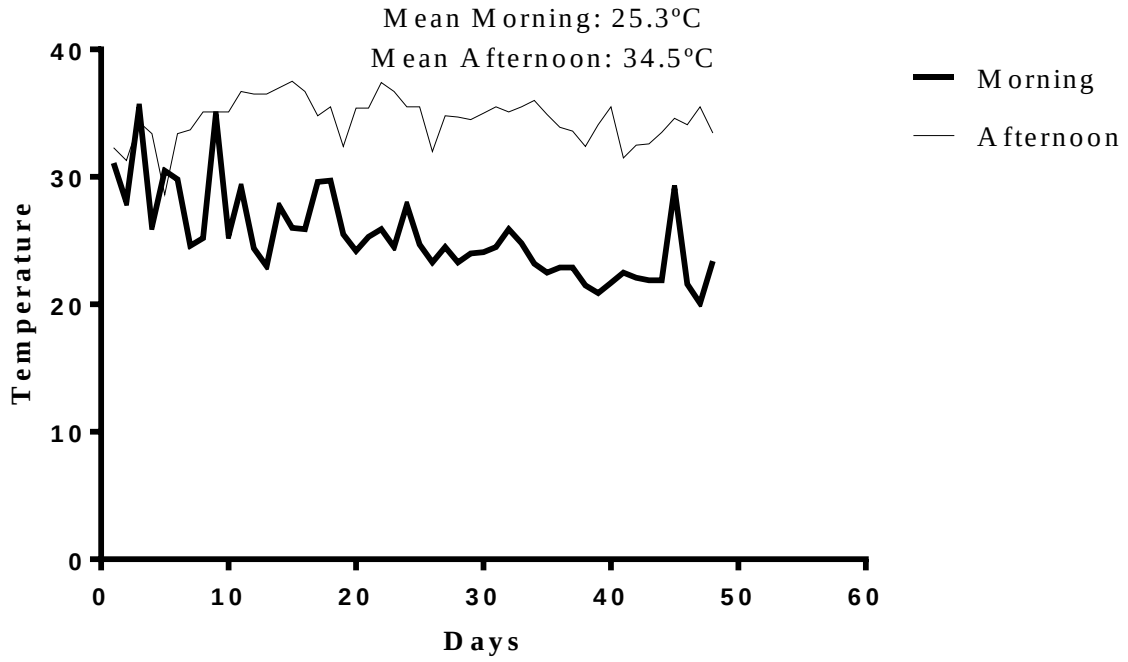
Appendix II: Daily relative humidities during the Dexamethasone study (May- June)



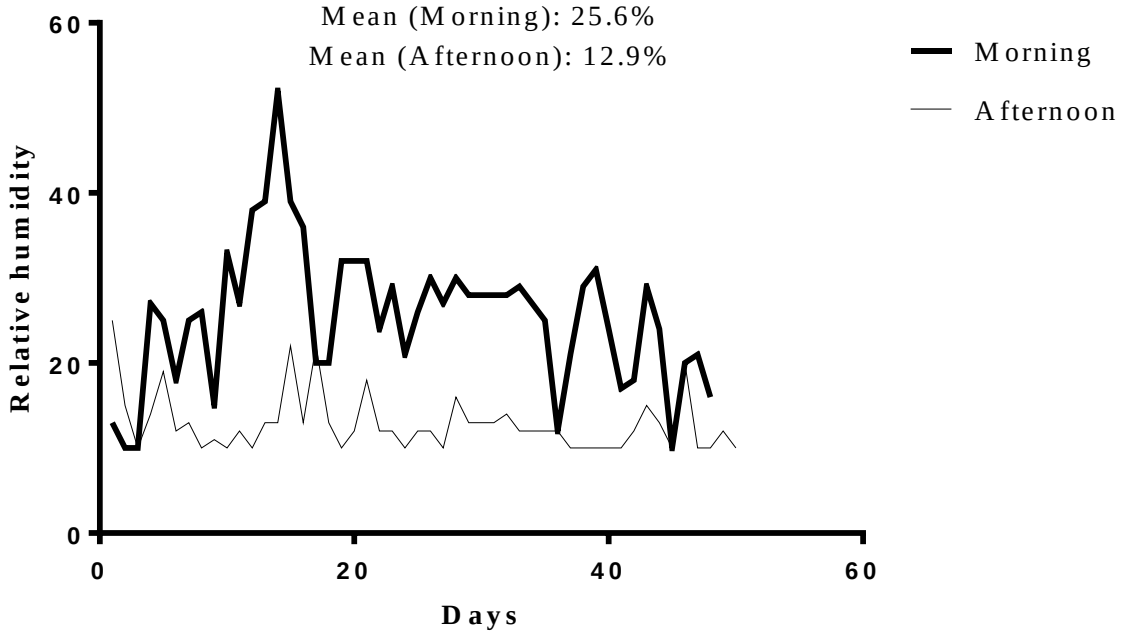
Appendix III: Daily temperatures during the 0.15% Betaine HCl study (August - September)



Appendix IV: Daily relative humidities during the 0.15% Betaine HCl study (August-September)



Appendix V: Daily temperatures during the 0.3% Betaine HCl study (November-December)



Appendix VI: Daily relative humidities during the 0.30% Betaine HCl study (November-December)