Effects of Dietary Zinc and Ascorbic Acid Supplementation and Thermal Environment on Physiological Responses and Performance of Ross Broilers Chicks.

By

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ABSTRACT

The studies reported in this thesis were performed to investigate the effect of dietary zinc and ascorbic acid supplementation and thermal environment on physiological responses and performance of broilers. In experiment 1, the effects of dietary ($ZnSO_4$) levels 250, 500, 750 and 1000 mg/kg on physiological responses and performance were investigated in unsexed broiler chicks. The rectal temperature (T_r) was not affected significantly by zinc level in the diet; T_r decreased with increase in age. The feed intake, body weight (BW) and feed conversion ratio (FCR) increased significantly in broilers receiving different levels of $ZnSO_4$ compared to the control. The packed cell volume (PCV) increased significantly with all levels of Zn supplementation and at all stages of growth. The plasma glucose level decreased significantly in broilers with all levels of Zn supplementation and at all stages of growth. The serum cholesterol level decreased significantly in broilers with all levels of Zn supplementation and at all stages of growth, and it decreased with advance of age. The serum levels of total protein and albumin were higher with all levels of Zn supplementation and at all stages of growth, and increased with advance of age. The serum levels of aspartate aminotransferase (ALT) and alanine aminotransferas (AST) decreased significantly in broilers with Zn supplementation and at all stages of growth. The serum Zn level increased significantly with all levels of Zn supplemented and at 4^{th} and 5^{th} week of age. In experiment 2, the effects of dietary supplementation of $ZnSO_4$ (50 mg/kg) and ascorbic acid (AA) (600 mg/kg) or their combination on physiological responses and performance

were investigated in unsexed broilers during summer and winter conditions. The rectal temperature (T_r) was not affected significantly by dietary Zn and AA supplementation or their combination during summer and winter conditions. T_r of control groups of broilers was significantly higher during summer compared to winter values at all stages of growth. The effects of Zn and AA or their combination on feed intake, BW and FCR were not significant throughout the experimental period. The mean values of feed intake, BW and FCR were significantly higher during winter at all stages of growth. The PCV was not affected significantly by Zn and AA or their combination during the course of experiment. The PCV of control groups was significantly higher during winter at all stages of growth. There was significant decrease in plasma glucose level with all treatments at the 4^{th} and 5^{th} weeks of age during summer, and a significant decrease in plasma glucose level in all treated experimental groups at the 4^{th} week of age during winter. At the 5^{th} and 6^{th} week of age, the plasma glucose level significantly decreased for broilers supplemented with combination of AA and Zn during winter. The serum cholesterol level decreased significantly with all treatments at the 5^{th} week of age during summer; it also decreased significantly at the 4^{th} and 6^{th} weeks of age in all treatments during winter. The serum total protein level increased significantly with all treatments at all stages of growth during summer; also it increased significantly in all treated groups at the 2^{nd}, 4^{th} and 6^{th} weeks of age during winter. The serum albumin level increased significantly for broilers supplemented with AA at the 6^{th} week of age during summer, and it increased significantly with all treatments

at the 4th, 5th and 6th week of age during winter. The serum AST level decreased significantly with all treatments at all stages of growth during summer. Also it decreased significantly with all treatments at the 2nd, 4th and 6th weeks of age during winter. The serum ALT level decreased significantly with all treatment groups at all stages of growth during summer. During winter, ALT level decreased significantly with all treatments at all stages of growth. The results which were related to thermoregulation and nutritional strategies adopted for alleviation of thermal stress in broilers were discussed in the light of previous findings reported in literature. The findings of the studies could have implications in improving the growth performance of broilers under tropical conditions.

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CHAPTER ONE
GENERAL INTRODUCTION

1.1 Effect of climatic and nutritional factors on poultry production under Sudan conditions

Poultry production in tropical countries faces a constant challenge. Heavy economic losses have been reported due to decreased productivity and increase in mortality due to climatic stress.

The Sudan remained dependent on the indigenous strains of domestic fowl as the main source of poultry meat and eggs up to the early fifties when the modern poultry farming was started by Khartoum North Government Unit. This unit served as a research station in the field of poultry breeding and extension. The research findings indicated the low productive potentialities of foreign breeds under local conditions in Sudan (Yassin, 1988).

During the last two decades, the poultry industry in the Sudan has witnessed a remarkable and rapid expansion. The demand for poultry meat and egg has increased tremendously due to the high prices of red meat, high rate of population growth and urbanization with dependence on industrial food production. The majority of poultry production units in the Sudan are based on the deep-litter system with open-sided housing designs. It is recognized that hot dry conditions prevail in northern Sudan most of year, and marked diurnal fluctuations in ambient temperature occur. However, most of poultry stocks are European breeds (such as Ross and Bovan). Exposure of such strains to the hot climatic conditions results in impairment

of physiological functions of the birds, reduction of food intake, and consequent depression of productivity (Berge, 1993).

Ration formulation needs to be changed under the tropical environments to allow adequate nutrient intake despite the thermal depression of appetite. A diet formulated to provide all necessary nutrients for productive efficiency at low environmental temperature become less adequate as ambient temperature rises (Njoku and Adeline, 1989). It was suggested that in hot environments the birds should be provided an opportunity for dietary selection (Blake et al., 1984).

Heat load on poultry can be alleviated by evaporative cooling and air conditioning techniques. However, because of the high cost of constructing closed housing system, the use of dietary manipulation is more feasible. Ration composition is a possible measure to influence internal heat production. The reduction of heat increment of the feed by fat supplementation or dietary inclusion of anti-stress minerals and vitamins may be used to minimize the severity of heat load.

1.2 Effect of thermal environment on poultry

1.2.1 Thermoregulation

The domestic fowl, like other homeotherms, developed some physical and metabolic mechanisms to maintain an approximately steady body-core temperature despite fluctuations in ambient temperature (Simmons et al., 1997). The hypothalamus is the centre for the integration of information on the thermal state of the bird (Timmons and Hillman, 1993). There are also

peripheral thermoreceptors and temperature sensitive neurons in the central nervous system involved in thermoregulation (Whittow, 1986).

Deeb and Cahaner (2002) reported that evaporative heat loss in birds occurs mainly through the respiratory tract and is initiated at a core temperature of 41 – 43°C in different species of birds. The onset of thermal panting was suggested to be consistently related to the increment in hypothalamic temperature and this relationship was influenced by both the peripheral and extracranial deep body temperature (Raup and Bottje, 1990). However, Wiernusz and Teeter (1996) reported that panting was initiated at an ambient temperature of 35°C without any significant increase in rectal temperature.

Metabolic heat production in birds is influenced by breed, age, sex, activity, insulation and nutritional status (Belay and Teeter, 1996). Metabolic rate is regulated by hypophyseal, thyroid, adrenal and pancreatic hormones (Darras et al., 2000). At low ambient temperature, the metabolic rate is elevated to compensate for the increased sensible heat loss, at high ambient temperature it rises due to activation of the cooling mechanism (Hai et al., 2000).

At high ambient temperature, birds alter their behaviour to maintain body temperature within normal limit. In hot environment, birds spread their wings away from their body to promote cooling by reducing body insulation, the domestic fowls may splash water on their combs and wattles in order to increase evaporative cooling from these surfaces (Timmons and Hillman, 1993), and chickens minimize muscular activity (Simmons et al., 1997),

reduce food intake and consume more water in order to compensate for water lost in evaporative cooling (May and Lott, 1992).

1.2.2 Heat balance

The chicken maintains its body-core temperature within narrow limits by establishing a balance between the total rate of heat gain and heat loss. A general heat balance equation for the chicken can be written as follow:

$$M - W = R_n + C + K + \lambda Er + \lambda Es + J$$

In this equation,

M = rate of heat gained by metabolism.

W = rate of external work done.

R_n = net rate of heat loss by radiation.

C = rate of heat loss by convection.

K = rate of heat loss by conduction.

λEr = rate of heat loss by evaporation from the respiratory tract.

λEs = rate of heat loss by evaporation from skin surface.

J = rate of storage of thermal energy in the body.

For a bird standing still, the rate of external work done (W) can be neglected, and the rate of heat loss by evaporation (λEr, λEs) can be summarized as λE so that the equation becomes:

$$M = R_n + C + K + \lambda E + J$$

A decrease in heat production is usually associated with a decline in basal metabolism, food intake and activity. Some extra heat may be produced as a result of panting and restlessness associated with increase in muscular

activities of respiration and acceleration of chemical reactions within the body (Geraert et al., 1996).

1.2.3 Heat balance and thermoneutrality

When the bird is at rest, the brain and the thoracic-abdominal viscera are chief centres of heat production. During activity, however, heat is also produced in the muscle. The intensive genetic selection for fast growth rate means that modern species of broiler chickens are very susceptible to heat stress (Deeb and Cahaner, 2002). Heat production is minimal over a specific range of ambient temperature, body temperature being regulated by non-evaporative physical processes alone. This range is the thermoneutral zone and it is equivalent to a zone of least thermoregulatory effort (Tzschentke et al., 1996). The thermoneutral zone is limited by a lower critical temperature below which the metabolism rises to maintain deep body temperature, and an upper critical temperature, above which the metabolism is increased with a concomitant rise in evaporative heat loss (Geraert et al., 1996).

1.2.4 Heat loss

Heat is transferred from the internal organs of bird where it is produced to the body surface by conduction from one molecule to another, by convection through blood stream and by countercurrent exchange in the limbs (Hillman et al., 1985). Heat exchange between a bird and its surrounding environment occurs through sensible and insensible heat loss.

The main pathway of heat dissipation for birds under hot environment is respiratory evaporation (Hillman et al., 1985), especially when ambient temperature approaches body temperature. When air temperature rises, the

breathing frequency of chickens is increased (Raup and Bottje, 1990; Wiernusz and Teeter, 1996), and the evaporative heat loss is significantly enhanced (Spiers, 1983; Chwalibog and Eggum, 1989; Wiernusz and Teeter, 1996). The amount of evaporative heat loss depends on air humidity and is suppressed when humidity rises (Chwalibog and Eggum, 1989). Panting constitutes the major avenue of heat loss by birds in hot environment. Panting involves an increase in the respiratory minute volume while the tidal volume is decreased.

1.2.5 Insulation

Thermal insulation in birds consists of internal insulation (R_I) and external insulation (R_E) superficial to the skin. The external insulation (R_E) includes the insulation offered by the feathers which is basically trapped still air, plus the insulation from the exposed surface to the boundary layer surrounding (Ingram and Mount, 1975). As the internal insulation (R_I) and external insulation (R_E) are in series,

$$\text{The total insulation } (R_T) = (R_I) + (R_E)$$

The bird feathers represent the most effective barrier to heat loss from the skin surface to the surrounding air. The down feathers trap air in which little convective movement occurs and the distal part of the contour feathers provide a wind proof covering (Lin et al., 2005). Yahav (2000) reported that the amount of feathering is an important determinant of heat production at environmental temperatures below about 34-35°C. Newly hatched chicks can reduce sensible heat loss by up to 60% by huddling (Wathes and Clark, 1981).

1.2.6 Effect of heat on the performance of broilers

Hai et al. (2000) reported that decrease in growth rate of birds during hot summer becomes more pronounced with age. High heat load in poultry generally results in a decrease in feed consumption and growth of immature fowl (Donkoh, 1989). Bollengier-Lee et al. (1998) showed that 63% of the reduction in growth rate was due to reduced feed intake. Adams and Rogler (1968) compared the growth of fast and slow growing breeds at 21°C and 29°C; the body weight gain was at lower rate at 29°C than at 21°C and the depression in growth was greater for the fast growing chicks than for the slow growing chicks. Optimal growth and feed utilization of chicks was obtained at 21°C and 60% RH (Lin et al., 2005). Nichelmann et al. (1991) observed that the maximal body weight in chicks was attained at ambient temperature of 18 - 24°C. Sahin et al. (2001) stated that normal feed consumption was found at or below 32.2°C but many birds ceased eating at 37.8°C.

1.2.7 Effect of heat on blood constituents

The flow of blood from the body core to the peripheral tissues plays a significant role in the transfer of heat to the surface for dissipation to the environment. In tropical environments heat stress alters cardiovascular function in birds (Donkoh, 1989). In chickens, high ambient temperature caused an increase in blood flow through the comb, wattles and shanks due to peripheral vasodilatation (Sahin et al., 2001).

Donkoh (1989) reported that heating of chicks increased plasma glucose concentrations, serum cholesterol, corticosteroids, sodium, and

significantly reduced plasma protein level; zinc and ascorbic acid ameliorated heat stress related responses. Depletion of hepatic and muscle glycogen was significantly increased during exposure of chicks to high ambient temperature; however, when exposed to cold, the plasma glucose level decreased in hens (Freeman, 1988).

Environmental stress has been demonstrated to cause an increase in oxidative stress and an imbalance in antioxidant status (Sahin et al., 2001). Moreover, it has been reported the effects of environmental stress such as heat ambient temperature reduced plasma protein concentration, antioxidant enzymes activities such as paraoxonase (PON1) (Sahin et al., 2002), and markedly increased blood glucose and cholesterol concentrations in broilers (Donkoh, 1989). The plasma antioxidant minerals and vitamins such as Zn levels, and vitamins C, E and folic acid declined oxidative damage increased in stressed poultry (Kafri and Cherry, 1984). Previous studies have shown that antioxidant nutrient supplementation, especially Zn and vitamin C are effective in protecting the oxidation of DNA, low density lipoprotein (LDL), and protein in vitro and in vivo, and that such nutrients could be included in the diet to prevent the negative effects of environmental stress (Mowat, 1994; Sahin et al., 2002).

1.3 Zinc requirement and functions in poultry production

Microelements play an important metabolic role and many of them must be balanced in the poultry diet. Different mineral supplements are currently applied, either organic (bioplexes, chelates) or inorganic (oxides, sulphates) such as Zn (Kidd et al., 1996). Their availability depends on

chemical form, feed composition, age and physiological state of bird and mineral interactions. The Zn requirement of the young broiler is approximately 35 to 40 mg/kg in semipurified diets containing isolated soy protein or casein (Bartlett and Smith 2003). Studies on corn-soybean meal and sesame meal diets suggest that the requirement is in excess of 40 mg/kg (Powell, 2000; Salgueiro et al., 2000). This conclusion was based primarily on low growth responses to Zn supplementation to the basal diets. A study by Stahl et al. (1998) showed that the tibia Zn concentration of chicks fed a corn soybean meal diet was increased markedly by dietary Zn supplementation, but did not provide an estimate of requirements.

The source of supplemental Zn used in most of the studies was Zn sulphate or Zn chloride. Availability of Zn varies among sources (Wedekind and Baker, 1990). Only tentative values are given for chicks after 3 weeks of age. Mineral requirements of layers in production are similar to mineral requirements of other poultry, with the exception of calcium. Zinc needs of turkey are known to depend on the levels of other dietary constituents. The recommended level of 70 mg/kg was determined with practical diets having phytic acid present, whereas 41 mg/kg were adequate in a purified diet where phytic acid was absent (Southern and Baker, 1983; Dewar and Downie, 1984).

Zinc has significant roles in birds probably because it is a cofactor of more than 200 enzymes. One of the most significant functions of Zn is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000). Zinc deficiency provokes oxidative damage through

the effects of free radical action (Powell et al., 1994; Salgueiro et al., 2000) and alters the status of antioxidant enzymes and substances (Prasad et al., 1993). The mechanism by which Zn exerts its antioxidant action is not well defined. However, it has been suggested that Zn increases the synthesis of metallothionein, a cysteine-rich protein which acts as a free radical scavenger (Prasad et al., 1993; Bales et al., 1994). Zinc is an essential component of both DNA and RNA polymerase enzymes and is vital to the activity of a variety of hormones including glucagon, insulin, growth hormone, and the sex hormones (Prasad et al., 1993; Bales et al., 1994).

1.3.1 Zinc metabolism

The mechanism and control of Zn absorption are still not fully understood. Zinc was absorbed at 14-67% depending on chemical form and concentrations of elements acting antagonistically (Cd, Cu, S, P and Mg) (Keen and Graham, 1989). Noy et al. (1994) evaluated the rate of absorption of several elements present in commercially mixed feed for hens and proved that the rate was about 30% for Zn and 60% for Se. Absorption is markedly affected by other dietary components. In poultry, phytate, for example, decreases Zn absorption, whereas low molecular weight binding ligands such as citrate, picolinate, ethylene diamine tetra acetic acid (EDTA) and amino acids such as histidine and glutamate enhance Zn absorption (Hambidge et al., 1986). Ascorbic acid consumed concurrently with Zn has been shown to increase Zn absorption (Baker and Ammerman, 1995).

The important portion of the Zn that comes from diet passes into blood and binds to proteins. In blood plasma, Zn is mostly carried by binding to

albumin (60-70%) and α-2macroglobulin (30-40%). A small amount is carried by transferrin and free amino acids (Prasad, 1978). Within the range of homeostatic regulation, the liver pool and storage of Zn is limited except in bone; storage increases only slightly as dietary Zn increases. Zinc concentration in bone has been used as a measure of Zn utilization and/or Zn status in growing birds. The liver is the primary organ involved in Zn metabolism. When hepatic Zn content is increased above normal levels, additional Zn is associated with metallothionein, a metal-binding protein thought to have a role in storage and detoxification of Zn, copper, cadmium and other metals (Prasad et al., 1993). Zinc is excreted primarily in the faeces as unabsorbed and endogenous Zn (Zago and Oteiza, 2001). Endogenous excretion varies according to the balance between true absorption and metabolic needs. Variable faecal excretion is one of the primary mechanisms for maintaining Zn homeostasis (Bales et al., 1994). Thus, both absorption and faecal excretion are important in regulating Zn balance.

Negative interactions can occur such that an excess of one trace mineral will interfere with another trace mineral's availability (Wedekind and Baker, 1990). The most common antagonism occurs between Zn and copper (Sandoval et al., 1997). High levels of dietary Zn will inhibit copper absorption, hepatic accumulation and deposition in the egg. Ratios greater than 4:1 of Zn: copper can be considered antagonistic. High levels of copper and iron can interfere with Zn availability and potentially could induce anaemia (Edwards and Baker, 1999).

1.3.2 Bioavailability of zinc

In birds, numerous factors have a marked effect on Zn absorption. The kind of chemical compound is one of them. Most studies on Zn bioavailability determine the relative bioavailability mainly related to Zn sulphate (Wedekind and Baker, 1990), and only a few studies address the absolute apparent bioavailability. The absolute bioavailability was established to be about 22% for Zn oxide, 23% for Zn sulphate and 19% for Zn acetate (Poulsen and Larsen, 1995; Poulsen and Carlson, 2001).

Ranking of criteria to assess bioavailability of Zn from different sources were described by Sandoval et al. (1997) for broilers. They showed that concentration of Zn in bones was closely related to the bioavailability, followed by Zn content in liver and pancreas.

Besides the chemical compound, numerous interactions between Zn and other feed components exist. Hexa- and penta phosphate derivatives of inositol (phytic acids) affect Zn absorption in non-ruminants, because insoluble Zn phytate complexes are formed. The absorbability of Zn depends not only on the concentration of phytate, but also of calcium, magnesium and phosphorus. In similar way a nickel oversupply leads to signs of Zn deficiency (Anke et al., 1995; 2002). It seems that an oversupply of divalent cations influences the metabolism of Zn. Consequently the reported recommendations for Zn may vary among studies owing to the differences in the absorption of supplemental Zn sources and the use of ingredients that interfere with absorption and/or utilization of Zn under study (Poulsen and Carlson, 2001).

1.3.3 Influence of zinc sulphate on the performance and blood constituents of broilers under hot environments

Zinc is an essential trace element that is required for growth, bone development, feathering, enzyme structure and function, and appetite for all avian species (Wedekind and Baker, 1990). Zinc is commonly added as a supplement to all formulated poultry diets. Currently there are two feed-grade Zn sources commonly used by the animal feed industry: Zn O (72% Zn) and Zn $SO_4.7H_2O$ (36% Zn). Of the supplemental Zn fed, 80 to 90% is Zn O, which is less bioavailable for poultry than reagent-grade or feed-grade Zn sulphate (Wedekind and Baker, 1990; Sandoval et al 1997; Edwards and Baker, 1999). However, the sulphates (acid salts) are highly water soluble, allowing reactive metal ions to promote free radical formation. This reaction can lead to the breakdown of vitamins and ultimately to the degradation of fats and oils, decreasing the nutrient value of the diet. Oxides are less reactive but also less bioavailable.

Belay and Teeter (1996) reported that heating of chicks increase plasma cholesterol, plasma glucose and decreased serum protein and albumin and decreased serum Zn ameliorated heat stress related responses.

Sahin and Kucuk (2003b) demonstrated that Zn supplementation increased feed intake, body weight and feed efficiency and reduced plasma glucose, serum cholesterol, and increased serum protein. Environmental stress has been shown to increase mineral excretion (Smith and Teeter, 1987).

It is proposed that Zn is the most important mineral substance among the other essential element after iron (Prasad et al., 1961). As the level of Zn in the diet increases, plasma, the liver, kidney, bone and muscle Zn levels also increase. In addition, bone is most sensitive tissue for Zn accumulation, liver and kidney come after (Shan, 1993; Stahl, 1989). Zinc is critical for proper immune function in broilers. Its deficiency has been shown to decrease cellular immunity, thymus and spleen development, and interleukin production (Fletcher, 1988).

In general, the mechanism of antioxidative action of Zn can be divided into acute and chronic effects. Chronic effects involve exposure of chicks to Zn on a long-term basis, resulting in induction of some other substance that is the ultimate antioxidant, such as metallothionein. The mode of action of Zn in antioxidant defense systems in vivo is yet to be elucidated (Garfinkel, 1986; Powell et al, 1994; Salgueiro et al., 2000; Zago and Oteiza, 2001).

1.3.4 Toxicity of zinc

Zinc is a nontoxic element (Dardenne and Bach, 1993). Nonetheless Zn toxicity has been reported to occur under non-experimental conditions (Kidd et al., 1996). But the soluble salts of Zn have major toxicity. Soluble Zn salts may cause acute poisoning, especially in the presence of acids and acidic substances (Ammerman et al., 1995). The toxicity of Zn clearly depends upon the Zn source, dietary level, the duration of feeding, and the levels of other minerals in the diet (Watkins and Southern, 1993).

In young chickens, the following concentrations (mg/kg diet) caused reduced growth: 800 mg as ZnO (Berg and Martinson, 1972), 1500 mg as

$ZnSO_4$ or $ZnCO_3$ (Roberson and Schaible, 1960), 3000 mg as $ZnSO_4$ (Jensen, 1975) and as ZnO (Johnson et al., 1962). Jensen (1975) observed also exudative diathesis and muscular dystrophy in his study at 2000 mg Zn/kg feed as Zn sulphate. In immature turkeys, 4000 mg Zn/kg diet as ZnO caused reduced growth (Vohra and Kratzer, 1968). It was proposed that the presence of zinc, 50-100 times of the normal level, in animal rations leads to decrease in the body weight gain due to a reduction in feed consumption (Watkıns and Southern, 1993; Ammerman et al., 1995). It is also stated that the addition of 4000 mg zinc to diet decreases the live weight, and also feed consumption rate and feed conversion ratio (Watkins and Southern, 1993).

1.4 Vitamin C (ascorbic acid, AA) requirement in poultry production

Birds are normally able to synthesize adequate amounts of AA. The levels of AA synthesized for physiological needs may only be sufficient when the environmental temperature does not cause any stress to the birds (Coates, 1985). However, there are many indications that under heat stress conditions birds cannot produce enough AA for their metabolic needs and they require dietary AA (Coates, 1985; Pardue and Thaxton, 1986). Under heat stress conditions, dietary supplemental AA was reported to alleviate the effect of heat stress on the performance of broilers chicks (Kafri and Cherry, 1984; McKee et al., 1997).

1.4.1 The chemistry and biosynthesis of ascorbic acid

L- ascorbic acid is a crystalline powder, optically active in water and melting at 192°C (McKee and Harrison, 1995). AA can be reversibly oxidize to dehydro-ascorbic acid and both the oxidized and reduced forms are

physiologically active. The physiological activity of AA appears to be associated with the reducing power and yet freshly oxidized solutions still retain their activity (Njoku, 1986).

The biosynthesis of AA in mammals and birds takes place either in the liver, kidney or in both, depending on the species. Kutlu and Forbes (1993) reported that the liver tissue of birds such as the chicken and the pigeon which are known to synthesize their own requirements failed to synthesize AA. In spite of the absence of anti-scorbutic substance in the egg yolk and egg white, the chick embryo develops normally in the egg (Sahin et al., 2003a). However, appreciable amounts of AA were present in the avian embryo after 4 days of incubation. The plasma AA concentration of the chick increased after the fifth day of age and reached the normal level at the age of 30 days (Horing and Frigg, 1979).

The mean concentration of AA in blood plasma averaged over all breeds, age and sexes approaches 14 mg/ml (Pardue et al., 1986). Other body tissues including the spleen, liver, intestine and testes contain AA at concentrations several times greater than the concentration in blood plasma. This suggests active transport of AA from blood to the tissues (Kutlu and Forbes, 1993). The avian adrenal gland is similar to that of mammals in that it contains a high level of AA (178 mg/100g) (Kutlu and Forbes, 1993). This led to much speculation of the possible role of AA in adrenal function and its relation with adrenocortical hormones synthesis and secretion (Sahin et al., 2002).

1.4.2 Influence of ascorbic acid on other nutrients

The effect of ascorbic acid (AA) on the poultry performance is highly dependent on the composition of the basal diet. Gonzalez-Vega-Aguirre et al. (1995) reported that AA stimulated growth in chicks when fed folic acid deficient diet; supplementation of the diet with folic acid reduces the growth stimulation due to AA. Also they showed that addition of AA to basal diet containing either tallow or cotton seed oil produced marked improvement in growth rate. AA was found to affect carbohydrates metabolism. This is related to its effect on corticosterone synthesis or glycogenesis impairment. McKee and Harrison (1995) reported that AA supplementation to a vitamin A-deficient diet fed to chicks, significantly reduced the liver glycogen content at 7 weeks of age.

AA promotes mineral mobilization from bones. Thornton (1970) showed that AA stimulates mobilization and excretion of both Ca^{45} and P^{32} and the effects were more specific for P^{32}. Sahin and Kucuk (2001) reported that the growth depression caused by cobalt, vanadium and selenium were reversed by AA supplementation at a rate of 200 – 2000 mg/kg diet. Orban et al. (1993) demonstrated that 5000 mg AA/kg diet reduced growth, lowered haemoglobin concentration and PCV value and increased mortality in chickens when added to a purified diet containing 8 mg Cu/kg. Increasing the level of Cu 3-5 fold counteracted the growth depression; AA intensifies the effect of copper deficiency (Orban et al., 1993). Lonnerdal (2000) reported that AA supplementation increased Zn absorption.

1.4.3 Influence of ascorbic acid on the performance of broilers under hot environment

There are numerous reports in the literature which support the importance of AA supplementation in poultry diets. Ladmakhi et al. (1997) suggested that AA had stimulating influence on the thyroid gland, it was found to increase food intake and oxygen consumption in cool environments and the reverse was observed in warm environments. Sahin et al. (2002) reported that AA supplementation could improve feed intake, body weight gain and efficiency of feed conversion in coccidiosis of broilers. AA was found to assist in counteracting the heat load and was helpful in maintaining body temperature and metabolic activities in broiler, their response being influenced by both age and breed (McKee et al., 1997).

Blood AA levels were inversely proportional to environmental temperature within the range of 21 to 31^{o}C (Ladmakhi et al., 1997). Within this temperature range, as the environmental temperature increases, the blood AA levels decrease. It has also been reported that excessive supplementation of AA can reduce the performance of broiler chicks, especially in the absence of stress (Kafri and Cherry, 1984; Kutlu and Forbes, 1993). Njoku (1986) concluded that 200 mg AA/kg produced the highest body weight gain in broilers when supplemented during the hot months.

Pardue et al. (1985a) demonstrated that AA supplementation at a rate of 1000 mg AA/kg to 4-week old chicks minimized the weight loss during exposure to 43ºC and 40% relative humidity. Ismail (1991) demonstrated that AA supplementation at a rate of 1000 mg/kg lowered body temperature in

broiler when supplemented during wet summer. He also reported that AA supplementation could improve body weight gain and feed conversion ratio in the 4th week of age, and efficiency of feed conversion was also improved in the 5th and 7th week of age during wet summer.

1.4.4 Influence of ascorbic acid on blood constituents under hot environment

Under hot conditions, birds increase skin blood flow by vasodilatation and reduction in vasomotor tone (Pardue et al., 1985b). The flow of blood increases in the skin and nasobuccal capillaries to increase heat loss and in the respiratory muscles to meet the energy cost of panting; accordingly blood flow is reduced in abdominal viscera, fat and non-respiratory muscles (Ladmakhi et al., 1997)

Sahin et al. (2001) reported that heating of chicks increased plasma levels of cholesterol, glucose and sodium and decreased potassium and serum protein levels; ascorbic acid ameliorated heat stress related responses. McDowell (1989) demonstrated that AA supplementation reduced plasma glucose, serum cholesterol and increased serum protein. Kutlu and Forbes (1993) reported that AA reduced the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, AA alleviates the negative effects of environmental stress such as heat stress-related depression in poultry performance (Pardue et al., 1985a). Environmental stress has been shown to increase mineral excretion (Smith and Teeter, 1987). Pardue et al. (1985b) reported that AA supplementation to chicks

reduced the high blood glucose produced by heat stress and significantly increased liver glycogen concentration above avitaminotic controls.

AA has been reported to affect general immune function in the fowl. Gonzalez-Vega-Aguirre (1995) noted that AA supplementation ameliorated the immunosuppression caused by cortisol administration and significantly increased primary agglutinin produced in response to injected sheep red cells. Heat associated immunosuppression has also been reported to be reduced by AA supplementation (Pardue et al., 1985).

1.5 Objectives

Broilers are exposed to thermal stress under local tropical conditions, and accordingly their physiological responses and performance are markedly influenced. Nutritional strategies could be beneficial in alleviating the stress and could be adopted in order to improve weight gain. Therefore, the studies described in this thesis were performed to investigate the following relationships:

(1) Effect of dietary zinc ($ZnSO_4$) supplementation on physiological response and performance of Ross broiler chicks during summer.

(2) Effect of dietary zinc ($ZnSO_4$) and ascorbic acid (vitamin C) supplementation on physiological responses and performance of Ross broiler chicks during summer and winter.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Experimental birds

A total of 400 one-day-old unsexed broiler chicks (Ross 308) were used in the studies. The chicks were obtained from Coral Company – Khartoum. The birds were vaccinated against Newcastle disease virus (NDV). Live Newcastle disease vaccine (Lasota strain**,** Amipharma**)** was administered via drinking water at 8 and 24 day of age.

2.2 Housing and management

This study was carried out during summer and winter at the Department of physiology, Faculty of Veterinary Medicine, University of Khartoum - Shambat. The experimental poultry house is provided with concrete, floor was cemented and covered with 5 cm-deep layer of fresh wood shavings, zinc roof, wire net sides, with dimensions of 24 m x 15 m x 3 m. The experimental house was divided into 25 wire cages, each with dimensions of 1 m^2 x 0.8 m height and 6 bird capacity. The poultry house was cleaned and disinfected with 40 % formalin. No further additions of wood shavings occurred during the trials. The room was divided into 25 identical 1 m^2 pens, with partitions of solid wire mesh between the pens, and manual feeding and drinking equipments were used. Light was provided for 24 hrs (natural and/or artificial light). Florescent light sources (100 watt/lamp) were located over each block to provide illumination of uniform intensity.

2.3 Experimental feeds

The feeds (starter and finisher) were formulated according to National Research Council (NRC, 1996) to meet or exceed the nutrients requirements of broiler chicks. Ingredients and chemical composition of the basal diets used in the experiments are shown in Tables 2-1, 2-2 and 2-3, respectively. The birds were fed the starter diet till 21 days of age, and they were fed the finishing diet up to the end of the experiment (42 days). Diet and water were offered ad libitum throughout the course of experiments.

2.4 Rectal temperature (T_r)

The rectal temperature of birds was measured by an electronic digital thermometer (Shwalk, China). The prope was inserted 5 cm into the rectum and read after 2 min.

2.5 Production parameters

2.5.1 Feed intake

Feed consumption was recorded weekly, and it was measured by an electronic digital balance (Mettler, Germany).

2.5.2 Body weight

During the experiments, the birds were weighed to the nearest 0.1g using an electronic digital balance (Mettler, Germany).

2.5.3 Weight gain

Mean weekly weight gain of birds was computed as follow:

Weight gain 1 = body weight-week 1 - initial body-weight

Weight gain 2 = body weight-week 2 - body weight-week 1

Table 2-1. Basal feed ingredients (100kg) of starter and finisher diet.

Nutrient	Starter diet (1-21 days)	Finisher diet (22-42 days)
Sorghum	**59.80**	**62.00**
Ground nut cakes	**30.00**	**20.00**
Wheat bran	**2.00**	**9.58**
Broiler concentrate*	**5.00**	**5.00**
Salt	**0.25**	**0.25**
Methionine	**0.09**	**0.06**
Di-calcium phosphate	**0.71**	**0.23**
Calcium carbonate	**0.28**	**0.50**
Lysine	**0.12**	**0.08**
Vegetable oil	**1.50**	**2.10**
Premix	**0.25**	**0.20**

*Broiler concentrates from Provimi Holland (Protein 38% ME 1825kcal/kg Phosphorus 7.5% Lysine 10.58% Methionine 4.25% Fat 3.25% Crude fiber 2%).

Table 2-2. Chemical composition of starter and finisher diets during (Summer).

Nutrient	Starter diet (1-21 days)	Finisher diet (22-42days)
Crude protein (%)	21.00	20.00
Lysine (%)	1.20	1.10
Methionine (%)	0.50	0.45
Calcium (%)	1.10	1.00
Phosphorus (%)	0.50	0.40
Crude fibre (%)	5.00	5.00
ME* (kcal/kg)	3000	3000

ME: metabolizable energy.

Table 2-3. Chemical composition of starter and finisher diets during (winter).

Nutrient	Starter diet (1-21 days)	Finisher diet (22-42days)
Crude protein (%)	23.00	21.00
Lysine (%)	1.20	1.10
Methionine (%)	0.50	0.45
Calcium (%)	1.10	1.00
Phosphorus (%)	0.50	0.40
Crude fibre (%)	5.00	5.00
ME* (kcal/kg)	3100	3173

ME: metabolizable energy.

2.5.4 Feed conversion ratio

Weekly feed conversion ratio (Nesheim et al., 1979) was computed from feed intake and the amount of weight gained as follows:

$$\text{Weekly feed conversion ratio (\%)} = \frac{\text{Mean feed consumed (g/week)}}{\text{Mean Wight gain (g/ week)}}$$

2.6 Blood analysis

Blood samples were collected from ten birds (two per replicate) randomly chosen from each group. The area of collection was scrubbed by 70% ethanol before the wing vein (*vena cuanea ulnaris*) was punctured. Then 2 ml blood samples were drawn using 1 ml disposable syringes. Immediately 0.5 ml of the blood was transferred to capped test tube containing ethylene diamine tetra-acetate acid (EDTA) (0.2 mg/ml of blood) as anticoagulant for the measurements of packed cell volume (PCV). Then 0.5 ml of blood was transferred to another test tube containing sodium fluoride as anticoagulant that inhibits the enzymatic reaction (Kelly, 1984) and was centrifuged at 3000 r.p.m. for 15 min. The plasma separated was used for glucose determination. The rest of the blood samples were allowed to stay for 2 hrs at room temperature and the serum was separated using a bench centrifuge (Gallenkamp Junior) operated at 3000 r.p.m for 15 min. Haemolysis-free serum samples were obtained and stored frozen for subsequent analysis.

2.6.1 Packed cell volume (PCV)

The PCV of erythrocyte, expressed as percentage of whole blood, was determined in capillary tubes using a microhaematocrit centrifuge (Hawksley-london) operated for 5min.

2.6.2 Plasma glucose

Plasma glucose level was determined by an enzymatic colorimetric method using a kit (Spinreact, S. A. Spain).

Principle:

The concentration of plasma glucose was determined by enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts with phenol and 4-aminophenazone (4-AP) to form a red violet dye as indicator.

$$\text{Glucose} \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O$$

Reagents:

Reagent (1): consists of buffer (TRIS 92 mmol/L pH 7.4, phenol 0.3 mmol/L) and enzymes (1500 U/L glucose oxidase (GOD), 1000 U/L peroxidase (POD) and 2.6 mmol/L 4- aminophenazone (4-AP)).

The glucose standard was prepared by dissolving 100 mg of glucose in 100 ml of distilled water.

Procedure:

1.0 ml of the glucose reagent was added to each of 3 test tubes. 10µl of plasma was added to one of the tubes to prepare sample tube, while 10µl of standard was added to the second tube to prepare standard tube, and the third tube without addition for blank tube.

The tubes were shaken well and kept for 15-20 min. at room temperature. Then the optical density (O.D.) of standard and samples were read at 520 nm using a colorimeter (Mitra and Bros. Ltd. London).

Calculation:

The concentration of plasma glucose was calculated as follows:

$$\text{Plasma glucose (mg/dL)} = \frac{\text{O.D. sample}}{\text{O.D. standard}} \times 100$$

2.6.3 Serum cholesterol

Serum cholesterol concentration was determined by enzymatic colorimetric method using a kit (Spinreact, S. A. Spain).

Principle:

Cholesterol and its esters are released from lipoprotein by detergents. Cholesterol esterase is hydrolyzed and the esters and H_2O_2 are formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase according to the following reaction:

$$\text{Cholesterol + Fatty acid} \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol ester + } H_2O_2$$

$$\text{Cholesten-3-ON + } H_2O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholesterol + } O_2$$

Reagents:

The buffer reagent (R1): consists of 90 mmol/L Pipes buffer (pH 6.9) and 26 mmol/L phenol.

The enzymatic reagent (R2): consists of 300 U/L cholesterol esterase, 300 U/L cholesterol oxidase, 1250 U/L peroxidase and 0.4 mmol/L 4-aminophenazone.

The cholesterol standard was prepared by dissolving 200 mg cholesterol in 100 ml distilled water.

Procedure:

The blank was prepared by adding 1.0 ml of R1 for the blank tube, 10 µl of serum was added to 1.0 ml of R1 to prepare sample tube. The standard tube was prepared by mixing 10 µl of cholesterol standard with 1.0 ml of R1.

The tubes were mixed well and incubated for 10 min at room temperature. Then the optical density for the samples and standard were read against the blank at 520 nm using a colorimeter (Mitra and Bros. Ltd. London).

Calculation:

The concentration of serum cholesterol level was calculated as follows**:**

$$\text{Serum cholesterol (mg/dL)} = \frac{\text{O.D. sample}}{\text{O.D. standard}} \times 200$$

2.6.4 Serum total protein

The concentration of serum total protein was determined using Biuret reagent as described by Coles (1974).

Principle:

Copper in alkaline solution reacts with the peptide bonds of amino acids in proteins producing a violet colour (Biuret).

Biuret: $NH_2 - \underset{\underset{O}{\|}}{C} - NH - \underset{\underset{O}{\|}}{C} - NH_2$

One copper atom complexes with 4 molecules of buiret, the linkage being to the central nitrogen atoms. The optical density varies with different protein concentrations.

Reagents:

Buiret reagent (stock solution)

9.0 g of sodium-potassium tartrate were dissolved in 500 ml of 0.2 N sodium hydroxide, 3.0g of copper sulphate($CuSO_4.7H_2O$) were dissolved, then 5.0g of potassium iodide were added and the volume was made up to 1 litre with 0.2 N sodium hydroxide.

Colour reagent:

This was prepared by dilution of 50 ml of stock solution to 250 ml with 0.2 N sodium hydroxide.

Procedure:

The blank was prepared by adding 3.0 ml of distilled water to 5 ml colour reagent. The standard was prepared by mixing 3 ml of standard bovine albumin (Sigma Chemical Company) to 5 ml colour reagent. The test tube was prepared by added 0.2 ml of plasma was made up to 3 ml with 2.8 ml of distilled water; the mixture was added to 5 ml of colour reagent.

Each of the three tubes containing the blank, standard and the test was mixed thoroughly and allowed to stand for 30 min at room temperature. The optical density was read at 540 nm using a colorimeter (Mitra and Bros. Ltd. London).

The concentration of total serum protein was calculated as follows:

$$\text{Total serum protein (g/dL)} = \frac{\text{O.D. test}}{\text{O.D. standard}} \times 7.5$$

2.6.5 Serum albumin

The serum albumin concentration was determined by the method of Barthalomew and Delaney (1966) that depends on dye binding. Bromocresol green (BCG) is the best binding reagent that gives green colour with albumin at pH 3.8 – 5.0.

Principle:

When albumin is added to BCG, the resulting change in colour is proportional to the amount of albumin present.

Reagents:

From IM sodium citrate solution 14.79g dehydrate sodium citrate in 50 ml distilled water; 17.3 ml was added to 32.7 ml M citric acid. Then 6 ml of stock solution of BCG (0.174g in 2.5 ml of 0.1 NaOH made up to 25 ml with distilled water) was added and the solution was made to 1 litre with distilled water and the pH was adjusted to 3.8.

The standard was prepared by dissolving 5.0 g bovine albumin (Sigma chemical company) in 100 ml distilled water.

Procedure:

The blank (B) was prepared by adding 4 ml of BCG reagent in a test tube. The standard was prepared by mixing 0.02 ml of standard bovine albumin to 4 ml of BCG reagent in a test tube. The sample was prepared by added 0.02 ml of serum to 4 ml of BCG reagent in a test tube.

The test tubes were well mixed and read in a colorimeter (Mitra and Bros. Ltd. London) at 637 nm.

The concentration of serum albumin was calculated as follows:

$$\text{Serum albumin (g/dL)} = \frac{\text{O.D. test}}{\text{O.D. standard}} \times 5$$

2.6.6 Serum aspartate aminotransferase (AST)

The serum AST activity was determined by enzymatic method using a commercially available kit (Randox Laboratory Ltd, London).

Principle:

AST is measured by monitoring the concentration of oxalacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine.

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate}$$

Reagents:

The buffer reagent (R1) was composed of 100 mmol/L of phosphate buffer (pH 7.4), 2.0 mmol/L of L-aspartate, and 2.0 mmol/L of α-oxoglutarate. The enzyme reagent (R2) was composed of 2.0 mmol/L of 2, 4-dinitrophenylhydrazine. The sodium hydroxide reagent (R3) was composed of 0.4 mol/L of NaOH solution. The standard consisted of 2 mmol/L of oxalaocetate.

Procedure:

The test solution was prepared by adding 0.1 ml of serum to 0.5 ml of reagent 1 (AST buffer). The blank was prepared by adding 0.1 ml of distilled water to 0.5 ml of reagent 1. The tubes were mixed and incubated for 30 min. at 37ºC. Then 0.5 ml of reagent 2 (2, 4-dinitrophenylhydrazine) was added and mixed and allowed to stand for 20 min. at 25ºC in a water bath. Then 5.0 ml of reagent 3 (NaOH) was added to each tube and mixed. The optical density (O.D.) of sample was read against the blank after 5 min.

Calculation:

The serum activity of AST was obtained from the Table.

2.6.7 Serum alanine aminotransferase (ALT)

The serum ALT activity was determined by enzymatic method using a commercially available kit (Randox Laboratory Ltd, London).

Principle:

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

α-oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + pyruvate

Reagents:

The buffer reagent (R1): was composed of 100 mmol/L of phosphate buffer (pH 7.4), 200 mmol/L of L-alanine and 2.0 mmol/L of α-oxoglutarate. The enzyme reagent (R2) was composed of 2.0 mmol/L of 2, 4-dinitrophenylhydrazine. The sodium hydroxide reagent (R3) was composed of 0.4 mol/L of NaOH solution. The standard consisted of 2 mmol/L of pyruvate.

Procedure:

The test solution was prepared by mixing 0.1 ml of serum with 0.5 ml of reagent 1 (ALT buffer). The blank was prepared by adding 0.1 ml of distilled water to 0.5 ml reagent 1. The tubes were mixed and incubated for 30 min. at 37ºC. Then 0.5 ml of reagent 2 (2, 4-dinitrophenylhydrazine) was

added to the sample and blank, then mixed and allowed to stand for 20 min. at 25ºC in a water bath. Then 5.0 ml of reagent 3 (NaOH) was added to each tube and mixed. The optical density (O.D.) of sample was read against the blank after 5 min.

Calculation:

The serum activity of ALT was obtained from the Table.

2.6.8 Serum zinc

Serum samples were diluted four times with deionized distilled water before zinc analysis. Zinc concentration was determined on a flame atomic absorption spectrophotometer (Vanish, Germany), at a wavelength of 213.9 nm. Zinc standard (15.3 mmol/L) (Sigma) was diluted with deionized distilled water to form a series of standards that ranged from 0 to 15.3 μmol zinc/L. 15 plastic tubes were used throughout the procedure to limit the binding of zinc to glassware. Five percent of samples were processed in duplicate to verify analytic quality. Sample sets were reanalyzed if more than 10% variation occurred in duplicate samples.

2.8 Statistical analysis

The statistical analysis was performed using Statistical Analysis System (SAS, 1999). Analysis of variance (ANOVA) test was carried out to examine the effect of dietary supplementation of zinc sulphate and ascorbic acid and season on physiological response of chicks. Mean separation was performed using Duncan Multiple Range Test. The results are presented as means ± standard deviation (S.D.).

2.7 General experimental plan

Experiment	No. of chicks	Parameters measured
(1) Effect of dietary zinc ($ZnSO_4$) supplementation on physiological responses and performance of broiler chicks during summer conditions.	150	Rectal temperature, feed intake, body weight, feed efficiency and blood analysis.
(2) Effect of dietary zinc ($ZnSO_4$) and ascorbic acid supplementations on physiological responses and performance of broiler chicks during summer and winter conditions.	125	Rectal temperature, feed intake, body weight, feed efficiency and blood analysis.

CHAPTER THREE

EFFECTS OF DIETARY SUPPLEMENTATION WITH ZINC SULPHATE ON PHYSIOLOGICAL RESPONSES AND PERFORMANCE OF BROILERS DURING SUMMER

3.1 Introduction

Under tropical conditions, high thermal load influences all types of poultry production. Feed intake, growth rate and mortality are adversely affected by heat stress (Bartlett and Smith, 2003). Broilers exposed to an environmental temperature of 34°C showed a significant decrease in feed intake (Geraert et al., 1996; Bartlett and Smith, 2003). Heat stress also increases excretion of minerals such as Zn, Cu, and Mn (Belay and Teeter, 1996). Moreover, stress causes accumulation of Zn in the liver, decreasing plasma Zn level; thus it may exacerbate a marginal Zn deficiency or an increased Zn requirement (Nishi, 1996; Belay and Teeter, 1996). The reduction in feed consumption and increase in excretion of minerals results in adverse effects on poultry performance, health status, and antioxidant system (Sahin et al., 2005). Zn retention by broilers exposed to heat stress is reduced with increased Zn excretion (Belay et al., 1992; Belay and Teeter, 1996). In birds kept under heat stress, Zn supplementation improved feed intake, body weight gain and feed efficiency as well as carcass weight and yield (Donmez et al., 2002).

Supplementation with Zn improved overall health, productivity, and performance of broiler as measured by body weight gain and feed efficiency (Sahin et al., 2005). Although National Research Council (NRC) (1996)

recommends a minimum of 40 mg of Zn /kg of diet, clinical signs of Zn deficiency have been observed even when dietary Zn was increased above the recommended minimum levels (Donmez et al., 2002). The apparent deficiency can be explained in part by reduced bioavailability resulting from dietary antagonists and interaction with other minerals.

Many studies showed that 40 mg Zn /kg diet is far less than the real requirement for broilers to improve production performance, immune competence and metabolite enzymes. (Park Waldroup, 1995; Cunxiao Sun, 1996; Kim and Patterson, 2004). Published research relative to the interactions between heat stress and Zn ($ZnSO_4.7H_2O$) in poultry is sparse (Belay and Teeter, 1996; Sahin et al., 2005). Adverse effects of heat stress on Zn metabolism are important and environmental concerns arise about excess Zn excretion in poultry manure (Kim and Pattreson, 2004).

This experiment was designed to evaluate the effects of Zn sulphate supplementation level on the physiological responses and performance of broilers chicks reared under tropical summer conditions.

3.2 Experimental plan

A total number of 150 one-day-old Ross broiler chicks were used in this study. The experiment was carried out under summer conditions (May- July, 2007). The prevailing climatic conditions during the experimental period are shown in Table 3-1. The birds were fed with starter diet till 21 days of age, then, they were fed with finishing diet up to the end of the experiment at day 42.

Table 3-1: Average weekly values of minimum, maximum and mean ambient temperature (Ta) and mean relative humidity (RH) during summer (May-July 2007).

Week	Ta (°C)			RH (%)
	Min.	Max.	Mean	Mean
Mean	**27**	**40**	**33.5**	**38**
1st	**26**	**41**	**33.5**	**31**
2nd	**28**	**42**	**35.0**	**33**
3rd	**26**	**39**	**32.5**	**43**
4th	**27**	**39**	**33.0**	**42**
5th	**27**	**40**	**33.5**	**35**
6th	**28**	**39**	**33.5**	**44**
Mean	**27**	**40**	**33.5**	**38**

The experiment was designed according to complete randomized. The chicks were randomly assigned to 5 groups of 5 replicates of 6 birds each (A, B, C, D and E). For each phase, the control group (A) was fed the basal diet while the treated groups (B, C, D and E) were fed the basal diet supplemented with increasing levels (250, 500, 750, 1000 mg/kg) of zinc sulphate ($ZnSO_4.7H_2O$) (BDH Chemical ltd Poole, England). The supplemented levels of $ZnSO_4$ were measured with a digital balance and mixed with the basal diet immediately before being offered to the chicks. During the experimental period, the birds were weighed on the first day, and then weekly. The rectal temperature (T_r), feed intake and body weight were measured weekly at 8:00 a.m. Blood samples were drawn weekly at 7:00 a.m. for analysis.

Analysis of variance (ANOVA) test was carried out to examine the effect of dietary supplementation level with $ZnSO_4$ on physiological responses of the birds. Mean separation was performed using Duncan Multiple Range Test. The results are presented as means ± standard deviation (S.D.).

3.3 Results

3.3.1 Rectal temperature (T_r)

The results of the effect of dietary supplementation level of $ZnSO_4$ on rectal temperature (T_r) of broiler chicks are shown in Table 3.2. There was a slight increase in Tr with rise in mean ambient temperature. However, the

Table 3-2. Effect of dietary zinc (ZnSO4.7H2O) supplementation on rectal temperature (°C) in Ross broiler chicks during summer. A: Control group , B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.

(n=10; mean±SD)

	Age						
Group	1st	2nd	3rd	4th	5th	6th	LS
A	$^{A}41.74\pm0.26^{b}$	$^{A}41.12\pm0.52^{a}$	$^{A}41.68\pm0.52^{b}$	$^{A}42.44\pm0.54^{c}$	$^{A}41.52\pm0.46^{b}$	$^{A}41.11\pm0.31^{a}$	*
B	$^{A}41.48\pm0.34^{b}$	$^{A}41.01\pm0.37^{a}$	$^{A}41.39\pm0.22^{ab}$	$^{A}42.06\pm0.49^{c}$	$^{A}41.27\pm0.55^{b}$	$^{A}41.07\pm0.41^{a}$	*
C	$^{A}41.60\pm0.52c^{b}$	$^{A}41.05\pm0.50^{a}$	$^{A}41.55\pm0.32^{b}$	$^{A}42.13\pm0.38^{c}$	$^{A}41.36\pm0.46^{b}$	$^{A}41.06\pm0.25^{a}$	*
D	$^{A}41.78\pm0.43^{b}$	$^{A}41.09\pm0.40^{a}$	$^{A}41.24\pm0.49^{b}$	$^{A}42.22\pm0.39^{c}$	$^{A}41.12\pm0.53^{a}$	$^{A}41.03\pm0.32^{a}$	*
E	$^{A}41.78\pm0.43^{b}$	$^{A}41.08\pm0.40^{a}$	$^{A}41.49\pm0.49^{b}$	$^{A}42.12\pm0.39^{c}$	$^{A}41.22\pm0.53^{b}$	$^{A}41.05\pm0.32^{a}$	*
LS	**NS**	**NS**	**NS**	**NS**	**NS**		**NS**

A,B,C : Mean values within the same column with similar superscripts (capital) are significantly different.

a,b,c : Mean values within the same row with different superscripts (small) are significantly different (*P<0.05).

dietary zinc level had no significant effect in rectal temperature (T_r). There was a significant ($P<0.05$) change in T_r with age in all groups.

3.3.2 Feed intake (FI)

Table 3.3. shows the effect of dietary $ZnSO_4$ level on FI. There was a significant ($P<0.05$) increase in FI of chicks receiving different levels of $ZnSO_4$ compared to the control in all weeks.

3.3.3 Body weight (BW)

The effect of dietary $ZnSO_4$ level on BW is shown in Table 3.4. There was a significant ($P<0.05$) increase in BW of chicks receiving different levels of $ZnSO_4$ compared to the control at 2^{nd}, 3^{rd}, 4^{th} and 5^{th} week of age and a significant ($P<0.01$) increase in BW of chicks receiving different levels of $ZnSO_4$ compared to the control at 1^{st} and 6^{th} week of age.

3.3.4 Feed conversion ratio (FCR)

The effect of dietary inclusion of $ZnSO_4$ on FCR is shown in Table 3.5. The results indicate that there was a significant ($P<0.05$) increase in FCR of chicks receiving different levels of $ZnSO_4$ compared to the control in all weeks.

3.3.5 Packed cell volume (PCV)

Table 3.6. shows that the PCV was increased significantly ($P<0.01$) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} week of age of birds.

3.3.6 Plasma glucose

Table 3.7 shows that the plasma glucose level decreased significantly ($P<0.01$) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E)

Table 3-3. Effect of dietary zinc ($ZnSO_4.7H_2O$) supplementation on feed intake (gm) in Ross broiler chicks during summer. A: Control group , B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD)

	Age					
Group	1st	2nd	3rd	4th	5th	6th
A	^{A}104.4±09.66	^{A}220.8±28.94	^{A}383.0±88.08	^{A}513.4±46.34	^{A}769.8±251.8	^{A}742.2±76.07
B	^{B}175.1±28.56	^{B}294.6±30.03	^{B}426.6±60.16	^{B}658.8±81.03	^{B}891.2±077.3	^{A}885.6±98.47
C	^{B}188.6±10.99	^{B}323.0±66.98	^{B}398.2±62.67	^{B}685.0±98.87	^{B}889.1±105.2	^{B}893.8±88.78
D	^{B}198.9±13.37	^{B}306.2±18.42	^{B}407.0±22.57	^{B}659.6±76.28	^{B}846.8±125.7	^{B}858.0±69.07
E	^{B}194.9±08.37	^{A}308.2±19.07	^{B}428.4±33.38	^{B}668.4±47.75	^{B}823.2±055.0	^{B}848.0±88.12
LS	*	*	*	*	*	*

A,B,C : Mean values within the same column with different superscripts are significantly different (* P<0.05).

SD: Standard deviation.

Table 3-4. Effect of dietary zinc (ZnSO4.7H2O) supplementation on body weight (gm) in Ross broiler chicks during summer. A: Control group , B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD)

	Age					
Group	1st	2nd	3rd	4th	5th	6th
A	^{C}109.0±9.43	^{A}251.4±33.15	^{A}469.2±48.82	^{B}717.8±98.83	^{A}1110.2±63.63	^{B}1396.6±136.6
B	^{B}121.2±5.31	^{B}291.8±34.62	^{B}554.6±48.12	^{A}871.8±71.15	^{B}1245.4±120.6	^{A}1633.2±109.1
C	^{B}119.4±7.02	^{AB}261.4±40.94	^{B}523.4±40.40	^{A}854.2±53.07	^{B}1203.4±43.03	^{A}1507.2±91.35
D	^{B}118.4±6.46	^{B}283.6±31.26	^{AB}509.4±57.93	^{AB}760.0±90.71	^{AB}1154.8±152.3	^{A}1457.0±36.67
E	^{A}131.4±3.58	^{B}280.6±07.79	^{B}513.2±32.43	^{A}832.2±54.19	^{B}1183.4±55.25	^{A}1435.0±104
LS	**	*	*	*	*	**

^{A,B,C} : Mean values within the same column with different superscripts (capital) are significantly different (*P<0.05, **P<0.01).

SD: Standard deviation.

LS: Level of significance.

Table 3-5. Effect of dietary zinc ($ZnSO_4.7H_2O$) supplementation on feed conversion ratio (%) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD)

	Age					
Group	1st	2nd	3rd	4th	5th	6th
A	^{B}1.49±0.13	^{B}1.58±0.14	^{B}1.86±0.58	^{B}1.87±1.14	^{B}1.96±0.54	^{B}2.22±0.44
B	^{A}2.45±0.27	^{A}2.45±0.17	^{A}2.42±0.15	^{A}2.79±0.43	^{A}2.85±0.64	^{A}3.08±0.35
C	^{A}2.36±0.07	^{A}2.73±0.67	^{A}2.49±0.21	^{A}2.83±0.09	^{A}2.91±0.39	^{A}3.01±0.65
D	^{A}2.59±0.27	^{A}2.64±0.20	^{A}2.61±0.22	^{A}2.66±0.42	^{A}2.98±0.30	^{A}3.06±0.81
E	^{A}2.26±0.15	^{A}2.71±0.18	^{A}2.65±0.20	^{A}2.71±0.22	^{A}2.80±0.39	^{A}3.03±0.79
	LS *	*	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 3-6. Effect of dietary zinc (ZnSO4.7H2O) supplementation on packed cell volume (%) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD).

	Age			
Group	2nd	4th	5th	6th
A	^{B}21.1±1.91	^{B}21.5±1.65	^{B}22.3±1.42	^{B}23.1±2.73
B	^{A}24.8±1.87	^{A}24.3±1.57	^{A}26.5±2.41	^{AB}24.7±1.89
C	^{A}24.7±2.06	^{A}25.4±3.03	^{A}25.6±2.37	^{AB}24.4±1.84
D	^{A}24.1±1.37	^{A}24.5±1.58	^{A}25.0±2.05	^{A}25.2±2.10
E	^{A}23.9±2.13	^{A}24.4±1.58	^{A}26.7±2.58	^{AB}25.1±1.52
LS	**	**	**	**

^{A,B} : Mean values within the same column with different superscripts are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

Table 3-7. Effect of dietary zinc (ZnSO4.7H2O) supplementation on plasma glucose level (mg/dL) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD).

	Age				
Group	2nd	4th	5th	6th	**LS**
A	^{A}200.5±10.91^{a}	^{A}197.0±16.45^{a}	^{A}186.2±9.19^{b}	^{A}174.3±11.96^{c}	**
B	^{B}178.8±10.33^{a}	^{B}176.7±03.09^{a}	^{B}162.2±9.12^{b}	^{B}150.2±07.89^{c}	**
C	^{B}176.9±03.54^{a}	^{B}177.7±04.14^{a}	^{B}164.5±5.04^{b}	^{B}152.1±06.45^{c}	**
D	^{B}179.2±04.15^{a}	^{C}171.1±02.85^{b}	^{C}148.4±3.81^{c}	^{B}151.2±04.42^{c}	**
E	^{B}176.4±02.79^{a}	^{BC}173.8±04.02^{a}	^{C}149.3±4.16^{b}	^{B}151.5±06.29^{b}	**
LS	**	**	**	**	

^{A,B,C} : Mean values within the same column with different superscripts (capital) are significantly different (**P<0.01).

^{a,b,c} : Mean values within the same row with different superscripts (small) are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

at the 2nd, 4th, 5th and 6th week of age of birds. Table 3.7 also shows that the plasma glucose level decreased significantly (P<0.01) in all groups A, B, C, D and E with increase in age.

3.3.7 Serum cholesterol

Table 3.8. shows that the serum cholesterol level decreased significantly (P<0.01) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2nd, 4th, 5th and 6th week of age of birds. The data also indicate that the serum cholesterol level increased significantly (P<0.01) in all groups (A, B, C, D and E) with increase in age.

3.3.8 Serum total protein

The effect of dietary $ZnSO_4$ level on serum total protein level is shown in Table 3.9. The total protein level increased significantly (P<0.01) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2nd, 4th, 5th and 6th week of age of birds.

3.3.9 Serum albumin

The effect of dietary $ZnSO_4$ level on serum albumin level is shown in Table 3.10. The albumin level increased significantly (P<0.01) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2nd, 4th, 5th and 6th week of age of birds.

3.3.10 Serum aspartate aminotransferase (AST)

The effect of dietary $ZnSO_4$ level on serum AST level is shown in Table 3.11. The AST level decreased significantly (P<0.01) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2nd, 4th, 5th and 6th week of age of birds.

Table 3-8. Effect of dietary zinc ($ZnSO_4.7H_2O$) supplementation on serum cholesterol level (mg/dL) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg. **(n=10; mean±SD)**

	Age				
Group	2nd	4th	5th	6th	**LS**
A	$^{A}149.6\pm10.21^{b}$	$^{A}158.3\pm9.39^{ab}$	$^{A}168.3\pm6.01^{a}$	$^{A}165.1\pm14.96^{a}$	**
B	$^{B}119.9\pm09.18^{b}$	$^{B}115.3\pm6.91^{b}$	$^{B}119.8\pm9.14^{ab}$	$^{B}127.0\pm15.87^{a}$	**
C	$^{B}105.7\pm11.41^{b}$	$^{B}113.9\pm7.50^{ab}$	$^{B}109.8\pm9.13^{a}$	$^{B}121.6\pm10.56^{a}$	**
D	$^{B}108.1\pm08.94^{b}$	$^{B}110.1\pm7.06^{b}$	$^{B}115.3\pm9.98^{b}$	$^{B}126.1\pm10.77^{a}$	**
E	$^{B}111.2\pm11.42^{b}$	$^{B}114.4\pm6.65^{ab}$	$^{B}116.2\pm8.89^{ab}$	$^{B}122.5\pm06.36^{a}$	**
LS	**	**	**	**	

^{A,B,C} : Mean values within the same column with different superscripts capital are significantly different (**P<0.01).

^{a,b,c} : Mean values within the same row with different superscripts small are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

Table 3-9. Effect of dietary zinc (ZnSO4.7H2O) supplementation on serum total protein (g/dL) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD).

	Age			
Group	2nd	4th	5th	6th
A	^{A}3.90±0.22	^{B}4.11±0.18	^{C}4.09±0.16	^{B}4.13±0.30
B	^{B}5.02±0.20	^{A}4.85±0.19	^{A}5.09±0.15	^{A}4.87±0.34
C	^{B}4.89±0.14	^{A}4.94±0.21	^{A}5.08±0.15	^{A}4.97±0.13
D	^{B}4.89±0.14	^{A}5.01±0.21	^{A}4.93±0.17	^{A}4.98±0.11
E	^{B}4.99±0.18	^{A}5.02±0.20	^{B}4.74±0.42	^{A}4.97±0.21
LS	**	**	**	**

^{A,B,C} : Mean values within the same column with different superscripts (capital) are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

Table 3-10. Effect of dietary zinc (ZnSO4.7H2O) supplementation on serum albumin (g/dL) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.
(n=10; mean±SD).

	Age			
Group	2nd	4th	5th	6th
A	^{B}1.42±0.10	^{B}1.44±0.08	^{B}1.51±0.04	^{B}1.59±0.17
B	^{A}1.89±0.08	^{A}1.95±0.06	^{A}1.98±0.04	^{A}1.92±0.13
C	^{A}1.87±0.06	^{A}1.98±0.06	^{A}2.00±0.04	^{A}1.95±0.06
D	^{A}1.86±0.07	^{A}1.98±0.04	^{A}2.01±0.03	^{A}1.96±0.06
E	^{A}1.85±0.09	^{A}1.97±0.05	^{A}2.02±0.04	^{A}1.95±0.07
LS	**	**	**	**

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

Table 3-11. Effect of dietary zinc (ZnSO4.7H2O) supplementation on serum aspartate aminotransferase (AST) level (U/L) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg. **(n=10; mean±SD)**.

	Age			
Group	2nd	4th	5th	6th
A	^{A}83.7±3.83	^{A}87.0±3.02	^{A}86.4±3.72	^{A}85.0±5.78
B	^{B}66.2±2.25	^{B}74.2±1.54	^{B}73.6±3.43	^{B}76.3±4.85
C	^{B}66.0±3.43	^{D}67.8±2.86	^{B}71.2±3.22	^{C}72.4±3.02
D	^{B}64.0±3.13	^{C}70.7±3.02	^{B}72.4±2.67	^{C}70.5±1.84
E	^{B}66.2±2.20	^{B}73.4±3.86	^{B}72.4±3.20	^{C}69.4±1.71
LS	**	**	**	**

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

3.3.11 Serum alanine aminotransferase (ALT)

The effect of dietary $ZnSO_4$ level on serum ALT level is shown in Table 3.14. The ALT level was decreased significantly ($P<0.01$) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} week of age of birds.

3.3.12 Serum zinc

The effect of dietary $ZnSO_4$ level on serum zinc level is shown Table 3.15. Serum Zn level increased significantly ($P<0.05$) in chicks receiving different levels of $ZnSO_4$ (groups B, C, D and E) at the 5^{th} and 6^{th} week of age of birds. There was gradual increase in serum zinc level with increase in the concentration of $ZnSO_4$ in the diet.

3.4 Discussion

In this experiment, $ZnSO_4$ was supplemented with graded increasing level in the diet of broiler chicks to investigate the effect on performance and physiological responses.

There was an increase in rectal temperature (T_r) with rise in the dietary inclusion of $ZnSO_4$ (Table 3.2), but this influence was not significant which might be related to adaptation of the chicks to the prevailing thermal environment. Similarly Donmez et al. (2002) reported that $ZnSO_4$ did not influence rectal temperature in broiler.

The body core temperature (T_r) is usually used as a reliable index of the body heat balance and heat content in birds (Richards, 1973). The rate of sensible heat loss from the body to the ambience depends on the feather insulation and movement of the feather and on the blood supply to the

Table 3-12. Effect of dietary zinc ($ZnSO_4.7H_2O$) supplementation on serum alanine aminotransferase (ALT) level (U/L) in Ross broiler chicks during summer. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg.

(n=10; mean±SD).

	Age			
Group	2nd	4th	5th	6th
A	^{A}9.6±1.17	^{A}9.7±0.95	^{A}9.8±1.03	^{A}10.5±3.31
B	^{B}6.6±0.84	^{B}7.0±0.82	^{B}6.8±0.92	^{B}7.1±1.10
C	^{B}6.3±1.16	^{B}6.9±0.99	^{B}6.8±0.79	^{B}7.0±0.82
D	^{B}6.9±0.99	^{B}7.0±0.82	^{B}6.9±1.10	^{B}7.4±1.07
E	^{B}6.7±0.94	^{B}6.9±1.10	^{B}6.6±0.84	^{B}7.2±1.14
LS	**	**	**	**

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.

Table 3-13. Effect of supplementation with different levels of zinc sulphate ($ZnSO_4.7H_2O$) on serum zinc level (mg/dL) in Ross broiler chicks under summer conditions. A: Control group, B: Zn 250 mg/kg, C: Zn 500 mg/kg, D: Zn 750 mg/kg, E: Zn 1000 mg/kg. **(n = 10; mean±SD)**

	Age	
Group	5th week	6th week
A	^{A}1.44±0.27	^{A}1.56±0.12
B	^{B}2.42±0.38	^{B}2.69±0.38
C	^{C}3.09±0.60	^{BC}3.14±0.39
D	^{C}3.25±0.38	^{C}3.54±0.53
E	^{C}3.55±0.50	^{C}3.85±0.38
LS	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different ($^{*}P<0.05$).

SD: Standard deviation.
LS: Level of significance.

appendages on the head and legs. Vasodilatation and vasoconstriction may alter the sensible heat loss from these appendages by 15 to 20% (Teeter and Belay, 1996).

In the present study, it was apparent that T_r in broilers decreased progressively with age. The progressive decrease in body temperature of chicks with age might result from decrease in metabolic rate influenced by the change in body size and relative increase in body surface area which facilitates sensible heat loss. Yahav et al. (1996) reported that newly hatched chicks attained the adult body temperature at an age of approximately 20 days. Donkoh (1989) indicated that heat loss from the feathered skin is less than from non-feathered parts such as comb, wattles and shank. Also the decrease in body temperature with age might be attributed to increase in carbohydrate metabolism as the variations in body temperature were paralleled closely by decrease in blood glucose level (Table 3.7).

In the present study, the feed intake (Table 3.3), mean body weight (BW) (Table 3.4) and feed conversion ratio (FCR) (Table 3.5) of chicks increased significantly with different levels of $ZnSO_4$ (groups B, C, D and E) at all stages of growth. Similarly, a number of studies reported that dietary zinc supplementation increased feed intake, growth rate, and feed efficiency in broilers (Roberson and Edwards, 1994; Kutlu et al., 1998) and the Japanese quail (Sahin and Kucuk, 2003b). The relation of $ZnSO_4$ to increased nitrogen retention (as source of amino acids) was reported by Kim and Patterson (2004) in broilers.

The reported responses of feed intake, BW and FCR may be related to improvement in nutrient digestibility of broilers under summer conditions. Sahin and Kucuk (2003b) reported that high environmental temperature suppressed nutrient digestibility in poultry. Wallis and Balnave (1984) found that the amino acids were decreased by high environmental temperature in broilers. Hai et al. (2000) reported that the activities of trypsin, chymotrypsin and amylase decreased significantly at a high temperature of 32°C. Zinc has a protective effect on pancreatic tissue against oxidative damage (Pond et al., 1995; Onderci et al., 2003), it may help the pancreas to function properly, including secretion of digestive enzymes, thus improving digestibility of nutrients. Onderci et al. (2003) reported that supplemental Zn ameliorated the decrease in digestibility of dry matter, crude protein and ether extract in laying hens reared under low temperature. However, Zn supplementation improved feed intake, body weight gain, and feed efficiency.

The findings reported in the present study, however, are not in agreement with other studies reported in literature. Cao et al. (2000) reported decreased feed intake and daily weight gain in chicks given 600 mg $ZnSO_4$/kg diet, while dietary $ZnSO_4$ concentration up to 400 mg/kg had no effect on feed intake and growth. Roberson and Schaible (1960) indicated that supplementation of 1500 mg/kg of $ZnSO_4$ depressed broiler growth, whereas this level of ZnO was tolerated to a greater degree than that of the sulphate or carbonate forms. Sandoval et al. (1998) also reported that the feed intake and BW of chicks fed 1500 mg/kg Zn as ZnSO4 were significantly depressed compared with those of chicks fed 0, 500, or 1,000 mg/kg Zn as

$ZnSO_4$. However, dietary supplementation of Zn as ZnO did not show any detrimental effect on broiler performance (Roberson and Schaible, 1960; Johnson et al., 1962; Sandoval et al., 1997).

The reported responses of feed intake, BW and FCR may be related to improvement in maturation of the intestinal and enzyme activity. Maturation of the intestinal tract in birds after hatching may account for the greater ability of homeostatic mechanisms to handle a load of Zn at an older age. Uni et al. (1995) reported that the gastrointestinal tract was immature at hatching, and that villus height and crypt depth continued to increase until day 7 after hatching. The structure of the mucosal surface matured dramatically during the first 2 to 3 weeks of life, and growth of the tract was generally four to five times as great as the proportional increase in body weight (Chesters, 1997). Embryonic enterocytes resemble adult cells histologically; however, digestive enzymes and mechanisms for active transport are absent from the luminal surface (Heintzelman and Mooseker, 1990). Moreover, activities of pancreatic enzymes, including amylase, lipase, trypsinogen, and chymotrypsinogen, have been reported to be depressed with zinc loading in the chick, with concomitant decline in starch digestibility of natural feed ingredients (Lu and Combs, 1988).

The PCV values reported in this study indicate that they are within the range recommended for healthy growing chicks suggested by Jain (1986). The significant increase in packed cell volume (PCV) in all treated groups (B, C, D and E) in this study (Table 3.6) could be attributed to the positive effect of the $ZnSO_4$ on improving plasma iron which is an important integral

part of the haemoglobin molecule. It is generally accepted that erythropoiesis and haemoglobin formation required micronutrients including iron, amino acids and vitamins.

In the present study, the decrease in plasma glucose level (Table 3.7) associated with $ZnSO_4$ supplementation might be related to improved pancreatic enzyme activities by increased Zn uptake of pancreas with dietary Zn supplementation. Lu and Combs (1988) reported that the decrease in plasma glucose level associated with $ZnSO_4$ supplementation may either partly result from the improved pancreatic enzyme activities by excess Zn or increased Zn uptake of pancreas with dietary Zn concentration. The putative effect of Zn on insulin metabolism is associated with increased glucose utilization (Keen and Graham, 1989).

The decrease in plasma glucose level with increasing age (Table 3.7) of broilers might be attributed to increase in storage of sugar as glycogen in the liver. Pearce (1983) reported that the domestic fowl, in common with other birds, is able to maintain the blood glucose level during stress. At early stage of growth, a chick is essentially poikilothermic and has few energy reserves, depending on its limited glycogen deposits, as it attempts to assimilate feed and water (Donaldson, 1995; Hazelwood, 2000). Uni and Ferket (2004) reported that the liver glycogen reserves are converted to blood glucose and provide the chick with an immediate energy source. This finding indicates that at early stage of growth, plasma glucose level is high.

The results indicate that the plasma glucose level was higher at early stage of growth. Klasing (1998) reported that, when dietary glucose proves

insufficient to meet metabolic demands, amino acids in the diet are utilized in the process of gluconeogenesis. The digestive tracts of broilers at early stage of growth may be limited in their ability to digest and utilize diets rich in proteins and carbohydrates (Uni and Ferket, 2004). Furthermore, during the transition from fat to protein and carbohydrate-based nutrition, chicks may have limited reserves of glycogen (Donaldson, 1995). Without expeditious nutrient intake, an energy imbalance can be created, and chicks may catabolize their own body tissues for use in the conversion to glucose (Donaldson, 1995; Hazelwood, 2000). In the present study, the chicks might have been more adapted to change in environmental temperature with age, and then decreased plasma glucose level. Thaxton and Puvaldolpirod (2000) reported that plasma glucose level can be used as a reliable indicator of a stress condition in broilers.

The increase in serum cholesterol level with increase in age might be attributed to an improvement in pancreatic secretion and in intestinal development. The gastrointestinal tract is the organ that undergoes most alterations. At the end of the first week of life, the intestinal tract will be five times as long as the whole body size and the intestinal villi will develop twice as much (Maiorka et al., 2004). The complete functional maturity is reached by 20 to 30 days of age (Kim and Patterson, 2004). In addition, chicks show low levels of pancreatic and intestinal enzymes at early stage of growth (Dechao Liu, 1995). However, an improvement in pancreatic secretion and in intestinal development can be induced by feeding chicks as early as possible. Shapiro et al. (1997) have indicated that the production of dissacharidases,

like maltases, is a limiting factor in the digestion of carbohydrates. Besides, young chicks are not able to digest fat, mainly saturated fat, due to low levels of bile salts (Lesson and Summers, 2001). In the present study, the increase in serum cholesterol level with age may be associated with increased ability to digest and absorb lipids. The capacity to digest and absorb lipids from diets after hatching is still not well developed, but it increases as the bird ages (Krogdahl, 1985).

The activity of pancreatic lipase was higher in birds fed diets containing high energy level (Maiorka et al., 2004). Krogdahl (1985) observed a 10-fold increment in lipase activity when the broiler diet was supplemented with high oil concentration. Krogdahl and Sell (1989) reported that pancreatic lipase activity could not be a limiting factor to fat digestion in the gut, as bile salts. The immature entero-hepatic circulation might be the most important limiting factor of the young bird to use lipids of the diet, since lipase enzyme concentration and activity can be modulated by the increments of lipids in the gut (Serafin and Nesheim, 1970; Carew et al., 1972).

In the present study, the decrease in serum cholesterol level with Zn supplementation (Table 3.8.) may be partly related to decreased lipid peroxidation. Zn deficiency causes increased lipid peroxidation and this can be inhibited by Zn supplementation (Sandoval et al., 1998).In addition, Zn supplementation decreased serum and liver malondialdhyde (MDA) levels in stressed birds (Sahin and Kucuk, 2003b). The reduced lipid peroxidation in Zn supplemented birds might be due to multifunctional roles of Zn, which

include the induction of metallothionein, modulation of the transition elements and its relationship with the antioxidant vitamins such as vitamin A and E (Salgueiro et al., 2000). Zn is a cofactor of the main antioxidant enzyme Cu Zn-superoxide dismutase; it may play a key role in suppressing free radicals and in inhibiting NADPH-dependent lipid peroxidation (Burke and Fenton, 1985). Zn can compete with iron and copper to bind to the cell membrane and decrease the production of free radicals, thus exerting a direct antioxidant action (Burke and Fenton, 1985; Tate et al., 1999; Girotti, 1985). Zn induces the production of metallothionein, an effective scavenger of hydroxyl radicals and it has been suggested that Zn-metallothionein complexes in the islet cells provide protection against immune-mediated free-radicals attack (Burke and Fenton, 1985; Shaheen and Abd El-Fattah, 1995).

In the present study, $ZnSO_4$ supplementation increased serum total protein and serum albumin levels (Tables 3.9 and 3.10). This could be attributed to the increase in feed intake in $ZnSO_4$ fed chicks at last stages of age associated with growth pattern observed. These finding agree with the results reported by Belay and Teeter (1996). Furthermore, the increase in serum total protein and albumin levels due to Zn observed in this study could be due to the improvement in protein absorption indicated by the high weight gain in treated groups. Dietary supplementation of $ZnSO_4$ was found to increase the concentration of protein as well as the activity of amylase and chymotrypsin in pancreatic homogenates in broilers (Sahin and Kucuk, 2003b).

In the present study, $ZnSO_4$ supplementation was associated with decreases of the serum enzymes ALT and AST (Tables 3.11 and 3.12, respectively). The aminotransferases are the most common indicators of cellular malfunction, they are found in small quantities in the serum, with higher values indicating a disease, malfunction in the liver or heat stress (Rosenthal, 1977). Although the values were different for the experimental groups, they were all within the normal range value for serum ALT in chicks which ranges from 1 - 37 U/L (Ker et al., 1982). Plasma ALT activity has been reported to be low in all tissues of chicks (Bogin and Israeli, 1976), but ALT activities often increase due to damage in many tissues (Zantop, 1997). Therefore, it has been suggested that the specific diagnostic value of these enzymes in birds is poor (Ker et al., 1982). The serum levels of ALT and AST were found to be higher in turkeys bred in tropical climates (Makinde and Fatunmbi, 1985).

Sandoval et al. (1998) reported that increasing dietary Zn supplementation to broilers resulted in higher Zn concentrations in the serum, bone, liver, kidney, and muscle at 1, 2, and 3 weeks of age. The current results indicate that the serum Zn level was stable with advance of age (Table 3.13). In relation to these results, Mohanna and Nys (1998) found that body Zn concentration in whole chicks, including feathers, supplemented with Zn (>100 mg/kg) changed mainly with age. Higher body Zn concentrations were observed at 4 and 11 days of age and these concentrations were lower and stable from 21 to 50 day of age. Subsequently, Mohanna and Nys (1999) reported that the whole body Zn concentration of 21-day-old chicks was

significantly lower in birds receiving 40 mg/kg of Zn supplementation than in birds receiving 170 mg/kg. The authors also found that when dietary Zn content was greater than the requirements for growth, an increase in the serum and tibia concentrations of Zn was observed up to dietary concentrations of 75 mg/kg of feed.

In the present study, the serum Zn level increased progressively with increase in dietary supplementation level of $ZnSO_4$ (Table 3.15). This is due to differences in endogenous $ZnSO_4$ loss, which has been reported to increase due to homeostatic mechanisms with increasing Zn absorption (Emmert and Baker, 1995). Zn homeostasis is regulated by a Zn binding protein, metallothionein (Cousins, 1985; Dunn et al., 1987). High Zn intake induces intestinal and liver metallothionein synthesis (Cao et al., 2000) which is associated with Zn absorption. The authors reported that chicks supplemented with high dietary concentrations of Zn might have had high amounts of intestinal metallothionein, which are associated with reduced Zn absorption. This protein influences the regulation of Zn absorption and possibly the bioavailability of Zn from the sources under study. The current theories regarding the physiological role of this unique metal binding protein include detoxification of certain heavy metals including Zn (Karin, 1985) and short-term storage of Zn for metabolic processes (Cousins, 1996, Coyle et al., 2002).

3.5 Summary

(1) The effects of dietary supplementation of different levels (250, 500, 750 and 1000 mg/kg) of $ZnSO_4$ have been investigated in unsexed broilers during summer.

(2) The rectal temperature (T_r) was not affected significantly by zinc level in the diet, T_r decreased with advance of age.

(3) The feed intake, body weight (BW) and feed conversion ratio (FCR) increased significantly in chicks receiving different level of $ZnSO_4$ compared to the control.

(4) The packed cell volume (PCV) increased significantly with all levels of zinc supplementation and at all stages of growth.

(5) The plasma glucose level decreased significantly in chicks with all levels of Zn supplementation and at all stages of growth.

(6) The serum cholesterol level decreased significantly in chicks with all levels of Zn supplementation and at all stages of growth, and it decreased with advance of age.

(7) The serum levels of total protein and albumin were higher with all levels of Zn supplementation and at all stages of growth, and increased with advance of age.

(8) The serum levels of ALT and AST decreased significantly in chicks with all levels of Zn supplementation and at all stages of growth.

(9) The serum Zn level increased significantly in chicks with all level of Zn supplemented at 4^{th} and 5^{th} week of age.

CHAPTER FOUR

EFFECTS OF DIETARY SUPPLEMENTATION OF ZINC SULPHATE AND ASCORBIC ACID (AA) ON PHYSIOLOGICAL RESPONSES AND PERFORMANCE OF BROILERS DURING SUMMER AND WINTER

4.1 Introduction

Tropical environment is of great concern in all types of poultry production. Feed consumption, growth rate, hatchability, mortality, and other important traits governing the prosperity of the industry are adversely affected by severe heat stress. Heat loss in broilers is limited by feathering and the absence of sweat glands (Salgueiro et al., 2000). When the temperature and relative humidity exceed the comfort level of a bird, it loses the ability to efficiently dissipate heat. This leads to physiological changes including a reduction in feed intake associated with reduced metabolic heat production (Teeter et al., 1985) and lower growth rate as well as reduction in feed efficiency (Geraert et al., 1996).

Thermal stress causes an increase in oxidative stress and an imbalance in antioxidant status (Halliwell and Gutteridge, 1989; Sahin et al., 2001). Moreover, it has been reported that environmental stress such as very high or low ambient temperature reduces serum total protein and albumin concentration (Sahin et al., 2001). Also marked changes in environmental temperature were associated with increase in blood glucose, cholesterol

concentrations and metabolite enzymes such as aspratate aminotransferase (AST) and alanine aminotransferase (ALT) (Donkoh, 1989).

The concentration of nutrients required to maintain health and productivity of the chicks is challenged due to the reduction in feed intake under tropical conditions. Studies have shown a redirection of nutrient flow to meet the metabolic requirements of immune or inflammatory responses (Bartlett and Smith 2003). There is evidence suggesting a redistribution of Zn during immunological stress. For example, plasma Zn level was greatly reduced and hepatic Zn was found to be more than four times the amount lost from plasma (Klasing, 1984). Belay and Teeter (1996) reported lower rate of Zn retention in broilers raised at high cycling ambient temperatures (24 > 40°C) compared with birds housed at 24°C. High temperatures affect availability of minerals, and BW gain is seriously compromised (Smith et al., 1995). It is therefore possible that the requirement for Zn is increased during exposure to tropical conditions. It is believed that Zn is essential in all aspects of immunity (Chandra and Dayton, 1982; Sherman, 1992) and functions through its association with the enzymes critical for the integrity of the cells involved in the immune response (Dardenne et al., 1985). There are conflicting results regarding the level of Zn required to maintain high performance of broiler (Belay and Teeter, 1996).

Variations in Zn requirement estimates are likely due to many factors, such as fibre, protein source, ascorbic acid, source of supplemental Zn used, and presence of other minerals in the diet that have been shown to influence Zn absorption (Baker and Ammerman, 1995).

A number of ways are available to alleviate the negative effects of high or low environmental temperature on the performance of poultry in terms of feed consumption, body weight gain and feed efficiency. Ascorbic acid (AA) could be used for reducing the negative effects of environmental stress because of the reported benefit of AA supplementation on poultry reared under heat or cold stress (McDowell, 1989; Kutlu and Forbes, 1993a; Sahin et al., 2001; Sahin et al., 2002b).

Poultry have an ability to synthesize ascorbic acid (AA) (Coates, 1984), but this ability is inadequate under stress conditions such as low or high environmental temperatures, humidity, high productive rate, and parasite infestation (Sykes, 1978; McDowell, 1989). Pardue and Thaxton (1986) have indicated that particular environmental stressors can alter AA use or synthesis in avian species. Previous studies reported that AA supplementation decreases blood concentrations of glucose, cholesterol, and metabolite enzymes such as AST and ALT and increases serum protein and albumin concentrations (Kutlu and Forbes, 1993a; Sandoval et al., 1998).

Broilers are faced with acute heat or cold exposures and seasonal changes in temperature characteristic of tropical environment in Sudan. Acclimatization may occur during the slow seasonal changes and the constant exposure to cyclic temperatures (Yahav et al., 1995). This experiment was designed to evaluate the effects of Zn sulphate ($ZnSO_4.7H_2O$) and vitamin C (ascorbic acid) supplementation on the physiological responses and performance of Ross broilers under tropical summer and winter conditions.

4.2 Experimental plan

A total number of 120 one-day-old Ross broiler chicks were used in this study. The experiment was carried out under summer (May- July 2006) and winter (January- February, 2006) conditions in the poultry house of the Department of Physiology. The prevailing climatic conditions during the experimental period are shown in Tables 4-1 and 4-2. The birds were fed a starter diet till 21 days of age, and a finishing diet up to the end of the experiment. The ingredients and chemical composition of the basal diets fed in summer are shown in Tables 2-1 and 2-2, respectively; while the ingredients and chemical composition of the basal diets fed in winter are shown in Tables 2-1 and 2-3, respectively.

In each season, the experiment was designed according to complete randomized design. The chicks were randomly assigned to 4 groups (A, B, C and D) of 5 replicates of 6 birds each. For each phase, the control group (A) was fed the basal diet while group B was fed the basal diet supplemented with 600 mg/kg of AA, group C was fed the basal diet supplemented with 50 mg/kg of Zn sulphate ($ZnSO_4.7H_2O$) and group D was fed the basal diet supplemented with a combination of Zn and AA supplemented as indicated for groups B and C. The added amounts of $ZnSO_4$ and AA were measured with a digital balance and mixed with the basal diet immediately before being offered to the chicks.

Table 4-1: Average weekly values of minimum, maximum and mean ambient temperature (T_a) and relative humidity (RH) during summer.

Week (%)	T_a (°C)			RH
	Min.	Max.	Mean	Mean
1	24	42	33	32
2	30	40	35	40
3	25	39	32	39
4	26	40	33	40
5	23	43	33	42
6	22	44	33	37
Mean	**25**	**41.3**	**33.2**	**38.3**

Table 4-2: Average weekly values of minimum, maximum and mean ambient temperature (T_a) and relative humidity (RH) during (January – February 2006) winter.

Weeks	T_a (°C)			RH (%)
	Min.	Max.	Mean	Mean
1	31	15	23	25
2	30	13	21.5	28
3	36	17	26.5	27
4	34	13	23.5	21
5	34	17	25.5	19
6	32	14	23	16
Mean	**32.8**	**14.8**	**23.8**	**22.7**

During the experimental period, the birds were weighed on the first day, and then weekly. The rectal temperature (T_r), feed intake and body weight (BW) were measured weekly at 8:00 a.m. Blood samples were drawn weekly at 7:00 a.m. for analysis.

4.3 Results

4.3.1 Rectal temperature (T_r)

The results of the effects of dietary Zn and AA supplementation on rectal temperature (T_r) of broilers during summer and winter are shown in Tables 4.3 and 4.4, respectively. The influence of dietary Zn and AA or their combination on T_r was not significant during summer and winter. The effect of season on T_r of control groups is shown in Table 4.5. T_r was significantly ($P<0.05$) lower during winter at all stages of growth.

4.3.2 Feed intake

The effect of dietary Zn and AA supplementation on feed intake during summer and winter is shown in Tables 4.6 and 4.7, respectively. The effects of Zn and AA supplementation or their combination on feed intake were not significant throughout the experimental period during summer and winter. The effect of season on feed intake of control groups is shown in Table 4.8. The mean feed intake of chicks was significantly ($P<0.05$) higher during winter at all stages of growth.

4.3.3 Body weight (BW)

The effect of dietary Zn and AA supplementation on BW during summer and winter is shown in Tables 4.9 and 4.10, respectively. The effect of Zn and AA or their combination on BW was not significant throughout the

Table 4-3. Effect of dietary Zn (ZnSO4.7H2O) and AA supplementation or their combination on rectal temperature (ºC) in Ross broilers during summer.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}41.74±0.26	^{A}40.97±0.52	^{A}41.78±0.52	^{A}41.03±0.54	^{A}41.82±0.46	^{A}41.51±0.31
B	^{A}41.48±0.34	^{A}40.86±0.37	^{A}41.96±0.22	^{A}41.06±0.49	^{A}41.67±0.55	^{A}41.27±0.41
C	^{A}41.60±0.52	^{B}40.90±0.50	^{A}41.98±0.32	^{A}41.13±0.38	^{A}41.46±0.46	^{A}41.46±0.25
D	^{A}41.78±0.43	^{A}41.35±0.40	^{A}41.88±0.49	^{A}41.22±0.39	^{A}41.42±0.53	^{A}41.53±0.32
LS	NS	NS	NS	NS	NS	NS

^{A}: Mean values within the same column with similar superscripts are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-4. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on rectal temperature (ºC) in Ross broilers during winter.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}40.62±0.16	^{A}40.92±0.17	^{A}40.97±0.19	^{A}40.20±0.17	^{A}40.06±0.19	^{A}40.05±0.22	
B	^{A}41.22±0.21	^{A}40.86±0.37	^{A}40.01±0.35	^{A}40.06±0.54	^{A}40.21±0.33	^{A}40.01±0.20	
C	^{A}41.00±0.15	^{A}40.17±0.37	^{A}41.16±0.11	^{A}40.98±0.31	^{A}40.07±0.16	^{A}40.25±0.17	
D	^{A}40.99±0.17	^{A}40.06±0.17	^{A}41.02±0.14	^{A}41.11±0.29	^{A}40.19±0.15	^{A}40.55±0.30	
LS	NS	NS	NS	NS	NS	NS	

^{A}: Mean values within the same column with similar superscripts are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-5.Effect of season on rectal temperature, T_r (ºC) of control groups of Ross broilers. (n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
Summer	^{A}41.74±0.26	^{A}40.97±0.52	^{A}41.78±0.52	^{A}41.03±0.54	^{A}41.82±0.46	^{A}41.51±0.31
Winter	^{B}40.62±0.16	^{B}40.92±0.17	^{B}40.97±0.19	^{B}40.20±0.17	^{B}40.06±0.19	^{B}40.05±0.22
LS	*	*	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-6. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on mean feed intake (gm/bird) in Ross broilers during summer.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}90.5±11.66	^{A}150.8±18.94	^{A}281.2±78.08	^{A}401.4±36.54	^{A}580.9±99.8	^{A}542.2±66.07
B	^{A}105.2±17.56	^{A}194.6±20.93	^{A}306.4±50.19	^{A}422.7±71.11	^{A}601.4±77.3	^{A}665.9±88.67
C	^{A}98.4±18.79	^{A}163.0±45.08	^{A}299.3±64.67	^{A}479.2±85.77	^{A}621.4±98.2	^{A}715.7±78.78
D	^{A}108.5±15.56	^{A}186.1±25.42	^{A}307.5±42.57	^{A}396.1±66.38	^{A}651.5±87.7	^{A}725.0±59.17
LS	NS	NS	NS	NS	NS	NS

^{A} : Mean values within the same column with similar superscripts are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-7. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on mean feed intake (gm/bird) in Ross broilers during winter.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}111.5±10.52	^{A}254.5±20.44	^{A}398.7±68.85	^{A}551.6±26.34	^{A}650.5±89.88	^{A}655.5±59.29
B	^{A}125.2±15.36	^{A}291.3±22.55	^{A}450.6±74.42	^{A}601.5±82.11	^{A}758.4±85.65	^{A}700.5±75.85
C	^{A}124.4±19.70	^{A}245.0±25.05	^{A}401.5±77.55	^{A}615.2±95.44	^{A}771.5±78.89	^{A}720.9±90.85
D	^{A}118.5±14.50	^{A}266.1±35.42	^{A}446.8±45.89	^{A}610.8±78.77	^{A}765.8±69.90	^{A}719.8±87.58
LS	NS	NS	NS	NS	NS	NS

^{A} : Mean values within the same column with similar superscripts are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-8.Effect of season on feed intake (gm/bird) of control groups of Ross broiler chicks. (n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
Summer	^{A}90.5±11.66	^{A}150.8±18.94	^{A}281.2±78.08	^{A}401.4±36.54	^{A}580.9±99.8	^{A}542.2±66.07
Winter	^{B}111.5±10.52	^{B}254.5±20.44	^{B}398.7±68.85	^{B}551.6±26.34	^{B}650.5±89.88	^{B}655.5±59.29
LS	*	*	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-9. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on mean body weight (gm) in Ross broilers during summer.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}80.0±6.89	^{A}205.6±24.21	^{A}317.4±37.08	^{A}692±34.99	^{A}939.2±63.55	^{A}1170±75.37
B	^{A}91.8±8.89	^{A}288.6±15.19	^{A}391.8±15.77	^{A}718±22.11	^{A}991.2±42.75	^{A}1295±96.41
C	^{A}90.2±2.77	^{A}310.4±2.70	^{A}413.8±13.18	^{A}764±40.37	^{A}1005.2±52.15	^{A}1248±63.64
D	^{A}93.2±6.69	^{A}321.8±21.51	^{A}418±33.34	^{A}712±76.29	^{A}987.2±95.18	^{A}1200±88.44
LS	NS	NS	NS	NS	NS	NS

^{A}: Mean values within the same column with similar superscript are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-10. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on mean body weight (gm) in Ross broilers during winter.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}115±10.84	^{A}321±43.19	^{A}583±90.10	^{A}973±94.43	^{A}1366±83.97	^{A}1799±084.66
B	^{A}119±05.50	^{A}326±32.18	^{A}599±50.40	^{A}999±59.18	^{A}1388±122.2	^{A}1858±124.80
C	^{A}116±13.50	^{A}321±40.43	^{A}591±68.31	^{A}988±72.45	^{A}1390±81.89	^{A}1899±105.12
D	^{A}117±07.90	^{A}321±31.70	^{A}588±50.78	^{A}974±85.78	^{A}1379±39.32	^{A}1895±73.68
LS	NS	NS	NS	NS	NS	NS

^{A}: Mean values within the same column with similar superscript are significantly different ($P<0.05$).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-11. Effect of season on mean body weight, BW (gm) of control groups of Ross broilers. (n = 10; mean±SD).

	Age (weeks)					
Season	1	2	3	4	5	6
Summer	^{A}80.0±6.89	^{A}205.6±24.21	^{A}317.4±37.08	^{A}692±34.99	^{A}939.2±63.55	^{A}1170±75.37
Winter	^{B}115±10.84	^{B}321±43.19	^{B}583±90.10	^{B}973±94.43	^{B}1366±83.97	^{B}1799±84.66
LS	*	*	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

experimental period during summer and winter. The effect of season on BW of control groups is shown in Table 4.11. The mean BW of chicks was significantly ($P<0.05$) higher during winter at all stages of growth.

4.3.4 Feed conversion ratio (FCR)

The effect of dietary Zn and AA on FCR during summer and winter is shown in Tables 4.12 and 4.13, respectively. The overall effect of dietary Zn and AA supplementation or their combination on FCR was not significant throughout the experimental period during summer and winter. The effect of season on FCR of control groups is shown in Table 4.14. The FCR was significantly ($P<0.05$) higher during winter at all stages of growth.

4.3.5 Packed cell volume (PCV)

Tables 4.15 and 4.16 show the effects of supplementation of Zn and AA on PCV of broilers during summer and winter, respectively. Dietary Zn and AA or their combination had no significant effect on PCV. The effect of season on PCV of control groups is shown in Table 4.17. The PCV was significantly ($P<0.05$) lower in chicks during winter at all stages of growth.

4.3.6 Plasma glucose

Table 4.18 shows that during summer, the plasma glucose level was significantly ($P<0.01$) lower in all treated groups of chicks (B, C and D) at the 4^{th} and 5^{th} week of age. Table 4.19 shows that during winter, the plasma glucose level decreased significantly ($P<0.01$) in all treated experimental groups at the 4^{th} week of age. On the 5^{th} and 6^{th} weeks of age, the plasma glucose level was significantly ($P<0.05$) lower for the chicks supplemented with combination of AA and Zn (group D).

Table 4-12. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on feed conversion ratio (%) in Ross broilers during summer.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}1.69±0.27	^{A}2.49±0.29	^{A}2.82±0.62	^{A}3.00±2.04	^{A}3.56±0.45	^{A}3.45±1.98
B	^{A}2.10±0.25	^{A}2.99±0.18	^{A}3.42±0.32	^{A}3.70±0.52	^{A}3.98±0.64	^{A}4.01±1.35
C	^{A}2.50±0.12	^{A}2.89±0.50	^{A}3.04±0.41	^{A}3.66±0.58	^{A}4.01±0.59	^{A}4.21±1.25
D	^{A}2.19±0.15	^{A}2.95±0.30	^{A}3.01±0.39	^{A}3.59±0.58	^{A}3.88±0.78	^{A}4.06±1.51
LS	NS	NS	NS	NS	NS	NS

^{A} : Mean values within the same column with similar superscript are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-13. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on feed conversion ratio (%) in Ross broilers during winter.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

	Age (weeks)					
Group	1	2	3	4	5	6
A	^{A}1.50±0.15	^{A}1.60±0.18	^{A}1.80±0.60	^{A}1.90±1.21	^{A}1.85±1.54	^{A}2.02±1.01
B	^{A}1.62±0.28	^{A}1.70±0.22	^{A}1.90±0.22	^{A}2.03±0.50	^{A}1.98±0.84	^{A}2.15±1.15
C	^{A}1.68±0.20	^{A}2.00±0.36	^{A}2.01±0.34	^{A}2.10±1.05	^{A}2.05±1.39	^{A}2.31±1.60
D	^{A}1.69±0.21	^{A}1.78±0.30	^{A}2.03±0.40	^{A}2.15±1.08	^{A}2.20±1.30	^{A}2.46±0.91
LS	NS	NS	NS	NS	NS	NS

^{A} : Mean values within the same column with similar superscript are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-14. Effect of season on feed conversion ratio, FCR (%) of control groups of Ross broilers. (n = 10; mean±SD).

	Age (weeks)					
Season	1	2	3	4	5	6
Summer	^{B}1.69±0.27	^{B}2.49±0.29	^{B}2.82±0.62	^{B}3.00±2.04	^{B}3.56±0.45	^{B}3.45±1.98
Winter	^{A}1.50±0.15	^{A}1.60±0.18	^{A}1.80±0.60	^{A}1.90±1.21	^{A}1.85±1.54	^{A}2.02±1.01
LS	*	*	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-15. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on packed cell volume (%) in Ross broilers during summer.

A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (weeks) 2	4	5	6
A	^{A}26.4±3.53	^{A}26.9±0.47	^{A}26.6±0.52	^{A}26.1±2.42
B	^{A}27.3±3.83	^{A}27.4±0.46	^{A}27.7±1.25	^{A}28.2±2.94
C	^{A}27.1±1.45	^{A}26.8±0.44	^{A}27.0±1.94	^{A}27.7±2.91
D	^{A}27.3±1.89	^{A}27.4±0.36	^{A}26.8±1.39	^{A}28.7±2.79
LS	NS	NS	NS	NS

^{A}: Mean values within the same column with similar superscript are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-16. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on packed cell volume (%) in Ross broilers during winter.

A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

	Age (weeks)			
Group	2	4	5	6
A	^{A}28.9±2.32	^{A}30.2±2.44	^{A}30.1±2.11	^{A}30.3±2.33
B	^{A}31.4±4.61	^{A}31.6±5.22	^{A}31.7±1.82	^{A}31.2±3.54
C	^{A}32.9±3.22	^{A}31.4±2.51	^{A}32.6±3.21	^{A}31.7±3.21
D	^{A}30.9±4.51	^{A}31.4±0.64	^{A}32.5±2.47	^{A}31.7±2.47
LS	NS	NS	NS	NS

^{A} : Mean values within the same column with similar superscript are not significantly different.

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-17. Effect of season on packed cell volume, PCV (%) of control groups of Ross broilers. (n = 10; mean±SD).

	Age (weeks)			
Season	2	4	5	6
Summer	^{A}26.4±3.53	^{A}26.9±0.47	^{A}26.6±0.52	^{A}26.1±2.42
Winter	^{B}28.9±2.32	^{B}30.2±2.44	^{B}30.1±2.11	^{B}30.3±2.33
LS	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-18. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on plasma glucose level (mg/dL) in Ross broilers during summer.

A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

	Age (weeks)			
Group	2	4	5	6
A	^{A}188.7±38.05	^{A}162.4±15.06	^{A}156.5±20.71	^{A}158.9±16.41
B	^{A}173.0±28.92	^{B}135.9±23.56	^{AB}145.5±12.62	^{A}151.4±21.69
C	^{A}179.2±23.25	^{B}130.8±11.93	^{B}135.6±14.38	^{A}145.6±19.59
D	^{A}173.9±26.32	^{B}133.6±21.96	^{B}133.4±15.09	^{A}142.1±16.49
LS	NS	**	**	NS

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (**P<0.01).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-19. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on plasma glucose level (mg/dL) in Ross broilers during winter.

A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

Group	Age (weeks)			
	2	4	5	6
A	^{A}190.8±29.81	^{A}189.9±22.32	^{A}183.6±13.54	^{A}180.1±14.80
B	^{A}188.5±31.21	^{B}178.0±10.42	^{A}170.2±14.43	^{A}170.6±15.56
C	^{A}187.7±23.18	^{B}173.2±21.45	^{A}171.9±21.93	^{A}169.3±15.20
D	^{A}180.6±26.74	^{C}169.9±10.91	^{B}162.7±22.25	^{B}164.7±11.54
LS	NS	**	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05, **P<0.01).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

4.3.7 Serum cholesterol

Table 4.20 shows the effect of dietary Zn and AA supplementation on serum cholesterol level during summer. At the 5th week of age the cholesterol level was significantly ($P<0.05$) lower in all treated groups. Table 4.21 shows the effect of dietary Zn and AA supplementation on serum cholesterol level during winter. At the 4th week of age, the serum cholesterol level was significantly ($P<0.05$) lower in all treated groups. At the 6th week of age, the serum cholesterol level was significantly ($P<0.01$) lower in all treated groups.

4.3.8 Serum total protein

The effect of dietary Zn and AA supplementation on serum total protein level during summer and winter conditions are shown in Tables 4.22 and 4.23, respectively. During summer, the serum total protein level was significantly ($P<0.05$) higher in all treated groups at the 2nd, 4th, 5th and 6th week of age. The effect of dietary Zn and AA supplementation on the serum total protein level under winter conditions is shown in Table 4.23. On the 2nd, 4th and 6th weeks of age the serum total protein level was significantly ($P<0.05$) higher in all treated groups.

4.3.9 Serum albumin

The effect of dietary Zn and AA supplementation on the serum albumin level during summer and winter is shown in Tables 4.24 and 4.25, respectively. During summer, serum albumin level was significantly ($P<0.05$) higher in all treated groups at the 2nd, 4th, 5th and 6th week of age. The effect

Table 4-20. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum cholesterol level (mmoL/L) in Ross broilers during summer.

A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

Group	Age (week) 2	4	5	6
A	^{A}3.67±0.98	^{A}3.40±0.47	^{A}3.62±0.52	^{A}3.58±0.47
B	^{A}3.74±0.56	^{A}3.27±0.46	^{B}3.07±0.49	^{A}3.33±0.46
C	^{A}3.53±0.34	^{A}3.50±0.44	^{B}2.86±0.27	^{A}3.24±0.62
D	^{A}3.83±0.33	^{A}3.59±0.36	^{B}2.87±0.39	^{A}3.38±0.56
LS	NS	NS	*	NS

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-21. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum cholesterol level (mmoL/L) in Ross broilers during winter. A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (weeks) 2	4	5	6
A	^{A}3.94±0.58	^{A}3.88±0.52	^{A}3.45±0.17	^{C}3.94±0.19
B	^{A}3.52±0.78	^{B}3.01±0.49	^{A}3.37±0.32	^{AB}3.53±0.18
C	^{A}3.34±0.61	^{B}3.21±0.22	^{A}3.34±0.39	^{A}3.44±0.19
D	^{A}3.32±0.39	^{B}3.11±0.52	^{A}2.99±0.21	^{B}3.00±0.25
LS	NS	*	NS	**

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05, **P<0.01).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-22. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum total protein level (g/dL) in Ross broilers during summer.
A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week) 2	4	5	6
A	^{C}3.81±0.27	^{B}3.49±0.34	^{B}3.56±0.37	^{B}3.42±0.26
B	^{AB}4.24±0.39	^{A}4.22±0.30	^{A}3.97±0.31	^{A}4.02±0.54
C	^{B}4.00±0.41	^{A}3.98±0.56	^{A}3.85±0.25	^{A}3.97±0.59
D	^{A}4.35±0.39	^{A}3.94±0.42	^{A}3.97±0.35	^{A}4.19±0.61
LS	*	*	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-23. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum total protein level (g/dL) in Ross broilers during winter.
A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week) 2	4	5	6
A	^{B}3.31±0.22	^{B}3.23±0.37	^{A}3.41±0.45	^{B}3.55±0.28
B	^{A}3.82±0.18	^{A}3.89±0.33	^{A}3.85±0.73	^{A}3.99±0.34
C	^{A}4.21±1.29	^{A}4.12±0.38	^{A}3.50±0.24	^{A}3.88±0.59
D	^{A}3.92±0.30	^{A}3.79±0.83	^{A}4.12±0.24	^{A}4.00±0.61
LS	*	*	NS	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-24. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum albumin level (g/dL) in Ross broilers during summer.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).

(n = 10; mean±SD).

	Age (week)			
Group	2	4	5	6
A	^{B}2.00±0.24	^{B}1.80±0.19	^{B}1.96±0.16	^{B}1.95±0.31
B	^{A}2.34±0.56	^{A}2.08±0.37	^{A}2.16±0.39	^{A}2.35±0.35
C	^{A}2.27±0.45	^{A}2.13±0.39	^{A}2.30±0.35	^{A}2.26±0.35
D	^{A}2.44±0.33	^{A}2.07±0.33	^{A}2.23±0.35	^{A}2.22±0.22
LS	*	*	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-25. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum albumin level (g/dL) in Ross broilers during winter.

A: Control group , B: AA 600 mg/kg, C: Zn 50 mg/kg,
D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week) 2	4	5	6
A	^{A}1.78±0.24	^{A}1.77±0.14	^{A}1.78±0.23	^{A}1.79±0.22
B	^{B}2.00±0.26	^{B}1.96±0.23	^{B}1.88±0.28	^{B}1.98±0.25
C	^{B}2.02±0.13	^{B}1.98±0.22	^{B}1.98±0.36	^{B}2.11±0.55
D	^{B}1.94±0.23	^{B}1.91±0.25	^{B}1.90±0.40	^{B}2.21±0.42
LS	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Of dietary Zn and AA supplementation on the serum albumin level under winter is shown in Table 4.25. The albumin level was significantly ($P<0.05$) higher in all treated groups at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} week of age.

4.3.10 Serum aspartate aminotransferase (AST)

The effect of dietary Zn and AA supplementation on the serum AST level during summer and winter is shown in Tables 4.26 and 4.27, respectively. Table 4.26 shows that during summer, AST level was significantly ($P<0.05$) lower in all treated groups at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} weeks of age. Table 4.27 shows that serum AST level was significantly ($P<0.05$) lower in all treated groups at the 2^{nd}, 4^{th} and 6^{th} weeks of age.

4.3.11 Serum alanine aminotransferase (ALT)

Tables 4.28 and 4.29 show the effect of dietary supplementation of Zn, AA on serum ALT of chicks raised under summer and winter conditions, respectively. During summer, at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} week of age, the ALT level was significantly ($P<0.05$) lower in all treated groups. During winter, ALT level was significantly ($P<0.05$) lower in all treated groups at the 2^{nd}, 4^{th}, 5^{th} and 6^{th} weeks of age.

4.4 Discussion

This study investigated the effects of dietary inclusion of Zn and AA or their combination on the physiological responses and the performance of Ross broilers during summer and winter conditions.

The thermoregulatory responses indicated that during natural summer or winter conditions, the rectal temperature (T_r) (Tables 4.3, and 4.4, respectively) tended to be lower with dietary inclusion of Zn and AA or their

Table 4-26. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum aspartate aminotransferase level (U/L) in Ross broilers during summer. A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA). **(n = 10; mean±SD)**.

Group	Age (week) 2	4	5	6
A	^{A}81.7±09.88	^{A}88.5±06.13	^{A}90.6±9.02	^{A}91.4±11.25
B	^{B}76.2±11.75	^{B}77.1±17.25	^{A}78.1±10.39	^{B}87.3±10.12
C	^{B}74.0±10.90	^{B}79.5±15.22	^{A}80.3±09.69	^{B}83.0±16.79
D	^{B}62.9±09.49	^{B}78.8±18.34	^{A}84.2±10.71	^{C}80.2±04.57
LS	*	*	NS	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.
NS: Not significant.

Table 4-27. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum aspartate aminotransferase level (U/L) in Ross broilers during winter. A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week)			
	2	4	5	6
A	^{A}90.9±16.2	^{A}80.1±11.23	^{A}94.9±16.26	^{A}89.4±10.12
B	^{B}76.9±12.4	^{B}73.6±19.91	^{B}76.9±12.46	^{B}79.3±12.02
C	^{B}77.2±9.04	^{B}75.4±12.12	^{B}80.2±09.04	^{B}81.0±16.79
D	^{B}80.3±17.9	^{B}77.4±13.84	^{B}82.3±17.97	^{C}83.2±11.57
LS	*	*	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-28. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum alanine aminotransferase level (U/L) in Ross broilers during summer. A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week) 2	4	5	6
A	^{A}8.82±1.64	^{A}9.71±2.11	^{A}9.62±1.17	^{A}8.94±1.26
B	^{B}7.74±1.95	^{B}7.93±2.42	^{B}7.52±0.53	^{B}7.24±1.03
C	^{B}6.93±2.56	^{B}7.84±1.48	^{B}8.02±0.82	^{B}7.72±0.94
D	^{B}7.43±1.43	^{B}7.08±1.63	^{B}7.71±0.67	^{B}7.83±1.62
LS	*	*	*	*

^{A,B,C} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

Table 4-29. Effect of dietary Zn ($ZnSO_4.7H_2O$) and AA supplementation or their combination on serum alanine aminotransferase level (U/L) in Ross broilers during winter. A: Control group, B: AA 600 mg/kg, C: Zn 50 mg/kg, D: Combination (Zn and AA).
(n = 10; mean±SD).

Group	Age (week)			
	2	4	5	6
A	^{A}8.84±1.22	^{A}9.75±2.31	^{A}9.86±1.23	^{A}9.94±2.36
B	^{B}7.18±1.37	^{B}8.15±2.89	^{B}7.19±1.34	^{B}7.78±1.34
C	^{B}7.74±0.68	^{B}7.94±1.29	^{B}7.76±0.68	^{B}7.89±1.24
D	^{B}8.17±1.40	^{B}6.91±2.13	^{B}7.44±1.43	^{B}7.63±2.42
LS	*	*	*	*

^{A,B} : Mean values within the same column with different superscripts are significantly different (*P<0.05).

SD: Standard deviation.
LS: Level of significance.

combination, but this influence was not significant. The response might be related to adaptation of the chicks to the prevailing thermal environment.

The higher T_r values of chicks measured during summer compared to winter (Table 4.5) is related to increase of ambient temperature (T_a) and relative humidity (RH) as shown in Tables 4.1 and 4.2, respectively. Lin et al. (2005) indicated that the elevation of rectal temperature at high ambient temperature was aggravated by humidity, indicating increased heat load with humidity compared with low temperature (Chwalibog and Eggum, 1989).

The feed intake, BW and FCR (Tables 4.6, 4.7, 4.9, 4.10, 4.12, 4.13, respectively) of broilers raised during summer and winter were not significantly influenced by Zn supplementation when considering the overall effect. In the present study Zn levels were apparently low to affect feed intake or weight gain. The lack of response to Zn supplementation is similar to the finding of Swinkels et al. (1994) who showed that diets low in Zn lead to depressed appetite resulting in lowered feed intake and reduced weight gain. Hess et al. (2001) reported that supplementing a corn-soy diet having 100 mg/kg of Zn from zinc-sulphate led to improved BW and FCR from 0 to 21 day of age; but these differences in growth were not apparent by 6 weeks of age.

The feed intake, BW and FCR of broilers raised under summer and winter were not significantly influenced by AA supplementation. The overall lack of response to AA supplementation in this study might be related to the ability of chicks to synthesize AA. The lack of response to AA supplementation is similar to the finding of Faruga (1975) who reported that

supplemental AA had no effect on the growth rate and development of broilers under heat stress (35°C). Nakaya et al. (1986) also reported that AA supplementation had no appreciable effect on food intake, body weight gain and efficiency of food conversion in broilers and White Leghorn chicks. However, McKee et al. (1997) reported increased gain in live weight and meat protein and a decrease in carcass fat in broilers given supplemental AA. Njoku (1986) showed that exogenous supplementation of 200 mg AA/kg food under tropical summer conditions produced a positive response in growth rate and feed utilization.

The pattern of response of chicks to AA was not consistent as regards feed intake, BW and feed efficiency. This might be ascribed to the progress in the innate ability of the chicks to synthesize ascorbic acid with age. An increase in the rate of synthesis of ascorbic acid with age has been reported (Horing and Frigg, 1979). Also the concentration of AA was shown to increase with age in heart muscle and spleen (Dorr and Nockels, 1971). Horing and Frigg (1979) suggested that exogenous AA is not critically needed in the late stages of growth of broilers, but it could be supplemented in the early stages because of the low biosynthetic ability.

The increase of feed intake, BW and decrease of FCR (Tables 4.8, 4.11 and 4.14, respectively) of control groups during winter compared to respective summer values may be related to favourable thermal environment during winter in Sudan for chicks, the mean ambient temperature was markedly lower in winter compared to summer value. Similarly the findings

of Gonzalez-Esquerra and Leeson (2005) indicated that broilers kept at 27°C exhibited higher performance than those at 32°C.

The lower feed consumption and BW observed in birds subjected to tropical summer environment agrees with previous reports (Leeson, 1986; Teeter and Belay, 1996; Yahav, 2000). The reduced productivity of these birds was closely associated with the severity of heat stress and duration of exposure (Tables 4.6, 4.9 and 4.13). Thus, in the present study, chicks kept during summer at mean ambient temperature 33.5°C exhibited lower performance compared to those under winter at mean ambient temperature 24°C, and the performance of broilers was more severely affected relative to controls in late, rather than early growth. However, it is likely that at some point birds adapt to tropical environment, which would curtail loss in performance (Teeter and Belay, 1996). Thus, the adaptation mechanisms in place would not allow birds stressed under summer conditions to perform similarly to controls under winter conditions, but would curtail further loss in performance. These findings support the results of Shannon and Brown (1969) who measured a decline in basal metabolic rate of the domestic fowl in response to an ambient temperature of 28°C, where stasis developed after just 3 days.

The PCV (Tables 4.15 and 4.16) was not affected by dietary Zn and AA supplementation or their combination. It could be speculated that in the present study, Zn was not supplemented at levels which may interfere with erythropoiesis and affect the PCV. However, Zn and AA were found to

increase absorption of iron which is an important integral part of haemoglobin (Hungerford et al., 1983).

The data summarized in Table 4.17 indicate that Ross broilers maintained higher PCV values during winter. This finding could be associated with higher feed intake in winter; also it could be related to relative haemoconcentration in winter compared to summer. Previous studies have found an increase in plasma volume of birds in hot environment (Burton and Smith, 1967; Jaeger and McGrath, 1974; Arieli et al., 1979; Hillman et al., 1985). The higher amounts of AA intake during winter associated with higher food intake could also be implicated in enhanced erythropoiesis during winter.

In the present study, the plasma glucose level (Tables 4.18 and 4.19) decreased significantly during summer and winter with Zn and AA supplementation. These results are consistent with the finding of McDowell (1989) who reported that there was a trend towards a hypoglycaemic state in AA-supplemented chicks. Previous studies indicated that glucocorticoids increase the concentration of plasma glucose in heat acclimated broiler and laying hens (Braganza et al., 1973). Ascorbic acid was shown to reduce glucocorticoid synthesis (McDowell, 1989). The increase in glucose concentration may be attributed to increased glucocorticoid secretion, which increases gluconeogenesis (McDowell, 1989). Dietary Zn and ascorbic acid may reverse these changes, presumably by reducing the secretion and/or synthesis of glucocorticoids. Similar to the current result, Sahin et al. (2005) reported that plasma glucose level was increased in broiler when both dietary

Zn and AA. Also, Kutlu and Forbes (1993a) reported that AA supplementation markedly decreased plasma glucose concentrations in heat-stressed broilers.

In the present study, the decrease in serum cholesterol level (Tables 4.20 and 4.21) during summer and winter associated with Zn may be related to decreased lipid peroxidation. The reduced lipid peroxidation in Zn supplemented birds might be due to multifunctional roles of Zn, which include the induction of metallothionein, modulation of the transition elements and its relationship with the antioxidant vitamins such as vitamin A and E (Halliwell and Gutteridge, 1989; Salgueri et al., 2000; Sahin et al., 2002). Furthermore, Zn is a cofactor of the main antioxidant enzyme Cu Zn-superoxide dismutase; it may play a key role in suppressing free radicals and inhibiting NADPH-dependent lipid peroxidation (Burke and Fenton, 1985) as well as preventing lipid peroxidation via inhibition of glutathione depletion (Sahin et al., 2002).

The decrease in serum cholesterol level (Tables 4.20 and 4.21, respectively) during summer and winter associated with AA supplementation may be related to decreased serum malondialdehyde. AA supplementation decreased serum and liver malondialdehyde levels in stressed birds (Shaheen and Abd El-Fattah, 1995; Sahin and Kucuk, 2003a).

The results indicate that Zn supplementation during summer and winter increased serum total protein (Tables 4.22 and 4.23) and serum albumin level (Tables 4.24 and 4.25). This increase could be attributed to the increase in feed intake in Zn fed chicks associated with increase in age of

birds. These findings are similar to the results reported by Belay and Teeter (1996). Furthermore, the increase in serum total protein and albumin levels associated with Zn observed in this study could be due to the improvement in protein absorption considering the weight gain among treated groups. Dietary inclusion of Zn was found to increase the concentration of protein as well as the activity of amylase and chymotrypsin in pancreatic homogenates (Sahin and Kucuk, 2003b). It has been postulated that the improved performance of poultry results from a decrease in protein-derived gluconeogenesis (McDowell, 1989). Furthermore, it has been reported that serum total protein and albumin concentrations increased when dietary Zn was supplemented (Sahin et al., 2002).

In both seasons, AA supplementation increased serum total protein (Tables 4.22 and 4.23) and serum albumin level (Tables 4.24 and 4.25). This increase could be attributed to the increase in feed intake in AA fed chicks associated with increase in age of birds. Similarly, it has been reported that serum total protein and albumin concentrations increased when dietary AA was supplemented (Cao et al., 2000).

Zn supplementation during summer and winter was associated with lower values of the serum enzymes ALT (Tables 3.26 and 3.27) and AST (Tables 3.28 and 3.29). Although the values of ALT were different for the experimental diets, they were all within the normal range value in chicks (Ker et al., 1982). The mechanism by which Zn exerts its influence on ALT and AST is not well defined (Rosenthal, 1977). Lower ALT and AST correlate with better health in birds. Zn was shown to reduce both of these

enzymes to the range of normal level, which represent the non-pathological metabolism of the liver and heart (Bogin and Israeli, 1976).

The current results indicate that AA supplementation during summer and winter was associated with lower values of the serum enzymes ALT (Tables 3.29 and 3.30) and AST (Tables 3.31 and 3.32). The mechanism by which AA exerts its influence on ALT and AST is not well defined (Makinde and Fatunmbi, 1985). Plasma ALT activity has been reported to be low in all tissues of chicks (Bogin and Israeli, 1976), but ALT activities often increase due to damage in many tissues of chicks (Zantop, 1997).

The results showed similar trends in the effects of Zn and AA for most parameters measured. The similar responses to Zn and AA could be attributed to similarity between the role of Zn and AA as antistress agents, indicating a possible additive effect of the two supplements.

4.5 Summary

(1) The effects of dietary supplementation of Zn, AA or their combination on physiological responses and performance were investigated in unsexed Ross broilers during summer and winter.

(2) The rectal temperature (T_r) was not affected significantly by dietary Zn and AA supplementation or their combination during summer and winter conditions. T_r of control groups of broilers was significantly higher during summer compared to winter values at all stages of growth.

(3) The effects of Zn and AA or their combination on feed intake, BW and FCR were not significant throughout the experimental period. The mean

values of feed intake and BW were significantly higher during winter at all stages of growth.

(4) The effects of Zn and AA or their combination on FCR was not significant throughout the experimental period. The mean value of FCR was significantly lower during winter at all stages of growth.

(5) The PCV was not affected significantly by Zn and AA or their combination during the course of experiment. The PCV of control groups was significantly higher during winter at all stages of growth.

(6) There was significant decrease in plasma glucose level with all treatments at the 4^{th} and 5^{th} weeks of age during summer, and significant decrease in plasma glucose level in all treated experimental groups at the 4^{th} week of age during winter. At the 5^{th} and 6^{th} weeks of age, the plasma glucose level was significantly lower for broilers supplemented with combination of AA and Zn during winter.

(7) The serum cholesterol level decreased significantly with all treatments at the 5^{th} week of age during summer. Also it decreased significantly at the 4^{th} and 6^{th} weeks of age with all treatments during winter.

(8) The serum total protein level increased significantly with all treatments at all stages of growth during summer. Also it increased significantly in all treated groups at the 2^{nd}, 4^{th} and 6^{th} weeks of age during winter.

(9) The serum albumin level increased significantly for chicks supplemented with AA at the 6^{th} week of age during summer, and it increased significantly with all treatments at the 4^{th}, 5^{th} and 6^{th} week of age during winter.

(10) The serum AST level decreased significantly with all treatments at all stages of growth during summer. Also it decreased significantly with all treatments at the 2^{nd}, 4^{th} and 6^{th} weeks of age during winter.

(11) The serum ALT level decreased significantly with all treatments at all stages of growth in both seasons.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

The changes in environmental temperature above the thermal comfort zone have a negative effect on bird performance. Broiler chick is a homeotherm that can live comfortably only in a relatively narrow zone of thermoneutrality (Halliwell and Gutteridge, 1989). High temperatures act in a negative way, thus adversely influencing the performance of broiler chickens. However, Manning and Wyatt (1990) proved that broiler chicks adapted more easily to lower than to higher temperature. The optimal temperature range for efficient production for broiler chicks over 4 weeks of age is 18 - 21˚C (Aengwanich and Simaraks, 2004). Shinder et al. (2002) reported that short-term cold conditioning of chicks at an early age could induce an improvement either in thermotolerance during cold challenge or in performance of chicks exposed to an optimal environmental temperature.

Dietary modifications are among the most preferable and practical methods to alleviate the effect of thermal load on poultry performance. Organic Zn sources such as Zn-methionine and Zn-propionate or inorganic Zn sources such as ZnO or $ZnSO_4$. H_2O were used to alleviate heat stress in broilers (Spears, 1989; Wedekind et al., 1992). Chicks exposed to stress cannot synthesize sufficient amount of ascorbic acid (AA) to cope with their physiological needs. The endogenous synthetic capacity of AA can be exceeded by metabolic demand during heat exposure. This may result in

lowered productivity coupled with increased mortality (Sahin and Kucuk, 2003a). The information delivered previously indicates that certain environmental and nutritional factors may influence Zn absorption and cause Zn deficiency in birds. The main objective of the experiments performed in this thesis was to improve our understanding of the effects of Zn and AA supplementation on the physiological responses and performance of broilers. The results presented in Chapter 3 indicate that Zn supplemented to broiler could be at least 250 gm/kg diet, and the results reported in Chapter 4 suggest that the optimal AA supplemented to broiler may be 600 gm/kg diet under tropical summer conditions.

The progressive increase in the level of Zn was associated with changes in feed intake, BW, plasma glucose, serum cholesterol, serum total protein and serum enzymes (ALT, AST). Seasonal changes in thermal environment were associated with marked changes in thermoregulation. The results also indicate that the changes in certain physiological responses were related to the age of broilers.

Chicks, like all homeothermic animals maintain a constant body temperature over wide range of environmental temperatures. The ability of the chicks to maintain homeothermy within normal range depends on a balance between the metabolic heat production and the rate of heat dissipation (Richards, 1971). The amount of internally produced heat depends on BW, feed intake and rate of heat dissipation which depend on the thermal environment. Accordingly the diurnal and seasonal changes in thermal load may influence the physiological and performance responses of

birds (Burke and Fenton, 1985). Under tropical summer conditions, the increase in the excretion rate of Zn could decrease digestibility in poultry (Wallis and Balnave, 1984). Previous studies showed that high thermal load was associated with a decrease in the serum Zn level in broiler (Klotz et al., 2003; Maret, 2003).

The results reported in Chapter 4 showed that the body temperature of chicks was influenced by seasonal changes in ambient temperature, with higher values of rectal temperature (T_r) measured during summer. The decrease in T_r in winter (Table 4.7) could be related to enhancement of heat loss due to the increase in thermal gradient during winter. The results also showed an increase in BW, feed intake and FCR during winter.

The physiological responses in Chapter 4 as regards changes in feed intake (Table 4.29) and body weight (Table 4.30) could be attributed to the effects of thermal load on the activity of the hypothalamic feeding and satiety centres. When the physiological and behavioural responses to high environmental temperature are inadequate, an elevation in body temperature occurs, causing a decrease in appetite, growth rate and productivity of birds (Ain Baziz et al., 1996; Geraert et al., 1996). As the thyroid hormones play a major role in the chemical regulation of homeothermy, the thyroid function is decreased under conditions of heat exposure in birds (Bowen et al., 1984). The ratio of feed intake to body weight gain could be influenced by environmental heat load which is reflected on the productivity of birds.

The results in Chapter 3 indicate that dietary supplementation with Zn could influence blood constituents. This has been indicate by changes in PCV

(Table 3.8). The results also showed decrease in plasma glucose level associated with Zn supplementation, because of the enhancement of the effect of Zn on insulin metabolism (Keen and Graham, 1989) indicating increased glucose utilization.

The current studies (Chapter 4) indicate that seasonal changes in the thermal environment could influence the haematologic responses in birds. This has been confirmed by the changes in PCV values (Table 4.31). Exposure of birds to hot environment is associated with peripheral vasodilation and haemodilution, whereas in cold environment, vasoconstriction and haemoconcentraction are encountered (Whittow, 1986). The increase in energy requirement as a result of exposure to low environmental temperature implies necessary changes in the cardiovascular system to accommodate the energy needs. Higher values of PCV have been observed in broiler chicks and turkeys exposed to low environmental temperature (Yahav 2002).

The absorption of the Zn is controlled by metallothionein. Zn homeostasis is regulated by Zn binding protein metallothionein (Cousins, 1985; Dunn et al., 1987). Under the influence of high dietary Zn, the intestine and liver increase metallothionein synthesis (Cao et al., 2000) which is associated with Zn absorption.

The serum zinc level could be influenced by change in the thermal environment. Change in environmental temperature may cause alteration in Zn excretion and in the serum concentration of Zn (Belay and Teeter, 1996). This pattern is particularly important under tropical conditions characterized

by marked seasonal changes in the thermal environment. Cold exposure in birds leads to higher growth rate and enhanced Zn absorption (Karin, 1985). Conversely, exposure to warm environment induced a significant increase in the rate of excretion of Zn (Klotz et al., 2003; Maret, 2003).

Future studies on birds should include measurements of serum level of Zn and AA. Also endocrine responses of bird which include thyroid hormones, thyroid stimulating hormone and cortciosterones should be monitored in order to evaluate the effect of changes in thermal environment with dietary supplementation of Zn and AA. Measurement of certain blood constituents including serum proteins and immunoglobulins could help in interpretation of responses related to nutritional as well as immunological changes. Also monitoring of the activities of specific enzyme which include creatine phosphokinase (CPK), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) should be considered in order to examine the effects of heat stress on tissue catabolism.

REFERENCES

Adams, R.L. and Rogler, J.C. (1968). The effect of environmental temperature on the protein requirements and responses to energy in slow and fast growing chicks. Poult. Sci., 47: 579-586.

Aengwanich, W. and Simaraks, S. (2004). Pathology of heart, lung, liver and kidney in broilers under chronic heat stress. Songklanakarin. J. Sci. Tech., 26:417-424.

Ain Baziz, H. A., Geraert, P. A., Padilha, J. C. F. and Guillaumin, S. (1996). Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poult. Sci., 75(4):505-513.

Ammerman, B.C., Baker, H.D. and Lewis, A.J. (1995). Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins. Academic Press. Inc., New York, pp: 367-375.

Anderson, R. A., Roussel, A. M., Zouari, N., Mahjoub, S., Matheau, J. and M., Kerkeni, A. (2001). Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J. Am. Coll. Nutr., 20(3):212-218.

Anke, M., Angelow, L., Glei, M., Müller, M. and Illig, H. (1995). The biological importance of nickel in the food chain. J. Anal. Chem., 152: 92-96.

Anke, M., Trüpschuch, A., Arnold, W., Dorn, W. and Hoppe, C. (2002). The effect of a nickel rich on the zinc, magnesium and manganese status of the hens and their eggs. Ber. 7. Tagung Schweine-und Geflügelernährung, Martin-Luther-University Halle-Wittenberg., 7: 210-212.

Arad, Z., Horowitz, M., Eylath, U. and Marder, J. (1989). Osmoregulation and body fluid compartmentalization in dehydrated heat-exposed pigeons. Am. J. Physiol., 257: R377–R382.

Arieli, A., Meltzer, A. and Berman, A. (1979). Seasonal acclimatization in the hen. Br. Poult. Sci., 20: 505–513.

Austic, R. E. (1985). Feeding poultry in hot and cold climates. In: Stress Physiology in Livestock. (Edited by Yousef, M. K.), pp: 123–136. Press, Boca Raton.

Baker, D. H., and Ammerman, C. B. (1995). Zinc bioavailability. In: Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. (Edited by Ammermann, C. B., Baker, D. H. and Lewis, A. J.), pp: 367−398. Academic Press, San Diego.

Bales, C. W., Disilvestro, R. A., Currie, K. L., Plaisted, C. S., Joung, H., Galanos, A. N. and Lin, P. H. (1994). Marginal zinc deficiency in older adults: Responsiveness of zinc status indicators. J. Am. Coll. Nutr., 13:455–462.

Bartholomew, R. J. and Delaney, A. M. (1966). Determination of serum albumin. Proc. Assoc. Clin. Biochem., 1: 214-218.

Bartlett, J. R. and Smith, M. O. (2003). Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci., 82:1580-1588.

Belay, T., Wiernusz, C. J. and Teeter, R. G. (1992). Mineral balance and urinary and faecal mineral excretion profile of broilers housed in thermoneutral and heat-distressed environments. Poult. Sci., 71:1043–1047.

Belay, T., and Teeter, R. G. (1996). Effects of environmental temperature on broiler mineral balance partitioned into urinary and faecal loss. Br. Poult. Sci., 37:423–433.

Berg, L. R. and Martinson, R.D. (1972). Effect of diet composition on the toxicity of zinc for the chick. Poult. Sci., 51: 1690-1694.

Berge, G. (1993). Heat stressing broiler-new trail results. Alpharma technical bulletin No. ARBO 12.

Blake, A.G., Mather, F.B. and Gleaves, E.N. (1984). Dietary self-selection of laying hens inadequate to overcome the effects of high environmental temperature. Poult. Sci., 63: 1346- 1349.

Blamberg, D.L., Blackwood, U.B., Supplee, W.C. and Combs, G.F. (1960). Effect of zinc deficiency in hens on hatchability and embryonic development. Proc. Soc. Exp. Bio. and Med., 104: 217-220.

Bogin, E. and Israeli, B. (1976). Enzymes profile of heart and skeletal muscle, liver and lung of rooster and geese. Zbl. Vet. Med., 23:152–157.

Bollengier-Lee S., Mitchell, M.A., Utomo, D.B., Williams, P. E. V. and Whitehead, C.C. (1998). Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Br. Poult. Sci., 39: 106–112.

Bowen, S. J., Washburn, K. W. and Huston, T. M. (1984). Involvement of the thyroid gland in the response of the young chicken to heat stress. Poult. Sci., 63: 66–69.

Braganza, A. F., Peterson, R. A. and Cenedella, R. J. (1973). The effect of heat and glucagon on the plasma glucose and free fatty acids of the domestic fowl. Poult. Sci., 52: 58-63.

Brandeo-Neto, J., Stefan, V., Mendonca, B., Bloise, W. and Castro, A. (1995). The essential role of zinc in growth. Nutr. Res., 15: 335-358.

Brown, K. H., Wuehler, S. E. and Peerson, J. M. (2001). The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr. Bull., 22:113–125.

Burke, J. P. and Fenton, M. R. (1985). Effect of a Zn-deficient diet on lipid peroxidation in liver and tumor cellular membranes. Proc. Soc. Exp. Bio. and Med., 179: 187-191.

Burton, R. R., and Smith, A. H. (1967). Effect of polycythaemia and chronic hypoxia on heart mass in the chicken. J. Appl. Physiol., 22: 782–785.

Buss, D. H., and Rose, H. J. (1992). Dietary intake of nutrient trace elements. Food Chem., 43: 209–212.

Cahaner, A. and Leenstra, F. (1992). Effects of high temperature on growth and efficiency of male and female broilers from lines selected for high weight gain, favourable feed conversion, and high or low fat content. Poult. Sci., 71: 1237-1250.

Cao, J., Henry, P. R., Guo, R., Holwerda, R. A., Toth, J. P., Littell, R. C., Miles, R. D. and Ammerman, C. B. (2000). Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci., 78: 2039-2054.

Carew, L.B., Machemer Jr, R.H. and Sharp Jr., R.W. (1972). Fat absorption by the very young chick. Poult. Sci., 51: 738-742.

Chandra, R. K., and Dayton, D. H. (1982). Trace element regulation of immunity and infection. Nutr. Res., 2: 721–733.

Chandra, R.K. and Au, B. (1980). Single nutrient deficiency and cell-mediated immune responses. 1. Zinc. Am. J. Clin. Nutr., 33: 736—738.

Chen, Y. and Maret, W. (2001). Catalytic selenols couple the redox cycles of metallothionein and glutathione. Eur. J. Biochem., 268: 3346–3353.

Chesters, J. K. (1997). Zinc. In: O'Dell, B. L. and Sunde, R. A. (Ed.) Handbook of Nutritionally Essential Mineral Elements. pp 185−230. Marcel Dekker, New York.

Chwalibog, A. and Eggum, B. O. (1989). Effect of temperature on performance, heat production, evaporative heat loss and body composition in chickens. Arch. Geflu¨ gelkd., 53:179– 184.

Coates, M.E. (1984). Metabolic role of the vitamins. In: Physiology and Biochemistry of the Domestic Fowl. (Edit by Freeman, B.W.), Vol.5 pp: 27-36. Academic Press, London.

Coles, E. H. (1974). Veterinary Clinical Pathology, 3rd ed., W. B. Saunders Co., Philadelphia, USA.

Cousins, R. J. (1985). Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol. Rev., 65:238̃-309.

Cousins, R. J. (1996). Zinc. In: Present Knowledge, in: Nutrition, 7th Edition. (Edited by Ziegler, E. E. and Filer, L. J., Jr.), pp: 293–306. Academic Press, London.

Coyle, P., Philcox, J. C., Carey, L. C. and Rofe, A. M. (2002). Metallothionein: The multipurpose protein. Cell Mol. Life Sci. 59:627–647.

Cunxiao Sun, (1996). Influence of dietary zinc levels on the activity of zinc-enzyme in livers of broiler chicken. Chin. J. Hus. and Vet., 31: 156-157.

Dardenne, M. and Bach, J.M. (1993). Rationale for the mechanism of zinc interaction in the immune system. In: Nutrient Modulation of the Immune Response (Edited by Cunningham-Rundles, S.), pp: 501-509. Marcel Dekker, New York.

Dardenne, M., Savino, W. Borrih, S. and Bach, J. F. (1985). Zinc dependent epitope of the molecule of thymulin, a thymic hormone. Proc. Natl. Acad. Sci., 82: 7035.

Darras, V. M., Van Der Geyten, S. and Kühn, E. R. (2000). Thyroid hormone metabolism in poultry. Biotechnol. Agron. Soc. Environ., 4: 13-20.

Dechao L. (1995). Research of true lysine and methionine requirement of broiler chicken. Chin. J. Hus. and Vet., 33: 178-181.

Deeb, N. and Cahaner, A. (2002). Genotype-by-environment interaction with broiler genotypes differing in growth rate. 3. Growth rate and water consumption of broiler progeny from weight-selected versus nonselected parents under normal and high ambient temperatures. Poult. Sci., 81:293–301.

Dewar, W. A. and Downie, J. N. (1984). The zinc requriement of broiler chicks and turkey poults fed on purified diets. Br. J. Nutr., 51: 467-477.

Donaldson, W. E. (1995). Carbohydrate, hatchery stressors affect. poult. survival. Feedstuffs, 67: 16-17.

Donkoh, A. (1989). Ambient temperature: a factor affecting performance and physiological responses of broiler chickens. Int. J. Biol., 33: 259-265.

Donmez, Z., Donmez, H. H., Keskin., E. and Celik, I. (2002). Effects of zinc supplementation to ration on some haematological parameters in broiler chicks. Biol. Trace Elem. Res., 87: 125-131.

Dorr, P. E. and Nockels, C. F. (1971). Effects of aging and dietary ascorbic acid on tissue ascorbic acid in the domestic fowl. Poult. Sci., 50: 1375-1382.

Dowd, P. S., Kelleher, J. and Guillou, P. J. (1986). T– lymphocyte subsets and interleukin-2 production in zinc–deficient rats. Br. J. Nutr., 55: 59-69.

Dunn, M. A., Blalock, T. L. and Cousins., R. J. (1987). Metallothionein. Proc. Soc. Exp. Biol. and Med., 185:107 (Abstr.).

Edwards, H. M. and Baker, D. H. (1999). Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. J. Anim. Sci., 77: 2730−2735.

Emmert, J. L. and Baker, D. H. (1995). Zinc stores in chickens delays the onset of zinc deficiency symptoms. Poult. Sci., 74: 1011–1021.

Faruga, A., (1975). Fattening chickens with standard feed supplemented with vitamin. Nutr. Abstr. Rev., 45: 732 (abstr.).

Feenster R. (1985). High temperatures decrease vitamin utilization. Misset Poult., 38: 38–41.

Fletcher, M. P., Gershwin, M. E., Keen, C. L. and Hurley, L. S. (1988). Trace element deficiencies and immune responsiveness. In: human and animal models. In: Nutrition and Immunology. (Edited by Chandra, R. K.), pp: 215–239. Alan Liss, New York.

Fletcher, M. P., Gershowin, M. E., Keen, C. L., and Hurley, L. S. (1998). Trace element deficiencies and immune responsiveness. In: human

and animal models. Nutrition and Immunology. (Edited by Chandra, R.K.), pp: 215-239. Alan liss, New York.

Food and Nutrition Board (2001). Dietary Reference Intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC.

Freeman, B. M. (1988). The domestic fowl in biomedical research: Physiological effects of environment. Poult. Sci., 44: 41-60.

Garfinkel, D. (1986). Is aging inevitable the intracellular zinc deficiency hypothesis of aging. Med. Hypotheses, 19: 117–137.

Geraert, P. A., Padilha, J. C. F. and Guillaumin, S. (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. Br. J. Nutr., 75:195–204.

Girodon, F., Galan, P., Monget, A. L., Boutron-Ruault, M. C., Brunet-Lecomte, P., Preziosi, P., Arnaud, J., Manuguerra, J. C. and Hercberg, S. (1999). Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: A randomized controlled trial. Arch. Intern. Med., 159:748–754.

Girotti, A. W. (1985). Mechanisms of lipid peroxidation. J. Free Radic. Biol. Med., 1: 87-95.

Gonzalez-Esquerra, R. and Leeson, S. (2005). Effects of acute versus chronic heat stress on broiler response to dietary protein. Poult. Sci., 84: 1562–1569.

Gonzalez-Vega-Aguirre, D., Contreras, B. P. A., Klein, R. and Bohmwald, H. (1995). Effect of vitamin C and E supplementation in the diet of broiler chicks on performance and immune response. Veterinaria, 26: 333-340.

Hai, L., Rong, D. and Zhang, Z. Y. (2000). The effect of thermal environment on the digestion of broilers. J. Anim. Physiol. Anim. Nutr., 83: 57-64.

Halliwell B. and Gutteridge, J. M. C. (1989). Lipid peroxidation: a radical chain reaction. In: Free radicals in biology and medicine. 2nd edition. pp: 188–218. Oxford University Press, New York.

Hambidge, K. M., Casey, C. E. and Krebs, N. F. (1986). Zinc. In: Trace elements in human and animal nutrition. (Edited by Mertz W.), vol. 2, pp: 1-137., San Diego.

Harvey, C., Scances, C. G. and Brown, K. I. (1986). Adrenals. In: Avian physiology 4th Edition. (Edited by P. D. Sturkie), pp: 379-393. springer-verlag. New York. Berlin. Heidelberg. Tokyo.

Hassan, A. and Al-Rawl, B. A. (1982). Relative important of environmental temperature and humidity on the physiological performance of layers. Rev. Anim. Prod., 18:343–348.

Hazelwood, R. L. (2000). Pancreas. In: Sturkie's Avian Physiology. 5th Edition. (Edited by G. C. Whittow), pp: 539–555. Academic Press, San Diego.

Heintzelman, M. B. and Mooseker, M. S. (1990). Structural and compositional analysis of early stages in microvillus assembly in the enterocyte of the chick embryo. Differentiation, 43: 175−182.

Hess, J. B., Blake, J. P., Norton, R. A., Downs, K. M., Kalinowski, A. and Corzo, A. (2001). Dehydrated poultry meal as a replacement for soybean meal in broiler diets. J. Anim. Sci., 79 (Suppl.1): 325. (Abstr.)

High, K. P. (1999). Micronutrient supplementation and immune function in the elderly. Clin. Infect. Dis., 28: 717–722.

Hill, C. (1979). Studies on the ameliorating effect of ascorbic acid on minerals toxicities in the chick. J. Nutr., 109: 84-90.

Hillman, P. E., Scott, N. R. and van Tienhoven, A. (1985). Physiological responses and adaptations to hot and cold environments. In: Stress Physiology in Livestock. (Edited by M.K. Yousef), pp 1–71. Inc Boca Koton, FL, CPC Press. Florida.

Horing, D. and Frigg, M. C. (1979). Effect of age on biosynthesis of ascorbate in chicks. Arch. Geflugelk, 43: 108-112.

Hortin, A. E., Oduho, G., Han, Y., Bechtel, P. J. and Baker., D. H. (1993). Bioavailability of zinc in ground beef. J. Anim. Sci., 71: 119–123.

Hungerford, Jr., D. M. and Linder, M. C. (1983). Interaction of pH and ascorbate in intestinal iron absorption. J. Nutr., 113: 2615-2622.

Hussein, L. and Bruggeman, J. (1997). Zinc analysis of Egyptian foods and estimated daily intakes among an urban population group. Food Chem., 58: 391–398.

Ingram, D. L. and Mount, L. E. (1975). Heat exchange between animal and environment. In: Man and Animals in Hot Environment, pp: 123-145. Springer-Verlag, New York.

Ismail, I. B. (1991). Effect of Ascorbic Acid (Vitamin C) on Broiler Under Hot Climate. M.V.Sc. Thesis, Unversity of Khartoum.

Jacob, C., Maret, W. and Vallee, B. L. (1999). Selenium redox biochemistry of zinc-sulfur coordination sites in proteins and enzymes. Proc. Natl. Acad. Sci., 96: 1910–1914.

Jaeger, J. J. and McGrath, J. J. (1974). Haematologic and biochemical effects of simulated high altitude on the Japanese quail. J. Appl. Physiol., 37: 357–361.

Jain, C. N. (1986). Haematological technique. In: Schalm's Veterinary Hematology, 4th Edition. (Edited by Lea and Febiger), pp: 443-678. Philadelphia.

Jensen, L. S. (1975). Precipitation of a selenium deficiency by high dietary levels of copper and zinc. Proc. Soc. Exp. Biol. and Med., 149: 113-116.

Jiang, G., Gong, Z., Li, X. F., Cullen, W. R. and Le, X. C. (2003). Interaction of trivalent arsenicals with metallothionein. Chem. Res. Toxicol., 16: 873–880.

Johnson, D., Jr, Mehring, Jr., A. L., Savino, F. X. and Titus, H. W. (1962). The tolerance of growing chickens for dietary zinc. Poult. Sci., 41: 311–317.

Kafri, I. and Cherry, J. A. (1984). Supplemental ascorbic acid and heat stress in broiler chicks. Poult. Sci., 63: 125-132.

Karin, M. (1985). Metallothioneins: Proteins in search of function Cell. J. Anim. Sci., 41: 9-10.

Keen, C. L. and Graham, T. W. (1989). Zinc. In: Clinical biochemistry domestic animals. 4th Edition. (Edited by Kaneko, J. J.), pp: 776-784.

Ker, G. R., Lae, E. S., Lan, M. K. M, Lamor, R. J., Randell, E. and Forhoter, R. (1982). Relationship between diet and biochemical measures of nutritional status. Am. J. Clin. Nutr. 35: 294 - 308.

Kidd, M. T., Ferket, P. R. and Qureshi, M. A. (1996). Zinc metabolism with special reference to its role in immunity. J. W. Poult. Sci., 52: 309—323.

Kim, W. K. and Patterson, P. H. (2004). Effects of dietary zinc supplementation on broiler performance and nitrogen loss from manure. Poult. Sci., 83: 34-38.

King, E. G. and Wooton, T. D. P. (1955). Determination of total protein in plasma or serum. In: Medical Biochemistry., pp: 133. Churchill. Ltd. London.

Klandorf, H. and Harvey, S. (1985). Food intake regulation of circulating thyroid hormones in domestic fowl. Gen. Comp. Endocrinol., 60: 162-170.

Klasing, K. C. (1984). Effects of inflammatory agents and interleukin-1 on iron and zinc metabolism. Am. J. Physiol., 247: R901–R904.

Klasing, K. C. (1998). Nutritional modulation of resistance to infectious diseases. Poult. Sci., 77: 1119-1125.

Klotz, L. A., Kro¨ncke, K. D., Buchczyk, D. P. and Sies, H. (2003). Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J. Nutr., 1448S–1451S.

Krogdahal, A. (1985). Digestion and absorption of lipids in poultry. J. Nutr., 115: 675-685.

Krogdahal, A. and Sell, J. L. (1989). Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poult. Sci., 68: 1561–1568.

Kutlu H. R. and Forbes J.M. (1993a). Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Proc. Nutr. Soc., 36: 335–350.

Kutlu, H. R. and Forbes, J. M. (1993b). Effect of changes in environmental temperature on self-selection of ascorbic acid in coloured feeds by broiler chicks. Proc. Nutr. Soc., 52: 29-32.

Kutlu, H.R., Görgülü, M. and Baykal, L. (1998). Effect of dietary zinc concentration on growth performance of broilers. National Zinc Congress, 12-16 May, pp: 671-676. Eskişehir, Turkey.

Ladmakhi, M. H., Buys, N., Dewil, E., Rahimi, G. and Decuypere, E. (1997). The prophylactic effect of vitamin C supplementation on broiler ascites incidence and plasma thyroid hormone concentration. Avian Pathol., 26: 33-44.

Lease, J. C., Barnett, B. D., Lease, E. J. and Turk, D.E. (1960). The biological unavailability to the chick of zinc in a sesame meal ration. J. Nutr., 72: 66-70.

Leeson, S. (1986). Nutritional considerations of poultry during heat stress. World's Poult. Sci. J., 42: 69-81.

Leeson S. and Summers J. D. (2001). Nutrition of the chicken. 4th Edition. pp: 112-125. Ontario: University Books.

Lesourd, B. M. (2001). Undernutrition: A factor of accelerated immune aging in healthy and diseased aged persons. In: Handbook of Nutrition in the Aged 3rd Edition. (Edited by R. R. Watson), pp: 145–158. CRC Press. Boca Raton.

Lewandowski, A. H. and Harrison, G. J. (1986). Clinical Avian Medicine and Surgery. W. B. Saunders, Philadelphia, PA.

Lin, H. Zhang, H. F., Du, R. Gu, X. H. Zhang, Z. Y., Buyse, J. and Decuypere, E. (2005). Thermoregulation responses of broiler chickens to humidity at different ambient temperatures. II. Four weeks of age. Poult. Sci., 84 (8): 1173-1178.

Lonnerdal, B. (2000). Dietary factors influencing zinc absorption. J. Nutr., 130: 1378–1383.

Lott, B. D., Simmons, J. D. and May, J. D. (1998). Air velocity and high temperature effects on broiler performance. Poult. Sci., 77: 391–393.

Lu, J. and Combs, G. F. (1988). Effect of excess dietary zinc on pancreatic exocrine function in the chick. J. Nutr., 118: 681−689.

Ma, J. and Betts, N. M. (2000). Zinc and copper intakes and their major food sources for older adults in the 1994–96 continuing survey of food intakes by individuals (CSFII). J. Nutr., 130: 2838–2843.

Maiorka, A., Da Silva, A. V. F., Santin, E., Pizauro, Jr., J. M. and Macari, M. (2004). Broiler breeder age and dietary energy level on performance and pancreas lipase and trypsin activities of 7-days old chicks. Int. J. Poult. Sci., 3: 234–237.

Makinde M. O. and Fatunmbi O. O. (1985). Some haematological and biochemical values of turkeys in Ibadan. Bull. Anim. Hlth. Prod. Afr. 33(3): 245-248

Manning R. O. and Wyatt R. (1990). Effect of cold acclimatization on the broilers chicks resistance to acute aflatoxicosis. Poult. Sci., 69: 388–396.

March, B. and Biely, J. (1953). The effect of ascorbic acid on the growth rate of chicks. Poult. Sci., 32:768-774.

Maret, W., Jacob, C., Vallee, B. L. and Fischer, E. H. (1999). Inhibitory sites in enzymes: Zinc removal and reactivation by thionein. Proc. Natl. Acad. Sci., 96:1936–1940.

Maret, W. (2000). The function of Zinc metallothionein: A link between cellular zinc and redox state. J. Nutr., 130: 1455S–1458S.

Maret, W. (2003). Cellular zinc and redox states converge in the metallothionein/thionein pair. J. Nutr., 133: 1460S–1462S.

May, J. D. and Lott, B. D. (1992). Feed and water consumption patterns of broilers at high environmental temperatures. Poult. Sci., 71:331–336.

McDowell, L. R. (1989). Vitamins in Animal Nutrition. In: Comparative Aspects to Human Nutrition. (Edited by McDowell, L.R.), pp: 93-131. Academic Press. London.

Mckee, J. S. and Harrison, P. C. (1995). Effect of supplemental ascorbic acid on the performance of broiler chikens exposed to multiple concurrent stressors. Poult. Sci., 74: 1772-1785.

McKee, J. S., Harrison, P. C. and Riskowski, G. L. (1997). Effects of supplemental ascorbic acid on the energy conversion of broiler chicks during heat stress and feed withdrawal. Poult. Sci., 76: 1278-1286.

Mohanna, C. and Nys, Y. (1998). Influence of age, sex and cross on body concentrations of trace elements (zinc, iron, copper and manganese) in chickens. Br. Poult. Sci., 39: 536–543.

Mohanna, C. and Nys, Y. (1999). Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. Br. Poult. Sci., 40:108–114.

Morrison, A. B. and Sarett, H. P. (1958). Studies on zinc deficiencies in the chick. J. Nutr., 65: 267-280.

Mowat, D. N. (1994). Organic chromium in animal nutrition. A new nutrient for stressed animals. In: Biotechnology in the feed industry. Proc. Asia-Pacific Lecture Tour. P: 31. Alltech Inc., Nicholasville, KY.

Nakaya, T., Suzuki, S. and Watanabe, K. (1986). Effect of high dose supplementation of ascorbic acid on chicks. Poult. Sci., 23: 276-283.

National Research Council. (1996). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Nesheim, R. C., Austic, R. E., and Card, L. E. (1979). Poultry Production. Twelfth, Edition. Lea and Fibeger. Philadelphia.

Nishi, Y. (1996). Zinc and growth. J. Am. Coll. Nutr., 15: 340-344.

Njoku, P. C. (1986). Effect of dietary ascorbic acid (vitamin C) supplementation on the performance of broiler chickens in a tropical environment. Anim. Feed Sci. and Tech.,16:17-42.

Njoku, P. C. and Adeline, O. U. N. (1989). Effect of dietary inclusion of ascorbic acid and palm oil on the performance of laying hens in a hot tropical environment. Br. Poult. Sci. 30: 831-840.

Nockels, C. F., Lopez, G. A. and Phillips, R. W. (1973). Influence of vitamin A and C on corticosterone and carbohydrate metabolism in chickens. Poult. Sci., 52:1261-1269.

Nordberg, M. (1998). Metallothioneins: Historical review and state of knowledge. Cell. Mol. Biol., 46:243–254.

Noy, Y., Frisch, N., Rand, M. and Sklan D. (1994). Trace mineral requiments in turkeys. Poult. Sci., 50: (3) 253-269.

O'dell, B. L. and Savage, J. E. (1957). Potassium, zinc and distillers dried solubles as supplements to a purified diet. Poult. Sci., 36: 459-460.

O'dell, B. L., Newberne, P. M. and Savage, J. E. (1958). Significance of dietary zinc for the growing chicken. J. Nutr., 65: 503-518.

O'dell, B. L. (1992). Zinc plays both structural and catalytic roles in metalloproteins. Nutr. Rev., 50: 48-50.

Oluymemi, J. A. (1979). Measures applied to combat thermal stress in poultry under practical tropical environment. Poult. Sci., 58: 967-973.

Onderci, M., Sahin, N., Sahin, K. and Kilic, N. (2003). The antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins and some minerals under a low ambient temperature. Biol. Trace Elem. Res., 92: 139-150.

Orban, J. I., Roland, D. A. Jr., Cummins, K. and Lovell, R.T. (1993). Influence of large doses of ascorbic acid on performance, plasma calcium, bone characteristics and eggshell quality in broiler and Leghorn hens. Poult. Sci., 72: 691-700.

Pardue, S. L., Thaxton, J. P. and Brake, J. (1985a). Influence of supplemental ascorbic acid on broiler performance following exposure to high environmental temperature. Poult. Sci., 64: 1334-1338.

Pardue, S.L., Thaxton, J.P. and Brake, J. (1985b). Role of ascorbic acid in chicks exposed to high environmental temperature. J. Appl. Physiol., 58:511-516.

Pardue S. L. and Thaxton, J. P. (1986). Ascorbic acid in poultry. A review. Poult. Sci., 42:107.

Park, W., (1995). Dietary nutrient allowances for poultry. Feedstuffs, 19: 67-76.

Pearce, J. (1983). Carbohydrate metabolism. In: Physiology and Biochemistry of Domestic fowl. 4th Edition. (Edited by B. M. Freeman), pp: 147-161. Academic Press, London.

Pond, W. G., Church, D. C. and Pond, K. R. (1995). Basic Animal Nutrition and Feeding. 4th Edition. John Wiley and Son, Inc., New York.

Poulsen, H.D. and Larsen, T. (1995). Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livestock Prod. Sci., 43: 235-242.

Poulsen, H.D. and Carlson, D. (2001). Bioavailability of zinc from different zinc sources. Proc. 52nd Ann.Meeting Eur. Ass. Anim. Prod., Budapest, Hungary. EAAP publications, p:123 (Abstr.).

Powell, S. R., Hall, D., Aiuto, L., Wapnir, R. A., Teichberg, S. and Tortolani, A. J. (1994). Zinc improves postischaemic recovery of isolated rat hearts through inhibition of oxidative stress. Am. J. Physiol., 266: H2497–H2507.

Powell, S. R. (2000). The antioxidant properties of zinc. J. Nutr., 130:1447–1454.

Prasad, A.S., Halsted, J.A. and Nadimi, M. (1961).Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med., 31: 532-546.

Prasad, A. S. (1978). Trace Elements and Iron Human Metabolism. 4th Edition. John Wiley and Sons, pp: 289-303. Great Britain.

Prasad, A. S., Fitzgerald, J. T., Hess, J. W., Kaplan, F., Pelen, J. and Dardenne, M. (1993). Zinc deficiency in elderly patients. Nutr., 9:218–224.

Raup, T. J. and Bottje, W. G. (1990). Effect of carbonated water on arterial pH, pCO2 and plasma lactate in heat-stressed broilers. Br. Poult. Sci., 31:377–384.

Richards, S. A. (1971). The significance of changes in the temperature of the skin and body-core of the chicken in the regulation of heat loss. J. Physiol. Lond., 216: 1-10.

Roberson, R.H. and Schaible, P.J. (1958). The zinc requirement of the chick. Poult. Sci., 37: 1321-1323.

Roberson, R. H. and Schaible, P. J. (1960). The tolerance of growing chicks for high levels of different forms of zinc. Poult. Sci., 39: 893–896.

Roberson, K. D., and Edwards, H. M. (1994). Effects of 1,25 dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. Poult. Sci., 73:1312–1316.

Rosenthal, P. (1977). Assessing liver function and hyperbilinemia in the new born. Clin. Chem., 43: 228 - 234.

Rucker, R. B., Lönnerdal, B. and Keen, C. L. (1994). Intestinal absorption of nutritionally important trace elements. In: Physiology of the Gastrointestinal Tract. (Edited by Johnson L. R.), pp: 2183-2202. Raven Press. New York.

Sahin, K., Sahin, N., Ondrci, M., Yaralioglu, S. and Kucuk, O. (2001). Protective role of supplementation vitamins E, A and some minerals concentrations of broilers reared under heat stress. Vet. Med. Czech., 46: 140-144.

Sahin, K. and Kucuk, O. (2001). Effects of vitamin C and Vitamin E on performance, digestion of nutrients and carcass characteristics of Japanese quails reared under heat stress (34^{o}C). J. Anim. Physiol. Anim. Nutr., 85: 335-342.

Sahin K., Kucuk, O., Sahin, N. and Sari, M. (2002a). Effects of vitamin C and vitamin E on lipid peroxidation status, some serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34°C). Int. J. Vit. Nutr. Res., 72: 91–100.

Sahin, K., Sahin, N. and Yaralioglu, S. (2002b). Effect of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites and mineral concentrations of laying hens reared under high ambient temperature. Biol. Trace Elem. Res., 85: 35-45.

Sahin, K. and Kucuk, O. (2003a). Heat stress and dietary vitamin supplementation of poultry diets. Nutr. Abstr. Rev. Ser. B. Livest. Feeds Feeding, 73: 41R–50R.

Sahin, K. and Kucuk., O. (2003b). Zinc supplementation alleviates heat stress in laying Japanese quail. J. Nutr., 133: 2808–2811.

Sahin, K., Smith, M. O., Onderci, M., Sahin, N., Gursu, M. F. and Kucuk, O. (2005). Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. Poult. Sci., 84: 882–887.

Salgueiro, M. J., Zubillaga, M., Lysionek, A., Sarabia, M. I., Caro, R., De Paoli, T., Hager, A., Weill, R. and Boccio, J. (2000). Zinc as essential micronutrient: A review. Nutr. Res., 20:737–755.

Sandercock, D. A., Hunter, R. R., Nute, G. R., Mitchell, M. A. and Hocking, P. M. (2001). Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. Poult. Sci., 80:418–425.

Sandoval, M., Henry, P. R., Ammermann, C. B., Miles, R. D. and Little, R.C. (1997). Relative bioavailability of supplemental inorganic zinc sources for chicks. J. Anim. Sci., 75: 3195-3205.

Sandoval, M., Henry, P. R., Luo, X. G., Littell, R. C., Miles, R. D. and Ammerman, C. B. (1998). Performance and tissue zinc and metallothionine accumulation in chicks fed a high dietary level of zinc. Poult. Sci., 77: 1354–1363.

SAS Institute. (1999). SAS User's Guide: Version 6.12. SAS Institute Inc., Cary, NC.

Savarino, L., Granchi, D. Ciapetti, G. Genni, E. Ravaglia, G. Forti, P. Maioli, F. and Mattioli, R. (2001). Serum concentrations of zinc and selenium in elderly people: Results in health nonagenarians/centenarians. Exp. Gerontol, 36: 327–339.

Schwarz, M. A., Lazo, J. S., Yalowich, J. C., Reynolds, I., Kagan, V. E., Tyurin, V., Kim, Y. M., Watkins, S. C. and Pitt, B. R. (1994). Cytoplasmatic metallothionein overexpression protects NIH 3T3 cells from tert-butyl hydroperoxide toxicity. J. Biol. Chem., 269: 15238–15243.

Scott, M. L. (1975). Environmental influences on ascorbic acid requirements in animals. Anim. Acad. Sci., 258: 151-155.

Seel, J. L. (1996). Physiological limitations and potential for improvement in gastrointestinal tract function of poultry. J. Appl. Poult. Res., 5: 96-101.

Serafin, J. A. and Nesheim, M. C. (1970). Influence of dietary heat-labile factors in soybean meal upon bile acid and turnover in the chick. J. Nutr., 100: 786-796.

Shaheen, A. A. and Abd El-Fattah, A. A. (1995). Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity in rat tissues. Int. J. Biochem. Cell Biol., 27: 89-95.

Shan, A.S. (1993). Effect of fibre and various zinc compounds on performance, blood biochemical parameters and zinc

concentrations in tissues of chicks. Acta Vet. ET Zoote. Sin., 24(1) 29-35.

Shannon, D. W. F. and Brown, W. O. (1969). The period of adaptation of the fasting metabolic rate of the common fowl to an increase in environmental temperature from 22°C to 28°C. Br. Poult. Sci., 10:13–18.

Shapiro, F., Mahagna, M. and Nir, I. (1997). Stunting syndrome in broilers: effect of glucose or maltose supplementation on digestive organs, intestinal disaccharidases, and some blood metabolites. Poult. Sci., 76:369–380.

Sherman, A. R. (1992). Zinc, copper and iron nutrition and immunity. J. Nutr., 122:604–609.

Shinder, D., Luger, D., Rusal, M., Rzepakovsky, V., Bresler, V. and Yahav, S. (2002). Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. J. Thermal Biol., 27: 517-523.

Shlosberg, A., Bellaiche, M., Zeitlin, G., Ya'acobi, M. and A. Cahaner, (1996). Haematocrit values and mortality from ascites in cold-stressed broilers from parents selected by haematocrit. Poult. Sci., 75:1–5.

Shlosberg, A., Zadikov, I., Bendheim, U., Handji, V. and Berman, E. (1992). The effect of poor ventilation, low temperatures, type of feed and sex of bird on the development of ascites in broilers. Physiopathological factors. Avian Pathol., 21: 369–382.

Simmons, J. D., Lott, B. D. and May, J. D. (1997). Heat loss from broiler chickens subjected to various wind speeds and ambient temperatures. Appl. Eng. Agric., 13(5):665–669.

Smith, M. O., Sherman, I. L., Miller, L. C. and Robbins, K.R. (1995). Relative biological availability of manganese from manganese proteinate, manganese sulfate, and manganese monoxide in broilers reared at elevated temperatures. Poult. Sci., 74: 702-707.

Smith, M. O. and Teeter, R. G. (1987). Potassium balance of the 5 to 8-week old boiler exposed to constant heat or cycling high temperature stress and the effects of supplemental potassium chloride on body weight gain and feed efficiency. Poult. Sci., 66: 487-492.

Southern, L. L. and Baker, D. H. (1983). Zinc toxicity, zinc deficiency and zinc-copper interrelationship in *Eimeria acervulina* infected chicks. J. Nutr., 113: 688-696.

Spears, J. W. (1989). Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci., 67:835–843.

Spiers, D. E. (1983). Temperature regulation in adult quail during acute thermal stress. Comp. Biochem. Physiol., 74: 369–373.

Stahl, J. L., Gregr, J. L. and Cook, M. E. (1989). Zinc, copper and iron utilization by chicks fed various concentrations of zinc. Br. Poult. Sci., 30: 123-134.

Stahl, J. L., Janet, L. and Grger, C. M. E. (1998). Zinc, copper and iron utilization by chicks fed various concentration of zinc. Br. Poult. Sci., 30:123-134.

Stojević, Z., Milinković-Tur, S. and Ćurčija, K. (2000). Changes in thyroid hormones concentrations in chicken blood plasma during fattening. Vet. Arhiv., 70: 31-37.

Subar, A. F., Krebs-smith, S. M. Cook, A. and Kahle, L. L. (1998). Dietary sources of nutrients among US adults. J. Am. Diet. Assoc., 98: 537–547.

Suso, F. A. and Edwards, H. M. (1968). A study of techniques for measuring Zn absorption and biological half-life in the chicken. Poult. Sci., 47: 991-999.

Swinkels, J. W., Kornegay, E. T. and Verstegen, M. W. (1994). Biology of zinc and biological value of dietary organic zinc complexes and chelates. Nutr. Res. Rev., 7: 129–149.

Sykes, A.H. (1978).Vitamin C for poultry: Some recent research. In: proceedings of the Roshe Symposium, pp: 5-15.

Tate, D. J., Miceli, M. V. and Newsome, D. A. (1999). Zn protects against oxidative damage in cultured human retinal pigment epithelial cells. Free Radic. Boil. Med., 26: 704-713.

Teeter, R. G., Smith, M. O., Owens, F. N. Arp, S. C., Sangiah, S. and Breazile, J. E. (1985). Chronic heat stress and respiratory alkalosis: Occurrence and treatment in broiler chicks. Poult. Sci., 64:1060–1064.

Terre´s, C., Navarro, M., Martı´n-Lagos, F., Gime´nez, R., Lo´pez, H. and Lo´pez, M. C. (2001). Zinc levels in foods from southeastern Spain: Relationship to daily intake. Food Addit. Contam., 18:687–695.

Thaxton, J. P. and Puvaldolpirod, S. (2000). Model of physiological stress in chickens 5. Quantitative evaluation. Poult. Sci., 79: 391–395.

Thaxton, P., Watt, R.D. and Hamilton, P.B. (1974). The effect of environmental temperature on parathyroid infection in the neonatal chicken. Poult. Sci., 53: 88-94.

Thornton, P. A. (1970). Influence of exogenous ascorbic acid on calcium and phosphorus metabolism in the chick. J. Nutr., 100: 1479- 1486.

Thornton, P. A. and Moreng, R. E. (1959). Further evidence on the value of ascorbic acid for maintenance of shell quality in warm environmental temperature. Poult. Sci., 38:594-599.

Thornton, P.A. (1961). Increased environmental temperature influences on ascorbic acid. Fed. Proc., 20: 210.

Timmons, M. B. and Hillman, P. E. (1993). Partitional heat losses in heat stressed poultry as affected by wind speed. 4th International Livestock Environment Symposium. London, England.

Ting, H. (1995). Research of zinc requirement in broilers' diet. Acta. Zoonutrimenta Sinica, 7: 2-9.

Todd, W.R., Elvehjem, C.A. and Hart, E.B. (1934). Zinc in the nutrition of the rat. Am. J. Physiol., 107: 146-156.

Tucker, H.F. and Salmon, W.D. (1955). Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med., 88: 613-616.

Tzschentke, B., Nichelmann, M. and Postel. T. (1996). Effects of ambient temperature, age and wind speed on the thermal balance of layer-strain fowl. Br. Poult. Sci., 37:501–520.

Uni, Z., Noy, Y. and Sklan, D. (1995). Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poult. Sci., 37:63−71.

Uni, Z. and Ferket, R.P. (2004). Methods for early nutrition and their potential. Worlds Poult. Sci. J., 60: 101-111.

Vallee, B. L. and Auld, D. S. (1990). The metallobiochemistry of zinc enzymes. In: Advances in Enzymology. Meister, A., pp. 283-429. New York.

Van den B. A. H. M. and Thoday, K. L. (1986). Skin disease in dogs associated with zinc deficiency: A report of five cases. J. Small Anim. Pract., 27:313–317.

Vohra, P. and Kratzer, F. H. (1968). Zinc, copper and managanese toxicities in turkey poults and their alleviation by EDTA. Poult. Sci., 47: 699-705.

Wallis, I. R. and Balnave, D. (1984). The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. Br. Poult. Sci., 25: 401–407.

Wallis, I. R. and Balnave, D. (1984). The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. Br. Poult. Sci., 25:401–407.

Wathes, C. M. and Clark, J. A. (1981). Sensible heat transfer from the fowl: thermal resistance of the pelt. Br. Poult. Sci., 22:175-183.

Watkins, K. L. and Southern, L. L. (1993). Effect of dietary sodium ziolet A on zinc utilization by chicks. Poult. Sci., 72:296-305.

Wedekind, K. J. and Baker, D. H. (1990). Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci., 68: 684-689.

Wedekind, K. J., Hortin, A. E. and Baker, D. H. (1992). Methodology for assessing zinc bioavailability: Efficacy estimated for zinc-methionine, zinc sulphate, and zinc oxide. J. Anim. Sci., 70: 178–187.

Weigand, E. and Kirchgessner, M. (1980). Total true efficiency of zinc utilization: Determination and homeostatic dependence upon the zinc supply status in young rats. J. Nutr., 110:469-480.

Whitehead, C. C. (1998). Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Br. Poult. Sci., 39: 106-112.

Whittow, G. C. (1986). Regulation of body temperature. In: Avian Physiology. 4th Edition. (Edited by E. D. Sturkie), pp: 221–252. Springer-Verlag, New York.

Wiernusz, C. J. and Teeter, R. G. (1996). Acclimation effects on fed and fasted broiler thermobalance during themoneutral and high ambient temperature exposure. Br. Poult. Sci., 37:677–687.

Xiuyun, W. (1995). Zinc requirement for broilers. Chin. J. Animal Sci., 31: 10

Yahav, S., Goldfield, S. I., Plavnik, I. and Hurwitz, S. (1995). Physiological responses of chickens and turkeys to relative humidity during exposure to high ambient temperature. J. Therm. Biol., 20: 245-253.

Yahav, S., and Hurwitz, S. (1996). Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci., 75: 402–406.

Yahav, S., Straschnow, A., Plavnik, I. and Hurwitz, S. (1996). Effect of diurnal cycling versus constant temperatures on chicken growth and food intake. Br. Poult. Sci., 37: 43-54.

Yahav, S., Straschnow, A., Plavnik, I. and Hurwitz S. (1997). Blood system response of chickens to changes in environmental temperature. Poult. Sci., 76: 627-633.

Yahav, S. (1999). The effect of constant and diurnal cyclic temperatures on performance and blood system of young turkeys. J. Therm. Biol., 24: 71-78.

Yahav, S. (2000). Domestic fowl-strategies to comfort evironmental conditions. Avian Poult. Biol. Rev., 11: 81-95.

Yahav, S. (2002). Limitations in energy intake affect the ability of young turkeys to cope with low ambient temperatures. J. Therm. Biol., 27: 103-108.

Yassin, O. E. (1988). Poultry industry in Sudan. In: International Symposium on the Development of Animal Resources (Programme and Abstract). On the occasion of the 50th anniversary of the Faculty of Veterinary Science, University of Khartoum – Sudan., pp. 37-39.

Yousef M. K., Egbunike, G. N. (1979). The relative important of dry- and wetbulb temperatures in the thermorespiratory function in the chicken. Zentralbl. Veterinarmed., 26:573–579.

Zago, M. and Oteiza, P. I. (2001). The antioxidant properties of zinc: Interactions with iron and antioxidants. Free Radic. Biol. Med., 31: 266–274.

Zantop, D. W. (1997). Principles and Applications. In: Avian Medicine. (Edited by Wingers P.), pp: 115–129. Lake Worth.

Zeigler, T.R., Leach, R.M., Norris, L.C. and Scott, M.L. (1961). Zinc requirement of the chick. Factors affecting requirement. Poult. Sci., 40: 1584-1593.

بسم الله الرحمن الرحيم

مستخلص الاطروحة

اجريت الدراسة لمعرفة تاثير سلفات الزنك وحامض الاسكوربيك (فيتامين ج) المضافة الي العليقة ، علي تنظيم درجة حرارة الجسم والانتاجية في الدجاج اللاحم. اثبتت الدراسة في التجربة الاولي، تاثيرات الزنك المضافة للعليقة علي الاستجابات الفسيولوجية والانتاجية في فراخ اللاحم في فصل الصيف. ان الزنك لم يؤثر تاثيراً واضحاً علي درجة حرارة جسم الفراخ. اما بالنسبة لكمية الغذاء، ووزن الجسم ونسبة تحويل الغذاء زادت بشكل ملحوظ بالنسبة للمجموعة التي اضيف الي عليقتها الزنك بتركيز 250، 500، 750، 1000 ملجم/ كجم في كل الاسابيع من العمر. لوحظ ان هنالك زيادة في حجم خلايا الدم المرصوص بكل مستويات الزنك وفي كل مراحل العمر. نقص مستوي جلوكوز البلازما بشكل ملحوظ في الفراخ بكل مستويات الزنك وفي كل مراحل النمو. نقص مستوي الكوليسترول بشكل ملحوظ في الفراخ بكل مستويات الزنك وفي كل مراحل النمو، وكذلك انخفض مع التقدم في العمر. مستويات البروتين الكلي والالبومين زادت بكل نسب الزنك المضافة للعليقة وفي كل مراحل النمو، كذلك زادت نسبهم مع التقدم في العمر. نقصت نسبة ال اي ال تي و ال اي اس تي بشكل ملحوظ بكل نسب الزنك وفي كل مراحل النمو. زادت نسب الزنك في المصل بشكل ملحوظ في الفراخ بكل مستويات الزنك المضافة الي العلف في الاسبوع الرابع والخامس من العمر. اثبتت الدراسة في التجربة الثانية ، تاثيرات الزنك والاسكوربيك اسيد المضافة للعليقة علي الاستجابات الفسيولوجية والانتاجية في فراخ الروس في فصلي الشتاء والصيف. ان الزنك والاسكوربيك لم يؤثران تاثيراً واضحاً علي درجة حرارة جسم الفراخ. زادت درجة حرارة الجسم بشكل ملحوظ في الصيف عن ماهو في الشتاء في كل مراحل النمو. كمية الغذاء، ووزن الجسم ونسبة تحويل الغذاء لم تتاثر تاثيرا ملحوظا بنسب الزنك والاسكوربيك المضافي الي العليقة وفي كل مراحل التجربة. كمية الغذاء، ووزن الجسم ونسبة تحويل الغذاء زادت بشكل ملحوظ في الشتاء عن ماهو في الصيف وفي كل مراحل النمو. حجم خلايا الدم المرصوص لم يتاثر بنسب الزنك وحامض الاسكوربيك المضافة الي العليقة وفي كل مراحل النمو. حجم خلايا الدم المرصوص زادت بشكل ملحوظ في الشتاء مقارنة مع الصيف.لوحظ نقصان مستوي جلوكوز البلازما في كل الاضافات

العلاجية المستخدمة في الاسبوع الرابع والخامس للعمر في الصيف، وكذلك نقصان في مستوي الجلوكوز في البلازما في كل المجموعات التجريبية المعالجة باضافة الزنك وحامض الاسكوربيك بشكل ملحوظ في الاسبع الرابع من العمر في الشتاء، في الاسبوع الخامس والسادس من العمر نقص مستوي الجلوكوز للفراخ في مجموعة المضاف اليها الزنك و حامض الاسكوربيك في الشتاء. نقص مستوي الكوليسترول بشكل ملحوظ في الفراخ بكل المجموعات المعالجة وفي الاسبوع الخامس من العمر في فصل الصيف، نقص ايضا في الاسبوع الرابع والسادس في جميع المعالجات في الشتاء. مستويات البروتين الكلي والالبومين زادت بكل المعالجات المضافة للعليقة وفي كل مراحل النمو في الصيف. ايضا زاد بشكل ملحوظ في مجموعة حامض الاسكوربيك في الاسبوع الرابع والسادس من العمر في الشتاء. زاد مستوي الالبومين في مجموعة حامض الاسكوربيك بشكل ملحوظ في الاسبوع السادس من العمر في الصيف، وايضا زاد بشكل ملحوظ بكل المعالجات في الاسبوع الرابع والخامس والسادس من العمر في الشتاء. نقصت نسبة ال اي اس تي بشكل ملحوظ بكل المجموعات المعالجة وفي كل مراحل النمو في الصيف. ايضا نقص بشكل ملحوظ بكل المجاميع المعالجة في الاسبوع الثاني والربع والسادس من العمر في الشتاء. ال اي ال تي نقص بشكل ملحوظ بكل الاضافات العلفية وفي كل مراحل النمو اثناء الصيف. في فصل الشتاء انخفض مستوي ال اي ال تي وبشكل ملحوظ بكل الاضافات العلفية المستخدمة وفي كل مراحل النمو. النتائج التي تعلقت بالتنظيم الحراري والاستراتيجات التغذية تبني لتخفيف الاجهاد الحراري في الدجاج اللاحم نوقشا علي ضوء النتائج السابقة المذكورة في الدراسات. نتائج الدراسات لها نتيجة في تحسين اداء النمو في الدجاج اللاحم تحت الشروط الاستوائية.