A comparison of inorganic and organic sources of zinc to improve bone mineralisation and growth in broilers subjected to high ambient temperatures

by

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DECLARATION

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

_____

A de Villiers
University of Pretoria
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LIST OF ABBREVIATIONS

3D	Three dimensional
AZ	*Availa*®Zn, organic treatment A
BHT	Butylated hydroxytoluene
Ca	Calcium
CF	Crude fibre
CFI	Cumulative feed intake
Chol	Cholesterol
Cl	Chlorine
CP	Crude protein
Cr	Chromium
Cu	Copper
ddH_2O	Distilled water
DM	Dry matter
DNA	Deoxyribonucleic acid
ED	Embryonic day
EE	Ether extract
FCR	Feed conversion ratio
FI	Feed intake
Fe	Iron
GLM	Generalized linear model
HSP	Heat shock protein
Ig	Immunoglobulin
IZ	*IntelliBond*®*Z*, organic treatment B
K	Potassium
MDA	Malondialdehyde
Mg	Magnesium
Mg/dL	Milligram per deciliter
Mg/kg	Milligram per kilogram
Mmol	Millimol
Mn	Manganese

mol/liter	Molar concentration per liter
MUC	Mucin gene
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate (Reduced Form)
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
Nm	Nanometer
NO	Nitric acid
NRC	National Research Council
O_2	Oxygen
OD value	Odometer reading
P	Phosphorous
Ppm	Parts per million
RNA	Ribonucleic acid
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TD	Tibial dyschondroplasia
Zn	Zinc
$ZnSO_4$	Zinc sulphate

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Abstract

This study investigated the effects of organic and inorganic Zinc (Zn) supplementation on heat stressed, male Ross broilers. Birds were randomly assigned to 12 treatment groups, consisting of 8 replications each. Each dietary treatment was provided to broilers subjected to either thermoneutral conditions or cyclic heat stress. Dietary treatments consisted of inorganic feed grade Zn sulphate, *Availa*®Zn (Zinpro Performance Minerals, Zinpro Corporation) or *IntelliBond*®*Z* (Micronutrients, Selko, Nutreco). Control treatments comprised of inorganic feed grade Zn sulphate, supplemented at 80 mg/kg and 120 mg/kg inclusion level. Treatment diets contained 40 mg/kg Zn sulphate supplemented with either 40 mg/kg or 80 mg/kg *Availa*®Zn or *IntelliBond*®*Z.* High temperature stress started on day 9 and simulated a cyclic heat wave.

Subjecting birds to high temperature stress resulted in significantly ($P < 0.05$) lighter body weight, lower feed intake and higher FCR compared to birds that were exposed to thermoneutral conditions. Birds supplemented with organic Zn presented with significantly ($P < 0.05$) heavier body weights and higher feed intakes, compared to inorganic Zn supplemented groups. Hepatic tissues were analysed for total malondialdehyde concentration and total antioxidative capacity at 35 days of age. No significant differences ($P > 0.05$) were found in malondialdehyde concentration or total antioxidative capacity between birds supplemented with different Zn sources and inclusion levels, under both temperature treatments. Blood samples were analysed for cholesterol concentration, but no significant differences ($P > 0.05$) were observed between birds supplemented with different Zn sources, at both inclusion levels and temperature treatments.

Tibial samples were analysed for dry matter percentage, ash percentage, Zn concentration in bone ash (mg/kg tibia ash) and Zn concentration in dry bone (g/kg dry tibia). Dry matter and ash percentage were not significantly affected by Zn source or Zn inclusion level. Birds supplemented with *Availa*®Zn presented with the highest concentrations of Zn in bone ash. Broilers presented with significantly ($P < 0.05$) higher tibia ash concentrations when organic, rather than inorganic, Zn were supplemented at 120 mg/kg inclusion level. The same was observed in Zn concentration in dry bone. Zn concentration in dry bone was the highest in birds supplemented with *Availa*®Zn and the lowest in Zn sulphate supplemented birds. At 120 mg/kg

inclusion level, under both temperature treatments birds presented with significantly higher ($P < 0.05$) Zn concentration in bone.

From our results we concluded that broilers supplemented with organic Zn, at 120 mg/kg inclusion level, offered a feasible, inexpensive way to alleviate the effects of hyperthermia and the performance losses associated with heat stress prevalent in sub-Saharan countries.

Chapter 1

Introduction

High ambient temperatures have been classified as a significant source of stress for birds across the world (Sahin *et al.,* 2005), adversely affecting animal welfare and production parameters (Quinteiro-Filho *et al.,* 2010). The northern region of South Africa reportedly has heat waves 8 to 12 times a year, where maximum temperatures exceed 30°C for 5 or more consecutive days (Schulze and Maharaj, 2007). In various studies poultry presented with severely depressed feed intake and growth rates when subjected to high temperature stress (Yalçin *et al.,* 1998; Sahin *et al.,* 2009; Quinteiro-Filho *et al.,* 2010; Syafwan *et al.,* 2011). High temperature stressed broilers presented with nutrient impairment (Sahin *et al.,* 2005), immunosuppression (Sohail *et al.,* 2015) and compromised intestinal mucosal barrier function (Burkholder *et al.,* 2008). Lower mineral absorption and increased levels of excretion were found in stressed birds (Sahin *et al.,* 2009) leading to increased mineral requirements and exacerbated marginal mineral deficiencies (Sahin *et al.,* 2002). The deteriorated mineral availability associated with hyperthermic stress in broilers (Sahin *et al.,* 2009) resulted in weak, poorly mineralised skeletal systems (Štofaníková *et al*., 2011) with porous bones and reduced calcification (Williams *et al.,* 2000; Świątkiewicz and Arczewska-Wlosek, 2012). This resulted in birds with increased incidences of bone abnormalities (Świątkiewicz and Arczewska-Wlosek, 2012), fractures (Blake and Fogelman, 2002; Molnár *et al.,* 2010; Štofaníková *et al.,* 2012) and locomotory issues (Oviedo-Rondón *et al.,* 2009). Broilers suffering from leg welfare disorders were unable to reach or consume feed and water, resulting in starvation, dehydration and death (Bradshaw *et al.,* 2002; Groves and Muir, 2011). Affected birds laid down more and reduced movement, lessening time spent feeding (Bradshaw *et al.,* 2002). This further reduced the already depressed body weight gain and growth seen in broilers during hyperthermic stress (Bradshaw *et al.,* 2002; Sahin *et al.,* 2009).

During high temperature stress cell membrane (lipid) peroxidation is induced, resulting in increased concentrations of malondialdehyde (Ismail *et al.,* 2015) and altered cell membrane integrities (Kucuk *et al.,* 2003). Malondialdehyde concentration is directly proportional to the degree of lipid peroxidation (Sehirli *et al.,* 2008; Yousef *et al.,* 2009) and increased concentrations signifies toxic cellular stress (Zhao *et al.,* 2014) and reactive oxygen species production (Yang *et al.,* 2010). High concentrations of malondialdehyde have been associated

with reductions of serum and liver antioxidative enzyme levels (Sahin *et al.*, 2001; Ma *et al.*, 2011). Reactive oxygen species leads to imbalanced oxidant and antioxidant defense systems and oxidative damage to DNA and proteins (Lin *et al.*, 2006) and biological molecules (Harsini *et al.*, 2012).

Studies have shown that supplementary minerals, especially antioxidant vitamins such as vitamin A, E and C, as well as Zn and Cr could successfully be used to alleviate high temperature stress in broilers (Sahin and Kucuk, 2001; Sahin *et al.*, 2002; Bartlett and Smith, 2003; Sahin *et al.*, 2009; Ismail *et al.*, 2015). Mineral and vitamin supplements acted to alleviate the marginal mineral and vitamin deficiencies induced by high temperature stress (Bartlett and Smith, 2003) and improved biological performance parameters (Nollet *et al*., 2007; Peric *et al*., 2007; Salim *et al.*, 2011). Significant differences were observed in tibia, liver and excreta Zn when birds were supplemented with Zn proteinate compared to Zn oxide, sulphide and carbonate (Idowu *et al.*, 2011). Feng *et al*. (2010) and Salim *et al*. (2012) found serum Zn and plasma Ca levels increased when broilers were supplemented with organic Zn, where Hudson *et al*. (2004) observed greater concentrations of skeletal Zn when organic Zn was supplemented.

The aim of this project was to find if supplementing Zn at an optimal level could present a feasible, inexpensive way to alleviate heat induced production losses through reducing locomotory issues and malondialdehyde, while simultaneously improving bone mineralisation and total antioxidant capacity in broilers.

Hypothesis: Zinc supplementation will alleviate high temperature stress and associated losses in poultry while improving skeletal strength and decreasing cellular stress parameters.

Chapter 2

Literature Review

2.1. Introduction

Animal well-being has recently become a top priority in the public eye, leading to adjustments in laws and welfare codes to protect production animals from distress and fear (Quinteiro-Filho *et al.,* 2010). High ambient temperatures are a significant source of stress for birds across the world (Sahin *et al.,* 2005). High temperatures in tropical and subtropical regions, such as South Africa, have led to broilers with reduced feed intakes (Sahin *et al.,* 2005) and poor weight gain (Kucuk *et al.,* 2003; Sahin *et al.,* 2009). Poultry subjected to high temperatures also presented with increased lipid peroxidation in cell membranes (Ismail *et al.,* 2015) and augmented mineral tissue mobilisation (Sahin *et al.,* 2002). Subjecting poultry with subclinical leg disorders to hyperthermic conditions resulted in birds with further depressed feed intakes as birds could not reach feed and water (Bradshaw *et al.,* 2002; Groves and Muir, 2011). Mortalities resulting from leg abnormalities in broiler flocks in the U.S. have been reported to be 1.1%, with a further 2.1% loss due to condemnations and downgrades during processing (Talaty *et al.,* 2009a). Bone ailments resulted in an estimated loss of several hundred million dollars per year (Rath *et al.,* 2000; Štofaníková *et al.,* 2012). Limiting production losses in poultry resulting from high temperature stress (Sahin *et al.,* 2009) and locomotory issues (Rath *et al.,* 2000; Štofaníková *et al.,* 2012) could therefore lead to significant savings in sub-Saharan countries such as South Africa.

2.2. The South African poultry industry

The South African poultry industry contributes 18% of the total agricultural gross value and 39% of animal product gross value (SAPA, 2016). Poultry is considered the most affordable source of animal protein in South Africa (Bureau for Food and Agricultural Policy and National Agricultural Marketing Council, 2016) and is the largest single contributor to the South African agricultural industry, slaughtering 927.1 million broilers in 2017 (SAPA, 2016). Egg production accounts for 24% of poultry production, where the other 76% of birds are used for meat (SAPA, 2016). The poultry industry is also the second

largest maize consumer in South Africa (SAPA, 2016). Rising feed costs and increased numbers of imports (53%) have, however, influenced the competitiveness of the South African poultry industry (Bureau for Food and Agricultural Policy and National Agricultural Marketing Council, 2016).

2.3. Heat stress in broilers

South Africa is classified as a subtropical country with warm temperature conditions, resulting from the altitude of the interior plateau and the neighbouring of ocean on 3 sides (South African year book, 2015/2016). The South African weather service defines a heat wave as temperatures that exceed the average maximum temperature in a particular area by 5℃ for 3 days or more (Weather SA, 2017). When the daily maximum temperature exceeds the 90th percentile during austral summer (December to February), it is considered a heat wave (Lyon, 2009). Heat waves of temperatures exceeding 35℃ for 3 or more days occur 8 to 10 times per annum in the North West region of South Africa and 2 to 4 times in northern and eastern Limpopo (Schulze and Maharaj, 2007). Heat waves, with maximum temperatures exceeding 30℃ for 5 or more consecutive days, occur annually 8 to 12 times in the northern and eastern regions of Limpopo, 6 to 10 times in the west and twice per annum over the eastern areas (Schulze and Maharaj, 2007).

High ambient temperatures are a significant source of stress for birds across the world (Sahin *et al.,* 2005). Hyperthermia is especially damaging in poultry due to birds' feather covering, an absence of sweat glands and a high body temperature (41.5℃) (Sahin *et al.,* 2009). High temperatures in tropical and subtropical regions predispose broilers to reduced feed intakes (Sahin *et al.,* 2005), poor weight gain (Kucuk *et al.,* 2003; Sahin *et al.,* 2009) and immunosuppression (Sohail *et al.,* 2015). Stressed broilers have been shown to suffer multiple physiological disturbances, including endocrine disorders (Sohail *et al.,* 2012), electrolyte imbalances (Teeter *et al.,* 1985) and systemic immune dysregulation (Sohail *et al.,* 2010). Hyperthermia symptoms can present in numerous ways, displaying as disrupted electrolyte balance, hyperpnoea, weakness and muscle tremors (Bogin *et al.,* 1996). Birds mainly use respiratory evaporative cooling to dissipate heat, which can lead to dehydration (Santos *et al.,* 2015), altered metabolic processes (Bogin *et al.,* 1996) and gut damage (Quinteiro-Filho *et al.,* 2010). Excessive panting could lead to polypnoea and a subsequent shift in the blood gas balance (Bogin *et al.,* 1996). Consequentially, respiratory alkalosis

would result from lost carbon dioxide and oxygen enrichment in the blood (Bowen and Washburn, 1985). A substantial increase in kidney enzyme activities have been found in heat stressed broilers, acting to compensate the blood pH change associated with panting and hyperventilation (Bogin *et al.,* 1996). If animals are unable to alleviate hyperthermia through compensatory mechanisms such as sweating or panting, death will likely result (Sahin *et al.,* 2009).

2.3.1. Consequences of heat stress on the broiler

2.3.1.1. Performance

Climatic, environmental, social, psychological and nutritional stress adversely affects animal welfare and production parameters (Quinteiro-Filho *et al.,* 2010). In multiple studies (Yalçin *et al.,* 1998; Sahin *et al.,* 2009; Quinteiro-Filho *et al.,* 2010; Syafwan *et al.,* 2011), broilers under hyperthermic stress presented with severely depressed growth rates and feed intakes. Broilers subjected to high temperature stress presented with reductions in feed efficiencies (Geraert *et al.,* 1996; Sahin *et al.,* 2005; Sahin *et al.,* 2009), nitrogen retention and protein digestibility (Sahin *et al.,* 2005). Poultry subjected to hyperthermic conditions presented with lower net yields (Yalçin *et al.,* 1998) and higher mortality (Syafwan *et al.,* 2011), especially close to slaughter (Arjona *et al.,* 1990) and during transportation (Mitchell and Kettlewell, 1998; Sahin *et al.,* 2009). Undesirable meat characteristics have been associated with rapid growth during heat stress in broilers (Sandercock *et al.,* 2001). Studies showed an association between pale, exudative meat (Owens *et al.,* 2000) and increased mortality resulting from high temperature stress in turkeys (Evans *et al.,* 2000). If the thermoneutral zone is exceeded and ambient temperatures surpass 20°C, a 17% reduction in feed intake could be expected for every 10°C increase in temperature (Bartlett and Smith, 2003). Broilers subjected to temperatures above 32°C post-hatch showed a 14% reduction in feed intake after four weeks, reducing to 24% after 6 weeks (Geraert *et al.,* 1996; Bartlett and Smith, 2003). Sahin *et al*. (2009) stated broilers exposed to 32°C presented a 14% reduction in feed intake. The reductions in feed intake were suggested to be responsible for the lower broiler growth rates during heat stress (Hurwitz *et al.,* 1980; Leksrisompong *et al.,* 2009). Bartlett and Smith (2003) stated broilers reduced their feed consumption to alleviate the metabolic heat increment associated with feeding (Teeter *et al.,* 1985). The decreased feed intake resulted in lower weight gains, feed efficiencies and depressed growth rates (Bartlett and Smith, 2003; Sahin *et al.,* 2009). Poultry under hyperthermic

conditions with subclinical leg disorders presented with further depressions in feed intakes (Bradshaw *et al.,* 2002; Groves and Muir, 2011). This was due to birds spending more time lying down and less time eating, as they suffered from walking constraints (Bradshaw *et al.,* 2002; Groves and Muir, 2011). Nutrient digestibility was also found to be severely suppressed in broilers under hyperthermic conditions (Sahin and Kucuk, 2003). Broilers subjected to 32°C showed significant reductions in activities of trypsin, chymotrypsin and amylase (Hai *et al.,* 2000). Reductions in starch, protein and fat digestibility were also found in heat stressed birds (Bonnet *et al.,* 1997; Sahin *et al.,* 2009). Broilers exposed to high ambient temperatures presented with alterations in blood levels of electrolytes, glucose, uric acid, calcium, inorganic phosphorous and thyroxine (Bogin *et al.,* 1996). Deyhim *et al*. (1995) found chicks exposed to high temperatures had hyperglycemia, with decreased plasma protein and serum triglycerides. Heat stressed chicks presented with decreased protein synthesis and nitrogen deposition, which led to reductions in serum protein, albumin and uric acid (Sonaiya *et al.,* 1989; Deyhim *et al.,* 1995).

Quinteiro-Filho *et al,* (2010) suggested that corticosterone was responsible for the reduced feed intake associated with high temperature stress. During hyperthermic conditions, cytokines such as interleukin-1 are released, stimulating immune cells to activate the hypothalamus and release corticotrophin-releasing hormone (Quinteiro-Filho *et al.,* 2010). Corticotrophin-releasing hormone stimulates the pituitary gland and adrenal cortex to release adrenocorticotropic hormone and corticosterone (Yang & Glaser, 2002). Increased corticosterone negatively influences the hypothalamic nuclei through regulation of food and intake satisfaction, resulting in reduced weight gain (Quinteiro-Filho *et al.,* 2010). Harsini *et al*. (2012) observed birds subjected to high temperature stress presented with elevated gluconeogenesis and corticosterone induced muscle catabolism. This was in agreement with Lin *et al*. (2004) who observed corticosterone induced gluconeogenesis and muscle proteolysis in broilers subjected to hyperthermic stress. Broilers with higher corticosterone levels presented with increased serum uric acid and glucose (Lin *et al.,* 2004) as amino acid to glucose conversion was amplified (Malheiros *et al.,* 2003). Depressed feed intake during heat stress could also be attributed to the release of certain interleukins (i.e. interleukin-1b) (Costa-Pinto *et al.,* 2009). Interleukin release, in response to heat induced intestinal inflammation, would induce sickness related behaviour in birds, such as reduced feed intake (Costa-Pinto *et al.,* 2009).

2.3.1.2. Mineral status

Broilers raised in high cycling temperatures, compared to thermoneutral conditions, showed higher mineral excretion (Sahin *et al.,* 2009) and lower retention of Zn, Ca, Fe, P, Na, K, Mg, S, Mn and Cu (Sahin *et al.*, 2001; 2005; 2009). Reductions in tissue and serum levels of antioxidant vitamins A, E and C (Sahin and Kucuk, 2003), as well as zinc and chromium were observed in broilers exposed to high temperatures (Sahin *et al.,* 2001; Sahin *et al.,* 2002). Sahin *et al.* (2002) observed increased tissue mobilisation of Cr and Zn in broilers during periods of high temperature stress. This increased broiler mineral requirement and exacerbated marginal Cr and Zn deficiencies (Sahin *et al.,* 2002). Reductions in Fe, Zn and Cr levels in serum and liver have been observed during hyperthermic conditions in broilers (Sahin *et al.,* 2001). Mineral reductions were attributed to either decreased feed intake, increased vitamin or mineral excretion, or because vitamins and minerals were broken down by the products of oxidative stress (Sahin *et al.,* 2005). The deteriorated mineral availability associated with heat stress in broilers (Sahin *et al.,* 2009) led to weak bones (Štofaníková *et al.,* 2011). Bone mineralisation affected bone strength (Štofaníková *et al.,* 2011) and poorly mineralised bones increased incidences of fractures (Blake and Fogelman, 2002; Molnár *et al.*, 2010).

2.3.1.3. Immune system

High temperatures in tropical and subtropical regions have been found to predispose broilers to immunosuppression (Sohail *et al.,* 2015). Birds exposed to high temperature stress presented with compromised cellular and humoral immune responses (Bartlett and Smith, 2003). Young birds showed impaired immune functions (Quinteiro-Filho *et al.,* 2010) and depressed specific humoral responses during high temperature stress (Sahin *et al.,* 2005). Animals under stress exhibited mild reductions in natural killer cells (Zorrilla *et al.,* 2001), increased overall leukocytosis (Quinteiro-Filho *et al.,* 2010) and decreased T-cell functions (Zorrilla *et al.,* 2001; Quinteiro-Filho *et al.,* 2010). Sahin *et al.* (2005) observed increased heterophil-to-lymphocyte ratios in broilers during high temperature stress. Broilers exposed to high temperatures presented with reduced antibody production (Sahin *et al.,* 2005) and significant reductions in lymphoid organ weights (Bartlett and Smith, 2003). Environmental stressors were found to increase the risk and severity of infections (Glaser and Kiecolt-Glaser, 2005; Quinteiro-Filho *et al.,* 2012). Electrolyte imbalances, decreased spleen weights and reductions in blood lymphocyte counts were

seen in avian species subjected to stress (Quinteiro-Filho *et al.*, 2010). Quinteiro-Filho *et al*. (2010) observed decreased macrophage activities, increased intestinal inflammation and enteritis in broilers exposed to acute and chronic heat stress.

2.3.1.4. Gastro intestinal health and gut integrity

The intestinal mucosal barrier is involved in the provision of adequate dietary nutrients, health maintenance and prevention of tissue injury and disease (Zhang *et al.*, 2012). The mucosal barrier is often described as the first line of defense in the bird (Zhang *et al.*, 2012), as food and microorganisms carrying heavy loads of antigenic molecules are continuously passed through the intestinal tract (Quinteiro-Filho *et al.*, 2012; Song *et al.*, 2013). Environmental stressors, such as hyperthermia, have been found to compromise intestinal mucosal barrier function (Burkholder *et al.*, 2008). The intestinal mucosa barrier contains epithelium that forms a continuous intact physical barrier with interspersing tight junctions (Zhang *et al.*, 2012). During stressful conditions, bacterial pathogens can transverse the mucosal epithelial cells into the underlying mucous layer (Opitz *et al.*, 2007). Bacteria will then alter mucin secretion (Zhang *et al.*, 2012), diminishing mucosa and lumen protection (Opitz *et al.*, 2007). Bacterial pathogens modify tight junction proteins (Zhang *et al.*, 2012) resulting in increased paracellular permeability and disrupted tight junction integrity (Parlesak *et al.*, 2000; Kucharzik *et al.*, 2001). Pathogens, bacterial endotoxins, macromolecules and antigens would consequently cross through the intestinal lumen into circulation (Parlesak *et al.*, 2000; Kucharzik *et al.*, 2001) resulting in systemic inflammation (Quinteiro-Filho *et al*. 2010; Quinteiro-Filho *et al.*, 2012). Increased antigen and bacterial passage in broilers with damaged intestinal mucosa were found to lead to imbalanced anti- and pro-inflammatory molecules (Song *et al.*, 2013). Increased intestinal inflammatory infiltrates were found to augment production of pro-inflammatory cytokines such as interleukin-1, contributing to diminished permeability of tight junctions (Quinteiro-Filho *et al.*, 2010).

Depressed nutrient absorption, nutrient supply and health have been found in animals with stress induced intestinal damage (Burkholder *et al.*, 2008; Zhang *et al.*, 2012). Destabilised microbial communities in broiler gastrointestinal tracts have been observed during disease, high temperature stress and dietary changes (Traub-Dargatz *et al.*, 2006; Sohail *et al.*, 2015). Broilers exposed to high temperatures presented with modified commensal microbiota activities, leading to pathogenic micro-organism colonisation (Brisbin *et al.*,

2008). Birds subjected to high temperatures presented with enhanced pathogen colonisation and shedding, negatively influencing food safety (Traub-Dargatz *et al.*, 2006; Sohail *et al.*, 2015). Horizontal pathogen transmission between heat stressed birds were seen (Quinteiro-Filho *et al.*, 2012), resulting in amplified pathogen shedding and contamination during processing (Jones *et al.*, 2001). High temperature stress has also been found to induce enteritis in broilers, disrupting microbiota even further (Quinteiro-Filho *et al.*, 2010).

Santos *et al*. (2015) stated intestinal damage could be used as a valuable tool to objectively evaluate damage caused to the intestine by elevated temperatures. Various methods are available to determine intestinal mucosa integrity and small intestinal function. Sucrase activity, villus height to crypt depth ratio (Lamb-Rosteski *et al.*, 2008; Zhang *et al.*, 2012), villus height, villus width, surface area and crypt depth could all be used to evaluate intestinal function (Song *et al.*, 2014). Intestinal lesions could be scored using the Chiu or Park scale (Quaedackers *et al.*, 2000) or histopathologically (Quinteiro-Filho *et al.*, 2010). Lesions would be classified as mild, moderate or severe, but scoring would only be semi-quantitative (Quinteiro-Filho *et al.*, 2010; Santos *et al.*, 2015). MUC2 is the major gene responsible for mucous expression and could be used to measure intestinal function through analysing the thickness of the intestinal mucous layer, thus indicating damage caused by high temperature stress (Zhang *et al.*, 2012).

2.3.1.5. Antioxidant system and production of reactive oxidative species

The principle components of the antioxidant enzymatic system, which act to neutralise oxidative stress and protect against DNA deterioration include Cu-Zn superoxide dismutase, catalase and glutathione peroxidase (Cadenas and Davies, 2000; Lu *et al.*, 2010; Ismail *et al.*, 2015). The non-enzymatic antioxidant system comprises of glutathione, selenium and vitamins C and E (Cadenas and Davies, 2000; Lu *et al.*, 2010). Superoxide dismutase utilises either catalase or glutathione peroxidase to convert highly cytotoxic, superoxide radicals into hydrogen peroxide, molecular oxygen or water (Ismail *et al.*, 2015). Catalase protects the mitochondrial membrane against reactive oxidative species (ROS) and removes peroxide from the body (Duzguner and Kaya, 2007; Jiang *et al.*, 2009; Zhao *et al.*, 2014). Glutathione peroxidase scavenges for ROS and acts in the conversion of lipids and hydroperoxidases into non-cytotoxic products (Ismail *et al.*, 2015). Conversion occurs through conjugation of reduced glutathione with xenobiotic substances, catalysed by glutathione-S transferase (El-Bahr *et al.*, 2013). Oxidised glutathione can

regenerate glutathione-S transferase, using the NADPH glutathione reductase system (El-Bahr *et al.,* 2013).

Hyperthermia induces physiological and biochemical changes that promote ROS formation (Mujahid *et al.,* 2007; Harsini *et al.,* 2012). This leads to lipid peroxidation, imbalanced oxidant and antioxidant defense systems and oxidative damage to DNA, proteins (Lin *et al.,* 2006) and biological molecules (Harsini *et al.,* 2012). Total antioxidant capacity is a contributor to active oxygen balance, which can be utilised to reflect antioxidant status in body fluids and serum (Zhao *et al.,* 2014). In the reduction of molecular oxygen to water, superoxide anions can be generated through electron leakage (Ismail *et al.,* 2015). Electron leakage could also occur during dismutation of superoxide anions in mitochondrial respiration, generating ROS (Lin *et al.,* 2006). During periods of stress, pro-oxidant and high free radical production can overwhelm the antioxidant system (Sies, 1991; Ismail *et al.,* 2015). Both of these will lead to oxidative stress, resulting in macromolecule, cellular, DNA and tissue damage (Sies, 1991; Ismail *et al.,* 2015). During high temperature stress cell membrane (lipid) peroxidation is induced by corticosterone and catecholamines (Ismail *et al.,* 2015). This leads to increased concentrations of malondialdehyde (MDA), a biomarker used to indicate lipid peroxidation and subsequent cellular stress (Ismail *et al.,* 2015). MDA causes toxic cellular stress (Zhao *et al.,* 2014) and has been found to be directly proportional to the degree of lipid peroxidation (Sehirli *et al.,* 2008; Yousef *et al.,* 2009). Halliwell and Gutteridge (1989), Sahin *et al.* (2002) and Naziroglu *et al.* (2000) found birds subjected to high temperature stress presented with augmented lipid peroxidation and free radical production. Free radicals were found to induce cellular peroxidation, leading to altered cell membrane integrities and abnormal polyunsaturated fatty acids (Kucuk *et al.,* 2003). Mujahid *et al.* (2009), Wang *et al.* (2009) and Harsini *et al*. (2012) found MDA concentration increased in skeletal muscle of heat stressed broilers, indicating lipid peroxidation. Elevated MDA concentrations were found in broilers (Sahin *et al.,* 2001; Sahin *et al.,* 2002), Japanese quails (Sahin *et al.,* 2002), and laying hens exposed to high ambient temperatures (Naziroglu *et al.,* 2000; Sahin and Kucuk, 2001). El-Shaieb *et al.* (2009) and Ismail *et al.* (2015) reported high MDA concentrations in broilers under hyperthermic conditions. Azad *et al.* (2010) reported higher concentrations of MDA in broilers during periods of acute, compared to chronic heat exposure. Increased concentrations of MDA were found to coincide with decreased concentrations of Cu-Zn superoxide dismutase, glutathione peroxidase and catalyse in serum and liver of stressed

broilers (Sahin *et al.,* 2001; Ma *et al.,* 2011). Altan *et al.* (2003) observed antioxidative enzymes increased consistently with increases in serum and liver MDA. Perceived increases were found to be directly dependent on ROS production (Yang *et al.,* 2010). Ramnath *et al.* (2008) observed increased liver Cu-Zn superoxide dismutase, without concurrent increases in glutathione peroxidase in broilers during acute heat stress. MDA concomitantly increased with antioxidant enzymes (Cu-Zn superoxide dismutase, catalase, glutathione peroxidase) in liver and serum of birds subjected to high temperature stress (Yang *et al.,* 2010). MDA and antioxidant enzymes, however, decreased progressively to almost pre-stress levels after high temperature removal at recovery temperatures of 25°C (Yang *et al.,* 2010). Human studies showed increased antioxidant enzyme activities when MDA and free radical concentrations increased during stressful conditions such as disease and non-damaging exercise (McArdle and Jackson, 2000). Azad *et al.* (2010) found Cu-Zn superoxide dismutase activities in skeletal muscles were two times higher in birds subjected to high temperatures compared to birds kept under thermoneutral temperatures. Results signified birds entered the initial stages of antioxidant changes in response to stress related ROS formation (Azad *et al.,* 2010). Cu-Zn superoxide dismutase and glutathione peroxidase activities have thus been suggested to analyse the response of the enzymatic scavenging systems to high temperature stress (Azad *et al.,* 2010). Lin *et al.* (2004) reported increases in thiobarbituric acid reactive substances (TBARS), a lipid peroxidation biomarker, in liver and plasma when broilers were subjected to acute heat stress. Higher concentrations of TBARS were found in hepatic compared to cardiac tissues, suggesting hepatic tissues were more susceptible to oxidative stress. Lin *et al*. (2006) attributed their results to the higher levels of unsaturated fatty acids present in hepatic tissue.

2.4. Methods to alleviate hyperthermic stress in broiler production systems

There are various methods to alleviate high temperature stress in avian species, with many focusing on improving the direct environment of the bird (Sahin *et al.,* 2009). Approaches focus on increasing air flow and improving ventilation, increasing evaporative cooling and reducing stocking densities. Changing housing, cooling and ventilation systems, however, have economic constraints, making it unfeasible under commercial conditions (Sahin *et al.,* 2009). Cheaper, alternative strategies to combat heat stress, such as nutritional and dietary manipulations are therefore being investigated (Yalçin *et al.,* 2001). Nutritional approaches, such as increasing nutrient densities and altering protein and energy nutrition, have been used during high temperature stress (Sahin *et al.,* 2009). Nutritional

manipulations such as provision of supplementary fat have been applied to increase poultry energy intake without the associated heat increment of feed (Sahin *et al.,* 2009). Supplying balanced amino acids and reducing protein content have been utilised to improve protein uptake during high temperature stress (Sahin *et al.,* 2009). Supplementary probiotics have been successful in reversing dysbiosis of the gastrointestinal bacteria, through promotion of beneficial bacterial growth in broilers subjected to stress (Sohail *et al.,* 2015). Mineral and vitamin supplementation, especially antioxidant vitamins such as vitamin A, E and C, as well as Zn and Cr, have proven successful as alleviators of heat stress in multiple studies (Sahin and Kucuk, 2001; Sahin *et al.,* 2002; Bartlett and Smith, 2003; Sahin *et al.,* 2009; Ismail *et al.,* 2015). Mineral and vitamin supplements acted to alleviate the marginal mineral and vitamin deficiencies induced by high temperature stress (Bartlett and Smith, 2003). Cool, electrolyte or mineral (Na, Cl, K, and $NaHCO_3$) enriched water has proven successful in ameliorating heat stress in poultry (Balnave and Zhang, 1993). Results were attributed to the corrective effect supplementation had on the acid-base balance (Nollet *et al.,* 2008).

2.5. The function of zinc in poultry

Zinc is an essential trace mineral acting as cofactor for multiple enzymes and metallo-enzymes such as carboxypeptidases (Star *et al.,* 2012). Growth, skeletal development, feathering (Pinion *et al.,* 1995; Idowu *et al.,* 2011; Salim *et al.,* 2011), as well as wound healing (Zhang *et al,* 2012), skin collagen synthesis and even appetite regulation (Batal *et al.,* 2001; Idowu *et al.,* 2011) require Zn (Salim *et al.,* 2011). Zn plays a role in carbohydrate and energy metabolism, as well as hormone production and protein synthesis (Star *et al.,* 2012). DNA and RNA synthesis, as well as DNA polymerases, are affected by Zn (Star *et al.,* 2012). Skin quality maintenance requires Zn (Downs *et al.,* 2000) due to the participation of Zn during skin keratin and nucleic acid synthesis (Close, 1999). Superoxide dismutase, a component of macrophage and heterophil integrity, requires Zn and Mn for optimal function (Cook-Mills *et al.,* 1993; Virden *et al.,* 2003). Zn suppresses free radicals and inhibits glutathione depletion (Prasad, 1997; Kucuk *et al.,* 2003). Zn exerts a direct antioxidant function in poultry by binding Fe and Cu binding sites in cellular membranes (Prasad and Kucuk, 2002; Sahin *et al.,* 2005), lipids, DNA and proteins (Powell, 2000; Kucuk *et al.,* 2003). This displaces the transition minerals, thereby successively reducing free radical production (Prasad and Kucuk, 2002; Sahin *et al.,* 2005). Zn plays a role in the structural integrity and is a cofactor for Cu-Zn superoxide dismutase (Bray and Bettger,

1990; Courdray *et al.,* 1992). Zn further inhibits NADPH-dependent lipid peroxidation, thereby suppressing free radical formation (Prasad and Kucuk, 2002; Sahin *et al.,* 2005). Zn inhibits lipid peroxidation through prevention of glutathione depletion (Prasad and Kucuk, 2002; Sahin *et al.,* 2005) and has been found to inhibit the Cu efflux transporter, enhancing cellular Zn uptake *in vitro* (Reeves *et al.,* 1998; Sahin *et al.,* 2002). Reports stated Zn exhibited similar behaviour to vitamin E (Sahin *et al.,* 2002). Both vitamin E and Zn have been seen to protect red blood cell membranes, as well as stabilise and protect against peroxidation induced changes and damage (Sahin *et al.,* 2002). Dietary Zn supplementation resulted in increased metallothionein synthesis in tissues (Sahin *et al.,* 2009), which protected organs and tissues against immune-mediated reactive oxygen species attack (Taylor *et al.,* 1997; Ohly *et al.,* 2000; Ma *et al.,* 2011). Pancreatic function and enzyme secretion were improved by Zn supplementation, promoting digestion and alleviating the reductions in enzyme activities associated with hyperthermic stress (Sahin *et al.,* 2009). Supplementing Zn also protected pancreatic tissue against oxidative damage (Sahin *et al.,* 2009). Zn has gastro-hepatic protective effects (Sturniolo *et al.,* 2002), enhances intestinal mucosal repair and maintains epithelial barrier integrity, as well as gastrointestinal tract function in broilers (Zhang *et al.,* 2012). Under a variety of conditions including malnutrition, disease, stress and enteral pathogen challenge, Zn proved effective in alleviating intestinal permeability (Roselli *et al.,* 2003).

Shelton and Southern (2007) and Scrimgeour *et al*. (2007) found altered mechanical properties of bone, reductions in bone integrity and density in Zn deficient birds. Bone length and compact bone formation also deteriorated (Scrimgeour *et al*., 2007; Shelton and Southern, 2007), with decreased growth disc activity (Štofaníková *et al.,* 2011). This led to modifications in biomechanical competency of bone tissue and density, reducing weight bearing ability (Štofaníková *et al.,* 2011). Redistribution of Zn during high temperature stress was seen as a fourfold increase in hepatic Zn, with simultaneous decreases in plasma Zn (Bartlett and Smith, 2003). Altered Zn distribution was suggested to be an immunological adaptation to stress in order to repartition nutrients towards immune function (Bartlett and Smith, 2003). Immune aspects, including cellular immune integrity (Prasad and Kucuk, 2002), interleukin production (Sahin *et al.,* 2009) and T-lymphocyte development (Dardenne and Bach, 1993) were negatively influenced in Zn deficient poultry (Sahin *et al.,* 2009). Adverse effects were seen on the thymus and spleen (Sahin *et al.,* 2009), with imbalanced T helper-1 and T helper-2 cell functions in broilers with Zn

deficiencies (Shankar and Prasad, 1998). Broilers exposed to Zn deficient conditions presented with physical and pathological changes, including depressed growth, depressed thymic hormone circulation, reduced skin quality and poor feathering (Star *et al.,* 2012; Favero *et al.,* 2013). Chylomicrons in the enterocyte became defective when broilers were subjected to Zn deficient conditions, impairing absorption of vitamin A and E (Sahin *et al.,* 2005). This led to a vitamin A and E deficiency, as well as increased oxidative damage (Sahin *et al.,* 2005).

2.6. Leg welfare in broilers

Genetic selection has led to modern broilers requiring a third of the time to reach slaughter weights of 1.8 kg, compared to 20 years ago (Havenstein *et al.,* 2003). Broilers display extremely rapid increases in body weight in the first 5 to 6 weeks post-hatch (Uni and Ferket, 2004). This increased mechanical load resulting from the extreme growth of musculature has caused severe pressure on the skeletal system (Dibner *et al.,* 2007; Favero *et al.,* 2013). Subsequently, broiler skeletal systems have presented with poor calcification and porous bones (Williams *et al.,* 2000; Świątkiewicz and Arczewska-Wlosek, 2012) which has led to increased incidences of bone abnormalities (Świątkiewicz and Arczewska-Wlosek, 2012), fractures (Štofaníková *et al.,* 2012) and locomotory issues (Oviedo-Rondón *et al.,* 2009). Leg welfare implications in broilers have been classified as an inability to walk, frustration, leg weakness and pain (Bradshaw *et al.,* 2002). Affected birds laid down more and reduced movement, lessening time spent feeding (Bradshaw *et al.,* 2002). Other birds disturbed sleeping and resting patterns of immobile birds, scratching them and damaging their skin (Groves and Muir, 2011). This further reduced the already decreased body weight gains and growth seen in broilers during hyperthermic stress (Bradshaw *et al.,* 2002). Scratches on birds also led to cellulitis and eventual death (Groves and Muir, 2011). Mortalities resulting from leg abnormalities in broiler flocks in the U.S. have been reported to be 1.1%, with a further 2.1% loss due to condemnations and downgrades during processing (Talaty *et al.,* 2009a). Processing carcasses with weak skeletal systems led to bone breakages that negatively affected meat grade (Štofaníková *et al.,* 2011), processing quality and food safety (Oviedo-Rondón *et al.,* 2009). Rath *et al*. (2000) estimated losses of several hundred million dollars per year resulting from bone ailments in the poultry industry (Štofaníková *et al.,* 2012).

2.6.1. Factors affecting bone health

Rapid growth rates, genetic mutations, nutritional imbalances, as well as disease have been found to negatively affect bone health (Oviedo-Rondón *et al.,* 2009). Bone disorders and lameness conditions have been found to vary among breeder strains (Oviedo-Rondón *et al.,* 2009). Both Groves and Muir (2011) and Oviedo-Rondón *et al.* (2009) stated environmental management conditions such as incorrect lighting, stocking density, lack of exercise and litter quality negatively influenced bone health and affected the incidence of skeletal disorders. Developmental, degenerative and infectious diseases have also been found to result in lameness conditions (Groves and Muir, 2011). Excessive and rapid growth rates are stated to be a major factor influencing leg problems and abnormalities in modern meat-type birds (Talaty *et al.,* 2009b). Reichmann and Connor (1977) stated bone strength was influenced by the level of bone mineralisation (Štofaníková *et al.,* 2011). This was supported by increased levels of fractures seen in poorly mineralised bones (Štofaníková *et al.,* 2011). Mazzuco and Hester (2005) observed tibia breakage increased with corresponding decreases in tibia mineral density in white Leghorns (Talaty *et al.,* 2009b). Tibia mineralisation and porosity in rapid-growing broiler lines were found to be similar to slow-growing broiler lines when growth was restricted through feed restriction (Williams *et al.,* 2003; Talaty *et al.,* 2009b). Growth rate, not genotype, was therefore the major contributor to poor bone quality (Williams *et al.,* 2003). Oviedo-Rondón *et al*. (2009) reported males presented with higher incidences of leg problems such as crooked toes and valgus deformities. This was attributed to the faster growth rates and heavier weights associated with males (Oviedo-Rondón *et al.,* 2009). Oviedo-Rondón *et al*. (2009) further stated that even within the same sex skeletal problems were more prevalent within birds with faster growth rates. This was in agreement with Talaty *et al*. (2009b) who found bone mineral density increased when purebred male and female broilers were feed-restricted to prevent rapid body weight gain. Unrestricted growth led to less mineral dense and potentially more porous bones, prone to abnormalities and breakage (Talaty *et al.,* 2009b). Bone mineralisation has thus been suggested as a valuable measure of skeletal health (Talaty *et al.,* 2009b).

Degenerative leg conditions have also been found to negatively affect each other (Groves and Muir, 2011). Broilers with rickets, for example, were seen to be predisposed to rotated tibias (Crespo and Shivaprasad, 2008). Birds with both rickets and tibial dyschondroplasia presented with increased occurrences of varus and valgus deformities later in life (Thorp *et*

al., 2008). Flocks with infectious stunting syndrome were shown to be predisposed to rickets and tibial dyschondroplasia (Groves and Muir, 2011).

2.6.2. Ways to improve bone development in broilers

2.6.2.1. Breeder nutrition

The embryo is completely dependent on maternal nutrition for all its requirements (Romero-Sanchez *et al.,* 2008). Trace mineral and vitamin supplementation to the breeder hen must therefore supply the hen as well as the embryonic requirements (Virden *et al.,* 2003; Calini and Sirri, 2007; Torres *et al.,* 2009). Embryonic development, as well as hatching traits, will be influenced by maternal mineral and vitamin deficiencies, imbalances or excesses (Surai *et al.,* 2002; Kidd, 2009; Moraesa *et al.,* 2011). Maternal feeding and nutrition during rearing and lay will affect fertile egg production, yolk nutrients and progeny viability (Robinson *et al.,* 2007; Romero-Sanchez *et al.,* 2008; Moraesa *et al.,* 2011). Egg composition stays relatively constant (Calini and Sirri, 2007), but will be influenced by egg weight, breeder genetic strain and age (Yadgary *et al.,* 2010). An egg will generally comprise of 65% to 75% albumin, containing 88% water and 12% protein (Yadgary *et al.,* 2010). Yolk will generally contain 50% water, 15% protein and 33% fat, with less than 1% carbohydrates (Yadgary *et al.,* 2010). Trace mineral and vitamin concentration in the egg is, however, dependent on maternal mineral supplementation (Moraesa *et al.,* 2011).

Chicks are typically only exposed to an external nutrient source 36 to 48 hours post-hatch (Yair and Uni, 2011). This makes yolk the only source of energy for organ and skeletal development until external nutrients become available (Yair and Uni, 2011). Internalisation of the yolk sac will begin from ED 19 and comprise 15% to 20% of the chicks' body weight at hatch (Yadgary *et al.,* 2010). Prior to feed exposure, chicks have been seen to use 60% of the residual yolk to supply requirements (Noy and Sklan, 1999). Deficiencies in the yolk would thus negatively affect chick vitality, growth rate and development, even after feed exposure (Yadgary *et al.,* 2010). Mineral and nutrient depletion below nutritional requirements have been found in embryos during late incubation (Yair *et al.,* 2012). Yair and Uni, (2011) found levels of Cu, Zn, P, Fe and Mn in residual yolk were too low to adequately support metabolic requirement at the end of incubation. As bone growth has been found to be the most rapid a few days pre- and post-hatch (Applegate and Lilburn,

2002), skeletal mineralisation, development and bone strength were negatively affected by mineral depletion (Yair *et al.,* 2012). Mineral and vitamin depletion also affected bone modelling and remodeling negatively (Oviedo-Rondón *et al.*, 2006; Shim and Pesti, 2011). Newly hatched chicks have immature gastro-intestinal tracts, which in combination with delayed post hatch feed availability negatively influenced feed consumption and absorption (Angel *et al.,* 2007). This contributed to the already negative bone growth and mineralisation observed in chicks (Yair *et al.,* 2012).

Maternal diets supplemented with high concentrations, or more bioavailable forms of Zn, Mn and Se, have been found to improve progeny livability, growth and immune function (Virden *et al.,* 2003; Kidd, 2009; Moraes *et al.,* 2011). Progeny from Zn deficient animals presented with chromosomal aberrations and poor viabilities at birth or hatch (Favero *et al.,* 2013). Broiler progeny displayed poor growth when parental flocks were subjected to Zn deficient diets (Favero *et al.,* 2013). Broiler and turkey breeder hens supplemented with amino acid complexed Zn presented progeny with enhanced cellular immune functions (Kidd *et al.,* 1992). Progeny from hens supplemented with organic Zn presented with thicker tibias and increased tibia calcification before hatch (Favero *et al.,* 2013). Chicks from Zn methionine supplemented breeders showed improved survival when challenged with *Escherichia coli* (Virden *et al.,* 2003). Hudson *et al*. (2004) found increased concentrations of Zn in eggs from breeder hens supplemented with organic Zn. Organic Mn and Zn, provided in combination to breeder hens improved progeny livability (Hudson *et al.,* 2004). Favero *et al*. (2013) found supplementing breeder hens with organic Zn led to increased yolk and albumin Zn concentrations, where inorganic supplemented broilers presented with no significant changes. Kidd *et al*. (1992) and Angel *et al*. (2007) found no alterations in egg mineral concentrations when supplementing breeders with inorganic minerals. The increased tibia weights seen in progeny from hens supplemented with organic minerals led to the conclusion that organic Zn was either better transferred into the yolk and embryo, or better absorbed than inorganic minerals (Kidd *et al.,* 1992; Favero *et al.,* 2013).

2.6.2.2. Incubation

Modern embryos from fast growing lines have higher metabolic rates compared to lines with slower growth rates (Tona *et al.,* 2004), leading to increase metabolic heat production (Oviedo-Rondón *et al.,* 2009). These increases in growth and heat production have led to

greater temperatures and lower oxygen concentrations in incubators (Oviedo-Rondón *et al.*, 2008). Oviedo-Rondón *et al*. (2009) found suboptimal incubation conditions negatively affected organic bone matrix ossification in embryos. Shim and Pesti (2011) suggested bone developmental abnormalities originated during incubation. Bone formation is initiated in the embryonic phase, along with growth plate differentiation (Ballock and O'Keefe, 2003). A critical time for embryonic bone development is between ED 4 and ED 7, when primary muscle fibres are laid down and limb bud formation occurs (Hammond *et al.*, 2007). Between ED 7.5 and ED 8, tibia and tarsus osteoid mineralisation ensue (Hammond *et al.*, 2007). Hammond *et al*. (2007) suggested altering incubation temperature as an approach to improve skeletal and limb tissue development, as well as an increase in the size of tibia and tarsus bones. Oviedo-Rondón *et al*. (2009) found altering incubation temperatures improved growth rates and decreased locomotory issues in high yielding broilers. Yalçin *et al*. (2007) observed incubation conditions affected the incidences of tibial dyschondroplasia. Changing incubation temperature was proposed to increase chick motility, thereby improving myoblast differentiation through muscle stretch (Otis *et al.*, 2005). Limb muscles play a crucial role in prevention of locomotory issues through limb stabilisation and support post hatch (Hammond *et al.*, 2007). Oviedo-Rondón *et al*. (2009) reported incubation conditions influenced bone development through altered functions of insulin-like growth factor 1, thyroid- and growth hormones. Thyroid hormones, for example, were seen to influence chondrocyte plate differentiation (Shao *et al.*, 2006). It was suggested that increased temperature led to a balanced alteration between proliferation and differentiation in the long bone cartilage model (Kronenberg *et al.*, 2003). One optimum value for setting time, incubation and house conditions are, however, difficult to establish, as incubation requirements differ across broiler strains, ages and egg sizes (Shim and Pesti, 2011).

2.6.2.3. Supplementation of zinc, copper and manganese

Bone abnormalities are most commonly observed in the long leg bones, as they carry the heavier and faster increasing body weights of modern broilers (Yair *et al.*, 2012). The tibia is considered the most rapidly growing long bone, making it more susceptible to mechanical stress (Favero *et al.*, 2013). The tibia is subsequently the bone selected for assays (Church and Johnson, 1964; Favero *et al.*, 2013). Zn is essential for skeletal development and has a regulatory role in bone development (Favero *et al.*, 2013). Zn forms a part of the carbonic anhydrase system (Mabe *et al.*, 2003; Favero *et al.*, 2013) and is

associated with gene transcription that leads to endochondral ossification (Oviedo-Rondón and Ferket, 2005; Dibner *et al.,* 2007; Yair and Uni, 2011). Bone tensile strength and elasticity is influenced by Cu, through its role on collagen and elastin crosslinking (Dibner *et al.,* 2007; Yair and Uni, 2011; Favero *et al.,* 2013). Gajula *et al*. (2011) observed complementary effects when supplementing Mn and Zn together. Tibia Zn content was increased with significant reductions in hepatic Cu uptake, resulting in higher levels of hepatic and tibia Zn (Gajula *et al.,* 2011). Mn is essential for skeletal development, chondrotin sulphate formation and forms a part of the bone cartilage model (Star *et al.,* 2012). Mn is also involved in compression resistance of the epiphyseal bone through proteoglycan formation (Favero *et al.,* 2013). Zn is crucial for mucopolysaccharide synthesis and carbonate formation, acting both as cofactor and component of the enzymes required (Mabe *et al.,* 2003; Idowu *et al.,* 2011). Carbonic anhydrase requires Zn and influences egg shell formation by supplying bicarbonate ions (Mabe *et al.,* 2003). Egg shell weight, as well as egg shell and egg shell membrane strength will thus be greatly reduced in cases of insufficient Zn availability (Nys *et al.,* 1999; Idowu *et al.,* 2011).

2.7. Organic compared to inorganic mineral supplementation

Accurate bioavailability values are required to ensure trace mineral supplementation is sufficient to maintain animal growth, production, reproduction and health (Star *et al.,* 2012). The bioavailability of a substance is the amount of that substance that reaches the target tissue after administration (O'Toole and Miller-Keane, 2003). Higher bioavailability values thus indicate greater amounts of a mineral would be available for use in the animal body (Gajula *et al.,* 2010). Bioavailability values are, however, determined experimentally and reflect a specific mineral utilisation and absorption (Sandoval *et al.,* 1997), under precise conditions (Fairweather-Tait *et al.,* 1987), influencing requirement levels (Gajula *et al.,* 2010). Bioavailability values could also be dependent on the animal the test was conducted on (Chesters *et al.,* 1997). Bioavailability values are therefore an estimation of a specific test, at a certain time and should be considered experimental and not as an inherent characteristic of a mineral source (Sandoval *et al.,* 1997).

Inorganic trace minerals appear to have lower bioavailability values compared to organic trace minerals (Viden *et al.,* 2003; Favero *et al.,* 2013). Ao *et al*. (2009) stated inorganic mineral sources, such as oxides and sulphates, had lower bioavailability compared to minerals provided as organic proteinates and amino acid chelates (Wedekind *et al.,* 1992;

Cao *et al.*, 2000). This suggested more of organic minerals were retained in the body for necessary functions (Star *et al.*, 2012). Organic minerals are classified as complexes, chelates or proteinates (Salim *et al.*, 2011; El-Husseiny *et al.*, 2012). These would include metal complexes, such as metal amino acid complexes and chelates, metal-organic acid complexes, metal proteinates, metalpolysaccharide complexes and metal-yeast complexes (Patton, 1990; Salim *et al.*, 2011; El-Husseiny *et al.*, 2012). Inorganic minerals can be classified as sulphates, oxides and carbonates (El-Husseiny *et al.*, 2012). Inorganic Zn will generally be provided as feed grade Zn sulphate, chloride, or oxide (Star *et al.*, 2012). Due to the lower bioavailability, inorganic trace minerals have wide safety margins and may be fed at levels 2 to 10 times above the NRC (1994) requirements (Zhao *et al.*, 2010; El-Husseiny *et al.*, 2012). This is to ensure mineral requirement for genetic growth potential is met and to prevent deficiencies (El-Husseiny *et al.*, 2012). Zhao *et al*. (2014) reported inorganic Zn concentrations supplemented at levels as high as 20 to 30 times over requirement (Bratz *et al.*, 2013). Supplementing such high, compensatory mineral levels led to mineral and nutrient interactions in the gastrointestinal tract (Suttle *et al.*, 2010). High mineral levels interfered with absorption of other minerals, increasing excretion (Aksu *et al.*, 2011) whilst also decreasing mineral retention (Mohanna and Nys, 1998) and absorption rates (El-Husseiny *et al.*, 2012). High mineral excretion is economically wasteful (El-Husseiny *et al.*, 2012) and can pollute the environment (Bao *et al.*, 2007). The use of high mineral manure fertiliser could adversely affect farm yields and production resulting from mineral build up in soil (Nollet *et al.*, 2007) and excess minerals can leach into underground and surface water contaminating reserves (Jackson *et al.*, 2003).

Organic minerals have higher solubility values and cause less mineral interactions in the gastrointestinal system (Salim *et al.*, 2011; El-Husseiny *et al.*, 2012; Favero *et al.*, 2013). Organic minerals have a ring structure, preventing antagonistic interactions and subsequent insoluble complex formation, improving absorption (Wedekind *et al.*, 1992, 1994; Ao *et al.*, 2009). Improved uptake has been found when supplementing chelated minerals, as each chelate stays intact in the gut, allowing absorption without interactions (Ashmead *et al.*, 1992). Chelates can be absorbed via different routes (Ao *et al.*, 2009), degrading at the required sites (Aldridge *et al.*, 2007). Chelated minerals have also been found to facilitate the absorption of other minerals in that chelate, improving overall absorption (Miles and Henry, 2000). Proteinates are formed when minerals are chelated to a peptide, amino acid or protein, giving it a constant bioavailability similar to that of the amino acids or peptides

(Salim *et al.*, 2011). This makes it a suitable form to accurately formulate diets that receive minimal trace element levels (Leeson *et al.*, 2003). Organic mineral supplementation has been suggested as a method to alleviate mineral environmental pollution and wastage (El-Husseiny *et al.*, 2012). Higher bioavailability values allow lower mineral concentrations to be used, without sacrificing animal health or performance (Bao *et al.*, 2009).

Nollet *et al*. (2007), Peric *et al*. (2007) and Salim *et al*. (2011) found improved biological performance parameters in broilers when supplementary inorganic minerals were replaced with the organic counterparts. Idowu *et al*. (2011) reported significant differences in tibia, liver and excreta Zn, with improved FCR in birds supplemented with Zn proteinate compared to Zn oxide, sulphide and carbonate. Results were in agreement with Hudson *et al*. (2004), where broilers presented with greater levels of bone and pancreatic Zn when organic Zn was supplemented. Ao *et al*. (2009) reported increased tibial, mucosal and plasma Zn, with reduced hepatic Cu in broilers given supplementary Zn. Star *et al*. (2012) compared tibia ash from broilers supplemented with inorganic and organic Zn and found organic Zn had 64% higher bioavailability values. Feng *et al*. (2010) and Salim *et al*. (2012) found serum Zn and plasma Ca levels increased when broilers were supplemented with organic Zn. Pimental *et al*. (1991) observed supplementing Zn methionine instead of ZnO to broilers significantly increased pancreatic Zn by 33%. Wedekind *et al*. (1992) observed bioavailability values of 177% to 206% for organic Zn. In contrast, Salim *et al*. (2011) found no significant differences in body weight gain, FCR, feed intake or mortality between broilers supplemented with organic or inorganic Zn. Rossi *et al*. (2007) observed no improvements in growth performance parameters or carcass yields when supplementing broilers with organic Zn. Pimentel *et al*. (1991) and Ammerman *et al*. (1995) found no differences in bioavailability between organic and inorganic mineral supplementation (Star *et al.*, 2012). Cao *et al*. (2000) observed very small differences in bioavailability between Zn sulphate and organic Zn, when supplementing a basal diet containing 58 mg/kg to 100 mg/kg Zn. These inconsistencies across studies were suggested to be resulting from the differing levels of Zn in the basal diet (Leeson and Summers, 2005). Nutritional values of mineral sources could be influenced by multiple factors, including feed mineral concentration, antagonistic effects and mineral interactions (Star *et al.*, 2012). Dietary ligands such as Ca and phytate could form insoluble complexes with Zn, preventing absorption (Salim *et al.*, 2011). Actual mineral absorption in the intestinal tract and bioavailability to the bird would also influence nutritional values (Salim *et al.*, 2008).

Compared to other minerals, Zn has been found to have extremely complex homeostatic and kinetic metabolisms (Sandoval *et al.*, 1998). Sandoval *et al*. (1998) suggested this was due to the structure of Zn containing no unpaired electrons and a filled 3D shell.

2.8. Evaluating zinc status and the efficacy of zinc supplementation on bone development in broilers

Plasma and serum Zn are the biomarkers used most widely to determine Zn status of an animal (Sian *et al.,* 1996). Plasma Zn will decrease when dietary Zn is insufficient and homeostasis cannot be established without functional reserve mobilisation (King, 1990). Reduced plasma Zn would therefore indicate Zn was lost from the skeletal system and hepatic tissues (Al-Daraji and Amen, 2011). Gajula *et al*. (2011) suggested using tissue Zn and Mn contents, as well as their enzyme-related activities such as metallothionein, to determine mineral status. Al-Daraji and Amen (2011) stated that plasma Zn could be used as a simple and accurate indicator of Zn status and according to Mitchell and Rose (1991) also as an estimator of reproductive status in poultry. In contrast, Štofaníková *et al*. (2011) stated serum and plasma Zn had poor sensitivities and imperfect specificities (Sian *et al.,* 1996). Gajula *et al*. (2011) found plasma Zn to be a poor predictor of Zn intake at all ages, as tissue mineral content would be reduced with chick age. As chicks aged, muscle size would increase, thereby diluting mineral concentrations (Gajula *et al.,* 2011). Using blood as an indicator of Zn status was suggested, but would only be effective if birds were exposed to long dietary abundances or deficiencies in Zn (Štofaníková *et al.,* 2011). Changes such as retardation of growth would become apparent before Zn blood level would change (Underwood and Suttle, 1999). Jongbloed *et al*. (2002) stated the best response criteria to assess biological value of Zn status in monogastric animals were bone and pancreatic Zn. Bone has been seen to act as a functional Zn reserve and could be mobilised during periods of deficiency and reductions in plasma Zn (Sandoval *et al.,* 1998). Sandoval *et al*. (1998) found pancreas and bone accumulated the majority of Zn during supplementation. In poultry, dietary Zn and Mn supplementation resulted in linear increases in tibio-tarsal bones and plasma Zn and Mn (Mohanna and Nys, 1999; Štofaníková *et al.,* 2011). The greatest Zn and Mn accumulations were observed in tibias, compared to broiler livers, indicating the tibia was more responsive to mineral accumulation (Berta *et al.,* 2004). Zn yolk content was suggested to be used to indicate mineral status (Kaya *et al.,* 2001). Yolk mineral content could, however, only be used to determine Zn status if minerals were

supplemented in their organic forms, as inorganic mineral supplementation would show no effects on egg yolk mineral contents (Kaya *et al.*, 2001; Al-Daraji and Amen, 2011; Favero *et al.*, 2013). Increased levels of metallothionein have been found in various tissues when animal Zn status was improved (Sandoval *et al.*, 1998). The greatest accumulations of metallothionein in broilers were found in the pancreas, with increases in hepatic tissues, kidneys and intestines (Sandoval *et al.*, 1998). Gajula *et al*. (2011) saw hepatic, kidney, gastrointestinal tract and pancreatic tissues presented with the greatest metallothionein concentrations. Metallothionein was, however, found to be an inadequate bioavailability estimator of Zn as levels were variable, even though there was accumulation to some extent after Zn supplementation (Sandoval *et al.*, 1998).

Mineral nutrition stays a complicated study, as mineral metabolism is highly dynamic and minerals are constantly redistributed throughout body compartments (Sandoval *et al.*, 1998). Zn gets used extremely diversely throughout the body, playing a role in multiple physiological processes, enzymatic systems and reactions. There are also constant homeostatic mechanisms acting in the body to redistribute minerals to where requirement is greatest, regardless of dietary intake (Sandoval *et al*., 1998).

Chapter 3

Materials and Methods

3.1. Facilities and husbandry

The trial was conducted at the Hillcrest experimental research farm, University of Pretoria, with appropriate animal ethical approval from the Animal Ethics Committee of the University of Pretoria (EC065-15). Only designated persons were allowed access to the research trial and facilities were locked at all times. Persons allowed entree had to be free from contact with avian species for a minimum of fourteen days. Movement into the broiler house was strictly controlled. The test facility was free from poultry, disinfected and cleaned for a minimum of fourteen days before new chicks were placed. Test facilities consisted of ninety-six environmentally controlled pens, sized 2.25m^2. The ninety-six pens were divided into two separate sides (within one house) consisting of forty-eight pens per side. The one side was kept under thermoneutral conditions, as recommended by the Ross 308 manual for broiler requirements, while the other side was subjected to cyclic hyperthermic stress, simulating a South African summer heat wave.

Nipple drinkers were flushed with water and tested individually before the start of placement. Pens were covered with clean pine shavings approximately 10 cm deep. Forty-eight hours before chick arrival, the broiler house was preheated to an ambient temperature of 36℃ and litter temperature of 34℃. Litter temperatures were recorded before chick arrival, using an infrared thermometer. Both sides of the house were also fitted with ambient temperature sensors. After chick arrival, litter temperatures were examined three times daily and recorded. This was done to ensure constant, uniform temperatures and to indicate any temperature abnormalities.

Two thousand four hundred vaccinated Ross 308 male chicks were sourced from a commercial hatchery (National Chicks, Boshkop, Pretoria) from 60-week-old breeders. Feather sexing was done at the hatchery and all chicks were checked again for sex upon arrival to remove any sexing errors. Visibly smaller chicks, as well as chicks with unhealed or black vents and deformities were removed during sexing. To ensure optimum flock uniformity, chicks that were deemed to be unhealthy or lethargic were also removed during sexing. Remaining chicks

were randomly assigned to pens, with 25 chicks per pen, tagged with numbered neck tags and body weight per pen recorded.

During the first seven days, supplementary broiler starter feed and water were provided. Feed was available *ad libitum* in hanging tube feeders, on papers and in feed pans, ensuring feed accessibility at all times. Feeders, supplementary feed pans and papers were cleaned twice daily. Supplemental drinkers were cleaned and water replaced every morning to ensure availability of fresh cool water at all times. The first 24 hours after placement chick condition and behaviour were monitored every four hours, as well as litter temperatures recorded. Chicks were picked up within that period and crops felt to ensure they were feeding and drinking. After 24 hours, chicks were examined every six hours.

Feed was allocated in hanging tube feeders, available *ad libitum* throughout the trial. Feed was weighed weekly on days 7, 14, 21, 28 and 35. Feed was weighed into labelled, colour coded, weighed bins, corresponding to pen number and treatment. Before new feed was weighed and added, leftover feed and bin weights were recorded, then feed discarded. New feed was added on the scale into bins, which were then used to refill the corresponding tube feeders. Great care was taken to ensure each pen and treatment number on the bins corresponded to the correct pen number. Feeders were shaken and cleaned twice daily for the duration of the trial, ensuring clean and fresh feed at all times whilst simultaneously improving intake.

3.2. Diet formulation

Dietary formulations were based on proximate and mineral analysis of raw materials (DM, ash, CP, EE Ca, P) in accordance with procedures described by the AOAC (2000). One basal diet was formulated and mixed for all dietary treatments. The basal diets were formulated using software from Format International, U.K. The basal diet for each phase was formulated with a premix containing no Zn. The basal diet was then divided into six parts. Each of the individual six parts were thoroughly mixed with the corresponding Zn treatment (treatment 1 to 6). Individual treatments were then placed into labelled, colour coded bags corresponding to treatment. This was done for each of the dietary phases, namely starter, grower and finisher phases.

3.3. Dietary treatment and temperature profiles

Dietary treatments consisted of feed containing different levels of either feed grade Zn sulphate, or a combination of Zn sulphate and *Availa*®Zn (Zinpro Performance Minerals, Zinpro Corporation) or *IntelliBond*®*Z* (Micronutrients, Selko, Nutreco). *Availa*®Zn is a metal amino acid complex, constituting of one metal ion bound to one amino acid ion. Chelating minerals to amino acids have been found to give minerals constant bioavailability similar to that of amino acids or peptides (Salim *et al.*, 2011). *IntelliBond*®*Z* (zinc hydroxychloride) is formed through the covalent bonding of an OH group to a hydroxy trace mineral such as Zn. Covalent bonds occur in a crystalline matrix, resulting in low solubility at neutral pH ranges (Arthington, 2015).

Dietary treatments were randomly assigned to pens and were supplemented with either 80 mg/kg or 120 mg/kg of Zn, as follows (shown in Table 3.1):

- Treatment 1 and 2 were supplemented with either 80 mg/kg or 120 mg/kg of an inorganic Zn source (Zn sulphate).
- Treatment 3 was supplemented with 40 mg/kg Zn sulphate and 40 mg/kg of the organic Zn source, *Availa*®Zn (Zinpro Performance Minerals, Zinpro Corporation) to a total of 80 mg/kg Zn inclusion.
- Treatment 4 was supplemented with 80 mg/kg Zn sulphate and 40 mg/kg *Availa*®Zn (Zinpro Performance Minerals, Zinpro Corporation) to a total of 120 mg/kg Zn inclusion.
- Treatment 5 was supplemented with 40 mg/kg Zn sulphate and 40 mg/kg of the organic Zn source, *IntelliBond*®*Z* (Micronutrients, Selko, Nutreco) to a total of 80 mg/kg Zn inclusion.
- Treatment 6 was supplemented with 80 mg/kg Zn sulphate and 40 mg/kg *IntelliBond*®*Z* (Micronutrients, Selko, Nutreco) to a total of 120 mg/kg Zn inclusion.

Each of the six dietary treatments were replicated eight times in a facility with thermoneutral temperatures and eight times in a facility where broilers were subjected to cyclic heat stress. Heat stress started on day 9 and lasted until day 35. Temperature surges simulated a cyclic heat wave, starting at 32°C (89.6°F) on day 9, increasing to 39°C (102.2°F) at 25 to 27 days. Temperatures were gradually increased daily at 8:00 am and 11:00 am, then decreased to thermoneutral (evening) conditions at 19:00 until the next morning at 8:00 am.

Table 3.1 The composition of dietary treatments and zinc supplementation levels

	Treatment inclusion levels					
	T1	**T3**	**T5**	**T2**	**T4**	**T6**
Zn Source	80 mg/kg			120 mg/kg		
Zn Sulphate	80	40	40	120	80	80
Availa®Zn	-	40	-	-	40	-
IntelliBond®*Z*	-	-	40	-	-	40
Total supplemental Zn	80	80	80	120	120	120

Table 3.2 Temperature profile for thermoneutral (control) and cyclic heat stress temperatures in ℃

	Morning (8:00-11:00)		Afternoon (11:00-19:00)		Evening (19:00-08:00)	
Days	Standard	Heat stress	Standard	Heat stress	Standard	Heat stress
0-3	33	33	33	33	33	33
3-6	31	31	31	31	31	31
6-9	29	29	29	29	29	29
9-12	27	27-32	27	32	27	30-22
12-15	26	25-32	26	32	26	32-26
15-19	25	25-36	25	36	25	36-25
20-24	24	24-36	24	36	24	36-24
25-27	23	23-39	23	39	23	39-23
28-31	21	21-34	21	34	21	34-21
31-35	20	21-34	20	34	20	34-20

Table 3.3 Feed ingredient (%) composition for starter, grower and finisher diets

Dietary ingredient	Starter	Grower	Finisher
Maize (yellow)	60.6	64.5	69.5
Soya oilcake meal	23.6	14.4	11.4
Full fat soya	7.7	13.5	12.2
Sunflower oilcake	3.0	3.0	3.0
Crude soya oil	1.0	1.0	1.0
Methionine-DL	0.34	0.32	0.25
Threonine	0.12	0.40	0.09
Lysine HCL	0.40	0.40	0.36
Feed lime	1.40	1.10	1.10
Mono-dicalciumphosphate	0.95	0.543	0.47
Salt (fine)	0.21	0.141	0.153
Sodium bicarbonate	0.32	0.35	0.33
Phytase feed enzymes (Axtra Phy 10000 P)	0.01	0.01	0.01
Salinomycin 12%	0.05	0.05	0.05
Olaquindox 10%	0.02	0.02	0.02
Mineral premix	0.30	0.25	0.20

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

	Starter		Grower		Finisher	
	Formulated	Analysed	Formulated	Analysed	Formulated	Analysed
Moisture	10.62	10.22	11.75	11.89	11.98	12.31
Dry matter	89.38	89.78	88.25	88.11	88.02	87.69
Metabolisable energy (MJ/kg)	12.21	11.99	12.73	12.80	12.88	13.00
Crude protein	20.59	19.20	18.85	19.0	17.00	17.3
Fat	4.42	5.00	5.41	5.69	5.32	5.45
Crude fibre	3.16	3.51	3.24	3.40	3.16	3.10
Ash	4.97	5.15	4.06	3.95	3.76	4.00
Calcium	0.97	0.99	0.79	0.81	0.75	0.79
Total phosphorous	0.53	0.55	0.42	0.50	0.39	0.42
Sodium	0.18	0.17	0.16	0.15	0.16	0.16

3.4. Special procedures during the research trial

Mortalities were collected twice daily and recorded on a mortality sheet containing pen number, tag number and body weight. Chicks were then necropsied and reason for mortality determined and recorded. Feed conversion ratio was adjusted for mortalities, calculated as broiler feed intake divided by broiler body weight gain of each pen. Litter temperatures were recorded three times daily and any sick, immobile or injured bird removed and humanely euthanised. For the duration of the study, drinker lines and individual nipples were closely monitored to ensure functionality and observe any leaks. Leaking nipples were immediately replaced and wet shavings replaced with new, dry shavings.

3.5. Feed sample analysis procedures

3.5.1. Dry matter and ash analysis

Dry matter and ash procedures were completed in accordance to methods by the AOAC (1995). Crucibles were dried in an oven at 105℃ for a minimum of 24 hours before use. Crucibles were removed, placed into desiccators for 30 minutes and then weights were recorded. After

empty dry weights were obtained, each sample was placed into a labelled crucible on the scale and the wet weight recorded. Thereafter, samples were placed into an oven to dry for 12 hours at 105℃. Crucibles were handled with tongs and care was taken not to touch crucibles, potentially influencing moisture levels. After 12 hours, crucibles were removed from the oven and placed into desiccators to cool for 30 minutes. Dry crucible and sample weights were then recorded. Dry matter was calculated as the difference between the sample and crucible after 24 hours of drying, minus the initial weight of the dried crucible containing the samples before placement into the oven. Dry matter value was then multiplied by 100, to express the value as a percentage.

Ash content was obtained by placing the dried crucibles, containing the samples, into a muffle furnace for 2 hours at 250℃ and then temperature was increased to 600℃ for 6 hours. Samples were allowed to cool in the sealed muffle furnace for 8 hours. Crucibles containing samples were removed with tongs and placed into desiccators for 30 minutes. Ash was calculated by finding the difference of the crucible containing the ash with the dry sample, from the DM analysis, divided by the mass of the sample. To express values as a percentage it was multiplied by 100.

3.5.2. Crude protein analysis

The Macro Kjeldahl method was used to determine nitrogen and obtain values for crude protein (CP). All procedures were completed in accordance with methods by the AOAC (1995). Sulphuric acid (25 mL, 98% concentrated H_2SO_4) was added to an Erlenmeyer flask. The dried sample (0.5g), 10 g of sodium sulphate and 0.4 g of elemental sulphur were added to the flask and boiled for 45 minutes. When the solution became clear it was removed and allowed to cool. Once the solution had cooled, 35 mL of boric acid solution, containing 40 g of boric acid in 10 mL methyl red and 25 mL methyl blue (made up to a volume of 1000 mL with distilled water) were added. Zinc granules, 350 mL distilled water and 100 mL NaOH (45%) were also added. The solution was then boiled until 200 mL of distillate remained. The distillate was titrated with 0.1 N of H_2SO_4. A blank sample was titrated to use for correction of values. Nitrogen in the sample was calculated using:

% N = F x (titration - blank) * 100 / sample mass

Where F was the factor associated with the strength of the H_2SO_4.

Dietary crude protein was then calculated as the percentage of N multiplied with 6.25

3.5.3. Crude fibre analysis

Crude fibre was determined using the AOAC Approved Procedure BA 6a-05, ANKOM Technology method 10. Filter bags were labelled with solvent free, acid resistant markers and weighed (W1). A blank bag was also weighed and included in the analysis to indicate particle loss and determine the blank bag correction value (C1). The samples to be analysed, approximately 1 gram per sample, were weighed into labelled filter bags (W2). The filter bags were then sealed with a heat sealer approximately 4 mm from the top. Bags containing samples and the blank bag were soaked in ether for 10 minutes. After air-drying the bags, samples were placed into the fibre analyser vessel of the ANKOM2000. The bags containing the samples were removed and placed into a beaker filled with acetone. The samples were completely submerged and soaked for 5 minutes. The samples were then removed and placed into an oven set to 102℃ for four hours. After four hours, the sample bags were taken out with tongs and placed into a desiccator for 30 minutes. Bags were then removed and weighed. The bags containing the samples were placed into dried crucibles and placed into a muffle furnace to be ashed (as described in 4.1.). After ashing, samples were weighed and used to calculate the organic matter weight loss of the samples (W3). Crude fibre percentage was determined using:

$$\% \text{ crude fibre} = 100 \times [W3 - (W1 \times C1)] / W2$$

Where W1 was the empty bag weight,
W2 was the sample weight,
W3 was the weight of the organic matter (loss of weight on ignition of bag and fibre),
C1 was the ash corrected blank bag factor.

3.5.4. Ether extract

Ether extract was determined using the official methods of analysis of the AOAC (2000) Official method of analysis 920.39 (17^{th} Edition) Volume I, using a Tecator Soxtec System HT 1043 extraction unit. Approximately 0.1 g of sample was weighed and placed into a dried, weighed cellulose thimble. Thimbles were dried for at least 5 hours at 100℃ before use. Extraction cups were placed into an oven for at least 5 hours at 100℃, removed, allowed to cool and weighed. The extractor was preheated and the condenser water switched on. The

extractor was used following the manufacturer instructions for operation. Thimbles containing samples were placed into the extractor columns, with corresponding extraction cups. Extraction cups were filled with enough diethyl ether to cover the test portion. Thimbles were lowered into the ether and boiled for 20 minutes. Thimbles were then raised out of the solvent in the extractor for 40 minutes. Extraction cups were removed and placed under a fume hood, until all the solvent evaporated. Extraction cups were then dried for 30 minutes at 102℃. Samples were removed, cooled in a desiccator and weighed. Ether extract was then calculated as

% crude fat = (weight of dried extraction cup containing fat - initial dry extractor cup weights)/ weight of thimble containing sample.

Result was multiplied by 100 to obtain percentage.

3.5.5. Calcium and phosphorous analysis

All laboratory equipment was used following the manufacturer instructions for operation. Feed samples were air dried as described in 3.5.1 and then placed into test tubes with 25 mL of HNO_3 (65%). Tubes were heated for approximately 10 minutes, until 50% of the HNO_3 had evaporated, where after 5 mL of HOCl was added and the solution was boiled for another 40 minutes. The solution was allowed to cool. Distilled water was then added to make a final sample volume of 50 mL.

Calcium

Calcium readings were obtained using a Perkin Elmer 5100PC Atomic absorption Spectrophotometer, following the method as described by Giron, H.C., 1973 Atomic absorption Newsletter 12.28 Perkin Elmer.

Phosphorous

Phosphorous readings were taken using an Analytic Jena Specord 200 Plus, following procedures as described by the AOAC, 2000. Official method of analysis 965.17 (17^{th} Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

3.6. Sample collection and analysis

Two birds with body weights closest to the average of their respective pens were selected for sampling. Average weights were calculated using 35-day body weights. Five birds were placed

into a crate and eight crates transported to the abattoir at a time. This was to minimise any potential discomfort and stress to birds.

3.6.1. Serum cholesterol ester hydrolase

Blood was collected into 10 mL non-clotting (heparin free) tubes through cardiac puncture, immediately after birds were stunned electrically. Samples were transported to the Department of Clinical Pathology (Faculty of Veterinary Sciences, Onderstepoort, University of Pretoria) every 30 minutes. Upon arrival, samples were analysed for serum cholesterol.

Cholesterol was measured colorimetrically, utilising the reaction where cholesterol esters are cleaved by cholesterol esterase, yielding free cholesterol and fatty acids. The reaction was catalysed by cholesterol oxidase to yield cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide affected oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye in the presence of peroxidase, measured at 500 nm (Allain *et al.*, 1974). Cholesterol concentration was determined through measuring the absorbance, as dye colour intensity was directly proportional to the cholesterol concentration. All samples were centrifuged before processing to remove precipitates before performing the assay. Samples and 50 g/mol of Serachol were added to 3.0 mL of phosphate buffer (0.10 mol/liter, pH 6.7). The phosphate buffer contained 3.0 mmol of sodium chelate, 0.8 mmol of 4-aminoantipyrine, 14 mmol of phenol, 120 U cholesterol oxidase, 0.17 mmol of Carbowax-6000 and 67 000 U of HPOD (horseradish source; donor: H_2O_2 oxidoreductase, EC 1.11.1.7) per liter. The solution was then incubated in a 1.0 cm cuvette at 37°C for 5 minutes. After incubation, 0.10 mL of cholesterol ester hydrolase solution, in phosphate buffer (pH 6.7; 0.10 mol/liter), was added. Change in rate of absorbance at 500 nm and 37°C was then obtained. One unit of cholesterol ester hydrolase activity was the amount of enzyme decomposing 1 μmol of cholesterol ester per minute at 37°C.

3.6.2. Liver, heart and fat pad sampling

Immediately after cardiac puncture, birds were humanely euthanised by cervical dislocation. Hepatic tissue was removed in a timely fashion and liver weight recorded. Directly thereafter, samples were placed into labelled, water- and airtight bags. Bagged hepatic samples were submerged in frozen carbon dioxide in airtight foam coolers. Frozen carbon dioxide ensured instant freezing, thereby inhibiting activity and breakdown of metabolic enzymes and pathways. After liver removal, cardiac tissue and fat pads were excised and weighed. Sample

organs were then discarded into biohazardous containers. Every hour liver samples were transported in the airtight cooler bags containing dry ice to the Department of Biochemistry (University of Pretoria) laboratory. At the laboratory, liver samples were transferred into a -80°C freezer, where samples remained frozen for approximately 60 days. Livers were analysed for malondialdehyde level and total antioxidant capacity. Reagent kits from Biocombiotech (K739-100 - Lipid Peroxidation (MDA) Colorimetric/Fluorometric assay kit (100 assays) and STA - 360, OxiSelect™ Total Antioxidant Capacity (TAC) assay kit (200 Assays) were utilised. All storage and analysis were carried out in accordance with the manufacturer's instructions.

3.6.2.1. Malondialdehyde

Analysis was performed in accordance with the manufacturers' instructions, as described in the Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit, Catalog # K739-100, Rev 12/14. Approximately 2 g of liver was homogenised on ice in 300 µL of MDA lysis buffer (with 3 µL BHT 100X), then centrifuged at 13,000 X *g* for 10 minutes to remove insoluble material. To obtain a MDA Standard Curve, 10 µL of MDA standard was diluted with 407 µL of ddH_2O to prepare a 0.1 M MDA solution. 20 ML of the 0.1 M MDA solution were diluted with 980 µL of ddH_2O to prepare a 2 mM MDA standard. Thereafter 0, 2, 4, 6, 8 and 10 µL of the 2 mM MDA standard were separated into microcentrifuge tubes and volumes adjusted with 200 µL ddH_2O. This generated 0, 4, 8, 12, 16 and 20 nmol standards for colorimetric analysis. 600 µL of TBA reagent were added into each vial containing the standard and sample. Vials were then incubated for 60 minutes at 95°C. Samples were cooled to room temperature by placing vials into an ice bath for 10 minutes, then 200 µL of sample solutions (from each 800 µL reaction mixture) were pipetted into 96-well microplates. Colorimetric analysis was performed by reading absorbance at 532 nm. A MDA Standard curve was plotted and used to determine the quantity of MDA in each sample. This was presented in nmol through interpolation from the standard curve. Sample values were corrected for any dilutions performed during specimen preparation.

3.6.2.2. Total antioxidant capacity (TAC)

Hepatic tissues (2 g) were homogenised on cold phosphate buffered saline and centrifuged at 10,000 x g for 10 minutes at 4°C. Supernatant was aliquoted for storage at -80°C for protein determination and subsequent TAC assay. Then 20 µL of diluted uric acid standards and samples were added to 96 well microtiter plates. Reaction buffer, 180 µL of 1X, were added to

each well using multichannel pipettes and mixed thoroughly. An initial absorbance reading was obtained at 490 nm. Thereafter the reaction was initiated through the addition of 50 µL of 1X copper ion reagent into each well. The tray was incubated for 5 minutes in an orbital shaker and then 50 µL of 1X stop solution was added to terminate reactions. Absorbance was obtained again at 490 nm. Net absorbance was calculated through subtraction of initial sample values and standard absorbance values from final absorbance values. Net absorbance was plotted against uric acid concentration for the uric acid standard curve. TAC of unknown samples were calculated through comparison of net OD 490 values of samples to the standard uric acid curve. Analyses was carried out using the manufacturers' instructions, as described in the OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit, Catalog Number STA-360, Cell Bio labs, Inc.

3.6.3. Tibia collecting and ashing

Left legs from sample birds were excised through proximal laceration at the femur. Great care was practiced and employees trained to inflict no damage to the ends of the tibias. Samples were placed into labelled bags and stored at room temperature for three days. After three days, tibia samples were manually defleshed, removing the cartilage cap and any tissue residue on the bone. Samples were transported to the Department of Animal Science (University of Pretoria) laboratory and stored at 2℃. Samples were stored for a maximum period of 4 days before defatting commenced.

Defatting was done following the official methods of analysis 960.39 of the AOAC (2000) using a Soxhlet fat extraction apparatus. Each tibia was placed into a mesh bag with an identifying tag containing the sample number. Mesh bags, containing tibia samples and tags, were placed in a Soxhlet fat extraction apparatus containing 40-60℃ ether. The defatting procedure was run for 24 hours, then tibia samples were removed. Samples were placed under a furnace hood for 30 minutes to allow ether evaporation.

After defatting and ether evaporation, wet tibia samples were placed into crucibles. Tibia samples were dried in accordance with the AOAC (2000) official methods of analysis 950.46B. Crucibles were dried in the oven at 105℃ for a minimum of 24 hours before use. Crucibles were removed, placed into desiccators for 30 minutes and weighed. After empty dry weight was obtained, each tibia was placed into a labelled crucible on the scale and the wet weight recorded. Thereafter, tibia samples were placed in a drying oven for 12 hours at 105℃. Crucibles were handled with tongs and care was taken not to touch crucibles and potentially

influence moisture levels. After removal from the oven, crucibles containing the tibias were placed into desiccators to cool for 30 minutes. Dry crucible and tibia weights were recorded. Dry matter was then calculated as the difference between the tibia sample and crucible after 24 hours of drying minus the initial weight of the dried crucible containing the tibia sample before placement in the oven. Dry matter value was then multiplied by 100 to express value as a percentage.

Tibia samples were ashed following the official procedures of the AOAC (2000) method 920.153. Dried crucibles containing tibia samples were placed into a muffle furnace for 2 hours at 250℃, where after temperature was increased to 500℃ for 6 hours. Tibias were allowed to cool in the muffle furnace for 8 hours and then placed into desiccators for 30 minutes. Crucibles containing the tibias were removed with tongs and weights were recorded. Bone ash was calculated by finding the difference of the crucible containing the ash with the dry sample, from the DM analysis, divided by the mass of the sample. To express values as a percentage it was multiplied by 100.

3.6.4. Determination of zinc concentration in bone ash

The ashed bone samples were ground into fine homogenous particles. To determine zinc in bone ash, 0.100 g of sample was weighed and transferred into a mineral digestion tube. Mineral digestion was done following the official methods as described by the AOAC (2000) method 935.13. 25 mL of nitric acid (HNO_3) was added to bone ash samples and boiled for 15 minutes at 240℃ on a preheated mineral block under a fume hood. Samples were taken out and cooled for 5 minutes under a fume hood, then 10 mL of Perchloric acid ($HClO_4$) was added and samples were boiled for another 35 minutes at 240℃. After 18 minutes, mineral digestion tubes containing the sample solutions were rotated 180˚ to ensure even heating on the mineral block. Samples were then taken out and placed under a fume hood, where cooling commenced until no more fumes were released. Samples were transferred quantitatively to 50 mL volumetric flasks, adding deionised water to make up final sample volumes of 50 mL. Samples were shaken to ensure thorough mixing and then transferred to 50 mL glass bottles. Zinc was analysed using GBS 905 AA, according to manufacturers' instructions.

3.7. Data collection and statistical analyses

A randomised complete block design was used where birds were randomly assigned to 12 treatment groups. Treatments consisted of 8 replications per treatment, in a 2 x 2 x 3 factorial design. Each house was divided into 4 blocks with 16 pens per block. Treatment replications were randomised and repeated twice within a block. Each dietary treatment was given to broilers that were subjected to thermoneutral or hyperthermic conditions.

Data was analysed statistically using a randomised block design with the GLM model (Statistical Analysis Systems, 2016) for average effects over time. Repeated measures analysis of variance with the GLM model were used for repeated weekly measures. Means and standard errors were calculated and the significance of difference ($p < 0.05$) between means determined by Fishers Test (Samuels, 1989). The linear model used is described by the following equation:

$$Y = \mu + T_i + S_j + L_k + B_l + e$$

Where:

T_i = effect of the i^{th} temperature

S_j = effect of the j^{th} source

L_k = effect of the k^{th} level

B_l = effect of the l^{th} block

e = error associated with each Y

Chapter 4

Results

4.1. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) from 0 - 35 days of age

As presented in Table 4.1, broilers supplemented with *Availa*®Zn (AZ) and *IntelliBond*®*Z* (IZ) compared to Zinc sulphate (ZS) presented with higher body weights within the first 7 days of the research trial. At the conclusion of the trial, at 35 days of age, broilers supplemented with AZ had significantly higher body weights ($P < 0.05$) compared to birds that received supplementary IZ and ZS. Birds provided with AZ had the heaviest body weights at weekly weighing, in contrast to birds provided with ZS, where broilers consistently presented with the lightest body weights. Broilers supplemented with IZ exhibited significantly heavier body weights ($P < 0.05$) compared to birds supplemented with ZS, but birds supplemented with AZ consistently presented significantly heavier body weights for the duration of the trial.

Birds supplemented at 120 mg/kg inclusion of zinc presented with significantly heavier body weights compared to birds at 80 mg/kg inclusion level. Body weights from birds at 120 mg/kg inclusion level remained significantly heavier for the duration of the trial ($P < 0.05$) compared to birds supplemented at 80 mg/kg inclusion level. Differences in body weight between broilers supplemented at 120 mg/kg inclusion were apparent from day 7, presenting broilers with significantly higher body weights for the remainder of the research period.

Broilers under high temperature stress exhibited significantly lower ($P < 0.05$) body weights compared to birds exposed to thermoneutral conditions. Significant differences ($P < 0.05$) in broiler body weights, under both temperature treatments, were found starting at day 14 and continued for the duration of the research trial. Body weights of birds measured at day 7 presented with no significant differences between groups, as heat stress only started on day 9. Birds subjected to high temperature stress presented with significantly lower ($P < 0.05$) body weights at 35 days of age, resulting in a difference of approximately 394 g at the conclusion of the research trial.

Table 4.1 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) from 0 - 35 days of age

Treatment	Days of age					
	0	7	14	21	28	35
Zn Source						
Availa®Zn	43.98	200.5^{a}	549.4^{a}	1049^{a}	1683^{a}	2373^{a}
IntelliBond®Z	43.73	199.6^{ab}	547.4^{b}	1042^{b}	1674^{b}	2368^{b}
Zn Sulphate	43.81	197.6^{b}	545.7^{b}	1030^{c}	1671^{b}	2361^{c}
SEM ±	0.122	0.801	0.662	1.044	1.329	1.105
Zn level (mg/kg)						
80	43.94	198.3^{b}	546.6^{b}	1038^{b}	1671^{b}	2365^{b}
120	43.74	200.1^{a}	548.4^{a}	1043^{a}	1682^{a}	2370^{a}
SEM ±	0.100	0.654	0.540	0.852	1.086	0.902
Temperature						
Normal	44.02	199.6	554.2^{a}	1085^{a}	1809^{a}	2565^{a}
Hot	43.66	198.9	540.8^{b}	995.2^{b}	1544^{b}	2171^{b}
SEM ±	0.100	0.654	0.540	0.852	1.086	0.902

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same column with no common superscript differ significantly ($P < 0.05$).

SEM - Standard error of the mean

4.1.1. The main and interactive effects of Zn source, Zn level and house temperature on broiler body weight (g) from 0 - 35 days of age

Main and interactive effects between Zn source, Zn inclusion level and house temperature for the different weeks are presented in Table 4.2. Zn source and Zn dosage level exhibited with significant interactive effects ($P < 0.05$) on broiler body weight from 7 to 14 days of age, remaining significant until day 28. No other interactive effects were seen on broiler body weight between Zn source and Zn dosage level. Zn source and Zn dosage level showed significant interactive effects on body weight for the duration of the trial, starting at day 7.

Significant interactive effects were found on body weight between house temperature and Zn source, as well as Zn source, level and house temperature. Interactive effects between house temperature and Zn source, as well as Zn dosage level, Zn source and house temperature were apparent for the duration of the trial, starting at day 14 and continuing until day 35. Zn dosage level and house temperature only presented significant interactive effects on body weight from day 28 to day 35.

Table 4.2 The main and interaction effects of Zn source[1], Zn inclusion level and temperature[2] effects on body weight (g) at day 0 - 35 days of age (P-values)

	Days of age				
	7	14	21	28	35
Zn Source	0.033	0.001	<0.001	<0.001	<0.001
Zn Level	0.050	0.027	<0.001	<0.001	<0.001
Zn Source x Level	0.758	0.831	0.001	0.043	0.648
Temperature	0.456	<0.001	<0.001	<0.001	<0.001
Zn Source x Temperature	0.461	0.029	<0.001	<0.001	<0.001
Zn Level x Temperature	0.545	0.512	0.354	0.002	<0.001
Source x Level x Temperature	0.438	0.009	<0.001	<0.001	<0.001

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.1.2. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) at 7 days of age

Tables 4.3 - 4.7 show the effects of Zn source, Zn inclusion level and house temperature on body weight of broilers for each week of the study period.

Significant differences ($P < 0.05$) were found between body weights of birds supplemented with AZ and ZS at 120 mg/kg inclusion level under thermoneutral conditions. Birds receiving IZ displayed no significant differences between body weights of AZ or ZS supplemented birds, as found in Table 4.3. The same was noted for mean body weights of birds in thermoneutral

conditions. Birds under high temperature stress, supplemented at 120 mg/kg inclusion level presented with significantly higher body weights compared to birds supplemented at 80 mg/kg inclusion level. No other significant differences were observed.

Table 4.3 The effect of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) at 7 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	201.0 (±1.603)	198.6 (±1.603)	197.3 (±1.603)	199.0 (±0.925)
120 mg/kg	202.4^{a} (±1.603)	200.4^{ab} (±1.603)	197.8^{b} (±1.603)	200.2 (±0.925)
Mean	201.7^{a} (±1.133)	199.5^{ab} (±1.133)	197.5^{b} (±1.133)	
Hot temperature				
80 mg/kg	198.9 (±1.603)	199.0 (±1.603)	195.1^{A} (±1.603)	197.7 (±0.925)
120 mg/kg	199.8 (±1.603)	200.3 (±1.603)	200.2^{B} (±1.603)	200.1 (±0.925)
Mean	199.4 (±1.133)	199.6 (±1.133)	197.6 (±1.133)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same column (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.1.3. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) at 14 days of age

Under thermoneutral temperatures at 80 mg/kg inclusion, AZ supplementation resulted in broilers with significantly higher body weights on day 14 ($P < 0.05$) compared to broilers supplemented with ZS. At 120 mg/kg inclusion under thermoneutral temperatures birds presented with no significant differences in body weight between different sources, as seen in Table 4.4. The same was noted in broilers under high temperatures at both inclusion levels, where different sources did not affect body weights of broilers. Under both temperature

treatments, broilers at 120 mg/kg inclusion compared to 80 mg/kg inclusion exhibited with significantly heavier body weights ($P < 0.05$).

Table 4.4 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) at day 14 of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	558.6^{a} (±1.324)	553.1^{b} (±1.324)	549.3^{cA} (±1.324)	553.6^{b} (± 0.764)
120 mg/kg	556.1 (±1.324)	555.5 (±1.324)	553.0^{B} (±1.324)	554.9^{a} (± 0.764)
Mean	557.3^{a} (±0.936)	554.3^{b} (±0.936)	551.1^{c} (±0.936)	
Hot temperature				
80 mg/kg	538.8^{B} (±1.324)	540.4 (±1.324)	539.8 (±1.324)	539.6^{b} (± 0.764)
120 mg/kg	544.1^{A} (±1.324)	540.6 (±1.324)	540.9 (±1.324)	541.9^{a} (± 0.764)
Mean	541.5 (±0.936)	540.5 (±0.936)	540.3 (±0.936)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

4.1.4. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) at 21 days of age

Broilers supplemented with AZ under thermoneutral conditions, at 80 mg/kg inclusion presented with significantly higher body weights ($P < 0.05$), as seen in Table 4.5. Under thermoneutral conditions at 120 mg/kg inclusion level, IZ and ZS supplemented broilers presented with body weights that differed significantly ($P < 0.05$) from broilers that received AZ. At both inclusion levels under high temperature stress, birds supplemented with AZ and IZ showed no significant differences between body weights of broilers, although body weights

were significantly higher ($P < 0.05$) than the ZS supplemented group. Under both temperature treatments, at 120 mg/kg inclusion level, birds exhibited significantly higher body weights ($P < 0.05$) compared to birds that received Zn at 80 mg/kg inclusion.

Table 4.5 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) at 21 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1102^{aA}(±2.088)	1081^{b} (±2.088)	1062^{cB} (±2.088)	1082^{b} (±1.206)
120 mg/kg	1093^{aB} (±2.088)	1085^{b} (±2.088)	1086^{bA} (±2.088)	1089^{a} (±1.206)
Mean	1098^{a} (±1.474)	1084^{b} (±1.474)	1075^{c} (±1.474)	
Hot temperature				
80 mg/kg	994.0^{aB} (±2.088)	999.7^{a} (±2.088)	985.3^{b} (±2.088)	993.0^{b} (±1.206)
120 mg/kg	1004^{aA} (±2.088)	1001^{a} (±2.088)	986.1^{b} (±2.088)	997.3^{a} (±1.206)
Mean	999.3^{a} (±1.474)	1000^{a} (±1.474)	985.7^{b} (±1.474)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same rows with no common superscript differ significantly ($P < 0.05$).

[A,B] Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

4.1.5. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) at 28 days of age

Body weights presented with significant differences between broilers supplemented with all three sources ($P < 0.05$) across both temperature treatments and inclusion levels, excluding inclusion level 80 mg/kg under thermoneutral conditions. As shown in Table 4.5, under thermoneutral conditions at 80 mg/kg inclusion level, broilers supplemented with organic sources had no significant differences between body weights. Body weights from birds supplemented with organic sources were significantly heavier ($P < 0.05$) than body weights

from birds that received supplementary ZS. At 120 mg/kg inclusion under thermoneutral conditions, AZ supplemented broilers exhibited the highest body weights, where ZS supplemented broilers displayed the lowest body weights. Heat stressed birds supplemented at both 80 mg/kg and 120 mg/kg inclusion level presented with significantly heavier ($P < 0.05$) body weights, where IZ supplemented birds had the lowest body weights. Birds supplemented with ZS under heat stressed conditions presented with the heaviest body weights, differing significantly from the two organic sources ($P < 0.05$). Body weights were significantly higher ($P < 0.05$) in broilers supplemented at 120 mg/kg inclusion level under thermoneutral temperatures. Supplementing AZ under heat stress, at 80 mg/kg inclusion resulted in significantly heavier body weights than at 120 mg/kg inclusion level. Supplementing ZS at 120 mg/kg inclusion, under high temperature stress resulted in broilers with significantly heavier ($P < 0.05$) body weights.

Table 4.6 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) form 28 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1807^{aB} (±2.660)	1812^{aB} (±2.660)	1772^{bB} (±2.660)	1797^{b} (±1.536)
120 mg/kg	1839^{aA} (±2.660)	1826^{bA} (±2.660)	1796^{cA} (±2.660)	1820^{a} (±1.536)
Mean	1823^{a} (±1.881)	1819^{a} (±1.881)	1784^{b} (±1.881)	
Hot temperature				
80 mg/kg	1548^{aA} (±2.660)	1531^{b} (±2.660)	1555^{cB} (±2.660)	1545 (±1.536)
120 mg/kg	1540^{aB} (±2.660)	1529^{b} (±2.660)	1563^{cA} (±2.660)	1544 (±1.536)
Mean	1544^{a} (±1.881)	1530^{b} (±1.881)	1559^{c} (±1.881)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

4.1.6. The effects of Zn source, Zn inclusion level and house temperature on broiler body weight (g) at 35 days of age

Under high temperature stress, organic source IZ and AZ supplementation at both 80 mg/kg and 120 mg/kg inclusion resulted in significantly higher ($P < 0.05$) body weights for the birds. This was also noted under thermoneutral conditions at 80 mg/kg inclusion, as found in Table 4.7. Under both temperatures and inclusion levels, broilers supplemented with AZ and IZ presented with no significant differences between body weights, but differed significantly ($P < 0.05$) with ZS supplemented birds. This was not found in broilers under thermoneutral conditions at 120 mg/kg inclusion. At 120 mg/kg inclusion under thermoneutral conditions, broilers supplemented with different sources displayed significant differences ($P < 0.05$) between body weights. Broilers supplemented with ZS had the lightest body weights, where birds supplemented with AZ showed the heaviest body weights. Birds supplemented at 120 mg/kg inclusion under heat stress presented with significantly ($P < 0.05$) heavier body weights than birds supplemented at 80 mg/kg inclusion level. Broilers under thermoneutral temperatures exhibited no significant differences between body weights from broilers under different inclusion levels. Birds supplemented with AZ and ZS, however presented with heavier body weights when 120 mg/kg rather than 80 mg/kg Zn was supplemented.

Table 4.7 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler body weight (g) at 35 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	2564^{aB} (±2.209)	2561^{a} (±2.209)	2571^{bB} (±2.209)	2565 (±1.275)
120 mg/kg	2573^{aA} (±2.209)	2564^{b} (±2.209)	2556^{cA} (±2.209)	2565 (±1.275)
Mean	2569^{a} (±1.562)	2562^{b} (±1.562)	2563^{b} (±1.562)	
Hot temperature				
80 mg/kg	2175^{a} (±2.209)	2172^{a} (±2.209)	2148^{bB} (±2.209)	2165^{b} (±1.275)
120 mg/kg	2180^{a} (±2.209)	2178^{a} (±2.209)	2171^{bA} (±2.209)	2176^{a} (±1.275)
Mean	2177^{a} (±1.562)	2175^{a} (±1.562)	2159^{b} (±1.562)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

4.2. The effect of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) from 0 - 35 days of age

From 0 - 7 days of age, broiler feed intake was the lowest in broilers supplemented with ZS compared to IZ supplemented groups, where broilers presented with significantly higher feed intakes ($P < 0.05$). Birds supplemented with AZ had significantly lower feed intakes ($P < 0.05$) from 14 - 21 days of age compared to groups that received ZS. This was also noted from 21 - 28 days of age, as found in Table 4.8. At 28 - 35 days of age, the two organic sources AZ and ZS did not differ significantly between feed intakes of broilers, although feed intakes were significantly lower ($P < 0.05$) than ZS supplemented birds.

From 0 - 7 days of age birds provided with supplementary Zn at 120 mg/kg inclusion presented with significantly higher feed intakes ($P < 0.05$) than birds supplemented at 80 mg/kg

inclusion. No other significant differences were observed in feed intakes between birds supplemented with Zn at either 80 mg/kg or 120 mg/kg inclusion level after 0 - 7 days of age.

Broilers exposed to high temperature treatments compared to thermoneutral conditions displayed significantly lower feed intakes ($P < 0.05$). Birds under thermoneutral conditions presented with significantly ($P < 0.05$) higher feed intakes at 7 - 14 days of age. Significant differences ($P < 0.05$) between broiler feed intakes under different temperature treatments were apparent from 14 days of age, as high temperature treatments commenced on day 9 of the research study. At the conclusion of the trial, birds subjected to high temperature treatments had approximately 196 g lower feed consumptions.

Table 4.8 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed intake (g) from 0 - 35 days of age

Treatment	Days of age				
	0 – 7	7 – 14	14 – 21	21- 28	28 – 35
Zn Source					
Availa®Zn	172.9^{ab}	449.7	722.3^{a}	1057^{a}	1304^{a}
IntelliBond®Z	173.0^{a}	447.5	723.5^{ab}	1058^{ab}	1308^{a}
Zn Sulphate	171.0^{b}	447.8	727.3^{b}	1064^{b}	1336^{b}
SEM ±	0.683	0.864	1.621	2.200	2.739
Zn level (mg/kg)					
80	171.3^{b}	448.4	723.9	1058	1318
120	173.3^{a}	448.3	724.8	1061	1314
SEM±	0.557	0.706	1.324	1.796	2.236
Temperature					
Normal	172.1	455.0^{a}	760.0^{a}	1183^{a}	1414^{a}
Hot	172.5	441.7^{b}	688.7^{b}	936.5^{b}	1218^{b}
SEM ±	0.557	0.706	1.324	1.796	2.236

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a,b]Means within the same column with no common superscript differ significantly ($P < 0.05$) S*EM* - Standard error of the mean

4.2.1. The main and interactive effects of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) from 0 - 35 days of age

The main and interactive effects between Zn source, Zn inclusion level and house temperature for the different weeks are presented in Table 4.9 Zn source and dosage level presented with significant interactive effects ($P < 0.05$) on broiler feed intake from 14 - 21 and 28 - 35 days of age. No other interactive effects were found between Zn source and Zn dosage level for feed intake for the duration of the study. Significant interactions between Zn source and Zn inclusion level were seen from 14 - 21 days of age. No other significant interactions were observed between Zn source and Zn inclusion level. Significant interactive effects between house temperature and Zn source, as well as Zn dosage level and house temperature, were found on broiler feed intake from 14 - 21 days of age, showing significant interactive effects for the remainder of the trial. Zn source, Zn dosage level and house temperature presented with significant interactive effects on broiler feed intake, starting at day 14 for the remainder of the research trial.

Table 4.9 The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on feed intake (g) in broilers from 0 - 35 days of age (P-values)

	Days of age				
	0-7	7-14	14-21	21-28	28-35
Zn Source	0.082	0.162	0.080	0.068	<0.001
Zn Level	0.010	0.860	0.616	0.308	0.277
Zn Source x Level	0.686	0.716	0.018	0.264	0.202
Temperature	0.591	<0.001	<0.001	<0.001	<0.001
Zn Source x Temperature	0.440	0.210	0.178	<0.001	<0.001
Zn Level x Temperature	0.915	0.763	0.495	<0.001	<0.001
Source x Level x Temperature	0.408	0.009	<0.001	<0.001	<0.001

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.2.2. The effect of Zn source, Zn level and house temperature on broiler feed intake (g) from 0 - 7 days of age

Tables 4.10 - 4.14 show the effects of Zn source, Zn inclusion level and house temperature on broiler feed intake for the duration of the study period.

No significant differences were observed in feed intakes between broilers supplemented with different sources across both inclusion levels and temperatures, as seen in Table 4.10. Under thermoneutral conditions, birds supplemented with AZ presented with significantly ($P < 0.05$) higher mean feed intakes compared to broilers supplemented with ZS. Mean feed intakes from broilers supplemented with AZ and ZS did not differ significantly from mean feed intakes of IZ supplemented broilers. Birds supplemented with ZS under high temperatures presented with higher feed intakes at inclusion level 120 mg/kg compared to inclusion level 80 mg/kg.

Table 4.10 The effect of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed intake (g) from 0 - 7 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	172.6 (±1.365)	170.5 (±1.365)	169.9 (±1.365)	171.0 (±0.788)
120 mg/kg	174.2 (±1.365)	174.2 (±1.365)	171.1 (±1.365)	173.2 (±0.788)
Mean	173.4^{a} (±0.965	172.3^{ab} (±0.965	170.5^{b} (±0.965	
Hot temperature				
80 mg/kg	172.1 (±1.365)	172.9 (±1.365)	169.5^{B} (±1.365)	171.5 (±0.788)
120 mg/kg	172.7 (±1.365)	174.3 (±1.365)	173.5^{A} (±1.365)	173.5 (±0.788)
Mean	172.4 (±0.965	173.6 (±0.965	171.5 (±0.965	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was

increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

4.2.3. The effect of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) from 7 - 14 days of age

Under thermoneutral conditions at 80 mg/kg inclusion, broilers provided with AZ presented with the highest feed intakes, where broilers displayed the lowest feed intakes in ZS supplemented birds. Feed intakes from broilers supplemented with IZ at 80 mg/kg inclusion did not differ significantly with feed intakes from birds supplemented with AZ and ZS under thermoneutral conditions. Broilers under thermoneutral conditions at 120 mg/kg inclusion showed no significant differences in feed intakes between different sources. This was also noted in heat stressed broilers at 80 mg/kg inclusion, as found in Table 4.11. Heat stressed broilers supplemented with AZ at 120 mg/kg inclusion had the highest feed intakes. The lowest feed intakes were found in broilers supplemented with ZS. At 120 mg/kg inclusion under high temperature stress, birds supplemented with organic sources AZ and IZ differed in feed intakes of broilers, although no significant differences were found with ZS supplemented birds. Birds supplemented with AZ at 120 mg/kg inclusion, under high temperature stress presented with significantly ($P < 0.05$) higher feed intakes compared to birds supplemented at 80 mg/kg inclusion. No other significant differences were found between feed intakes of broilers, across both temperature treatments and inclusion levels.

Table 4.11 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed intake (g) from 7-14 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	458.9^{a} (±1.728)	455.1^{ab} (±1.728)	451.6^{b} (±1.728)	455.2 (±0.998)
120 mg/kg	455.0 (±1.728)	454.4 (±1.728)	454.8 (±1.728)	454.7 (±0.998)
Mean	457.0^{a} (±1.222)	454.8^{ab} (±1.222)	453.2^{b} (±1.222)	
Hot temperature				
80 mg/kg	440.0^{B} (±1.728)	441.2 (±1.728)	444.0 (±1.728)	441.7 (±0.998)
120 mg/kg	445.1^{aA} (±1.728)	439.4^{b} (±1.728)	441.0^{ab} (±1.728)	441.8 (±0.998)
Mean	442.5 (±1.222)	440.3 (±1.222)	442.5 (±1.222)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

4.2.4. The effect of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) at 14 - 21 days of age

Under thermoneutral conditions at 80 mg/kg inclusion, birds supplemented with AZ presented with significantly ($P < 0.05$) higher feed intakes compared to birds supplemented with IZ and ZS. Under both 80 mg/kg and 120 mg/kg inclusion, broilers supplemented with IZ and ZS did not differ significantly in feed intakes, although feed intakes differed significantly ($P < 0.05$) from broilers supplemented with AZ. Broilers under thermoneutral temperatures, supplemented with AZ at 120 mg/kg inclusion presented with the lowest feed intakes ($P < 0.05$). This was also noted under heat stressed conditions at 80 mg/kg inclusion, as seen in Table 4.12. Heat stressed broilers supplemented with ZS at 80 mg/kg inclusion exhibited the highest feed intakes ($P < 0.05$).

Significantly higher feed intakes ($P < 0.05$) were obtained in broilers supplemented with AZ at 120 mg/kg, compared to 80 mg/kg inclusion under high temperature stress. Under thermoneutral conditions, AZ supplemented broilers had significantly higher ($P < 0.05$) feed intakes when supplemented at 80 mg/kg inclusion level. ZS supplemented broilers exhibited significantly higher ($P < 0.05$) feed intakes under thermoneutral conditions at 120 mg/kg inclusion level, compared to broilers supplemented at 80 mg/kg inclusion. Heat stressed broilers showed significantly higher ($P < 0.05$) feed intakes when ZS was supplemented at 80 mg/kg inclusion rather than 120 mg/kg inclusion. Under both temperature treatments no significant differences ($P < 0.05$) were found between feed intakes of broilers under different level means.

Table 4.12 Effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed intake (g) for 14 - 21 days (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	776.2^{aA} (±3.242)	754.3^{b} (±3.242)	750.0^{bB} (±3.242)	760.2 (±1.871)
120 mg/kg	744.1^{aB} (±3.242)	759.7^{b} (±3.242)	775.7^{bA} (±3.242)	759.8 (±1.871)
Mean	760.2 (±2.292)	757.0 (±2.292)	762.8 (±2.292)	
Hot temperature				
80 mg/kg	674.7^{aB} (±3.242)	690.3^{b} (±3.242)	697.8^{cA} (±3.242)	687.6 (±1.871)
120 mg/kg	694.2^{A} (±3.242)	689.5 (±3.242)	685.8^{B} (±3.242)	689.8 (±1.871)
Mean	684.5^{a} (±2.292)	689.9^{ab} (±2.292)	691.8^{b} (±2.292)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.2.5. The effect of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) at 21 - 28 days of age

IZ supplementation under thermoneutral conditions, at 80 mg/kg inclusion resulted in significantly ($P < 0.05$) higher feed intakes in broilers. At 80 mg/kg inclusion level, AZ and ZS supplemented broilers did not differ in feed intake, although feed intakes were significantly lower ($P < 0.05$) than IZ supplemented birds. Birds presented with the highest feed intakes when ZS was supplemented, at both inclusion levels under high temperature stress. As found in Table 4.13, broilers subjected to high temperature stress supplemented with AZ and IZ, at both 80 mg/kg and 120 mg/kg inclusion levels had significantly lower ($P < 0.05$) feed intakes. AZ and IZ did not differ in feed intakes between broilers, although intakes were significantly lower ($P < 0.05$) than that of the ZS supplemented group.

Supplementary AZ at 120 mg/kg compared to 80 mg/kg inclusion level, under thermoneutral conditions resulted in significantly higher ($P < 0.05$) feed intakes in broilers. AZ supplementation at 80 mg/kg inclusion, under high temperature stress resulted in broilers with significantly higher ($P < 0.05$) feed intakes compared to broilers supplemented at 120 mg/kg inclusion level. Supplementing at 120 mg/kg inclusion level, under thermoneutral conditions led to significantly higher ($P < 0.05$) feed intakes in broilers, where heat stressed broilers had higher intakes when 80 mg/kg inclusion rather than 120 mg/kg was provided.

Table 4.13 The effects of Zn source[1], Zn level and Temperature[2] on broiler feed intake (g) for 21-28 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1160^{aB} (±4.40)	1197^{b} (±4.40)	1155^{a} (±4.40)	1171^{b} (±2.540)
120 mg/kg	1214^{aA} (±4.40)	1209^{b} (±4.40)	1160^{c} (±4.40)	1194^{a} (±2.540)
Mean	1187^{a} (±3.11)	1203^{b} (±3.11)	1157^{c} (±3.11)	
Hot temperature				
80 mg/kg	947.1^{aA} (±4.40)	914.7^{a} (±4.40)	975.6^{b} (±4.40)	945.8^{a} (±2.540)
120 mg/kg	907.3^{aB} (±4.40)	909.8^{a} (±4.40)	964.5^{b} (±4.40)	927.2^{b} (±2.540)
Mean	927.2^{a} (±3.11)	912.3^{b} (±3.11)	970.1^{c} (±3.11)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.2.6. The effect of Zn source, Zn inclusion level and house temperature on broiler feed intake (g) at 28 - 35 days of age

As found in Table 4.14, AZ and IZ supplementation at both 80 mg/kg and 120 mg/kg inclusion under thermoneutral conditions resulted in broilers with significantly lower ($P < 0.05$) feed intakes. Under thermoneutral conditions, at both 80 mg/kg and 120 mg/kg inclusion, broilers presented with significantly higher ($P < 0.05$) feed intakes when supplementary ZS was provided. The same was noted at 120 mg/kg inclusion level in broilers exposed to high temperature stress. Broilers supplemented with AZ and ZS at inclusion level 80 mg/kg compared to 120 mg/kg under thermoneutral conditions displayed significantly higher ($P < 0.05$) feed intakes. At 120 mg/kg inclusion in heat stressed birds, different Zn sources resulted in significant differences ($P < 0.05$) between broiler feed intakes. Birds given supplementary

ZS presented with the lowest feed intakes, where IZ supplemented birds had the highest feed intakes.

Supplementing ZS and AZ at 80 mg/kg inclusion, under thermoneutral temperatures, resulted in broilers with significantly higher ($P < 0.05$) feed intakes. Broilers under high temperature stress, however, displayed with higher feed intakes when ZS was supplemented at 120 mg/kg. Broilers supplemented at 80 mg/kg inclusion level presented with significantly higher ($P < 0.05$) feed intakes under thermoneutral conditions, where heat stressed broilers had higher feed intakes when Zn was supplemented at 120 mg/kg inclusion level.

Table 4.14 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed intake (g) at 28 - 35 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1407^{aA} (±5.477)	1394^{a} (±5.477)	1489^{bA} (±5.477)	1430^{a} (±3.162)
120 mg/kg	1377^{aB} (±5.477)	1392^{a} (±5.477)	1424^{bB} (±5.477)	1398^{b} (±3.162)
Mean	1392^{a} (±3.873)	1393^{a} (±3.873)	1457^{b} (±3.873)	
Hot temperature				
80 mg/kg	1210^{a} (±5.477)	1218^{b} (±5.477)	1188^{cB} (±5.477)	1205^{b} (±3.162)
120 mg/kg	1220^{a} (±5.477)	1228^{a} (±5.477)	1244^{bA} (±5.477)	1231^{a} (±3.162)
Mean	1215 (±3.873)	1223 (±3.873)	1216 (±3.873)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.3. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 35 days of age

As seen in Table 4.15, broilers supplemented with IZ from 0 - 7 days of age presented with significantly higher ($P < 0.05$) cumulative feed intakes compared to broilers supplemented with ZS. Birds given supplementary AZ presented with significantly higher ($P < 0.05$) cumulative feed intakes than broilers that received IZ or ZS from 0 - 14 and 0 - 21 days of age. Broilers displayed no significant differences in cumulative feed intakes between IZ and ZS supplemented birds, although sources were significantly lower ($P < 0.05$) than broilers supplemented with AZ. From 0 - 28 days of age no significant differences were found in cumulative feed intakes between broilers supplemented with AZ and ZS, although both groups differed significantly ($P < 0.05$) with broilers supplemented with IZ. Broilers supplemented with IZ exhibited the lowest cumulative feed intakes from 0 - 28 days of age. At 0 - 35 days of age cumulative feed intakes did not differ between birds supplemented with organic sources IZ and AZ. ZS supplemented birds presented with the highest cumulative feed intakes at 0 - 35 days of age, differing significantly ($P < 0.05$) from broilers supplemented with AZ and IZ.

Broilers supplemented with Zn at 120 mg/kg inclusion level consistently presented with significantly higher ($P < 0.05$) cumulative feed intakes than birds supplemented at 80 mg/kg inclusion level. Cumulative feed intakes from broilers supplemented at 80 mg/kg inclusion level consistently exhibited lower values. Significant differences in cumulative feed intakes were apparent from 0 - 7 days of age, continuing for the remainder of the research trail.

Broilers under thermoneutral compared to high temperature stress presented with significantly higher ($P < 0.05$) cumulative feed intakes. Significant differences ($P < 0.05$) in cumulative feed intakes, under both temperature treatments were found starting at day 14, continuing for the duration of the research trial. Cumulative feed intakes measured at day 7 showed no significant differences between groups, as heat stress only started on day 9. At the end of the research period at 35 days of age, birds subjected to heat stress presented with significantly lower ($P < 0.05$) cumulative feed intakes, displaying a difference of 530 g less overall feed consumed.

Table 4.15 The effects of Zn source[1], Zn inclusion level and house temperature[2] on cumulative feed intake (g) in broilers at 0-35 days of age

Treatment	Days of age				
	0 - 7	0 - 14	0 - 21	0 - 28	0 – 35
Zn Source					
Availa®Zn	172.9[ab]	622.7[a]	1350[a]	2407[a]	3712[a]
IntelliBond®Z	173.0[a]	620.5[b]	1344[b]	2402[b]	3709[a]
Zn Sulphate	171.0[b]	618.9[b]	1347[b]	2410[a]	3746[b]
SEM ±	0.683	0.749	1.370	1.822	2.155
Zn level (mg/kg)					
80	171.3[b]	619.7[b]	1344[b]	2402[b]	3719[b]
120	173.3[a]	621.6[a]	1350[a]	2411[a]	3725[a]
SEM ±	0.557	0.612	1.119	1.488	1.759
Temperature					
Normal	172.1	627.0[a]	1390[a]	2573[a]	3987[a]
Hot	172.5	614.3[b]	1303[b]	2240[b]	3457[b]
SEM ±	0.557	0.612	1.119	1.488	1.759

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn Sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

SEM - Standard error of the mean

4.3.1. The main and interaction effects of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) at 0 - 35 days of age

The main and interactive effects between Zn source, Zn inclusion level and house temperature for the different weeks are presented in Table 4.16. No significant interactive effects were observed between Zn source and Zn dosage level on cumulative feed intakes for the duration of the trial. Zn source and house temperature presented with significant interactions on cumulative feed intakes for the duration of the trial, starting at 0 - 14 days of age. Zn dosage level and house temperature presented with no significant interactive effects from 0 - 21 days

of age. After day 21, at 0 - 28 and 0 - 35 days of age, significant interactive effects were found between Zn dosage level and house temperature for cumulative feed intakes. Interactive effects between Zn source, Zn level and temperature were observed starting at 0 - 14 days of age. Significant interactive effects between Zn source, Zn inclusion level and house temperature were seen on cumulative feed intakes for the remainder of the research trail.

Table 4.16 The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 35 days of age (P-values)

	Days of age				
	0 – 7	0 - 14	0 - 21	0 - 28	0 – 35
Zn Source	0.082	0.003	0.008	0.007	<0.0001
Zn Level	0.010	0.035	.0002	<0.0001	0.026
Zn Source x Level	0.686	0.800	0.311	.3999	0.349
Temperature	0.591	<0.0001	<0.0001	<0.0001	<0.0001
Zn Source x Temperature	0.440	0.032	<0.0001	<0.0001	<0.0001
Zn Level x Temperature	0.915	0.839	0.222	<0.0001	0.046
Source x Level x Temperature	0.408	0.013	<0.0001	0.005	<0.0001

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.3.2. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 7 days of age

The effects of Zn source, Zn inclusion level and house temperature on cumulative feed intake in broilers for each week of the study period are found in Tables 4.17 to 4.21.

No significant differences were obtained in broiler cumulative feed intake between sources, Zn inclusion levels and temperature treatments, as seen in Table 4.17. Broilers under thermoneutral temperatures presented with significantly higher ($P < 0.05$) mean cumulative feed intakes when AZ compared to ZS was supplemented. No significant differences were observed between mean cumulative feed intakes with IZ supplemented birds. Birds

supplemented at 120 mg/kg inclusion level, under high temperature stress exhibited significantly higher cumulative feed intakes compared to birds that were supplemented at 80 mg/kg inclusion level.

Table 4.17 The effects of Zn source[1], Zn inclusion level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 7 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	172.6 (±1.370)	170.5 (±1.370)	169.9 (±1.370)	171.0 (±0.788)
120 mg/kg	174.2 (±1.370)	174.2 (±1.370)	171.1 (±1.370)	173.2 (±0.788)
Mean	173.4[a] (±0.965)	172.3[ab] (±0.965)	170.5[b] (±0.965)	
Hot temperature				
80 mg/kg	172.1 (±1.370)	172.9 (±1.370)	169.5[B] (±1.370)	171.5 (±0.788)
120 mg/kg	172.7 (±1.370)	174.3 (±1.370)	173.5[A] (±1.370)	173.5 (±0.788)
Mean	172.4 (±0.965)	173.6 (±0.965)	171.5 (±0.965)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.3.3. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 14 days of age

Broilers subjected to high temperature stress at both inclusion level 80 mg/kg and 120 mg/kg presented with no significant differences ($P < 0.05$) in cumulative feed intakes between broilers supplemented with different Zn sources, as found in table 4.18. The same was noted in birds under thermoneutral conditions at 120 mg/kg inclusion. Under thermoneutral temperatures at 80 mg/kg inclusion level, birds supplemented with AZ presented with significantly higher (*P*

< 0.05) cumulative feed intakes. Birds supplemented with IZ and ZS presented with no significant differences between cumulative feed intakes, where AZ supplemented birds had significantly lower (*P < 0.05*) cumulative feed intakes.

Supplementing ZS to heat stressed birds at 120 mg/kg inclusion resulted in birds with higher cumulative feed intakes compared to inclusion level 80 mg/kg. Temperature stressed broilers supplemented with AZ, at 120 mg/kg inclusion presented with higher cumulative feed intakes than birds supplemented at 80 mg/kg inclusion level.

Table 4.18 The effects of Zn source[1], Zn inclusion level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 14 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	631.5^{a} (±1.500)	625.6^{b} (±1.500)	621.6^{bB} (±1.500)	626.2 (±0.865)
120 mg/kg	629.2 (±1.500)	628.6 (±1.500)	625.9^{A} (±1.500)	627.9 (±0.865)
Mean	630.3^{a} (±1.060)	627.1^{b} (±1.060)	623.7^{c} (±1.060)	
Hot temperature				
80 mg/kg	612.2^{B} (±1.500)	614.1 (±1.500)	613.5 (±1.500)	613.3 (±0.865)
120 mg/kg	617.8^{A} (±1.500)	613.7 (±1.500)	614.5 (±1.500)	615.3 (±0.865)
Mean	615.0 (±1.060)	613.9 (±1.060)	614.0 (±1.060)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly (*P < 0.05*).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly (*P < 0.05*)

SEM - Standard error of the mean

4.3.4. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 21 days of age

Broilers supplemented at 80 mg/kg and 120 mg/kg inclusion level, under thermoneutral conditions presented with significant differences ($P < 0.05$) in cumulative feed intakes between all three Zn sources, presented in table 4.19. AZ supplementation under thermoneutral conditions, at 80 mg/kg inclusion resulted in significantly higher ($P < 0.05$) cumulative feed intakes for the birds. Birds provided with supplementary ZS at 80 mg/kg inclusion exhibited significantly lower ($P < 0.05$) cumulative feed intakes compared to both organic sources AZ and IZ. Supplementary ZS at 120 mg/kg inclusion, under thermoneutral conditions resulted in broilers with significantly higher ($P < 0.05$) cumulative feed intakes, where birds given IZ presented with significantly lower ($P < 0.05$) cumulative feed intakes. At 80 mg/kg inclusion under high temperature stress, broilers supplemented with AZ and IZ had no significant differences in cumulative feed intakes, although broilers presented with significantly higher ($P < 0.05$) cumulative feed intakes compared to ZS supplemented birds. At 120 mg/kg inclusion level, AZ and IZ did not differ in cumulative feed intakes of broilers, although cumulative feed intakes were significantly higher ($P < 0.05$) than in broilers supplemented with ZS.

AZ supplementation at 80 mg/kg inclusion, under thermoneutral temperatures resulted in broilers with higher cumulative feed intakes compared to the 120 mg/kg inclusion level. The same was noted when supplementary ZS was provided during heat stress. Broilers supplemented with IZ and ZS, however, had significantly higher ($P < 0.05$) cumulative feed intakes when 120 mg/kg Zn inclusion was given. This was also noted in broilers supplemented with AZ under high temperatures.

Table 4.19 The effects of Zn source[1], Zn inclusion level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 21 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1408^{aA} (±2.740)	1380^{bB} (±2.740)	1372^{cB} (±2.740)	1386^{b} (±1.582)
120 mg/kg	1394^{aB} (±2.740)	1388^{bA} (±2.740)	1402^{cA} (±2.740)	1395^{a} (±1.582)
Mean	1401^{a} (±1.938)	1384^{b} (±1.938)	1387^{b} (±1.938)	
Hot temperature				
80 mg/kg	1287^{aB} (±2.740)	1304^{a} (±2.740)	1311^{bA} (±2.740)	1301 (±1.582)
120 mg/kg	1312^{aA} (±2.740)	1303^{b} (±2.740)	1300^{bB} (±2.740)	1305 (±1.582)
Mean	1299^{a} (±1.938)	1304^{ab} (±1.938)	1306^{b} (±1.938)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.3.5. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 28 days of age

Under thermoneutral conditions, AZ and IZ supplementation at 80 mg/kg inclusion resulted in significantly higher ($P < 0.05$) cumulative feed intakes for the birds. Cumulative feed intakes from broilers supplemented with AZ and IZ did not differ, but displayed significantly higher ($P < 0.05$) cumulative feed intakes than broilers supplemented with ZS, as presented in Table 4.20. Cumulative feed intakes differed significantly ($P < 0.05$) between broilers supplemented with different sources at 120 mg/kg inclusion under thermoneutral conditions. Birds supplemented with AZ presented with significantly higher ($P < 0.05$) cumulative feed intakes, where broilers supplemented with ZS showed significantly lower ($P < 0.05$) cumulative feed intakes. Under high temperature stress, at both 80 mg/kg and 120 mg/kg inclusion levels broilers exhibited the highest cumulative feed intakes when ZS was supplemented. The lowest

cumulative feed intakes were found in broilers supplemented with IZ. During heat stress at inclusion level 120 mg/kg, the two organic sources AZ and IZ did not differ in cumulative feed intakes of broilers, although cumulative feed intakes were significantly lower ($P < 0.05$) than in broilers supplemented with ZS.

Birds presented with significantly higher ($P < 0.05$) cumulative feed intakes when Zn was supplemented at 120 mg/kg compared to 80 mg/kg inclusion levels, regardless of source. Under heat stressed conditions, supplementing broilers at 80 mg/kg inclusion rather than 120 mg/kg inclusion resulted in significantly higher ($P < 0.05$) cumulative feed intakes in broilers, regardless of source.

Table 4.20 Effects of Zn source[1], Zn level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 28 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	2568^{aB} (±3.645)	2577^{aB} (±3.645)	2526^{bB} (±3.645)	2557^{b} (±2.104)
120 mg/kg	2608^{aA} (±3.645)	2597^{bA} (±3.645)	2562^{cA} (±3.645)	2589^{a} (±2.104)
Mean	2588^{a} (±2.577)	2587^{a} (±2.577)	2544^{b} (±2.577)	
Hot temperature				
80 mg/kg	2234^{aA} (±3.645)	2219^{bA} (±3.645)	2287^{cA} (±3.645)	2247^{a} (±2.104)
120 mg/kg	2219^{aB} (±3.645)	2213^{aB} (±3.645)	2265^{bB} (±3.645)	2232^{b} (±2.104)
Mean	2227^{a} (±2.577)	2216^{b} (±2.577)	2276^{c} (±2.577)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same column (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.3.6. The effect of Zn source, Zn inclusion level and house temperature on cumulative feed intake (g) in broilers at 0 - 35 days of age

Under thermoneutral conditions, ZS supplementation at 80 mg/kg inclusion level resulted in broilers with significantly higher ($P < 0.05$) cumulative feed intakes, as found in table 4.21. The same was noted in broilers at 120 mg/kg inclusion under heat stress. Supplementing IZ and AZ at 80 mg/kg inclusion level, resulted in no significant differences in cumulative feed intakes between broilers, but cumulative feed intakes were significantly ($P < 0.05$) higher in ZS supplemented birds. This was also noted under thermoneutral conditions at 120 mg/kg inclusion. ZS supplementation, at 120 mg/kg inclusion level resulted in significantly higher ($P < 0.05$) cumulative feed intakes than broilers supplemented with organic sources AZ and IZ.

No significant differences were observed between cumulative feed intakes in broilers supplemented with different sources at 80 mg/kg. Supplementing AZ at 120 mg/kg compared to 80 mg/kg inclusion level, under thermoneutral temperatures resulted in significantly higher ($P < 0.05$) cumulative feed intakes in broilers. The same was observed in broilers supplemented with ZS under high temperature stress. Supplementary IZ and ZS at 120 mg/kg inclusion, in broilers under thermoneutral conditions presented broilers with significantly higher cumulative feed intakes compared to groups supplemented at 80 mg/kg inclusion level. Heat stressed broilers presented with higher cumulative feed intakes when Zn was supplemented at 120 mg/kg rather than 80 mg/kg inclusion levels.

Table 4.21 The effects of Zn source[1], Zn level and house temperature[2] on cumulative feed intake (g) in broilers at 0 - 35 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	3975^{aB} (±4.309)	3971^{aA} (±4.309)	4016^{bA} (±4.309)	3987 (±2.488)
120 mg/kg	3988^{aA} (±4.309)	3990^{aB} (±4.309)	3985^{bB} (±4.309)	3988 (±2.488)
Mean	3981^{a} (±3.047)	3980^{a} (±3.047)	4001^{b} (±3.047)	
Hot temperature				
80 mg/kg	3444 (±4.309)	3437 (±4.309)	3475^{B} (±4.309)	3452^{b} (±2.488)
120 mg/kg	3440^{a} (±4.309)	3439^{a} (±4.309)	3508^{bA} (±4.309)	3463^{a} (±2.488)
Mean	3442^{a} (±3.047)	3438^{a} (±3.047)	3492^{b} (±3.047)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same column with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.4. The effect of Zn source, Zn level and house temperature on feed conversion ratio in broilers at day 0 - 35 of age

As seen in Table 4.22, for the first 7 days of the research trial feed conversion ratio (FCR) in birds supplemented with AZ were significantly higher than FCR in IZ and ZS supplemented broilers. Feed conversion ratios between birds, supplemented with IZ and ZS did not differ significantly between sources, although FCR were significantly higher ($P < 0.05$) than in birds supplemented with AZ. At 0 - 14 days of age, FCR between broilers supplemented with organic sources AZ and IZ did not differ significantly ($P < 0.05$) between sources, but were significantly lower ($P < 0.05$) compared to birds receiving supplementary ZS. Birds supplemented with ZS at 0 - 21 days of age presented with the highest FCR, where birds supplemented with AZ exhibited the lowest FCR. Significant differences were seen between FCR from birds supplemented with different sources. Birds supplemented with AZ consistently

exhibited the lowest FCR, with significantly higher ($P < 0.05$) values in IZ supplemented birds. ZS supplementation, however, resulted in birds with FCR that were significantly higher ($P < 0.05$) than in IZ and AZ supplemented birds. This was found at 0 - 21 days of age and was evident for the remainder of the research trial.

At both 80 mg/kg and 120 mg/kg inclusion levels, no significant differences were observed in FCR between broilers from 0 - 21 days of age. Birds supplemented with Zn at 80 mg/kg inclusion presented with significantly higher FCR in comparison to birds supplemented at 120 mg/kg inclusion level. This was noted from 0 - 28 through 0 - 35 days of age.

Broilers subjected to high temperature stress, compared to birds in thermoneutral conditions presented with significantly higher ($P < 0.05$) FCR. Significant differences ($P < 0.05$) in FCR between broilers were seen under both temperature treatments, starting at 0 - 7 days of age. Significant differences between broilers under different temperature treatments were observed for the remainder of the research trial. At day 0 - 35, birds subjected to high temperature stress displayed with significantly higher ($P < 0.05$) FCR compared to birds that were exposed to thermoneutral conditions.

Table 4.22 The effects of Zn source[1], Zn inclusion level and house temperature[2] on feed conversion ratio in broilers at 0 - 35 days of age

Treatment	Days of age				
	0 – 7	0 - 14	0 - 21	0 - 28	0 – 35
Zn Source					
Availa®Zn	0.862[a]	1.133[a]	1.288[a]	1.431[a]	1.565[a]
IntelliBond®Z	0.867[b]	1.134[a]	1.290[b]	1.435[b]	1.567[b]
Zn Sulphate	0.866[b]	1.134[b]	1.308[c]	1.443[c]	1.589[c]
SEM±	0.124	0.165	0.732	0.662	0.542
Zn level					
80 mg/kg	0.864	1.134	1.296	1.439[a]	1.574[a]
120 mg/kg	0.866	1.134	1.295	1.434[b]	1.573[b]
SEM±	0.101	0.134	0.597	0.540	0.442
Temperature					
Normal	0.862[b]	1.131[b]	1.281[b]	1.423[b]	1.555[b]
Hot	0.867[a]	1.136[a]	1.309[a]	1.450[a]	1.593[a]
SEM±	0.101	0.134	0.597	0.540	0.442

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same column with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

SEM - Standard error of the mean

4.4.1. The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed conversion ratio at 0 - 35 days of age

The main and interactive effects between Zn source, Zn inclusion level and house temperature for the different weeks are presented in Table 4.23. Zn source and dosage level presented with significant interactive effects ($P < 0.05$) on FCR in broilers from 0 - 14 days of age. Significant interactive effects for FCR were observed for the remainder of the research trial. Significant

interactive effects were found between temperature and Zn source for FCR, starting at 14 - 21 days of age. Effects remained significant for the remainder of the trial. No interactive effects for temperature treatments were found on FCR before 14 days of age, as high temperature treatments commenced on day 9. Zn dosage level and house temperature exhibited interactive effects on FCR at 0 - 7 and 0 - 14 days of age. No significant interactions were found between Zn dosage level and house temperature after 14 - 21 days of age. Zn dosage level and house temperature showed interactive effects from days 0 - 21, showing significant interactions on FCR for the duration of the trial. Zn source, Zn inclusion level and temperature displayed no significant interactions for the first 14 days of the trial, where after significant interactions were found on FCR for the remainder of the research trial.

Table 4.23 The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed conversion ratio at 0 - 35 days of age (P-values)

	Days of age				
	0 - 7	0 - 14	0 – 21	0 - 28	0 - 35
Zn source	0.023	0.039	<0.0001	<0.0001	<0.0001
Zn level	0.086	0.342	0.333	<0.0001	0.036
Zn source x Level	0.209	0.669	<0.0001	<0.0001	0.038
Temperature	0.001	<0.0001	<0.0001	<0.0001	<0.0001
Zn source x Temperature	0.947	0.935	<0.0001	<0.0001	<0.0001
Zn Level x Temperature	0.044	0.001	0.506	<0.0001	0.004
Source x Level x Temperature	0.699	0.379	<0.0001	<0.0001	0.006

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.4.2. The effect of Zn source, Zn inclusion level and house temperature on feed conversion ratio in broilers from 0 - 7 days of age

Tables 4.30 - 4.35 show the effects of Zn source, Zn inclusion level and house temperature on broiler FCR for each week of the study period

Under thermoneutral conditions at 120 mg/kg inclusion level, significant differences ($P < 0.05$) were found in FCR between broilers supplemented with AZ and IZ, but no significant differences were seen with ZS supplemented birds. The same was noted in broilers under heat stress conditions for mean FCR, as found in table 4.24. Under heat stress at 120 mg/kg inclusion level, FCR from AZ and IZ supplemented birds were significantly lower ($P < 0.05$) than FCR from birds that received ZS. Birds presented with significantly lower ($P < 0.05$) FCR when IZ was provided at 80 mg/kg compared to 120 mg/kg under thermoneutral conditions. No other significant differences were observed.

Table 4.24 Effects of Zn source[1], Zn inclusion level and house temperature[2] on feed conversion ratio in broilers at 0 - 7 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	0.859 (±0.247)	0.859^{B} (±0.247)	0.861 (±0.247)	0.860^{b} (±0.143)
120 mg/kg	0.861^{a} (±0.247)	0.869^{bA} (±0.247)	0.865^{ab} (±0.247)	0.865^{a} (±0.143)
Mean	0.860 (±0.175)	0.864 (±0.175)	0.863 (±0.175)	
Hot temperature				
80 mg/kg	0.865 (±0.247)	0.869 (±0.247)	0.869 (±0.247)	0.868 (±0.143)
120 mg/kg	0.864^{a} (±0.247)	0.871^{a} (±0.247)	0.867^{b} (±0.247)	0.867 (±0.143)
Mean	0.865^{a} (±0.175)	0.870^{b} (±0.175)	0.868^{ab} (±0.175)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.4.3. The effect of Zn source, Zn level and temperature on feed conversion ratio in broilers at 0 - 14 days of age

As seen in Table 4.25, birds supplemented with ZS under thermoneutral conditions, at 80 mg/kg inclusion presented with significantly higher ($P < 0.05$) FCR. Birds provided with organic sources AZ and IZ showed no significant differences in broiler FCR between sources, although birds supplemented with ZS presented with significantly higher ($P < 0.05$) FCR. No significant differences were found between broilers given different sources under high temperature treatments, at both 80 mg/kg and 120 mg/kg inclusion levels. The same was noted in broilers at 120 mg/kg inclusion level under thermoneutral conditions.

Birds under high temperature stress supplemented at 80 mg/kg with AZ and IZ, exhibited significantly higher ($P < 0.05$) FCR compared to birds supplemented at 120 mg/kg inclusion level. Birds supplemented with AZ, under thermoneutral conditions presented with significantly lower ($P < 0.05$) FCR when birds were supplemented at 80 mg/kg rather than 120 mg/kg inclusion level.

Table 4.25 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed conversion ratio from 0 - 14 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1.131^{aB} (±0.333)	1.131^{ab} (±0.333)	1.132^{b} (±0.333)	1.131^{b} (±0.190)
120 mg/kg	1.132^{A} (±0.333)	1.132 (±0.333)	1.132 (±0.333)	1.132^{a} (±0.190)
Mean	1.131^{a} (±0.233)	1.131^{ab} (±0.233)	1.132^{b} (±0.233)	
Hot temperature				
80 mg/kg	1.136^{A} (±0.333)	1.137^{A} (±0.333)	1.137 (±0.333)	1.137^{a} (±0.190)
120 mg/kg	1.135^{B} (±0.333)	1.135^{B} (±0.333)	1.136 (±0.333)	1.136^{b} (±0.190)
Mean	1.136 (±0.233)	1.136 (±0.233)	1.136 (±0.233)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same rows with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.4.4. The effect of Zn source, Zn inclusion level and house temperature on feed conversion ratio in broilers at 0 - 21 days of age

Under thermoneutral conditions, AZ and IZ supplementation at 80 mg/kg inclusion resulted in birds with FCR significantly lower ($P < 0.05$) than ZS supplemented birds. At 80 mg/kg inclusion, the two organic sources did not differ in FCR of broilers, although FCR were significantly ($P < 0.05$) lower than that of the ZS supplemented groups. Under thermoneutral conditions at 120 mg/kg inclusion level, different Zn sources resulted in significant differences ($P < 0.05$) in FCR between broilers. This was also noted under heat stressed conditions, at both 80 mg/kg and 120 mg/kg inclusion levels, as seen in Table 4.26. Under thermoneutral conditions, broilers supplemented with AZ at 120 mg/kg inclusion presented with significantly lower ($P < 0.05$) FCR compared to birds receiving supplementary IZ and ZS. Compared to birds supplemented with the organic sources AZ and IZ, birds that received ZS had significantly higher ($P < 0.05$) FCR. This was also noted in birds subjected to high temperature stress at both 80 mg/kg and 120 mg/kg inclusion levels, as well as under thermoneutral conditions at 120 mg/kg inclusion level. Significantly lower ($P < 0.05$) FCR were found in broilers supplemented with AZ at 80 mg/kg inclusion under hyperthermic conditions. Broilers receiving supplementary IZ under high temperature stress presented with the lowest FCR. Birds supplemented with ZS presented the highest FCR, across both temperature treatments and inclusion levels.

Birds subjected to high temperatures, supplemented with AZ at 80 mg/kg inclusion presented with significantly ($P < 0.05$) lower FCR compared to birds at 120 mg/kg inclusion. Heat stressed birds supplemented with ZS exhibited significantly lower ($P < 0.05$) FCR when Zn was supplemented at 120 mg/kg rather than 80 mg/kg inclusion level. No other significant differences were observed for FCR between levels, across Zn sources and temperature treatments.

Table 4.26 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed conversion ratio at 0 - 21 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1.278^{a} (±0.146)	1.276^{a} (±0.146)	1.291^{b} (±0.146)	1.281 (±0.845)
120 mg/kg	1.274^{a} (±0.146)	1.279^{b} (±0.146)	1.290^{c} (±0.146)	1.281 (±0.845)
Mean	1.276^{a} (±0.104)	1.277^{a} (±0.104)	1.290^{b} (±0.104)	
Hot temperature				
80 mg/kg	1.295^{aB} (±0.146)	1.305^{b} (±0.146)	1.331^{cA} (±0.146)	1.310 (±0.845)
120 mg/kg	1.306^{aA} (±0.146)	1.302^{b} (±0.146)	1.319^{cB} (±0.146)	1.309 (±0.845)
Mean	1.300^{a} (±0.104)	1.303^{b} (±0.104)	1.325^{c} (±0.104)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.4.5. The effect of Zn source, Zn inclusion level and house temperature on feed conversion ratio in broilers at 0 - 28 days of age

Birds under thermoneutral conditions presented with significant differences ($P < 0.05$) in FCR between birds supplemented with AZ and ZS at 80 mg/kg inclusion. Broilers supplemented with IZ showed no significant differences in broiler FCR between AZ or ZS supplemented broilers, as seen in Table 4.27. Significant differences ($P < 0.05$) were found in FCR between broilers supplemented with different sources at 120 mg/kg inclusion under thermoneutral conditions. This was also noted at 80 mg/kg inclusion under heat stress. Birds supplemented with AZ at 120 mg/kg inclusion, under thermoneutral conditions exhibited the lowest FCR, where ZS supplemented birds had the highest FCR. This was also noted in broilers under high temperature stress at 80 mg/kg inclusion. Birds exposed to high temperatures, at 120 mg/kg

inclusion level presented no significant differences in FCR between IZ and ZS supplemented broilers, but sources were significantly higher ($P < 0.05$) than AZ supplemented birds.

Birds supplemented with ZS presented with significantly lower ($P < 0.05$) FCR when 80 mg/kg rather than 120 mg/kg inclusion level was supplemented. This was also noted in level means in heat stressed broilers. No other significant differences were observed between different Zn sources.

Table 4.27 The effects of Zn source[1], Zn inclusion level and house temperature[2] on feed conversion ratio at 0 - 28 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1.421^{a} (±0.132)	1.422^{ab} (±0.132)	1.425^{b} (±0.132)	1.423 (±0.764)
120 mg/kg	1.418^{a} (±0.132)	1.422^{b} (±0.132)	1.427^{c} (±0.132)	1.422 (±0.764)
Mean	1.420^{a} (±0.936)	1.422^{a} (±0.936)	1.426^{b} (±0.936)	
Hot temperature				
80 mg/kg	1.443^{a} (±0.132)	1.449^{b} (±0.132)	1.471^{cB} (±0.132)	1.454^{b} (±0.764)
120 mg/kg	1.441^{a} (±0.132)	1.448^{b} (±0.132)	1.449^{bA} (±0.132)	1.446^{a} (±0.764)
Mean	1.442^{a} (±0.936)	1.448^{b} (±0.936)	1.460^{c} (±0.936)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.4.6. The effect of Zn source, Zn inclusion level and house temperature on feed conversion ratio in broilers at 0 - 35 days of age

As found in Table 4.28, organic source AZ and IZ supplementation at 80 mg/kg inclusion resulted in significantly lower FCR for the birds. This was also noted under heat stressed conditions at both 80 mg/kg and 120 mg/kg inclusion levels. At 80 mg/kg inclusion level, the

two organic sources did not differ significantly in FCR between broilers, although birds supplemented with ZS presented with significantly higher FCR. The same was noted in heat stressed broilers at both 80 mg/kg and 120 mg/kg inclusion levels. Birds supplemented with ZS, under both temperature treatments had significantly higher ($P < 0.05$) FCR at both 80 mg/kg and 120 mg/kg inclusion levels. Birds supplemented at 120 mg/kg inclusion level, under thermoneutral conditions exhibited significant differences ($P < 0.05$) in FCR between Zn sources. Birds that received AZ presented with significantly lower ($P < 0.05$) FCR than broilers that received supplementary IZ and ZS.

IZ and ZS supplementation under thermoneutral conditions, at 120 mg/kg inclusion level resulted in birds with significantly higher ($p < 0.05$) FCR compared to birds supplemented at 80 mg/kg inclusion level. This was in contrast to broilers subjected to high temperatures, where AZ and IZ supplementation at 80 mg/kg resulted in higher FCR. Supplementing Zn at inclusion level 120 mg/kg under heat stress resulted in broilers with significantly lower ($P < 0.05$) FCR.

Table 4.28 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler feed conversion ratio at 0 - 35 days of age (± standard error of the mean)

	Zinc Source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	1.550^{a} (±0.108)	1.551^{aB} (±0.108)	1.562^{bB} (±0.108)	1.554 (±0.625)
120 mg/kg	1.550^{a} (±0.108)	1.556^{bA} (±0.108)	1.559^{cA} (±0.108)	1.555 (±0.625)
Mean	1.550^{a} (±0.766)	1.553^{b} (±0.766)	1.561^{c} (±0.766)	
Hot temperature				
80 mg/kg	1.583^{aA} (±0.108)	1.582^{aA} (±0.108)	1.618^{b} (±0.108)	1.594^{a} (±0.625)
120 mg/kg	1.578^{aB} (±0.108)	1.579^{aB} (±0.108)	1.616^{b} (±0.108)	1.591^{b} (±0.625)
Mean	1.581^{a} (±0.766)	1.581^{a} (±0.766)	1.617^{b} (±0.766)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).

[A,B]Means within the same columns (within temperature treatment) with no common superscript differ significantly ($P < 0.05$)

4.5. The influence of Zn source, Zn level and house temperature on serum cholesterol concentration and liver, heart and fat pad weight in broilers at 35 days of age

Parameters measured in Table 4.29, namely liver, heart and fat pad weights presented with no significant differences between birds supplemented with different Zn sources. This was also noted when organs were expressed as a percentage of body weight. Birds did not exhibit significant differences in liver weight or percentage of liver weight when supplemented with different Zn sources, although birds supplemented with ZS presented the lightest liver weight. Liver weight and percentage of liver weight were found to be highest in birds supplemented with AZ. Broilers supplemented with ZS had the heaviest heart weight and heart weight expressed as percentage of body weight. Birds supplemented with IZ had the lowest heart weight and percentage of heart weight. The same was noted for fat pad weight and percentage.

Broilers supplemented at 80 mg/kg or 120 mg/kg inclusion presented with no significant differences between liver, heart or fat pad weight. The same was noted for organs expressed as percentage of body weight. All measured parameters, excluding cholesterol concentration, were significantly lower in birds subjected to high temperatures ($P < 0.05$). No significant differences were observed in broiler cholesterol concentrations between different Zn sources, under both inclusion levels and temperature treatments.

Table 4.29 The effect of Zn source[1], Zn level and temperature[2] on serum cholesterol concentration and liver, heart and fat pad weight[3] and percentage[4] in broilers at 35 days of age

Treatment	Liver (g)	Liver (%)	Heart (g)	Heart (%)	Fat pad (g)	Fat (%)	Chol (μmol/L)
Zn Source							
Availa®Zn	50.94	2.137	12.93	0.543	30.85	1.301	3.828
IntelliBond®Z	50.56	2.129	12.90	0.542	29.46	1.245	3.771
Zn Sulphate	49.57	2.095	13.35	0.565	31.17	1.321	3.860
SEM ±	0.788	0.033	0.221	0.009	1.024	0.044	0.053
Zn level (mg/kg)							
80	50.43	2.124	13.08	0.551	30.47	1.288	3.853
120	50.27	2.116	13.04	0.549	30.51	1.289	3.785
SEM ±	0.644	0.027	0.836	0.008	0.084	0.036	0.043
Temperature							
Normal	56.33[a]	2.196[a]	14.58[a]	0.569[a]	32.81[a]	1.279[b]	3.791
Hot	44.38[b]	2.045[b]	11.53[b]	0.531[b]	28.17[b]	1.298[a]	3.848
SEM ±	0.644	0.027	0.180	0.007	0.836	0.036	0.043

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

SEM - Standard error of the mean

[3]Total wet/fresh weight

[4]Expressed as a percentage of live body weight

4.5.1. The main and interaction effects of Zn source, Zn inclusion level and house temperature on serum cholesterol concentration and liver, heart and fat pad weight in broilers at 35 days of age

The main and interactive effects between Zn source, Zn inclusion level and house temperature for the different measured parameters are presented in Table 4.30. Zn source and Zn dosage level presented with no significant interactive effects on liver, heart and fat pad weight. The

same was noted when organs were expressed as percentage of body weight. Zn source and Zn dosage level, Zn source and house temperature, as well as Zn dosage level and house temperature showed no significant interactive effects on any of the measured parameters. Significant interactive effects (*P < 0.05)* were found between Zn source, Zn dosage level and house temperature for liver weight and liver weight percentage. Significant interactions were found for house temperature on fat pad weights. No other significant interactive effects were observed in measured parameters.

Table 4.30 The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on serum cholesterol concentration and liver, heart and fat pad weight[3] and percentage[4] in broilers at 35 days of age (P-values)

Treatment	Liver (g)	Liver (%)	Heart (g)	Heart (%)	Fat (g)	Fat (%)	Chol (µmol)
Zn Source	0.450	0.623	0.274	0.156	0.457	0.464	0.492
Zn Level	0.861	0.837	0.897	0.856	0.973	0.979	0.271
Zn Source x Level	0.510	0.432	0.609	0.573	0.997	0.994	0.787
Temperature	<0.001	0.002	<0.001	0.001	0.002	0.717	0.351
Zn Source x Temperature	0.416	0.399	0.216	0.131	0.949	0.988	0.789
Zn Level x Temperature	0.260	0.317	0.451	0.543	0.514	0.565	0.923
Source x Level x Temperature	0.020	0.026	0.275	0.294	0.848	0.831	0.689

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[3]Total wet/fresh weight

[4]Expressed as a percentage of live body weight

4.5.2. The effects of Zn source, Zn inclusion level and house temperature on serum cholesterol concentration and liver, heart and fat pad weight in broilers at 35 days of age

Shown in Table 4.31, broilers under thermoneutral conditions at 80 mg/kg inclusion level presented with no significant differences between cholesterol concentration, liver, heart and fat pad weight. The same was noted in organ weight expressed as percentage of body weight. Liver weight and percentage of liver weight were found to be higher in AZ supplemented birds at 80 mg/kg inclusion level, although differences were not statistically significant. Broilers presented with significantly heavier ($P < 0.05$) liver weight percentage under thermoneutral compared to hyperthermic conditions when AZ and ZS were supplemented at 80 mg/kg inclusion level. Significantly lower liver weight was found in broilers for all three Zn sources when birds were exposed to high temperature stress. No significant differences were observed between broiler heart weights in birds supplemented with different sources, at 80 mg/kg inclusion level under both temperature treatments. Broiler heart weight was significantly higher ($P < 0.05$) in birds under thermoneutral temperatures supplemented at 80 mg/kg inclusion level, across all Zn sources. Heart weight as percentage of body weight was found to be significantly heavier ($P < 0.05$) in birds supplemented with AZ, at 80 mg/kg inclusion level under thermoneutral compared to heat stressed conditions. The same was noted for fat pad weights. Birds supplemented with IZ under thermoneutral conditions, at 80 mg/kg inclusion level presented with the lightest fat pad weights, where heat stressed birds exhibited the heaviest fat pad weights when ZS was supplemented. Differences were, however, not statistically significant. No significant differences were found in cholesterol concentration between birds supplemented with different Zn sources, across inclusion levels and temperature treatments. Birds presented with significantly higher liver, heart and fat pad weight when exposed to thermoneutral compared to high temperature treatments. The same was noted in liver and heart percentages.

Broilers supplemented at 120 mg/kg inclusion level, under thermoneutral conditions displayed significantly heavier ($P < 0.05$) liver weight when IZ compared to ZS was supplemented. No significant differences were observed in birds supplemented with AZ. No significant differences were found in broiler liver weight between Zn sources in birds subjected to high temperatures. Birds supplemented with organic source IZ and AZ, at 120 mg/kg inclusion level presented with significantly higher ($P < 0.05$) liver weight under thermoneutral compared to heat stressed conditions. The same was noted in broiler livers when expressed as percentage of body weight. Heart weight and heart weight percentage were significantly heavier in birds

supplemented at 120 mg/kg inclusion level under thermoneutral compared to hyperthermic conditions. Birds under thermoneutral conditions, at 120 mg/kg inclusion level presented with no significant differences in heart weight between Zn sources, where heat stressed birds had significantly heavier heart weights when ZS was supplemented rather than organic sources AZ and IZ. No significant differences were found between broiler fat pad weights at 120 mg/kg inclusion, across Zn sources and temperature treatments. Liver and heart weights, as well as liver and heart percentages were significantly lower ($P < 0.05$) in birds exposed to high temperature compared to thermoneutral conditions.

No significant differences were found for cholesterol concentration between birds supplemented at 80 mg/kg and 120 mg/kg inclusion levels under both temperature treatments. Birds supplemented at 80 mg/kg presented with significantly higher level means for liver and heart weight, as well as liver and heart percentages in birds under thermoneutral conditions. Birds under high temperature stress, at 120 mg/kg inclusion level presented with lighter liver and heart weights, as well as fat pad percentage.

4.5.3. The main effects of Zn source and house temperature on serum cholesterol concentration and liver, heart and fat pad weights in broilers at 35 days of age

In Table 4.32, the effects of Zn source and temperature treatment on broiler liver, heart and fat pad weight, as well as liver, heart and fat pad as percentage of body weight and serum cholesterol concentration can be seen. No significant differences were observed in liver, heart, and fat pad weight between broilers supplemented with different Zn sources, under both thermoneutral and high temperature treatments. The same was noted for liver, heart and fat pad weight expressed as a percentage of body weight. All measured parameters were negatively influenced by high temperature stress. Broilers presented with significantly higher ($P < 0.05$) liver, heart and fat pad weights, as well as liver and heart percentages when exposed to thermoneutral compared to high temperature conditions. Broilers presented with no significant differences in serum cholesterol concentration between Zn sources and both temperature treatments.

Table 4.31 The effects of Zn source[1], Zn inclusion level and house temperature[2] on broiler serum cholesterol concentration and liver, heart and fat pad weight[3] and percentage[4] in broilers at 35 days of age

Treatment	Temperature													
	Normal							Hot						
	Liver (g)	Liver (%)	Heart (g)	Heart (%)	Fat (g)	Fat (%)	Chol (µmol/L)	Liver (g)	Liver (%)	Heart (g)	Heart (%)	Fat (g)	Fat (%)	Chol (µmol/L)
Zn level 80 mg/kg														
Zn Source														
Availa®Zn	58.78^{A}	2.293^{A}	14.91^{A}	0.582^{A}	34.02^{A}	1.327	3.853	44.74^{B}	2.056^{B}	11.17^{B}	0.514^{B}	27.54^{B}	1.266	3.901
IntelliBond®Z	55.25^{A}	2.158	14.54^{A}	0.568	31.52	1.231	3.788	45.49^{B}	2.094	11.45^{B}	0.527	27.35	1.259	3.851
Zn Sulphate	56.73^{A}	2.207^{A}	14.64^{A}	0.596	34.01	1.323	3.824	41.62^{B}	1.937^{B}	11.74^{B}	0.546	28.41	1.323	3.903
SEM±	1.577	0.066	0.442	0.019	2.048	0.089	0.106	1.577	0.066	0.442	0.019	2.048	0.089	0.106
Level Mean	56.92^{A}	2.219^{A}	14.70^{A}	0.573^{A}	33.18^{A}	1.294	3.822	43.95^{B}	2.209^{B}	11.45^{B}	0.529^{B}	27.77^{B}	1.283	3.885
Level *SEM*	0.910	0.038	0.255	0.573	1.183	0.051	0.061	0.910	0.038	0.255	0.573	1.183	0.051	0.061
Zn level 120 mg/kg														
Zn Source														
Availa®Zn	56.66^{abA}	2.201^{A}	14.18^{A}	0.551	32.49	1.262	3.718	43.57^{B}	1.999^{B}	11.44^{abB}	0.525^{a}	29.37	1.348	3.838
IntelliBond®Z	57.48^{aA}	2.242^{A}	14.74^{A}	0.575^{A}	31.51	1.229	3.663	44.02^{B}	2.021^{B}	10.87^{bB}	0.499^{Aa}	27.45	1.261	3.782
Zn Sulphate	53.05^{bA}	2.076	14.49^{A}	0.567	33.34	1.304	3.898	46.86^{B}	2.159	12.54^{aB}	0.578^{b}	28.92	1.332	3.814
SEM±	1.577	0.066	0.442	0.019	2.048	0.089	0.106	1.577	0.066	0.442	0.019	2.048	0.089	0.106
Level mean	55.73^{A}	2.173^{A}	14.47^{A}	0.564	32.45	1.265^{A}	3.760	44.82^{B}	2.060^{B}	11.61^{B}	0.534	28.58	1.314^{B}	3.811

Level *SEM*±	0.910	0.038	0.255	0.255	1.183	0.051	0.061	0.016	0.910	0.255	0.573	0.051	1.183	0.061

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32℃ (89.6℉) and was increased up to 39℃ (102.2℉). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

SEM - Standard error of the mean

[3]Total wet/fresh weight

[4]Expressed as a percentage of live body weight

Table 4.32 The main effects of Zn source[1] and house temperature[2] on serum cholesterol concentration and liver, heart and fat pad weight[3] and percentage[4] in broilers at 35 days of age

Treatment	Temperature													
	Normal							Hot						
	Liver	Liver	Heart	Heart	Fat	Fat	Chol	Liver	Liver	Heart	Heart	Fat	Fat	Chol
Zn Source	(g)	(%)	(g)	(%)	(g)	(%)	(µmol/L)	(g)	(%)	(g)	(%)	(g)	(%)	(µmol/L)
Availa®Zn	57.72^{A}	2.247^{A}	14.55^{A}	0.566^{A}	33.25^{A}	1.295	3.786	44.16^{B}	2.028^{B}	11.31^{B}	0.519^{B}	28.45^{B}	1.307	3.869
IntelliBond®Z	56.36^{A}	2.200^{A}	14.64^{A}	0.571^{A}	31.52^{A}	1.223	3.725	44.75^{B}	2.058^{B}	11.16^{B}	0.513^{B}	27.40^{B}	1.260	3.817
Zn Sulphate	54.89^{A}	2.141	14.56^{A}	0.568	33.67^{A}	1.314	3.861	44.24^{B}	2.048	12.14	0.562	28.66^{B}	1.327	3.859
SEM±	1.115	0.047	0.312	0.013	1.448	0.063	0.075	1.115	0.047	0.312	0.013	1.448	0.063	0.075

[1]Zn sources were either Zn Sulphate or a combination of Zn Sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn Sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn Sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

SEM - Standard error of the mean

[3]Total wet/fresh weight

[4]Expressed as a percentage of live body weight

4.6. The effects of Zn source, Zn inclusion level and house temperature on malondialdehyde concentration and total antioxidant capacity in livers of broilers at 35 days of age

No significant differences were observed between malondialdehyde (MDA) concentration in broilers supplemented with different Zn sources, as seen in Table 4.33. The same was noted for total antioxidant capacity (TAC). The highest MDA concentrations were observed in birds supplemented with ZS, where the lowest MDA concentrations were found in broilers supplemented with IZ. Broilers supplemented with ZS presented with the lowest TAC and birds supplemented with IZ exhibited the highest TAC. No significant differences were, however, found between birds receiving any of the three Zn sources. Birds supplemented at both inclusion level 80 mg/kg and 120 mg/kg presented no significant differences in MDA concentration or TAC. Broilers supplemented at inclusion level 120 mg/kg presented with slightly elevated TAC and MDA concentration. Birds presented with no significant differences in TAC or MDA concentration across both temperature treatments. Elevated MDA concentration were found in broilers under thermoneutral conditions, where TAC concentration were found to be higher under in birds exposed to high temperatures.

Table 4.33 Effects of Zn source[1], Zn inclusion level and house temperature[2] on Malondialdehyde concentration and total antioxidant capacity in broiler livers at 35 days of age

Treatment	Malondialdehyde (mol/L)	Total antioxidant capacity (mol/L)
Zn Source		
Availa®Zn	2.793	14.78
IntelliBond®Z	2.651	15.05
Zn Sulphate	2.814	13.70
SEM±	0.087	0.687
Zn level (mg/kg)		
80	2.720	14.37
120	2.786	14.65
SEM±	0.071	0.561
Temperature		
Normal	2.777	14.22
Hot	2.728	14.80
SEM±	0.071	0.561

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z

[2]Heat stress started at 32℃ (89.6℉) and was increased up to 39℃ (102.2℉). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

SEM - Standard error of the mean

4.6.1. The main and interaction effects of Zn source, Zn inclusion level and house temperature on malondialdehyde concentration and total antioxidative capacity in broiler livers at 35 days of age

The main and interactive effects between Zn source, Zn inclusion level and house temperature are presented in Table 4.34. Zn source and dosage level presented with no significant interactive effects between MDA concentration or TAC in broilers. Zn source and temperature, as well as Zn level and house temperature also exhibited no significant interactions in broiler MDA concentration and TAC. The same was noted for Zn source, Zn inclusion level and house temperature interactions.

Table 4.34 The main and interaction effects of Zn source[1], Zn inclusion level and house temperature[2] on malondialdehyde concentration and total antioxidant capacity in broiler livers at 35 days of age

Treatment	Malondialdehyde (mol/L)	Total antioxidant capacity (mol/L)
Zn Source	0.363	0.339
Zn Level	0.512	0.719
Zn Source x Level	0.800	0.471
Temperature	0.628	0.463
Zn Source x Temperature	0.119	0.510
Zn Level x Temperature	0.722	0.843
Source x Level x Temperature	0.898	0.721

[1] Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.6.2. The effect of Zn source, Zn inclusion level and house temperature on malondialdehyde concentration in broiler livers at 35 days of age

As found in Table 4.35, broilers under thermoneutral conditions at both 80 mg/kg and 120 mg/kg inclusion levels presented with no significant differences in MDA concentration. The lowest MDA concentration were observed in broilers supplemented with ZS, where broilers that received organic Zn presented with insignificant differences between MDA concentrations. At both 80 mg/kg and 120 mg/kg inclusion levels, broilers subjected to high temperature stress presented with no significant differences in MDA concentration between Zn sources. Birds supplemented with ZS under high temperature stress exhibited significantly higher ($P < 0.05$) source mean MDA concentrations compared to birds supplemented with IZ. Birds that received AZ presented with no significant differences in MDA concentration between birds supplemented with IZ and ZS. No significant differences were found in MDA concentration between birds supplemented at different inclusion levels, although birds supplemented with Zn under thermoneutral conditions, at 120 mg/kg inclusion level had the highest MDA concentration.

Table 4.35 Effects of Zn source[1], Zn level and house temperature[2] on malondialdehyde concentration in broiler livers at 35 days of age (± standard error of the mean)

	Zinc source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	2.782 (±0.174)	2.733 (±0.174)	2.664 (±0.174)	2.726 (±0.101)
120 mg/kg	2.861 (±0.174)	2.872 (±0.174)	2.751 (±0.174)	2.828 (±0.101)
Mean	2.821 (±0.123)	2.803 (±0.123)	2.707 (±0.123)	
Hot temperature				
80 mg/kg	2.826 (±0.174)	2.429 (±0.174)	2.884 (±0.174)	2.713 (±0.101)
120 mg/kg	2.702 (±0.174)	2.571 (±0.174)	2.957 (±0.174)	2.743 (±0.101)
Mean	2.764^{ab} (±0.123)	2.500^{a} (±0.123)	2.920^{b} (±0.123)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

4.6.3. The effect of Zn source, Zn inclusion level and house temperature on total antioxidative capacity in broiler livers at 35 days of age

As found in Table 4.36, at both 80 mg/kg and 120 mg/kg inclusion levels and temperature treatments, no significant differences were found in broiler TAC between different sources. Under thermoneutral conditions at 80 mg/kg inclusion, IZ supplemented broilers presented with the highest TAC. This was also noted in broilers under thermoneutral conditions at 120 mg/kg inclusion level and in heat stressed broilers at 80 mg/kg inclusion. Heat stressed broilers supplemented with AZ, at 120 mg/kg inclusion presented with the highest TAC. The lowest values were consistently found in broilers that received supplementary ZS, excluding birds supplemented at 80 mg/kg inclusion under high temperature stress, where AZ supplemented birds presented with the lowest TAC.

Table 4.36 The effects of Zn source[1], Zn inclusion level and house temperature[2] on total antioxidative capacity in broiler livers (mol/L) at 35 days of age (± standard error of the mean)

	Zinc source			
	Availa®Zn	IntelliBond®Z	Zn Sulphate	Mean
Normal Temperature				
80 mg/kg	13.34 (±1.373)	14.90 (±1.373)	13.75 (±1.373)	14.00 (±0.793)
120 mg/kg	14.36 (±1.373)	15.07 (±1.373)	13.89 (±1.373)	14.44 (±0.793)
Mean	13.85 (±0.971)	14.99 (±0.971)	13.82 (±0.971)	
Hot temperature				
80 mg/kg	14.70 (±1.373)	16.08 (±1.373)	13.46 (±1.373)	14.74 (±0.793)
120 mg/kg	16.74 (±1.373)	14.18 (±1.373)	13.68 (±1.373)	14.87 (±0.793)
Mean	15.72 (±0.971)	15.12 (±0.971)	13.57 (±0.971)	

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.7. The effect of Zn source, Zn inclusion level and house temperature on broiler tibia parameters

Broilers supplemented with AZ presented tibias with the highest dry matter values, as found in Table 4.37. Birds supplemented with AZ and IZ differed significantly ($P < 0.05$) in tibia dry matter (DM) values, although no significant differences were found with ZS supplemented birds. No significant differences were observed in bone ash values between broilers receiving different Zn sources. Significantly higher Zn concentrations (mg/kg) were observed in tibias from broilers supplemented with AZ. Broilers supplemented with ZS presented with significantly lower tibia Zn concentrations compared to birds that received AZ and IZ. Total Zn (g) in dry bone was found to be significantly higher in broilers supplemented with organic sources AZ and IZ. The two organic sources did not differ in total tibia Zn (g) between broilers, although broilers supplemented with organic Zn sources presented with significantly higher tibial Zn concentrations (g) compared to broilers supplemented with ZS.

Dry matter and ash percentages were not significantly different between broilers supplemented at 80 mg/kg or 120 mg/kg inclusion level. Supplementation at 120 mg/kg inclusion resulted in birds with significantly higher Zn concentrations (mg/kg) in tibia ash. The same was noted for total tibial Zn. Birds supplemented at inclusion level 80 mg/kg presented with significantly lower Zn concentrations in both bone ash and total Zn in the tibia. Broilers were significantly affected by temperature treatment, where birds under high temperature stress presented with higher DM percentage compared to birds kept under thermoneutral conditions.

Table 4.37 The effects of Zn source[1], Zn inclusion level and house temperature[2] on tibia measurements in broilers at 35 days of age

Treatment	Tibia dry matter (%)	Tibia ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)
Zn Source				
Availa®Zn	52.15[a]	25.93	423.7[a]	1.658[a]
IntelliBond®Z	51.20[b]	25.60	405.6[b]	1.604[a]
Zn Sulphate	51.46[ab]	25.46	382.8[c]	1.519[b]
SEM±	0.326	0.499	4.109	0.033
Zn level				
80	51.52	26.09	382.7[b]	1.486[b]
120	51.68	25.24	425.4[a]	1.701[a]
SEM±	0.266	0.407	3.355	0.027
Temperature				
Normal	52.21[a]	25.49	402.15	1.598
Hot	51.00[b]	25.84	405.94	1.590
SEM±	0.266	0.407	3.355	0.027

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

[a-c]Means within the same column with no common superscript differ significantly ($P < 0.05$). *SEM* - Standard error of the mean

4.7.1. The main and interaction effects of Zn source, Zn inclusion level and house temperature on broiler tibia parameters

Presented in Table 4.38 are the main and interactive effects between Zn source, Zn inclusion level and house temperature for the different weeks on the measured broiler bone parameters. No interactive effects were observed for Zn source and Zn dosage level, Zn source and house temperature and Zn level and temperature for DM in broiler tibias. Zn source, inclusion level and house temperature interaction presented with no interactive effects on tibial DM. This was also noted in bone ash percentage. Zn source and dosage level presented with significant interactions in ash percentage and Zn in dry bone. No other significant interactions were observed for Zn in dry bone and Zn concentration in bone ash. Zn source and house temperature, Zn level and temperature as well as Zn source, Zn dosage level and temperature presented with no significant interactions on either Zn bone ash concentrations or total Zn in dry bone.

Table 4.38 The main and interaction effects of Zn source[1], Zn level and house temperature[2] effects on broiler tibia parameters at 35 days of age (P-values)

	Dry matter (%)	Ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)
Zn Source	0.113	0.795	<0.001	0.016
Zn Level	0.688	0.140	<0.001	<0.001
Zn Source x Level	0.061	0.601	<0.001	0.008
Temperature	0.002	0.553	0.427	0.833
Zn Source x Temperature	0.501	0.149	0.915	0.317
Zn Level x Temperature	0.356	0.183	0.725	0.333
Source x Level x Temperature	0.589	0.496	0.837	0.547

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

4.7.2. The effect of Zn source, Zn inclusion level and house temperature on broiler tibia parameters at 35 days of age

As shown in Table 4.39, AZ supplementation at 80 mg/kg inclusion, under thermoneutral conditions resulted in birds with significantly higher DM in the tibias of birds. ZS supplemented birds did not differ between AZ or IZ in tibia DM of broilers, although tibia DM from AZ supplemented broilers were significantly higher ($P < 0.05$) than the IZ supplemented groups. At 120 mg/kg inclusion level under thermoneutral conditions, no significant differences between tibia DM were found in broilers supplemented with different Zn sources. This was also noted at 120 mg/kg inclusion in broilers under high temperature stress. Heat stressed birds supplemented with ZS, at 80 mg/kg inclusion presented with significantly lower ($P < 0.05$) tibia DM than birds supplemented with AZ. Birds supplemented with IZ presented with no significant differences between tibia DM of broilers that received IZ and ZS, even though AZ and ZS broilers exhibited significant differences ($P < 0.05$) in tibia DM.

No significant differences were observed in tibia ash percentage between broilers supplemented with different Zn sources, across both temperature treatments and inclusion levels. Broilers under thermoneutral conditions at 80 mg/kg inclusion, presented with no significant differences between Zn concentrations in bone ash. The same was noted at 80 mg/kg inclusion in broilers under heat stress. Broilers subjected to thermoneutral conditions and supplemented with organic sources AZ and IZ, presented significantly higher ($P < 0.05$) Zn concentrations in tibia ash compared to broilers supplemented at 120 mg/kg inclusion. Zn concentration in tibia ash, from broilers supplemented with IZ and AZ did not differ significantly between groups, although Zn concentrations were significantly lower ($P < 0.05$) in ZS supplemented birds. The same was noted in heat stressed birds at 120 mg/kg inclusion. Under both thermoneutral and heat stressed conditions, birds supplemented with AZ and IZ at 120 mg/kg inclusion presented with significantly higher tibia Zn concentrations.

At 80 mg/kg inclusion under thermoneutral conditions, broilers supplemented with different sources displayed no significant differences in total tibial Zn concentration. At 120 mg/kg inclusion level, broilers supplemented with the two organic sources AZ and IZ did not differ in total tibial Zn concentration, although total Zn concentration was significantly higher ($P < 0.05$) than total Zn concentration from ZS supplemented broilers. Birds supplemented with AZ and IZ, at 120 mg/kg compared to 80 mg/kg inclusion presented with significantly higher ($P < 0.05$) total tibial Zn concentrations. This was also noted in broilers supplemented with IZ under

high temperature stress. At 120 mg/kg inclusion under high temperature stress, total Zn concentration in tibias of broilers differed significantly ($P < 0.05$) between AZ and IZ supplemented birds, but no significant differences were observed with ZS supplemented broilers. Heat stressed birds supplemented at 120 mg/kg exhibited significantly higher ($P < 0.05$) total tibial Zn concentrations in AZ supplemented birds and significantly lower ($P < 0.05$) total Zn in ZS supplemented birds. IZ supplemented birds displayed no significant differences in total tibial Zn concentrations between birds supplemented with AZ and ZS. Birds under thermoneutral conditions, supplemented at 120 mg/kg rather than 80 mg/kg inclusion level presented with significantly higher ($P < 0.05$) total Zn concentrations in bone. The same was observed in birds under high temperature stress.

4.7.3. The main effects of Zn source and house temperature on broiler tibia parameters at 35 days of age

Presented in Table 4.40 are the measured bone parameters from broilers supplemented with different sources under both temperature treatments. No significant differences were seen in DM and ash percentages between broilers supplemented with different sources, under both temperature treatments. Broilers under thermoneutral conditions presented with significant differences ($P < 0.05$) in Zn concentration in bone ash (mg/kg) between all three sources. Birds supplemented with AZ presented with the highest Zn concentrations (mg/kg), where ZS supplemented birds had the lowest values. Birds supplemented with IZ had significantly higher ($P < 0.05$) total tibial Zn concentrations than ZS supplemented birds, but significantly lower values than AZ supplemented birds. Broilers under high temperature stress displayed no significant differences in Zn concentration in bone ash (mg/kg) between birds supplemented with organic Zn sources AZ and IZ, although broilers had significantly higher ($P < 0.05$) concentrations of Zn (mg/kg) compared to ZS supplemented birds.

Total tibial Zn was found to be significantly higher ($P < 0.05$) in IZ, compared to ZS supplemented birds under thermoneutral conditions. Broilers supplemented with ZS and IZ did not differ significantly in total tibial Zn concentration with AZ supplemented birds. Broilers under heat stress presented with significant differences ($P < 0.05$) in total tibia Zn concentration between broilers that received ZS and AZ. AZ supplemented birds presented with significantly higher total tibial Zn concentrations compared to ZS supplemented birds. Broilers that received IZ exhibited no significant differences in total tibial Zn concentrations between ZS and AZ supplemented birds.

Table 4.39 The effects of Zn source[1], Zn level and house temperature[2] on broiler tibia parameters at 35 days of age

Treatment	Temperature							
	Normal				Hot			
	DM (%)	Ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)	DM (%)	Ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)
Zn level 80 mg/kg								
Zn source								
Availa®Zn	52.98^{a}	27.67	393.9^{B}	1.452^{B}	52.41^{a}	25.19	395.7^{B}	1.590^{a}
IntelliBond®Z	50.98^{b}	25.52	375.3^{B}	1.484^{B}	50.92^{ab}	27.18	375.3^{B}	1.396^{bA}
Zn Sulphate	51.92^{ab}	25.74	375.6	1.478	49.95^{b}	25.27	380.2	1.515^{ab}
Level Mean	51.96	26.31^{a}	381.6^{b}	1.471^{b}	51.09	25.88	383.7^{b}	1.500^{b}
SEM±	0.651	0.997	2.218	0.067	0.651	0.997	2.218	0.067
Zn level 120 mg/kg								
Zn source								
Availa®Zn	52.23	24.93	452.6^{aA}	1.822^{aA}	50.97	25.93	452.7^{aA}	1.766^{a}
IntelliBond®Z	52.31	23.77	430.2^{aA}	1.815^{aA}	50.60	25.95	441.5^{aA}	1.722^{abB}
Zn Sulphate	52.84	25.34	385.2^{b}	1.536^{b}	51.12	25.50	390.2^{b}	1.547^{b}
SEM±	0.651	0.997	2.218	0.067	0.651	0.997	2.218	0.067
Level	52.46	24.68^{b}	422.7^{a}	1.724^{a}	50.90	25.79	428.14^{a}	1.679^{a}
SEM±	0.376	0.576	4.745	0.039	0.376	0.576	4.745	0.039

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

SEM - Standard error of the mean

Table 4.40 The main effects of Zn source[1] and house temperature[2] on measured broiler tibia parameters at 35 days of age

Treatment	Temperature							
	Normal				Hot			
	DM (%)	Ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)	DM (%)	Ash (%)	Zn (mg/kg tibia ash)	Zn (g/dry tibia)
Zn Source								
Availa®Zn	52.60	26.30	423.3[a]	1.637[ab]	51.69	25.56	424.2[a]	1.678[a]
IntelliBond®Z	51.65	24.65	402.8[b]	1.650[a]	50.76	26.56	408.4[a]	1.559[ab]
Zn Sulphate	52.38	25.54	380.4[c]	1.507[b]	50.54	25.39	385.2[b]	1.531[b]
SEM±	±0.460	±0.705	±5.811	±0.047	±0.460	±0.705	±5.811	±0.047

[1]Zn sources were either Zn sulphate or a combination of Zn sulphate and Availa®Zn or IntelliBond®Z. Level 80: 40 mg/kg Zn sulphate and 40 mg/kg Availa®Zn or IntelliBond®Z. Level 120: 80 mg/kg Zn sulphate with 40 mg/kg Availa®Zn or IntelliBond®Z.

[2]Heat stress started at 32°C (89.6°F) and was increased up to 39°C (102.2°F). Temperature was increased at 8:00am and 11:00am and then decreased to thermoneutral conditions at 19:00.

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

[A,B]Means within the same rows (within temperature treatment) with no common superscript differ significantly ($P < 0.05$).

SEM - Standard error of the mean

Chapter 5

Discussion

5.1. The effects of zinc source, zinc inclusion level and house temperature on broiler performance

In this study, broiler body weight ($P < 0.05$) was significantly higher in broilers supplemented at 120 mg/kg compared to the 80 mg/kg inclusion level. Birds supplemented with organic Zn sources presented with significantly higher ($P < 0.05$) body weights, where *Availa*®Zn (AZ) supplementation resulted in broilers with the heaviest ($P < 0.05$) body weights under both temperature treatments. The lightest body weights ($P < 0.05$) were consistently observed in broilers that received supplementary Zinc sulphate (ZS) under both thermoneutral and heat stress conditions. Body weight was significantly ($P <0.05$) affected by Zn source from day 7 onwards. Interactions between Zn source and Zn inclusion level ($P < 0.05$) were significant for broiler body weight from 21 to 28 days of age. Zn inclusion level was also significant ($P < 0.05$) for body weight for the duration of the trial. Interactions between Zn source, Zn dosage level and house temperature were significant for broiler body weight ($P < 0.05$) from 7 to 14 days of age, for the duration of the trial. Our results were in agreement with Wedekind *et al.* (1992) and Kidd *et al.* (1996) who found organic Zn was more bioavailable to broilers than inorganic Zn. Suo *et al.* (2015) reported supplementing Zn-methionine complexes to broilers resulted in higher average daily gains, compared to Zn provided as inorganic Zn sulphide. Sahin *et al.* (2005) found quails supplemented with Zn picolinate chelate performed better than quails supplemented with Zn sulphate.

In the current study, broiler body weight was severely depressed by high temperature stress. Our results coincided with Geraert *et al.* (1996), Bartlett and Smith (2003) and Sahin *et al.* (2005) where they observed depressed body weight gains in broilers subjected to hyperthermic stress. Kutlu and Forbes (1993) reported body weight of broilers exposed to high temperature stress was reduced by 91%, where Azad *et al.* (2010) observed 80% reductions in broiler body weight gain when birds were subjected to constant hyperthermic stress. In agreement with our findings, Kucuk

et al. (2003) reported that Zn supplementation during high temperature stress resulted in broilers with heavier live weight gains and carcass traits. Sahin *et al.* (2005) found supplementing Zn picolinate to heat stressed quail improved feed efficiency and subsequent body weight gain. At temperatures above or below the thermoneutral zone, increased corticosteroid secretion has been found in response to stress (Brown and Nestor, 1973). In our study, we did not test for corticosterone levels, but muscle breakdown was likely induced by increased corticosterone. Corticosterone-induced gluconeogenesis and muscle proteolysis have been observed during hyperthermic stress (Lin *et al.*, 2004). The combination of increased corticosterone levels inducing muscle breakdown, decreased feed intakes and feed efficiencies potentially contributed to the lighter carcass weights observed in the current study.

Feed conversion ratio was found to be significantly higher in birds that received ZS compared to birds that received organic sources, where *IntelliBond®Z* (IZ) supplemented birds exhibited significantly higher feed conversion ratio than birds supplemented with AZ. Coinciding with our results, Idowu *et al*. (2011) found significant improvements in feed conversion ratio in birds supplemented with Zn proteinate compared to Zn oxide, sulphide and carbonate. Supplementing broilers with organic Zn resulted in significantly higher ($P < 0.05$) feed intake and cumulative feed intake compared to broilers supplemented with inorganic ZS across both temperature treatments. No significant differences were found in broiler feed intake and cumulative feed intake between the two organic sources, IZ and AZ. Cumulative feed intake over the 35 day rearing cycle was significantly higher ($P < 0.05$) in broilers supplemented with Zn at 120 mg/kg rather than 80 mg/kg inclusion level across temperature treatments. The same was observed for feed conversion ratio. Similar to the current study, Kucuk *et al.* (2003) reported that Zn supplementation during high temperature stress resulted in broilers with improved feed intake and feed efficiency. Sahin *et al.* (2005) found supplementing Zn to heat stressed quail improved feed intake and efficiency, which could have been attributed to the role Zn plays in appetite control (Suo *et al.*, 2015). Sahin *et al.* (2005) and Ao *et al.* (2007) reported increased feed intake and feed efficiency in poultry when supplementary Zn was provided.

In this study, broilers subjected to high temperature stress presented with significantly depressed feed intake and cumulative feed intake ($P < 0.05$). Our results coincided with studies by Ensminger

et al. (1990), Siegel, (1995) and Kucuk *et al.* (2003) who all found severely reduced feed intakes in broilers under hyperthermic stress. Sahin *et al.* (2005) reported reductions in feed intake as well as nutrient impairments in poultry under hyperthermic stress. Donkoh (1989) and Lu *et al.* (2007) observed feed intake, feed efficiency and growth rates of broilers were significantly depressed under constant high temperatures. Results coincided with Yalçin *et al.* (1998), Sahin *et al.* (2009), Quinteiro-Filho *et al.* (2010) and Syafwan *et al.* (2011), where broilers under hyperthermic stress presented with severely depressed feed intakes and growth rates. Geraert *et al.* (1996) found ambient temperatures above 34°C resulted in significant reductions in broiler feed intake and subsequent body weight gain. Reductions in feed intake were suggested to be the main mechanism reducing body weight during high temperature stress (Hurwitz *et al.,* 1980; Kucuk *et al.,* 2003). It was suggested birds reduced intake as an adaptive mechanism to alleviate metabolic heat production (Geraert *et al.,* 1996; Sahin *et al.,* 2001; Sahin *et al.,* 2009). Depressed feed intake in broilers exposed to high temperatures was also attributed to disruptions in body homeostasis resulting from hyperthermic stress (Geraert *et al.,* 1996; Sahin *et al.,* 2009).

5.2. The effects of zinc source, zinc inclusion level and house temperature on broiler liver, heart and fat pad weight

No significant differences were found in overall liver, heart or fat pad weights in broilers supplemented with different Zn sources. The same was observed when organs were expressed as percentage of body weight. Our results were in agreement with Salim *et al*. (2011) where no significant differences were found in broiler organ weights between birds supplemented with organic and inorganic Zn. Relative liver weight were higher when organic Zn sources rather than ZS was supplemented, while heart and fat pads were heavier in birds supplemented with ZS. Birds subjected to hyperthermic conditions had significantly lower liver and heart weight.

5.3. The effects of zinc source, zinc inclusion level and house temperature on malondialdehyde concentration and total antioxidative capacity in broiler livers

Total antioxidant capacity (TAC) is a contributor to active oxygen balance and can be utilised to reflect antioxidant status in body fluids and serum (Zhao *et al.,* 2014). Malondialdehyde (MDA) is a biomarker used to indicate lipid peroxidation (Ismail *et al.,* 2015) and is directly proportional to the degree of lipid peroxidation, thus indicating damage (Sehirli *et al.,* 2008; Yousef *et al.,*

2009). In the current study, neither Zn source nor Zn level in the diet had and significant effect on MDA concentrations or TAC in the broilers. Similarly, heat stress also showed no effect on MDA concentrations and TAC either. In agreement with our results, Sahin *et al*. (2005) found supplementing Zn sulphate under thermoneutral conditions had no effect on serum and liver MDA concentrations. Lin *et al.* (2006) observed no obvious changes in Cu-Zn superoxide dismutase between thermoneutral and acutely heat stressed birds. A study by Morrison *et al.* (2005) showed similar results, where rats displayed no antioxidant changes in the liver when subjected to acute heat stress. In contrast to our findings, several other studies did find that Zn source affected serum MDA concentrations in broilers. Ma *et al.* (2011) found supplementing Zn-glycine to broilers resulted in reduced MDA concentrations in hepatic tissues. Duzguner and Kaya (2007) found MDA was significantly reduced when broilers were supplemented with Nano Zn Oxide. Effects were, however, not significant with prolonged supplementation (Duzguner and Kaya, 2007; Zhao *et al.,* 2014).

Lin *et al.* (2004) reported increased thiobarbituric acid reactive substances in liver and plasma when broilers were subjected to acute heat stress. Lin *et al.* (2006) found higher concentrations of TBARS in hepatic compared to cardiac tissues. Elevated MDA concentrations were found in Japanese quails (Sahin *et al.,* 2002), as well as broilers exposed to high ambient temperatures (Sahin *et al.,* 2001; Sahin *et al.,* 2002). This coincided with results by El-Shaieb *et al.* (2009) and Ismail *et al.* (2015) who reported high MDA concentrations in broilers under hyperthermic conditions. Halliwell and Gutteridge (1989), Sahin *et al.* (2002) and Naziroglu *et al.* (2000) found induction of lipid peroxidation and free radical production when broilers, as well as other animals, were subjected to high temperatures. Azad *et al.* (2010) observed no changes in skeletal MDA in broilers under cyclic heat stress, but found MDA concentration increased 1.2 to 1.5 times in broilers exposed to acute heat. Broilers exposed to acute heat for 3 to 5 hours presented with fourfold increases in MDA in the pectoralis majors muscle (Wang *et al.,* 2009). This agreed with results by Mujahid *et al.* (2009), where a twofold increase of MDA in skeletal muscles were found in broilers subjected to high temperatures for 12 hours. Yang *et al.* (2010) observed MDA concentrations concomitantly increased with antioxidant enzymes in liver and serum of heat stressed birds. Altan *et al*. (2003) found increased MDA concentrations (2.01 to 2.59) in serum and livers of broilers after heat exposure, with concomitant increases in superoxide dismutase,

catalase and glutathione peroxidase activities. MDA and antioxidant enzymes were reported to decrease progressively to almost pre-stress concentrations after high temperature removal, when birds were exposed to recovery temperatures of 25℃ (Yang *et al.*, 2010). The absence of significant differences between MDA and TAC concentrations obtained in our study could possibly have been attributed to the heat profile being cyclic rather than acute. On the final day when birds were euthanized, sampling commenced at 5 am when broilers had been in the low recovery temperature cycle since 19:00 the previous evening. This possibly allowed birds to completely recover from the cyclic heat stress from the previous day. Although we did not analyse corticosterone concentration in our study, increased corticosterone concentration could have had an influence on MDA and TAC concentrations obtained in our study. Lin *et al.* (2004) observed chronic corticosterone administration led to lipid peroxidation and significantly increased plasma concentrations of TBARS and superoxide dismutase.

The utmost care was taken to handle broilers humanely and produce little stress and discomfort for the duration of the trial. Prior to slaughter, however, broilers were deprived of feed, as well as placed into crates and transported. Transportation to the abattoir lasted approximately 5 minutes, where a short waiting period commenced prior to humane euthanasia. Fasting has been said to be a major stressor in poultry husbandry (Gross, 1992; Lin *et al.*, 2006) and has been found to alter oxidative status in mammals (Marczuk-Krynicka *et al.*, 2003). Marczuk-Krynicka *et al.* (2003) observed significant reductions in catalase and Cu-Zn superoxide dismutase in rat livers after feed withdrawal of 36 hours. A simultaneous increase in free radical production was perceived in the rat livers (Marczuk-Krynicka *et al.*, 2003). This was in agreement with Di Simplicio (1997) who observed reductions in glutathione and superoxide dismutase in mice fasted for 18 hours (Lin *et al.*, 2006). In the current study, birds were subjected to a shorter fast, but the combined effects of fasting, handling and transport could have led to increased oxidative stress across treatments and temperatures. The combination of all of these stressful factors could therefore have acted as acute stressors, increasing oxidative stress across all treatments and birds. The differences in MDA and TAC concentrations between temperature treatments could therefore possibly have been masked by these stressors, resulting in statistical insignificance.

5.4. The effect zinc source, zinc inclusion level and house temperature on serum cholesterol concentration in broilers

In our study, serum cholesterol concentration in broilers were not significantly influenced by Zn sources, Zn inclusion levels or temperature treatments. Slight elevations in serum cholesterol concentration were observed in birds under thermoneutral conditions, but results were statistically insignificant. This was in contrast to findings by Kucuk *et al*. (2003) who found cholesterol concentration significantly decreased in heat stressed birds when supplementary Zn was given, separately or in combination with vitamin A. Sahin *et al*. (2005) reported serum cholesterol concentrations decreased linearly with increases in dietary ZS and Zn picolinate supplementation in quail under heat stress. Results were found to be greater in broilers supplemented with Zn picolinate (Sahin *et al.*, 2005). Al-Daraji and Amen (2011) found significant increases in plasma cholesterol, glucose and protein in broiler breeders supplemented with Zn. This was suggested to be due to the role Zn has on synthesis of prostaglandins, sex and steroid hormones (Favier, 1992; Brown and Pentland, 2007). Plasma cholesterol concentration has been found to be affected by the amount of cholesterol ingested daily, where increased dietary cholesterol consumption has led to slight elevations in plasma cholesterol in broilers (Al-Daraji and Amen, 2011). A diet high in saturated fat has also been seen to increase blood cholesterol (Al-Daraji and Amen, 2011). We did not analyse our diets for cholesterol concentration, which could have contributed to the broiler cholesterol concentration obtained in this study. The fate of cholesterol concentration in liver is determined by the degree of activation of specific enzymes responsible for lipid metabolism (Guyton and Hall, 2006). Zinc participates in multiple reactions, which may influence cholesterol concentration (Al-Daraji and Amen, 2011). Steroid hormones and bile acid synthesis require body cholesterol (Sastry, 2008), thereby influencing cholesterol concentration in serum. Cholesterol concentration in plasma has been stated to be positively correlated with corticosterone concentration (Al-Daraji and Amen, 2011). This was in agreement with Sahin *et al*. (2005) who reported serum cholesterol concentration increased parallel with serum corticosterone concentration. This was suggested to be due to corticosterone having a catabolic effect, thereby increasing glucose and cholesterol concentration in serum (Sahin *et al*. 2005). Thyroid hormones have also been found to increase blood cholesterol concentration, where excess thyroid hormones would result in reduced cholesterol concentrations (Al-Daraji and Amen, 2011). Thyroid hormones, corticosterone concentration and dietary cholesterol could all have potentially

influenced the concentration of cholesterol obtained in our research trial, thus leading to the insignificant differences between sources, Zn inclusion levels and temperature treatments.

5.5. The effects of zinc source, zinc inclusion level and house temperature on tibial parameters

Tibias from broilers supplemented with organic and inorganic Zn did not differ in dry matter (DM), however, birds supplemented with AZ presented with significantly higher DM in the tibias. Birds that received supplementary IZ displayed significantly lower tibial DM. Birds under high temperature stress presented with significantly lower tibial DM compared to birds kept under thermoneutral conditions. Tibial ash percentage was not affected by Zn level, source or house temperature. Yuan *et al*. (2011) reported no significant differences between dry weight, ash weight and ash percentage in the metatarsus of broilers supplemented with either organic or inorganic Zn and Mn. This was in agreement with our results, however, it should be noted that we sampled broiler tibias and not metatarsal bones.

Bone has been shown to be the most sensitive indicator of Zn bioavailability in poultry (Suo *et al.,* 2015). Approximately 35% of whole body Zn is stored in bone, although based on dry body weight, bone only comprises of 19% of the total bird weight (Mavromichalis *et al.,* 2000; Richards *et al.,* 2015). Birds supplemented with AZ presented with significantly higher ($P < 0.05$) Zn concentration in bone ash and dry bone. Zn concentration in bone ash and dry bone were found to be significantly lower ($P < 0.05$) in broilers supplemented with ZS. This was in agreement with a study by Wedekind *et al.* (1992), where they found higher Zn concentration in bone when Zn methionine, compared to Zn sulphate was supplemented. Salim *et al.* (2010) observed significant increases in tibia, collagen and skin Zn concentrations in response to supplementary Zn. Significantly higher values were obtained when supplementary organic, compared to inorganic, Zn were provided (Salim *et al.,* 2010). Favero *et al*. (2013) reported greater tibia strength and stiffness when broilers were supplemented with minerals in their organic forms. Higher tibia moment inertia was found in broilers supplemented with organic Cu, Mn and Zn (Favero *et al.,* 2013). In the present study, supplementing Zn at 120 mg/kg inclusion level resulted in significantly higher ($P < 0.05$) Zn concentrations in bone ash and dry bone, compared to broilers supplemented at 80 mg/kg inclusion. Suo *et al.* (2015) reported when supplementing increasing levels of dietary

Zn, broilers presented with linear increases in bone and pancreatic Zn concentrations. Ao *et al.* (2007) found linear increases in Zn concentration in liver, tibia and tibia ash in broilers supplemented with Zn. Increased bone thickness and higher calcification were observed in 18-day old embryo femurs from hens supplemented with organic Zn (Favero *et al.*, 2013). No significant differences were found in Zn concentration in dry bone and bone ash in broilers across both temperature treatments.

Chapter 6

Conclusion

Broiler body weight was significantly depressed when birds were exposed to high temperature stress. Supplementing organic Zn at 120 mg/kg inclusion level, under hyperthermic conditions resulted in broilers with significantly improved body weight. The same was found in broilers under thermoneutral conditions. Broiler body weight was significantly higher when organic Zn, specifically AZ was provided, across temperature treatments. Birds that received ZS consistently presented the lowest body weights, across Zn inclusion levels and temperature treatments.

Feed intake and cumulative feed intake were significantly lower in birds under high temperature stress compared to birds exposed to thermoneutral conditions. Feed conversion ratio was found to be significantly higher in birds exposed to high temperature stress. Supplementing organic Zn at 120 mg/kg inclusion level, under hyperthermic conditions resulted in broilers with significantly improved feed conversion ratio, while decreasing feed intake and cumulative feed intake. Feed intake and cumulative feed intake were the highest in birds that were supplemented with Zn sulphate and the lowest when organic Zn was supplemented, under both heat stress and thermoneutral temperatures. The same was found for feed conversion ratio. Broilers had the highest feed intakes under thermoneutral conditions when Zn inclusion level was 80 mg/kg, compared to heat stressed conditions when 120 mg/kg yielded higher feed intakes. Supplementing Zn at 120 mg/kg during high temperature conditions resulted in higher cumulative feed intake for the birds, where birds under thermoneutral conditions presented with no differences in cumulative feed intake between inclusion levels. Feed conversion ratio from birds supplemented at different Zn inclusion levels presented with no significant differences under thermoneutral temperatures, where heat stressed birds had lower feed conversion ratio when Zn was supplemented at 80 mg/kg.

No significant differences were observed between liver, heart and fat pad weights for broilers supplemented with different sources and at different inclusion levels of Zn. Malondialdehyde and cholesterol concentration, as well as total antioxidative capacity were not significantly influenced by Zn sources, Zn inclusion levels or temperature treatments.

Tibia ash and dry matter values were not significantly influenced by Zn sources, Zn inclusion levels or temperature treatments. Zn concentration in tibias were found to be significantly higher in birds supplemented with organic Zn. Birds supplemented with AZ presented with the highest bone Zn concentrations, where birds that received ZS displayed the lowest values. Zn concentration in broiler tibias were found to be higher when broilers were supplemented at 120 mg/kg inclusion level rather than 80 mg/kg inclusion, across both temperature treatments.

Based on our results we concluded that supplementing broilers with 120 mg/kg of organic Zn offered a feasible, inexpensive way to alleviate hyperthermia and associated losses in performance and leg health associated with high temperature stress prevalent in sub-Saharan countries. Supplementing organic Zn (*Availa*®Zn) to birds in thermoneutral conditions was an efficient way to improve performance parameters and should be supplemented at 80 - 120 mg/kg inclusion level to improve bone strength parameters and overall broiler performance.

Critical review and recommendations

Broilers were handled minimally on the sampling day and any contact was humane and made to be as stress free as possible for the birds. Prior to slaughter, however, broilers were deprived of feed, as well as placed into crates and transported. Sampling commenced at approximately 5 am and finished at approximately 12 pm, where birds were in a constant state of fasting or feed withdrawal. As the sampling period lasted for approximately 7 hours, it resulted in birds being subjected to fasting conditions from 1 to 7 hours. Allowing birds to go that long without feed possibly influenced malondialdehyde concentrations and total antioxidative capacity. The elevated stress from fasting could have potentially masked any differences between broilers subjected to different Zn sources, levels and temperature treatments. The combined effects of fasting, handling and transport would have been extremely stressful for the birds, even though every possible measure was taken to keep birds as calm and comfortable as possible. The combination of all these stressful factors could therefore have acted as acute stressors, increasing oxidative stress across all treatments and birds, which very likely masked any significant differences obtained in malondialdehyde concentration and total antioxidative capacity between birds. Insignificant differences obtained in malondialdehyde concentration and total antioxidative capacity could also have resulted due to the cessation of temperature treatments the previous day. Temperatures were returned to thermoneutral conditions at 17:00 the evening before sampling commenced, allowing birds time to recover from the high temperature stress of the previous day. Broilers were subjected to cyclic heat stress, which also could have influenced malondialdehyde concentration and total antioxidative capacity.

No significant differences were observed in broiler tibial dry matter and tibia ash, across Zn levels, Zn sources and temperature treatments. This could have possibly resulted from inconsistencies in sampling and processing, or laboratory and human errors. Broilers were selected to be as close as possible to the average broiler size. This was to ensure uniform sampling, which could possibly have resulted in broilers that were so similar in size that any differences in tibia weights and sizes were masked. If broilers were slaughtered at a later age, differences would possibly have been statistically different.

Liver, heart and fat pad weights were significantly lower in broilers subjected to high temperatures, but no other significant differences were observed. The lack of differences could possibly have resulted from broilers being exposed to cyclic instead of acute heat stress. Birds were subjected to thermoneutral temperatures from 17:00 until 8:00, allowing broilers time to recover from the high temperature stress that occurred during the previous day. Even though cyclic heat stress negatively affected broiler performance parameters, such as body weight and feed intake, liver, heart and fat pad weight had small statistically insignificant differences. If the trial was continued for a longer time period differences between organ weights would possibly have presented with significant differences between broilers supplemented with different sources and inclusion levels. Sampling errors could possibly have occurred, especially with the fat pad removal. Although staff were trained to follow specific, written instructions that were thoroughly discussed and explained with examples beforehand, sampling errors could have occurred due to human error as well as inconsistent sample removal. Fat pads were removed manually and by different staff members. This could have potentially resulted in significant differences between fat pad sizes, as some of the fat pad could have remained and was thus not properly excised.

There is potential for further valuable research using organic Zn supplementation to alleviate other stressful conditions in poultry, such as disease, pathological conditions, immunological challenges, gut health challenges and other environmental stressors. Further research could be conducted on the effect Zn has on the skeletal system, in both broiler and breeder nutrition, as well as embryo development. Zn could also be used in antioxidative response trials during stressful conditions.

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