Effects of incubation and the trace minerals, zinc and manganese in organic form, on ascites incidence in broiler chickens

by

Thabani Prince Mbule

Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Science: (Production Management) in the

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

Supervisor: Dr. C. Jansen van Rensburg

Co-supervisor: Dr. P.W. Plumstead

February 2017

PREFACE AND DECLARATION

I, Thabani Prince Mbule declare that:

The research in this dissertation, except where otherwise indicated, is my own original research work.

This dissertation has not been previously submitted for any degree purposes at any other university.

This dissertation does not by any means contain other person's data, unless precisely acknowledged as being sourced from other persons. Where other written sources have been quoted, then: their words have been rewritten but the information they provided has been referenced and acknowledged.

This dissertation does not contain text, pictures, graphics or tables copied and pasted from the internet, unless specifically acknowledged and the source being detailed in the dissertation and in the reference section.

Signed: _____ Date: _____

DEDICATION

This thesis is dedicated to my loving mother, Rebecca Mokoena. Through her courage, supervision, love, belief and support I was able to complete this project. A very strong, smart and respectful woman who always worked very hard so she supports me financially during my college years. She is indeed, my hero!

BIOGRAPHY

THABANI PRINCE MBULE, the son of Mfanafuthi Simon Mbule and Rebecca Mokoena was born in South Africa on the 31st of August 1989 in KwaZulu Natal, Newcastle. Thabani attended his high school years in Phendukani high school in Newcastle. In 2006 he enrolled with the University of Pretoria where he obtained a BSc (Agric) Animal Sciences degree. After his undergraduate studies in 2010, he then decided on furthering his studies with the University of Pretoria for MSc (Agric) degree under the supervision of Dr Christine Jansen van Rensburg and Dr Peter William Plumstead.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank the following individuals, companies, and institutions for their pronounced contribution to the success of this thesis.

A word of appreciation is firstly dedicated to my parents, Rebecca Mokoena and Mfanafuthi Mbule for their prayers, love, motivation and their never ending support throughout my study years. Without their input, this research would have not been a success.

Chemunique International (Pty) Ltd, especially Terry Wiggill, for financial support towards my Master's degree and a Prof Rob Gous scholarship.

My supervisor, Dr C. Jansen van Rensburg for the study guidance, financial arrangements, practical training, and professional supervision.

My Co-supervisor, Dr P.W. Plumstead for my research planning, technical assistance, constructive criticism, and life lessons most of all.

I will also like to extend my words of appreciation to the poultry student research team at the University of Pretoria for their assistance during my research. Special thanks to K.E. Brannan for her extreme participation, patience, and guidance.

I extend my gratitude to the farm manager, Mr Roelf Coertzee for statistical analysis of data.

University of Pretoria, Onderstepoort campus (Clinical Pathology Laboratory) for blood samples analysis and their enduring professionalism.

To all my friends especially Philani Khathide, Sandla Sakhe Sikho Nomvete, and Siphesihle Kunene for their love, moral support, and motivation. I am also thankful for their patience, faithfulness, and lasting friendship.

And finally, To God be the glory, without the Lord this research would have not been a success.

I can do all things through Christ who strengthens me (Philippian 4:13)

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

O_2	-	Oxygen
CO_2	-	carbon dioxide
Zn	-	zinc
Mn	-	manganese
Cu	-	copper
P		phosphorus
FCR	-	feed conversion ratio
mL	-	millilitres
°C	-	degrees Celsius
BW	-	body weight
EST	-	eggshell temperature
YFBM	-	yolk free body mass
RV	-	right ventricle
TV	-	total ventricles
g	-	gram
HCT	-	haematocrit
PHS	-	pulmonary hypertension syndrome
RH	-	relative humidity
CP	-	crude protein
CF	-	crude fibre
DM	-	dry matter
GE	-	gross energy
MJ	-	mega joules

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Recently, intense broiler selection for rate of body weight gain and improved feed conversion has been an area of focus for poultry breeders. Modern broilers achieve slaughtering age at 60% less time than 40 years ago. However, this increased growth rate has resulted in complications that ultimately resulted in metabolic diseases. Pulmonary hypertension syndrome (also known as "ascites") is a very common disease that is caused by an increased metabolic rate due to increased growth rate. Other factors such as incubation conditions, nutrition, lighting programme, altitude and temperature had proven to exacerbate this metabolic disease. Ascites syndrome is characterised by the accumulation of abdominal fluid, liver damage, pericardial effusion and inflammation of the right ventricle of the heart.

Zn is responsible for > 300 enzyme processes and is also required for the synthesis of metallothionine that is responsible for antioxidant activity. Mn also operates as a cofactor for superoxide dismutase (SOD) enzymes that provide oxidative stress resistance through formation of non-proteinaceous manganese-based antioxidants.

Five thousand eight hundred and eight (5808) eggs from parent stock (Ross 308) were incubated at either normal (37.5-38.0 °C) or high (38.5-39.0 °C) temperatures. Chicks from both these incubation temperatures were randomly allocated to one of four dietary treatments to create a 4x2 factorial design with 8 treatments replicated in 12 pens each.

One thousand eight hundred and twenty four (1824) Ross 308 feather sexed males were placed in a 96-pen broiler facility, with each pen (1.5m×1.5m) containing 19 chicks. Four dietary treatments were fed *ad libitum* from day-old until 42 days of age. Treatment 1 contained 80 mg/kg of $ZnSO_4$ and 80 mg/kg of $MnSO_4$ while Treatment 2 contained 120 mg/kg of each. Treatment 3 contained 80 mg/kg of $ZnSO_4$ and 80 mg/kg $MnSO_4$ and 40 mg/kg Availa Zn/Mn (Zinpro Corporation, Eden Prairie, Minnesota, USA). Finally, Treatment 4 contained 40 mg/kg of $ZnSO_4$ and 40 mg/kg of $MnSO_4$ and 80 mg/kg Availa Zn/Mn.

Weekly body weights, feed intake and feed conversion ratio (RCR) were recorded per pen. Daily mortalities were collected, recorded and dissected to determine if the cause of death was attributable to ascites syndrome. At 21 and 42 days of age, two birds per pen were sampled for haematocrit values and necropsied for right ventricle to total ventricle weight (RV/TV) ratios, and ascites syndrome related symptoms.

Birds incubated at normal temperature generally performed better than the birds that were incubated at high temperatures. Chicks that were incubated at normal temperatures showed lower mortalities (general and

ascites related) and greater carcass weights at the end of the growing period (42 days of age). However, the RV/TV ratios were not significantly different between incubation treatments at all ages.

Treatment 1 contained 80 mg/kg inorganic Zn/Mn (sulphates), which represented the lower end inclusion rate of inorganic zinc in poultry diets as an industry standard. Treatment 2 contained 120 mg/kg inorganic Zn/Mn that represented an upper end inclusion rate of inorganic zinc in commercial poultry diets and to observe the level response between Treatment 1 and 2. Treatment 3 contained 80 mg/kg inorganic Zn/Mn and 40 mg/kg organic Zn/Mn, which is the commercial optimum replacement rate of inorganic Zn with organic Zn based on literature to observe performance benefits from organic Zn and Mn. Treatment 4 contained 40 mg/kg Inorganic Zn/Mn and 80 mg/kg organic Zn/Mn, this was to observe any further performance benefits from increasing organic Zn/Mn relative to inorganic Zn/Mn in the diet.

Treatment 3 and 4 (Availa Zn and Mn) exhibited improved body weight gain, less mortality (general and ascites related), improved carcass weights, and better flock uniformity at 42 days of age as compared to chicks from Treatment 1 and 2 ($ZnSO_4$ and $MnSO_4$) at the same inclusion levels. The ascites syndrome incidence was also lower in Treatment 3 and 4 as compared to Treatment 3 and 4.

These research findings suggest that high incubation temperatures are detrimental to broiler health and welfare and may ultimately increase the incidence of ascites syndrome. However, 40% replacement of inorganic Zn and Mn with their organic sources alleviated ascites incidence and improved overall broiler performance.

CHAPTER 1
GENERAL INTRODUCTION

The modern broiler (*Gallus domesticus*) has been intensely selected for fast early growth and is subjected to feeding and management procedures that support a high growth rate (Lorenzoni *et al*., 2006; Baghbanzadeh & Decuypere, 2008). Currently, broilers are able to achieve their target market weight in approximately 60% less time than broilers 40 years ago (Baghbanzadeh & Decuypere, 2008). This has led to various welfare concerns and metabolic problems such as ascites syndrome (Pulmonary Hypertension Syndrome; PHS) (Bessei, 2006; Lesson, 2007). Ascites is one of the most widespread and costly metabolic diseases in poultry (Balog, 2003) and inflicts serious economic concern due to carcass condemnations and considerable mortalities of heavy, fast growing broilers (Olkowski *et al*., 1996; Maxwell & Robertson, 1998; Gupta, 2011).

In the World Broiler Ascites Survey conducted in 1996, information on 18 countries from 4 continents confirmed that ascites syndrome affected 4.7% of live broilers worldwide, with economic losses estimated to cost the global poultry industry in excess of US$ 1 billion per year (Maxwell & Robertson, 1997; Currie 1999). An estimated 40 billion broilers are produced annually worldwide (Baghbanzadeh & Decuypere, 2008), with mortality rates of up to 5% of broilers and 20% of roaster birds due to ascites syndrome (Balog, 2003).

Although the occurrence of ascites is more noticeable at the end of the growing period, it is assumed that the aetiology of this metabolic problem might have existed early in embryonic development as a consequence of inappropriate incubation conditions that affect cardiovascular and pulmonary development of a chick (Decuypere *et al*., 2000; Lekrisompong *et al*., 2007; Herrera *et al*., 2013). Studies have shown a reduction in heart development at hatch by 28 to 30% after exposure to high incubation temperatures and an increase of ascites incidence by 3.8% during the growing period (Leksrisompong *et al*., 2007; Molenaar *et al*., 2011a). Incubation at high altitudes may be a further pre-disposing factor due to the decreased O_2 pressures that creates hypoxic conditions in tissues that increase the occurrence of ascites syndrome (Villamor *et al*., 2004; Herrera *et al*., 2013).

Studies at high altitudes reported an improvement in performance and a reduction in ascites incidence when inorganic sources of Zn and Mn were partially replaced with their organic sources (Arce-Menocal *et al*., 2004; Virden *et al*., 2004). This might be due to higher bioavailability of organic sources as compared to their inorganic counterparts (Lu *et al*., 2006; Lui *et al*., 2011).

In this study the dietary treatments were designed as follows: Treatment 1 contained 80 mg/kg inorganic Zn/Mn (sulphates), which represented the lower end inclusion rate of inorganic zinc in poultry diets as an industry standard. Treatment 2 contained 120 mg/kg inorganic Zn/Mn that represented an upper end inclusion rate of inorganic zinc in commercial poultry diets to observe any possible level response between Treatment 1 and 2. Treatment 3 contained 80 mg/kg inorganic Zn/Mn and 40 mg/kg organic Zn/Mn, which is the commercial optimum replacement rate of inorganic Zn with organic Zn based on literature to observe performance benefits from organic Zn and Mn. Treatment 4 contained 40 mg/kg inorganic Zn/Mn and 80 mg/kg organic Zn/Mn, this was to observe any further performance benefits from increasing organic Zn/Mn relative to inorganic Zn/Mn in the diet.

The aim of the study was to evaluate the effects of an organic source of Zn and Mn (Availa Zn and Mn, Zinpro Corporation, Eden Prairie, Minnesota, USA) on broilers that were exposed to ascites inducing conditions, i.e. incubation at high temperatures and rearing under low brooding temperatures.

Hypotheses:

Incubation trial

H_0: High incubation temperature from embryonic day 11 (E11) until hatching does not increase the incidence of ascites syndrome in broilers during the rearing period.

H_1: High incubation temperature from embryonic day 11 (E11) until hatching increases the incidence of ascites syndrome in broilers during the rearing period.

Broiler trial

H_0: Dietary supplementation with organic Zn and Mn does not alleviate ascites syndrome in broilers during the rearing period.

H_1: Dietary supplementation with organic Zn and Mn does alleviate ascites syndrome in broilers during the rearing period.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

A number of metabolic problems arise in chickens due to intensive selection to manifest their genetic potential for fast growth and feed conversion (Leeson, 2007). One such metabolic disease, ascites syndrome (PHS; Pulmonary Hypertension Syndrome) is one of the major causes of mortality and morbidity in modern broiler production today (Gupta, 2011). A worldwide survey conducted in 1996 showed that in 18 countries from 4 continents, the ascites incidence was approximately 4.7% in broilers (Maxwell & Robertson, 1998). This condition inflicts financial losses to farmers at a global cost of around US$1 billion per annum (Maxwell & Robertson, 1998; Currie, 1999). Ascites syndrome has become a worldwide noticeable non-infectious metabolic disease that causes bird mortalities and carcass condemnations of 5-7% in the broiler industry (Gupta, 2011). Ascites syndrome, like several other metabolic disorders, is a multifactorial syndrome, caused by a number of interacting factors such as environment, physiology, and genetics (Baghbanzadeh & Decuypere, 2008). The pathogenesis of this disease can be related to high basal metabolic rates induced by cold, overeating, activity, hyperthyroidism and accelerated muscle mass (Currie, 1999). Ascites is associated with increased fluid accumulation in the peritoneal spaces with or without an increase of fibrin proteins in the fluid (Julian, 2003). Normal meat type chickens frequently have 1 to 3 mL of fluid in the pericardial sac at 6 to 8 weeks of age but any quantities beyond 4 mL is considered to be abnormal (Julian, 1993).

2.2 Pulmonary hypertension syndrome (ascites syndrome or “waterbelly”)

Genetic selection for growth and feed conversion in broilers has resulted in high metabolic rates (fast growth) and high oxygen demands such that it exceeds the cardiopulmonary capacity of heart and lungs to supply oxygen (Gupta, 2011). However, ascites is not a disease; it is a sign or lesion that may result from one or more physiological changes that cause an increased production or decreased removal of peritoneal lymph (Julian, 1993). It might be caused by an obstruction of lymph drainage, decreased plasma osmotic pressure, increased vascular permeability, and increased hydrostatic pressure in the vascular system due to (Currie, 1999):

1 Hepatic pathology
2 Pathology of the right atrioventricular valve
3 Pulmonary hypertension
4 Miscellaneous cardiac pathologies

The high metabolic rate increases oxygen demands such that the chicken can barely be able to meet the supply that ultimately results in a series of events, including peripheral vasodilatation, increased cardiac output and pulmonary arterial pressure and right ventricular hypertrophy (Gupta, 2011). The peak incidence of ascites occurs after 4 weeks of age, typically in the 5th or 6th weeks of the growing period (Gupta, 2011).

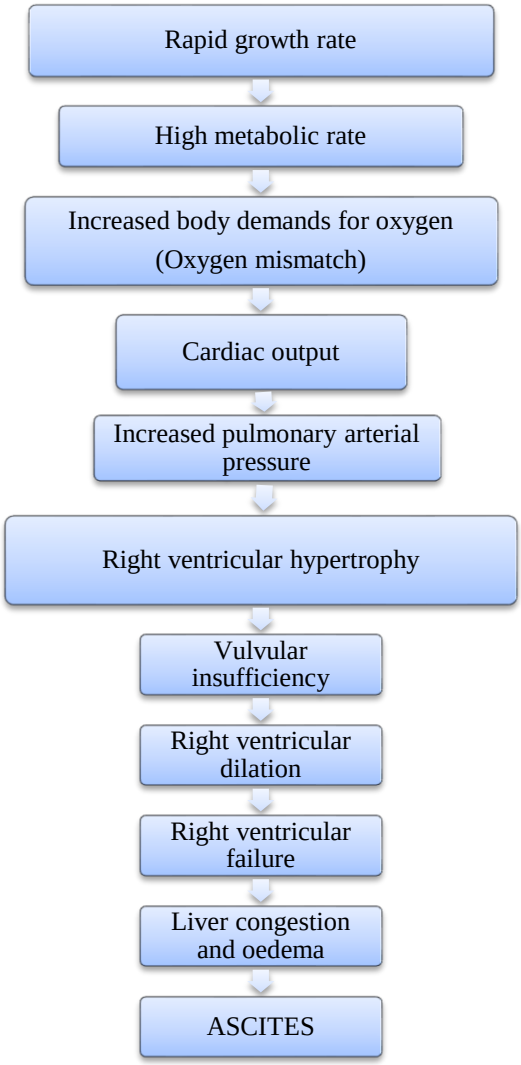


Figure 2.1 Ascites aetiology in rapid growing broilers (Julian, 1993)

The lymphatic system functions to collect the fluids, proteins, other macromolecules and cells, before returning them to the main circulatory system (Tamsma *et al.*, 2001). The lymphatic system consists of one layer of endothelium that drains into the lymphatic capillary. The basement membrane may be present at this level but mostly is interrupted in ascetic birds due to other affecting factors e.g. free radicals, bacterial or viral infection and chemical toxins (Tamsma *et al.*, 2001; Singh *et al.*, 2011). Plasma proteins are responsible for blood osmotic pressure, in ascites-sensitive and ascetic birds these proteins in the blood are reduced (Singh *et al.*, 2011).

The lowered concentration of these proteins may be due to loss in high protein lymph from the liver and the reduction in feed intake due to right ventricular hypertrophy (Julian, 1993). The loss of proteins may change the fluid dynamics and draw water to an area, resulting in fluid accumulation in peritoneal spaces due to damage (Julian, 1993). Increased vascular hydraulic pressure is caused by the dilation of the blood capillaries to accommodate the increase in blood flow to fulfil the oxygen demands of the body (Julian, 1998). This tends to increase the myocardial contractility due to elevated preload that leads to pulmonary vascular resistance and pressure overload resulting in ventricular hypertrophy (Singh *et al.*, 2011). The overload in the ventricles and the resultant haemodynamic pressure ultimately distorts the atrioventricular valves and result in blood regurgitation causing disturbances in the haemodynamic (Currie, 1999).

2.2.1 Hydraulic pressure and liver damage

The increased fluid in the capillary bed and sinusoids of the liver or other parts of the vascular system is the cause of fluid accumulation in the peritoneal spaces (Julian, 1993). The sinusoids of the liver are fenestrated allowing high protein lymph to escape easily. The pressure increases can affect the liver resulting in liver atrophy and fibrosis in the efferent vessel leading to obstruction in venous return, causing ascites (Currie, 1999).

2.2.2 Pulmonary hypertension

The pulmonary hypertension is caused by abnormalities at the respiratory and cardiovascular system levels (Julian, 2000; Baghbanzadeh & Decuypere, 2008). The small stature of modern broilers with large, heavy breast muscles creates pressure from the abdominal contents on air sacs and the small lung volume predisposes the birds to increased incidence of pulmonary hypertension syndrome (Julian, 1998; Wideman *et al.*, 2013). An increased blood flow is necessary to supply oxygen demand required in fast growing birds that creates an increased blood pressure necessary to pump the blood through the blood capillaries in the lungs (Julian, 1993; Julian, 1998; Currie 1999). The blood and air capillaries form a network that allows small blood capillaries of the lung to dilate slightly in order to accommodate increased blood flow (Julian, 1998; Currie, 1999; Baghbanzadeh & Decuypere; 2008). The pressure created by cardiac output causes increased blood flow in the circulation that results in increased stroke volume and eventually leads to increased right ventricular pressure

(Julian 1990, Currie, 1999, Hassanzadeh, 2009). This phenomenon is clearly demonstrated by Poiseuille's equation. Poiseuille's law relates volume flow (Q) to the pressure drop (P1-P2) along a tube of radius (r) and length (L) during steady flow as follows (Currie, 1999; Baghbanzadeh & Decuypere, 2008):

$Q = (P_1\text{-}P_2) \times \frac{\pi r^4}{8\mu L}$ (π is blood viscosity)

Vascular resistance (R) will be calculated as:

$$R = \frac{P1-P2}{Q}$$

The slight change of the radius of the pipe or a vessel will significantly influence the flow and resistance in the vessel (Baghbanzadeh & Decuypere, 2008). An increased pulmonary vascular resistance leads to pressure overload in the right ventricles and ventricular hypertrophy, which is one of the common lesions in ascetic birds (Baghbanzedeh & Decuypere, 2008). The distortions in the atrioventricular valves result in ventricular hypertrophy and the valves become thicker and leaky (Currie, 1999). The leaking valves regurgitate flow due to leakiness and the resultant haemodynamic pressure imbalance creates ascites syndrome (Currie, 1999).

2.3 Factors causing ascites syndrome ("Pulmonary Hypertension Syndrome")

2.3.1 Genetics

Genetic selection for fast growth rates and improved feed conversion rate leads to high oxygen demands in a broiler in order to support the high metabolic rate (Aftab & Khan, 2005; Leksrisompong *et al*., 2007; Malan *et al*., 2007; Hassanzadeh, 2009; Singh *et al*., 2011). Modern broilers, especially male parent lines, tend to be more prone to ascites than females (Arce-menocal *et al*., 2004; Singh *et al*., 2011).

2.3.2 Nutrition

Broiler growth rate is directly related to ascites susceptibility (Singh *et al*., 2011). Major nutritional factors, including high nutrient density rations, high feed intake, and feed form are known to increase growth rate and increase the incidence of ascites in poultry (Singh *et al*., 2011). High energy diets and pelleted feed increase the incidence of ascites syndrome (Bolukbasi *et al*., 2005; Baghbanzadeh & Decuypere, 2008). Diets containing high sodium and diets low in phosphorus, vitamin E, selenium, and vitamin C can also predispose broilers to ascites (Gupta, 2011).

2.3.3 Incubation

An oxygen deficit is one of the major factors that triggers ascites syndrome in broilers during incubation (Gupta, 2011). Low oxygen concentrations result in hypoxaemia and ultimately ascites due to an increase in pulmonary arterial pressures (Baghbanzadeh & Decuypere, 2008). Increased growth rate during embryonic stages due to high incubator temperature increases oxygen consumption, cardiac output and blood flow that might result in increased pulmonary arterial pressure primarily by increasing metabolic demands for oxygen (Baghbanzadeh & Decuypere, 2008). Leksrisompong (2005) also confirmed that increased incubation temperature impaired heart development, reduced live performance, and damaged oxygen-exchange mechanisms in broilers.

2.3.4 Blood pH

Poor ventilation and exchange of gases in the lungs increase carbon dioxide in the vascular system which might decrease the pH of the blood (Baghbanzadeh & Decuypere, 2008). Acidic conditions result in vasoconstriction of the vessels and alkali conditions cause vasodilation (Baghbanzaden & Decuypere, 2008). The acidity of the blood affects pulmonary arterial pressure, pulmonary hypertension, and also the oxygen binding affinity of haemoglobin (Baghbanzadeh & Decuypere, 2008).

2.3.5 Erythropoiesis

Broilers have a thick respiratory membrane that negatively influences their ability to move oxygen into haemoglobin (Baghbanzadeh & Decuypere, 2008). Research indicated that fast growing birds have lower haemoglobin oxygen saturation than slow growing birds (Baghbanzaden & Decuypere, 2008). This resulted in high red blood cell production (partially immature) to compensate for the reduced oxygen saturation (Baghbanzaden & Decuypere, 2008). Due to the large amounts of immature red blood cells produced, the development of hypoxaemia was further aggravated by their reduced ability to bind oxygen (Currie, 1999).

2.3.6 Antioxidants

An elevated production of reactive oxygen in broilers prone to ascites may aggravate the disease (Baghbanzadeh & Decuypere, 2008). In broilers, the first line of defence against reactive oxygen is endogenous antioxidants such as tocopherols, glutathione, uric acid, and ascorbic acid (Currie, 1999). Ascorbic acid and glutathione levels are reduced in broilers reared in an ascites-promoting environment, signifying their utilisation in the tissues due to high reactive oxygen levels (Baghbanzadeh & Decuypere, 2008).

2.4 Environmental/management factors that may cause ascites or contribute towards the development of ascites

2.4.1 Altitude

High altitudes are associated with low partial pressures of oxygen (Currie, 1999), which results in the constriction of blood vessels and increased pulmonary resistance (Baghbanzadeh & Decuypere, 2008). This immediately increases pulmonary arterial pressure and causes right ventricular hypertrophy and eventually ascites syndrome (Baghbanzadeh & Decuypere, 2008).

2.4.2 Lighting

The use of a near continuous light schedule in broiler production systems stimulates feed consumption and growth rate (Bolukbasi *et al*., 2005). Increased growth rate imposes increased oxygen requirement on the birds to maintain their high metabolic rate and failure to fulfil the requirements may cause ascites (Malan *et al*., 2003). The reduction in light regimens may help reduce feed consumption, growth, activity and reduce the overall oxygen required (Singh *et al.*, 2011).

2.4.3 Cold temperature

Cold ambient temperatures cause production of triiodothyronine (T3) that is required for the generation of extra metabolic heat to maintain body temperature in cooler environments (Gupta, 2011). This increase in metabolic rate causes an increase in blood pressure to supply oxygen, which leads to pulmonary hypertension and right ventricular failure (Julian, 1991).

2.4.4 Air quality and ventilation

A constant supply of oxygen in the broiler house and ventilation for exchange of carbon dioxide is essential for normal physiology of broilers (Currie, 1999; Leksrisompong *et al*., 2007). Ammonia formation and dust increase the incidence of ascites (Gupta, 2011; Franciosini *et al*., 2012). High levels of ammonia predispose broilers to ascites syndrome by reducing cilia motility favouring the appearance of respiratory diseases (Franciosini *et al*., 2012). Disease-causing micro-organisms can be transferred through dust particles, causing irritation or infection to lungs leading to reduced oxygen transfer between the birds and the environment (Mcgovern *et al*., 1999; Gupta, 2011).

2.4.5 Toxins

Hepatotoxins, mycotoxins, and high levels of furazolidone in the feed may cause ascites in broilers (Julian, 1993; Currie, 1999; Gupta, 2011). Liver damage was caused by aflatoxin or by toxin contamination in broiler chicken feed and ultimately resulted in ascites syndrome (Julian, 1993, Gupta, 2011).

2.4.6 Diseases

Aspergillus species, infectious bronchitis virus, *E. coli*, and avian leucosis virus are all potential inducers of ascites in modern broilers (Huchzermeyer & De Ruyck, 1986; Gupta, 2011). Zefra *et al*. (2008) demonstrated the occurrence of ascites syndrome following fungal *Aspergillus* disease, which led to the destruction of pulmonary tissue. Adenovirus was also isolated from affected broilers and was the cause of myocardial damage that resulted in ascites syndrome (Julian, 1993).

2.5 Embryonic requirements and incubation

2.5.1 Introduction

The intensification of poultry production resulted in the replacement of the brooding hen with small air incubators that were later replaced by forced-draught incubator technology (Molenaar *et al.*, 2010b). During incubation of chicken embryos, environmental conditions, such as temperature, relative humidity, gaseous exchange, and egg turning must be closely monitored to meet embryonic requirements that vary during the different phases of embryonic development (Molenaar *et al.*, 2010b). Due to intense selection for high growth rate and a subsequent reduction in the overall growing period of the broiler, the incubation period has become a large part of the broiler life time (Hulet, 2007). This suggested that the incubation conditions need to be maintained at optimum in order to maximise broiler performance (Tazawa & Whittow, 2000; Hulet, 2007; Molenaar *et al*., 2010b; Barri *et al*., 2011).

Incubation is a process that has several critical points that can be monitored and controlled to produce healthy and well-developed hatchlings (Hulet, 2007). During incubation, embryonic and eggshell temperatures need to be maintained between 37.5 and 38.0°C throughout incubation to optimise hatchability, hatchling quality, and subsequent performance (Molenaar, 2010). An embryo consumes oxygen and excretes carbon dioxide through the eggshell to the outside environment (Tazawa & Whittow, 2000). Robertson (1961) showed that high relative humidity (75-80%) increased mortalities during the first 10 days of incubation causing disruptions in the development of the embryo. Elibol & Brake (2006) showed that the incidence of malposition embryos was increased by a reduced turning angle and then corrected by a concomitant increase in turning frequency.

2.5.2 Temperature

Traditionally, heat transfer from the hens' body to the egg was the most vital contribution to the success of the chick survivability. Hens sometimes develop a seasonal naked patch of the skin on the thorax and abdomen that allows for maximum heat transfer to the eggs (Tazawa & Whittow, 2000). The lower the incubation temperature, the slower the development of the chick, while high temperatures will result in fast chick

development, increased yolks retained, and earlier hatching (Leksrisompong, 2005). In the growing embryo, metabolic heat increases with development from about 35mW on E12 to 130-140mW on E17-18 (Tazawa & Whittow, 2000). This implies that embryonic temperature increases beyond that of ambient temperature by 1-2 °C (Tazawa & Whittow, 2000). This indicates the importance of measuring embryo temperature using eggshell temperature (EST) and not only by machine temperature, as machine temperature differs from the temperature experienced by the embryo. In chickens, the changes in egg temperature are easily sensed by the hen and she adjusts the heat by standing up or increasing the blood flow for extra heat transfer. When artificial incubators are used, they require proper temperature monitoring for the success of chick viability (Leksrisompong *et al.*, 2007).

Temperature fluctuates within an egg as the bottom part of an egg is in contact with the nest material and the upper part is in contact with the hens' plumage (Molenaar *et al.*, 2010b). In artificial incubators, one egg differs from the next in eggshell temperature readings even though the machine is run uniformly as one unit (Hulet, 2007). Embryonic temperature is of importance but since it can only be determined after breaking of the egg, EST is used as a close approximate value of embryonic temperature (Molenaar *et al.*, 2010b). It was found that the EST only deviated from the target embryonic temperature by 0.1-0.2°C (Molenaar *et al.*, 2010b). In many studies it has been shown that EST of about 37.5-38.0 °C yielded high hatchability and hatchling quality (Leksrisompong *et al.*, 2007). The difference between embryonic temperature and EST is caused by a difference in heat production of an embryo and heat transfer between the egg shell and the surroundings i.e. air flow (Lourens *et al*., 2005).

Molenaar *et al*. (2011a) found that high incubation temperature resulted in the reduction of heart size by 28%, and this might have been a possible cause of ascites during the growing period. Overheating towards the end of the incubation period resulted in reduced hatchling development as indicated by a reduced yolk-free body mass, a larger residual yolk, a shorter chick length, and a poorer navel condition compared to broilers that were incubated at a normal EST (Lourens *et al*., 2005). These chicks also exhibited a reduction in weights of hearts, gizzards, proventriculus and small intestines (Hulet, 2007; Leksrisompong *et al.*, 2009). High incubating temperatures resulted in poor hatchling quality and subsequent chick performance (Lourens *et al.*, 2011).

Incubation temperature is regarded as the single most important physical factor determining or influencing embryonic development and hatchability (Molenaar *et al*., 2010a; Willemsen *et al*., 2010; Lourens *et al*., 2011). The optimal development of the broiler embryo occurs within a temperature range of 37-38ºC (Decuypere & Bruggeman, 2007). However, the development can still be maintained in temperatures as low as 27ºC (French, 1997; Feast *et al*., 1998). The incubation temperature in commercial hatcheries is normally maintained at 37.0-

37.5 ºC for a 21 day period until hatch to achieve good hatchability, chick body weight (BW) and low mortalities (French, 1997; Feast *et al*., 1998; Hulet, 2007; Molenaar *et al*., 2010b; Molenaar *et al*., 2011a).

During incubation the embryo produces metabolic heat, which increases with development (Tazawa & Whittow, 2000). EST normally increases by the end of incubation due to increased heat production of the embryo (Lourens *et al*., 2005; Willemsen *et al*., 2010; Molenaar *et al*., 2011a). High EST (≥38.9°C) during the second half of incubation reduces hatchling quality, exhibited as a lower yolk-free body mass (YFBM), a shorter chick length, and a poorer navel condition, feed intake and feed efficiency (Leksrisompong *et al*., 2007; Willemsen *et al*., 2010; Barri *et al*., 2011; Molenaar *et al*., 2011a). Studies have revealed that high EST resulted in poor organ developments, which caused a reduction in heart weights by 28% (Lourens *et al*., 2005; Leksrisompong *et al*., 2007; Molenaar *et al*., 2011a). Under high temperatures, chicks presented white down feathers instead of yellow coloured feathers due to an inadequate yolk absorption as a result of poor intestinal development (Leksrisompong *et al*., 2007; Barri *et al*., 2011). It was also found that high EST caused slower growth and poorer feed conversion efficiency during rearing (Table 2.1; Hulet, 2007).

Table 2.1 Effects of eggshell temperature on post hatch broiler body weight, feed conversion, and percentage mortality (Hulet, 2007)

	Eggshell Temperature			
	37.5ºC	**38.6ºC**	**39.7ºC**	**SEM**
BW (g) 44 d	2213.8[b]	2263.3[a]	2165.7[c]	9.77
Feed conversion (1 to 21 d)	1.56[b]	1.55[b]	1.60[a]	0.26
Feed conversion (0 to 44 d)	1.91	1.86	1.87	0.01
Mortality (%) (1 to 21 d)	2.21	2.55	3.99	0.41

[a-c] Means within a row with no common superscripts differ significantly (P<0.05).

2.5.3 Humidity

It is important that the machine humidity be properly controlled to promote successful embryonic development (Peebles *et al*., 2001; Decuypere & Bruggeman, 2007). The rate of water loss from the egg can be regulated by incubator relative humidity (RH) using the wet bulb thermometer as a guide (Peebles *et al*., 2001). The water evaporates from the egg and it is continuously replaced by gas molecules and this causes an increase in air cell size that occupies 15% of the egg by the end of the incubation period (Molenaar *et al*., 2010a). It is important that relative humidity be kept at 60% and ventilation at optimum to prevent excess removal of humidity from the environment (Decuypere & Bruggeman, 2007; Hulet, 2007). Water loss from an egg occurs via pores; the amount of water loss from the egg at transfer should amount to 12-14% of the mass of the freshly laid egg (Decuypere & Bruggeman, 2007; Molenaar *et al*., 2010d).

Water loss from the egg is important for the air cell to reach adequate size to promote the start of lung ventilation (Tazawa & Whittow, 2000; Molenaar *et al*, 2010b). Egg moisture is determined by the amount of water loss from the egg and the amount of metabolic water added from the oxidation of yolk lipids (Molenaar *et al*., 2010b). The drier the surrounding air, the more water is lost from the egg to the environment (Tazawa & Whittow, 2000). The water vapour conductance is a function of number of pores and the thickness of the shell. Thicker shells and smaller pores reduce water vapour conductance (Tazawa & Whittow 2000). Higher incubation humidity of about 63% resulted in increased embryo mortalities due to increased water vapour in the air that reduced conductance of oxygen closer to hatching when the demand was higher (Bruzual *et al.*, 2000; Molenaar *et al*., 2010b). Molenaar *et al*. (2010b) confirmed that reducing humidity from 57 to 43% between E3 and E18 resulted in early hatching due to loss in extra embryonic water. It is important that relative humidity is kept between 40 and 65% with maximum hatchability around 50% RH (Robertsons, 1961; Bruzual *et al*., 2000). Lower RH during incubation resulted in reduced body weight (BW) at hatch due to increased moisture loss by embryos (Bruzual *et al*., 2000).

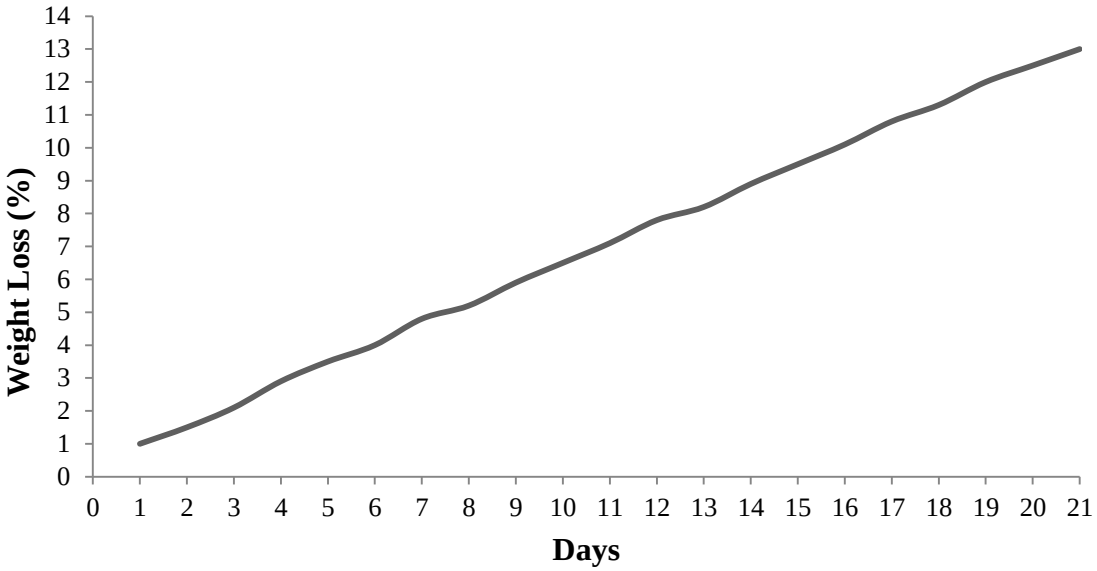


Figure 2.2 Optimum egg weight loss during the incubation period from 0 to 21 days of age (Cobb, 2008)

2.5.4 Gaseous exchange

Oxygen (O_2) and carbon dioxide (CO_2) exchange is of fundamental importance for embryonic development during incubation (Chan & Burggren, 2005; Mortola, 2009; Molenaar *et al*., 2010b) and the exchange is via the chorio-allantoic membrane (Wangensteen, 1972). The embryo utilises O_2 and releases CO_2 which is dissolved across the eggshell and excreted to the environment (Tazawa & Whittow, 2000). The oxygen concentration of air is 21% at any altitude (Clauer, 2009), but the partial pressures of oxygen decreases at high altitudes, which affects the bird's ability to oxygenate their haemoglobin at altitudes of 2500-3000m (Molenaar *et al*., 2010b; Sahan *et al*., 2011). Birds at high altitudes demonstrate impaired cardiovascular development, high blood haematocrit and vasoconstriction (Sahan *et al*., 2011). In contrast, an increase in oxygen during incubation can result in increased embryonic growth and survival rates (Sahan *et al*., 2011). Oxygen uptake increases with embryonic development and during the second week of incubation the lungs are already fully functional (Tazawa *et al*., 2000; Lourens *et al*, 2005; Sahan *et al*., 2011).

Molenaar *et al*. (2010b) reported a gradual increase of CO_2 concentration in the single stage incubator from about 0.05% at the beginning of incubation. Maximal CO_2 concentration in the incubator depended on the number of fertile eggs and ventilation rate but did not normally exceed 0.50% (Molenaar *et al*., 2010b). During the late embryonic stage between the start of breathing and hatching, O_2 uptake increases by 60% and CO_2 production increases accordingly which might bring about hypoxic conditions (Molenaar *et al*., 2010b; Sahan *et al*., 2011). The reduction of O_2 and the increase in CO_2 during late incubation, especially at high altitudes, result

in increased production of thyroid hormones that trigger earlier than expected hatching (Wangensteen, 1972; Decuypere *et al*., 2006; Malan *et al*., 2007; Sahan *et al*., 2011). Embryos at high altitudes exhibited increase plasma T_3 and T_4 concentrations with an increase in T_3:T_4 ratio (Sahan *et al*., 2011). Several studies also showed that a gradual increase in the CO_2 concentration from 0.7% to 1.5% during early stages of incubation accelerated embryonic development and improved hatchability (Molenaar *et al*., 2010b).

2.5.5 Egg turning

During natural incubation, parent birds have been observed to be actively turning the eggs in the nest (Tazawa & Whittow, 2000; Tona *et al*., 2003). Egg turning during incubation involves many parameters such as frequency of turning, axis of setting and turning, turning angle and planes of rotation (Tona *et al*., 2005; Elibol *et al*., 2006). It is a common practice in artificial incubation to turn the eggs once per hour at an angle of 90º until E18 of incubation to aid and assist in cooling as the heat production increases (Tona *et al*., 2005). Egg turning reduce embryo malpositions, prevents the adhering of embryo to shell and shell membranes, encourages timely closure of the chorioallantois at the small end of the eggs, and ensures optimal utilisation of the albumen (Robertson, 1961; Tona *et al*., 2003; Decuypere & Bruggeman, 2007). Lack of turning can result in many complications during embryonic development such as poor hatchability, irregular hatching period, embryonic fluid formation, poor development of chorioallantois for gaseous exchange, and decreased embryonic growth (Tazawa & Whittow; 2000, Elibol *et al*., 2006).

Table 2.2 Recommended hatchery ventilation, temperature, relative humidity and air pressure for optimum chick production (Cobb, 2008)

Hatchery ventilation, temperature, area pressure and relative humidity				
Areas	Ventilation Rate (m^3/hr)	Temperature (°C)	Relative Humidity (%)	Area pressure in relation to atmosphere (H_2O)
Egg Receiving	(5 minute air exchange to room)	19-21	60-65	Neutral to +0.01
Egg Holding Area	3.38	19-21	60-65	Neutral to +0.01
Setter Room	13.5	24-27	55-62	+0.015 to +0.02
Hatcher Room	28.7	24-27	55-62	+0.005 to +0.01
Chick Holding Rooms	67.6	22-24	65-70	Neutral
Chick Take-off	(0.5 minute air exchange to room)	22-24	65-70	-0.015 to -0.025
Wash Room	(0.5 minute air exchange to room)	22-24	65-70	-0.015 to -0.025
Clean Equipment Room	(1 minute air exchange to room)	22-24	N/A	Positive
Hallways	(5 minute air exchange to room)	24	N/A	Neutral

Pressure conversion (0.01 in H_2O = 2.5 Pascal, 0.025 mbar, 0.255 mm H_2O)

2.6 The role of incubation in the prevalence of ascites

2.6.1 Introduction

Suboptimal incubation conditions may adversely affect the development and survival of chicken embryos (Molenaar, 2010). The development of the embryo must be closely monitored throughout the incubation period in order to yield quality chicks (Leksrisompong *et al*., 2009). There are five environmental conditions that need to be controlled to ensure optimum development of the embryo (Leksrisompong *et al*., 2009). These conditions are temperature, air exchange, turning, humidity, and hygiene (Leksrisompong *et al*., 2009). The most important factors for proper survivability and quality of the chicks are eggshell temperature (EST) and oxygen (O_2)

availability for the embryo relative to temperature to ensure adequate utilisation of the nutrients in the egg (Molenaar, 2010).

The chicken embryo grows and develops without any interference from direct maternal effects and solely depends on the nutrients available in the egg throughout the entire 21 days of incubation (Molenaar *et al*., 2011a). The nutrients stored in the egg consists of large amounts of protein and fat but only a small amount (<1%) of carbohydrates (Molenaar *et al*., 2011a). During the first week of incubation glucose is the main source of energy since the chorioallantois is not sufficiently developed to supply adequate O_2 to oxidise fatty acids (Molenaar *et al*., 2010c). However, during the second half of incubation fatty acids are utilised and O_2 is fully supplied for adenosine triphosphate (ATP) production and proteins are deposited (Molenaar *et al*., 2010c).

During the first half of incubation, the temperature is the most important environmental condition for embryonic development (Molenaar, 2010). The temperature gradient of an artificial incubator is kept within narrow limits and should be in the range of 36 to 38 °C throughout incubation (Molenaar, 2010). High EST reduced hatchling development compared to normal EST, as demonstrated by lower yolk free body mass (YFBM), shorter chick length, greater residual yolk weight, and lower protein and total energy content (Molenaar *et al*., 2010d).

The O_2 availability is the second most important environmental factor during incubation that affects survival and chick development (Lourens *et al*., 2005). Oxygen availability for the embryo depends on the gas exchange across the shell and shell membranes by diffusion and the partial pressure gradient between the inside and the outside of the egg (Molenaar, 2010). Embryonic O_2 consumption increases exponentially after E9 of incubation and reached a plateau phase from E15-19 of incubation (Lourens *et al*., 2005).

There is an interaction between eggshell temperature and oxygen, with temperature being more important in the first half of incubation and oxygen requirement of the embryo increasing exponentially during the second half of the incubation period (Molenaar, 2010). Temperature and oxygen depend on each other, increasing temperature during incubation will increase oxygen requirements.

2.6.2 Temperature

The temperature experienced by the developing embryo is dependent on three factors: 1) the incubator temperature, 2) the ability of heat to pass between the incubator and the embryo, and 3) the metabolic heat production of the embryo (French, 1997). Eggshell temperature (EST) is used to measure embryo temperature and temperature often increases at the end of incubation because of the greater heat production of the embryos and due to inefficient cooling and air velocity in the incubators (Lourens *et al*., 2005). Evidence has shown that

high embryo temperatures during incubation can lead to reduced chick growth during the subsequent brooding period due to heat-stressed chicks being less alert and more sensitive to poor post hatch brooding conditions (Leksrisompong *et al*., 2009). There is a point where the increase in incubation temperatures above optimal not only accelerate growth rates of avian embryos but also negatively affected hatchability, feed efficiency, body weight, and general post hatch chick live performance (Leksrisompong *et al*., 2009). Altered air cell, blood gases and a retarded hatching process further indicated reduced growth of embryos exposed to higher incubation temperatures during the latter part of incubation (Willemsen *et al*., 2010). Embryos subjected to a high temperature treatment consumed less yolk, as revealed by the greater remaining yolk weight (Willemsen *et al*, 2010).

A study conducted by Molenaar *et al*. (2011a) (Table 2.3) showed a greater mortality of 4.1% in the high EST group compared to the normal EST treatment. The ratio between the right and total ventricle (RV:TV) was 1.1% greater in the high EST compared with the normal EST treatment at slaughter age (Molenaar *et al*., 2011a).

2.6.3 Oxygen

An oxygen deficit during embryonic development may result in ascites related problems in commercial flocks (Sahan *et al*., 2011). Oxygen availability depends on gas exchange across the shell and shell membranes via diffusion (Molenaar, 2010). Leghorns were better able to oxygenate their haemoglobin fully at 2500 to 3000 m above sea level, whereas in broilers there was a reduction in arterial blood oxygen even at low altitude (Sahan *et al*., 2011). Beker *et al*. (2003) found that ascites heart ratio (AHR), ascites score (AS), right ventricular mass (RVM), and haematocrit (HCT) all increased at high altitude ($P<0.01$). Beker *et al*. (2003) found 19.6% atmospheric O_2 as a minimal allowable level to which broilers can thrive with no cardiac or HCT changes related to ascites being observed.

At high altitudes, the incubation period is often shorter than at lower altitudes due to low oxygen partial pressures (Sahan *et al*., 2011). At higher altitudes, plasma T_3, T4 as well as T_3:T_4 ratios were found to be increased (Sahan *et al*., 2011). However, the T_3:T_4 ratio was decreased as O_2 was supplemented (Sahan *et al*., 2011). It is generally accepted that haematological characteristics play an important role in the pathophysiology of ascites in chicks that are incubated at high altitudes (Scheele *et al*., 2003). The differences in O_2 concentration during incubation affect the development of adaptive mechanisms, which influence nutrient utilisation and body development (Molenaar, 2010). Body composition and the efficiency of nutrient utilisation in the post-hatch period are influenced by the O_2 concentration that the embryo experiences during the incubation period (Molenaar *et al*., 2010d).

Table 2.3 Percentages of infertile eggs, hatchability of fertile eggs, second-grade chickens, embryonic mortality, malposition embryos, and hatchling measurements for eggs incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from E 7-E 21 of incubation (Molenaar *et al*., 2011a)

	n	Normal EST	High EST	P > F
		Incubation[1]		
Infertile eggs[2] (%)	20	2.6 ± 0.45	3.4 ± 0.56	0.26
Hatchability of fertile eggs[3] (%)	20	94.2 ± 0.57	92.5 ± 1.04	0.12
Second-grade chickens[4] (%)	20	0.2 ± 0.11[b]	0.9 ± 0.30[a]	0.02
		Embryo mortality[3] (%)		
Week 2	20	1.5 ± 0.27	1.1 ± 0.29	0.34
Week 3	20	1.9 ± 0.48	3.1 ± 0.68	0.17
Malposition embryos[3] (%)		1.4 ± 0.41	2.4 ± 0.67	0.24
		Hatchlings[5]		
Body weight (g)	100	40.6 ± 0.39[a]	37.2 ± 0.41[b]	<0.001
YFBM[6] (g)	100	36.9 ± 0.33[a]	33.9 ± 0.33[b]	<0.001
Chick length (cm)	100	19.5 ± 0.07	19.7 ± 0.07	0.14
Residual yolk (g)	100	3.7 ± 0.15[a]	3.2 ± 0.18[b]	0.05
Navel condition[7]	100			
1		50	18	
2		48	80	0.002
3		2	2	
Heart weight (g)	100	0.38 ± 0.00[a]	0.28 ± 0.00[b]	<0.001

[a, b] Means followed by different letters within a row are significantly different ($P \leq 0.05$).
[1] Tray was the experimental unit.
[2] Expressed as a percentage of the total number of eggs.
[3] Expressed as a percentage of fertile eggs.
[4] Expressed as a percentage of hatched chickens.
[5] Chick was the experimental unit.
[6] Yolk-free body mass.
[7] Percentage of chickens per treatment group that were scored with a navel condition of 1, 2, or 3, where 1 = good, 2 = moderate, and 3 = poor.

2.7 Dietary and management strategies to reduce the incidence of ascites

2.7.1 Introduction

The modern chicken is intensely selected for higher growth rates and indirectly selected for high rate of protein synthesis, which requires more oxygen (Julian, 1998; McGovern *et al*., 1999; Baghbanzadeh & Decuypere, 2008). This faster growth rate has resulted in mortalities related to cardiovascular diseases, including pulmonary hypertension syndrome, due to a mismatch between oxygen supply and demand (Julian, 1998; Solis de los Santos *et al*., 2005; Cangar *et al*., 2007). Despite the selection of faster growth rates in modern broilers, the pulmonary and cardiac capacity is still similar to that of the old broiler, which forces the cardiopulmonary system to function at its' physiological limits (Baghbanzadeh & Decuypere, 2008). The lungs in broilers are smaller relative to body weight, and unable to contract and relax, as compared to mammalian lungs and therefore cannot increase in volume during periods of hypoxia (Julian, 1998; Baghbanzadeh & Decuypere, 2008).

Ascites is a multi-factorial syndrome caused by interactions between genetics, physiological, environmental (high altitude), and management (ventilation, air quality, and disease status) factors (Singh *et al*., 2011). Research confirmed that ascites is not caused by an increased oxygen requirement of fast growth rate as such, but an impaired oxygen supply to sustain the faster growth rate (Baghbanzadeh & Decuypere, 2008). At high altitudes, where oxygen partial pressure is severely reduced like in Mexico, Peru, Colombia, and Bolivia, the oxygen demands can be easily mismatched in a fast growing broiler, which may result in a cascade of events that results in hypoxia and eventually ascites syndrome (Julian, 1998; Luger *et al*., 2001; Comacho-Fernandez *et al*., 2002; Baghbanzadeh & Decuypere, 2008). Ascites symptoms in broiler chickens include generalised oedema, fluid accumulation in the pericardium, epicardial fibrosis, lung oedema, enlarged and flaccid heart, increased right ventricle to total ventricles ratio (RV:TV ratio), and hypertrophy and dilation of the heart, especially of the right ventricle (Singh *et al*., 2011). During ascites, the fluid in the abdomen is not found in the air sacs, but the pressure this fluid exerts on the air sacs results in difficulty of breathing and eventual death (Julian, 1998).

Providing thermoneutral environments, limiting growth rate, and the use of feed additives can be employed to reduce the incidence of ascites in flocks (Singh *et al*., 2011).

2.7.2 Nutrition and ascites

To reduce the incidence of ascites syndrome, qualitative and quantitative nutritional strategies need to be practised, including the use of feed additives (Julian, 1998; McGovern *et al*., 1999; Balog *et al*., 2000b; Comacho-Fernandez *et al*., 2002; Camacho *et al*., 2004; Cangar *et al*., 2007; Baghbanzadeh & Decuypere, 2008; Ozkan *et al*., 2010). The gut is a highly metabolically active organ and requires a high supply of oxygen and

nutrients (Solis de los Santos *et al*., 2005). Under restricted feeding the activity of this organ is reduced and the oxygen demands are also lower, which may explain the concurrent lower demand on the heart and lungs for oxygen supply (Solis de los Santos *et al*., 2005).

By restricting feed intake, growth rate can be reduced, which allows the cardiopulmonary organs to keep up with the oxygen demands (Isaacs *et al*., 2010). However, feed restriction can reduce the availability of nutrients and pigmentation precursors from the feed and this affects BW, muscle mass, and final product quality (Baghbanzadeh & Decuypere, 2008). The timing, duration, and the age at which feed restriction is applied has an impact on whether the bird will be able to attain the desired final BW through compensatory growth (Balog *et al*., 2000b; Cangar *et al*., 2007).

2.7.3 Feed form

Diet form can be manipulated to reduce growth rate (Bolukbasi *et al*., 2005). Most broilers are fed crumbled or pelleted diets that support high growth rates related to ascites syndrome (Bendheim *et al*., 1992; Bolukbasi *et al*., 2005; Brickett *et al*., 2007; Baghbanzadeh & Decuypere, 2008). It was observed that the first outbreak of ascites in Bolivia occurred concurrently with the first introduction of pelleted feed 20 years ago (Lamas da Silva *et al*., 1988). This is because the pellets are more dense and digestible to a bird and normally their intake is higher compared to mash feeds (Bangbanzadeh & Decuypere, 2008). Brickett *et al. (*2007) proposed that feeding mash feed reduced feed intake in broilers and the degree of reduction was even more severe when the diet was of low energy density. Feeding mash feeds instead of pelleted feed in the first four weeks can indirectly alleviate ascites by reducing growth rate without degrading market parameters (Isaacs *et al*., 2010).

Table 2.4 showed that feeding pelleted feed in a cold environment resulted in increased ventricular dilatations, increased ratio of right ventricle to total ventricles (RV/TV) and increased haematocrit values (Bolukbasi *et al*., 2005). The haematocrit value of the birds consuming pelleted feeds was greater compared to mash-fed birds (Bolukbasi *et al*., 2005).

Table 2.4 The effect of house temperature and feed form on mortality rate due to ascites, haematocrit, ratio of RV/TV and weight of heart relative to body weight in broilers (Bolukbasi *et al*., 2005)

Treatments	Mortality %	Haematocrit %	RV/TV	Heart (g/100g BW)
Pellet control[1]	6.0[b]	35.0 ± 6.0[a]	0.26 ± 0.007[c]	0.567 ± 0.01[c]
Pellet cold[2]	13.3[a]	35.8 ± 2.6[a]	0.32 ± 0.008[a]	0.611 ± 0.008[a]
Mash control[3]	0.0[b]	31.8 ± 1.3[b]	0.26 ± 0.004[c]	0.552 ± 0.01[d]
Mash cold[4]	3.3[b]	33.5 ± 1.3[b]	0.30 ± 0005[b]	0.584 ± 0.004[b]
Significance	*	*	**	**

*P<0.05; **: p< 0.01

[a, b] Means within the same column with no common superscript differ significantly.

[1] Pellet: birds received feed in pellet form in control house (25°C, 24 °C and 23 °C at the 4th, 5th and 6th weeks of the experiment respectively).

[2] Birds received feed in pellet form in cold house (16 °C after 21 d.).

[3] Birds received feed in mash form in control house (25°C, 24 °C and 23 °C at the 4th, 5th and 6th weeks of the experiment respectively).

[4] Birds received feed in mash form in cold house (16 °C after 21 d.)

2.7.4 Nutrient density

Feeding diets with a reduced nutrient density has been one of the strategies used to control ascites by decreasing growth rate (Madrigal *et al*., 2002; Brickett *et al*., 2007; Baghbanzadeh & Decuypere, 2008). The concentration of feed nutrients can be lowered to control growth rate especially during 0-21 days of age (Baghbanzadeh & Decuypere, 2008). However, broilers tend to consume more of the low energy diet and eventually require more oxygen for digestion (Julian, 2000). Hence, the reduction of protein concentration in the feed is important as proteins have a higher oxygen requirement for metabolism that results in more oxygen being required to convert excess proteins to energy and excreted excess nitrogen (Julian, 2000). The selection of feed ingredients is critical as some ingredients increase metabolic rate and subsequently ascites syndrome while others contain some anti-nutrients that might reduce growth rate and ascites incidence (Julian, 2000).

2.7.5 Limited access feeding

The use of near continuous light schedules to maximise feed intake and growth rate have elevated the metabolic rate, which required an increased oxygen supply (Singh *et al*., 2011). Under continuous lighting, feed intake was increased and resulted in high growth rates and ascites mortalities (Brickett *et al*., 2007, Ozkan *et al*., 2010). Intermittent lighting programmes improved feed conversion ratio (FCR), reduced abdominal fat, reduced metabolic rate, and reduced ascites (Buys *et al*., 1998; Aftab & Khan, 2005, Bolukbasi *et al*., 2005).

"Step down-Step up" lighting programmes (Table 2.5) have been used to control feed intake in broilers (Qiao, 2007). During the early stages of broiler rearing, lighting should not be limited too severely as longer photoperiods are required to stimulate feed intake and maintain high growth rates (Qiao, 2007)

Table 2.5 "Step-down" lighting program for broilers (Qiao, 2007)

	Bird Age (days)	Hours of light
Example 1	0-4	23
	4-10	8
	10-14	10
	14-18	14
	18-23	18
	23-market	23
Example 2	0-4	23
	4-14	8
	14-market	23
Example 3	0-4	23
	4-14	Natural Day Length
	14-18	18
	18-market	23

2.7.6 Antioxidants

The elevated production of reactive oxygen as a result of increased metabolic rate in broilers prone to ascites may potentiate the development of the disease or aggravate the disease as it occurs (Singh *et al*., 2010). These major free forming reactive species includes superoxide radicals, hydroxyl radicals, hydrogen peroxide, peroxyl radicals, hypochlorous acid, and nitrogen dioxide radical (Currie, 1999). Ascites or oedema may result from fluid leakage secondary to increased vascular permeability following oxidative or chemical damage (Julian, 2005). Reactive oxygen species (ROS) produced by lipid oxidation may destroy cardiac and pulmonary muscles and cause pulmonary hypertension and subsequently ascites syndrome (Aftab & Khan, 2005; Rajani *et al*., 2011).

In broilers, the first line of defence against these reactive oxygen radicals is endogenous antioxidants such as tocopherols, glutathione, ascorbic acid (Singh *et al*., 2011), uric acid, cysteine, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase (Currie, 1999). The levels of glutathione and α-and γ-tocopherol are reduced in the mitochondria of ascetic birds, which indicate that they are greatly utilised against

reactive oxygen species (Singh *et al.*, 2011). It has been suggested that the presence of these compounds either in the circulation or at the level of the respiratory membrane may have reduced cellular damage, prevented the induction of hypoxia or reduced the incidence of ascites (Currie, 1999). Broilers that received a vitamin E implant that released a total of 15 mg α-tocopherol from 0 to 3 weeks of age exhibited significantly reduced ascites-induced mortality compared to the control group (Singh *et al.*, 2011).

2.7.7 Omega-3 fatty acids

Omega-3 fatty acids have an erythrocyte deforming ability during periods of hypoxia that promotes ease of blood flow and this characteristic has proven to reduce ascites in flocks fed flax and fish oil (Baghbanzadeh & Decuypere, 2008). The administration of flax oil (50 g per kg diet) to broilers under hypobaric conditions reduced right ventricular hypertrophy, haematocrit, and haemoglobin as erythrocyte deformability was increased and whole blood viscosity was reduced (Table 2.6; Aftab & Khan, 2005). Flax oil is rich in α-linolenic acid, which is a precursor of eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Aftab & Khan, 2005).

Table 2.6 The effect of flax oil and animal/vegetable oil (A/V) blend on the performance and ascites parameters of broilers at 28 days of age (Aftab & Khan, 2005)

Treatment	Body weight gain (g)	Right ventricle/Total ventricle	Haemoglobin (g/L)	Blood viscosity (Cps)	Filtration index (s)
A/V blend 50 g per kg feed	558	0.0331[a]	110[a]	3.1[a]	23.5[a]
Flax oil 50 g per kg feed	542	0.305[b]	94.3[b]	2.8[b]	16.3[b]

[a-b] Means followed by different superscripts within a column differ significantly (P<0.05)

Studies have shown that erythrocyte deformity can be increased by supplementing omega-3 fatty acids from flax oil and fish oils (Baghbanzadeh & Decuypere, 2008). High levels of unsaturated fatty acids increased the fluidity of erythrocyte membranes, reduced blood viscosity, and improved blood flow (Baghbanzadeh & Decuypere, 2008). Easier flow of erythrocytes through the capillaries resulted in improved oxygen transport and decreased ascites incidence (Singh *et al.*, 2011).

2.7.8 L-arginine and L-carnitine

Dietary arginine is a precursor of nitric oxide (NO), which is a potent vasodilator during hypoxic conditions to prevent pulmonary hypertension syndrome (Izadinia *et al*., 2010). Soybean meal has more arginine

than canola meal and feeding soybean based diets can result in a reduction of ascites syndrome in a flock (Aftab & Khan, 2005). L-carnitine is involved in free radical scavenging that may be beneficial to the reduction of ascites incidence (Baghbanzadeh & Decuypere, 2008). It was found that L-carnitine supplemented chickens were more resistant to the development of ascites due to an improved cardiac output (Baghbanzadeh & Decuypere, 2008).

2.7.9 Medicinal control

All factors that can reduce pulmonary resistance and increase vascular capacity of the lungs can reduce ascites (Currie, 1999). For example, Diaoxinxueng (Chinese medicine) improved blood circulation, reduced oxygen consumption by cardiac myofibrils, and improved hydraulic pressure of the blood in the heart (Aftab & Khan, 2005). An addition of 0.2% aspirin in the diet resulted in reduced final BW and reduced incidence of ascites syndrome (Aftab & Khan, 2005). However, it was unclear whether this was due to a direct effect of aspirin on BW or an indirect effect on ascites development (Aftab & Khan, 2005).

Prostaglandins are produced in the lungs, liver, digestive tract, kidneys, and reproductive tract of mammals where they are responsible for vasoconstriction, vasodilation, and blood clotting (Aftab & Khan, 2005). Aspirin was also found to inhibit the effects of prostaglandins on vasoconstriction and blood clotting that may cause ascites (Balog *et al.*, 2000b).

Dietary electrolytes have also been used as a preventative measure for ascites as the inclusion of 1000 mg potassium carbonate per litre of drinking water increased blood oxygenation (Isaac *et al.*, 2011). Other drugs like furosemide, a diuretic, can be used to reduce mortalities in ascites (Gupta, 2011). The addition of the diuretic furosemide at 0.001%, 0.005% and 0.01% significantly reduced the incidence of ascites without reducing the final broiler BW (Currie, 1999). Clenbuterol, a β_2- agonist, has been used experimentally to reduce mortality due to ascites. It acts on specific receptors within the bronchi causing dilation, and hence increases lung ventilation (Currie, 1999). Also, feed supplemented with Coenzyme Q_{10} (CoQ_{10}) at 40 mg per kg of diet reduced the right ventricle to total ventricle ratio (RV/TV), erythrocyte osmotic fragility, and pulmonary arterial diastolic pressure when compared to the negative control (Aftab & Khan, 2005).

2.8 Blood parameters

In hypoxic situations, it has been long-established that oxygen tension reduced capillary blood flow, and in combination with polycythaemia, ascites development was accelerated (Balog *et al*, 2000a). The decreased oxygen supply or increased oxygen demand triggered the increase in blood viscosity that caused pulmonary hypertension and ultimately ascites syndrome development (Balog *et al.*, 2000b). In ascetic broilers, there was an increased intracellular calcium level and reduced Ca^{2+}-ATPase activity in erythrocytes, which might have

been a reason for reduced erythrocytes deformability (Li *et al.*, 2011). Lower plasma T_3 and T_4 concentrations in ascetic broilers have been confirmed to affect the control of metabolism (Ozkan *et al.*, 2006). Plasma T_4 concentration in feed-restricted broilers was greater than those in the *ad libitum* birds at 30 days of age (Ozkan *et al.*, 2006).

2.9 Zinc and manganese requirement, availability and absorption in broilers

2.9.1 Introduction

Essential trace minerals are important to a wide variety of physiological processes in all animals (Yuan *et al*., 2011). Several enzymes require the presence of minerals for their activity (Yuan *et al*., 2011). The National Research Council (NRC, 1994) has suggested nutrient requirement levels for all livestock species, including poultry. However, the animals 40 years ago were much different in terms of their productive potential from animals existing today due to intense genetic selection for higher growth rates (Huang *et al*., 2007). Trace minerals and vitamin requirements may have been underestimated, for instance NRC recommendations for zinc (Zn) and manganese (Mn) have been estimated to be 40 mg/kg and 60 mg/kg of feed, respectively (NRC, 1994). Most of this research was carried out using purified or semi-purified diets with growth as the only requirement criterion (Huang *et al*., 2007). Furthermore, the diets used were free of phytate and fibre, which may not be applicable to conventional diets (Huang *et al*., 2007). It should not be ignored that trace mineral uptake from feed depends on the species, chemical form of a mineral, interactions with other elements, and assimilation characteristics in the digestive tract (Nunez-Noguera *et al*., 2007). Chelates of Zn and Mn are known to have higher bioavailability than inorganic salts because of their stability and low molecular weight (Apines *et al*., 2003). Organic trace minerals have been shown to enhance mineral uptake, improve BW gain, and reduce mineral excretion as compared to their inorganic counterparts (Yuan *et al*., 2011).

Modern broiler is fast growing and the requirements for Zn and Mn are so high that the body have not adjusted to the new requirements (Lorenzoni *et al*., 2006). The homeostatic control of Zn and Mn metabolism is dependent on availability or absorbable forms of these minerals (Huang *et al*., 2007). Due to high requirements of these minerals and genetically induced deficiency in modern broilers, even when supplied in excess will still be extensively absorbed (Underwood & Suttle, 1999).

The requirement for nutrients is defined as the minimum dietary concentration required in achieving optimum performance (Huang *et al*., 2007). Zn is an essential mineral for poultry but also a toxic metal to the environment when it is supplied in excess (Huang *et al*., 2007). The natural zinc concentration in feed is inadequate for the daily Zn requirement of broilers because of the high variation in dietary Zn and Zn antagonists in feed ingredients (calcium, phytate, and fibre) (Schlegel *et al*., 2009). Antioxidant effects of Zn have been

demonstrated where it is involved in the increased synthesis of metallothionien, a cysteine-rich protein that acts as a free radical scavenger (Karamouz *et al*., 2010). Also lactate dehydrogenase was studied where increased activity in the serum of bull calves was noted when Zn was added to the diet (Karamouz *et al*., 2010).

Supplementation of Mn is also important in broilers. This is the 12th most common element and the 4th most abundant metal in the earth's crust and waters (Baden and Eriksson, 2006). Mn is an essential trace element in animals, with particular importance in fast growing poultry (Lu *et al*., 2006). Deficiency of Mn in chick feeds results in leg disorders such as slipped tendon or perosis, chondrodystrophy, and bone shortening and bowing (Lyons and Insko, 1937). Mn is a component of superoxide dismutase (MnSOD), which functions as an antioxidant in animal bodies (Lu *et al*., 2006).

Zn and Mn are involved in the scavenging of reactive oxygen species (ROS) and can reduce oxidative stress and lipid peroxidation related to ascites syndrome (Rajani *et al*., 2011). Birds' requirement for Mn is higher than other species as they have high body temperature and oxygen requirements (Watson, 1937). Research showed that tissues with the highest uptake (liver and kidney) of intraperitoneal injected radioactive Mn were also rich in mitochondria (Watson, 1937). About one-half of the Mn activity found in these tissues was found to be located in the mitochondria demonstrating its role as a respiratory cofactor (Watson, 1937).

2.9.2 Role of zinc

Zinc is an important trace mineral in poultry nutrition for growth, bone development, appetite, feathering, and enzyme structure (Collins and Moran, 1999; Virden *et al*., 2004). Complexed Zn also appears to play an important role in wound healing as demonstrated by improvements shown in skin quality and pododermatitis (Saenmahayak, 2007). The other more significant functions are in cellular respiration, cellular utilisation of oxygen, DNA replication and transcription, reproduction, maintenance of cell membrane integrity, and sequestration of free radicals (Collins & Moran, 1999).

Zn deficiency has been shown to decrease cellular immunity, thymus and spleen development, and interleukin production (Virden *et al*., 2004). Research with other animal species have shown that Zn can act as a protective agent in heart muscles with deficient blood supply by producing reactive oxygen intermediates (Virden *et al*., 2004).

Zn contributes to carbonic anhydrase function, a metalloenzyme that catalyses the reversible hydration of CO_2 and dehydration of bicarbonate during cellular respiration (Lukaski, 2005). This facilitates the excretion of CO_2 and prevents the accumulation of CO_2 inside cells (Lukaski, 2005). Rahman (1961) reported gasping

respiration signs in broilers with significantly reduced red blood cell total carbonic anhydrase activity when Zn was deficient in the diet.

2.9.3 Role of manganese

The Mn requirement is quite variable, being affected by factors such as the species, breed of the animal being considered, the age of the animal, the chemical form of Mn fed, and the level of other elements in the diet that may have an antagonistic (interacting) nature to Mn utilisation (Watson, 1970). Mn is important in poultry during growth and skeleton development and is involved in the synthesis of cartilage mucopolysaccharides (Saenmahayak, 2007). Mn is also essential for normal bone formation, enzyme function, and amino acid metabolism in poultry (Ji *et al*., 2006a). Watson (1970) showed that since birds required more Mn than other species and had a higher body temperature with greater oxygen consumption, Mn was perhaps involved in oxidation-reduction processes as a respiratory cofactor.

However, dietary Mn deficiency in animals results in a wide variety of structural and physiological defects, including growth retardation, skeleton and cartilage malformations, impaired reproductive function, congenital ataxia due to abnormal inner ear development, optic nerve abnormalities, impaired insulin metabolism, abnormal glucose tolerance, alterations in lipoprotein metabolism, and an impaired oxidant defence system (Luo *et al*., 2007). Mn supplementation at low levels increased haemoglobin content in the blood, which implied an improvement in oxygen transportation necessary to prevent ascites syndrome (Martinez & Diaz, 1996).

2.9.4 Availability and absorption of zinc

It is well known that Zn is involved in a myriad of critical reactions in broilers (Collins and Moran, 1999). Bioavailability, in reference to trace minerals, is defined as the proportion of the ingested element that is absorbed, transported to the site of action, and converted to a physiologically active form, therefore bioavailability not only involves absorption but also the utilisation for a specific function (Owens *et al*., 2009). The ileum is the main site of Zn absorption and the mechanism involved is a non-saturable diffusion (Yu *et al*., 2008). Zn can be obtained from both organic and inorganic sources with organic form being more bioavailable (Owens *et al*., 2009). However, organic trace mineral supplements vary with regards to the type of ligand or ligands used to form the metal complex or chelate (Owens *et al*., 2009). Theoretically, the supplemental concentration of Zn should be reduced when an organic mineral source is used as a supplement in the diet (Ao *et al*., 2011). Superior Zn utilisation depends on the elaborated homeostatic regulation in absorption, storage and secretion of Zn within the whole body (Yu *et al*., 2008).

There are two feed grade sources of inorganic Zn widely used in the poultry industry, which are zinc oxide (72% Zn) and zinc sulphate (36% Zn), and they accumulate principally in the skin (10%), bones (30%), and muscles (55%) (Dolegowska *et al*., 2003), and are subsequently released for use during a period of Zn deficiency (Yu *et al*., 2008). Zinc sulphates are highly water soluble reactive metal ions that promote free radical formation (Saenmahayak, 2007). Sources of Zn from amino acid complexes were reported to be more bioavailable in poultry species than Zn from inorganic sources (Hudson *et al*., 2005).

Dietary factors reducing Zn bioavailability in monogastrics have been mainly identified as phytate, non-starch polysaccharides, and excessive dietary contents of Fe and Cu (Mohanna & Nys, 1999). Other minerals fed with Zn will affect its absorption due to competition at the absorption site (Ao *et al*., 2011). Physico-chemical conditions and transit time in the digestive tract of broilers may also contribute to different Zn solubility in the digestive tract, and in turn, to different Zn bioavailability in broilers (Schlegel *et al*., 2009). An excessive supply of dietary Cu reduces Zn bioavailability due to their competition at the same absorption sites (Schlegel *et al*., 2009). The intestinal pH plays a major role in the absorption of Zn with absorption being lower in weak acidic to neutral pH environments (Mohanna & Nys, 1999). The process whereby Zn is transferred from the lumen in the 3 intestinal segments to the surrounding vasculature may involve a transcellular pathway via brush border transport, intracellular diffusion and basolateral transport (Yu *et al*., 2008). The process may differ between the duodenum, jejunum and ileum, with absorption in both the duodenum and jejunum dependent on a saturated, carrier-mediated active pathway (Yu *et al*., 2008). Zn absorption was regulated by a non-saturated, diffusive process in the ileum and appeared to be a preferred site of Zn transport in the small intestine (Yu *et al*., 2008).

2.9.5 Availability and absorption of manganese

Mn is an essential micronutrient in animal nutrition that is involved in metabolism of carbohydrates, amino acids, and cholesterol as a constituent and an activator of other enzymes involved in respiration (Luo *et al*., 2007). Mn is a component of superoxide dismutase (MnSOD), which functions as an antioxidant against free radicals in animal species (Virden *et al*., 2004). Oxygen derived free radicals are involved in the lipid peroxidation and tissue damage that is associated with ascites syndrome in broilers (Rajani *et al*., 2011).

It is generally accepted that maize-soybean meal based diets need to be supplemented with Mn because of relatively low availability of Mn (Attia *et al*., 2010). Organic amino acid sources of Mn have a higher bioavailability compared to inorganic sources due to less interaction with other minerals (Attia *et al*., 2010). Mn requirement of poultry is higher than in mammalian species because of a relatively inefficient intestinal absorption of this mineral (Collins and Moran, 1999).

Excess calcium (Ca) and phosphorus (P) adversely affects Mn utilisation in chicks and results in poor absorption in the gut due to chemical interactions (Ji *et al*., 2006b). In commercial poultry, the dietary inclusion levels of these inorganic minerals are relatively high mainly because of their inexpensive sources with low bioavailability (Bao *et al*., 2007). Therefore, the excretion levels are high which is not only wasteful but also harmful to the environment (Bao *et al*., 2007). Replacing the inorganic trace minerals with organically complexed trace minerals may decrease environment pollution because of their greater bioavailability (Bao *et al*., 2007). Studies consistently showed that the absorption of Mn was greater in the ileum than in the duodenum or jejunum of broilers (Ji *et al*., 2006a). The absorption of "complexed" or "chelated" organic Mn was much greater than inorganic Mn in the small intestinal segments of broilers, which might have been due to different absorption modes for organic Mn (Ji *et al*., 2006a).

2.10 Function of zinc and manganese related to ascites aetiology

Recent studies have shown that reactive oxygen species can damage heart cells (cardio-myocytes) and lung lining, which may ultimately cause or aggravate ascites syndrome as it occurs (Bottje *et al*., 1998; Xi *et al*., 2012). Zn was shown to be an anti-apoptotic agent in heart muscle (Powell, 2000). It was shown that Zn/Mn has antioxidant activity that reduces the cell membrane damage due to free radicals (Karamouz *et al*., 2011). Mn also operates as a cofactor for superoxide dismutase (SOD) enzymes that provide oxidative stress resistance through formation of non-proteinaceous Mn-based antioxidants (Aguiree & Culotta, 2012). Zn increased the synthesis of metallothionine and had a significant role in the reduction of malondialdehyde (MDA) levels as an indicator of lipid peroxidation in tissues and serum (Karamouz *et al*., 2011). The inclusion of organically complexed Zn and Mn in the diet significantly reduced the incidence of ascites syndrome (Table 2.7; Arce-Menocal *et al*., 2004)

Table 2.7 Live productive parameters obtained at 56 days of age when AAMC[1] was added to a basal broiler diet (Arce-Menocal *et al*., 2004)

Treatments	Body weight (g)	Feed intake (g)	Feed conversion ratio (g/g)	Mortality (%)	
				General	Ascites
Control	**2735 ± 75**	**5763 ± 106**	**2.13 ± 0.06**	**17.3 ± 30[a]**	**11.4 ± 30[b]**
MZn[2]	**2818 ± 70**	**5676 ± 125**	**2.03 ± 0.05**	**15.1 ± 30[ab]**	**11.8 ± 30[a]**
MMn[3] + MZn[2]	**2809 ± 79**	**5664 ± 138**	**2.04 ± 0.06**	**12.2 ± 30[b]**	**8.9 ± 20[b]**

[*] Mean ± SD

[1] AAMC= specific amino acid metal compound

[2] MZn= methionine-zinc

[3] MMn= methionine-manganese

[ab] Means with different superscripts within treatments in columns differ significantly (P<0.05)

[cd] Means with different superscripts within sexes in columns differ significantly (P<0.01)

CHAPTER 3
MATERIALS AND METHODS

3.1 Incubation trial

3.1.1 Incubation of eggs and development of ascites model

Ethical approval for the trial was obtained from the Animal Ethics Committee (project number: ec081-13), University of Pretoria, South Africa. Five thousand eight hundred and eight (5808) broiler hatching eggs from parent stock (Ross 308) of 41 weeks of age were obtained from National Chicks, Astral Operations (Gauteng, South Africa) and were placed in 40 trays immediately after arrival at the hatchery, Experimental Farm, Hatfield, University of Pretoria, Pretoria (RSA). Eggs selected for incubation were placed as such so that all trays were kept within a 5 g difference. The 40 trays were randomly assigned and set into four identical incubators, assuring that each machine had the same weight of eggs.

Four identical single-stage incubators were used for the incubation study (Chick Master, Model 1056, Medina, OH 44258, USA). Three days before eggs were placed in the incubator; all the machines were switched on and allowed to reach an air temperature of 37.5 to 38.0 $^{\circ}$C. Egg rotation was set to occur hourly through an angle of 90°. The machines were monitored twice daily and readings for dry bulb temperature and relative humidity recorded. Each machine was loaded with 10 trays of 132 eggs each. Each tray was divided into 3 sections (front, middle and back). One egg per section, i.e. three eggs per tray was marked as reference eggs.

Before ESTs were recorded, a heated tent was constructed over all four incubators in order to measure the eggshell temperatures of the reference eggs without risking a decrease in incubator temperature during measurements. Three electric heaters were switched on inside the plastic tent in order to stabilise the temperature around the incubators to prevent heat escaping from the machines to the outside environment. The eggshell temperature (EST) of all reference eggs was measured daily from embryonic day (E) 11-18 with a Braun digital Thermoscan infrared thermometer (Braun GmbH, Kronberg, Germany). Before ESTs were measured, the Braun thermometer was placed on the floor of the incubator for 15 minutes before use to equilibrate. ESTs were taken at the equator of each egg. The four machines were divided into 2 groups (treatments), a normal incubation temperature (machine 1 and 4) and a high incubation temperature (machine 2 and 3). Between E 11 and E 18, machine temperatures were adjusted in order to achieve ESTs of 37.5 °C (normal group) and 39.0 °C (hot group). The temperature settings of the machines for the hot incubation treatment were gradually increased from E 10 to reach 39.0 °C at E 15. This temperature was then maintained

until the end of the incubation period at E 21.5. The ESTs in the normal treatment were maintained between 37.5 and 38.0 °C from E 15-21.5 (Table 3.1).

Table 3.1 Average eggshell temperature (EST) during the incubation period

Embryonic day (E)	Normal	Hot
E 0-10	37.5	37.5
E 10-15	37.5	38.8-39ºC
E 15- until hatch	37.5-38.0 ºC	39ºC

The egg weight of each tray was determined at setting and again at E 18 of incubation. These weights were used to calculate egg weight loss up to E18. All eggs were candled at E 18 and those with evidence of living embryos were transferred to hatching baskets. All infertile and eggs containing dead embryos were removed and recorded as such during candling. The hatching basket was separated into four quarters using mesh material and the eggs from each quarter of each tray were transferred to that respective quarter in the basket. This was to prevent that the hatched chicks from different quarters do not mix before placement. Each basket was placed in the same position and machine as the tray from which the eggs were transferred. As shown in Table 3.1, the temperature treatments were continued until day of hatch (E 21.5). All the eggs removed during candling were broken out to determine infertile eggs or stage of embryonic death. True fertility was calculated as a percentage of eggs set and also for early, middle and late embryonic mortality. Early mortality (E 0-7 of incubation) was characterised by the development of the primitive streak with the end of this stage marked by the appearance of the egg tooth on the beak. Middle mortality (E8-14 of incubation) was defined primarily by the number of somites, embryos having an egg tooth and eyes, and no obvious feather development. Late mortality (E 15-21 of incubation) was defined by morphological changes in feathers, beak, fully formed organs and body size.

3.1.2 Hatching

At hatch, all the hatching baskets were removed from the machines. Baskets with the least number of hatched chicks at E 21.5 were removed last to provide extra hatching time. The hatchlings were sorted as first-grade, second-grade, or culls. A chick was classified as first-grade when it was clean, dry, and without deformities or lesions. Small chicks and those with red hocks, pale colour or wet and dirty chicks were classified as second grade chicks. Limping, malformed chicks or those with open navels were classified as culls. Only first grade chicks were selected for use in this trial. The percentage of first-grade chicks, second-grade chicks, and culls were expressed as a percentage of fertile eggs.

Chicks from each quarter and basket were counted, weighed, and sexed using the feather-sexing method. Three chicks per basket in each machine were necropsied to determine BW and the weight of the yolk sac, heart, liver, proventriculus, and gizzard. The chicks were selected from different quarters, levels and weighed in groups of 19, with a BW difference of not more than 8 g between groups. This was to ensure that each group consists of chicks from all quarters and position within the machine. After weighing, the 19 chicks were identified using neck tags and placed in floor pens with wood litter shavings according to treatments.

3.2 Broiler performance

3.2.1 Housing

An environmentally controlled house was used for the rearing of chicks. The house was separated into two sides with 48 pens per side (house 1 and house 2) and 96 pens in total. Each pen was equipped with 1 tube feeder and 6 nipple drinkers to provide feed and water for *ad libitum* consumption. During the first 7 days, chick fountain drinkers were supplied for supplemental water and a paper flat was put on the floor for supplemental feeding. The facility was preheated for 3 days to obtain a litter temperature of 33 ºC before chick placement (Table 3.2). The chicks were reared to 6 weeks of age (42 days). During the brooding period (0-7 d of age) the temperature was set to be 1-3 ºC below recommended temperature (Ross, 2009) to promote the development of ascites.

Table 3.2 Recommended litter temperatures for Ross 308 broilers and temperatures used in this trial

Age (days)	Recommended litter temperature (°C) (Ross, 2009)	Litter temperature used in this trial (°C)
0	30	33
7	27	25
14	24	24
21	22	22
28	20	19
35	20	20
42	20	20

Litter temperatures were recorded daily during the brooding period with a Traceable infrared thermometer gun (Fisher Scientific, Control Company, Friendswood, TX 77546) by taking readings at two dry spots in each pen in the morning and late afternoon. These temperatures were used to adjust the air temperature each day. Air temperatures were monitored with two high-low mercury recording thermometers placed at bird level in each

room. Figure 2 in the appendix shows the actual daily litter temperature for the two houses achieved from day 0 to 42 day of the trial.

3.2.2 Hygiene and biosecurity

The broiler house was cleaned, washed, and disinfected with Vet GL 20 (Immuno-vet services, Kya Sand, Randburg, South Africa) before placing the chicks. The wood litter shavings were evenly spread on the concrete floor in each of the pens. Foot baths (Vet Fluid-O, Immuno-vet services) were available at the entrance to the broiler house. The broiler houses had their own feed bins to ensure optimum biosecurity. All the farm visits, truck deliveries and pests were monitored to promote maximum biosecurity. In case of water leakages, wet wood litter shavings were replaced to keep the house ammonia levels and humidity at acceptable levels. Mortalities were collected, weighed, and recorded accordingly on a daily basis. Dead and culled birds were removed from the broiler house for post-mortem examination and stored in the freezing store room until incinerated.

3.2.3 General health management and vaccinations

On the day of hatch the chicks were removed from the incubators and placed in the disinfected broiler house. A scheduled vaccination programme was followed. The chicks were vaccinated against infectious bursal disease (Gumboro) at 14 days of age and at 18 days of age they were vaccinated for both Gumboro and Newcastle disease. At 28 days of age, birds were again vaccinated against Newcastle disease. All vaccinations were applied by spraying a fine mist spray onto the broilers.

3.2.4 Feasibility, risk assessment and fall-back scenario

The broiler research was conducted with high caution, accountability and manipulatively. Birds were monitored 3 times daily. Research assistants were available on a daily basis for the supervision of the research trial. The permission for animal use was granted by the University of Pretoria's Animal Ethics Committee (project number: ec081-13) prior to the start of the trial. Whenever an individual bird was suffering from a disability or illness, it was culled by cervical dislocation under the supervision of a qualified broiler technician or veterinarian.

3.2.5 Lighting programme

The lighting programme was 24 hours of light from day 0-1 to promote feeding and drinking of the chicks. After the first day, the lights were gradually reduced until 18 hours of light and 6 hours of darkness was reached when the birds were 7 days of age. This lighting schedule was maintained from 7 until 42 days of age (Table 3.3).

Table 3.3 Lighting programme used in this trial

Age (Days)	Daylength (hours)
0-1	24 hours light
2-7	23 light, 1 darkness
7- 42	18 light, 6 darkness

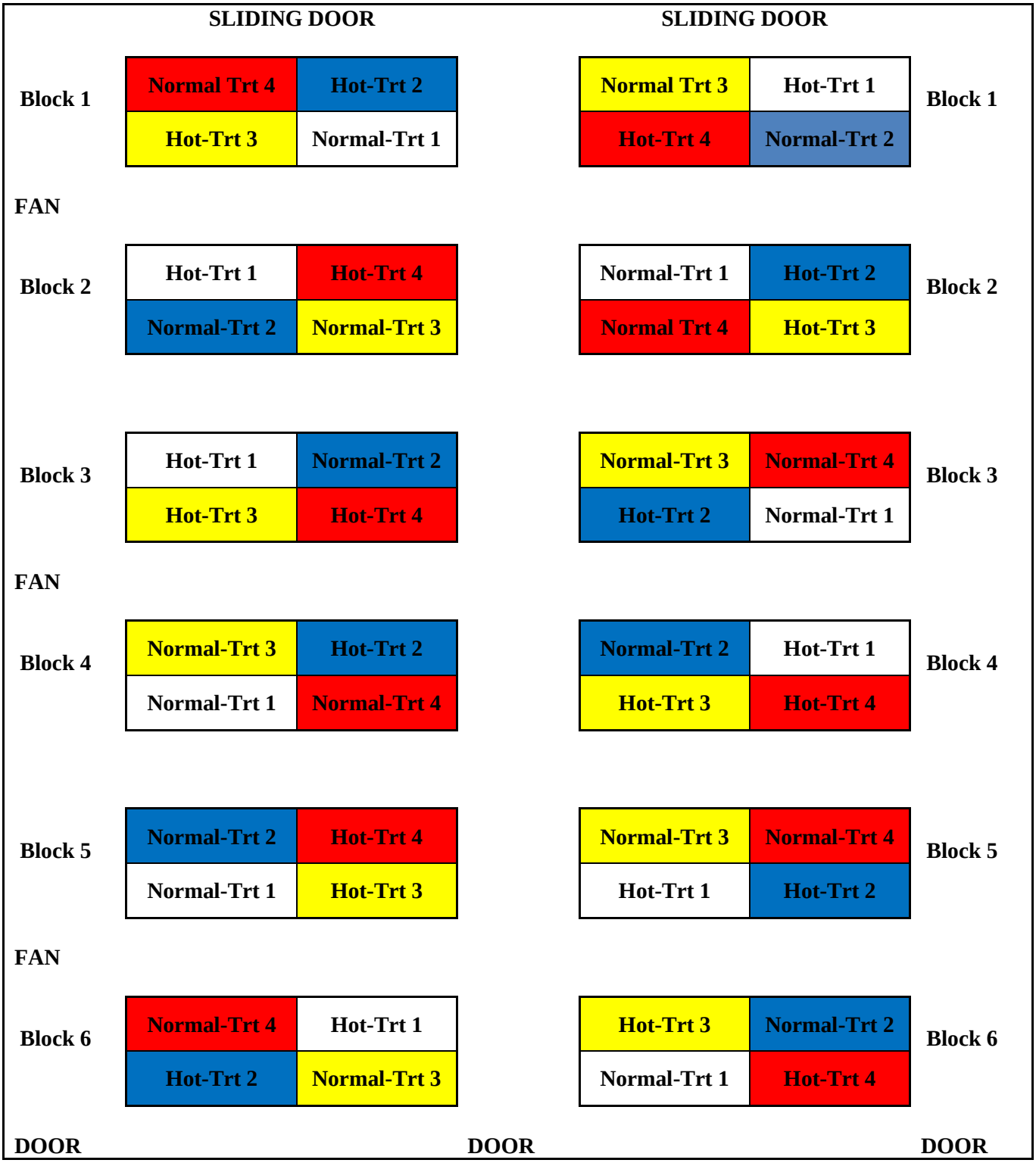


Figure 3.1(a) Layout of house 1 of the experimental broiler facility showing the randomisation of treatments (incubation x feed)

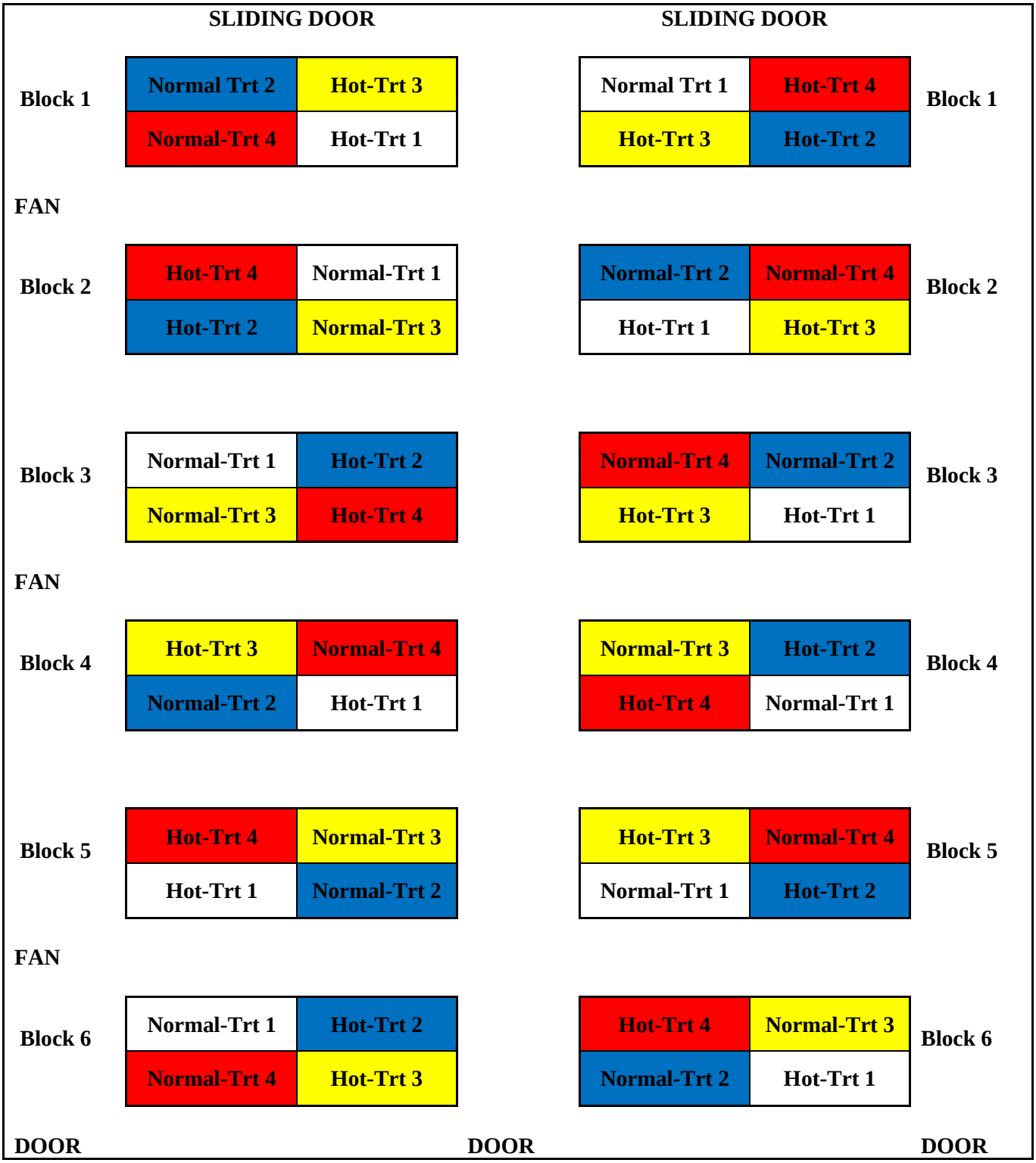


Figure 3.2(b) Layout of house 2 of the experimental broiler facility showing the randomisation of treatments (incubation x feed)

3.2.6 Dietary treatments

Birds were fed a crumbled starter diet from 0-14 d of age, a pelleted grower diet from 15-28 d of age, and a pelleted finisher diet from 29-42 days of age. The dietary formulation of raw materials was computed using Format formulation software (Format International, Surrey, UK) as shown in Table 3.4. The diets for the trial were mixed by Pennville (Pty) Ltd (Pretoria, South Africa). Representative samples from each bag of starter, grower, and finisher feeds were collected and analysed for gross energy, crude protein, crude fat, ash, crude fibre and also the levels of total Zn and Mn.

Table 3.4 Percentage (%) inclusion levels for raw materials in a basal diet (starter, grower and finisher)

Ingredient	Starter (0-14 days)	Grower (15-28 days)	Finisher (29-42 days)
	%		
Yellow maize (fine)	58.60	62.00	66.40
Soya oilcake meal	25.80	18.40	13.50
Full fat soya	5.60	10.00	11.90
Maize gluten 60 % CP	3.00	3.00	2.00
Sunflower oilcake meal	3.00	3.00	3.00
Limestone	1.60	1.34	1.20
Monocalcium phosphate	0.95	0.71	0.46
Salt (fine)	0.43	0.43	0.43
Lysine-HCl	0.31	0.29	0.28
DL-Methionine	0.23	0.28	0.22
L-Threonine	0.06	0.05	0.05
Salinomycin[1]	0.05	0.05	0.05
Phyzyme XP 10 000 TPT[2]	0.01	0.01	0.01
Premix[3]	0.30	0.25	0.25
Calculated nutrient analysis (as is basis)			
DM,%	89.90	89.90	89.40
CP, %	22.20	20.50	19.00
GE, MJ/kg	11.50	12.00	12.30
Ca, %	0.94	0.83	0.72
Total P, %	0.79	0.69	0.63
Avl. P, %	0.45	0.38	0.33
Na, %	0.20	0.19	0.18
Cl, %	0.30	0.30	0.30

[1]Salinomycin 12% included at 500 mg/kg
[2]Phyzyme TPT at 1000 FTU
[3]The basal vitamin-mineral premix included as indicated in Table 3.5. The Zn and Mn were included as indicated in Table 3.5.

The starter, grower, and finisher diets of the different treatments were manufactured from the same raw materials and the 4 dietary treatments were only differentiated by the source and levels of Zn and Mn included in the premix.

Table 3.5 Inclusion levels and source of supplemental zinc and manganese of the four dietary treatments in the broiler performance trial

	Zinc (mg/kg)		Manganese (mg/kg)	
Treatment	**Inorganic[1]**	**Organic[2]**	**Inorganic[1]**	**Organic[2]**
1	80	0	80	0
2	120	0	120	0
3	80	40	80	40
4	40	80	40	80

[1] Zinc and manganese supplemented to the basal diet using the inorganic forms, zinc sulphate and manganese sulphate, respectively.

[2] Zinc and manganese supplemented to the basal diet using the organic mineral products, Availa Zn and Availa Mn (Zinpro Corporation, Minnesota, USA), respectively.

There were two sources (Organic and Inorganic) of Zn and Mn that were used in the four dietary treatment groups. The commercial inorganic $ZnSO_4$ and $MnSO_4$ and the organic (Availa®) Zn and Mn supplied by Zinpro (Zinpro Corporation, Minnesota, USA). The inorganic $ZnSO_4$ and $MnSO_4$ had 36 and 32% available Zn and Mn, respectively. The Availa-Z/M from Zinpro is built on a unique, patented molecule that consists of one metal ion bound to one amino acid ion called a metal amino acid complex. This unique metal amino acid molecule is readily available (Zinpro Corporation, Minnesota, USA).

As indicated in Table 3.5, Treatment 1 contained 80 mg/kg inorganic Zn/Mn (sulphates), which represented the lower end inclusion rate of inorganic zinc in poultry diets as an industry standard. Treatment 2 contained 120 mg/kg inorganic Zn/Mn which represented an upper end inclusion rate of inorganic zinc in commercial poultry diets and to observe the level response between Treatment 1 and 2. Treatment 3 contained 80 mg/kg inorganic Zn/Mn and 40 mg/kg organic Zn/Mn, this is the commercial optimum replacement rate of inorganic Zn with organic Zn based on literature to observe performance benefits from organic Zn and Mn. Treatment 4 contained 40 mg/kg Inorganic Zn/Mn and 80 mg/kg organic Zn/Mn, this was to observe any further performance benefits from increasing organic Zn/Mn relative to inorganic Zn/Mn in the diet.

3.2.7 Chemical analyses of feed

The feed samples from all four treatments and three phases (starter, grower, and finisher) were analysed for dry matter, ash, crude protein, crude fibre, crude fat, gross energy, zinc, and manganese content. The nutrient content for different treatment phases were analysed according to specific methods described by the Association of Official Analytical Chemists (AOAC, 2000) (Table 3.6).

Table 3.6 Analysed nutrient content of feed for the starter, grower and finisher diets (as is basis)

Treatments	Ash (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Gross energy (MJ/kg)	Zinc (mg/kg)	Manganese (mg/kg)
			Starter diet				
1	5.6	21.2	3.5	3.5	17.8	108.0	111.6
2	5.5	22.6	4.6	3.7	17.7	134.1	134.6
3	6.0	22.6	3.9	3.9	17.7	141.9	165.9
4	6.2	22.4	4.3	4.2	18.1	183.0	173.8
			Grower diet				
1	5.1	20.5	4.4	4.2	18.0	122.8	121.3
2	5.0	18.2	4.6	4.1	17.9	147.5	136.3
3	5.3	21.3	4.6	4.5	17.9	150.8	157.3
4	4.9	20.4	4.4	4.4	18.0	179.6	169.1
			Finisher diet				
1	4.7	17.7	5.0	4.8	17.9	113.4	108.7
2	4.5	18.1	4.8	4.7	18.0	163.5	153.4
3	4.4	19.1	5.0	4.8	18.1	169.2	154.5
4	4.6	19.0	4.9	4.7	18.1	168.3	164.5

Treatment 1= Inorganic Zn/Mn= 80 mg/kg
Treatment 2= Inorganic Zn/Mn= 120 mg/kg
Treatment 3=Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
Treatment 3=Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

i. Dry matter and ash determination

The determination of dry matter and ash in the feed samples (starter, grower, and finisher) was performed at UP Nutrilab (Department of Wildlife and Animal Sciences, University of Pretoria, Pretoria, South Africa) according to AOAC (2000; method 934.01).Initially, 2 ± 0.05 g of each sample was weighed into a ceramic crucible and placed inside the oven for approximately 24 hours. After 24 hours, the samples were removed from the oven and allowed to cool for 30 minutes in a desiccator. The dry matter of the sample was determined by using the difference between initial sample + crucible weight and sample + crucible weight after 24 hours in the oven.

$$\text{Dry matter (\%)} = \frac{\text{Dry sample + Crucible weight}}{\text{Wet sample+Crucible weight}} \times 100$$

The ash percentage of the samples was determined by incineration of the dried sample in a muffle furnace at 550 °C for 4 hours (AOAC, 2000; method 942.05). After 4 hours the muffle furnace was switched off. The samples were kept in the oven for 2 hours to allow them to cool down before they were transferred to the desiccator. The ash percentage was determined using the formula below.

$$\text{Ash (\%)} = \frac{\text{Ash+Crucible weight}}{\text{Dry sample+Crucible weight}} \times 100$$

ii. Gross energy

The gross energy was determined using a bomb calorimeter (MC-1000 Modulator Calorimeter) according to the method described by the operators' manual. Approximately 0.5 g of each sample was weighed and placed in a clean, dry crucible attached with a cotton thread. The labelled sample together with the crucible was placed inside the bomb calorimeter for 5 minutes and allowed to ignite. The resultant gross energy (GE) of the sample was recorded.

iii. Crude protein

All the dried feed samples were analysed for crude protein using a nitrogen analyser (Leco nitrogen analyser, model FP-428, Leco Corporations, MI, USA). The resultant nitrogen percentage was converted to crude protein by multiplying by a factor of 6.25 (AOAC, 2000; method 968.06).

Initially, 10 g sodium sulphate and 0.4 g of elemental sulphur were added to 0.5 g of feed sample. This mixture was added to 25 mL concentrated (98%) sulphuric acid in an Erlenmeyer flask. After mixing, the solution was placed inside a heated oven and allowed to boil for approximately 45 minutes until the solution

became clear. Afterwards, the solution was removed from the oven and placed outside to cool. Then, 35 mL boric acid solution (40 g boric acid in 10 mL methyl red and 25 mL methyl blue were added to a volume of 1000 mL with distilled water) was added. 350 mL of distilled water, 100 mL of NaOH (45%) and zinc granules were also added. This solution was again heated in an oven and allowed to boil for a further 10 minutes until 200 mL of distillate remained. The remaining distillate was titrated with 0.1 N of H_2SO_4 and the values were corrected by the titration of a blank sample. The percentage nitrogen in the sample was calculated as follows:

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

Where F is a factor associated with H_2SO_4 strength

The total percentage crude protein in the diet was then calculated by multiplying the percentage N by 6.25.

iv. **Crude fibre**

Crude fibre percentage of the feed samples was analysed using a Tecator Fibertec system (AOAC, 2000; method 962.09). Prior to the analysis, the samples were air dried and milled to pass through a sieve with circular 1 mm diameter openings and were also defatted with petroleum ether. The sintered glass crucibles were cleaned with a brush to remove any adhering debris and were rinsed thoroughly with Ca 5% vol/vol HCl solution.

$$\% \text{ Crude fibre} = \frac{(\text{Residue in crucible after drying}) - (\text{Residue in crucible after ashing})}{\text{Sample weight}} \text{ x } 100$$

v. **Crude fat**

The Tecator Soxtec System 1034 (FOSS, Högänas, Sweden) extraction unit with petroleum ether (40-60 ° C) was used for the fat extraction (AOAC, 2000; method 920.39). Approximately 2.00 g of a milled and air dried sample was weighed into a thimble for crude fat analysis. The thimble containing the test portion was immersed into the boiling ether solvent for 20 min. the intermixing of matrix with hot solvent that ensured rapid solubilisation of extracts. The thimble was raised above the solvent and the test portion was further extracted by a continuous flow of condensed solvent. After 20 min, the solvent was evaporated and recovered by condensation. The resulting crude fat residue was determined gravimetrically after drying.

$$\% \text{ Crude fat} = \frac{(\text{weight of cup+ fat residue}) - (\text{weight of empty cup})}{\text{Sample weight}} \text{ x } 100$$

vi. Zinc and manganese concentration in the feed

Preparation: Acid digestion for mineral analysis

Approximately 0.5 g of air dried, milled feed sample were place in a digestion tube followed by the addition of 25 mL nitric acid [HNO_3 (65%)]. The tube was heated to 240 °C for 15 minutes, followed by the addition of 10 mL perchloric acid ($HClO_4$) after approximately 10 minutes when half of the HNO_3 had boiled off. The solution was cooled and diluted with deionised water to a volume 50 mL. After cooling, the solution was transferred to a 50 mL volumetric flask and again filled to a volume of 50 mL with deionised water.

Zinc analysis

The feed samples analysis for zinc percentage was analysed using an atomic absorption spectrophotometer (Model 905AA, GBC Scientific Equipment, Braeside, Austria) (AOAC, 2000; method 999.11).

Manganese analysis

The feed samples were analysed for manganese using a Varian Spectra atomic absorption spectrophotometer (Model AA50, Spectralab Scientific Inc., Palo Alto, USA) (AOAC, 2000; method 999.11).

3.2.8 Chick performance and physiological parameters

i. Organ weights at hatch

Immediately after the chicks were removed from the incubators, 3 chicks per tray from all four incubators were randomly selected and culled. The heart, yolk sac, liver, gizzard, and proventriculus of all the chicks were weighed to determine the effect of incubation treatment on embryonic development.

ii. Growth measurements

The weighing scales were calibrated before the commencement of the study to ensure accuracy. The birds were closely monitored to ensure *ad libitum* feeding and water intake at all times. Weekly measurements in each pen (7, 14, 21, 28, 35 and 42 d) for body weights (BW), feed intake (FI) , mortalities, and feed conversion ratio (FCR) were recorded. Birds per pen were counted and weighed in crates. The feed in the bin was also weighed to determine the amount of feed that was consumed during a weekly period. The feed and BW measurements were used to calculate the weekly FCR adjusted for mortalities in each pen. The FCR (weight of feed consumed divided by live weights per pen) was adjusted for mortalities by considering the number and weekly weights of mortalities before the weighing day.

iii. **Ascites and pulmonary hypertension**

All the mortalities were removed, recorded, and weighed on a daily basis for the duration of the trial. Additionally, two birds per pen were culled at 21 and 42 days of age to measure the right ventricle to total ventricles ratio. All mortalities and culled birds were necropsied and visually inspected for macroscopic lesions related to pulmonary hypertension (ascites) syndrome such as massive accumulation of fluid in the peritoneal/abdominal cavity ("water belly"), right heart ventricular dilatations and liver cirrhosis. The right heart ventricle was excised and weighed, followed by the added weight of the left ventricle and septum to obtain the total heart weight (Julian, 1988). The ascites heart ratio (AHR) was calculated as ratio between the right ventricles to total ventricles (RV/TV ratio). The AHR values above 0.29 were considered as an indication of right ventricular hypertrophy and consequently pulmonary hypertension syndrome (Huchzermeyer & De Ruyck, 1986).

iv. **Packed cell volume (PCV)**

At 21 and 42 d of age, the blood was collected from the right jugular vein into heparinised micro capillary tubes at 21 and 42 d of age. The blood samples were immediately placed on ice and transported to the laboratory for determining haematocrit by centrifugation at 16 099g for 5 minutes. Haematocrit (HCT) values were used as an indication of pulmonary hypertension syndrome.

v. **Tibia bone ash in day old chick**

During the day of hatch (E 21.5), three chicks per basket within the machine were culled and the tibia was severed for ash determination. The tibia was defleshed, cleaned of all cartilage and other connective tissue using a sharp scalpel blade followed by air drying (AOAC, 2000; method 934.01). After drying, the bones were wrapped in a cheese cloth and defatted with diethyl ether by the Soxhlet extraction method (AOAC, 1990; method 963.15). The bones were transferred into a fume hood to evaporate excess ethyl ether and allowed to dry before ashing.

vi. **Zinc and manganese contents of metatarsal bones**

At 21 and 42 d of age, metatarsal bones were collected for the analysis of zinc and manganese content. The metatarsi were defleshed, cleaned of scales, cartilage and other connective tissue using a sharp scalpel blade followed by air drying (AOAC, 2000; method 934.01). After drying, bones were wrapped in a cheese cloth and defatted with diethyl ether by the Soxhlet extraction method (AOAC, 1990; method 963.15). The bones were

transferred into a fume hood to evaporate excess ethyl ether and allowed to dry before the ashing procedure was completed.

- **Bone ashing**

A ceramic crucible was placed in an oven at 100 °C for at least an hour to dry completely after which it was allowed to cool in a desiccator before weighing. The dry bones were weighed into dry ceramic crucibles and transferred into a muffle furnace for ashing at 600 °C (AOAC, 1990; method 932.16).

$$\% \text{ Defatted bone ash} = \frac{\text{Dry bone ash weight}}{\text{Dry defatted bone weight}} \times 100$$

- **Zinc and manganese analysis in bone**

Digestion of samples was carried out using a microwave digestion system (MarsXpress 5, CEM Corporation) according to the method described in the operators' manual. Approximately 0.1g of bone ash was weighed into a digestion tube followed by the addition of 10 mL nitric acid (HNO_3). The samples were heated up to 240 °C followed by the addition of 2.5 mL of perchloric acid ($HClO_4$) when approximately half of the HNO_3 had boiled away. Thereafter, the solution was allowed to cool and transferred to a 50 mL volumetric flask and filled up to volume.

The zinc content of the ash was determined using an atomic absorption spectrophotometer (Model 905AA, GBC Scientific Equipment, Braeside, Austria) (AOAC, 2000; method 999.11) Manganese content was determined using a Varian Spectra atomic absorption spectrophotometer (Model AA50, Spectralab Scientific Inc., Palo Alto, USA) (AOAC, 2000; method 999.11).

3.3 Statistical analysis

Data was analysed as a factorial treatment structure in a randomized complete block design. The incubation trial was a 2×3 factorial design with 2 temperature treatments (hot and normal) and 3 position treatments within the machine (top, middle, and bottom) as main effects. The broiler performance trial had 4 dietary treatments that were fed from 1-42 d of age. The trial design was a 2×4 factorial design with 2 temperature treatments and 4 dietary treatments. The 8 treatment combinations were randomly assigned within each block, which consisted of 8 pens. The broiler house had two rooms (house 1 and 2) with each room having 6 blocks and 48 pens. The 2 broiler houses had 96 pens in total and each dietary treatment was replicated in 24 pens. The analysis of variance (ANOVA) was determined using PROC MIXED procedure of SAS (SAS 9.1). Treatment means were compared using fisher's protected LSD. The pdmix800 macro (Saxton, 1998) was used to

assign letters of statistical significance between the treatment means. All mortality data was arcsine transformed before analysis. Unless otherwise stated, all means where $P<0.05$ were of statistical significance.

CHAPTER 4
RESULTS

4.1 Hatchability and chick quality

The hatchability and chick quality results for the different incubation temperatures (normal and hot) and positions (top, middle and bottom) in the machines are shown in Table 4.1. At day of hatch (E 21), all the unhatched eggs were counted per machine and all the hatched chicks were graded as first grade, second grade or culls. There were no significant differences in number of hatched eggs between eggs incubated at high temperature and eggs incubated at normal temperature ($P>0.05$). However, there were significant differences in the number of unhatched eggs ($P<0.05$) for different positions within the incubator. The bottom baskets had more unhatched eggs when compared to the top and middle baskets ($P<0.05$). There were significant differences for the first grade chicks when middle baskets were compared to the bottom baskets within the incubator. The number of second grade chicks differed significantly between the positions within the incubator, where the bottom baskets had more second grade chicks as compared to the top and middle baskets ($P<0.05$). The number of culled chicks were also significantly different between the positions within the incubator ($P<0.05$) as the bottom baskets had more culls as compared to the top baskets. Figure 4.1 presents the temperatures observed in the normal and the hot treatment incubators from E 10-18.

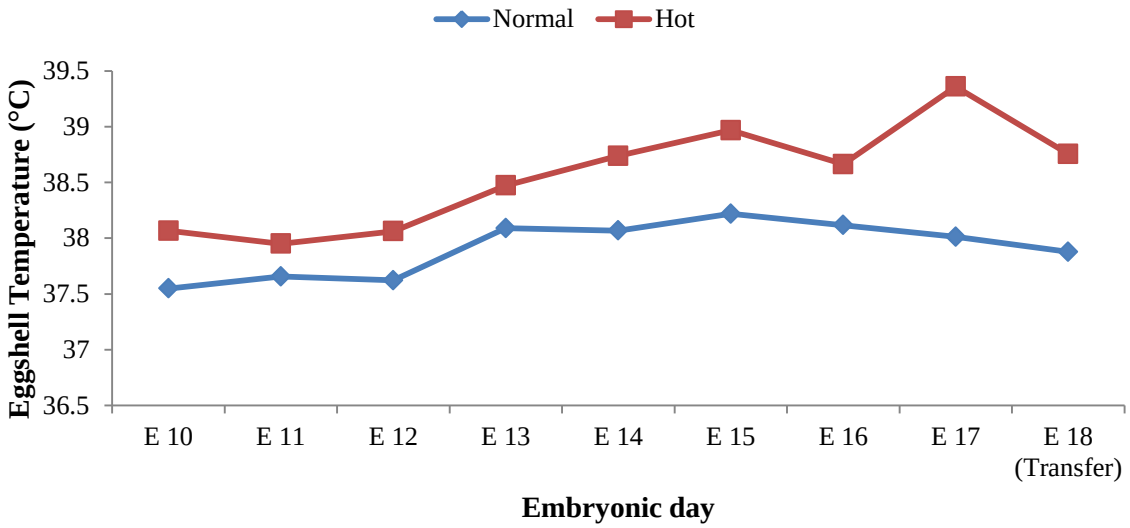


Figure 4.1 Eggshell temperature of eggs incubated at normal and hot temperatures from 10-18 days of incubation

4.2 Pre-hatch mortalities

As shown in Table 4.2, there were no significant differences in the number of early and middle stage mortalities of embryos for the temperature and position treatments ($P>0.05$). However, the number of late stage mortalities was significantly different between the positions within the incubator ($P<0.01$). The bottom baskets had more late hatchings than the top and middle baskets ($P<0.05$). At hatch, the number of pipped eggs were significantly different between the positions within the incubator ($P<0.05$). The bottom baskets had more pipped eggs than the top and middle trays.

The number of malposition embryos were significantly different for the position within the incubator, where the bottom baskets had more malposition embryos than the top and middle baskets ($P<0.01$). The number of abnormal embryos was also not affected by temperature and position within the incubator ($P>0.05$).

4.3 Organ weights of chicks at hatch (E. 21.5)

As indicated in Table 4.3, there were no significant differences in organ weights at hatch between the temperature treatments ($P>0.05$). However, the yolk free body mass (YFBM) was significantly different between the different positions within the incubator ($P<0.01$). The top exhibited greater YFBM as compared to the baskets located in the middle and bottom position within the incubator (Table 4.3). The heart weights were not significantly different between the temperature treatments and positions within the incubator ($P>0.05$). Differences in yolk weights were significant ($P<0.01$), where chicks from the bottom baskets had more retained yolk as compared to the chicks from the top and middle baskets. Liver weights were also significantly different between different position within the incubator ($P<0.01$) as the top and middle baskets produced chicks with heavier heart weights as compared to chicks in the bottom baskets. Gizzard weights were also affected significantly by the position of the tray within the incubator ($P<0.01$) as the gizzard of the chicks in the top and middle baskets weighed more than chicks from the bottom baskets. The proventriculus weights were significantly different between the positions in the incubator ($P>0.01$) as the proventriculus of the chicks in the top and middle baskets weighed more as compared to the chicks in the bottom baskets (Table 4.3).

Table 4.1 Number (mean ± standard error of the mean) of unhatched eggs, first grade chicks, second grade chicks, culled chicks, dead chicks, and hatched chicks as a percentage of total eggs and fertile eggs

	Unhatched eggs	1st grade chicks	2nd grade chicks	Culled chicks	Dead chicks	Hatch % (total eggs)	Hatch % (fertile eggs)
			Temperature				
Normal[1]	13.58 ± 2.12	92.92 ± 4.18	6.42 ± 1.16	0.63 ± 0.26	3.80 ± 3.38	79.4 ± 1.35	87.09 ± 1.91
Hot[1]	10.88 ± 2.12	91.41 ± 4.18	7.59 ± 1.16	1.00 ± 0.26	0.25 ± 3.38	81.17 ± 1.35	88.90 ± 1.91
			Position				
Top[2]	7.88^{B} ± 1.97	98.69^{ab} ± 4.44	4.75^{B} ± 1.23	0.38^{b} ± 0.24	5.75 ± 3.55	83.12^{a} ± 1.43	91.36^{A} ± 1.79
Centre[3]	10.13^{B} ± 2.62	93.74^{a} ± 6.28	5.38^{B} ± 1.75	0.88^{ab} ± 0.32	0.13 ± 5.00	80.68^{ab} ± 2.03	89.29^{A} ± 2.38
Bottom[4]	18.69^{A} ± 1.98	87.93^{b} ± 4.44	10.88^{A} ± 1.23	1.19^{a} ± 0.24	0.19 ± 3.55	77.08^{b} ± 1.43	83.34^{B} ± 1.79
			Temperature × Position treatment				
Normal × Top	7.75 ± 2.80	95.62 ± 6.28	4.63 ± 1.75	0.25 ± 0.34	11.25 ± 5.02	84.56 ± 2.03	92.49 ± 2.53
Normal × Centre	11.00 ± 3.72	93.50 ± 8.88	6.00 ± 2.47	0.50 ± 0.46	0.00 ± 7.06	80.11 ± 2.86	88.68 ± 3.36
Normal × bottom	7.75 ± 2.80	90.24 ± 6.28	8.63 ± 1.75	1.13 ± 0.34	0.13 ± 5.02	73.58 ± 2.03	80.09 ± 2.53
Hot × Top	8.00 ± 2.80	94.62 ± 6.28	4.88 ± 1.75	0.50 ± 0.34	0.25 ± 5.02	81.68 ± 2.03	90.23 ± 2.53
Hot × Centre	9.25 ± 3.72	94.00 ± 8.88	4.75 ± 2.47	1.25 ± 0.46	0.25 ± 7.06	81.25 ± 2.86	89.89 ± 3.36
Hot × bottom	15.38 ± 2.80	85.62 ± 6.28	13.13 ± 1.75	1.25 ± 0.34	0.25 ± 5.02	80.59 ± 2.03	86.59 ± 2.53
			Source of variation				
P > F							
Temp[5]	0.45	0.48	0.48	0.40	0.52	0.37	0.56
Position	0.00030	0.0037	0.0030	0.036	0.48	0.019	0.0034
Temp[5]**× Position**	0.37	0.96	0.32	0.69	0.48	0.063	0.16

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)
[A-B] Means in the same column with no common superscript differ significantly (P<0.01)
[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Top-trays in the top section of the machine
[3] Centre-trays in the middle section of the machine
[4] Bottom-trays in the bottom section of the machine
[5] Temperature (Temp)

Table 4.2 Number (mean ± standard error of the mean) of early-, mid- and late-embryonic mortalities as well as number of pipped, malposition embryos and abnormal chicks

	Early mortality	Middle mortality	Late mortality	Pipped eggs	Malposition embryos	Abnormal chicks
			Temperature			
Normal[1]	4.13 ± 0.53	0.29 ± 0.15	8.67 ± 1.79	3.46 ± 0.95	0.79 ± 0.67	0.29 ± 0.15
Hot[1]	4.58 ± 0.53	0.42 ± 0.15	8.04 ± 1.79	2.04 ± 0.95	1.46 ± 0.67	0.42 ± 0.15
			Position			
Top[2]	4.13 ± 0.55	0.13 ± 0.13	6.31^{B} ± 1.43	0.44^{B} ± 1.01	0.19^{B} ± 0.58	0.38 ± 0.16
Centre[3]	4.50 ± 0.76	0.38 ± 0.38	7.88^{AB} ± 1.69	1.88^{B} ± 1.42	0.13^{B} ± 0.72	0.25 ± 0.22
Bottom[4]	4.44 ± 0.55	0.56 ± 0.16	10.88^{A} ± 1.43	5.94^{A} ± 1.01	3.06^{A} ± 0.58	0.44 ± 0.16
			Temperature × Position treatment			
Normal × Top	3.24 ± 0.77	0.13 ± 0.22	5.88 ± 2.03	0.63 ± 1.42	0.13 ± 0.82	0.50 ± 0.22
Normal × Centre	5.50 ± 1.08	0.25 ± 0.31	8.00 ± 2.39	1.75 ± 2.01	0.00 ± 1.02	0.25 ± 0.31
Normal × Bottom	3.63 ± 0.77	0.50 ± 0.50	12.13 ± 2.03	8.00 ± 1.42	2.25 ± 0.82	0.13 ± 0.22
Hot × Top	5.00 ± 0.77	0.13 ± 0.22	6.75 ± 2.03	0.25 ± 1.42	0.25 ± 0.82	0.25 ± 0.22
Hot × Centre	3.50 ± 1.08	0.50 ± 0.31	7.75 ± 2.39	2.00 ± 2.01	0.25 ± 1.02	0.25 ± 0.31
Hot × Bottom	5.25 ± 0.78	0.63 ± 0.22	9.63 ± 2.03	3.88 ± 1.42	3.88 ± 0.82	0.75 ± 0.22
			Source of variation			
P > F						
Temp[5]	0.59	0.55	0.83	0.30	0.55	0.55
Position	0.89	0.16	0.0042	0.0016	<0.0001	0.79
Temp[5] **× Position**	0.11	0.90	0.42	0.32	0.44	0.15

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Top-trays in the top section of the machine

[3] Centre-trays in the middle section of the machine

[4] Bottom-trays in the bottom section of the machine

[5] Temperature (Temp)

Table 4.3 Organ weights (mean ± standard error of the mean) at hatch of chicks incubated at different temperatures and positions in the incubator

	Organs						
	Body weight (g)	**YFBM[1]**	**Heart**	**Yolk**	**Liver**	**Gizzard**	**Proventriculus**
				(g/100g)			
				Temperature			
Normal[2]	40.13 ± 1.09	91.95 ± 1.15	1.06 ± 0.034	8.13 ± 1.15	3.90 ± 0.071	5.59^{b} ± 0.11	1.21 ± 0.071
Hot[2]	38.64 ± 1.09	91.87 ± 1.15	0.98 ± 0.034	8.04 ± 1.15	3.86 ± 0.071	5.94^{a} ± 0.11	1.14 ± 0.071
				Position			
Top[3]	39.74^{A} ± 0.88	93.04^{A} ± 0.92	1.00 ± 0.036	6.96^{B} ± 0.92	4.03^{A} ± 0.076	6.31^{A} ± 0.11	1.31^{A} ± 0.065
Middle[4]	37.57^{B} ± 1.04	92.97^{B} ± 1.08	1.02 ± 0.051	7.03^{B} ± 1.08	4.01^{A} ± 0.11	6.09^{B} ± 0.16	1.29^{A} ± 0.086
Bottom[5]	40.86^{A} ± 0.88	89.73^{B} ± 0.85	1.04 ± 0.036	10.27^{A} ± 0.92	3.60^{B} ± 0.076	4.90^{C} ± 0.11	0.92^{B} ± 0.065
				Temperature × Position treatment			
Normal × Top	40.41 ± 1.25	93.26 ± 1.29	0.99 ± 0.051	6.73 ± 1.29	4.11 ± 0.11	6.16 ± 0.16	1.38 ± 0.092
Normal × Middle	38.52 ± 1.48	93.11 ± 1.52	1.06 ± 0.073	6.89 ± 1.52	4.05 ± 0.15	5.96 ± 0.22	1.32 ± 0.12
Normal × Bottom	41.45 ± 1.25	89.50 ± 1.29	1.10 ± 0.051	10.50 ± 1.29	3.55 ± 0.11	4.65 ± 0.16	0.92 ± 0.092
Hot × Top	39.06 ± 1.25	92.82 ± 1.29	1.00 ± 0.051	7.18 ± 1.29	3.95 ± 0.11	6.45 ± 0.16	1.23 ± 0.092
Hot × Middle	36.62 ± 1.46	92.83 ± 1.52	0.96 ± 0.073	7.17 ± 1.52	3.97 ± 0.15	6.22 ± 0.22	1.26 ± 0.12
Hot × Bottom	40.26 ± 1.25	89.97 ± 1.29	0.97 ± 0.051	10.03 ± 1.29	3.66 ± 0.11	5.14 ± 0.063	0.94 ± 0.092
				Source of variation			
P > F							
Temp[6]	0.44	0.96	0.12	0.96	0.68	0.026	0.58
Position	0.0072	0.0004	0.66	0.00040	0.00060	<0.0001	0.0001
Temp[6] × position	0.93	0.84	0.38	0.84	0.48	0.76	0.57

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)
[A-B] Means in the same column with no common superscript differ significantly (P<0.01)
[1] YFBM (yolk-free body mass)
[2] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[3] Top-trays in the top section of the machine
[4] Centre-trays in the middle section of the machine
[5] Bottom-trays in the bottom section of the machine
[6] Temperature (Temp)

4.4 Weekly average body weight

At hatch (E 21.5), there were significant differences in the average BW of chicks that were placed as a normal and that were placed as a hot temperature treatment ($P<0.01$). There were no significant differences in BW between the chicks incubated at higher temperatures and chicks incubated at normal temperatures at day 7 of age ($P>0.05$) (Table 4.4).

At 14 days of age, there were no statistical differences between average BW of chicks incubated at normal temperatures and chicks incubated at high temperatures ($P>0.05$). There were also no significant differences in average BW in chicks fed different dietary treatments ($P>0.05$).

At 21 days of age, there were no significant differences in average BW for the chicks incubated at normal temperatures and chicks that were incubated at higher temperatures ($P>0.05$). However, there were significant differences in BW for chicks fed different dietary treatments at 21 days of age ($P<0.01$). Chicks from dietary Treatments 1, 3 and 4 exhibited greater BW as compared to those from dietary Treatment 2 at 21 days of age.

At 28 days of age, there were no statistical differences in average BW for chicks incubated at normal temperatures and chicks incubated at high temperatures ($P>0.05$). There were no significant differences in average BW for the different dietary treatments fed ($P>0.05$).

At 35 days of age, there were no significant differences in average BW for the chicks incubated at normal temperatures as compared to chicks incubated at high temperatures ($P>0.05$). However, the dietary treatment had a significant effect on average BW at 35 days of age ($P<0.05$). Chicks from dietary Treatment 3 and 4 (organic zinc, 120 mg/kg) weighed more than those chicks fed dietary Treatment 2 (inorganic zinc; 120 mg/kg).

At day 42, there were significant differences in average BW for chicks incubated at normal temperatures and chicks incubated at high temperatures ($P<0.01$). Chicks incubated at normal temperatures weighed more than chicks incubated at high temperatures.

4.5 Weekly average body weight gain

As shown in Table 4.5, there were no significant differences in weekly average BW gain for chicks incubated at normal and those incubated at high temperatures ($P>0.05$). However, there were significant differences in weekly average BW gain for chicks fed different diets at day 21-28 ($P<0.01$).Chicks from dietary Treatment 3 weighed more than chicks that were fed Treatment 2 and 4.

4.6 Cumulative average body weight gain

As shown in Table 4.6, there were no significant differences for average BW gain in chicks that were incubated at normal temperatures as compared to chicks that were incubated at high temperatures at 7 days of age ($P>0.05$). At 28 days of age, there were significant differences in chicks that were fed different dietary treatments ($P<0.01$). Chicks that were fed dietary Treatment 3 were heavier than those chicks that were fed dietary Treatment 2 and 4. At 35 days of age, there were significant differences in chicks that received an organic Zn and Mn diet compared to chicks that received inorganic Zn and Mn diet ($P<0.05$). Chicks from Treatment 3 (organic Zn and Mn) gained more BW as compared to chicks that received dietary Treatment 1 and 2 (inorganic treatment). At 42 days of age, there were significant differences in BW gain in chicks that were incubated at normal temperatures as compared to chicks that were incubated at high temperatures ($P<0.01$). Chicks that were incubated at high temperatures gained more BW than chicks that were incubated at normal temperatures.

4.7 Weekly average feed intake

At day 7-14, there were significant differences in weekly average feed intake between chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures ($P<0.05$) (Table 4.7). There were no significant differences in weekly average feed intake between chicks that were fed different dietary treatments ($P>0.05$).

4.8 Cumulative average feed intake

Incubation temperature did not significantly affect feed intake during the first 7 days of age ($P>0.05$). However, at 14 days of age, there were significant differences in feed intake between the chicks that were incubated at normal temperatures and those that were incubated at high temperatures ($P<0.01$). Chicks that were incubated at normal temperatures consumed more feed as compared to chicks that were incubated at high temperatures (Table 4.8).

4.9 Weekly adjusted (mortality) feed conversion ratio

At day 15-21, there were significant differences in FCR between chicks that were incubated at normal and those chicks that were incubated at high temperatures ($P<0.01$) (Table 4.9). Chicks that were incubated at high temperatures had a better FCR than chicks that were incubated at normal temperatures. At day 22-28 of age, there were significant differences in FCR between the chicks that were incubated at high temperatures and chicks that were incubated at normal temperatures ($P<0.01$). Chicks that were incubated at normal temperatures had a better FCR as compared to chicks that were incubated at high temperatures.

At day 29-35, there were significant differences in FCR between chicks that were fed different dietary treatments (P<0.05). Chicks that were fed dietary Treatment 3 had a better FCR as compared to chicks that were fed Treatment 2 and 4. At day 36-42, there were also significant differences in FCR between the chicks that were fed different dietary treatments (P<0.05). Chicks that received dietary Treatment 4 had a better FCR as compared to chicks that were fed Treatment 1.

4.10 Cumulative adjusted (mortality) feed conversion ratio

At day 14, there were significant differences in FCR between chicks that were incubated at normal and those that were incubated at high temperatures (P<0.05) (Table 4.10). Chicks that were incubated at high temperatures had a better FCR as compared to chicks that were incubated at normal temperatures. At day 0-28, there were significant differences in FCR for chicks that received different dietary treatments (P<0.05). Chicks that received dietary Treatment 3 had a better FCR as compared to chicks that received Treatment 2 and 4. Also, chicks that were fed dietary Treatment 1 had a better FCR as compared to chicks that were fed dietary Treatment 2.

4.11 Cumulative ascites mortality rate

As shown in Table 4.11, there were no significant differences in ascites related mortalities between chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures from 0-21 days of age (P>0.05). At 28 days of age, chicks that were incubated at normal temperatures had significantly lower ascites related mortalities as compared to chicks that were incubated at high temperatures (P<0.01). At 35 days of age, chicks that were incubated at high temperatures exhibited a significantly greater ascites related mortality rate as compared to chicks that were incubated at normal temperatures (P<0.01). At 42 days of age, there were significantly greater ascites related mortalities in chicks that were incubated at hot temperatures as compared to chicks that were incubated at normal temperatures (P<0.01).

At day 0-21, there were significant differences for the interaction between temperature and dietary treatment (P<0.01). Chicks that were incubated at hot temperatures and fed dietary Treatment 2 had more ascites related mortalities as compared to chicks that were incubated at normal temperatures and fed dietary Treatment 2.

4.12 Cumulative non-ascites mortality rate

There were no significant differences in non-ascites related mortalities between chicks that were incubated at normal temperatures and those that were incubated at high temperatures (P>0.05) (Table 4.12). Also, there were no significant differences in non-ascites mortality between chicks that were fed different dietary treatments (P>0.05).

4.13 Cumulative total (ascites + non-ascites) mortality rates

At 7 days of age (Table 4.13), there were significantly greater total mortality for the chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures ($P<0.01$). Chicks that were incubated at normal temperatures had a high mortality rate as compared to chicks that were incubated at hot temperatures. At 28 days of age, chicks that were incubated at normal temperatures exhibited a significantly lower total mortality rate as compared to chicks that were incubated at hot temperatures ($P<0.01$). Chicks that were incubated at normal temperatures had a 5% lower total mortality rate as compared to chicks that were incubated at high temperatures. At 35 days of age, the normal incubation treatment exhibited lower mortalities as compared to the hot incubated treatment ($P<0.01$). Chicks from the normal incubation temperature treatment had an 8% lower mortality rate as compared to chicks from the hot incubation treatment.

At 42 days of age, there were significant differences for total mortalities between the normal and high temperature incubated treatment groups ($P<0.01$). The chicks from the normal temperature treatment had 11% lower mortalities as compared to chicks incubated at high temperatures.

4.14 Body weight variation

At 42 days of age, there were no significant differences in chicks that were incubated at normal temperatures and those that were incubated at high temperatures ($P>0.05$). However, there were significant differences in BW variation per pen between chicks that were fed different diets ($P<0.05$). Chicks that received Treatment 1 (inorganic Zn/Mn at 80 mg/kg) had an increased within-pen BW variation as compared to chicks that received Treatment 2 (Inorganic Z/M at 120 mg/kg) and Treatment 4 (organic Zn/Mn at 120 mg/kg) (Table 4.14).

4.15 Right ventricular weight to total ventricular weight (RV/TV) ratio

As indicated in Table 4.15, the incubation temperature treatment did not affect the RV/TV ratio of chicks that were incubated at normal and chicks that were incubated at hot temperatures ($P>0.05$). The dietary treatments also did not show significant differences in RV/TV ratio between chicks that were fed different levels and sources of Zn/Mn ($P>0.05$).

4.16 Haematocrit values

At 41 days of age (Table 4.16) there were significant differences in haematocrit values between chicks that were incubated at normal and high temperatures ($P<0.05$). However, there were no significant differences in haematocrit values between chicks that received different levels and sources of Zn/Mn ($P>0.05$).

Table 4.4 Weekly average body weight of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
	(g)						
	Temperature						
Normal[1]	40.11^{A}	158.36	4703.99	913.00	1610.35	2360.05	3330.11^{A}
Hot[1]	38.68^{B}	164.61	471.85	894.09	1620.51	2390.77	3200.10^{B}
SEM[6]	0.21	3.21	2.60	12.43	17.59	12.95	19.86
	Diet						
Treatment 1[2]	39.45	157.93	471.29	904.99	1640.05^{AB}	2340.84^{b}	3200.53
Treatment 2[3]	39.19	160.29	473.37	886.64	1560.06^{C}	2330.68^{b}	3240.98
Treatment 3[4]	39.51	158.65	471.97	918.01	1660.32^{A}	2440.20^{a}	3310.08
Treatment 4[5]	39.42	169.08	475.04	904.55	1590.31^{AB}	2380.91^{ab}	3300.55
SEM[6]	0.23	4.49	3.18	13.16	23.46	17.37	28.08
	Temperature × Dietary treatment						
Normal × Treatment 1	40.21	157.03	472.72	911.52	1620.82	2360.42	3140.93
Hot × Treatment 1	38.68	158.83	469.85	900.60	1660.27	2320.27	3270.13
Normal × Treatment 2	39.82	158.25	476.58	900.60	1560.49	2310.31	3230.41
Hot × Treatment 2	38.55	162.32	470.16	872.67	1550.62	2360.04	3260.55
Normal × Treatment 3	40.30	158.34	473.07	933.77	1660.42	2440.16	3240.71
Hot × Treatment 3	38.73	158.97	470.88	902.24	1670.21	2450.27	3380.76
Normal × Treatment 4	40.09	159.83	473.60	906.11	1600.67	2340.31	3180.17
Hot × Treatment 4	38.77	178.33	476.49	903.00	1570.94	2420.51	3180.94
SEM[6]	0.28	6.32	4.11	14.52	32.13	23.87.	39.71
	Source of variation						
P > F							
Temperature	<0.00010	0.18	0.41	0.15	0.76	0.42	0.0089
Diet	0.41	0.33	0.75	0.46	0.0049	0.0185	0.39
Temperature × Diet	0.84	0.26	0.65	0.29	0.75	0.52	0.60

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)
[A-B] Means in the same column with no common superscript differ significantly (P<0.01)
[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg
[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg
[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg
[6] SEM= Standard error of the mean

Table 4.5 Weekly average body weight gain of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 7-14	Day 15-21	Day 22-28	Day 29-35	Day 36-42
	(g)					
Temperature						
Normal[1]	118.93	310.63	450.01	690.35	750.70	830.06
Hot[1]	125.93	310.23	440.25	710.42	770.26	950.33
SEM[6]	3.20	1.92	13.50	25.01	22.41	14.64
Diet						
Treatment 1[2]	118.48	310.35	440.71	730.05^{AB}	700.80	860.69
Treatment 2[3]	121.10	310.08	430.26	650.42^{C}	780.62	910.30
Treatment 3[4]	120.65	310.32	450.03	740.31^{A}	780.89	860.14
Treatment 4[5]	120.10	310.96	440.51	670.76^{C}	790.60	920.64
SEM[6]	4.48	2.45	13.92	29.17	29.66	20.71
Temperature × Dietary treatment						
Normal × Treatment 1	120.82	310.69	450.80	700.29	740.61	780.51
Hot × Treatment 1	120.15	310.02	440.61	760.81	660.99	940.86
Normal × Treatment 2	120.43	320.33	430.02	650.89	750.82	920.10
Hot × Treatment 2	130.77	310.84	430.51	650.95	800.42	900.51
Normal × Treatment 3	120.03	310.73	460.70	720.685	790.74	800.78
Hot × Treatment 3	120.24	310.91	440.36	770.97	780.03	930.51
Normal × Treatment 4	120.74	310.76	440.51	690.57	740.64	840.86
Hot × Treatment 4	140.56	320.16	440.50	650.94	740.57	840.43
SEM[6]	6.32	3.22	15.71	37.03	48.60	29.29
Source of variation						
P > F						
Temperature	0.088	0.086	0.19	0.45	0.56	0.043
Diet	0.24	0.89	0.26	0.011	0.063	0.82
Temperature × Diet	0.25	0.097	0.24	0.48	0.12	0.60

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.6 Cumulative average body weight gain of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
	(g)					
Temperature						
Normal[1]	120.93	430.49	880.89	1570.24	2320.05	3160^{B}
Hot[1]	120.25	430.79	870.41	1580.83	2350.68	3290^{A}
SEM[6]	3.19	2.46	11.62	18.59	31.22	39.86
Diet						
Treatment 1[2]	120.48	430.54	870.54	1600.59^{AB}	2300.85^{b}	3160.08
Treatment 2[3]	120.10	430.74	870.45	1520.87^{C}	2290.04^{b}	3200.80
Treatment 3[4]	120.13	430.17	880.49	1620.80^{A}	2410.20^{a}	3270.83
Treatment 4[5]	120.65	440.01	880.12	1550.88^{BC}	2340.36^{ab}	3260.12
SEM[6]	1.28	3.41	13.34	24.46	37.19	53.09
Temperature × Dietary treatment						
Normal × Treatment 1	116.82	430.41	880.30	1580.60	2320.09	3100.72
Hot × Treatment 1	120.15	430.66	870.78	1620.59	2280.80	3230.45
Normal × Treatment 2	120.42	440.80	870.77	1520.67	2270.71	3190.59
Hot × Treatment 2	130.77	430.67	860.12	1520.07	2320.37	3220.00
Normal × Treatment 3	120.03	430.45	900.47	1620.12	2400.39	3200.64
Hot × Treatment 3	120.24	430.90	870.51	1630.49	2410.01	3340.03
Normal × Treatment 4	120.74	430.31	870.02	1560.59	2300.20	3140.08
Hot × Treatment 4	140.56	440.70	870.22	1540.17	2380.52	3370.17
SEM[6]	1.62	4.23	14.66	32.14	47.09	73.13
Source of variation						
P > F						
Temperature	0.088	0.78	0.22	0.72	0.39	0.0082
Diet	0.24	0.72	0.48	0.05	0.019	0.39
Temperature × Diet	0.25	0.64	0.29	0.75	0.53	0.60

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.7 Weekly average feed intake of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 7-14	Day 15-21	Day 22-28	Day 29-35	Day 36-42
	(g)					
Temperature						
Normal[1]	150.91	400.40[A]	870.32	1100.82	1305.83	1840.67
Hot[1]	160.66	390.65[B]	840.01	1120.22	1380.30	1850.52
SEM[6]	3.05	4.55	11.16	25.26	19.56	79.97
Diet						
Treatment 1[2]	150.81	400.34	870.16	1070.41	1360.03	1840.22
Treatment 2[3]	160.89	390.34	860.53	1130.81	1330.61	1800.62
Treatment 3[4]	160.62	400.34	840.28	1100.96	1380.32	1840.00
Treatment 4[5]	160.82	400.65	840.69	1130.91	1400.29	1900.55
SEM[6]	3.33	5.57	15.79	31.04	26.51	85.36
Temperature × Dietary treatment						
Normal × Treatment 1	150.60	411.27	870.27	1070.76	1350.14	1860.25
Hot × Treatment 1	150.03	390.27	880.04	1060.06	1370.93	1820.18
Normal × Treatment 2	160.17	400.01	860.71	1140.80	1330.42	1820.02
Hot × Treatment 2	160.61	380.68	860.36	1120.82	1330.79	1790.21
Normal × Treatment 3	160.29	410.24	830.91	1080.42	1340.06	1830.52
Hot × Treatment 3	160.95	400.44	850.65	1130.49	1420.57	1850.47
Normal × Treatment 4	160.57	410.08	800.39	1100.31	1380.57	1850.47
Hot × Treatment 4	160.07	390.23	800.99	1160.52	1420.02	1950.21
SEM[6]	4.04	7.01	22.33	40.19	37.65	97.89
Source of variation						
P > F						
Temperature	0.62	0.0012	0.083	0.40	0.14	0.76
Diet	0.35	0.14	0.31	0.25	0.22	0.53
Temperature × Diet	0.99	0.96	0.42	0.54	0.70	0.68

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.8 Cumulative average feed intake of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
	(g)					
Temperature						
Normal[1]	150.91	560.31[A]	1400.63	2500.45	3840.75	5680.42
Hot[1]	160.66	550.32[B]	1410.33	2530.55	3910.38	5770.90
SEM[6]	2.98	3.87	11.57	26.28	32.01	64.35
Diet						
Treatment 1[2]	150.81	550.58	1430.74	2490.16	3850.19	5690.40
Treatment 2[3]	160.89	550.23	1410.76	2540.57	3870.18	5670.79
Treatment 3[4]	160.62	560.95	1400.24	2500.20	3880.51	5720.51
Treatment 4[5]	160.89	560.47	1400.16	2530.08	3920.37	5820.92
SEM[6]	3.33	5.47	16.36	35.85	42.79	79.11
Temperature × Dietary treatment						
Normal × Treatment 1	150.60	560.87	1430.14	2490.91	3840.05	5700.30
Hot × Treatment 1	150.03	550.30	1430.34	2490.40	3850.33	5670.51
Normal × Treatment 2	160.17	550.18	1420.89	2560.69	3880.11	5700.13
Hot × Treatment 2	160.61	540.28	1400.64	2520.46	3850.25	5640.46
Normal × Treatment 3	160.29	570.53	1390.44	2470.87	3810.93	5640.45
Hot × Treatment 3	160.95	550.38	1400.04	2530.53	3960.10	5810.58
Normal × Treatment 4	160.57	560.65	1370.04	2460.35	3840.91	5640.80
Hot × Treatment 4	160.07	550.30	1430.29	2590.81	4000.83	5950.04
SEM[6]	3.94	7.74	23.14	47.56	59.83	102.42
Source of variation						
P > F						
Temperature	0.62	0.0020	0.37	0.27	0.082	0.18
Diet	0.35	0.33	0.57	0.70	0.65	0.37
Temperature × Diet	0.99	0.97	0.38	0.28	0.24	0.23

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.9 Weekly feed conversion ratio (adjusted for mortalities) of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 7-14	Day 15-21	Day 22-28	Day 29-35	Day 36-42
			(g/g)		
			Temperature		
Normal[1]	1.23	1.29^{A}	1.90^{B}	1.60	1.82
Hot[1]	1.27	1.26^{B}	2.00^{A}	1.58	1.89
SEM[6]	0.029	0.011	0.053	0.028	0.051
			Diet		
Treatment 1[2]	1.27	1.29	2.00	1.54^{BC}	2.03^{a}
Treatment 2[3]	1.28	1.25	2.00	1.69^{A}	1.74^{b}
Treatment 3[4]	1.30	1.29	1.87	1.50^{C}	1.85^{ab}
Treatment 4[5]	1.30	1.27	1.91	1.62^{AB}	1.80^{b}
SEM[6]	0.031	0.016	0.062	0.040	0.070
			Temperature × Diet		
Normal × Treatment 1	1.26	1.31	1.96	1.59	1.91
Hot × Treatment 1	1.25	1.27	2.03	1.49	2.14
Normal × Treatment 2	1.31	1.25	2.00	1.71	1.77
Hot × Treatment 2	1.25	1.25	2.02	1.67	1.71
Normal × Treatment 3	1.31	1.31	1.78	1.50	1.73
Hot × Treatment 3	1.29	1.27	1.96	1.50	1.96
Normal × Treatment 4	1.28	1.30	1.85	1.60	1.85
Hot × Treatment 4	1.30	1.30	2.00	1.65	1.75
SEM[6]	0.035	0.022	0.076	0.056	0.096
			Source of variation		
P > F					
Temperature	0.26	0.04	0.02	0.55	0.28
Diet	0.21	0.26	0.11	0.06	0.02
Temperature × Diet	0.40	0.66	0.57	0.56	0.14

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.10 Cumulative feed conversion ratio (adjusted for mortalities) of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
	(g/g)					
Temperature						
Normal[1]	1.29	1.29[a]	1.60	1.60	1.66	1.80
Hot[1]	1.27	1.26[b]	1.63	1.62	1.68	1.76
SEM[6]	0.029	0.0082	0.020	0.024	0.019	0.021
Diet						
Treatment 1[2]	1.26	1.28	1.64	1.58[bc]	1.68	1.81
Treatment 2[3]	1.28	1.26	1.63	1.68[a]	1.69	1.77
Treatment 3[4]	1.30	1.30	1.59	1.54[c]	1.63	1.76
Treatment 4[5]	1.29	1.28	1.60	1.68[ab]	1.68	1.79
SEM[6]	0.031	0.013	0.025	0.034	0.027	0.028
Temperature × Dietary treatment						
Normal × Treatment 1	1.26	1.30	1.63	1.60	1.66	1.85
Hot × Treatment 1	1.25	1.27	1.64	1.56	1.69	1.77
Normal × Treatment 2	1.31	1.27	1.63	1.69	1.71	1.79
Hot × Treatment 2	1.25	1.25	1.63	1.66	1.67	1.75
Normal × Treatment 3	1.31	1.31	1.55	1.53	1.59	1.76
Hot × Treatment 3	1.29	1.28	1.62	1.56	1.67	1.75
Normal × Treatment 4	1.28	1.29	1.57	1.58	1.67	1.81
Hot × Treatment 4	1.30	1.26	1.62	1.71	1.69	1.78
SEM[6]	0.035	0.018	0.033	0.048	0.038	0.040
Source of variation						
P > F						
Temperature	0.26	0.054	0.11	0.46	0.38	0.13
Diet	0.21	0.27	0.28	0.027	0.41	0.55
Temperature × Diet	0.40	0.97	0.50	0.27	0.37	0.87

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.11 Cumulative ascites mortalities of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
	%					
Temperature						
Normal[1]	0.11	0.30	0.66	2.40^{B}	5.36^{B}	9.54^{B}
Hot[1]	0.10	0.18	0.97	5.53^{A}	11.4^{A}	19.3^{A}
SEM[6]	0.0099	0.020	0.022	0.024	0.039	0.033
Diet						
Treatment 1[2]	0.084	0.59	0.93	4.75	8.56	14.48
Treatment 2[3]	0.00	0.00	1.16	3.35	7.56	15.00
Treatment 3[4]	0.084	0.44	0.57	3.21	8.23	14.38
Treatment 4[5]	0.084	0.18	0.64	4.02	8.23	12.43
SEM[6]	0.014	0.024	0.027	0.032	0.045	0.039
Temperature × Dietary treatment						
Normal × Treatment 1	0.15	0.93	1.34^{AB}	5.32	8.36	12.6
Hot × Treatment 1	0.04	0.33	0.59^{B}	4.21	8.76	16.5
Normal × Treatment 2	0.00	0.00	0.14^{B}	1.09	2.96	9.05
Hot × Treatment 2	0.00	0.04	3.12^{A}	6.79	14.0	22.2
Normal × Treatment 3	0.15	0.33	0.33^{B}	1.23	6.46	10.9
Hot × Treatment 3	0.04	0.55	0.88^{AB}	6.06	10.1	18.3
Normal × Treatment 4	0.33	0.44	1.27^{AB}	2.98	4.41	6.23
Hot × Treatment 4	0.00	0.04	0.22^{B}	5.20	13.1	20.4
SEM[6]	0.0056	0.0091	0.014	0.016	0.018	0.018
Source of variation						
P > F						
Temperature	0.08	0.51	0.44	0.008	0.001	<0.0001
Diet	0.34	0.06	0.72	0.78	0.98	0.83
Temperature × Diet	0.5	0.45	0.0088	0.13	0.13	0.25

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.12 Cumulative non-ascites mortalities of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
			%			
Temperature						
Normal[1]	0.15	0.33	0.69	0.95	1.32	1.32
Hot[1]	0.02	0.08	0.42	1.81	2.64	2.70
SEM[6]	0.011	0.014	0.017	0.018	0.019	0.019
Diet						
Treatment 1[2]	0.04	0.27	0.83	2.73	3.15	3.15
Treatment 2[3]	0.08	0.27	0.83	1.43	1.67	1.67
Treatment 3[4]	0.15	0.15	0.27	0.79	1.46	1.46
Treatment 4[5]	0.04	0.08	0.38	0.83	1.63	1.74
SEM[6]	0.015	0.019	0.024	0.026	0.027	0.027
Temperature × Dietary treatment						
Normal × Treatment 1	0.15	0.73	1.53	2.62	3.18	3.18
Hot × Treatment 1	0.04	0.04	0.33	2.84	3.12	3.12
Normal × Treatment 2	0.15	0.44	1.09	1.53	1.53	1.53
Hot × Treatment 2	0.04	0.15	0.59	1.34	1.81	1.81
Normal × Treatment 3	0.15	0.15	0.22	0.22	0.84	0.84
Hot × Treatment 3	0.15	0.15	0.33	1.70	2.23	2.23
Normal × Treatment 4	0.15	0.15	0.33	0.33	0.44	0.44
Hot × Treatment 4	0.00	0.04	0.44	1.53	3.57	3.89
SEM[6]	0.022	0.028	0.034	0.036	0.038	0.039
Source of variation						
P > F						
Temperature	0.12	0.15	0.44	0.15	0.08	0.07
Diet	0.78	0.79	0.55	0.13	0.42	0.45
Temperature × Diet	0.78	0.68	0.63	0.52	0.35	0.3

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.13 Cumulative total mortalities (arcsine transformed) of chicks incubated at different temperatures and fed different zinc and manganese levels and sources from 0-42 days of age

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35	Day 0-42
	%					
Temperature						
Normal[1]	0.52[A]	1.20	2.09	4.41[B]	8.43[B]	13.1[B]
Hot[1]	0.05[B]	0.43	2.28	9.31[A]	16.1[A]	24.1[A]
SEM[6]	0.014	0.022	0.025	0.025	0.041	0.033
Diet						
Treatment 1[2]	0.23	1.53	2.89	8.70	13.5	20.0
Treatment 2[3]	0.08	0.38	3.29	6.94	11.4	18.8
Treatment 3[4]	0.38	0.86	1.20	5.07	11.7	18.2
Treatment 4[5]	0.23	0.51	1.68	6.13	11.6	16.1
SEM[6]	0.019	0.028	0.031	0.033	0.047	0.038
Temperature × Dietary treatment						
Normal × Treatment 1	0.59	2.89	4.21	8.71	13.1	17.7
Hot × Treatment 1	0.04	0.59	1.81	8.70	13.9	22.5
Normal × Treatment 2	0.15	0.44	1.46	3.72	6.00	12.9
Hot × Treatment 2	0.04	0.33	5.82	11.1	18.3	25.7
Normal × Treatment 3	0.59	0.93	1.09	2.10	9.24	14.3
Hot × Treatment 3	0.22	0.79	1.32	9.24	14.3	22.5
Normal × Treatment 4	0.93	1.09	2.15	4.25	6.23	8.35
Hot × Treatment 4	0.00	0.15	1.27	8.33	18.3	25.9
SEM[6]	0.027	0.037	0.041	0.046	0.057	0.048
Source of variation						
P > F						
Temperature	0.009	0.07	0.8	0.0024	0.0004	<0.0001
Diet	0.68	0.29	0.18	0.43	0.89	0.66
Temperature × Diet	0.49	0.50	0.065	0.25	0.14	0.14

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= standard error of the mean

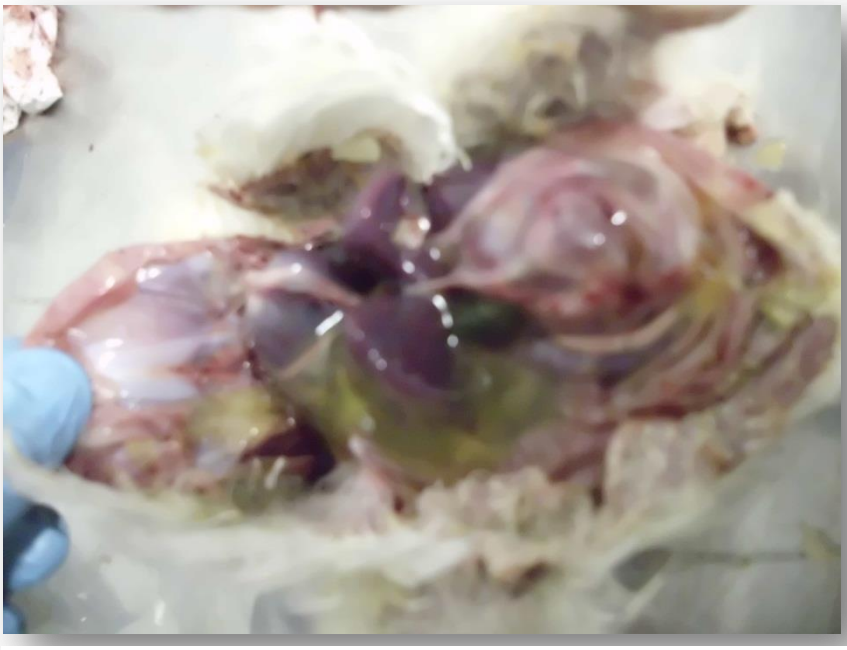


Figure 4.2 Ascetic broiler showing pericardial effusion and liver damage at 20 days of age

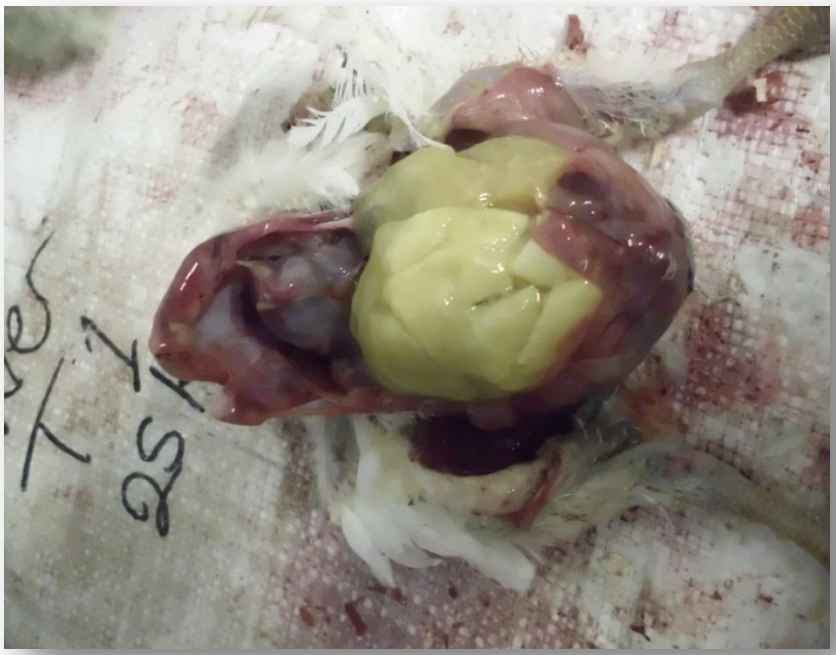


Figure 4.3 Fibrin clots and pericardial effusion in an ascetic broiler at 22 days of age

Table 4.14 Average variation within pen body weight of chicks incubated at different temperatures and fed different zinc and manganese levels and sources at 42 days of age

	Variance
Temperature	
Normal[1]	0.18
Hot[1]	0.19
SEM[6]	0.024
Diet	
Treatment 1[2]	0.25[a]
Treatment 2[3]	0.15[b]
Treatment 3[4]	0.19[ab]
Treatment 4[5]	0.14[b]
SEM[6]	0.032
Temperature × Dietary treatment	
Normal × Treatment 1	0.25
Hot × Treatment 1	0.25
Normal × Treatment 2	0.14
Hot × Treatment 2	0.16
Normal × Treatment 3	0.21
Hot × Treatment 3	0.17
Normal × Treatment 4	0.12
Hot × Treatment 4	0.17
SEM[6]	0.043
Source of variation	
P > F	
Temperature	0.78
Diet	0.03
Temperature × Diet	0.70

[a-b] Means in the same column with no common superscript differ significantly (P<0.05)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.15 Average right ventricular weight to total ventricular weight (RV/TV) ratio of chicks incubated at different temperatures and fed different zinc and manganese levels and sources at different days of age

	Days of age							
	D 17	D 18	D 19	D 20	D 38	D 40	D 41	D 42
				Temperature				
Normal[1]	0.23	0.22	0.22	0.22	0.22	0.21	0.22	0.22
Hot[1]	0.25	0.22	0.22	0.22	0.22	0.21	0.21	0.21
SEM[6]	0.011	0.0072	0.0054	0.0076	0.011	0.010	0.011	0.011
				Diet				
Treatment 1[2]	0.25	0.22	0.22	0.21	0.22	0.20	0.21	0.21
Treatment 2[3]	0.24	0.24	0.22	0.22	0.23	0.22	0.20	0.22
Treatment 3[4]	0.24	0.22	0.22	0.22	0.23	0.22	0.23	0.22
Treatment 4[5]	0.23	0.22	0.22	0.22	0.22	0.21	0.22	0.21
SEM[6]	0.014	0.0090	0.0076	0.011	0.015	0.014	0.014	0.016
				Temperature × Dietary treatment				
Normal × Treatment 1	0.25	0.22	0.22	0.22	0.21	0.19	0.22	0.21
Hot × Treatment 1	0.26	0.23	0.23	0.20	0.23	0.21	0.19	0.22
Normal × Treatment 2	0.22	0.25	0.22	0.21	0.22	0.22	0.19	0.22
Hot × Treatment 2	0.26	0.23	0.23	0.23	0.24	0.23	0.21	0.22
Normal × Treatment 3	0.23	0.22	0.24	0.20	0.26	0.23	0.24	0.24
Hot × Treatment 3	0.25	0.21	0.20	0.24	0.20	0.20	0.23	0.20
Normal × Treatment 4	0.24	0.21	0.22	0.23	0.20	0.22	0.24	0.23
Hot × Treatment 4	0.23	0.22	0.23	0.22	0.23	0.20	0.21	0.20
SEM[6]	0.0011	0.00035	0.00037	0.00075	0.0014	0.0011	0.00077	0.0016
				Source of variation				
P > F								
Temperature	0.36	0.10	0.73	0.63	0.80	0.78	0.19	0.37
Diet	0.82	0.11	0.98	0.95	0.80	0.61	0.19	0.98
Temperature × Diet	0.56	0.48	0.05	0.19	0.14	0.46	0.46	0.60

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg
[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg
[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg
[6] SEM= Standard error of the mean

Table 4.16 Percentage packed cell volume (% PCV) of chicks incubated at different temperatures and fed different zinc and manganese levels and sources at different days of age

	Days of age							
	D 17	D 18	D 19	D 20	D 38	D 40	D 41	D 42
				Temperature				
Normal[1]	34.3	35.8	30.5	32.8	35.4	36.2	38.2[A]	37.0
Hot[1]	35.6	35.1	31.3	33.6	38.9	35.3	34.7[B]	35.1
SEM[6]	1.17	0.74	0.88	0.85	0.64	0.77	0.87	0.93
				Diet				
Treatment 1[2]	36.1	37.0	33.2	32.9	34.9	35.9	35.1	35.2
Treatment 2[3]	33.3	35.4	31.4	32.8	35.1	35.7	36.0	36.1
Treatment 3[4]	35.7	34.2	28.9	32.7	37.2	35.3	36.8	35.8
Treatment 4[5]	35.5	35.0	30.0	34.2	35.4	36.1	37.8	37.3
SEM[6]	1.57	0.0093	1.25	1.14	0.91	1.09	1.20	1.31
				Temperature × Dietary treatment				
Normal × Treatment 1	35.5	37.8	32.4	33.0	33.1	36.8	35.8	34.6
Hot × Treatment 1	37.8	36.3	34.1	32.8	36.8	35.0	34.5	35.8
Normal × Treatment 2	32.8	34.1	30.8	32.1	35.2	35.2	36.8	38.0
Hot × Treatment 2	33.8	36.8	32.1	33.6	35.1	36.2	35.3	34.2
Normal × Treatment 3	37.0	35.2	28.2	32.3	38.6	35.6	39.3	36.0
Hot × Treatment 3	34.3	33.2	29.6	33.2	35.9	35.0	34.3	35.6
Normal × Treatment 4	32.7	36.0	30.5	33.7	34.9	37.3	41.0	39.6
Hot × Treatment 4	36.3	34.0	29.5	34.8	35.9	34.9	34.5	35.0
SEM[6]	0.027	0.013	0.018	1.61	1.29	1.54	1.67	1.85
				Source of variation				
P > F								
Temperature	0.44	0.54	0.50	0.48	0.61	0.40	0.009	0.16
Diet	0.64	0.20	0.12	0.76	0.30	0.96	0.46	0.71
Temperature × Diet	0.56	0.28	0.86	0.97	0.13	0.71	0.33	0.37

[A-B] Means in the same column with no common superscript differ significantly (P<0.01)
[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg
[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg
[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg
[6] SEM= Standard error of the mean

4.17 Carcass yields and portion weights

There were significant differences in carcass weights between chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures ($P<0.05$). In chicks that were incubated at normal temperatures, the carcass weights were heavier at slaughter as compared to those that were incubated at high temperatures. There were also significant differences in carcass weights for chicks that received different levels and sources of Zn/Mn ($P<0.05$). Chicks that were fed dietary Treatment 3 and 4 had heavier carcass weights at slaughter as compared to chicks that were fed Treatment 2. Carcass yields and portion weights are shown in Table 4.17.

There were no significant differences in fat pad weight between chicks that were incubated at high temperatures and those that were incubated at normal temperatures ($P>0.05$). Also, there were no significant differences between chicks that were fed different levels and sources of zinc ($P>0.05$). There were no significant differences in abdominal fat pad weight between chicks that were incubated at normal and high temperatures ($P>0.05$).

There were no significant differences in thigh weights between chicks that were incubated at normal temperatures and those that were incubated at high temperatures ($P>0.05$). Breast muscle weights between chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures was not significantly different ($P>0.05$). There were no significant differences in thigh weights between chicks that were incubated at normal temperatures and those that were incubated at high temperatures ($P>0.05$). The levels and sources of Zn/Mn did not significantly affect the wing weights in broilers ($P>0.05$). There were no significant differences in wing weights between chicks that were incubated at normal temperatures and those that were incubated at high temperatures ($P>0.05$) (Table 4.17).

Table 4.17 Carcass yields and portion weights (g) of broilers incubated at different temperatures and fed different zinc and manganese levels and sources at 42 days of age

	Carcass	Abdominal fat pad	Thigh	Breast (plus bone)	Breast (no bone)	Wing
	(g)	(g/100g)				
Temperature						
Normal[1]	2135.94[a]	1.76	28.02	34.42	28.62	10.19
Hot[1]	2094.17[b]	1.68	27.6	34.62	28.86	10.07
SEM[6]	87.07	0.070	0.18	0.27	0.18	0.086
Diet						
Treatment 1[2]	2112.29[ab]	1.78	27.71	34.93	28.96	10.12
Treatment 2[3]	2076.46[b]	1.78	27.60	34.29	28.36	10.08
Treatment 3[4]	2145.21[a]	1.65	27.97	34.57	29.18	10.12
Treatment 4[5]	2126.25[a]	1.66	27.92	34.31	28.46	10.19
SEM[6]	87.78	0.088	0.25	0.27	0.25	0.10
Temperature × Dietary treatments						
Normal × Treatment 1	2117.92	1.74	28.05	34.53	28.63	10.31
Hot × Treatment 1	2106.67	1.82	27.37	35.32	29.29	9.93
Normal × Treatment 2	2100.42	2.00	28.07	34.27	28.57	10.12
Hot × Treatment 2	2052.50	1.56	27.13	34.31	28.15	10.03
Normal × Treatment 3	2158.33	1.66	27.97	34.60	28.83	10.04
Hot × Treatment 3	2132.08	1.65	27.97	34.59	29.53	10.21
Normal × Treatment 4	2167.08	1.63	28.00	34.54	28.46	10.29
Hot × Treatment 4	2085.42	1.70	27.85	34.33	28.48	10.09
SEM[6]	89.17	0.012	0.34	0.35	0.35	0.13
Source of variation						
P > F						
Incubation	0.021	0.44	0.06	0.37	0.32	0.13
Diet	0.009	0.31	0.65	0.15	0.06	0.80
Incubation × Diet	0.42	0.05	0.46	0.50	0.32	0.12

[a-d] Means in the same column with no common superscript differ significantly (P<0.05)

[A-C] Means in the same column with no common superscript differ significantly (P<0.01)

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg

[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg

[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg

[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg

[6] SEM= Standard error of the mean

Table 4.18 Tibia ash percentage of chicks incubated at different temperatures and positions within the machine at hatch (E 21.5 of incubation)

	Tibia ash (%)
Temperature	
Normal[1]	39.0± 0.75
Hot[1]	38.1 ± 0.77
Position	
Top[2]	38.8 ± 0.67
Centre[3]	38.4 ± 0.94
Bottom[4]	38.5 ± 0.71
Temperature × Position	
Normal × Top	38.8 ± 0.96
Normal × Centre	39.9 ± 1.29
Normal × Bottom	38.3 ± 0.98
Hot × Top	38.7 ± 0.94
Hot × centre	36.9 ± 1.36
Hot × Bottom	38.8 ± 1.03
Source of variation	
P > F	
Temperature	0.49
Position	0.92
Temperature × Position	0.25

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Top-Trays in the top section of the machine

[3] Centre-Trays in the middle section of the machine

[4] Bottom-Trays in the bottom section of the machine

Table 4.19 Concentration of Zn within tibia ash (g Zn/kg ash ± SEM) of chicks incubated at different temperatures and positions within the machine at hatch (E 21.5 of incubation)

	Tibia ash (g Zn/kg ash)
Temperature	
Normal[1]	39.2 ± 0.72
Hot[1]	38.3 ± 0.72
Position	
Top[2]	39.1 ± 0.68
Centre[3]	38.7 ± 0.90
Bottom[4]	38.6 ± 0.68
Temperature × Position	
Normal × Top	39.5 ± 0.96
Normal × Centre	39.9 ± 1.27
Normal × Bottom	38.3 ± 0.96
Hot × Top	38.7 ± 0.96
Hot × centre	37.5 ± 1.27
Hot × Bottom	38.8 ± 0.96
Source of variation	
P > F	
Temperature	0.46
Position	0.83
Temperature × Position	0.39

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)

[2] Top-Trays in the top section of the machine

[3] Centre-Trays in the middle section of the machine

[4] Bottom-Trays in the bottom section of the machine

Table 4.20 Metatarsal ash percentage of chicks incubated at different temperatures and fed different zinc and manganese levels and sources at 21days of age

	Tibia ash (%)
Temperature	
Normal[1]	49.50
Hot[1]	49.35
SEM[6]	0.12
Diet	
Treatment 1[2]	49.05[c]
Treatment 2[3]	49.34[bc]
Treatment 3[4]	49.77[a]
Treatment 4[5]	49.55[ab]
SEM[6]	0.16
Temperature × Dietary treatment	
Normal × Treatment 1	49.06
Hot × Treatment 1	49.03
Normal × Treatment 2	49.43
Hot × Treatment 2	49.24
Normal × Treatment 3	49.81
Hot × Treatment 3	49.74
Normal × Treatment 4	49.71
Hot × Treatment 4	49.38
SEM[6]	
Source of variation	
P > F	
Temperature	0.28
Diet	0.0033
Temperature × Diet	0.88

[a-c] Means in the same column with no common superscript differ significantly (P<0.05)
[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg
[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg
[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg
[6] SEM= Standard error of the mean

Table 4.21 Concentration of Zn within metatarsal ash (mg Zn/kg ash) of chicks incubated at different temperatures and fed different zinc and manganese levels and sources at 21days of age

	Metatarsal zinc (mg/kg ash)
Temperature	
Normal[1]	464.92
Hot[1]	457.58
SEM[6]	7.26
Diet	
Treatment 1[2]	463.88
Treatment 2[3]	459.81
Treatment 3[4]	462.19
Treatment 4[5]	459.13
SEM[6]	8.19
Temperature × Dietary treatment	
Normal × Treatment 1	466.83
Hot × Treatment 1	460.92
Normal × Treatment 2	463.84
Hot × Treatment 2	455.78
Normal × Treatment 3	461.38
Hot × Treatment 3	463.00
Normal × Treatment 4	467.62
Hot × Treatment 4	450.64
SEM[6]	9.79
Source of variation	
P > F	
Temperature	0.18
Diet	0.92
Temperature × Diet	0.68

[1] Eggshell temperature (EST): Normal (37.5 °C) and Hot (38.5 °C)
[2] Treatment 1= Inorganic Zn/Mn= 80 mg/kg
[3] Treatment 2= Inorganic Zn/Mn= 120 mg/kg
[4] Treatment 3= Inorganic Zn/Mn= 80 mg/kg; Organic Zn/Mn= 40 mg/kg
[5] Treatment 4= Inorganic Zn/Mn= 40 mg/kg; Organic Zn/Mn= 80 mg/kg
[6] SEM= Standard error of the mean

4.18 Percentage of tibia ash in chicks at hatch (day 21.5)

There were no significant differences in tibia ash between broilers that were incubated at normal temperatures and those that were incubated at high temperatures (P>0.05) (Table 4.18). Also, there were no significant differences in tibia ash for chicks that were incubated at different positions within the machine (P>0.05).

4.19 Concentration of Zn within tibia ash at hatch (day 21.5)

There were no significant differences in the concentration of Zn within tibia between chicks that were incubated at normal temperatures and those that were incubated at high temperatures (P>0.05) (Table 4.19). Also, there were no significant differences in the concentration of Zn within tibia ash between chicks that were incubated at different positions within the machine (P>0.05).

4.20 Ash percentage in the metatarsal bone of chicks at 21 days of age

There were no significant differences in percentage metatarsal ash between broilers that were incubated at normal temperatures and those that were incubated at high temperatures (P>0.05) (Table 4.20). However, there were significant differences in metatarsal ash percentage of broilers that received different levels and sources of zinc and manganese from 0-21 days of age (P<0.05). Treatment 4 (ZI40+80 MI40+80) had increased metatarsal ash percentage when it was compared to Treatment 1 (ZI80 MI80).

4.21 Zinc percentage in the metatarsal bone of broilers at 21 days of age

There were no significant differences in percentage metatarsal zinc in broilers that were incubated at normal temperatures and broilers that were incubated at hot temperature (P>0.05) (Table 4.21). Foot zinc percentage was also not significantly different between broilers that received different levels and sources of Zn/Mn (P>0.05).

CHAPTER 5
DISCUSSION

5.1 Hatchability and chick quality

Poor incubation conditions affect the development, chick quality, and the ultimate performance of chicks (Molenaar, 2010; Louren *et al*., 2011; Ipek *et al*., 2014). In this trial, there were no observed differences in hatchability between eggs that were incubated at normal and those that were incubated at high temperatures. However, the percentage hatchability was affected by the basket position within the machine. The bottom baskets which were evidently cooler during the incubation process exhibited increased percentage unhatched eggs and reduced percentage hatch as compared to the top and middle baskets. When the incubation temperature was too low during the incubation period, embryonic mortality was increased, and therefore hatchability and chick quality were decreased (Ipek *et al*., 2014). The bottom trays had a lower number of first grade chicks and a higher number of second grade and culled chicks at hatch. This was due to lower ESTs during incubation in the bottom trays that affected the rate of embryonic development and ultimately chick quality.

5.2 Pre-hatch mortalities

Yildirim & Yetisir (2004) found an increased number of late embryonic mortalities when incubation temperature was decreased below optimum. The reduction in incubation temperature resulted in extended incubation periods, poor quality hatchlings and increased embryonic mortalities. This was in agreement with the present findings where increased late embryonic mortalities were observed due to lower EST. Willemsen *et al*. (2010) also found that there was increased embryonic mortality and decreased hatchability when incubation temperature was reduced. A reduction in incubation temperature in the bottom trays was also associated with a decreased % hatch and increased % pipped eggs with dead embryos.

Wilson (1991) found that a reduction in incubation temperature below optimum resulted in increased number of malposition embryos. This was also observed in the present research where the bottom trays had decreased EST and increased malposition embryos.

5.3 Chick body weight at hatch

Incubation temperature is the first limiting factor that needs to be closely monitored in order to yield good quality chicks at hatch (Ipek *et al*., 2014). Chick embryos are poikilotherms (internal temperature varies according to incubator temperature) at the beginning of incubation and the EST influences oxygen utilisation and embryonic growth (Romanoff, 1960). In different studies, the optimum incubation temperature required to attain

the best hatchability and hatchling quality was achieved at 37.5 °C (Leksrisompong *et al*., 2009; Molenaar *et al*., 2011a). Yalcin *et al*. (2008) also recorded a decrease in BW when chicken embryos were subjected to increased EST. In this study, the BW between the normal and hot incubation temperature treatment was significantly different at hatch. The reduction in yolk free body mass (YFBM) for the hot incubation temperature was a result of a shorter hatching window and poor yolk absorption (Lourens *et al*., 2005). Chicks that were incubated at high temperatures exhibited lower BW, poor yolk absorption, and light yellow feathers. This BW reduction was caused by an increased retained yolk that was not absorbed by the body. Chicks that hatched in the middle trays weighed less at hatch and this was explained by poor yolk absorption due to limited oxygen supply. This finding was in agreement with Ipek *et al*. (2014) where it was shown that oxygen supply is essential to metabolise fat sources in the yolk sac to initiate body growth (Ipek *et al*., 2014).

5.4 Organ weights of chicks at hatch

The heart is responsible to pump the blood in order to supply vital tissues and organs with oxygen and nutrients necessary for growth (Romanoff, 1960). Leksrisompong *et al*. (2009) observed a 28% reduction in heart weights when EST was increased beyond optimum (37.5-38.0 °C). In this trial, there were no reduced heart sizes observed from chicks that were incubated at high incubation temperatures as compared to chicks that were incubated at normal temperatures. In general, the heart weights at hatch were greater than 1 % of the body weight. This problem might have been a result of low incubation temperatures that results in greater heart weights at hatch (Leksrisompong, 2005)

According to Leksrisompong (2005), a decreased EST affected the rate of cell division and organ development at hatch. In this trial, the bottom section of the machine had a lower EST due to inadequate heat circulation by the machine as compared to the top and middle sections within the machine. This explained a reduction in gizzard and proventriculus weights of the chicks from the bottom baskets.

5.5 Weekly body weight

In broiler operations, the quality and viability of the day old chicks are vital in order to achieve good post-hatch live performance. Proper development of the immune system, thermoregulatory system, and digestive tract is important, all of which had a marked effect on BW and post-hatch live performance (Leksrisompong, 2005). At 7 days of age, the chicks from the normal treatment were significantly heavier than those from the hot treatment due to better organ development at hatch which improved nutrient utilisation and ultimate performance. At 42 days of age, broilers from the normal incubation temperature weighed more than the hot treatment. Hulet *et al*. (2007) also reported that high temperature during the late incubation phase affected embryonic development and chick quality, which influenced post-hatch performance.

Broilers that received organic Zn and Mn in their diets weighed more when compared to chicks that received inorganic Zn. This was also confirmed by Virden *et al*. (2004) where chicks that received diets that were supplemented with organic minerals were heavier at slaughter as compared to the chicks from treatments supplemented with inorganic minerals.

5.6 Average body weight gain

At 42 days of age, broilers from the high incubation treatment had gained more BW than the hot incubation treatment. These findings were not in agreement with Leksrisompong (2005), where it was shown that hot incubation affected organ development and performance. However, average BW gain was affected by the level and source of Zn and Mn fed to broilers. Broilers that received diets supplemented with organic minerals (Treatment 3 and 4) gained more BW as compared to those that were supplemented with inorganic minerals (Treatment 1 and 2). These research findings were in agreement with Star *et al.* (2012) where it was shown that chicks fed diets supplemented with organic minerals gained more BW when compared to chicks that were supplemented with inorganic minerals.

5.7 Cumulative feed intake

At 14 days of age, chicks from the normal incubation treatment had consumed more feed as compared to those from the hot incubation treatment. This was in agreement with Leksrisompong *et al*. (2009) where it was shown that chicks incubated at normal temperatures were active and consumed more feed earlier at placement as compared to the hot incubation chicks that tended to huddle together and spend less time consuming feed.

The dietary source of Zn and Mn did not affect feed intake between chicks that were supplemented with either organic or inorganic Zn and Mn. This was in agreement with Star *et al.* (2012) where it was shown that there were no differences in feed intake between chicks that were supplemented with either organic or inorganic minerals.

5.8 Mortality adjusted feed conversion ratio

Chicks that were incubated at high temperatures exhibited a better FCR during 0-14 days of age as compared to those that were incubated at normal temperatures. Our findings were different from Leksrisompong (2005) where it was shown that chicks incubated at normal temperatures exhibited a better FCR as compared to those incubated at high temperatures. At 22-28 days of age, broilers that were incubated at normal temperatures exhibited a better FCR as compared to broilers that were incubated at high temperatures. At 36-42 days of age, broilers that received diets supplemented with organic Zn and Mn exhibited a better FCR as compared to broilers that received diets supplemented with inorganic Zn and Mn.

Arce-Menocal *et al.* (2004) did not find significant differences in FCR in chicks that received diets supplemented with either organic or inorganic Zn.

5.9 Post-hatch mortalities

Chicks that were incubated at high temperatures had a greater ascites related mortality rate as compared to those that were incubated at normal temperatures. This was in agreement with Hulet *et al*. (2007) and Leksrisompong *et al*. (2009) who found higher mortality rates in chicks that were incubated at high temperatures as compared to those that were incubated at normal temperatures. Poor organ and heart development predisposed these chicks to poor blood and oxygen circulation, which ultimately resulted in ascites. Lourens *et al*. (2005) shown that post hatch growth and organ function were impaired when chicks were incubated at high temperatures. At hatch, the heart weights for chicks that were incubated at hot temperatures were reduced and this resulted in poor oxygenated blood circulation and ultimately ascites syndrome.

Chicks that received diets that were supplemented with organic Zn and Mn had lower ascites related mortalities and total mortality. This was also in agreement with Arce-Menocal *et al*. (2004) where it was shown that chicks that were fed an organic Zn supplemented diet exhibited a lower mortality rate as compared to chicks that were supplemented with inorganic Zn.

5.10 Body weight variation within pen (uniformity)

Chicks that were incubated at normal temperatures and chicks that were incubated at high temperatures did not show differences in within-pen BW variation. However, chicks that were fed inorganic Zn at a lower end inclusion rate (80 mg/kg) exhibited high within-pen BW variation. This was explained by the varying ability of chicks to absorb and utilise inorganic Zn and Mn in the diet. Inorganic minerals are less bioavailable to chicks and result in poor BW uniformity (Mohanna & Nys, 1999).

5.11 Right ventricular weight to total ventricular weight (RV/TV) ratio

Right ventricle-to-total ventricle ratio (RV/TV) of more than 0.29 is considered as an accurate indicator of the onset of ascites (Huchzermeyer and De Ruyck, 1986). According to Malan *et al*. (2003) and Wideman *et al*. (2013) broilers prone to and affected by pulmonary hypertension syndrome expressed significantly greater right ventricles (RV) to total ventricles (TV) ratio. In our research findings the RV/TV ratio was between 0.21-0.22. In future, it might be required that the study be conducted for a longer period in order to observe differences in RV/TV ratio in broilers.

5.12 Packed cell volume (PCV)

An increase in haematocrit values were observed in broilers that were prone to ascites syndrome during week 5 of growing (Luger *et al*., 2001). Shlosberg *et al*. (1996) and Scheele *et al. (*2003) found high haematocrit values in broilers prone to and affected by ascites syndrome. However, this was not observed in our research finding where broilers that were incubated at normal temperatures had higher haematocrit values as compared to broilers that were incubated at high temperatures. Bolukbasi *et al.* (2005*)* also found increased haematocrit scores to be associated with increased ascites mortality rate. However, chicks that were incubated at normal temperatures had a lower mortality rate related to ascites as compared to those incubated at high temperatures. This showed that the high haematocrit values in normal incubated chicks was a reflection of increased red blood cell count necessary to transport oxygen, not necessarily as an indication of the onset of ascites.

5.13 Carcass traits at slaughter

Chicks that were incubated at optimal temperatures had an ability to perform better as compared to chicks that were incubated at high incubation temperature (Decuypere & Bruggeman, 2007). This was in agreement with our research findings where improvements in carcass weights were observed in broilers that were incubated at normal temperatures as compared to those that were incubated at high temperatures.

Organic minerals are highly bioavailable as compared to inorganic minerals fed to broilers (Lu *et al*., 2006; Lui *et al*., 2011). This was shown by improvements in carcass weights when organic Zn and Mn were supplemented in broiler diets. This was in agreement with our research findings, where the carcass weights were heavier for broilers that were supplemented with organic Zn and Mn treatments compared to broilers that were supplemented with inorganic Zn and Mn. These research findings suggest that the high bioavailability of organic minerals improved growth and ultimately yield heavier carcass weights at slaughter. Although carcass portions were not different at slaughter, but the carcass weights were heavier for the organic mineral treatment.

5.14 Percentage of tibia ash in chicks at hatch (E 21.5)

There were no significant differences in tibia ash between chicks that were incubated at normal and those that were incubated at high temperatures. However, chicks at normal incubation deposited numerically higher percentage ash than those chicks incubated at high temperatures. This was due to optimal incubation conditions for the normal incubated chick that improved the absorption of minerals from the yolk. The chicks that were incubated at high temperatures had impaired development and a shorter hatching period due to high metabolic rate as compared to chicks that were incubated at normal temperatures.

5.15 Concentration of Zn within tibia ash at hatch (E 21.5)

Zinc concentration in tibia was also affected by the incubation temperature that the chicks were exposed to. The normal incubation treatment had more Zn within tibia as compared to chicks that were incubated at high temperatures. At normal incubation temperature chicks had an ability to optimally absorb Zn from the yolk and the hatching period was sufficient to allow adequate Zn uptake.

5.16 Percentage ash in the metatarsal bone of chicks at 21 days of age

There were significant differences in percentage ash within the metatarsal bones of broilers that were supplemented with different levels and sources of Zn and Mn. Broilers that were supplemented with high levels of organic Zn and Mn had more ash content in the metatarsal bones as compared to broilers that were supplemented with inorganic Zn and Mn. According to Mohanna & Nys (1999) and Owens *et al*. (2009) this was due to a higher bioavailability of the organic minerals as compared to inorganic minerals in broiler diets.

5.17 Percentage zinc in the metatarsal bone of broilers at 21 days of age

Broilers that were incubated at normal temperatures exhibited a greater Zn content in the metatarsal bones as compared to broilers that were incubated at high temperatures. This was due to the optimum hatching window that allowed the broilers to sufficiently absorb Zn from the yolk and deposit on the bone.

CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

Ascites syndrome is a multifactorial disease that affects fast growing commercial broiler flocks. Various breeding programs, incubation methods, nutritional improvements, and management have been used to reduce the incidence of pulmonary hypertension syndrome ("ascites"). Broiler breeding companies have been focused on improving the growth rate of broiler birds while disregarding the cardiovascular system of these birds. This has resulted in complications related to broiler physiology that may have caused the metabolic rate of the broiler to function beyond its physiological limit. Metabolic diseases like 'ascites' syndrome has been observed since the success of breeding for growth rate was achieved. A modern broiler has the ability to reach slaughter BW in 60% less time than broilers 40 years ago. This growth has been achieved at an expense of welfare, increased carcass condemnations, and ascites mortalities. Improved management practises are required in order to improve broiler production such as proper chick incubation conditions and the use of highly bioavailable organic minerals to improve broiler performance.

Eggshell temperature (EST) should be closely monitored on a daily basis during incubation since it is a close estimate of true embryo temperature. Therefore, EST is a useful tool for determining whether or not the machine temperature should be adjusted. This will improve the incubation process, yield first grade chicks and alleviate ascites mortalities during the broiler rearing period. Incubation at optimum temperatures (37.8-38.0 °C) improved chick BW at hatch and reduced ascites syndrome mortalities during the rearing period.

A partial replacement of inorganic minerals also improved broiler live performance during the rearing period. A 30-40 % replacement of $ZnSO_4$ and $MnSO_4$ in the diet is a beneficial and economical strategy to improve broiler performance. The inclusion rate of 120 mg/kg of Zn/Mn in sulphate forms is an upper industry standard and replacing 40 mg/kg with Availa Zn and Mn improved BW gains, reduced mortalities and increased carcass weights slaughter. The effects of organic Zn and Mn were low in terms of performance in this study. This might have been due to other major factors interacting with Zn and Mn or other factors that might have affected the absorption. This was confirmed by no differences in percentage metatarsal ash at 21 days of age between the organic and inorganic treatments.

However, more research on a stepwise replacement of inorganic minerals at different environments is still required in order to understand the exact upper levels at which the replacement of inorganic minerals will be beneficial and cost effective. The largest dietary problem in South Africa are high levels Ca, P and K that might interact with Zn and Mn absorption causing them less effective in alleviating ascites.

For future researchers, a proper ingredient analyses for other trace minerals like Ca, P and K will be required to eliminate mineral interaction before studies with Zn and Mn are conducted. Proper raw material analyses for mineral content will be crucial when studies of mineral absorption are to be conducted.

CHAPTER 7
REFERENCES

Aftab, U. & Khan A.A., 2005. Strategies to alleviate the incidence of ascites in broilers: A review. Braz. J. Poult. Sci. 7(4), 199-204.

Ao, T., Pierce, J.L., Pescatore, A.J., Cantor, A.H., Dawson, K.A., Ford, M.J. & Paul, M., 2011. Effects of feeding different concentration and forms of zinc on the performance and tissue mineral status of broiler chicks, Br. Poult. Sci. 52(2), 466-471.

AOAC, 1990. Vitamin D3 in poultry feed supplements. Method 932.16. In Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists, Arlington, VA. 1094-1095.

AOAC, 1990. Vitamin D3 in poultry feed supplements. Method 963.15. In Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists, Arlington, VA. 1094-1095.

AOAC, 2000. Official methods of analysis (17th ed.). Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA.

Apines, M.S., Satoh, S., Kiron, V., Watanabe, T. & Fujita, S., 2003. Bioavailability and tissue distribution of amino acid-chelated trace elements in rainbow trout *Oncorhynchus mykiss*. Fish. Sci. 69, 722-730.

Arce-Menocal, J.A., Gonzalez, E.A., Coello, C.L., Fankler, T.M., Rapp, C.J., Ward, T.L. & Guillermo Vela, S., 2004. Use of zinc-methionine and manganese-methionine in broilers diets: productive parameters and ascites syndrome incidence. Tec. Pecu. Mex. 42(1), 113-119.

Attia, Y.A., Qota, E.M., Bovera, F., Tag El-Din, A.E. & Mansour, S.A., 2010. Effect of amount and source of manganese and/or phytase supplementation on productive and reproductive performance and some physiological traits of dual purpose cross-bred hens in the tropics, J. Br. Poult. Sci. 51(51), 235-245.

Baden, S.P. & Eriksson, S.P., 2006. Role, routes and effects of manganese in crustaceans. J. Oceanogr. Mar. Biol. 44, 61-83.

Baghbanzadeh, A. & Decuypere, E., 2008. Ascites syndrome in broilers: physiological and nutritional perspectives. J. Avian Pathol. 37(2), 117-126.

Balog, J.M., Anthony, N.B., Cooper, M.A., Kidd, B.D., Huff, G.R., Huff, W.E. & Rath, N.C., 2000a. Ascites syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. J. Poult. Sci. 79, 318-323.

Balog, J.M., Huff, G.R., Rath, N.C. & Huff, W.E., 2000b. Effect of dietary aspirin on ascites in broilers raised in a hypobaric chamber. J. Poult. Sci. 79, 1101-1105.

Balog, J.M., 2003. Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: Are we seeing light at the end of the tunnel? Avian Poult. Biol. Rev. 14(3), 99-126.

Bao, Y.M., Choct, M., Iji, P.A. & Bruerton, K., 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Appl. Poult. Res. 16, 448-455.

Barri , A., Honaker, C.F., Sottosanti, J.R., Hulet, R.M. & McElroy, A.P., 2011. Effect of incubation temperature on nutrient transporters and small intestine morphology of broiler chickens. J. Poult. Sci. 90, 118-125.

Beker, A., Vanhooser, S.L., Swartzlander, J.H. & Teeter, R.G., 2003. Graded atmospheric oxygen level effects on performance and ascites incidence in broilers. J. Poult. Sci. 82, 1550-1553.

Bendheim, U., Berman, E., Zadikov, I. & Shlosberg, A., 1992. The effects of poor ventilation, low temperatures, type of feed and sex of bird on the development of ascites in broilers. Production parameters, J. Avian Pathol. 21(3), 383-388.

Bessei, W., 2006. Welfare of Broilers: A review. J. Poult. Sci. 62, 455-466.

Bölükbasi, S.C., Sinan Aktas, M. & Güzel, M., 2005. The effect of feed regimen on ascites induced by cold temperatures and growth performance in male broilers. Int. J. Poult. Sci. 4(5), 326-329.

Bottje, W.G., Wang, S., Kelly, F.J., Dunster, C., Williams, A. & Mudway, I., 1998. Antioxidant defences in lung lining fluid of broilers: impact of poor ventilation conditions. J. Poult. Sci. 77, 516-522.

Brickett, K.E., Dahiya, J.P., Classen, H.L., & Gomis, S., 2007. Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. J. Poult. Sci. 86, 2172-2181.

Bruzual, J.J., Peak, S.D., Brake, J. & Peebles, E.D., 2000. Effects of relative humidity during incubation on hatchability and body weight of broiler chicks from younger breeder flocks. J. Poult. Sci. 79, 827-830.

Buys, N., Buyse, J., Hassanzadeh-Ladmakhi, M. & Decuypere, E., 1998. Intermittent lighting reduces the incidence of ascites in broilers: an interaction with protein content of feed on performance and the endocrine system. J. Poult. Sci. 77, 54-61.

Camacho, M.A., Suarez, M.E., Herrera, J.G., Cuca, J.M. & Garcia-Bojalil, C.M., 2004. Effect of age of feed restriction and microelement supplementation to control ascites on production and carcass characteristics of broilers. J. Poult. Sci. 83, 526-532.

Cangar, O., Aerts, J.M., Vranken, E. & Berckmans, D., 2007. Online growth control as an advance in broiler farm management. J. Poult. Sci. 86, 439-443.

Chan, T. & Burggren, W., 2005. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (*Gallus gallus*). Respir. Physiol. Neurobiol. 145, 251-263.

Clauer, P. J., 2009. Incubating Eggs. Virginia Polytechnic Institute and State University. Department of Animal and Poultry Sciences.

COBB hatchery guide. 2008. [Online available] www. cobb-vantress.com.

Collins, N.E., & Moran, E.T., 1999. Influence of supplemental zinc and manganese on live performance and carcass quality of broilers. J. Appl. Poultry Res. 8, 222-227.

Currie, R.J.W., 1999. Ascites in poultry: Recent investigations, Avian Pathol. 28(4), 313-326

Decuypere E., Buyse, D. & Buyse, N., 2000. Ascites in broiler chicken: Exogenous and endogenous structural and functional causal factors. World's Poult. Sci. J. 56, 367-376.

Decuypere, E. & Bruggeman, V., 2007. The endocrine interface of environmental and egg factors affecting chick quality. J. Poult. Sci. 86, 1037-1042.

Decuypere, E., Onagbesan, O., De Smit, L., Tona, K., Everaert, N., Witters, A., Debonne, M., Verhoelst, E., Buyse, J., Hassanzadeh, M., De Baerdemaeker, J., Arckens, L. & Bruggeman, V., 2006. Hypoxia and hypercapnia during incubation of chicken eggs: effects on development and subsequent performance. In XII European Poultry Conference 10-14 Sep, Verona, Italy.

Dolegowska, B., Machoy, Z. & Chlubek, D., 2003. Changes in the content of zinc and fluoride during growth of the femur in chicken. Biol. Trace Elem. Res. 91, 67-76.

Elibol, O. & Brake, J., 2006. Effect of egg turning angle and frequency during incubation on hatchability and incidence of unhatched broiler embryos with head in the small end of the egg. J. Poult. Sci. 85, 1433-1437.

Feast, M., Noble, R.C., Speake, B.K & Ferguson, M.W.J., 1998. The effect of temporary reductions in incubation temperature on growth characteristics and lipid utilisation in the chick embryo. J. Anat. 193, 383-390.

Franciosini, M.P., Tacconi, G. & Leonardi, L., 2012. Ascites syndrome in broiler chickens. Vet. Sci. Res. 3(1), 60-66.

French, N.A., 1997. Modelling incubation temperature: The effects of incubator design, embryonic development, and egg size. J. Poult. Sci. 76, 124-133.

Gupta, A.R., 2011. Ascites syndrome in poultry: A review. World's Poult. Sci. J. 67, 457-468.

Hassanzadeh, M., 2009. New approach for the incidence of ascites syndrome in broiler chickens and management control the metabolic disorders. Int. J. Poult. Sci. 8(1), 90-98.

Herrera, E.A., Salinas, C.E., Blanco, C.E., Villena, M. & Giussani, D.A., 2013. High altitude hypoxia and blood pressure dysregulation in adult chickens. J. Dev Orig. Health. Dis. 4(1), 69-76.

Huang, Y.L., Lu, L., Luo, X.G. & Liu, B., 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. J. Poult. Sci. 86, 2582-2589.

Huchzermeyer, F.W. & De Ruyck, A.M.C., 1986. Pulmonary hypertension syndrome associated with ascites in broilers. Vet. Rec. 119(94), 113-114

Hudson, B.P., Dozier, W.A. & Wilson, J.L., 2005. Broiler live performance response to dietary zinc source and the influence of zinc supplementation in broiler breeder diets. Anim. Feed Sci. Tech. 118, 329-335.

Hulet, R. M., 2007. Managing Incubation: Where Are We and Why? J. Poult. Sci. 86, 1017-1019.

Ipek, A, Sahan, U., Baycan, S.C. & Sozcu A. 2014. The effects of different eggshell temperatures on embryonic development, hatchability, chick quality and first-week broiler performance. J. Poult. Sci. 93, 464-472.

Isaac, Y.M., Abraham, J., Sreeparvathy, George, J. & Balusami. C., 2010. Managemental practices to control ascites in a flock. Vet. World 5(3), 250-252.

Izadinia, M., Nobakht, M., Khajali, F., Faraji, M., Zamanib, F., Qujeq, D. & Karimi, L., 2010. Pulmonary hypertension and ascites as affected by dietary protein source in broiler chickens reared in cool temperature at high altitudes. Anim. Feed Sci. Tech. 155, 194-200.

Ji, F., Luo, X.G., Lu, L., Liu, B. & Yu, S.X., 2006a. Effect of manganese source on manganese absorption by the intestine of broilers. J. Poult. Sci. 85, 1947-1952.

Ji, F., Luo, X.G., Lu, L., Liu, B. & Yu, S.X., 2006b. Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broilers' intestinal segments. J. Poult. Sci. 85, 1217-1225.

Julian, R.J., Summers, J. & Wilson, J.B., 1986. Right ventricular failure and ascites in broiler chickens caused by phosphorus-deficient diets. Avian Dis. 30(3), 453-459.

Julian, R.J., 1988. Ascites in meat-type ducklings. Avian Pathol. 17, 11-21.

Julian, R.J., McMillan, I. & Quinton, M., 1989. The effect of cold and dietary energy on right ventricular hypertrophy, right ventricular failure and ascites in meat-type chickens, Avian Pathol., 18(4), 675-684.

Julian, R.J., 1990. Pulmonary hypertension: A cause of right heart failure in meat type chickens. Feedstuffs 78, 19-22.

Julian, R.J., 1993. Ascites in poultry, Avian Pathol. 22(3), 419-454.

Julian, R.J., 1998. Rapid Growth Problems: Ascites and skeletal deformities in broilers. J. Poult. Sci. 77, 1773-1780.

Julian, R.J., 2000. Physiological, management and environmental triggers of the ascites syndrome: A review: Avian Pathol. 29(6), 519-527.

Julian, R.J., 2005. Production and growth related disorders and other metabolic diseases of poultry: A review. Vet. J. 169, 350-369.

Karamouz, H., Ghalehkandi, J.G., Zadeh Adam Nazhad, H., Ebrahim Nezhad, Y & Maheri Sis, N., 2011. Effect of different levels of zinc oxide supplement on mucosal sucrase enzyme activity in small intestine of male broiler chicks. Int. J. Anim. Vet. Adv. 3(2), 54-57.

Lamas da Silva, J.M., Dale, N. & Luchesi, J. B., 1988. Effect of pelleted feed on the incidence of ascites in broilers reared at low altitudes. Avian Dis. 32, 376-378.

Leeson, S., 2007. Metabolic Challenges: Past, Present, and Future. J. Appl. Poult. Res. 16, 121-125.

Leksrisompong, N., 2005. Effect of temperature during incubation and brooding on broiler chickens. Master's thesis, Poultry Science, Graduate Faculty of North Carolina State University, N.C., USA.

Leksrisompong, N., Romero-Sanchez, H., Plumstead, P.W., Brannan, K.E. & Brake, J., 2007. Broiler incubation. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. J. Poult. Sci. 86, 2685-2691.

Leksrisompong, N., Romero-Sanchez, H., Plumstead, P.W., Brannan, K.E., Yahav, S. & Brake, J., 2009. Broiler incubation. 2. Interaction of incubation and brooding temperatures on broiler feed consumption and growth. J. Poult. Sci. 88, 1321-1329.

Lorenzoni, A.G. & Ruiz-Feria, C.A., 2006. Effects of vitamin E and arginine on cardiopulmonary function and ascites parameters in broiler chickens reared under subnormal temperatures. J. Poult. Sci. 85, 2241-2250.

Lourens, A., van den Brand, H., Meijerhof, R. & Kemp, B., 2005. Effect of eggshell temperature during incubation on embryo development, hatchability and posthatch development. J. Poult. Sci. 84, 914-920.

Lourens, A., Meijerhof, R., Kemp B. & van den Brand, H., 2011. Energy partition during incubation and consequences for embryo temperature: A theoretical approach. Poult. Sci. 90, 516-523.

Lu, L., Ji, C., Lu, X.G., Liu, B. & Yu, S.X., 2006. The effect of supplemental manganese in broiler diets on abdominal fat deposition and meat quality. J. Anim. Feed Sci. Tech. 129, 49-59.

Luger, D., Shinder, D., Rzepakovsky, V., Rusal, M. & Yahay, S., 2001. Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. J. Poult. Sci. 80, 965-971.

Luger, D., Shinder, D. & Yahav, A., 2002. Hyper- or hypothyroidism: its association with the development of ascites syndrome in fast growing chickens. Gen. Comp. Endocrinol. 127, 293-299.

Lukaski, H.C., 2005. Low dietary zinc decreases erythrocyte carbonic anhydrase activities and impairs cardiorespiratory function in men during exercise. Am. J. Clin. Nutr. 81, 1045-51.

Luo, X.G., Li, S.F., Lu, L., Liu, B., Kuang, X., Shao, G.Z. & Yu, S.X., 2007. Gene expression of manganese-containing superoxide dismutase as a biomarker of manganese bioavailability for manganese sources in broilers. J. Poult. Sci 86, 888-894.

Lyons, M & Insko, W.M., 1937. Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen. Kentucky Agricultural Experimental Station Bulletin 371, pp 61-75.

Madrigal, S.E., Watkins, S.E., Anthony, N.B., Wall, C.E., Fritts, C.A. & Waldroup, P.W., 2002. Effect of dietary modifications designed to reduce early growth rate on live performance and on incidence and severity of ascites in two commercial broiler strains when maintained under low ventilation or low temperature models. Int. J. Poult. Sci. 1(5), 150-157.

Malan, D.D., 2007. Ascites susceptibility as affected by dietary lysine to energy ratios in interaction with broiler genotypes. Arch.Geflügelk. 71, 247-257.

Malan, D.D., C. W. Scheele, J. Buyse, C. Kwakernaak, F.K. Siebrits, J.D. Van Der Klis & E. Decuypere. 2003. Metabolic rate and its relationship with ascites in chicken genotypes. J. Br. Poult. Sci. 44, 309-315.

Martinez, D.A. & Diaz, G.J., 1996. Effect of graded levels of dietary nickel and manganese on bloodhaemoglobin content and pulmonary hypertension in broiler chickens, Avian Pathol. 25(3), 537-549.

Maxwell, M,H & Robertson, G.W., 1998. UK survey of broiler ascites and sudden death syndromes in 1993. J. Br. Poult. Sci. 39(2), 203-215.

Maxwell, M.H., Spence, S., Robertson, G.W. & Mitchell, M.A., 1990. Haematological and morphological responses of broiler chicks to hypoxia, Avian Pathol. 19(1), 23-40.

McGovern, R.H., Feddes, J.J.R., Robinson, F.E. & Hanson, J.A., 1999. Growth performance, carcass characteristics, and the incidence of ascites in broilers in response to feed restriction and litter oiling. J. Poult. Sci. 78, 522-528.

Mohanna, C. & Nys, Y., 1999. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. Anim. Feed Sci. Tech. 77, 241-253.

Molenaar, R., 2010. Perinatal development and nutrient utilization in chickens: Effects of incubation conditions. P.h.D thesis, Wageningen University, Netherlands.

Molenaar, R., Reijrink, I.A.M., Meijerhof, R. & van den Brand, H., 2010a. Meeting embryonic requirements of broilers throughout incubation: A Review. Braz. J. Poult. Sci. 12, 137-148.

Molenaar, R., Reijrink, I.A.M., Meijerhof, R. & van den Brand, H., 2010b. Meeting embryonic on hatchability and body weight of broiler chicks from young breeder flocks. J. Poult. Sci. 79, 827-830.

Molenaar , R., de Vries, S., van den Anker, I., Meijerhof, R., Kemp, B. & van den Brand, H., 2010c. Effect of eggshell temperature and a hole in the air cell on the perinatal development and physiology of layer hatchlings. J. Poult. Sci. 89, 1716-1723.

Molenaar, R., Meijerhof, R., van den Anker, I., Heetkam, M.J.W., van den Born, J.J.G.C., Kemp, B., & van den Brand, H., 2010d. Effect of eggshell temperature and oxygen concentration on survival rate and nutrient utilization in chicken embryos. J. Poult. Sci 89, 2010-2021.

Molenaar, R., Hulet, R., Meijerhof, R., Maatjens, C.M., Kemp, B. & van den Brand, H., 2011a. High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens. J. Poult. Sci. 90, 624-632.

Molenaar, R., van den Anker, I., Meijerhof, R., Kemp, B. & van den Brand, H., 2011b. Effect of eggshell temperature and oxygen concentration during incubation on the developmental and physiological status of broiler hatchlings in the perinatal period. J. Poult. Sci. 90, 1257-1266.

Mortola J.P., 2009. Gas exchange in avian embryos and hatchlings. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 153(4), 359-77.

National Research Council (NRC), 1994. Nutrient Requirements of Poultry. 9th reviewed ed., National Academy Press, Washington, DC.

Nunez-Nogueira, G., Rainbow, P.S. & Smith, B.D., 2006. Assimilation efficiency of zinc and cadmium in the decapods crustacean *Penaeus indicus*. J. Exp. Mar. Bio. Ecol. 332, 75-83.

Olkowski, A.A., Kumor, L. & Classen, H.L. 1996. Changing epidemiology of ascites in broiler chickens. Can. J. Anim. Sci. 76, 135-140.

Owens, B., McCann, M.E.E. & Preston, C., 2009. The effect of substitution of inorganic zinc with proteinated or chelated zinc on broiler chick performance. J. Appl. Poult. Res. 18, 789-794.

Ozkan, S., Plavnik, I. & Yahav, S., 2006. Effects of early feed restriction on performance and ascites development in broiler chickens subsequently raised at low ambient temperature. J. Appl. Poult. Res. 15, 9-19.

Peebles, E.D., Burnham, M.R., Gardner, C.W., Brake, J., Bruzual, J.J & Gerard, P.D., 2001. Effects of incubational humidity and hen age on embryo composition in broiler hatching eggs from young breeders. J. Poult. Sci. 80, 1299-1304.

Powell, S.R., 2000. The antioxidant properties of zinc. J. Nutr. 130, 1447-1454.

Qiao, G., 2007. Reduction of early mortality in broiler chickens through nutrition and management. Sales and service, Champion Feed Services Ltd. pp 1-2.

Rahman M.W., Davies R.E., Deyoe C.W., Reid B.L., Couch J.R., 1961. Role of zinc in the nutrition of growing pullets. J. Poultry Sci. 40, 195-200.

Rajani, J., Torshizi, M.A.K. & Rahimi, S.H., 2011. Control of ascites mortality and improved performance and meat shelf-life in broilers using feed adjuncts with presumed antioxidant activity. J. Anim. Feed Sci. Tech. 170, 239-245.

Robertson, I.S., 1961. Studies in the effect of humidity on the hatchability of hen's eggs. The determination of optimum humidity for incubation. J. Agric. Sci. 57, 185-194.

Romanoff, A.L., 1960. The influence of environment on early development. In the avian embryo: Structural and functional development. Macmillan company, New York. pp. 195-207.

Ross Broiler Management Manual. Newbridge, UK: Aviagen Limited, 2009.

Rossi, P., Rutz, F., Anciuti, M.A., Rech, J.L. & Zauk, N.H.F., 2007. Influence of graded levels of organic zinc on growth performance and carcass traits of broilers. J. Appl. Poult. Res. 16, 219-225.

Saenmahayak, B., 2007. Complexed trace mineral supplementation of broiler diets. Master's Degree Thesis. Auburn University, Alabama. USA.

Şahan, U., İpek, A., Yilmaz-Dıkmen, B., Aydin, C. & Kederli, E., 2011. Effect of oxygen supplementation in the hatcher at high altitude on the incubation results of broiler eggs laid at low altitude. J. Br. Poult. Sci. 52(3), 388-394.

SAS Institute. 2004. SAS/STAT User's Guide. Version 9.1. SAS Inst. Inc., Cary, NC.

Saxton, A.M., 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, 1243-1246.

Scheele, C.W., Van Der Klis, J.D., Kwakernaak, C., Buys, N. & Decuypere, E., 2003. Haematological characteristics predicting susceptibility for ascites. 1. High carbon dioxide tensions in juvenile chickens. J. Brit. Poult. Sci. 44(3), 476-483.

Schlegel, P., Nys, Y. & Jondreville, C., 2009. Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source. The animal consortium, Anim. 4(2), 200-209.

Shlosberg, A., Bellaiche, M., Zietlin, G., Ya'Acobi, M. & Cahaner, A., 1996. Heamatocrit values and mortality from ascites in cold-stressed broilers from parents selected for heamatocrit. J. Poult. Sci. 75, 1-5.

Singh, P.K., Shekhar, P., & Kumar, K., 2011. Nutritional and managemental control of ascites syndrome in poultry. Int. J. Livest. Prod. 2(8), 117-123.

Solis de los Santos, F., Farnell, M.B., Tellez, G., Balog, J.M., Anthony, N.B., Torres-Rodriguez, A., Higgins, S., Hargis, B.M. & Donoghue, A.M., 2005. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. J. Poult. Sci. 84, 1092-1100.

Star, L., van der Klis, J.D., Rapp, C. & Ward, T.L., 2012. Bioavailability of organic and inorganic zinc sources in male broilers. J. Poult. Sci. 91, 3115-3120.

Tamsma, J.T., Keizer, H.J. & Meinders, A.E., 2001. Pathogenesis of malignant ascites: Starling's law of capillary hemodynamics revisited. Ann. Oncol 12, 1353-1357.

Tazawa, H. and Whittow, G.C., 2000. Incubation physiology, in: Sturkie's Avian Physiology 2000 (G.C. Whittow 5th ed). London, Academic Press. pp. 617-634.

Tona, K.,Onagbesan, O., De Ketelaere, B., Decuypere, E. & Bruggeman, V., 2003. Effects of turning duration during incubation on corticosterone and thyroid hormone levels, gas pressures in air cell, chick quality, and juvenile growth. J. Poult. Sci. 82, 1974-1979.

Tona, K., Onagbesan, O., Bruggeman, V., Mertens, K & Decuypere, E., 2005. Effects of turning duration during incubation on embryo growth, utilization of albumen, and stress regulation. J. Poult. Sci. 84, 315-320.

Underwood, E. J & Suttle, N. F., 1999. The mineral nutrition livestock. 3rd Edition. CABI Publishing, Oxon.

Villamor, E., Kessels, C.G.A., Ruijtenbeek, K., van Suylen, R.J., Belik, J. & Blanco, C.E., 2004. Chronic in ovo hypoxia decreases pulmonary arterial contractile reactivity and induces biventricular cardiac enlargement in chicken embryo. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287, 642-651.

Virden, W,S., Yeatman, J.B., Barber, S.J., Willeford, K.O.,Ward, T.L., Fakler, T.M., Wideman, R.F. & Kidd, M.T., 2004. Immune system and cardiac functions of progeny chicks from dams fed diets differing in zinc and manganese level and source. Poult. Sci. 83, 344-351.

Wangensteen, O.D., 1972. Gas exchange by a bird's embryo. Respir. Physiol. 14(12), 64-74.

Watson, L.T., 1970. Manganese utilisation by ruminants and poultry. P.h.D Thesis, University Of Florida. USA.

Wideman, R.F., Rhoads, D.D., Erf , G.F. & Anthony, N.B., 2013. Pulmonary arterial hypertension (ascites syndrome) in broilers: A review. J. Poult. Sci. 92, 64-83.

Willemsen, H., Kamers, B., Dahlke, F., Han, H., Song, Z., Ansari Pirsaraei, Z., Tona, K., Decuypere, E. & Everaert, N., 2010. High- and low-temperature manipulation during late incubation: Effects on embryonic development, the hatching process, and metabolism in broilers. J. Poult. Sci. 89, 2678-2690.

Wilson, H.R. 1991. Physiological requirements of the developing embryo: Temperature and turning. Avian Incubation. S. G. Tullet, ed. Butterworth-Heinemann, London. pp.145-156.

Xi, Z., Yang, S., Liu, D., Wu, l., Liu, X., Zhao, J & Guo, D., 2012. ROS induce cardiomyocyte apoptosis in ascitic broiler chickens. Pak. Vet. J. 32(4), 613-617.

Yalcın, S., Abuk, M.C., Bruggeman, V., Babacanog Lu, E., Buyse, J., Decuypere, E. & Siegel, P.B., 2008. Acclimation to heat during incubation. 1. Embryonic morphological traits, blood biochemistry, and hatching performance. J. Poult. Sci. 87, 1219-1228.

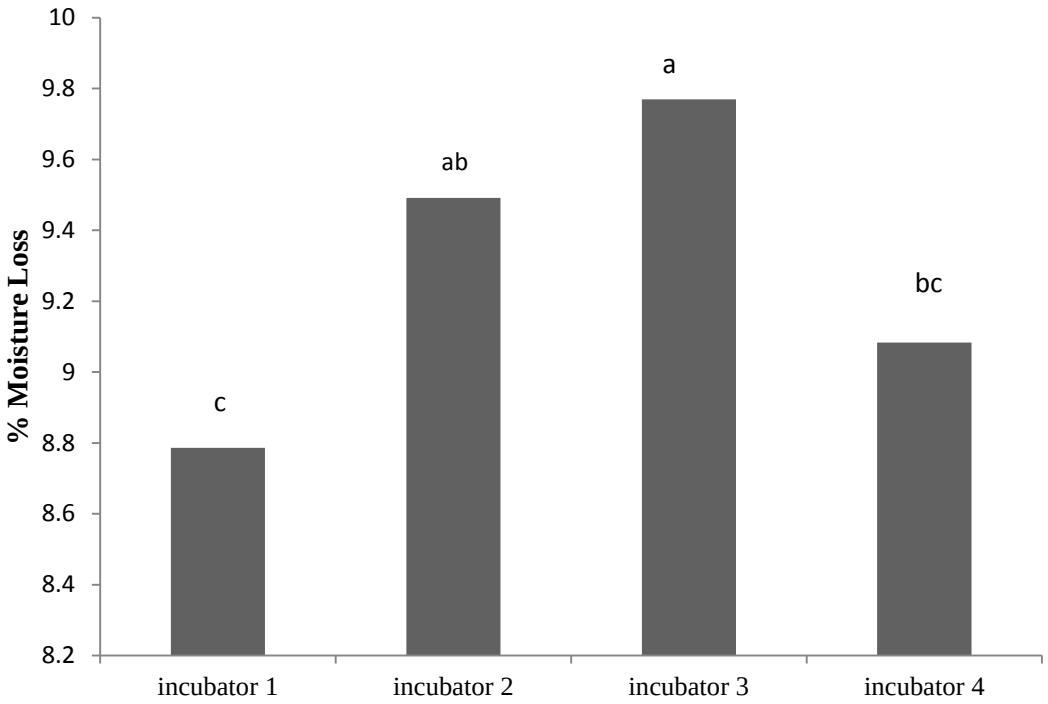
Yildirim, I. & Yetisir, R., 2004. Effects of different hatcher temperatures on hatching traits of broiler embryos during the last five days of incubation. S. Afr. J. Anim. Sci. 34(4), 211-216.

Yu, Y., Lu, L., Luo, X.G. & Liu, B., 2008. Kinetics of zinc absorption by in situ ligated intestinal loops of broilers involved in zinc transporters. J. Poult. Sci. 87, 1146-1155.

Yuan, J., Xu, Z., Huang, C., Zhou, S. & Guo, Y., 2011. Effect of dietary Mintrex-Zn/Mn on performance, gene expression of Zn transfer proteins, activities of Zn/Mn related enzymes and faecal mineral excretion in broiler chickens. Anim. Feed Sci. Tech. 168, 72-79.

Zefra R., Pérez J., Pérez-Écija R.A., Borge C., Bustamante R., Carbonero A. & Tarradas C. 2008. Concurrent aspergillosis and ascites with high mortality in a farm of growing broiler chickens. Avian Dis. 52, 711-713.

APPENDIX



[a-c] means with no common alphabets differ significantly (P<0.05)

Figure 1 Moisture loss from eggs set in four different incubators during the first 18 days of incubation

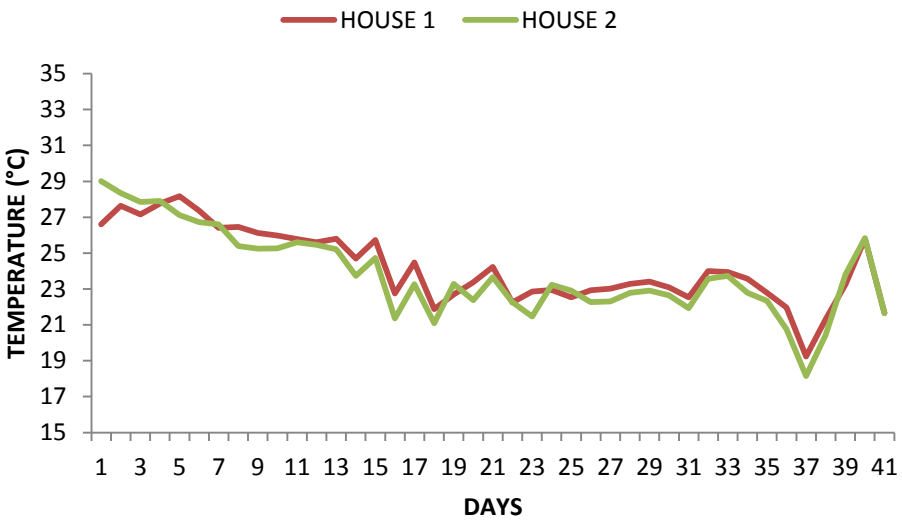


Figure 2 Daily brooding litter temperatures (°C) in house 1 & 2

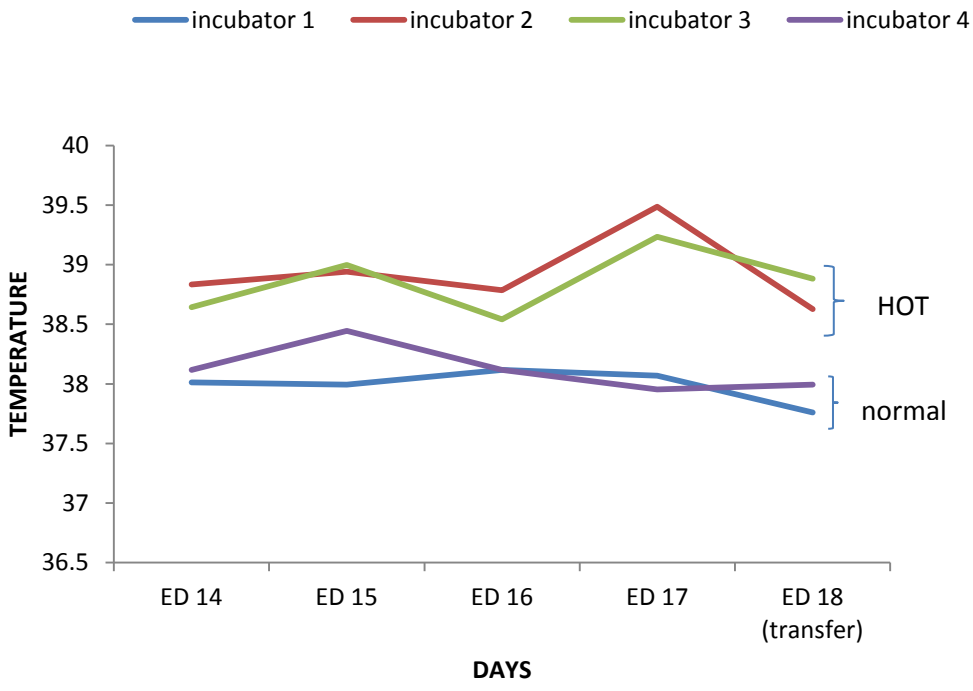


Figure 3. Eggshell temperatures for the 4 incubator treatments

Table 1 Machine 1 daily machine air temperature and humidity records (Normal treatment)

		AM			PM		
DAY	**DATE**	**TEMP °C**	**HUMD %**	**TURN**	**TEMP °C**	**HUMD %**	**TURN**
0	25-Jul	37.5	24	→	35.5	48	←
1	26-Jul	37.4	51	←	37.3	55	→
2	27-Jul	37.6	53	→	37.3	54	←
3	28-Jul	37.6	60	←	37.5	40	→
4	29-Jul	37.6	52	→	37.6	55	←
5	30-Jul	37.6	55	←	37.6	53	→
6	31-Jul	37.6	54	←	37.4	54	→
7	1-Aug	37.6	53	→	37.4	55	→
8	2-Aug	37.5	53	←	37.3	55	→
9	3-Aug	37.6	55	→	37.6	55	←
10	4-Aug	37.3	54	→	37.4	54	←
11	5-Aug	37.5	53	←	37.5	53	←
12	6-Aug	37.4	54	←	37.6	40	→
13	7-Aug	37.1	46	→	37.3	54	→
14	8-Aug	37.4	53	→	37.1	56	→
15	9-Aug	37.2	54	←	37.1	59	→
16	10-Aug	37.1	55	←	37.1	51	←
17	11-Aug	37.1	60	→	36.9	51	→
18	12-Aug	36.9	58	←	36.8	56	→
19	13-Aug	36.7	59	→	36.8	23	
20	14-Aug	36.8	34		36.9	26	
21	15-Aug	36.8	52		36.1	87	
22	16-Aug	37.1	34				

→/← - direction of turning

Table 2 Machine 2 daily machine air temperature and humidity records (Hot treatment)

		AM			PM		
DAY	**DATE**	**TEMP °C**	**HUMD %**	**TURN**	**TEMP °C**	**HUMD %**	**TURN**
0	25-Jul	37.5	21	→	33.4	60	←
1	26-Jul	37.4	55	←	37.6	60	→
2	27-Jul	37.4	38	→	37.5	51	→
3	28-Jul	37.4	70	←	37.2	46	←
4	29-Jul	37.7	51	→	37.7	45	→
5	30-Jul	37.4	54	←	37.4	55	→
6	31-Jul	37.5	53	←	37.6	55	→
7	1-Aug	37.2	58	→	37.4	54	→
8	2-Aug	37.5	53	←	37.2	55	→
9	3-Aug	37.2	55	→	37.6	55	→
10	4-Aug	37.3	52	→	37.6	48	→
11	5-Aug	37.6	57	→	37.5	53	←
12	6-Aug	37.2	54	→	37.4	54	←
13	7-Aug	37.3	55	←	37.6	55	→
14	8-Aug	37.6	55	→	37.7	55	→
15	9-Aug	37.9	53	←	38	51	→
16	10-Aug	37.9	55	←	38	55	→
17	11-Aug	37.9	58	→	38.1	57	→
18	12-Aug	37.8	54	←	37.9	46	→
19	13-Aug	37.9	63	→	37.9	31	
20	14-Aug	38	39		38	51	
21	15-Aug	35.3	84		38.1	39	
22	16-Aug	38	42				

→/← - direction of turning

Table 3 Machine 3 daily machine air temperature and humidity records (Hot treatment)

		AM			PM		
DAY	**DATE**	**TEMP °C**	**HUMD %**	**TURN**	**TEMP °C**	**HUMD %**	**TURN**
0	25-Jul	37.5	21	←	35.5	61	→
1	26-Jul	37.3	60	→	37.6	60	←
2	27-Jul	37.3	55	→	37.6	51	←
3	28-Jul	37.5	28	←	37.4	55	→
4	29-Jul	37.4	55	←	37.4	55	→
5	30-Jul	37.4	55	→	37.4	53	←
6	31-Jul	37.6	53	→	37.6	53	←
7	1-Aug	37.5	58	←	37.6	53	→
8	2-Aug	37.2	46	→	37.2	55	→
9	3-Aug	37.4	54	←	37.4	53	←
10	4-Aug	37.4	55	→	37.5	55	→
11	5-Aug	37.3	54	←	37.4	53	→
12	6-Aug	37.5	55	←	37.4	50	→
13	7-Aug	37.7	42	→	37.8	48	←
14	8-Aug	37.7	54	←	37.7	55	←
15	9-Aug	37.7	53	→	38	55	←
16	10-Aug	38	42	←	37.9	55	←
17	11-Aug	38	44	←	38	70	←
18	12-Aug	38.2	55	→	38.2	57	←
19	13-Aug	38.2	55	←	38.1	31	
20	14-Aug	38.2	39		38.2	51	
21	15-Aug	38.1	82		38.2	47	
22	16-Aug	38.1	51				

→/← - direction of turning

Table 4 Machine 4 daily machine air temperature and humidity records (Normal treatment)

		AM			PM		
DAY	DATE	TEMP °C	HUMD %	TURN	TEMP °C	HUMD %	TURN
0	25-Jul	37.5	24	→	36.6	55	←
1	26-Jul	37.5	58	←	37.3	52	→
2	27-Jul	37.2	51	→	37.4	47	→
3	28-Jul	37.6	60	→	37.6	40	←
4	29-Jul	37.5	55	→	37.4	55	←
5	30-Jul	37.4	55	←	37.4	55	←
6	31-Jul	37.3	51	←	37.4	60	←
7	1-Aug	37.4	58	→	37.3	45	←
8	2-Aug	37.6	53	→	37.3	46	←
9	3-Aug	37.4	46	←	37.3	58	←
10	4-Aug	37.3	60	→	37.4	48	→
11	5-Aug	37.4	54	←	37.4	57	→
12	6-Aug	37.4	54	←	37.4	50	→
13	7-Aug	37.3	54	→	37.6	53	←
14	8-Aug	37.6	57	→	37.6	57	→
15	9-Aug	37.6	57	→	37.4	60	→
16	10-Aug	37.4	50	←	37.4	60	→
17	11-Aug	37.4	54	→	37.1	58	←
18	12-Aug	37.1	51	←	37	55	←
19	13-Aug	37	51	←	36.9	35	
20	14-Aug	36.9	32		37	38	
21	15-Aug	37.9	56		36.4	90	
22	16-Aug	37	38				

→/← - direction of turning