Influence of incubation temperature on chorio-allantoic membrane vascularization, heart size and ascites incidence in broilers (*Gallus domesticus*)

by

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In the Department of Production Animal Studies,
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DECLARATION

I hereby declare that this dissertation, which I hereby submit for the MMedVet (Altil) to the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, is my own work and has not been previously presented by me for degree purposes at any other tertiary institution.

……

O. M. LUKHELE

DEDICATION

To my wife, Lena, and two children, Keratile and Thato, who continued to support and love me despite very little time I've spent with them during my study years.

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Doctor Theunis Botha of the Department of Mechanical and Aeronautical Engineering at the University of Pretoria developed the software in order to score blood vessels on the CAM. His contribution has been invaluable.

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SUMMARY

INFLUENCE OF INCUBATION TEMPERATURE ON CHORIO-ALLANTOIC MEMBRANE VASCULARIZATION, HEART SIZE AND ASCITES INCIDENCE IN BROILERS

By

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Supervisor: **Dr. D. B. R. Wandrag**

Co-supervisor: **Prof. P. T. Thompson**

Ascites or pulmonary arterial hypertension (PAH) syndrome is a significant cause of mortality in modern fast-growing broilers that are raised at high altitude. Damage caused by temperature to the cardio-vascular system during embryo development is often overlooked as an important predisposing factor to ascites experienced on the farm.

Eggs from a 38 week-old Ross 308 flock were exposed to three temperature treatments. One group was exposed to 36.8^{o}C (cool), the second group to 37.5^{o}C (control) and the third group to 38.2^{o}C (hot). Relative humidity ranged from 55-59% in all groups. These treatments were used throughout the incubation period.

Vascularisation of the chorio-allantoic membrane (CAM) was used as an indirect measure of embryonic blood vessel reaction to temperature insult. Vascular fraction (VF), density and fractal dimension (FD) and branching of blood vessels were assessed using a stereo microscope. The control had significantly lower

($p<0.001$) VF and FD compared to both hot and cool groups suggesting that high and low temperatures in incubators and hatchers trigger vascular compensation. High temperature during embryonic development resulted in significantly lower heart mass in both the control ($p<0.002$) and the hot ($p<0.001$) groups compared to the cool treatment. A smaller heart will limit the ability of the fast growing broiler to compensate for low oxygen levels at high altitude and thus be prone to ascites.

Formation of the embryo muscle mass was significantly reduced in hot ($p<0.001$) and the control ($p<0.007$) groups compared to the cool treatment. In this study embryo mass was reported as yolk-free body mass (YFBM). The hot treatment had significantly ($p<0.001$) lower (1 843g) body mass at 35 days of age when slaughtered compared to the cool and control groups that averaged 2 107g and 2 130, respectively.

There was, however, no statistical difference ($p<0.178$) on heart mass (HM) to YFBM ratio amongst all three temperature treatment groups.

Mortality due to ascites was double (53%) in the hot group compared to the control and cool treatments which were similar. The difference in mortality was significant at $p<0.001$. This very high mortality in the hot group is likely to have skewed the right ventricle to total ventricular (RV: TV) ratio which was the same to that of the cool treatment at 0.26. This falls within the range of a normal fowl. Birds in the control group had higher (0.28) RV: TV ratio which indicated susceptibility to ascites.

Feed conversion for the control group was 2.94% better (1.517) than the cool group (1.563) and 5.2% better than the hot treatment (1.601), the differences were not significant between the hot and control group and slaughter.

The combined farm performance variables expressed in terms of an efficiency factor (PEF) showed that the control group (292) performed 4.8% better than the cool group (278) and 47.6% better than the hot group (153).

Bacterial growth in yolks from eggs in the hot treatments was recorded in 19.2% of the samples, with 12% being gram-negative bacteria. Only 3% and 3.7% of samples from the control and cool groups, respectively, had bacterial growth. Of the bacteria isolated in the cool treatment group, all were gram-negative isolates. As the incubation and setter temperature increased, so has the number of positive samples. Statistically, bacterial growth was significantly ($p<0,024$) higher in the hot treatment compared to the cool and control groups combined.

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ABBREVIATIONS

ADG	Average daily gain
ANOVA	Analysis of variance
bFGF	Basic fibroblast growth factor
CAM	Chorio-allantoic membrane
CHF	Congestive heart failure
CO_2	Carbon dioxide
DBRU	Deon Barnard Research Unit
ED	Embryonic day
EET	Epoxyeicosatrienoic acids
EP	External pipping
FD	Fractal dimension
IP	Internal pipping
NO	Nitric oxide
PAH	Pulmonary arterial hypertension
PEF	Performance efficiency factor
RV	Right ventricle
SD	Standard deviation
T_3	Triiodothyronine
T_4	Thyroxine
TV	Total ventricle
VEGF	Vascular endothelial growth factor
VF	Vascular fraction
YFBM	Yolk-free body mass

CHAPTER 1: BACKGROUND

1.1. Introduction

In order to meet consumer needs and stay commercially viable, the poultry industry has been increasingly producing broilers efficiently through genetic selection for feed conversion, livability and growth. The latter has improved by 300-370% and feed utilization efficiency by 50% over the past 55-60 years (Zuidhof et al., 2014). As a result of this, some undesirable traits such as pulmonary arterial hypertension, PHS, (Julian, 1998), ascites (Wideman et al., 2013) or "water belly" (Decuypere et al., 2000) have arisen.

It is estimated that 5% of the 40 billion broilers produced world-wide annually die as a result of ascites. This figure could be as high as 30% (Closter et al., 2012). Thus, the economic loss due to ascites is significant (Baghbanzadeh & Decuypere, 2008). Though the peak incidence of ascites in broilers occurs during the growth phase, usually week 5 to 6, the condition can be initiated during the bird's embryonic stage (Baghbanzadeh & Decuypere, 2008). The embryo grows rapidly during its last seven days of incubation, resulting in a 60% increase in oxygen consumption from the start of pulmonary breathing to hatching (Baghbanzadeh & Decuypere, 2008). Important variables during incubation are temperature, turning of eggs, humidity and gas concentration (Verhoelst et al., 2011). As the final ontogeny of the thermoregulatory neuro-endocrine axis also takes place during this last phase of incubation, incubation temperature is the single most important factor influencing embryo development (Willemsen et al., 2010). The financial cost of ascites could be devastating to poultry producers.

Triggers of ascites in hatchers and incubators include temperature as well as low oxygen availability, especially between embryonic day 16-21 (ED 16-21), referred to as the plateau stage. Low conductance of the egg shell and extra-embryonic membranes limits heat loss and oxygen diffusion across the chorio-allantoic membrane (CAM) during the plateau phase.

The CAM harbours important development, growth and survival functions of the embryo such as respiration (Blacher et al., 2005), water-salt homeostasis and thermal exchange (Maskimov et al., 2006). Being the primary organ for gas exchange from day 8 to 19 it has an extensive network of vessels; arteries, veins and capillaries. The CAM also responds to injury

with complete inflammatory reaction (Leighton et al., 1983). It is recognized that angiogenesis and vascular activity in the CAM may also be stimulated by chemical variables such as epoxyeicosatrienoic (EET) acids, which are biochemical products of bacterial lipopolysaccharides (Verhoelst, 2011; Dunn et al., 2005).

Plausible parameters that can be monitored during embryonic growth to assess optimum incubation conditions include embryonic weight, organ weight and angiogenesis in the CAM (Verhoelst et al., 2011). These parameters, as well as insufficient lungs and/or pulmonary blood vessel development (Decuypere et al., 2000; Hassanzadeh, 2010) will predispose broilers to ascites.

The right ventricle to total ventricular (RV: TV) proportion is regarded as a sensitive index for ascites or PAH in broilers (Cueva et al., 1974; Peacock et al., 1990; Wideman & French, 1999; Lorenzoni & Ruiz-Feria, 2006; Closter et al., 2009). The RV: TV ratio of a healthy fowl is between 0.15 and 0.27 and that of a bird with ascites is above 0.27-0.285 (Cueva et al., 1974; Huchzermeyer & Deruyck, 1986; Huchzermeyer et al., 1988; Julian, 1988; Julian, 1993; Lubritz et al., 1995; Owen et al., 1995a; and Wideman & French, 1999; Wideman, 2001; Daneshyar et al., 2009; Ozkan et al., 2010).

1.2. Literature review

Ascites or pulmonary arterial hypertension (PAH) syndrome occurs as a result of increased cardiac output in response to increased oxygen demand experienced under cold temperature, rapid growth (e.g. when birds are fed pelleted feed and allowed to sleep for few hours), low oxygen availability (e.g. high altitude, insufficient ventilation in poultry houses or during incubation), increased pulmonary resistance (e.g. lung disease conditions) and heart valvular insufficiency that culminates in right ventricular dilation and failure (Cueval et al., 1974; Julian, 1998; Decuypere et al., 2000; Druyan, 2010).

Despite rapid growth and high oxygen demand of a modern broiler, its cardio-pulmonary capacity remains very similar to that of old broiler strains. This means that the gas exchange system works close to the bird's physiological limit (Lorenzoni, 2006). Another anatomical constrains are that lungs of birds are smaller as a percentage of body weight compared to

mammals and, that bird's lungs are fixed to the thoracic cavity, thus do not expand and contract during breathing (Julian, 1998).

In order to meet increased oxygen demand in rapidly growing broilers raised both at sea level and high altitude, adaptive efforts include increased heart rate that could lead to increased heart size, vascular changes and more erythrocyte production to help transport the required amount of oxygen by blood (Hassanzadeh, 2010). This is in line with the concept of symmorphosis, which suggest that a structural design (of an organ system) must match functional demand in an economical manner (Weibel et al., 1991). It is, however, inevitable that the rapid increase in modern broiler chick mass cannot be sustained without traumatic increase in functional capacity of the heart and lungs (Wideman et al., 2013). The cardiac output must increase from 8ml/min for a 40g day-old chick to 800ml/min for a 4-kg broiler at 8 weeks of age, while the venous blood returning to the heart must also match the cardiac output. This means that pulmonary blood vessels post-hatch must develop proportionally and that the increased cardiac work load will result in enlarged right ventricle or elevated right-to-total ventricular weight ratio (Wideman et al., 2013).

Nain et al. (2009) found that ascites may occur as a result of cardiogenic causes associated with pathological remodeling of the aorta and brachiocephalic arteries, which were flaccid and lacked elasticity. These researchers estimated that 15-25% of fast growing broilers with congestive heart failure (CHF) and severe heart pathology on post-mortem, do not develop ascites. This means that pathological changes in the heart could be associated with ascites but is not always predictive of ascites. However, all broilers with ascites had pathology of both the heart and major blood vessels, a finding that indicates that blood vessel pathology plays significant role in the pathogenesis of ascites.

Broilers with restrictive pulmonary vascular capacity are susceptible to pulmonary hypertension syndrome (Wideman et al., 2013) and show high innate pulmonary vascular resistance which is the key predisposing factor to PAH syndrome development (Chapman & Wideman, 2001; Rojas et al., 2011). Pulmonary vasoconstriction (Olkowski & Classen, 1998) can also be triggered by tissue hypoxia resulting in PAH syndrome.

The development of the CAM occurs between ED5-11 (Druyan et al., 2012) reaching its peak mitogenic activity and angiogenic response by ED10 (Druyan et al, 2012). During this period

(ED10-13), about half of the embryo's cardiac output goes to the CAM (Mueller et al., 2015). The embryo uses the CAM from ED8-19 as a primary organ for gaseous exchange with the outside environment. Closer to hatching (ED16-19), less (17-40%) blood is channelled to organs and tissues of the growing embryo (Mueller et al., 2015) and the lungs are used to breathe after internal pipping (IP). During this last week of pulmonary breathing and hatching, the chicken embryo grows rapidly, which result in increased oxygen consumption (Baghanzadeh & Decurypere, 2008) and heat production that reaches plateau phase at approximately 140mW for a 62g egg (Molenaar et al., 2010). Egg shell temperature above 38oC during the last day of incubation has an adverse effect on embryo development and maturation of the organ system, including the gizzard and intestines (Wineland & Oviedo, 2009).

The CAM is an affordable and useful biomaterial in angiogenesis and anti-angiogenesis studies of a variety of natural and synthetic compounds (Ribatti et al., 1996; Ribatti et al., 2006). Angiogenesis and vascular activity in the CAM can be stimulated by non-chemical factors such as high temperature and hypoxia (Druyan et al., 2012), as well as chemicals like epoxyeicosatrienoic acids, EETs, (Verhoelst et al., 2011), nitric oxide (Bowen et al., 2005; Lorenzoni & Ruiz-Feria, 2006) and adenosine (Dusseau et al., 1986). Vascular structures of the CAM may have major effects on the embryo metabolism and growth. Druyan et al., (2012) found an association of matrix metalloproteinase gene, which is involved in angiogenesis of the CAM and in the entire embryo, following exposure to elevated incubation temperatures. This finding suggests that changes in CAM vascularization may also be indicative of changes within the chick embryo's blood vessel system.

Hypoxia induces angiogenesis through the stimulation of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) production. As reported by Druyan et al. (2012), in these cases the heart will be larger in domestic chickens and hatchlings of the Canadian goose (Branta canadensis). The enlarged heart due to hypoxia is likely to be a result of normal physiological adaptive responses such as increased cardiac output, polycythemia, increased haemoglobin production, increased angiogenesis and vasculogenesis, as well as redistribution of blood from peripheral to vital organs such as the brain, heart and adrenal glands (Druyan et al., 2012).

Delayed external pipping (EP) might extend the hypoxic environment, thereby evoking ascites. Chronic hypoxia has also been reported to trigger the release of adenosine which causes vasodilation of the CAM (Dusseau et al., 1986).
Thyroid hormones (T3 and T4) were higher in the ascites-resistant (AR) broiler line compared to the ascites-susceptible (AS) strain. High thyroid hormone levels resulted in a shorter duration between the start of pulmonary respiration to hatching (Dewil et al., 1996).

Phospholipids from gram-negative bacteria, for an example, are bio-transformed into arachidonic acid through the enzymatic help of cytochrome P450 and then to epoxyeicosatrienoic acids (EETs) which are vasodilative and angiogenic (Verhoelst et al., 2011). Nitric oxide (NO) is a potent endogenous vasodilator produced in the pulmonary endothelial cells from arginine (Bowen et al., 2005; Lorenzoni and Ruiz-Feria, 2006). However, increased levels of arginine in the diet did not significantly reduce the RV/TV ratio (Lorenzoni and Ruiz-Feria, 2006) and thus the susceptibility of treated birds to ascites.

CHAPTER 2: HYPOTHESIS

2.1. Problem

Ascites remains a costly metabolic disease in modern broilers especially at high altitude. Hearts of modern broilers become flabby and compromised even though the chickens may survive to reach the slaughter plant. A compromised heart is likely to reduce growth, thereby limiting the genetic potential of modern broilers. Farm production efficiencies and profitability will be negatively impacted.

In broiler hatcheries, samples of incubated eggs are assessed at day-10 for fertility and stages at which the embryo died. It is also standard practice that the hatch residue are analyzed for fertility, stages at which embryos died, quality of the shells, signs of stress during hatching, bacterial and/or fungal contamination. The heart will also be assessed at hatch for its size relative to the yolk free body mass (YFBM). Despite the CAM being a vital organ for gaseous exchange before EP it is seldom, if at all, assessed for signs of abnormalities.

2.2. Hypothesis

High incubation and hatcher temperature will result in increased vascularization of the CAM and smaller hearts. These changes will result in predisposition of modern broilers to PAH syndrome – commonly known as ascites.

2.3. Objectives

Objectives of this research were to prove that:

2.3.1. There is a difference in CAM vascularization at embryonic day 14 (ED14) between eggs incubated at 36.8^{o}C, 37.5^{o}C and 38.2^{o}C.

2.3.2. Incubation temperatures of 36.8^{o}C, 37.5^{o}C and 38.2^{o}C affect heart mass to yolk free body mass (YFBM) proportion at hatch (first grade day-old chick).

2.3.3. Incubation temperatures of 36.8^{o}C, 37.5^{o}C and 38.2^{o}C affect percent mortality, feed conversion and body mass of broilers at slaughter (35 days).

2.3.4. Incubation temperatures of 36.8^{o}C, 37.5^{o}C and 38.2^{o}C affect right ventricle to total ventricular proportion of broilers at slaughter age (35 days).

CHAPTER 3: EXPERIMENTAL DESIGN

3.1. Materials and methods

3.1.1 Materials

3.1.1.1. Three groups of Ross 308 eggs (n = 700 each).

3.1.1.2. Three Easyhatch FRD-6T incubator-hatcher machines, each with 880 egg-carrying capacity. These incubators were supplied by Easyhatch Incubation Equipment based on Plot 96, Vaalfontein, Vanderbjpark, South Africa.

3.1.1.3. The 3 incubator-hatcher machines were kept at Deon Barnard Research Unit (DBRU) which was built at 1 508 meters above sea level. This facility is situated at Plot 36, 12th Avenue in Rietvlei, Pretoria, South Africa.

3.1.2 Methods

A total of 2 100 fertile eggs were collected from a Ross 308 broiler breeder flock that was 38 weeks of age. These eggs were divided into three groups of 700 per machine. One group of eggs was incubated and hatched at 36.8^{o}C (cool), the second group was incubated and hatched at 37.5^{o}C (control) and the third group was incubated and hatched at 38.2^{o}C (hot). Relative humidity (RH) in all machines was maintained at 55-59%.

The egg sample size was predetermined per treatment group in order to avoid the difference in egg mass per machine, air movement and heat generation inside the incubators.

The experimental design is summarised in Table 1.

Table 1: Treatment groups and sampling

Relative Humidity (55-59%)	**Temperature Treatments**		
	Cool	**Control**	**Hot**
	(36.8^{o}C)	(37.5^{o}C)	(38.2^{o}C)
Altitude of hatchery and farm (meters)	1 508	1 508	1 508
Number of eggs incubated per group	700	700	700
Breed	Ross 308	Ross 308	Ross 308
Eggs sampled at Day 14 of incubation	36	36	36
Chorio-allantoic membrane (CAM) harvested	27	33	26
Yolks swabbed	27	33	26
Chicks sampled at Day 21 of incubation (Hatch)	36	36	36
Yolk free body mass (YFBM)	36	36	36
Hearts	36	36	36
Broilers sampled at slaughter (35 Days)	36	36	36
Hearts	36	36	36

At embryonic day 14 (ED14), 36 eggs were sampled from each of the treatment groups. Of the 36 eggs sampled per treatment group, 27 eggs had embryo development in the cool (36.8^{o}C) treatment, 33 embryos developed in the control (37.5^{o}C) sample and 26 embryos were found in the hot (38.2^{o}C) treatment. As a result, the number of chorio-allantoic membranes and the inside of the yolk sacs that were swabbed was the same as the number of embryos found in eggs that were sampled per group.

3.1.2.1. Bacteriology

The eggs were carefully opened at the blunt (air space) end and the embryo, yolk and albumin removed making sure that the CAM remained intact. The inside of the yolk sacs was aseptically swabbed with cotton buds attached to a plastic shaft. Each sample was immediately placed into its own plastic sleeve, sealed and labeled (as shown in the first left hand columns of Appendices 4, 5 and 6 titled “Specimen ID”). All samples were dispatched the same day to Onderstepoort Poultry Diagnostic Laboratory, Bacteriology Section.

3.1.2.2. Stereomicroscopy

The inside of the egg shells was rinsed with an isotonic saline solution and immediately filled with 10% formalin for retention of the erythrocytes inside the CAM blood vessels. After overnight fixation, the eggs were emptied and allowed to dry. Each egg was then divided into three bands; top (next to blunt end opening), middle and bottom (sharp end). The top and middle bands were further divided into six equal size pieces using a diamond blade mounted on a drill (Dremel).

Twelve pieces of shell per egg were imaged using an Olympus SXZ16 stereomicroscope, and further analyzed by a computer software that was adapted from Verhoelst et. al. (2011). The software was used to measure blood vessel density (vascular fraction, VF) as well as the degree of branching (fractal dimension, FD) of the CAM. The stereoscopic image was converted into a binary image and thinned or skeletonized by well-defined skeletonization algorithms.

The degree of FD was measured using the box-counting method. A relative value was acquired by assigning the negative of the least squared regression gradient of a fit to the log of number of boxes with vessels versus the log of the box size. The R^2 of the regression slope of the log-log plot, was measured to be higher than 0.98 and therefore confirmed the fractality of the system.

The VF was measured by employing a grid-intersection method. It was calculated by the number of intersections for a 16 x 16 pixel box size grid over the image multiplied by the box size (possible number of intersections).

3.1.2.3. Metrics

Broiler chicks were removed from each machine at hatch and then graded. Three groups of 36 first grade chicks were sampled from each treatment group. Yolk-free body mass (YFBM), heart mass and heart-to-YFBM ratio of these chicks were measured and compared.

The chicks' abdomen was cut across and below the apex of the breast bone. The skin and underlying muscles were lifted in order to expose the abdominal viscera. The yolk was gently

pulled out using fingers. Chicks, without the yolk, were then weighed with a micro pocket scale (Micro P. S. 408) and the mass recorded as YFBM. Hearts were removed with small pair of scissors from the cut chicks. Major blood vessels were trimmed off, excess blood manually squeezed out of the atria and hearts then weighed on the Micro P. S. 408 pocket scale.

The rest of the as-hatched first grade chicks were randomly allocated to 16 cages with 8 birds per cage for each temperature treatment group. All birds had access to the same feed on scratch trays and water through nipple lines. All groups of birds were subjected to the same vaccination, temperature, ventilation and lighting programmes.

These birds were slaughtered at 35 days of age at a registered processing plant using the technique of electric stunning and throat-bleeding with a knife as is the case with commercial broilers. Thirty-six (36) hearts per treatment group were sampled to measure the RV: TV proportion. The body mass, feed conversion and mortality of all three groups were measured and compared.

All variables that were analysed statistically were done with Stata 14 (StataCorp, College Station, TX, U.S.A.). Statistical significance was assessed at $p<0.05$. Mortality, heart mass (HM), yolk-free body mass (YFBM) and HM-to-YFBM were compared between groups using the analysis of variance (ANOVA) The Shapiro-Wilk statistical test was used to analyse data on VF and FD for normality and, an appropriate power transformation was selected for each CAM variable for use in the mixed models. Body mass, average daily gain (ADG), feed conversion and performance efficiency factor (PEF) were each compared between groups using linear mixed models. The Bonferroni adjustment was used for multiple comparisons.

CHAPTER 4: RESULTS

4.1. Embryonic Day 14 (ED14)

4.1.1 Vascular Fraction (VF) and Fractal Dimension (FD)

Both density VF and degree of branching FD of the CAM were increased in eggs that were incubated at higher (38.2^{o}C) and lower temperatures (36.8^{o}C), when compared to the control group (37.5^{o}C). The results are depicted in Table 2 below:

Table 2: Vascular fraction and fractal dimension at embryonic day 14 (ED14)

Treatments	Sample Size (n)	Vascular Fraction (VF)		Fractal Dimension (FD)	
		*Mean	SD	*Mean	SD
Cool (36.8^{o}C)	27	0.0095^{a}	0.0031	1.29^{a}	0.92
Control (37.5^{o}C)	33	0.0079^{b}	0.0029	1.24^{b}	0.10
Hot (38.2^{o}C)	26	0.0100^{ac}	0.0033	1.30^{ac}	0.10

*Within a column means with different superscripts are significantly different (95% confidence interval, CI).

Vascular fraction (VF) was significantly lower ($p<0.001$) in control compared to both cool and hot temperatures inside setters and hatchers, but did not differ significantly ($p=0.115$) between cool and hot temperatures. These findings are graphically presented in Figure 1.

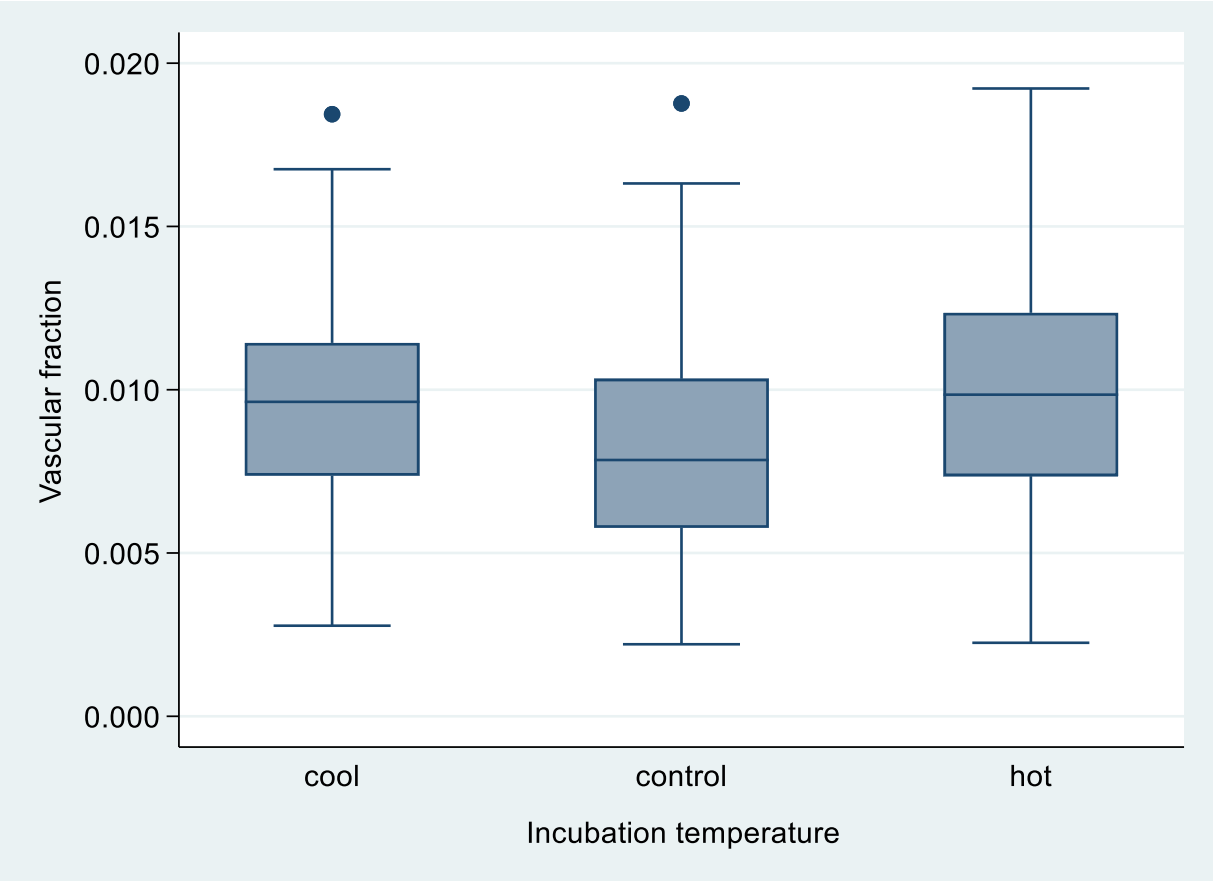

Figure 1: Comparative vascular fraction (VF) at Embryonic Day 14 (ED14)

Fractal dimension was significantly lower ($p<0.001$) in the control compared to both cool and hot treatments, but did not differ significantly ($p=0.103$) between cool and hot setter and hatcher temperatures. Figure 2 and Table 2 depict these findings.

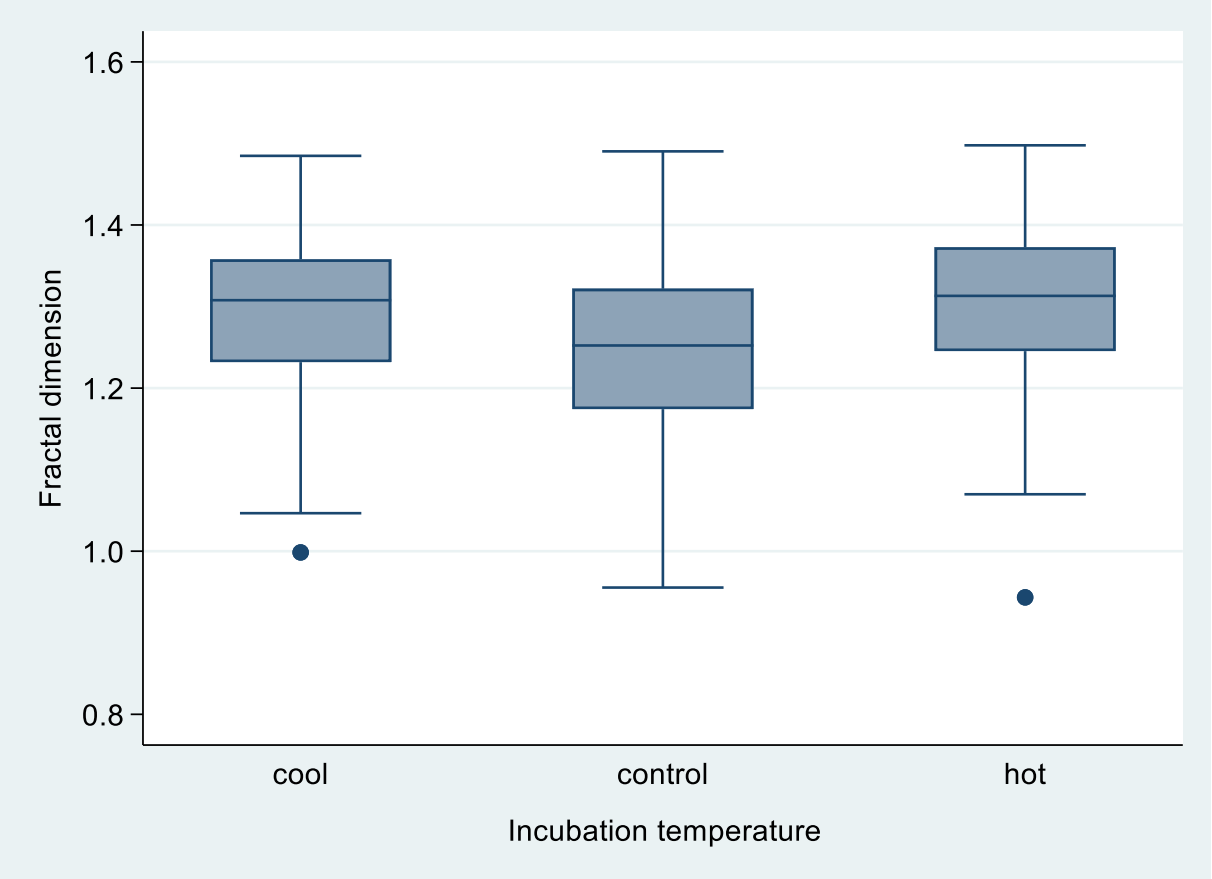

Figure 2: Comparative fractal dimension (FD) at Embryonic Day 14 (ED14)

Plate 1 shows the original image of CAM blood vessels that were captured using an Olympus SXZ16 stereomicroscopy. The fractal dimension (FD) and vascular fraction (VF) were determined from the stereoscopic image that was converted into a binary image and thinned or skeletonized by well-defined skeletonization algorithms (Plate 2).

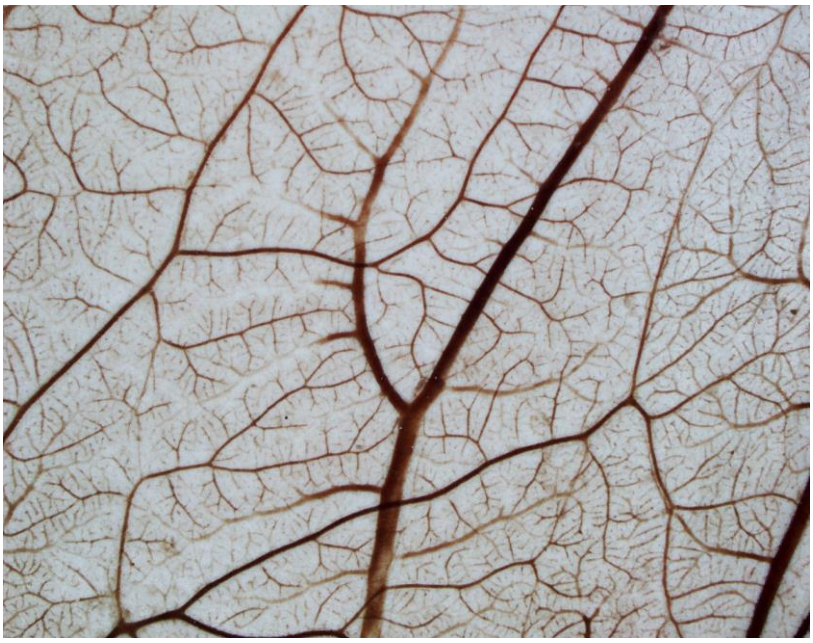

Plate 1: Original stereomicroscopic image of the CAM

Plate 2: Skeleton of original CAM image

4.1.2 Bacteriology of the yolk

During ED14, yolks that were sampled aseptically to assess bacterial load showed that more (19.2%) yolk samples contained bacteria in the hot (38.2^{o}C) group compared to 3% in the control (37.5^{o}C) and 3.7% in the cool (37.5^{o}C) group. Of the 19.2% of the positive samples in the hot group, 60% were positive for gram-negative bacteria. The control group had one positive sample for a gram-negative bacterium. Yolks sampled from eggs incubated cooler only had one sample that tested positive for a gram positive bacterium. These results are summarized in Table 3.

Table 3: Bacterial isolates from yolks of 14 days old (ED14) chick embryos

Treatment	**Sample Size**	**Bacterial Isolates**	**% of Positive Samples**	**% of Samples with Gram-Negative Bacteria**
Cool (36.8^{o}C)	27	*Micrococcus species*	3.7	0
Control (37.5^{o}C)	33	*Pseudomonas fluorescens*	3.0	3
		Enterococcus species		
		Staphylococcus species		
Hot (38.2^{o}C)	26	*Stenotrophomonas maltophilia*	19.2	12
		Escherichia vulneris		

4.2. Embryonic Day 21 (ED21)

4.2.1 Heart mass

Heart mass was significantly greater in the cool group compared to the control (p=0.002) and the hot group (p<0.001). However, the heart mass did not differ significantly between control and hot (p=0.699) groups. Figure 3 highlights these findings.

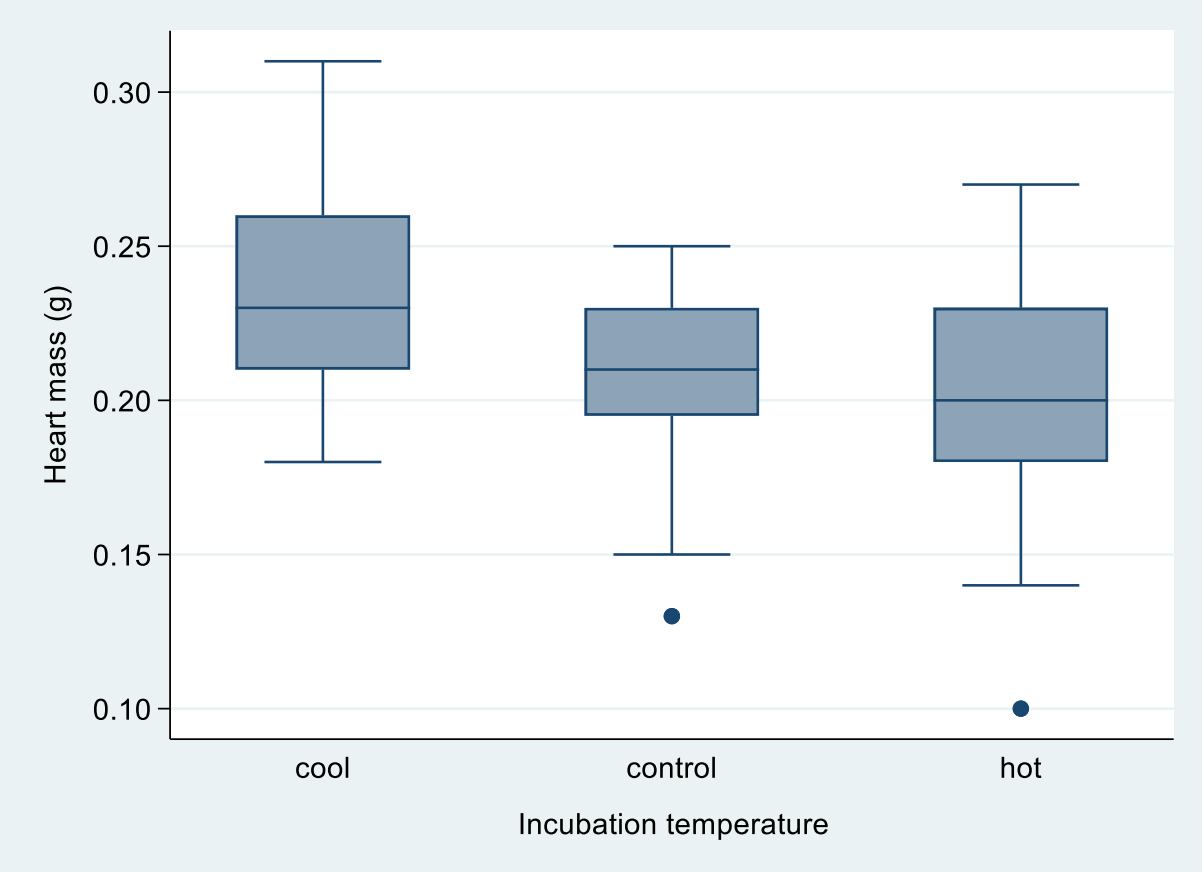

Figure 3: Impact of incubation and hatcher temperature on embryonic heart mass

4.2.2 Yolk-free body mass

Yolk-free body mass was significantly greater in the cool group than in the control group ($p=0.007$) and the hot group ($p<0.001$). Also, YFBM was significantly greater in the control compared to the hot ($p=0.042$).

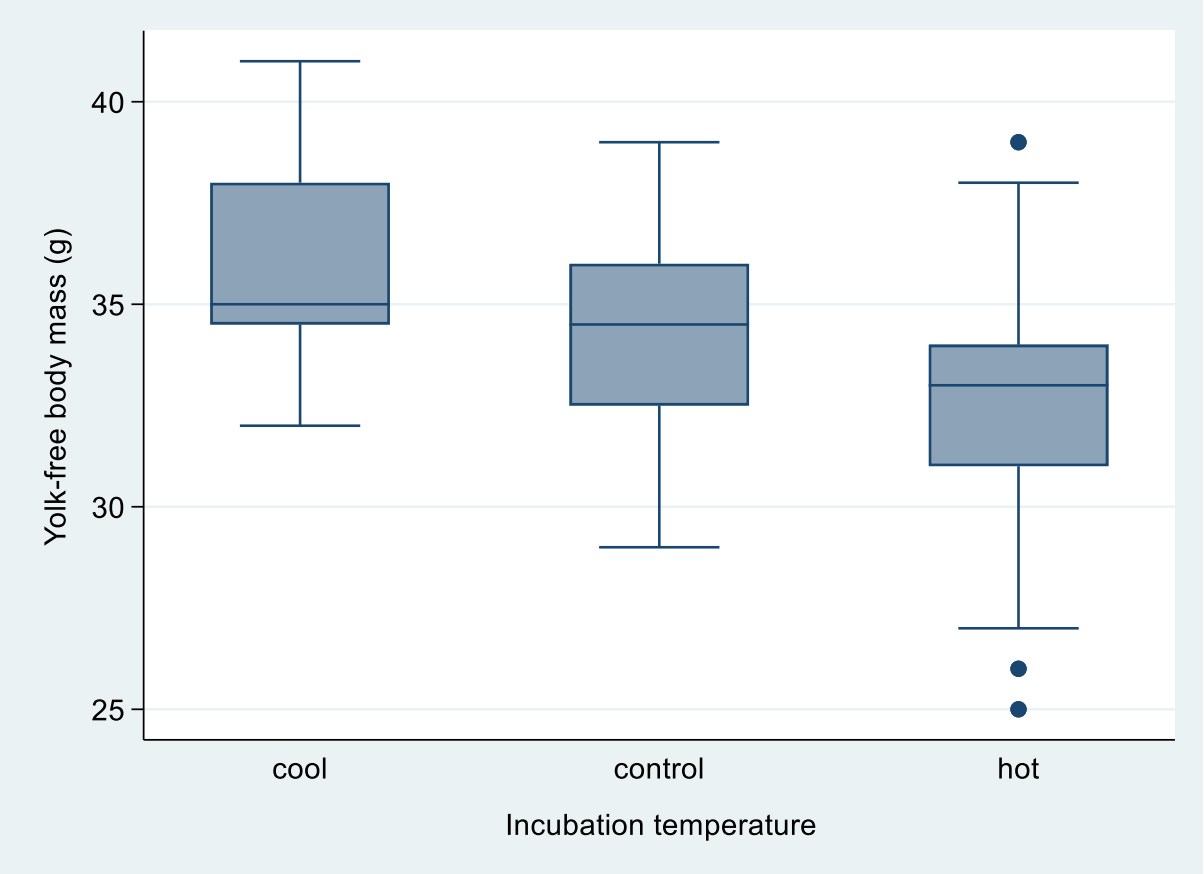

Figure 4: Effect of incubation and hatcher temperature on embryonic yolk-free body mass

4.2.3 Heart mass to Yolk-free body mass ratio

No statistically significant differences were observed in HM: YFBM between groups (p=0.178).

A graphical summary of the statistical analysis of HM: YFBM is illustrated in Figure 5.

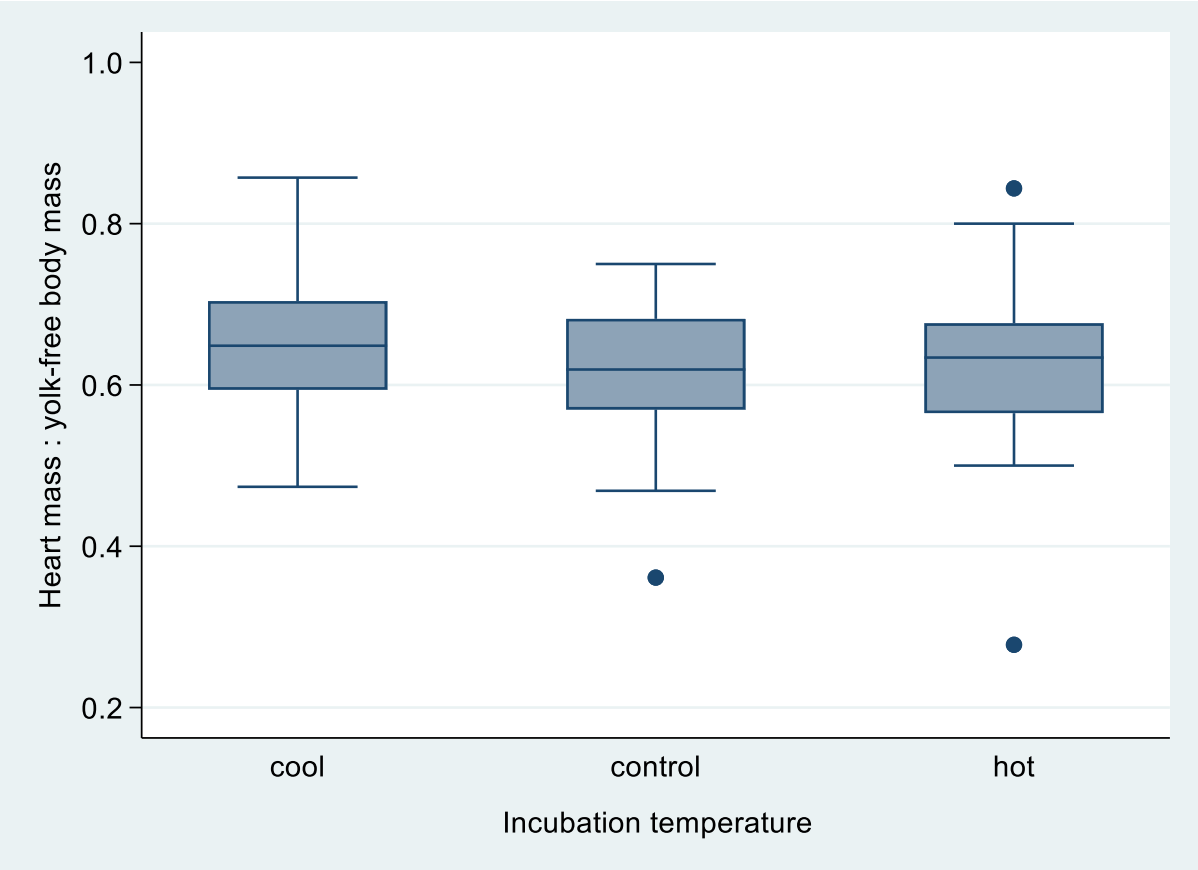

Figure 5: Effect of incubation and hatcher temperature on HM: YFBM

Increasing incubation temperature resulted in smaller hearts and lower YFMB. The mean heart mass to YFBM ratio was statistically not significant between all three treatment groups. Table 4 below shows a summary of these results.

Table 4: Heart mass and yolk-free body mass of first-grade chicks at hatch (ED21)

Parameter	**Sample size (n)**	**Cool (36.8°C)**		**Control (37.5°C)**		**Hot (38.2°C)**	
		***Mean**	**SD**	***Mean**	**SD**	***Mean**	**SD**
Heart mass (HM)	36	0.24^{a}	0.032	0.21^{bc}	0.028	0.20^{c}	0.034
Yolk Free Body Mass (YFBM)	36	36^{a}	2.38	34^{b}	2.13	33^{c}	3.10
HM: YFBM	36	0.66	0.09	0.62	0.09	0.62	0.10

*Within a row means with different superscripts are significantly different (95% confidence interval, CI).

Raw data on heart mass and yolk-free body mass of all three treatment groups is shown in Appendix 1.

4.3. Weekly performance including Day 35

Weekly performance of broiler chicks from the cool, control and hot treatment groups are depicted in graphical form and on tables below.

High incubator and hatcher temperature resulted in poor broiler performance compared to control and cool treatment groups.

4.3.1 Mortality

The incubators used for the three different heat treatments were stationed in the same high altitude facility, suggesting that broiler mortalities were mainly due to ascites. However, mortality was almost double in the high temperature group if compared to the control ($p<0.001$) and cool ($p<0.001$) treatment groups. Figure 6 illustrates the trend in mortality.

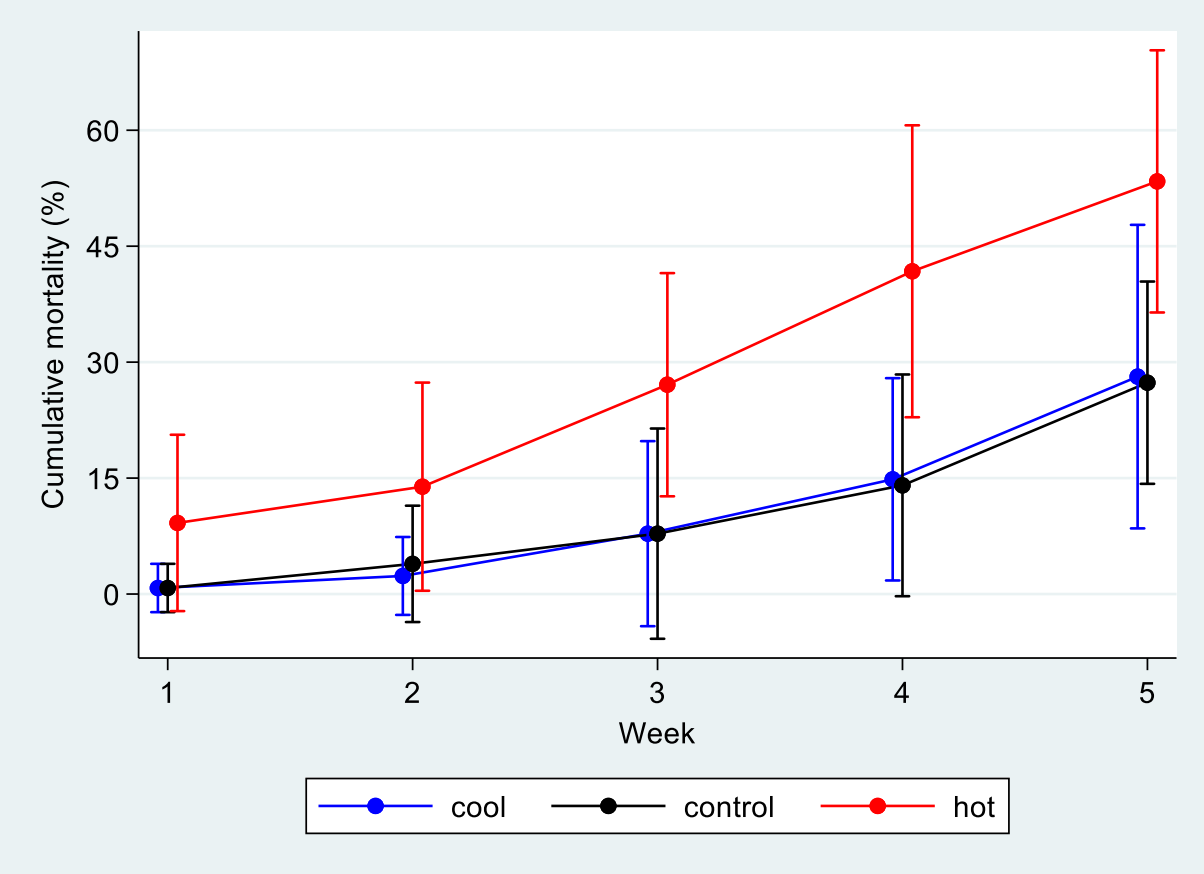

Figure 6: Effect of incubation and hatcher temperature on mortality due to ascites

4.3.2 Body mass

Body mass of broilers from the cool and control groups was significantly ($p<0.05$) greater than that of the hot group. Weekly trend between the three groups is shown in Figure 7.

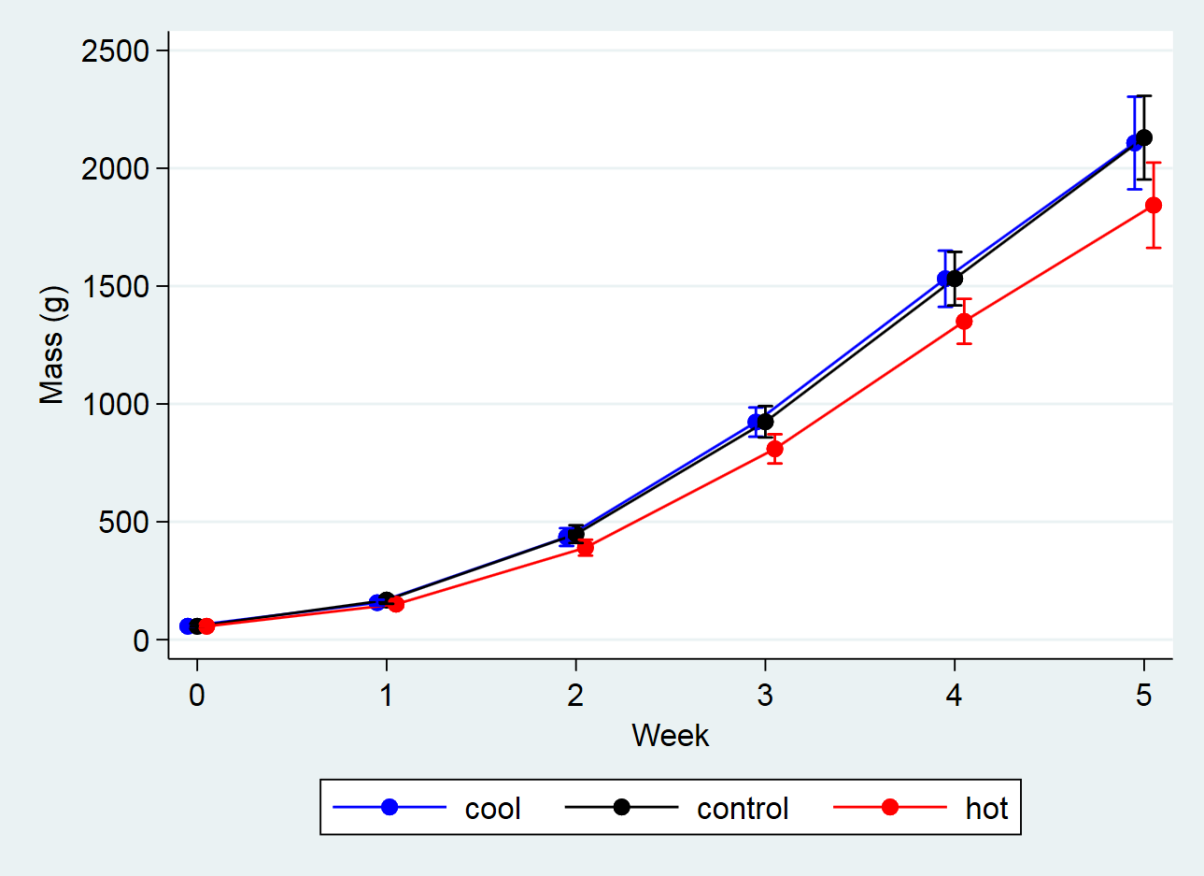

Figure 7: Effect of incubation and hatcher temperature on body mass (mean ± SD)

A weekly comparison in average body mass and standard deviation from the mean is shown in Table 5.

Table 5: Weekly average broiler body mass between the three different treatment groups

Body Mass						
Age	**Cool** (36.8^{o}C)		**Control** (37.5^{o}C)		**Hot** (38.2^{o}C)	
(weeks)	*Mean	SD	*Mean	SD	*Mean	SD
0	43	0,7	42	0,8	42	1,9
1	157	12,5	168	16,1	150	14,6
2	435	37,7	448	37,7	390	33,7
3	923^{a}	62,1	924^{a}	66,6	809^{b}	62,0
4	1531^{a}	119,3	1532^{a}	113,4	1351^{b}	95,3
5	2107^{a}	196,5	2130^{a}	177,6	1843^{b}	181,0

*Within a row means with different superscripts are significantly different (95% confidence interval, CI).

SD = Standard Deviation.

Broiler body mass of cool and control treatment groups was significantly greater than that of the hot treatment group at the age of week3 ($p<0.003$), week 4 ($p<0.001$) and week 5 ($p<0.001$).

4.3.3 Average daily gain (ADG)

In terms of ADG, the cool and control treatment groups gained significantly ($p<0.001$) more mass than the hot treatment group during week 5 of age as shown in Figure 8 and Table 7.

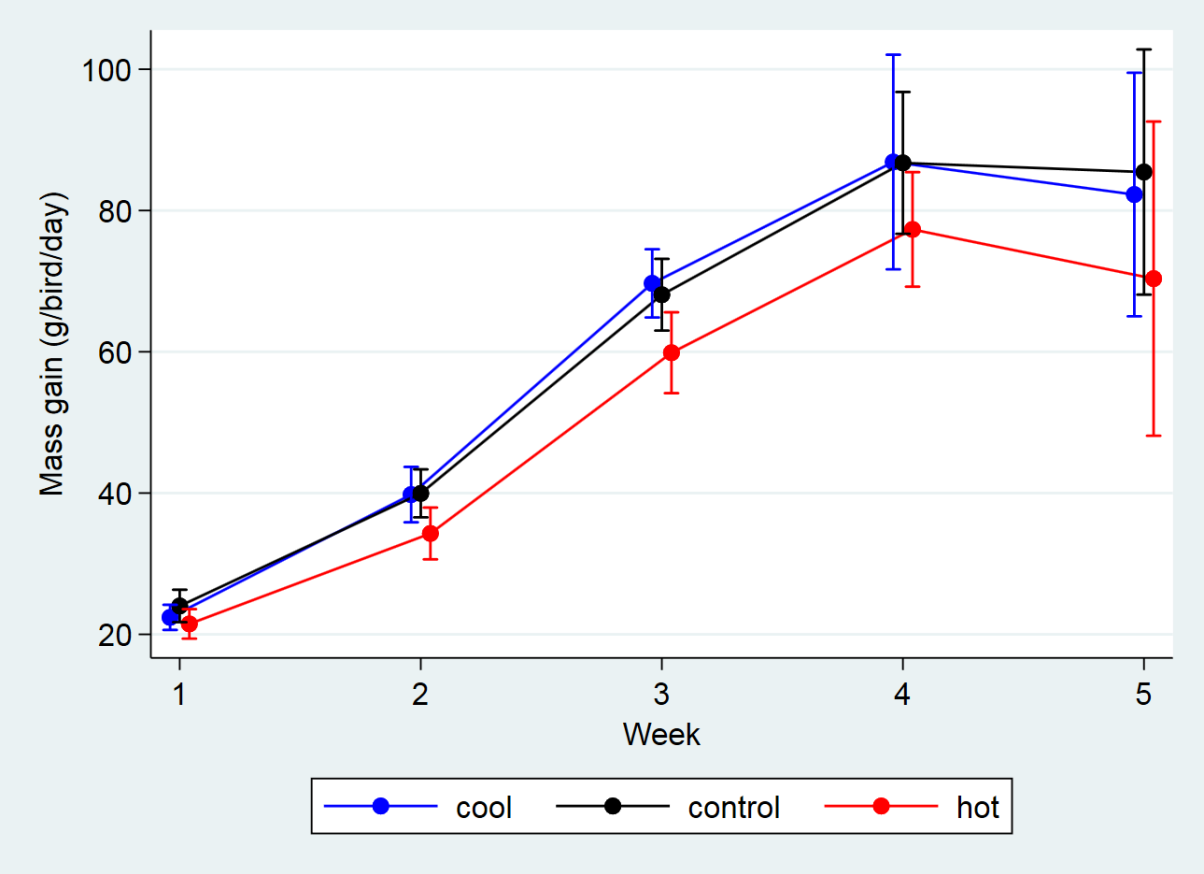

Figure 8: Effect of incubation and hatcher temperature on average daily gain (mean ± SD)

A weekly comparison in ADG and standard deviation from the mean is shown on Table 6.

Table 6: Weekly average daily gain between groups

Average Daily Gain (ADG)						
Age	**Cool** (36.8^{o}C)		**Control** (37.5^{o}C)		**Hot** (38.2^{o}C)	
(weeks)	*Mean	SD	*Mean	SD	*Mean	SD
1	22	1.8	24	2.3	21	2.1
2	40	3.9	40	3.4	34	3.7
3	70	4.8	68	5.1	60	5.7
4	87	15.2	87	10.0	77	8.1
5	82^{a}	17.2	85^{a}	17.4	70^{b}	22.2

*Within a row means with different superscripts are significantly different (95% confidence interval, CI).

SD = Standard Deviation.

4.3.4 Feed conversion ratio (FCR)

The difference in FCR for broilers between the cool and hot treatment group and between control and hot treatment groups was not significant throughout the entire 5-week grower period. Figure 9 show the trend per week of feed conversion until week 5 of broiler growth.

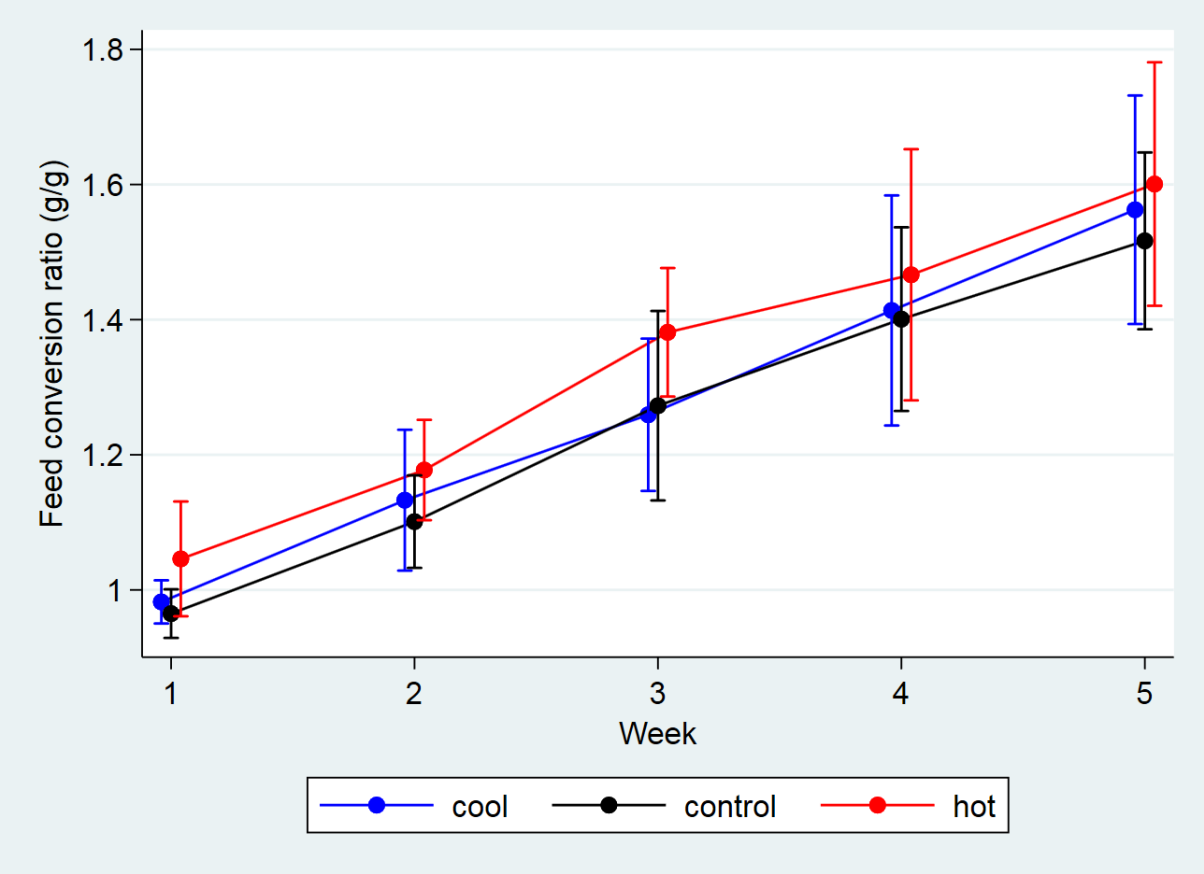

Figure 9: Effect of incubation and hatcher temperature on feed conversion (mean ± SD)

Weekly feed conversion values and standard deviation are shown in Table 7.

Table 7: Weekly feed conversion ratio between groups

Feed Conversion Ratio (FCR)						
Age	**Cool** (36.8^{o}C)		**Control** (37.5^{o}C)		**Hot** (38.2^{o}C)	
(weeks)	*Mean	SD	*Mean	SD	*Mean	SD
1	0.98	0.03	0.96	0.04	1.05	0.08
2	1.13	0.10	1.10	0.07	1.18	0.07
3	1.26	0.11	1.27	0.14	1.38	0.10
4	1.41	0.17	1.40	0.14	1.47	0.19
5	1.56	0.17	1.52	0.13	1.60	0.18

*Within a row means with different superscripts are significantly different (95% confidence interval, CI).

SD = Standard Deviation.

There was no statistical difference in feed conversion between the cool and the hot group and, between the control and the hot treatment.

4.3.5 Performance Efficiency Factor (PEF)

The performance efficiency factor (PEF) of the broilers from the control group, during the 5 weeks up to slaughter, was statistically higher ($p<0.001$) than the PEF for broilers from the hot treatment group. The same trend was recorded between the cool and hot treatment groups, where broilers from the cool treatment group showed higher ($p<0.05$) PEF than the broilers from the hot treatment group. At slaughter age the PEF for cool treatment birds had a significance level of less than 0.001. Figure 10 illustrates this trend with specific values shown in Table 8.

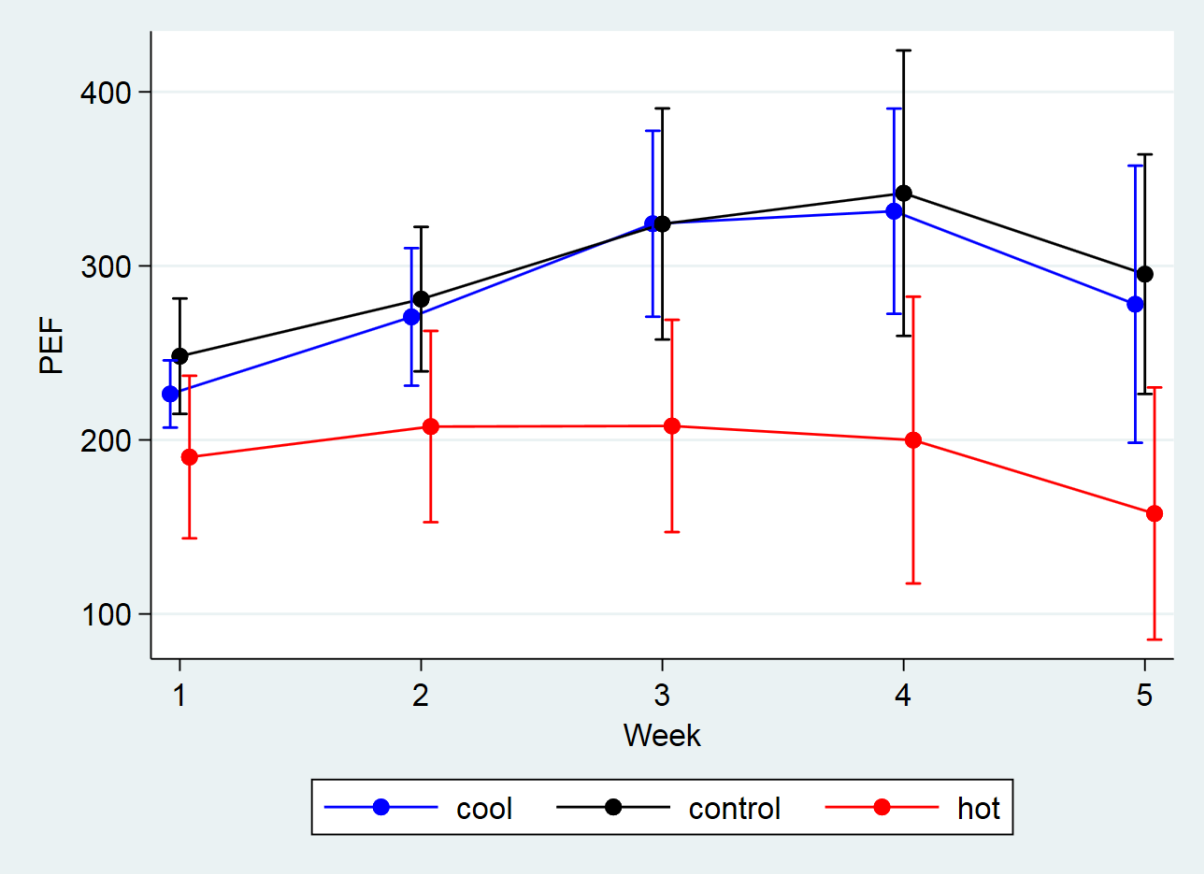

Figure 10: Effect of incubation and hatcher temperature on performance efficiency factor (mean ± SD)

A weekly comparison in PEF and standard deviation (SD) is shown in Table 8.

Table 8: Weekly performance efficiency factor between groups

Performance Efficiency Factor (PEF)						
Age	**Cool** (36.8^{o}C)		**Control** (37.5^{o}C)		**Hot** (38.2^{o}C)	
(weeks)	*Mean	SD	*Mean	SD	*Mean	SD
1	226^{a}	19.3	248^{a}	33.2	190^{ab}	46.7
2	271^{a}	39.5	281^{a}	41.5	208^{b}	55.0
3	324^{a}	53.4	324^{a}	66.4	208^{b}	61.0
4	331^{a}	59.0	342^{a}	82.0	200^{b}	82.4
5	278^{a}	79.6	295^{a}	68.8	158^{b}	72.5

*Within a row means with different superscripts are significantly different (95% confidence interval, CI).

Table 9 shows broiler performance figures between the cool, control and hot groups in a consolidate format.

Table 9: Broiler performance at 35 days of age (processing)

Performance parameters	**Cool** (36.8^{o}C)	**Control** (37.5^{o}C)	**Hot** (38.2^{o}C)	**Cool vs Hot**	**Control vs Hot**
				(p value)	(p value)
Body mass (g)	2 107	2 130	1 843	<0.001	<0.001
Feed conversion (g/g)	1.563	1.517	1.601	<1.000	<0.536
Cumulative mortality (%)	28	27	53	<0.001	<0.001
PEF	278	292	153	<0.001	<0.001

4.3.6 Right ventricle to total ventricular proportion

Thirty six heart ventricles from each treatment group were compared in terms of mass to assess susceptibility of birds to ascites at 35 days of age. The values obtained are depicted in Table 10.

Table 10: Heart ventricular mass and ratios at 35 days of age (processing)

Treatments	Sample Size (n)	Total Ventricle (TV) mass	Right Ventricle (RV) mass	RV: TV Proportion	Normal Range
Cool (36.8^{o}C)	36	7.96g	2.07g	0.26	0.15-0.27
Control (37.5^{o}C)	36	7.97g	2.28g	0.28	0.15-0.27
Hot (38.2^{o}C)	36	7.42g	1.89g	0.26	0.15-0.27

The ratio between the right heart ventricles to total ventricle of the broilers from the control group was 7% higher than those of the cool and hot groups which were the same at 0.26.

CHAPTER 5: DISCUSSION AND CONCLUSION

Molenaar et al. (2011) found that the heart mass was 26% lower at hatch in broiler chicks from the high temperature treatment (38.9^{o}C) if compared to chicks hatched from eggs at normal temperature (37.8^{o}C) treatment. In this study though, heart mass was significantly higher in the cool (36.8^{o}C) treatment group compared to both the control (37.5^{o}C) and the hot treatment groups (38.2^{o}C). Heart mass did not differ between control and hot treatment groups. High incubation temperature increased the incidence of ascites which may have been as a result of reduced heart development at hatch (Molenaar et al., 2011). This supports our findings that mortality due to ascites was significantly higher in broilers from the hot treatment group if compared to both control and cool treatment groups.

Further, Maatjens et al. (2014b) found that regardless of carbon dioxide (CO_2), high (38.9^{o}C) egg shell temperature resulted in smaller chick and heart mass compared to low (36.7^{o}C) and control (37.8^{o}C) temperature treatments. These results suggest that embryo growth and development is probably temperature dependent and, that broilers are more sensitive to high incubation temperature than to low temperatures (Willemsen et al., 2010).

As high (>37^{o}C) incubator temperature depresses yolk utilization (Wineland et al., 2006), yolk-free body mass (YFBM) was used as one of the chick embryo variables that could be affected by temperature. Results from our study showed that YFBM was the highest for the cool treatment group, followed by the control, with the lowest YFBM in eggs from the hot treatment group. This is consistent with findings by Molenaar at al. (2011) that the YFBM was 3g lower in high (38.9^{o}C) compared with normal (37.8^{o}C) treatment. Small deviations of 1.5^{o}C from egg shell temperatures of 37.8^{o}C could affect embryonic development causing YFBM to decrease by 10% at hatch (Lourens et al., 2010). Thus, as the incubation and hatcher temperatures are increased, the YFBM is negatively affected.

Body mass of cool and control treatment groups were 2 107g and 2 130g, respectively, and both heavier than the hot treatment group that weighed 1 843g. The ADG of 82g for the cool treatment group and 85g for the control treatment groups were both significantly more than 70g for the hot group during week 5 of age. These findings indicate that high incubation

temperatures had a negative effect on growth of modern broilers. This is supported by Molenaar et al. (2011).

Results showed a numerical difference between the cool, control and hot treatment groups in terms of feed conversion. The control had a lower (1.52) feed conversion compared to the cool (1.56) treatment which performed better than the hot (1.60) treatment group which contradict Molenaar et al. (2011), who found no difference in feed conversion between the high and normal treatment groups. The difference between the two studies is likely to have been as a result of high mortality in the hot group where birds continued to die from ascites late in their fattening life. Mortality in the hotter group was almost double that of the control and cool groups. This high mortality in the hot group may also have resulted in lower heart ventricular ratio in the hot group at slaughter.

Overall PEF between three temperature treatment groups was better in the control (295) group compared to the cool (278) and hot (158) treatments. Broiler chicks from the hot treatment group performed poorly relative to the other two treatments.

Both density (VF) and degree of branching (FD) of the CAM were increased in eggs that were incubated at higher and lower temperatures, when compared to the control group. This increase in CAM vascularisation is likely as a result of temperature insult to CAM blood vessels (Druyan et al., 2012). As blood vessel pathology plays a significant role in the pathophysiology of ascites, increased vascularisation may be an indicator of predisposition of modern broilers to ascites during their fattening phase on the farm.

The conclusion is that the genetic advancement on broiler growth rate and feed utilization efficiency pose a unique and significant hatchery and on-farm management challenge at high altitude. The cardio-vascular health starts during embryonic stage. An ideal setter environment, including temperature, is required to support and match rapid growth of birds placed on farms located at high altitude where there is lower oxygen availability.

The CAM is an interim respiratory organ of the chicken embryo for more than half of its embryonic life. Though CAM vascularisation was increased as a result of hot and cool temperature treatments, it couldn't be concluded from this study how much effect gram-negative (EETs) bacteria may have had on the vascularisation of the CAM between the three

different treatment groups. However, insults such as too high temperatures and bacterial contamination, especially gram negatives, to the CAM's vascular network must be eliminated or minimized.

Broiler performance in terms of mortality due to ascites or pulmonary arterial hypertension (PAH), feed conversion and live mass can be improved through establishment of an ideal incubation and hatcher temperature. The current industry standard is that eggs are incubated at 37.5^{o}C at 55-70% relative humidity for 18-19 days. Eggs are transferred to hatchers that are 36-37.5^{o}C inside and 60% relative humidity is maintained in passages of rooms where hatchers are housed. Dumpers of hatcher machines are opened wider as more chicks hatch.

In order to minimise vascular damage and achieve bigger YFBM and heart size in modern broiler chicks at high altitude (1 508 meters), eggs must be incubated at 37.5^{o}C with 55-59% relative humidity. Hatcher temperature must be maintained at 36.8^{o}C and the relative humidity maintained at 60% in passages of rooms where hatchers are housed. Dumpers must be opened wider as more chicks hatch. This practice will help improve broiler body mass at term, reduce mortalities caused by ascites and feed conversion. The latter is likely to be improved as a result of reduced mortality that occurs late in the broiler's fattening life.

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APPENDICES

Appendix 1

Raw data of heart mass and yolk-free body mass of first-grade chicks at hatch hatched at 36.8°C (Cool group)

Treatment	**Cool (36.8°C)**			
Chick No.	**Chick Mass (g)**	**Yolk Mass (g)**	**Heart Mass, HM (g)**	**YFBM (g)**
1	42	8	0,23	35
2	38	6	0,24	32
3	41	7	0,20	32
4	41	7	0,20	34
5	42	7	0,26	34
6	45	6	0,23	38
7	47	10	0,18	38
8	46	9	0,24	37
9	45	8	0,24	37
10	44	9	0,20	35
11	46	10	0,23	35
12	42	7	0,24	35
13	43	8	0,27	35
14	51	10	0,22	41
15	44	8	0,22	36
16	44	8	0,22	35
17	46	9	0,21	35
18	43	8	0,24	37
19	46	9	0,21	37
20	45	8	0,26	36
21	47	9	0,26	38
22	45	7	0,25	38
23	52	10	0,24	41
24	50	12	0,26	38
25	50	10	0,23	40
26	44	8	0,21	35
27	44	8	0,28	35
28	41	6	0,20	34
29	44	11	0,22	33
30	43	9	0,21	34
31	42	8	0,18	33
32	47	9	0,30	38
33	42	6	0,30	35
34	49	9	0,23	38
35	48	9	0,31	40
36	39	6	0,26	33
Average	**45**	**8**	**0,24**	**36**

Appendix 2

Raw data of heart mass and yolk-free body mass of first-grade chicks at hatch hatched at 37.5°C (Control group)

Treatment	**Control (37.5°C)**			
Chick No.	**Chick Mass (g)**	**Yolk Mass (g)**	**Heart Mass, HM (g)**	**YFBM (g)**
1	43	7	0,21	36
2	42	7	0,21	35
3	41	9	0,18	32
4	45	9	0,18	36
5	44	8	0,13	36
6	46	9	0,21	37
7	43	9	0,20	34
8	44	10	0,25	34
9	44	9	0,23	35
10	40	8	0,24	32
11	44	7	0,23	37
12	41	6	0,25	35
13	44	7	0,21	37
14	44	8	0,25	36
15	43	8	0,18	35
16	39	8	0,18	31
17	44	7	0,19	36
18	42	7	0,23	34
19	43	9	0,24	33
20	42	7	0,20	35
21	48	11	0,24	37
22	43	8	0,25	35
23	46	10	0,21	35
24	41	8	0,15	32
25	41	9	0,22	32
26	41	8	0,21	33
27	40	6	0,20	33
28	38	6	0,20	32
29	40	6	0,23	34
30	40	5	0,21	34
31	40	6	0,23	31
32	48	9	0,19	39
33	40	10	0,18	29
34	40	7	0,24	32
35	42	7	0,21	35
36	41	7	0,21	33
Average	**42**	**8**	**0,21**	**34**

Appendix 3

Raw data of heart mass and yolk-free body mass of first-grade chicks at hatch hatched at 38.2°C (Hot group)

Treatment	Hot (38.2°C)			
Chick No.	**Chick Mass (g)**	**Yolk Mass (g)**	**Heart Mass, HM (g)**	**YFBM (g)**
1	43	7	0,10	36
2	40	8	0,18	32
3	42	8	0,26	34
4	42	7	0,25	35
5	42	8	0,20	34
6	42	9	0,18	33
7	41	8	0,21	33
8	43	7	0,18	36
9	48	11	0,19	37
10	45	7	0,24	38
11	37	6	0,21	31
12	39	12	0,18	27
13	38	9	0,20	29
14	41	8	0,21	33
15	44	7	0,21	37
16	40	10	0,20	30
17	36	7	0,17	29
18	42	11	0,24	31
19	44	10	0,22	34
20	33	6	0,14	26
21	42	11	0,18	31
22	40	8	0,20	32
23	40	7	0,17	33
24	41	7	0,24	34
25	39	7	0,20	32
26	43	9	0,18	34
27	42	8	0,23	34
28	39	8	0,18	31
29	40	7	0,21	33
30	44	9	0,23	35
31	42	8	0,21	34
32	36	11	0,20	25
33	38	5	0,18	33
34	46	7	0,25	39
35	42	8	0,23	34
36	40	8	0,27	32
Average	**41**	**8**	**0,20**	**33**

Appendix 4

Raw data of bacteriology on yolk sac samples taken from eggs that were incubated at 36.8°C (Cool group)

BACTERIOLOGY TEST REPORT			DVTD BACTERIOLOGY LABORATORY Private Bag X04 Onderstepoort 0110 Republic of South Africa Tel (012) 529-8225/8 Fax (012) 529-8312 http://www.up.ac.za Faculty of Veterinary Science Dept of Veterinary Tropical Diseases *E-mail: johan.gouws@up.ac.za*
Lab case number	**:**	**B1042/2017**	
PRC number	**:**	**459/2017**	
Order no	**:**	**None stated**	
Date samples received	**:**	07/04/2017	
Date samples completed	**:**	19/04/2017	
Date of report	**:**	19/04/2017	
Department	**:**	Department of Production Animal Studies	
Clinic	**:**	Section of Poultry Health	
		Faculty of Veterinary Science	
		Private Bag x04	
		Onderstepoort	
		110	
Telephone number	**:**	012 529 8334/8224	
Fax number	**:**	012 529 8306	
Attention	**:**	Dr DBR. Wandrag	
Client / Owner	**:**	**Dr Lukhele/Dr B Wandrag**	
Farm	**:**	Dr Lukhele	
Site	**:**	None stated	
House	**:**	None stated	
Species	**:**	Chicken: Not specified	

SPECIMEN ID	Treatment (°C) COOL	SAMPLE CONDITION	RESULT
ML 14	36,8	Good	*Micrococcu* s species
ML12	36,8	Good	Negative
BLc	36,8	Good	Negative
MR 9	36,8	Good	Negative
BR 19	36,8	Good	Negative
BL 20	36,8	Good	Negative
BR 1-1	36,8	Good	Negative
TRm	36,8	Good	Negative
BRm	36,8	Good	Negative
TL 4	36,8	Good	Negative
TL 6	36,8	Good	Negative
MRm	36,8	Good	Negative
BL 22	36,8	Good	Negative
ML 13	36,8	Good	Negative
BL 23	36,8	Good	Negative
MR 11	36,8	Good	Negative
MLm	36,8	Good	Negative
BR 17	36,8	Good	Negative
ML15	36,8	Good	Negative
TL 5	36,8	Good	Negative
TLm	36,8	Good	Negative
TR 2	36,8	Good	Negative
MR 8	36,8	Good	Negative
TR 3	36,8	Good	Negative
MLc	36,8	Good	Negative
TL 7	36,8	Good	Negative
BR 18	36,8	Good	Negative

Appendix 5

Raw data of bacteriology on yolk sac samples taken from eggs that were incubated at 37.5oC (Control group)

BACTERIOLOGY TEST REPORT			DVTD BACTERIOLOGY LABORATORY Private Bag X04 Onderstepoort 0110 Republic of South Africa Tel (012) 529-8225/8 Fax (012) 529-8312 http://www.up.ac.za Faculty of Veterinary Science Dept of Veterinary Tropical Diseases E-mail: johan.gouws@up.ac.za
Lab case number	**:**	**B1042/2017**	
PRC number	**:**	**459/2017**	
Order no	**:**	**None stated**	
Date samples received	**:**	07/04/2017	
Date samples completed	**:**	19/04/2017	
Date of report	**:**	19/04/2017	
Department	**:**	Department of Production Animal Studies	
Clinic	**:**	Section of Poultry Health	
		Faculty of Veterinary Science	
		Private Bag x04	
		Onderstepoort	
		110	
Telephone number	**:**	012 529 8334/8224	
Fax number	**:**	012 529 8306	
Attention	**:**	Dr DBR. Wandrag	
Client / Owner	**:**	**Dr Lukhele/Dr B Wandrag**	
Farm	**:**	Dr Lukhele	
Site	**:**	None stated	
House	**:**	None stated	
Species	**:**	Chicken: Not specified	

SPECIMEN ID	Treatment (^{o}C) CONTROL	SAMPLE CONDITION	RESULT
TL 5	37,5	Good	*Pseudomonas* fluorescens
MR 1-1	37,5	Good	Negative
MR 11	37,5	Good	Negative
MLm	37,5	Good	Negative
TR 2	37,5	Good	Negative
MR 1-1	37,5	Good	Negative
MLc	37,5	Good	Negative
TLc	37,5	Good	Negative
MR 8	37,5	Good	Negative
MR 10	37,5	Good	Negative
ML14	37,5	Good	Negative
TR 1	37,5	Good	Negative
BR 18	37,5	Good	Negative
TL 7	37,5	Good	Negative
ML13	37,5	Good	Negative
TR 1-1	37,5	Good	Negative
MR 10	37,5	Good	Negative
BLc	37,5	Good	Negative
TL 6	37,5	Good	Negative
BL 22	37,5	Good	Negative
TR 3	37,5	Good	Negative
BR 1-1	37,5	Good	Negative
BR 17	37,5	Good	Negative
BR 16	37,5	Good	Negative
TLm	37,5	Good	Negative
BLm	37,5	Good	Negative
BR 19	37,5	Good	Negative
BL 21	37,5	Good	Negative
BLm (Duplicate)	37,5	Good	Negative
TRm	37,5	Good	Negative
ML 15	37,5	Good	Negative
BL 20	37,5	Good	Negative
MR 9	37,5	Good	Negative

Appendix 6

Raw data of bacteriology on yolk sac samples taken from eggs that were incubated at 38.2°C (Hot group)

BACTERIOLOGY TEST REPORT			DVTD BACTERIOLOGY LABORATORY Private Bag X04 Onderstepoort 0110 Republic of South Africa Tel (012) 529-8225/8 Fax (012) 529-8312 http://www.up.ac.za Faculty of Veterinary Science Dept of Veterinary Tropical Diseases E-mail: johan.gouws@up.ac.za
Lab case number	**:**	**B1042/2017**	
PRC number	**:**	**459/2017**	
Order no	**:**	**None stated**	
Date samples received	**:**	07/04/2017	
Date samples completed	**:**	19/04/2017	
Date of report	**:**	19/04/2017	
Department	**:**	Department of Production Animal Studies	
Clinic	**:**	Section of Poultry Health	
		Faculty of Veterinary Science	
		Private Bag x04	
		Onderstepoort	
		110	
Telephone number	**:**	012 529 8334/8224	
Fax number	**:**	012 529 8306	
Attention	**:**	Dr DBR. Wandrag	
Client / Owner	**:**	**Dr Lukhele/Dr B Wandrag**	
Farm	**:**	Dr Lukhele	
Site	**:**	None stated	
House	**:**	None stated	
Species	**:**	Chicken: Not specified	

SPECIMEN ID	Treatment (°C) HOT	SAMPLE CONDITION	RESULT
MLm	38,2	Good	*Enterococcus* species
MR 11	38,2	Good	*Staphylococcus* species
TRm	38,2	Good	*Stenotrophomonas maltophilia*
BR 1-1	38,2	Good	*Stenotrophomonas maltophilia*
BL 23	38,2	Good	*Escherichia vulneris*
TL 5	38,2	Good	Negative
MLc	38,2	Good	Negative
TL7	38,2	Good	Negative
BR 17	38,2	Good	Negative
MRm	38,2	Good	Negative
TLm	38,2	Good	Negative
BR 1-1	38,2	Good	Negative
MRm	38,2	Good	Negative
ML 14	38,2	Good	Negative
BL 20	38,2	Good	Negative
TR 0	38,2	Good	Negative
BLc	38,2	Good	Negative
BLm	38,2	Good	Negative
BR16	38,2	Good	Negative
MR 9	38,2	Good	Negative
MR1-1	38,2	Good	Negative
TLc	38,2	Good	Negative
TL 4	38,2	Good	Negative
TR1-1	38,2	Good	Negative
BL 21	38,2	Good	Negative
TR 1	38,2	Good	Negative

ADDENDA: Animal Ethics Committee Approval

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Animal Ethics Committee

PROJECT TITLE	**Influence of incubation temperature on chorio-allantoic membrane (CAM) vascularisation, heart size and ascites in broilers**
PROJECT NUMBER	**V102-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr OM Lukhele**

STUDENT NUMBER (where applicable)	**UP_96302918**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet (Altil)**

ANIMAL SPECIES	**Broilers**	
NUMBER OF ANIMALS	**2640**	
Approval period to use animals for research/testing purposes		**February 2016-February 2017**
SUPERVISOR	**Dr. B Wandrag**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	5 February 2016
CHAIRMAN: UP Animal Ethics Committee	Signature	

S4285-15