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Effect of High Incubation Temperature on Embryo Livability, Mortality, Hatchability, and Chick Quality in Commercial Layers

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Abstract: This research was conducted to determine the effect of high incubation temperature profiles on embryo livability, mortality, hatchability, and chick quality in commercial layers. Incubation temperature profiles were: Control (day 0-4: 37.8°C, day 5-9: 37.2°C, day 10-11: 37.0°C, day 12-13: 36.8°C), moderately high (day 0-4: 38.5°C, day 5-9: 38.2°C, day 10-11: 37.5°C, day 12-13: 37.0°C) and extremely high (day 0-4: 39.5°C, day 5-9: 39.0°C, day 10-11: 38.0°C, day 12-13: 37.5°C). The general linear model procedure in Minitab 17 was used to analyze data, whereas the mean separation was conducted using Fisher's LSD test ($p<0.05$). Temperature profiles influenced ($p<0.05$) livability, day 0-10, total moisture loss, body weight, and total mortality. The embryo livability was higher in a moderately high incubation temperature profile (93.31%) followed by control (86.55%) and extremely high incubation temperature profile (72.61%). Total moisture loss was higher ($p<0.05$) in the extremely high incubation temperature profile (16.55%) than in other temperatures. Dry chicks were greater in the extremely high incubation temperature profile (99.21%) compared to control (97.76%) and moderate high incubation temperature profile (93.19%). First-grade female yield was higher ($p<0.05$) in control (46.10%) compared to the other temperature treatments. The total hatch of a fertile was higher in the moderate-high incubation temperature profile (96.15%) compared to the control (93.63%) and extremely high incubation temperature profile (89.62%). Total embryo mortality was higher ($p<0.05$) in an extremely high incubation temperature profile (10.38%), followed by the control (6.37%), and then by a moderately high incubation temperature profile (3.69%). Moderate high incubation temperature profile showed great promise as a tool to increase embryo, livability, hatchability, and chick quality and reduce embryo mortality.

Keywords: Embryo Mortality, Hatchability, Yolk Weight

Introduction

South Africa's poultry industry continues to be the most significant sole contributor to the agricultural industry, employs more than 110,000 people, and accounts for 65.6% of domestically produced animal protein (South African Poultry Association, 2021). The average individual's consumption of eggs and meat of poultry has increased in the past few years (Association, 2020; 2021), as a result, millions of eggs are incubated throughout South African hatcheries to meet the growing demand. During incubation, temperature affects the growth and development of the embryo (Mesquita *et al*., 2021; Agyekum *et al*., 2022; Yalcin *et al*., 2022; Sözcü and van den Brand, 2022). In the recent past, relatively modest changes (1-1.5°C below or above) from the optimal incubation temperature (37.5-37.8°C) have been extensively studied in examining its effect on embryo development, hatchability, and chick quality (Yalçin *et al*., 2007; van der Pol *et al*., 2013; Yalcin *et al*., 2022).

The success of a hatchery is determined by the quantity of salable chicks available at the pulling of the hatch to sell. Hatchability, embryo mortality and chick quality are affected by different factors such as flock age (Elibol *et al*., 2002; Yilmaz and Sahan, 2009; Koppenol *et al*., 2015; Iqbal *et al*., 2016), egg storage (Nasri *et al*., 2020; Abioja *et al*., 2020) and Temperature (Nideou *et al*., 2019; Agyekum *et al*., 2022; Yalcin *et al*., 2022).

Chicken embryos are more susceptible to increased temperatures during incubation than to decreased incubation temperatures (Nideou *et al*., 2019). Low or high incubation temperatures (<36.7 or 38.4-39°C, respectively) during the first and/or second stage (0-10 days; 11-14 days) of incubation affects embryo development Nideou *et al*., hatchability (Noiva *et al*., 2014; Narinç *et al*., 2016; Sgavioli *et al*., 2016; Bednarczyk *et al*., 2021), mortality (Nideou *et al*., 2019; Agyekum *et al*., 2022; Yalcin *et al*., 2022), chick quality (Maatjens *et al*., 2016; Hamidu *et al*., 2018) and post-hatch performance (Yalçin *et al*., 2007; Groves and Muir, 2014; Ipek *et al*., 2014). In line with that, it is evident that incorrect temperature during incubation contributes greatly to embryo livability, mortality, hatchability, and chick quality and consequently, the success and profitability of a hatchery. As a result, the entire incubation process aims to achieve maximum hatchability and low mortality post-hatch as well as to maximize hatchery profit. Line and strain variations influence the tolerance to changes from the optimal incubation temperature (Nideou *et al*., 2019).

Incubation of eggs using different incubation temperatures at different incubation periods in broiler breeds is well documented (Janisch *et al*., 2015; Piestun *et al*., 2015; Muir and Groves, 2018; Nyuiadzi *et al*., 2020; Dayan *et al*., 2020) and also layers (Huth and Archer, 2015; Sgavioli *et al*., 2016). Noteworthy, much of the information discovered in the literature on incubation temperatures in layer chicken is not as rich as that on the same subject concerning broilers. There is no consensus amongst researchers, on whether incubating eggs at a higher temperature should be done during the first and/or second stages of incubation. There is a paucity of information on the impact of high incubation temperature profiles in the early stages and second stages of incubation on poultry breeders. Therefore, this research was conducted to determine the effect of high incubation temperature profiles on embryo livability, mortality, hatchability, and chick quality in commercial layers.

Materials and Methods

Study Area

The study was carried out at Bergvlei Chicks Hatchery of Quantum Foods, in Bronkhorstspruit Gauteng Province, South Africa.

Experimental Temperature Profiles

Three different temperature profiles were used in this research. The incubation temperature profiles are presented in Table (1). The control temperature profile is used as a standard temperature profile at the hatchery and has yielded better results over the years as and is currently referred to as the industry standard and is in line with findings by several authors who defined optimum temperature for embryonic development ranging between 36.8-39.3°C (Piestun *et al*., 2015; Muir and Groves, 2018; Dayan *et al*., 2020; Nyuiadzi *et al*., 2020;). As a tool for continued improvement, this research used the standard temperature profile and increased it to two different temperature profiles. In determining the moderate and extreme temperature profiles, this research gradually increased temperature by an overall 2.4°C from the control temperature. Whereas for the extremely high-temperature profile, the moderate high-temperature profile was further increased by 2.8°C.

Flock Management

Lohman brown lite laying birds were housed in open-laying houses with curtains to control ventilation. The laying house floors were concrete floors coated with wood shavings to a depth of 3-5 cm. The stocking density utilized was approximately 7.16 birds/m^2, according to the Lohman Parent Stock Management Guide (Lohmann Breeders Parent Stock Management Guide, 03/19, n.d). The capacity of the laying house is 4500 females and 450 males, representing a 1:10 male-to-female mating ratio. House conditions, animal rights, and welfare were adhered to following quantum foods hatchery protocols. Feed and water were provided ad libitum.

Table 1: The incubation temperature treatments

	Temperature profiles treatments		
Days of incubation	Control temperature	Moderate-high temperature	Extreme high temperature
Day 0-4	37.8°C	38.5°C	39.5°C
Day 5-9	37.2°C	38.2°C	39.0°C
Day 10–11	37.0°C	37.5°C	38.0°C
Day 12-13	36.8°C	37.0°C	37.5°C
Day 14-15	36.5°C	36.5°C	36.5°C
Day 18–21	36.3°C	36.3°C	36.3°C

Data Collection

A total of 16200 hatchable eggs were used for this research. Upon collection of hatching eggs from the breeder house, all eggs were placed on setter trays and farm trolleys and fumigated at the central egg room as per company Standard Operating Procedures (SOP) at the farm. Fumigation was done using paraformaldehyde at the farm. Eggs were stored overnight after fumigation at the farm`s central egg room and were transported to the hatchery the following day. Eggs were stored at 18-19°C at the hatchery for 2-3 days before setting was done per each temperature treatment and humidity of at least 70%. In total, 5400 hatchable eggs were randomly allocated to the three incubation temperature profile treatments. All eggs per temperature profile were pre-heated for 6 h (approximately 25°C) before the actual incubation started.

This research used the single-stage Petersime Airstreamers 4AS setters and hatchers. Briefly, each setter is connected to the central computer that records all machine proceedings. The setter had a screen outside the machine called the console which records and displays all proceedings (temperature, turning times) in the machine. Some of the probes and sensors installed in the setter were: (i) Two temperature probes recording temperature separately inside the setter with low/high-temperature alarms should there be any deviations from the set point, (ii) Pneumatic Turner which turns setter trolleys automatically within 60 min intervals to either left, right and middle. Should the trolley not turn within 2 h; this triggers an alarm. The turning system was connected to the central air compressor which supplied compressed air for turning purposes as and when required by the machine during incubation. (iii) Three ovoscan sensors that read and record the eggshell temperature during incubation. The readings were recorded as an average of 12 eggs with each sensor reading 4 eggs at a time. (iv) Spray nozzles for humidity purposes connected to pneumatic air pressure and activate when humidity is required in the setter. It sprays humidity in a mist form in the setter when activated depending on the set point. In the setter, relative humidity was 55% until day 18 of incubation. The setter had one humidity sensor inside the machine.

In the hatchers, the humidity sprays, sensors, and probes were the same as in the setters except that the hatcher does not have a turning system and over scan sensors. Relative humidity was 56% from day 18 of incubation until the pulling of the hatch. All setters were equipped with a 2.5-day calibration alarm and were thus calibrated at 2.5 days. The alarm will go off until the machine has either been calibrated or the alarm is cleared off. As a hatchery SOP, all hatchers were calibrated 8 h after the transfer of eggs.

All eggs were candled on day 10 to remove the clear/unfertile eggs. The average egg fertility of control, moderate, and extremely high incubation temperatures were 95.05-96.53 and 94.42%, respectively. The eggs that had not hatched were opened to visually identify the stage of embryonic mortality following the description provided by Reijrink *et al*. (2009). Embryonic mortality was determined at two stages, (i) At day 10 of incubation after candling, eggs break out, and (ii) At hatch day break out. Briefly, early-stage embryonic mortality: Is 1-9 d of incubation (pigment eye visible, embryo without feathers), middle-stage embryonic mortality: Is 10-17 d of incubation (small embryo with feathers), and late-stage embryonic mortality: Is 18-21 d (full grown embryo with yolk out or full-grown embryo dead or alive with yolk subtracted). The chick weight, length, and chick yolk percentage were also determined. Chick weight, chick length, rectal temperature, and chick yolk %. All hatched chicks were graded based on the following parameters: Active two legs and can walk straight with two eyes, straight beak, fully feathered, completely healed navels. All birds with unhealed navels and big belly buttons crossed beaks, twisted beaks, limps malformations and body malformations, crooked toes, and spraddled legs, not fully feathered, closed eyes, sticky chicks and chicks smeared with albumen were considered culls or reject chicks. All these were separated from the first-grade chicks and euthanized using a high-speed macerator at the hatchery used to expose all culled birds. First-grade chicks were weighed individually using a chick scale. The weight was recorded individually per each temperature profile. Birds were weighed as males and females separately however a representative sample per crate of about 10% was weighed on pulling of the hatch. Chick length was also taken from a representative sample wherein a chick was measured with a ruler from the tip of the beak to the end of the toe to determine the chick length in centimeters. Yolk % was determined by comparing birds from the representative sample. Sampled birds were euthanized by dislocation of the neck by trained and certified personnel to determine the yolk %, the residual yolk was removed from the euthanized birds and weighed to determine the yolk % as a percentage of body weight. However, yolk weights and yolk % were only determined from male chicks as birds had to be euthanized as all females were sent through to the rearing phase for commercial egg production while males were euthanasia as per hatchery operating procedures.

All unhatched eggs including pipped eggs from the hatcher trays position are removed and used as a sample for hatch daybreak out. The procedure involves breaking out of eggs and classifying them into the following categories: Late death: Pre-turn, turned, internal pips, external pips, infections, mal-formed and mal-positions. The total per each category was presented as a percentage of the total eggs for each setter tray and the percentage was compared to the

breed-acceptable performance guidelines. This info is then used to address any hatchability challenges either with the breeder flock or at the Hatchery. For this trial, all eggs that were removed at the pulling of the hatch were regarded as dead eggs and were all used for breakout analysis.

All chicks hatched were color sexed to distinguish between males and females. All females were brown while males were white.

The following parameters were determined using the following equations:

$$\text{Hatchability rate (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of eggs set}} \times 100$$

$$\text{Mortality rate (\%)} = \frac{\text{Total number of dead embryos}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Infertile eggs \%} = \frac{\text{Number of infertile eggs}}{\text{Total eggs per tray}} \text{ X } 100$$

$$\text{Day } 0 - 10 \text{ Incubation moisture loss} = \frac{\text{Egg weight at setting} - \text{Egg weight at candling}}{\text{Weight of eggs set}} \text{ X } 100$$

$$\text{Day } 11 - 18 \text{ incubation moisture loss} = \frac{\text{Egg weight after candling} - \text{Egg weight at transfer}}{\text{Total weight of eggs after candling}} \text{ X } 100$$

$$\text{Hatchability of fertile eggs (\%)} = \frac{\text{Total chicks hatched}}{\text{Total fertile eggs}} \text{ X } 100$$

$$\text{Pullets percentage (\%)} = \frac{\text{Total pullets chicks hatched}}{\text{Total fertile eggs}} \text{ X } 100$$

$$\text{Yolk percentage (\%)} = \frac{\text{Residual yolk weight}}{\text{Chick weight}} \text{ X } 100$$

Statistical Analysis

The data were analyzed using the General Linear Model procedures (GLM) of the MiniTab 17 (MiniTab 17 Statistical Software, 2017), whereas the mean separation was conducted using Fisher's LSD test ($p<0.05$). For the analyses of the body weight and chick length, the sex of chicks was fitted as a covariate.

The following statistical model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where, Y_{ij} = measurement of response (moisture loss, hatchability, and mortality rates, yolk weight, yolk percentage, etc.,) of the i^{th} higher incubation temperature profile (control, moderate-high temperature, and extreme high-temperature profile), μ = overall mean, T_i = fixed effect of incubation temperature profile (control, moderate-high temperature, and extreme high-temperature profile), ε_{ij} = random error.

Results

The Least-Square Means (LSM) for hatchability and chick quality at different temperature profile treatments are presented in Table (2). The temperature profiles statistically ($p<0.05$) influenced livability, 0-10 days' moisture loss, total moisture loss, and body weight.

Table 2: Least square means and their Standard Errors (SE) for hatchability and chick quality at different temperature profiles

Parameters	Temperature profiles treatments		
	Control Temperature	Moderate high temperature	Extreme high temperature
Livability	86.55^{b}±0.94	93.31^{a}±0.94	72.61^{c}±0.94
Moisture loss			
Moisture loss: Day 0-10	6.41^{b}±0.20	6.23^{b}±0.20	9.91^{a}±0.20
Moisture loss: Day 11-18	6.85^{a}±0.22	6.35^{a}±0.22	6.64^{a}±0.22
Total moisture loss	13.27^{b}±0.24	12.58^{c}±0.24	16.55^{a}±0.24
Hatchability traits			
Wet chicks (%)	2.24^{b}±0.36	6.81^{a}±0.36	0.78^{c}±0.36
Dry chicks (%)	97.76^{b}±0.36	93.19^{c}±0.36	99.21^{a}±0.36
1st grade female yield (%)	46.10^{a}±0.39	44.99^{b}±0.39	42.29^{c}±0.39
Male (%)	52.66^{a}±0.42	52.99^{a}±0.42	50.35^{b}±0.42
2nd grade female yield (%)	1.24^{b}±0.28	2.02^{b}±0.28	7.36^{a}±0.28
Total pullets (%)	47.34^{b}±0.42	47.01^{b}±0.42	49.65^{a}±0.42
Total hatch of fertile (%)	93.63^{b}±0.62	96.15^{a}±0.62	89.62^{c}±0.62
Total hatch of all set (%)	85.24^{b}±0.74	89.72^{a}±0.74	73.20^{c}±0.74
Chick quality traits			
*Body weight (g)	37.85^{a}±0.23	37.22^{a}±0.23	35.66^{b}±0.23
*Chick length (mm)	17.30^{a}±0.06	16.93^{b}±0.06	17.28^{a}±0.06
#Yolk weight (g)	3.37^{a}±0.134	3.19^{a}±0.134	3.19^{a}±0.134
#Yolk (%)	8.79^{a}±0.360	8.49^{a}±0.360	8.92^{a}±0.360

[a,b, c]Row means with different superscripts differ significantly ($p<0.05$);*sex of chicks was fitted as a covariate on the analysis; #only male chicks were used

The livability of the embryos in a moderately high incubation temperature profile was higher (93.31%) followed by the control (86.55%) and lastly by an extremely high incubation temperature profile (72.61%). The moisture loss between days 0-10 was higher ($p<0.05$) in Temperature 2 (9.91%), whilst similar moisture loss was observed ($p>0.05$) between the control temperature profile (6.41%) and moderate high incubation temperature profile (6.23%). The Moisture Loss: 11-18 days, was similar ($p>0.05$) between the temperature profiles. The average total moisture loss was higher ($p<0.05$) in an extremely high incubation temperature profile (16.55%) and lowest in a moderately high incubation temperature profile (12.58%). For hatchability traits, an extremely high incubation temperature profile yielded a greater ($p<0.05$) percentage of dry chicks (99.21%) compared to the control profile (97.76%) and moderately high incubation temperature profile (93.19%). The moderate high incubation temperature profile yielded a lower ($p<0.05$) percentage of dry chicks (93.19%). The percentage of wet chicks for moderately high incubation temperature profile, control profile, and extremely high incubation temperature profile was 6.81, 2.24, and 0.78%, respectively. The 1st-grade female yield was greater ($p<0.05$) in control (46.10%) compared to the moderate-high incubation temperature profile (44.99%) and extreme high incubation temperature profile (42.29%). The male percentage was greater ($p<0.05$) in the control temperature profile (52.66%) and moderate high incubation temperature profile (52.99%) compared to the extremely high incubation temperature profile (50.35%). The 2nd-grade female and total pullet percentage were higher ($p<0.05$) in the extremely high incubation temperature profile (2nd-grade female: 7.36% and total pullet: 49.65%) compared to the control profile (2nd-grade female: 1.24% and total pullets: 47.34%) and moderate high incubation temperature profile (2nd-grade female: 2.02% and total pullets: 47.01%). Control and extremely high incubation temperature profiles yielded similar 2nd-grade females and total pullet percentages. The total hatch of a fertile was higher ($p<0.05$) in moderate-high incubation temperature profile (hatch of a fertile: 96.15% and hatch of all eggs set: 89.72%), followed by control profile (hatch of a fertile: 93.63% and hatch of all eggs set: 85.24%) and lastly by extreme high incubation temperature profile (hatch of a fertile: 89.62% and hatch of all eggs set: 73.20%). For the chick quality, the body weight was higher ($p<0.05$) in control temperature profile (37.85 g) and moderate high incubation temperature profile (37.22 g) compared to Temperature profile 2 (35.66 g). However, the control temperature profile (17.30 mm) and extremely high incubation temperature profile (17.28 mm) yielded chicks of a longer length than the moderately high incubation temperature profile (16.93 mm). The chick length was similar between the control temperature profile (17.30) and the extremely high incubation temperature profile (17.28 mm). The yolk weight and yolk percentage were similar ($p>0.05$) between the temperature profiles, although this was only measured on male chicks as all females had to be sent further to the rearing phase and egg production phase thereafter. Hatchery sales are determined by all saleable females.

The incubation temperature profiles significantly ($p<0.05$) influenced all the embryo mortality in different stages under study except for the upside down (Table 3).

Table 3: Least square means and their Standard Errors (SE) for chick embryo mortality at different temperature profiles treatments

Mortality parameters	Temperature profiles treatments		
	Control temperature	Moderate high temperature	Extreme high temperature
Early-stage embryo mortality			
24 h	$1.28^{b}\pm0.192$	$0.55^{c}\pm0.192$	$2.56^{a}\pm0.192$
48 h	$1.54^{b}\pm0.150$	$0.94^{c}\pm0.150$	$2.33^{a}\pm0.150$
Blood ring	$1.26^{b}\pm0.179$	$1.61^{ab}\pm0.179$	$1.94^{a}\pm0.179$
Pigmented eye	$1.30^{b}\pm0.139$	$0.65^{c}\pm0.139$	$1.83^{a}\pm0.139$
Early-stage total embryo mortality	$5.37^{b}\pm0.383$	$3.78^{c}\pm0.383$	$8.67^{a}\pm0.383$
Middle-stage embryo mortality			
Middle-stage embryo mortality	$1.41^{b}\pm0.222$	$0.90^{b}\pm0.222$	$5.95^{a}\pm0.222$
Late-stage embryo mortality			
Internal pips	$1.59^{a}\pm0.169$	$0.62^{b}\pm0.169$	$0.95^{b}\pm0.169$
External pips	$0.81^{b}\pm0.117$	$0.38^{c}\pm0.117$	$1.19^{a}\pm0.117$
Malformed	$0.37^{b}\pm0.077$	$0.00^{c}\pm0.077$	$0.68^{a}\pm0.077$
Upside down	$0.47^{a}\pm0.010$	$0.38^{a}\pm0.010$	$0.33^{a}\pm0.010$
Infection	$0.70^{a}\pm0.106$	$0.57^{a}\pm0.106$	$0.14^{b}\pm0.106$
Late-stage total embryo mortality	$1.28^{b}\pm0.334$	$1.34^{b}\pm0.334$	$6.05^{a}\pm0.334$
Total embryo mortality	$6.37^{b}\pm0.621$	$3.69^{c}\pm0.621$	$10.38^{a}\pm0.621$

[a b, c] Row means with different superscripts differ significantly ($p<0.05$)

During the early stages of incubation, an extremely high incubation temperature profile led to the higher ($p<0.05$) 24 h (2.56%), 48 h (2.33%), blood ring (1.94%), pigment eye (1.83%) and early-stage mortality percentage (8.67%) as compared to the control temperature profile. The moderate high incubation temperature profile yielded consistently lower ($p<0.05$) embryo mortality at 24 h (0.55%), 48 h (0.94%) pigment eye (0.65%) and overall early-stage embryo mortality (3.78%) compared to both the other temperature profiles. Noteworthy, the middle and late-stage embryo mortality was similar ($p>0.05$) between the control temperature profile (middle-stage embryo mortality: 1.41% and late-stage embryo mortality: 1.28%) and moderate high incubation temperature profile (middle-stage embryo mortality: 0.90% and late-stage embryo mortality: 1.34%), whilst extreme high incubation temperature profile yielded higher ($p<0.05$) middle-stage embryo mortality (middle-stage embryo mortality: 5.95% and late-stage embryo mortality: 6.05%). The internal pips were higher ($p<0.05$) in the control (1.59%) as compared to the moderate-high (0.62%) and extremely high incubation temperature profile (0.95%). Furthermore, the internal pips were similar ($p<0.05$) between moderate high incubation temperature profile (0.62%) and 2 (0.95%). The moderate high incubation temperature profile yielded the lower ($p<0.05$) external pips (0.38%) and malformed (0.00%) followed by the control temperature profile (external pips: 0.81% and malformed: 0.37%) and then extremely high incubation temperature profile (external pips: 1.19% and malformed: 0.68%). The mortality caused by the infection was similar ($p<0.05$) between the control (0.70%) and moderate high incubation temperature profile (0.57%), but higher ($p<0.05$) than the extreme high incubation temperature profile (0.14%). The total embryo mortality was higher ($p<0.05$) in temperature 2 (10.38%), followed by the control (6.37%), and the lowest was recorded for the moderate-high incubation temperature profile (3.69%).

Discussion

The vast majority of research conducted focused on higher temperatures within the initial stages (Joseph *et al*., 2006; Nideou *et al*., 2019) or second stages of incubation (Yildirim and Yetisir, 2004; Tzschentke and Halle, 2010; Maatjens *et al*., 2017; Agyekum *et al*., 2022), whereas a combination of the two is less investigated. It has been well-documented that embryo livability, hatchability, and chick quality are dependent on incubation temperatures (Hamidu *et al*., 2018; Agyekum *et al*., 2022; Yalcin *et al*., 2022). The present study showed that a moderately high incubation temperature profile during incubation increased ($p<0.05$) hatchability and decreased mortality. These observations align with the results obtained by other researchers (Agyekum *et al*., 2022; Yalcin *et al*., 2022) that high incubation temperature significantly ($p<0.05$) increases livability, hatchability, and decreased mortality. The findings that the extremely high incubation temperature profile resulted in decreased livability and hatch of fertile depicts that developing chick embryos are temperature sensitive during incubation.

Incubating broiler eggs at a higher temperature (39.6°C) for a period of 6h per day resulted in decreased hatchability (Narinç *et al*., 2016). On the other hand, Sgavioli *et al*. (2016) discovered that increased temperatures do not affect embryo mortality and hatchability. Lower hatchability and increased embryo mortality are not solely linked to incubation temperatures, they are also dependent on egg storage periods (Grochowska *et al*., 2019; Okasha *et al*., 2023), humidity (Noiva *et al*., 2014), flock age Grochowska *et al*., and the biological value of the egg (Pawłowska and Sosnówka-Czajka, 2019; Biesek *et al*., 2023). Both incubation temperature profiles had greater mortality rates in the first week of incubation. These findings are comparable with those observed by Noiva *et al*. (2014), that embryo mortality occurs within the first four days of incubation. The findings that the late death mortality was higher in the extremely high incubation temperature profile, could be attributed to the fact that higher temperatures at the end of the incubation period result in temperature stress (Mesquita *et al*., 2021; Sözcü and van den Brand, 2022). Temperature stress could consequently lead to mortality. The high embryo mortality was significantly higher in the extremely high incubation profile. These findings are in line with those reported by Gonzales (2012), who reported that the main influence of temperature in embryo mortality is in the final incubation period. The malformed embryos were higher in eggs incubated at the extremely high-temperature profile. These results conform to the earlier reports (Noiva *et al*., 2014; Butcher and Nilipour, 2003; Tesarova *et al*., 2021) that embryos incubated at high temperatures had increased abnormality rates. The findings that the total mortality was higher in the extremely high-temperature profile, followed by the control temperature profile may be due to limited loss of heat by the egg itself, so disrupting embryo homeostasis, or excessive loss of water from eggs, generating increased late mortality percentage due to desiccation/drying up (Ono *et al*., 1994).

In multi-stage incubators, moisture loss may range between 10-13%, while in single-stage incubators, it may range from 9.5-12.5% of the initial egg mass (Green, 2017). Lohman breeders summarized that a layer-hatching egg needs to lose about 11-13% moisture until 18.5 days of incubation (Lohmann Tierzucht: Management Guide Hatchery 04/13, n.d). This research used the single-stage Petersime Airstreamers 4AS setters and hatchers. During 18 days of incubation, eggs should

lose between 9.5-13% of their initial weight as moisture (Green, 2017; Lohman Hatchery Management Guide, 04/13, n.d). Higher moisture loss was observed on eggs exposed to extremely high-temperature profiles could be because of higher evaporation of water from the embryos (Sgavioli *et al*., 2016; Boleli *et al*., 2016) and higher shell conductance (Boleli *et al*., 2016; Sözcü and van den Brand, 2022). Eggs that are fertile absorb heat during the very first phase of the period of incubation, but embryos lose heat in the second phase of the period of incubation (Tazawa and Whittow, 2000; Tona *et al*., 2022).

Moisture loss in a moderately high-temperature profile was within the range of the optimum moisture loss percentage suggested by Green (2017); Lohman breeders. Notably, the moisture loss in the control and the extreme high-temperature profiles was above the optimum moisture loss suggested by Green (2017). In view of this, several researchers (Gregorich *et al*., 2022) indicate that the chick quality will be affected negatively if the moisture loss is insufficient (<10.5%) and, consequently, poor post-hatch performance. This will in turn result in increased production costs (Maatjens *et al*., 2016; Abera *et al*., 2017; Souza da Silva *et al*., 2021). On the other hand, extreme moisture loss can result in dehydrated chicks and death. In broiler chickens, Ipek *et al*. (2014) recorded different egg weight loss between the three temperature ranges of 11% from chicks hatched in a temperature range of between 33.3˚C-36.7˚C, while 12,3% egg weight loss was recorded from chicks hatched on the temperature range of 37.8-38. 2˚C. The highest weight loss of 13.8% was recorded from a temperature range of 38.9-40˚C. The rate of moisture loss by the egg influences embryo development (van der Pol *et al*., 2013; Noiva *et al*., 2014).

To attain an ideal chick quality and hatchability, incubation conditions must be modified to match the embryonic requirements (Maatjens *et al*., 2016). Chick quality is determined both qualitatively and quantitatively. The qualitative methods are based on the visual assessment which is called the Tona score Tona *et al*. (Narinç *et al*., 2016; Narinç and Aydemir, 2021) and Pasgar score (Tona *et al*., 2003; Narinç and Aydemir, 2021). The quantitative methods encompass such morphological measurements, among others including chick weight, chick length, and yolk weight and percentage (Narinç and Aydemir, 2021; Agyekum *et al*., 2022). This research employed the quantitative methods. Yolk percentage and weight were similar across the different incubation temperature treatments. Similar observations were made by Agyekum *et al*. (2022) who observed no significant difference across the different temperature treatments. Therefore, these authors provided minimal evidence to corroborate their findings that different incubation temperatures do not influence the yolk weight and percentage.

On the other hand, the chicks were heavier in the control and had extremely high-temperature profiles. In the extremely high-temperature profile, chicks hatched were of lower body weight. In line with our findings, higher incubation temperatures resulted in chicks with lower body weight (Leksrisompong *et al*., 2007; Barri *et al*., 2011). Contrary to our findings, several researchers (Ipek *et al*., 2014; Agyekum *et al*., 2022) observed similar chick weight across different incubation temperatures, although birds hatched in temperature ranges of 38.9-40.0°C were heavier after 1-week post-hatch. It is also reported that day-old weight reflects in the chick's growth (Al-Nedawi *et al*., 2019) and this association, however, is not entirely understood because of inconsistent findings. Furthermore, it is suggested that the weight of a day-old chick is connected to egg weight rather than chick growth (Farhadi and Hosseini, 2014; Kalia *et al*., 2017). As the incubation temperature decreases from 37.8-36.6°C, the chick length also gets reduced (Joseph *et al*., 2006). The chick length might be a good predictor of chick quality for males but not for females (Molenaar *et al*., 2023).

Conclusion

A moderate high-temperature profile during incubation demonstrated enormous promise as a technique to increase livability and hatchability while decreasing embryo mortality in commercial flocks. It urged that the temperature during incubation be adjusted to meet the needs of the embryo for the purpose of increasing embryo livability and hatchability simultaneously minimizing embryo mortality. There is a need to evaluate the impact of high incubation temperatures on the post-hatch performance of layer chicks.

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Author's Contributions

Masia Khathutshelo Simon: Conceptualization of the research idea, collected and analyzed the data, and wrote the manuscript.

Takalani Judas Mpofu and Khathutshelo Agree Nephawe: Conceptualization of research idea and design, data collection and analysis, interpretation of data, and

review of the manuscript.

Willie Janse van Rensburg, Bohani Mtileni, Mamokoma Catherine Modiba, Jabulani Nkululeko Ngcobo and Keabetswe Tebogo Ncube: Critical revision of the manuscript. All authors read and approved the final manuscript.

Ethics

The study was approved by the Animal Research Ethics Committee of Tshwane University of Technology (AREC202306003) and Quantum Foods South Africa. The authors declare that they have no conflict of interest.

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