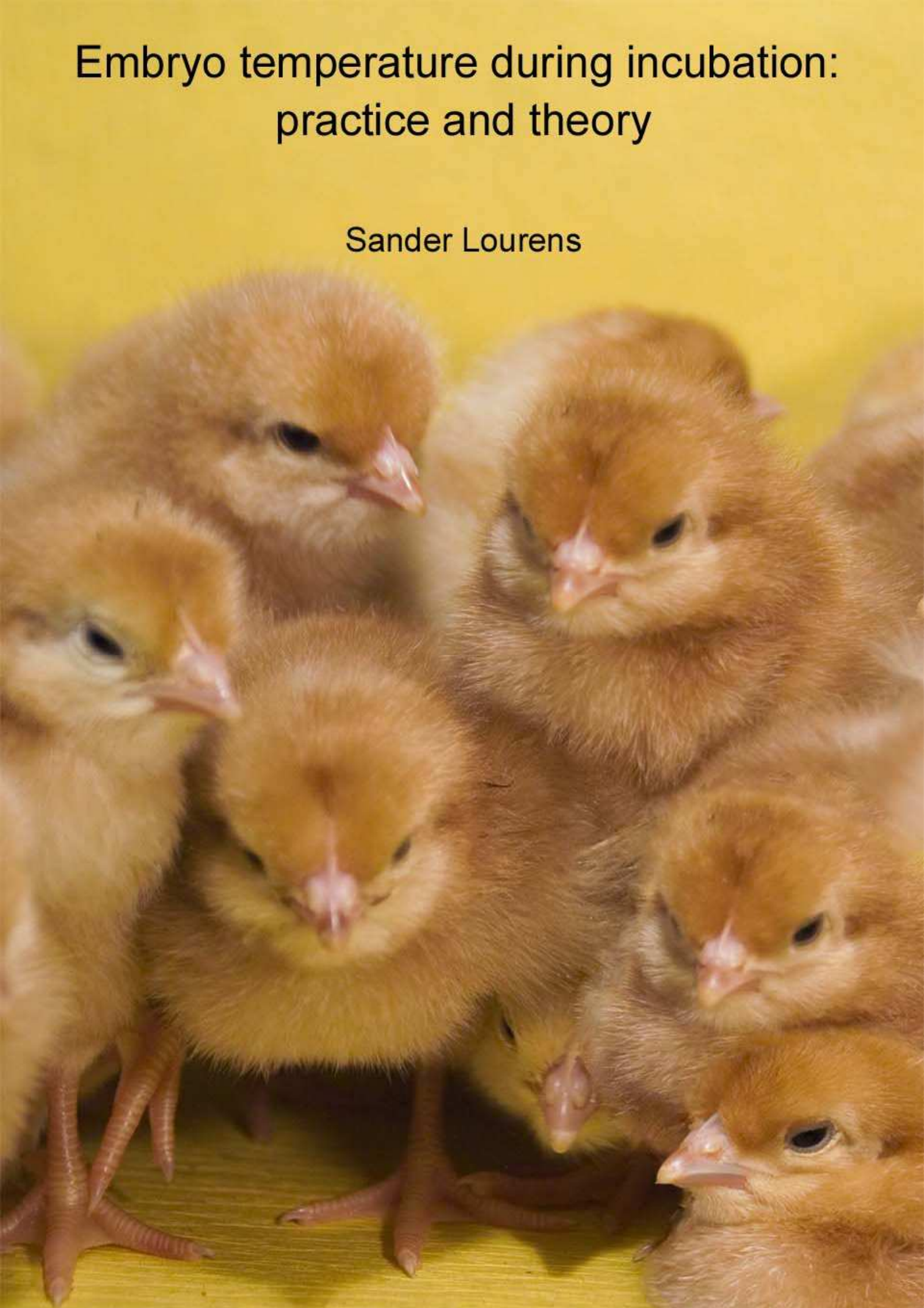
Embryo temperature during incubation: practice and theory

Sander Lourens



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EMBRYO TEMPERATURE DURING INCUBATION: PRACTICE AND THEORY

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Abstract

Until recently, all incubator studies were performed using a constant machine temperature (MT). But it is embryo temperature (ET) that is of importance to the embryo, and not MT. In practice, MT is often measured at one location within the incubator, while ET can vary between eggs within an incubator. Furthermore, ET is the result of the balance between heat production (HP) and heat loss, and if HP or heat loss is affected it may have consequences for ET. Aim of this dissertation was to identify the causes of variable ET and to describe the consequences of variable ET on embryonic development, hatchability, HP and chick quality. Because the direct measurement of ET is destructive, it was chosen in this dissertation to use eggshell temperature (EST) measurements as a reflection of ET.

Long term deviations of 1.1ºC away from a constant EST of 37.8ºC decreased embryonic growth, development, hatchability, and the ability of young chicks to maintain high body temperatures after hatching, especially under cold stress. HP was considered to be positively related to embryonic development, because when more energy is used for growth, HP during incubation will increase and chicks will subsequently hatch with a larger yolk free body and with a lower amount of energy left over in the residual yolk. Within the EST zone of 1.0ºC below and above 37.8ºC it was observed that HP increased linearly with short term EST increments, and the response of the embryos to EST variations was identical in young, mid term and late term embryos. Maximizing HP based on metabolic responses to EST fluctuations will therefore increase EST above the studied EST zone, leading to decreased embryonic growth and increased embryonic mortality. High EST increases the demand for oxygen, so oxygen availability was expected to limit HP and embryonic growth more at higher EST profiles than at EST of 37.8ºC. However, despite the fact that HP at day 18 was highest for the combination of high EST with high oxygen concentration, embryonic development did not show the same relationship. At EST above 37.8ºC, the amount of energy utilized from the egg content remained the same, but the efficiency of energy transfer (E_{YFB}) between egg and embryo decreased. Factors as egg size, breed, and oxygen availability affected HP through changes in energy utilization, and had no effect on E_{YFB}.

In this thesis, the importance was shown to measure and control ET during incubation and not MT. Factors were identified that affect ET through changes in HP and heat loss. When ET is controlled and maintained at a constant level of 37.8ºC, embryonic development may be improved by measures that increase energy utilization through increments in gas exchange, which will increase HP.

(*Key words:* incubation, embryo temperature, embryonic development, heat production, heat loss)

ABBREVIATIONS

ET	Embryo temperature (ºC)
MT	Machine temperature (ºC)
EST	Eggshell temperature (ºC)
DT	Difference between MT and EST (ºC)
HT (chapter 2)	Housing temperature (ºC)
RT	Rectal temperature (ºC)
HP	Heat production ($mW.egg^{-1}$)
BW	Body weight (g)
YFB	Yolk free body (g)
RY	Residual yolk (g)
E_{YFB}	Efficiency of energy utilization (%)
CL	Chick length (cm)
HW	Heart weight (g)
LW	Liver weight (g)
CRC	Climate respiration chamber
HT (chapter 5)	Hatching time (d)

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Chapter 1

GENERAL INTRODUCTION

FROM MACHINE TEMPERATURE TO EMBRYO TEMPERATURE

The earliest reference to artificial incubation occurs in Aristotle's *Historia Animalium*, written in the 4th century BC. A brief survey of the history of incubation from that time to 1990 is given by Visschedijk (1991). Visschedijk ended his review "Physics and physiology of incubation" with the question: "The future – have all problems been solved?" and concluded: "theoretically, the answer is a qualified yes". Indeed, the basic key factors for successful artificial incubation are well known. During incubation, the eggs have to be turned, and temperature, humidity and ventilation have to be controlled. From the climatic conditions, temperature is recognized as the most important one (Romanoff, 1960; Lundy, 1961; Deeming and Fergusson, 1991; Wilson, 1991; Decuypere and Michels, 1992).

The air temperature in modern incubators is often measured at one spot in the incubator. At this point, air temperature can be controlled very precisely, and adjustments are made on the level of tenths of a degree Celsius. It is questionable however, whether the temperature that is measured and controlled represents the key factor for the development of the embryo. Basically, the temperature that is important to the embryo is the temperature inside the egg: the embryo temperature (ET). What is measured and controlled in the modern incubators is air temperature or machine temperature (MT) and not ET (Meijerhof and van Beek, 1993; French 1997). Direct measurement of ET is destructive, so indirect but continuous and noninvasive eggshell temperature (EST) measurements as a reflection of ET may be more appropriate.

Romijn and Lokhorst (1951, 1956, 1960) measured EST during incubation for eggs incubated at a constant MT of 37.5ºC throughout incubation in still air incubators. In these studies, EST was about 0.1ºC lower than MT during the first 9 days of incubation. After 9 days of incubation, EST rose gradually to 1.3 – 1.5ºC above the MT set point at day 18 of incubation. In large scale forced air incubators often step-down MT programs are used to prevent overheating of eggs during the final stages of incubation. However, EST can vary largely between different places within one incubator (Lourens, 2001; Van Brecht, 2003). It was shown by Lourens (2001) that on average, the EST was close to 37.8ºC, but varied between 36.2 and 40.2ºC, depending on place and time. Similar as in the studies by Romijn and Lokhorst (1951, 1956, 1960), EST rose gradually to higher values from 10 days onwards. At d18 of incubation, MT was set at 37.2ºC, but at some places EST reached over 39.0ºC, which lead to a higher incidence of late embryonic mortality and decreased hatchability (Lourens, 2001).

Because MT can not be considered the same as ET or EST, it may have some consequences for the optimization of the incubation process and for the interpretation of the

results of incubator studies. Variation of ET in an incubator can be explained by the effect of increasing heat production (HP) in time due to the increasing age and mass of the embryo, and by local differences in heat transfer between egg and its environment. Next to fixed local differences in ET, also changes in HP, or changes in heat loss will affect ET. The relationships between HP and ET with embryonic development in time are unclear, and a better understanding of the factors that affect HP and heat loss is highly desired.

HEAT PRODUCTION AND HEAT LOSS

Briedis and Seagrave (1984) showed the temperature and heat exchange situation for the standard chicken egg during incubation, using the results of the measurements by Romijn and Lokhorst (1951, 1956, 1960). During the first 9 days of incubation when EST was lower than MT, heat was gained by the egg by convection, whereas after 9 days of incubation when EST was higher than MT, heat was lost by convection. It was observed that HP during incubation increased with embryonic development in time, parallel to the gradual increase of EST from day 9 onwards. Also in studies by Nichelmann et al. (1998) and Janke et al. (2002), it was observed that ET and HP increased parallel with embryonic development in time and HP and ET were linearly related. Using short term MT increments from 37.5 to 39.0ºC at different days of incubation, it was noticed that HP increased linearly with ET until ET reached 39.5ºC. When ET increased above 39.5ºC, HP instantly decreased (Janke et al., 2002). This effect was observed in late term embryos and not in younger embryos.

The gradual increase of ET and HP with embryonic age from day 9 or 10 onwards may not be a prerequisite for optimal embryonic development. It can be expected that HP will increase with embryonic age and growth anyway, even when ET is kept constant. The concept that HP and ET may not necessarily run parallel with embryonic age is new, and brings challenging questions for further research. This concept requires an excellent control of ET, so the factors that affect ET need to be known. ET is the result of a balance between heat production (HP) and heat loss (Meijerhof and Van Beek, 1993). When during the incubation process HP or heat loss is affected either directly or indirectly, it will affect ET as well. This may benefit or harm embryonic development, depending on the direction the measure has on ET.

The optimum ET during incubation for best embryonic development and hatching results is not known, and, within limits, it may vary between different batches of eggs. Instead of exploring the optimum ET using trial and error, metabolic responses of embryos to external factors may direct the conditions for best embryonic development and hatching results. It has for example been observed that embryonic HP decreases when incubation conditions are not

favourable for the embryo (Harun et al., 2002). It can therefore be hypothesised that maximizing HP may be an effective way to increase embryonic development and hatchability.

The relation between ET and HP is therefore of particular interest. The principle of on-line monitoring of CO_2 concentration using MT steps in order to increase CO_2 production and hatchability was first described by Hulet (2001) and Hulet and Meijerhof (2001). Their approach was primarily to avoid overheating of eggs: the effects of temperature increments on CO_2 production and hatchability were not yet investigated. Temperature may be the main factor of interest to affect embryonic development and HP (Nichelmann et al., 1998; Janke et al., 2002), but other factors affecting for example gas exchange will influence HP and embryonic development as well. However, if oxygen availability is the limiting factor for HP during the plateau phase of incubation (Rahn et al., 1974), an increase in oxygen availability will not only stimulate embryonic development and increase HP, but increase ET as well. Changes in ET may affect the response of embryos more than changes in gas exchange, which puts the optimistic conclusion by Visschedijk (1991) into other perspectives. Similar confounding effects by changes in ET with changes in HP can be expected to occur in studies measuring HP in eggs from different size or breed. It may become clear that ET will play an important, central role in the incubation process, and that ET can be affected directly or indirectly by many factors.

AIM AND OUTLINE OF THE DISSERTATION

The idea that ET and HP need to develop parallel with embryonic age was exchanged for the concept to control ET by balancing HP with heat loss. Aim of this dissertation was to identify the causes and consequences of variable ET on embryonic development, HP, hatchability and chick quality.

In Chapter 2 the effects of EST, as reference for ET, on embryonic development, hatchability and post hatch growth and development are described. EST was measured and MT was adjusted in order to create the different experimental EST profiles. The same technique is used in Chapter 3, to incubate eggs from different size at the same constant EST of 37.8ºC to examine the effect of egg size on HP, embryonic development and hatchability without any confounding effects of ET. A constant ET may not always lead to best hatching results, and metabolic responses to ET variations may provide a better direction. Therefore, in Chapter 4 an experiment is described where the relationship between MT and CO_2 production (large scale hatchery trials) or EST and HP (small scale laboratory trials) are examined during different periods in the incubation process. Aim of these experiments is to identify the

possibilities for an on-line monitoring system based on changes in CO_2 production by changes in temperature. Because embryonic development may be limited by a combination of factors related to gas exchange and ET, the effect of oxygen availability in relation to EST on HP and embryonic development was investigated in Chapter 5. An overview of the experiments that were performed in this thesis is shown in the following Table.

Table. Overview of experiments in this thesis.

Chapter	EST treatment			Experimental set-up
2	Week 1	week 2	week 3	Effects of EST
	36.7ºC	37.8ºC	37.8ºC	2 batches of eggs
	36.7ºC	37.8ºC	38.9ºC	2 housing temperatures
	37.8ºC	37.8ºC	37.8ºC	
	37.8ºC	37.8ºC	38.9ºC	
3	EST = 37.8ºC			Effects of egg size
4	EST varied between 36.7–38.9ºC during 3 different periods of incubation			Metabolic responses of embryos on EST fluctuations
5	EST = 37.8ºC or 38.9ºC between d9-19			Effects of EST O_2 concentrations of 17, 21 and 25%

In Chapter 6, differences in HP due to differences in egg characteristics or incubation conditions are explained by differences in energy utilization and efficiency of energy transfer between egg and hatchling. Aim of this theoretical approach was to identify the factors that may be responsible for temperature variations, and to calculate the theoretical highest possible HP and ET. In the General Discussion, the results reported in the Chapters 2-6 are discussed and evaluated with respect to the importance to control ET during incubation instead of MT. Factors that affect HP and factors that affect heat loss are evaluated, and the effects of changes in HP or heat loss on ET and subsequent embryonic development are further discussed. Directions for further improvement of embryonic development and hatchability are presented.

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Chapter 2

EFFECT OF EGGSHELL TEMPERATURE DURING INCUBATION ON EMBRYO DEVELOPMENT, HATCHABILITY AND POST-HATCH DEVELOPMENT

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ABSTRACT

An experiment was conducted to study the effect of different eggshell temperature (EST) profiles during incubation on embryo mortality, hatchability and embryo development. Furthermore, chicks from different EST profiles were reared under low and high housing temperatures to investigate subsequent post hatch growth and rectal temperature. Two batches of eggs were used in this experiment. Hatching eggs were subjected to 36.7 or 37.8ºC EST during the first wk, to 37.8ºC EST during the second wk and to 37.8 or 38.9ºC EST during the third wk of incubation. Post hatch housing temperature decreased from 35ºC at day 1 to 30ºC at day 7 (high) or decreased from 30ºC at day 1 to 25ºC at day 7 (low). The difference between machine temperature and EST (DT) was used to illustrate the effect of EST on heat production during incubation. DT differed per batch, and was smallest when eggs were incubated at 36.7ºC instead of 37.8ºC during wk 1. High EST during wk 3 of incubation (38.9ºC instead of 37.8ºC) reduced DT only in batch 2. Embryo development was most retarded in eggs incubated at 36.7ºC EST compared to 37.8ºC during the first wk of incubation. However, highest hatchability and embryo development were always found when EST was maintained at 37.8ºC constantly throughout incubation. Chicks that hatched from eggs incubated at low EST during wk 1 of incubation had lower body temperature after hatching, especially under low housing temperatures, and this effect lasted until 7 days post hatch in batch 1. Highest body temperatures were always found in chicks incubated at 37.8ºC EST constantly throughout incubation. Eggs and chicks from different batches require different environmental conditions for optimal embryo development, hatchability, and post hatch growth. Not only rearing temperature, but also incubation conditions affect the ability of young chicks to maintain their rectal temperature during the first wk post hatch.

(*Key words*: eggshell temperature; embryo development, hatchability, post hatch performance, rectal temperature)

INTRODUCTION

Temperature is a very important factor affecting embryo development (Romanoff, 1972), hatchability (Deeming and Fergusson, 1991; Wilson, 1991), and post hatch performance (Lundy, 1961; Wilson, 1991). In incubation trials often air temperature is used as treatment applied to the eggs (French, 1997). It can be questioned if internal egg temperature (embryo temperature) would be more relevant than air temperature, because air temperature is not simply equal to embryo temperature and can vary independently (Meijerhof and Van Beek, 1993). It can be assumed that embryo development and hatchability is more influenced by embryo temperature than by air temperature. However, measuring embryo temperature requires destructive methods that will influence embryo development and hatchability. Using eggshell temperature (EST) as a reflection of embryo temperature can solve this problem. Lourens (2001) found an average EST of 37.8ºC in commercial single stage incubators. However, a fluctuation of 4ºC in EST was observed, depending on stage of development and position of the egg in the machine (Lourens, 2001). Especially in multi-stage machines a relatively low and high EST at the start and end of incubation, respectively, can be expected, as a result of the imbalance between embryonic heat production and heat transfer (Meijerhof, 2002).

Because the influence of varying EST on embryo development and hatchability is not known, a trial was conducted to evaluate the effect of low EST (36.7ºC) during the first week and high EST (38.9ºC) during the third wk of incubation on embryo development, hatchability and post hatch performance. Eggs in the control group were incubated at 37.8ºC EST constantly. Because parent stock age is an important factor for embryo development and hatchability (Gladys et al., 2000), the trial was done twice with eggs of parent stock of different age. Beside effects of EST on embryo development and hatchability, also post hatch performance was determined in chicks housed at different house temperatures.

MATERIALS AND METHODS

Experimental setup

The experiment was set up to examine the effect of low EST in the first wk of incubation and the effect of high EST during the last wk of incubation on embryonic mortality, hatchability and embryo development in different batches of eggs. During wk 1 of incubation, EST was set at 36.7ºC or 37.8ºC and during wk 3 EST was set at 37.8ºC or 38.9ºC. EST during wk 2 was for all treatments set at 37.8ºC, resulting in 4 different EST treatments. Eggs were used from a

young parent stock of 28 wks of age (batch 1), and from an old parent stock of 60 wks of age (batch 2). The two different batches were incubated in two subsequent periods.

Incubation

Four identical digital Petersime 84 incubators[1] with a maximum capacity of 8,400 eggs were used. During the first two wks of incubation, only two incubators were used. At d 14 of incubation, eggs were split per treatment across four incubators. At d 18 of incubation eggs were transferred to hatching baskets, and hatching baskets were put back in the same incubator. Each incubator resembled one treatment.

Eggs were divided across 16 incubator trays before incubation. The 16 incubator trays were randomly split across four different EST treatments. At one egg in the centre of each incubator tray, a thermistor was attached in heat conducting paste (Shaffner[2]) and covered with regular cello tape (Tesa[3]). As a result, EST was measured at 4 eggs per treatment. On d 7, 14 and 18 of incubation these eggs were confirmed to contain living embryos. EST were read daily outside the incubators and accordingly, machine temperature (MT) was adjusted in order to achieve or maintain the desired EST in each treatment.

In all EST treatments, relative humidity was maintained constant at 55 % throughout incubation. To ensure sufficient and uniform air speed across all eggs and to avoid interaction between incubator trays and eggs, only 67 eggs instead of 150 were placed at each incubator tray, and every other egg place remained empty and every other (empty) incubator tray was removed.

All eggs originated from the same breed (Hybro G) but from different parent stock farms, and eggs were stored for a maximum of 1 wk. A total of 1,072 eggs per batch were incubated to determine embryonic mortality and hatchability (268 eggs per treatment per batch; equally divided across 4 incubator trays). Additionally, 360 extra eggs per batch were incubated (90 per treatment per batch; equally divided across 2 extra incubator trays) and opened at different stages to determine embryo development.

1 Petersime NV, Belgium.

2 Schaffner Holding AG, Switzerland.

3 Tesa SA-NV, Brussels, Belgium

Embryonic mortality and hatchability

At d 7 of incubation, all eggs were candled and infertile eggs and eggs with dead embryos were removed and counted per incubator tray. All clear eggs were opened and evaluated visually to determine true fertility as percentage of eggs set and to determine early embryonic mortality as percentage of fertile eggs. At d 18 of incubation, eggs were transferred to hatching baskets. Each hatching basket referred to an incubator tray. On the day of hatch, first and second grade chicks were counted per hatching basket. Second grade chicks were all chicks that were not able to stand straight up or chicks that showed visible signs of sub-optimal incubation conditions as red hocks or rough navels. Eggs that failed to hatch were counted and opened assigned by eye to determine the stage of embryonic mortality. As a result, embryonic mortality could be categorized by early dead (wk 1), mid dead (wk 2) or late dead (wk 3).

Embryo development

A total of 360 eggs per batch were incubated and subjected to analyses for embryo or chick weight, chick length, heart weight and residual yolk weight. On d 7, 14, and 18, a total of 30 eggs per treatment were removed from the incubator to measure yolk free embryo weight and embryo length. On the day of hatch at d 21.5, 30 chicks per treatment were killed to determine chick length, yolk free chick weight and residual yolk.

Post hatch development

Per batch, a total of 400 chicks (100 chicks per treatment) were wing tagged and placed in grow-out facilities under two different house temperature (HT) regimes. HT decreased from 35ºC at placement to 30ºC at d 7 (warm), and from 30ºC at placement to 25ºC at day 7 (cold). Feed and water was provided at lib and chicks were reared under continuous light. At placement and at 7 d post hatch, rectal temperature, chick length and weight was recorded from 15 chicks per treatment per batch.

Statistical analyses

The two different batches of eggs were incubated in two succeeding experiments that were set up as a 2 x 2 x 2 factorial design with two EST settings in the first wk of incubation, two EST settings in the last wk of incubation and two house temperature regimes during the first week post hatch. Fertility, embryo mortality and hatchability of second and first grade chicks were analyzed using a Generalized Linear Mixed Model (GLMM) procedure for a binomial distribution with a logit link function (Genstat 6.1, 2002). The GLMM model produced

log transformed values for the means, and back transformed means were used for further discussion. Embryo mortality and hatchability were analyzed as percentage of the fertile eggs, with incubator tray as experimental unit. The significance of differences between means was determined with the PDIFF option of the LSMEANS statement of Genstat software (Genstat 6.1, 2002). Embryo development was analyzed by three-way ANOVA with the general linear models procedure of Genstat software (Genstat 6.1, 2002), with egg as experimental unit.

The model was: $Y_{ijkl} = \mu + B_i + W1_j + W3_k + HT_l$ + interactions + ε_{ijkl}, where Y_{ijkl} is embryo mortality in wk 1, 2 or 3 of incubation, hatchability of second and first grade chicks, the percentage of chicks found dead in the hatcher baskets, or embryo development (yolk free embryo weight, embryo length, and residual yolk), or post hatch development (chick weight, chick length and rectal temperature). In this model, μ is the overall mean, B_i is batch (i= 1, 2), $W1_j$ is EST during wk 1 (j=36.7, 37.8ºC), $W3_k$ is EST during wk 3 (k=37.8, 38.9ºC), HT_l is house temperature (l=warm, cold) and ε_{ijkl} is the residual error term.

RESULTS

Eggshell temperature and machine temperature

To obtain the different EST profiles, different MT profiles were needed. EST values did not fluctuate more than 0.2ºC away from the mean EST set point. The difference between MT and EST (DT) is shown in Figure 1. Eggs from the different batches needed to be incubated at different MT in order to control EST. EST during wk 1 had a profound effect on DT, especially in batch 2 where MT for eggs incubated at 37.8ºC EST had to be decreased at an earlier stage (3 to 4 d) and to lower values thereafter, compared to eggs incubated at 36.7ºC during wk 1. In batch 1, this effect was less profound and MT needed to be decreased substantially only after d 7. In batch 2, high EST during wk 3 decreased DT by 0.2ºC at d 16, whereas at EST of 37.8ºC, DT remained constant at around –1.0ºC. This effect was observed both in eggs incubated at 36.7ºC and 37.8ºC during wk 1 (Figure 1). In batch 1, EST in wk 3 did not affect DT.

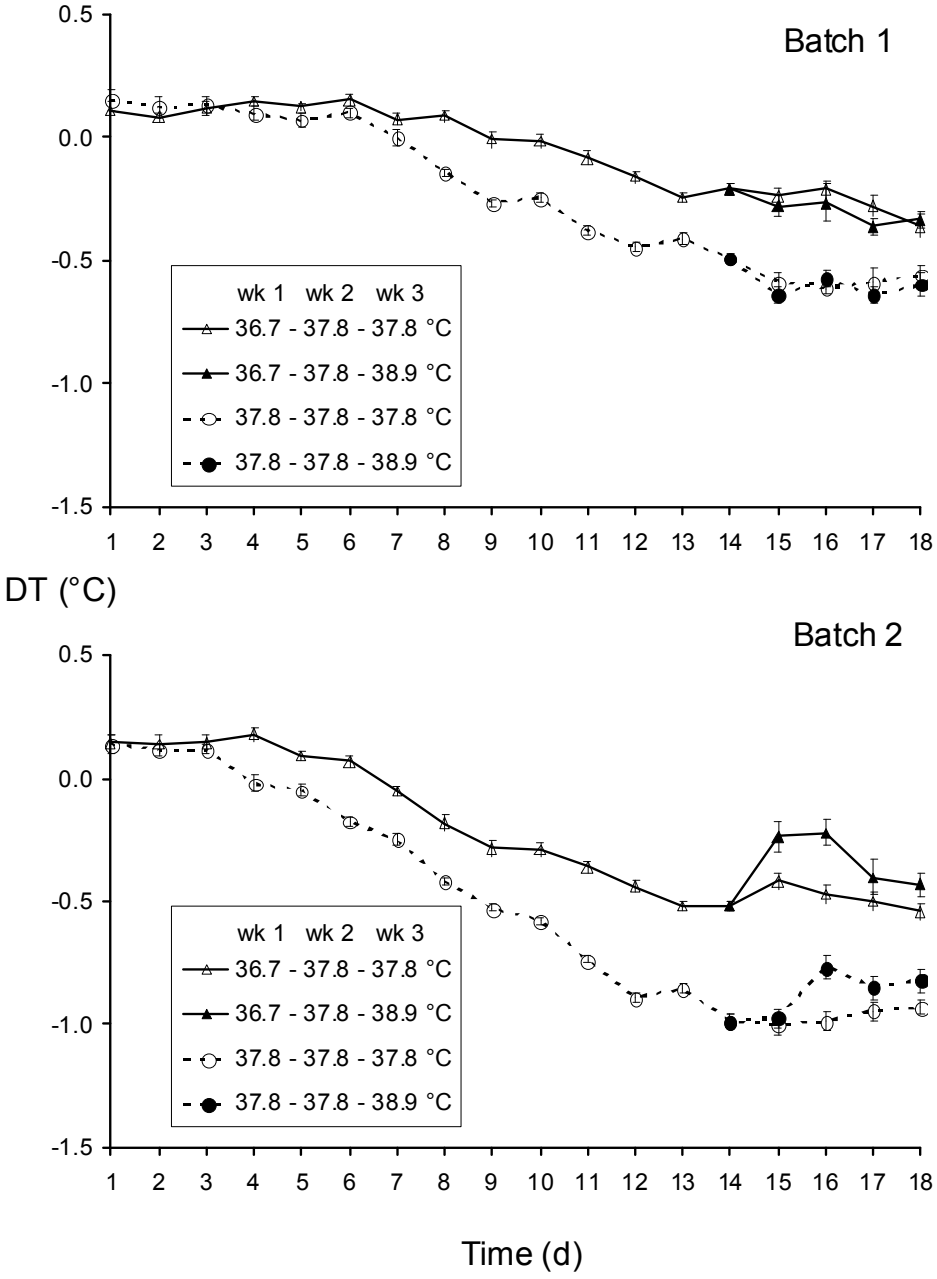

FIGURE 1. Difference (DT=MT-EST) between machine temperature (MT) and eggshell temperature (EST) in batch 1 (top) and batch 2 (bottom). During d 1 to 14, EST is indicated by the straight line with open triangles (wk 1 = 36.7ºC; wk 2 = 37.8ºC); or by a dotted line with open circles (wk 1 = 37.8ºC; wk 2 = 37.8ºC). During d 14 to 18, EST is indicated by open triangles (wk 1 = 36.7ºC; wk 2 = 37.8ºC; wk 3 = 37.8ºC) or open circles (wk 1 = 37.8ºC; wk 2 = 37.8ºC; wk 3 = 37.8ºC), closed triangles (wk 1 = 36.7ºC; wk 2= 37.8ºC; wk 3 = 38.9ºC) or closed circles (wk 1 = 37.8ºC; wk 2 = 37.8ºC; wk 3 = 38.9ºC). Error bars represent SEM.

Embryonic mortality and hatchability

EST during the first wk of incubation had no significant effect on embryonic mortality and hatchability. Both in batch 1 and batch 2, high EST during wk 3 increased late embryonic mortality, and, as a result, decreased hatchability of first grade chicks (Table 1). A significant interaction was observed between EST during wk 1 and EST during wk 3. The lowest wk 3 embryonic mortality and highest hatchability of first grade chicks was found when eggs were incubated at a constant EST of 37.8ºC. In batch 2, also the percentage of second grade chicks was lowest at a constant EST of 37.8ºC. In both batches, any deviation from 37.8ºC EST resulted in decreased hatchability of first grade chicks.

Embryo development

EST during the first wk of incubation significantly influenced embryo development (Table 2). In both batches, embryo length and yolk free body weight (YFB) was reduced on d 7, 14, 18 and 21.5 in embryos that were incubated at low EST during the first wk of incubation. High EST during the third wk of incubation had similar effects and reduced embryo length on d 18 and 21.5 in both batches. An interaction was observed between EST during wk 1 and EST during wk 3 with regard to embryo length on d 18. The largest embryos at day 18 were found in eggs incubated at 37.8ºC constantly through incubation. The effect of low EST during wk 1 on embryo length at d 18 of incubation was larger than the effect of EST during wk 3, which can be explained by the differences in exposure time and recovery time. Comparable effects and interactions were observed for YFB. Highest YFB was observed in embryos incubated at a constant EST of 37.8ºC, and deviations from 37.8ºC in the first or third wk of incubation resulted in decreased embryo development. On d 21.5 of incubation, the interactions between EST during wk 1 and 3 for YFB and embryo length disappeared, and only the main effects of EST during wk 1 and EST during wk 3 remained (Table 2). The amount of RY differed between batches (3.1 and 5.2 g respectively in batch 1 and 2), but did not differ between EST treatments.

Post hatch development

In batch 1 and under warm HT compared to cold HT, average chick weight at d 7 post hatch was 142.4 g and 136.6 g and chick length at d 7 post hatch was 26.1 and 26.1 cm respectively. Chicks in batch 1 responded differently with regard to EST treatment and HT. Chicks reared under warm HT reached highest first wk body weight only when incubated at a constant 37.8ºC EST (Table 3). Chicks reared under cold HT had lower first wk body weights,

and no effects of EST profiles could be observed. There were no significant effects of rearing conditions or EST profiles on chick length in batch 1 (Table 3).

In batch 2, and under warm HT compared to under cold HT, average chick weight at d 7 post hatch was 168.9 g and 154.4 g and chick length at d 7 post hatch was 27.1 and 26.6 cm respectively. HT not only affected post hatch development in batch 2, but also interacted with EST during incubation. Under warm HT, first wk body weight was reduced in chicks that hatched from eggs incubated at low EST during the first wk of incubation (164.2 g) compared to chicks incubated at 37.8ºC during the first wk of incubation (173.5 g), see Table 3.

Under cold HT, first wk body weight was highest in chicks that hatched from eggs incubated at 37.8ºC constantly (158.4 g) and differed significantly from chicks incubated at 36.7ºC during the first wk of incubation and at 37.8ºC (152.4 g) or 38.9ºC (152.6 g) during the third wk of incubation, but not from chicks incubated at 37.8ºC during the first wk of incubation and 38.9ºC during the third wk of incubation (154.2 g).

TABLE 1. Embryo mortality and hatchability in two different batches incubated at 36.7ºC or 37.8ºC eggshell temperature (EST) during wk 1, at 37.8ºC EST during wk 2 and at 37.8ºC or 38.9ºC EST during wk 3 of incubation.

	Embryo mortality			Hatchability	
	Week 1	Week 2	Week 3	2nd grade	1st grade
Batch 1					
EST wk 1					
36.7ºC	4.8	0.6	7.8	0.0	86.7
37.8ºC	5.1	1.1	6.3	0.0	87.5
EST wk 3					
37.8ºC	5.1	1.3	5.5	0.0	88.1
38.9ºC	4.8	0.4	8.6	0.0	86.2
EST wk 1 x 3					
36.7 x 37.8ºC	4.9	0.8	7.9[a]	0.0	86.4[b]
36.7 x 38.9ºC	4.7	0.4	7.7[a]	0.0	87.1[b]
37.8 x 37.8ºC	5.3	1.8	3.1[b]	0.0	89.8[a]
37.8 x 38.9ºC	4.9	0.4	9.4[a]	0.0	85.2[b]
Batch 2					
EST wk 1					
36.7ºC	11.0	1.1	14.2	3.3[a]	70.4
37.8ºC	8.9	3.4	11.8	1.0[b]	74.8
EST wk 3					
37.8ºC	9.6	1.4	11.2	1.9	76.0
38.9ºC	10.4	3.2	14.9	2.4	69.2
EST wk 1 x 3					
36.7 x 37.8ºC	10.2	0.0	13.7[a]	3.8[a]	72.2[b]
36.7 x 38.9ºC	11.8	2.3	14.7[a]	2.8[a]	68.5[b]
37.8 x 37.8ºC	8.9	2.8	8.6[b]	0.0[b]	79.7[a]
37.8 x 38.9ºC	8.9	4.1	15.0[a]	2.0[a]	69.9[b]
Pooled SEM	1.7	1.5	1.4	0.5	1.6
Source of variation[1]					
Batch	***	*	***	NS	***
wk 1	NS	NS	NS	NS	NS
wk 3	NS	NS	*	NS	*
wk1 x wk3	NS	NS	*	NS	*
Batch x wk1	NS	NS	NS	*	NS
Batch x wk3	NS	NS	NS	NS	NS
Batch x wk1 x wk3	NS	NS	NS	*	NS

[a,b] Means within a column and batch with no common superscript differ significantly

[1] NS = No significance; *** = P<0.001; * = P<0.05

Post hatch rectal temperature

At d 1 post hatch and under warm HT in batch 1, RT was highest in chicks that hatched from eggs incubated at 37.8ºC constantly during incubation (40.2ºC). RT was significantly lower in chicks that hatched from eggs incubated at 36.7ºC during the first wk of incubation and at 37.8ºC or at 38.9ºC during the third wk of incubation (38.8ºC and 39.2ºC respectively). The high EST during the third wk of incubation increased RT to 39.9ºC in chicks that hatched from eggs incubated at 37.8ºC during the first wk of incubation, and did not significantly differ from RT in chicks that hatched from eggs incubated at a constant EST of 37.8ºC. At d 1 post hatch and under cold HT in batch 1, RT was lowest in chicks that hatched from eggs incubated at 36.7ºC during the first wk and at 37.8ºC during the third wk of incubation (38.3ºC), and differed significantly compared to the other EST regimes during incubation.

At d 1 post hatch and under warm HT in batch 2, there were no significant differences in RT between chicks that hatched from egg incubated at the different EST profiles. At day 1 and under cold HT in batch 2 however, chicks had lowest RT when incubated at 36.7ºC during the first wk and at 37.8ºC during the third wk of incubation (38.8ºC), and differed significantly from RT in chicks incubated at 37.8ºC during the first wk of incubation and at 37.8ºC or 38.9ºC during the third wk of incubation (both 39.7ºC), see Table 3.

At d 7 post hatch and under warm housing conditions and as well in batch 1 and batch 2, no effects of EST profile on RT were observed (Table 3). At d 7 post hatch and under cold HT in batch 1 however, RT in chicks that hatched from eggs incubated at 36.7ºC during the first wk and at 37.8ºC during the third wk of incubation was significantly lower (39.3ºC) compared to chicks that hatched from eggs incubated at 37.8ºC constantly (39.9ºC). High EST during the third wk of incubation increased RT in chicks incubated at either 36.7ºC or 37.8ºC during the first wk of incubation (39.6ºC and 39.5ºC, respectively), see Table 3. At d 7 post hatch and under cold housing conditions in batch 2, no effects of EST profile on RT were observed.

TABLE 2. Embryo development in two different batches of eggs incubated at 36.7ºC or 37.8ºC eggshell temperature (EST) during wk 1, at 37.8ºC EST during wk 2 and at 37.8ºC or 38.9ºC EST during wk 3 of incubation.

	Embryo length (cm)				Yolk free embryo weight (g)				Residual yolk (g)
Day of incubation:	7	14	18	21,5	7	14	18	21.5	21.5
Batch 1									
EST wk 1									
36.7ºC	1.6^{b}	11.4^{b}	16.9	17.9^{b}	0.6^{b}	12.5^{b}	29.0	31.2^{b}	2.9
37.8ºC	1.7^{a}	11.9^{a}	17.6	18.8^{a}	0.8^{a}	13.6^{a}	30.4	34.6^{a}	3.2
EST wk 3									
37.8ºC	-	-	17.5	18.5^{a}	-	-	30.9	33.1^{a}	3.1
38.9ºC	-	-	17.0	18.2^{b}	-	-	28.5	32.7^{b}	3.1
EST wk 1 x 3									
36.7 x 37.8ºC	-	-	17.0^{b}	18.2	-	-	30.4^{ab}	31.8	3.1
36.7 x 38.9ºC	-	-	16.8^{b}	17.7	-	-	27.6^{b}	30.6	2.8
37.8 x 37.8ºC	-	-	17.9^{a}	18.8	-	-	31.4^{a}	34.4	3.1
37.8 x 38.9ºC	-	-	17.3^{ab}	18.7	-	-	29.4^{ab}	34.9	3.4
Batch 2									
EST wk 1									
36.7ºC	1.8^{b}	10.8^{b}	16.7	19.9^{b}	0.7^{b}	12.4^{b}	33.7	42.4^{b}	5.6
37.8ºC	2.0^{a}	11.4^{a}	17.0	20.3^{a}	0.9^{a}	13.4^{a}	34.9	42.9^{a}	4.7
EST wk 3									
37.8ºC	-	-	17.0	20.2^{a}	-	-	34.2	42.8^{a}	5.2
38.9ºC	-	-	16.7	19.9^{b}	-	-	34.5	42.5^{b}	5.1
EST wk 1 x 3									
36.7 x 37.8ºC	-	-	16.6^{b}	19.9	-	-	33.1^{b}	42.6	5.5
36.7 x 38.9ºC	-	-	16.8^{b}	19.8	-	-	34.3^{b}	41.9	5.8
37.8 x 37.8ºC	-	-	17.2^{a}	20.3	-	-	35.1^{a}	43.1	4.8
37.8 x 38.9ºC	-	-	16.8^{ab}	20.1	-	-	34.7^{ab}	42.8	4.7
Pooled SEM	0.0	0.1	0.3	0.3	0.0	0.2	0.7	0.9	0.8
Source of variation[1]									
Batch	***	***	***	***	NS	NS	***	***	***
wk 1	***	***	**	***	***	***	***	***	NS
wk 3	-	-	*	***	-	-	*	*	NS
wk1 x wk3	-	-	**	NS	-	-	**	NS	NS
Batch x wk1	NS	NS	NS	NS	NS	NS	NS	NS	NS
Batch x wk3	-	-	NS	NS	-	-	NS	NS	NS
Batch x wk1 x wk3	-	-	NS	NS	-	-	*	NS	NS

[a,b] Means within a column and batch with no common superscript differ significantly

[1] NS = No significance; *** = P<0.001; ** = P<0.01; * = P<0.05

TABLE 3. Development of chicks at 7 days post hatch in two different batches of eggs, incubated at 36.7ºC or 37.8ºC eggshell temperature (EST) during wk 1, and at 37.8ºC or 38.9ºC EST during wk 3 of incubation. Chicks were reared at two different temperature regimes (warm and cold) and rectal temperatures were measured at d 1 and d 7.

	Chick weight (g)		Chick length (cm)		Rectal temperature (°C)			
					d 1		d 7	
	warm	cold	warm	cold	warm	cold	warm	cold
Batch 1								
EST wk 1								
36.7ºC	140.4	135.3	26.0	25.9	39.0	38.5	40.1	39.4
37.8ºC	144.4	137.9	26.1	26.3	40.0	39.0	40.3	39.7
EST wk 3								
37.8ºC	144.7	138.3	26.0	26.3	39.5	38.7	40.1	39.6
38.9ºC	140.1	134.9	26.1	25.9	39.5	38.8	40.3	39.6
EST wk 1 x 3								
36.7 x 37.8ºC	140.8[b]	136.7	25.9	26.0	38.8[b]	38.3[b]	40.0	39.3[b]
36.7 x 38.9ºC	140.0[b]	134.0	26.2	25.7	39.2[b]	38.8[a]	40.2	39.6[ab]
37.8 x 37.8ºC	148.6[a]	139.9	26.2	26.5	40.2[a]	39.1[a]	40.2	39.9[a]
37.8 x 38.9ºC	140.3[b]	135.9	26.0	26.1	39.9[ab]	38.9[a]	40.3	39.5[ab]
Batch 2								
EST wk 1								
36.7ºC	164.2[b]	152.5	27.1	26.4	40.1	39.1	40.0	40.0
37.8ºC	173.5[a]	156.3	27.1	26.8	40.3	39.7	40.5	40.1
EST wk 3								
37.8ºC	167.6	155.4	27.2	26.7	40.2	39.3	40.4	40.0
38.9ºC	170.3	153.4	27.0	26.6	40.2	39.5	40.1	40.1
EST wk 1 x 3								
36.7 x 37.8ºC	161.9	152.4[b]	27.1	26.4	40.1	38.8[b]	40.1	40.0
36.7 x 38.9ºC	166.5	152.6[b]	27.0	26.4	40.1	39.3[ab]	39.9	39.9
37.8 x 37.8ºC	173.2	158.4[a]	27.3	26.9	40.4	39.7[a]	40.7	40.0
37.8 x 38.9ºC	173.8	154.2[ab]	27.0	26.7	40.3	39.7[a]	40.3	40.2
Pooled SEM	3.8	3.6	0.3	0.5	0.2	0.3	0.4	0.3
Source of variation[1]								
Batch	***	***	***	***	***	***	NS	***
wk 1	**	NS	NS	NS	***	***	NS	NS
wk 3	**	NS	NS	NS	NS	NS	NS	NS
wk1 x wk3	***	NS	NS	NS	***	***	NS	**
Batch x wk1	NS	NS	NS	NS	**	NS	NS	NS
Batch x wk3	NS	NS	NS	NS	NS	NS	NS	NS
Batch x wk1 x wk3	*	*	NS	NS	*	*	NS	*

[a,b] Means within a column and batch with no common superscript differ significantly

[1] NS = No significance; *** = P<0.001; ** = P<0.01; * = P<0.05

DISCUSSION

EST is influenced by heat production and heat transfer (Meijerhof and Van Beek, 1993), where MT is one of the factors influencing heat transfer. The goal of this experiment was to study the effect of EST, using the MT as a method to control EST. The results show that eggs that were subjected to lower EST during the first wk of incubation required a higher MT (lower DT) in the second and third week. This can be explained by a lower heat production in this period. Low temperatures early in incubation not only appear to have an effect on embryonic heat production, but are reported to have also effects on embryo development and post hatch development (Moreng and Bryant, 1954, 1956; Geers et al., 1983; Sarpong and Reinhart, 1985).

Embryonic metabolic rate and thus heat production changes with incubation temperature have been shown by Nichelmann et al. (1998). Early in incubation, metabolic rate increased with incubation temperature, whereas prior to pipping, metabolic rate of chicken and duck embryos decreased as the internal egg temperature exceeded 40.0ºC (Janke et al., 2002). In the present study, this effect was already observed at lower internal egg temperatures, because internal egg temperature in the present study could not exceed EST by more than 0.2 – 0.3ºC (Meijerhof and Van Beek, 1993). In both trials, the group having a higher EST in the third week of incubation required a smaller DT. However, in the second batch MT needed to be increased relatively more at higher EST than in the first batch, indicating that different batches of eggs can respond differently.

Embryo development could be expressed in terms of embryo length and yolk free embryo weight (Hill, 2001) and was always highest in eggs incubated at a constant EST of 37.8ºC. In the present trial, effects of low EST during wk 1 and high EST during wk 3 of incubation on embryo length and yolk free embryo weight were observed. Highest hatchability and best post hatch performance was observed when eggs were incubated at a constant EST of 37.8ºC, which is in agreement with Lourens and Van Middelkoop (2001).

Schmalhausen (1930) hypothesized that post hatch growth and organ function will be impaired if growth rates during embryonic development deviates from optimum. Development of organs and physiological systems begin in the first wk of embryonic development (Lilja and Olsson, 1987), and continue after hatch. An overview of physiological systems that start to mature during the last wk of incubation and during the first wk post hatch is documented by Christensen (2001). After hatching, the chick gradually transforms into a homeotherm organism that can regulate its body temperature within certain limits by increasing or decreasing heat production. On average, this transition period lasts about 3-4 d and the duration depends mainly on the size of the chicken and the age of the breeder flock

(Weyntjens et al., 1999). Chicks from young parent stock are more sensitive with regard to the control of RT in relation to HT (Weyntjens et al., 1999). The results of this experiment indicate that EST profiles during the first wk of incubation influences the control of body temperature during the first wk post hatch. Delayed development of thermoregulation in combination with decreased heat output may have been responsible for the lower RT, especially in chicks that were reared under cold HT. Chicks with decreased heat output as a result of low EST early in incubation may benefit from increased hatcher temperatures. Highest first wk body weights and highest RT were observed in chicks that hatched from eggs incubated at a constant EST of 37.8ºC, but rearing conditions appeared to play an important role as well. Under warm HT in batch 1, best results were observed in chicks that hatched from eggs incubated at a constant EST of 37.8ºC. In batch 2 however, the positive effect of a constant EST profile of 37.8ºC was only observed when chicks were reared under cold HT; under warm HT, the first wk EST profile was of more importance for post hatch growth.

It can be concluded that different batches of eggs require different MT settings in order to incubate at the same EST. Furthermore, relatively small deviations in EST result in decreased HP, retarded embryo development, increased late embryonic mortality, increased percentage of second grade chicks, decreased hatchability, decreased post hatch growth and decreased ability to maintain RT in the first week post hatch, especially under low HT. As EST can vary independent from MT, factors that influence either heat transfer or heat production should be taken into account for optimizing incubation conditions. Controlling incubator conditions by controlling only MT can result in sub-optimal incubation through an uncontrolled influence on EST. EST variation within incubators can therefore be responsible for an undesirable increase in variation of the response of embryos in incubation experiments or chicks in grow-out experiments.

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Chapter 3

EFFECT OF EGG SIZE ON HEAT PRODUCTION AND THE TRANSITION OF ENERGY FROM EGG TO HATCHLING

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ABSTRACT

An experiment was conducted to study the effect of egg size on embryo development, heat production (HP) and energy partitioning between egg and hatchling. Small (56.1 ± 0.12 g SEM) and large (70.0 ± 0.11 g SEM) hatching eggs were incubated in climate respiration chambers and eggshell temperature (EST) was maintained constant at 37.8ºC in both egg weight classes by adjusting machine temperature (MT). Dry matter, ash, protein, and fat contents were determined in albumen, yolk, yolk free body (YFB) and residual yolk (RY) and carbohydrate contents and caloric values were calculated. To achieve a constant EST, MT needed to be set lower from d 15 onwards compared to small eggs, coinciding with increased HP in large eggs compared to small eggs. Selective nutrient uptake resulted in higher fat content and lower protein content in RY in chicks that hatched from small eggs compared to large eggs. RQ in small and large eggs was the same, and embryos in small and large eggs were equally efficient in the transfer of energy from egg to YFB. The surplus availability of nutrients in large eggs was therefore held responsible for the absolute and relative higher weight of RY in chicks that hatched from large eggs compared to small eggs.

(*Key words*: egg size; eggshell temperature; heat production; yolk free body; residual yolk; energy utilization; efficiency)

INTRODUCTION

It has been reported that during incubation, large eggs produce more heat than small eggs (Rahn et al., 1974; Hoyt, 1987; Vleck et al., 1980; Vleck and Vleck, 1987; Meijerhof and Van Beek, 1993). Large eggs also face more difficulties to remove the surplus heat from the egg (French, 1997), as a result of the decreasing ratio between egg surface and egg content with increasing egg size (Vogel, 1984), and the reduced air velocity over the eggs in commercial incubators (French, 1997). If large eggs and small eggs are incubated under similar conditions, the higher heat production (HP) and increased difficulties to remove heat in large eggs will result in higher embryo temperatures in these eggs (Meijerhof and Van Beek, 1993, Meijerhof, 2002).

The influence of embryo temperature on embryo development and hatchability is shown by Lourens et al (2005), who used egg shell temperature (EST) as reflection of embryo temperature. As egg size influences embryo temperature through heat production and heat transfer, experiments studying the effect of egg sizes on embryo development between species (Ricklefs, 1987) or late embryonic mortality within one species (Hagger et al, 1986; Reinhart and Moran, 1979) will be influenced by differences in embryo temperature, if incubator conditions are not adjusted to obtain an equal embryo temperature.

To our knowledge, the effect of egg size on embryo development and hatchability is never studied independent of embryo temperature. Therefore, an experiment was conducted to incubate eggs from two different size classes at an equal EST. The goal of the experiment was to investigate the effect of egg size on HP, embryo development and energy transition between egg and hatchling, when eggs of different sizes were kept on the same EST of 37.8ºCthroughout incubation.

MATERIALS AND METHODS

Experimental set up

In four trials, small and large eggs were incubated separately at a constant EST of 37.8 ºC in one of two identical climate respiration chambers. The EST from 5 individual fertile eggs was measured continuously and machine temperature (MT) was adjusted automatically every 5 minutes, if the median EST drifted away from 37.8ºC. Heat production (HP) was calculated from oxygen consumption and carbon dioxide production. The amount of energy available in the egg albumen and egg yolk before incubation and the distribution of energy between the yolk free body (YFB) and residual yolk (RY) at hatch was determined by chemical analyses.

Hatching eggs and incubation

First grade hatching eggs from one Hybro G grand parent stock were divided into two different weight classes: small (54.0 – 56.0 g) and large (70.0 – 72.0 g) and eggs were stored between 5 and 7 d. Per trial, 30 small eggs and 30 large eggs were incubated in one of two identical small open circuit climate respiration chambers. Additionally, from both groups, five eggs per trial were used to determine egg constituents.

Both climate respiration chambers (267 l) contained an automatic tray turning system that turned the eggs every 30 minutes at an angle of 90°. Two different fans mixed fresh air with recirculated air and provided a consistent air flow across the eggs. One temperature sensor (Pt100) measured MT, a Vaisala sensor measured relative humidity. Another five sensors (Pt500) measured individual EST of five different eggs. Sensors were attached with tape (Tesa) in heat conducting paste (Schaffner) at the eggshell at the equator of the egg. All temperature sensors were compared after the experiments at different temperature levels between 36 and 40ºC. Differences between individual sensors and the mean were maximal 0.1ºC. Relative humidity was set at 55 % constantly during the first 18 d. EST was measured every minute, and MT was automatically adjusted according the median EST of 5 different eggs in order to maintain EST at 37.8ºC. Eggs were candled at 7 d of incubation and infertile eggs and eggs containing dead embryos were removed. At 18 d of incubation, all eggs were reweighed to determine egg weight loss during incubation, and eggs were transferred to hatching baskets. EST was measured again and MT was set at the constant value that corresponded to a constant EST of 37.8ºC. For the remaining time until hatching, EST was allowed to increase.

Heat production

Oxygen and carbon dioxide concentrations were measured every 9 minutes in both chambers and in fresh air. Carbon dioxide concentration was measured with a non dispersive infrared CO_2 analyzer of Hartmann & Braun, type Uras 3G. Oxygen concentration was measured with a paramagnetic oxygen analyzer type ADC7000. The refreshed air volume was 2 l per minute during the first 18 d of incubation and 3 l per minute from d 18 onwards. HP was calculated using the formula of Romijn and Lokhorst (1961) and adjusted for fertility and embryo mortality, based on the break-out analysis described below.

Embryo mortality, hatchability and hatchling measurements

Clear eggs (candled at 7 d of incubation) and dead in shell eggs (hatch debris) were opened to determine true fertility and pattern of embryonic mortality. For the correction for

mortality in the HP calculations, day of mortality was classified by the characteristics described in Table 1, and further in more detail to the estimated day of mortality by the size of the dead embryo. HP was expressed per living, fertile embryo (egg) based on fertility and mortality pattern. Hatchability was expressed as the percentage chicks that hatched from the true fertile eggs, based on the break out analysis. At 21.5 d of incubation, all hatched chicks were sacrificed with a mixture of CO_2 and O_2. All chicks were weighed and total chick lengths were measured by stretching chicks along a ruler and taking the length between the top of the beak and the tip of the middle toe of the right feet according to Hill (2001) or Lourens et al. (2005). Next, RY was removed and weighed.

TABLE 1. Classification of true fertility and embryo mortality.

Time	Characteristics
0 day	unfertilised; no signs of development
1 day	area vasculosa of 1 cm Ø
2 days	area vasculosa of 2.5 – 3.0 cm Ø
3 days	blood ring / presence of sub embryonic fluid
4 –10 days	eye present
11 – 17 days	feathers present
18- 20 days	yolk remains outside the embryo
21 days	residual yolk retracted inside the embryo; embryo ready to hatch

Energy partitioning during the transfer from egg to hatchling

A total of 40 fresh eggs (5 eggs per trial per egg weight class) were boiled and albumen and yolk were separated and weighed. The eggshell was dried for 24 hours at room temperature and weighed. From 40 chicks per egg weight class (5 chicks per trial per egg weight class), YFB and RY were separated for analysis. Albumen, yolk, residual yolks and the yolk free bodies were frozen at –18 ºC. At a later stage, dry matter (ISO 3332, 1983), ash (ISO 5984, 1978), protein (ISO 5983, 1989) and fat contents (ISO 6492, 1999) were determined. Carbohydrate content was estimated as 1000 – ash – protein – fat content (g/kg DM). Finally, the energy content in the different parts was calculated using energy densities for protein, fat and carbohydrates of respectively 16.8, 37.8 and 16.8 MJ/kg DM (International System of

Units, 1998). The efficiency in energy transfer from available energy to YFB (E_{YFB}) was calculated as:

$$E_{YFB} = \frac{YFB(kJ)}{Albumen(kJ) + Yolk(kJ) - RY(kJ)} * 100\%$$

Statistical analyses

The nonlinear, sigmoid curves of HP and MT were analyzed in Genstat 6.1 (2002) with a REML-procedure according to the following model for repeated measurements: $Y_{ijk} = \mu + E_i + T_j + (E \times T)_{ij} + D_k$ + interactions + ε_{ijk}, where Y_{ijk} is HP or RQ, μ is the overall mean, E_i is egg size (i = small, large), T_j is trial (j = 1,2,3,4), and D_k are the 21 days of incubation (k = 1,…,21), and ε_{ijk} is the residual error term. The interaction between treatment and trial $(E*T)_{ij}$ represents the random effect within trial between day numbers and is used as error term to test the effects of E_i and T_j.

A Generalized Linear Mixed Model (GLMM) procedure for a binomial distribution with a logit link function (Genstat 6.1, 2002) was used for hatchability data. Egg and chick related factors were analyzed by two-way ANOVA with the general linear model procedure of Genstat software (Genstat 6.1, 2002). In both procedures, a group of 30 eggs in one climate respiration chamber was used as experimental unit. The model was: $Y_{ij} = \mu + E_i + T_j + (E_i * T_j) + \varepsilon_{ij}$, where Y_{ij} is the dependent variable, μ is the overall mean, E_i is egg size (i = small, large), T_j is trial (j = 1,2,3,4) and ε_{ij} is the residual error term. In all analyses, non-significant interactions were deleted from the model. Data of DM, ash, protein, carbohydrates and energy content were analyzed according to the latter model. Unfortunately, data of yolk free body and residual yolk in trial four were lost, so results and conclusions with regard to energy contents and changes were based on only the first three trials.

RESULTS

Incubation temperature and heat production

For MT, an effect of time ($P<0.001$) and an interaction between time and egg size ($P=0.032$) was observed. From d 15 onwards, MT needed to be decreased more compared to small eggs (Figure 1). During the course of incubation, an effect of time ($P<0.001$), egg size ($P=0.001$) and an interaction between time and egg size ($P=0.040$) on HP was observed. HP in large eggs was higher compared to small eggs from d 15 onwards (Figure 2).

Hatching eggs, incubation parameters and hatchling characteristics

Hatching egg characteristics, incubation related parameters and hatchling characteristics per egg size category are summarized in Table 2. Shell weight of small eggs was lower than shell weight of large eggs, and absolute weight loss during incubation was lower in small eggs compared to large eggs. At d 18, small eggs produced 137 mW per egg (2.5 mW/g) and large eggs produced 155 mW per egg (2.2 mW/g), see Table 2. RQ decreased between day 5 and 9 from 1.08 to 0.68, remained constant thereafter and did not differ between small and large eggs. Embryo mortality (EM) in week 1, 2 or 3 and hatchability was similar for both egg size classes (Table 2). Chicks that hatched from small eggs weighed less and were shorter compared to chicks that hatched from large eggs.

Chemical analyses and energy content in eggs and hatchlings

Egg and hatchling constituents in both egg weight classes are shown in Table 3. Fresh albumen and yolk weights were lower in small eggs compared to large eggs. Also, YFB and RY weight were lower in chicks that hatched from small eggs compared to large eggs. Dry matter content in albumen, yolk, YFB and RY did not differ between egg size classes. Relative ash content (g/kg DM) in RY of chicks that hatched from small eggs was higher than in RY of chicks that hatched from large eggs. RY of chicks that hatched from small eggs also contained relatively more fat (g/kg DM) than RY of chicks that hatched from large eggs. On the contrary, RY of chicks that hatched from small eggs contained relatively less protein (g/kg DM) than RY of chicks that hatched from large eggs. No differences were observed in relative carbohydrate contents (g/kg DM) in albumen, yolk, YFB or RY between egg size classes. Also relative energy content (MJ/kg DM) in albumen, yolk, YFB or RY did not differ between egg size classes (Table 3).

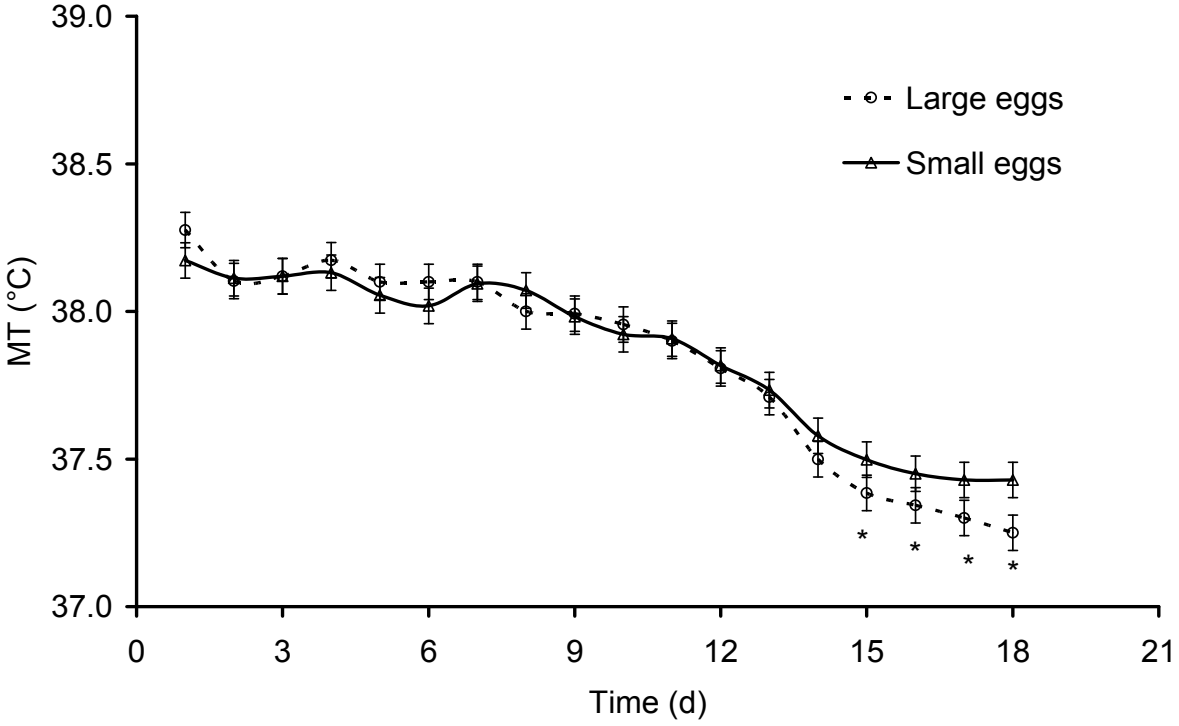

FIGURE 1. Machine temperature (MT) required to incubate eggs at 37.8ºC eggshell temperature (EST) in small and large eggs (overall SEM = 0.06ºC)

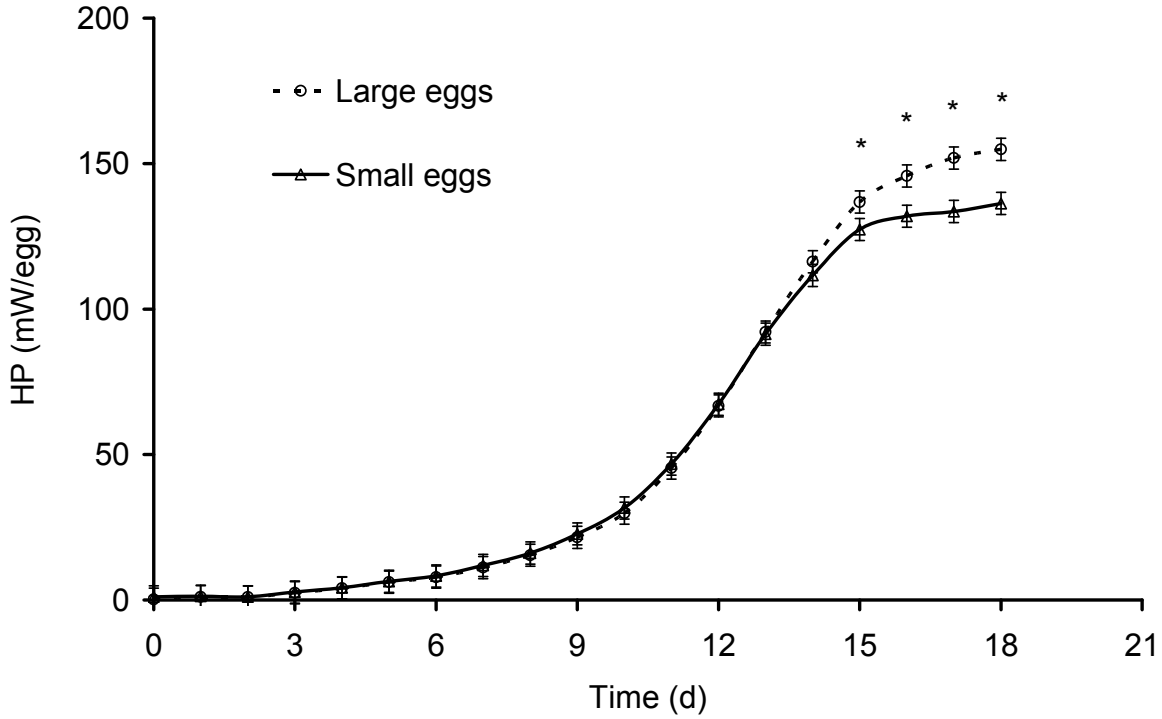

FIGURE 2. Heat production per egg (mW) in small and large eggs (SEM = 3.8 mW)

TABLE 2. Characteristics of hatching eggs, incubation process and hatchlings in small and large eggs.

	Small eggs	Large eggs	SEM
Hatching eggs			
Egg (g)	55.7[b]	70.4[a]	0.6
Shell (g)	6.5[b]	7.8[a]	0.4
Incubation process			
Weight loss (g)	8.0[b]	9.4[a]	0.4
HP d 18 (mW.egg^{-1})	137[b]	155[a]	3.8
Fertility (%)[1]	89.2	89.2	4.7
EM (%)[2]	15.9	17.8	5.6
- EM wk 1 (%)[2]	11.2	11.2	4.4
- EM wk 2 (%)[2]	0.9	0.9	1.2
- EM wk 3 (%)[2]	5.6	3.7	2.4
Hatchability (%)[2]	82.2	84.1	5.6
Hatchlings			
Chick weight (g)	36.0[b]	46.4[a]	0.6
Chick length (cm)	19.7[b]	20.3[a]	0.1

[1] Fertility expressed as percentage of eggs set;

[2] Embryo mortality (EM) and hatchability expressed as percentage of true fertile eggs;

[a,b] Significant differences are indicated by different letters (n=4; $P<0.05$)

TABLE 3. Weight, dry matter (DM), ash, fat, protein, carbohydrate and energy contents in albumen and yolk of small and large eggs and in yolk free body and residual yolk of hatchlings that hatched from small and large eggs.

	Weight[1]	DM[2]	Ash[3]	Fat[3]	Protein[3]	Carbohydrates[3]	Energy[4]
Albumen							
Small eggs	32.2[b]	134.4	58.2	2.6[a]	881.3	57.9	15.9
Large eggs	41.6[a]	134.5	52.4	2.2[b]	888.3	57.1	16.0
SEM	*0.7*	*2.0*	*2.5*	*0.1*	*3.0*	*5.1*	*0.0*
Yolk							
Small eggs	17.3[b]	514.3	41.6	618.2	317.6	22.5	29.1
Large eggs	20.8[a]	525.6	45.7	630.3	318.4	5.5	29.3
SEM	*0.6*	*6.8*	*2.9*	*11.6*	*3.4*	*14.0*	*0.2*
Yolk free body							
Small eggs	34.0[b]	225.8	77.8	224.3	683.0	14.9	20.2
Large eggs	41.5[a]	211.4	81.4	229.7	678.5	10.4	20.3
SEM	*0.6*	*7.1*	*1.8*	*9.9*	*11.4*	*4.2*	*0.2*
Residual yolk							
Small eggs	2.3[b]	501.8	98.5[a]	380.1[a]	470.4[b]	51.0	23.1
Large eggs	4.5[a]	509.3	62.6[b]	298.6[b]	576.0[a]	62.8	22.0
SEM	*0.2*	*6.0*	*6.0*	*26.3*	*26.2*	*8.7*	*0.7*

[1] fresh weight (g); [2] $g.kg^{-1}$ fresh weight; [3] $g.kg^{-1}$ dry matter; [4] $MJ.kg^{-1}$ dry matter

[a,b] Significant differences are indicated by different letters (n=3; $P<0.05$)

Caloric values (kJ in fresh product) in albumen, yolk, YFB and RY in small and large eggs are summarized in Table 4. The total energy content in both albumen and yolk of small eggs was less than in large eggs. The total energy content of the YFB in chicks that hatched from small eggs was less than in chicks that hatched from large eggs. The total energy content of RY in chicks that hatched from small eggs was about half the energy content of RY in chicks that hatched from large eggs. In albumen, the energy content of protein and carbohydrates was higher in large eggs compared to small eggs.

TABLE 4. Caloric values (kJ) in small and large hatching eggs (albumen and yolk), hatchlings (yolk free body or YFB and residual yolk or RY), the amount of energy lost, and the efficiency in energy transfer between egg and YFB (E_{YFB})

	Hatching egg (kJ)		Hatchling (kJ)		Lost (kJ)	E_{YFB} (%)
	Albumen	Yolk	YFB	RY		
Protein energy						
Small eggs	64.1[b]	47.4[b]	88.2[b]	9.2[b]	14.2[b]	86.2
Large eggs	83.6[a]	58.4[a]	99.9[a]	22.1[a]	20.1[a]	83.3
SEM	*3.0*	*1.9*	*2.5*	*1.6*	*1.6*	*1.3*
Fat energy						
Small eggs	0.4	207.8[b]	65.3[b]	16.4[b]	126.6[b]	34.0
Large eggs	0.5	260.7[a]	76.5[a]	26.0[a]	158.7[a]	32.5
SEM	*0.1*	*11.0*	*4.6*	*3.4*	*3.6*	*1.5*
Carbohydrate energy						
Small eggs	4.2[b]	3.4	1.9	1.0	4.7	28.8
Large eggs	5.4[a]	1.0	1.6	2.4	2.4	39.4
SEM	*0.5*	*2.0*	*0.6*	*0.4*	*1.6*	*9.5*
Total energy						
Small eggs	68.8[b]	258.7[b]	155.4[b]	26.5[b]	145.5[a]	51.7
Large eggs	89.5[a]	320.2[a]	178.0[a]	50.6[a]	181.1[b]	49.6
SEM	*3.1*	*12.3*	*6.7*	*3.9*	*5.1*	*1.3*

[a,b] Significant differences are indicated by different letters (n=3; $P<0.05$)

No differences were observed in energy contents of fats. In yolk, the energy content of protein and fat was higher in large eggs compared to small eggs, and no differences were observed in energy contents of carbohydrates. In YFB and RY, the energy content of protein and fat was higher in large eggs compared to small eggs, and no differences were observed in energy contents of carbohydrates.

Also the amount of energy lost during incubation from proteins and fats was higher in large eggs compared to small eggs, and no differences were observed in amount of energy lost from carbohydrates. The relative energy distribution between albumen and yolk in small and large eggs was the same: on average 21.4 % of the energy was allocated in the albumen and 78.6 % in the yolk. In both egg weight classes, on average 44.3 % of the available energy in

the egg was lost during incubation. The energy content of YFB and RY however differed between egg weight classes. Hatchlings that hatched from small eggs contained 47.5 % of the available energy in YFB compared to 43.4 % in large eggs (SEM = 0.6 %). RY in hatchlings that hatched from small eggs contained 8.1 % of the available energy, compared to 12.3 % in hatchlings that hatched from large eggs (SEM = 1.2 %). The efficiency (E_{YFB}) of the process of transferring energy from egg to YFB, was similar between small and large eggs with regard to protein, fat, carbohydrates and total energy.

DISCUSSION

Heat production and eggshell temperature

From the moment that large eggs produced more heat, MT needed to be decreased more for large eggs than for small eggs. In our study we observed a higher HP and lower MT in large eggs from d 15 onwards, which suggests that embryonic growth in large eggs increased more from that moment onwards. This is in accordance to the review by Wilson (1991), who concluded that embryo weight is not correlated with egg weight during the first half of the incubation period. If MT was not adjusted in the present study, EST would have increased more in large eggs than in small eggs from d 15 onwards, as shown in Romijn and Lokhorst (1956) or calculated by Meijerhof and van Beek (1993). Adjusting MT avoids adverse effects of high EST during the last week of incubation on embryo development (Lourens et al., 2005). HP initially increase with higher temperatures (Nichelmann et al., 1998; Janke et al., 2002) but too high temperatures decrease HP during the final stages of incubation (Janke et al., 2002). The effects of high EST on embryo development and HP can also be present in the studies of for example Romijn and Lokhorst, (1956, 1960); Tullett and Deeming, (1982); Tullett and Burton, (1982); Janke et al., (2004) and O'Dea et al. (2004), who incubated eggs at a constant MT of 37.8ºC. In these studies, HP at 18 days of incubation ranged between 2.0 and 2.6 mW/g, comparable to the results of the present study (2.5 mW/g in small eggs and 2.2 mW/g in large eggs). Higher HP of 2.8 to 3.2 mW/g was observed by Hulet and Meijerhof (2001) who adjusted MT based on the response of the embryos on varying MT. These high values were not confirmed in the present study, which may be due to the fact Hulet and Meijerhof (2001) used eggs of high yielding broilers, whereas in the present study eggs of grand parents of high yielding broilers were used. Breed may influence HP, as in Tona et al. (2004), very large differences in HP were observed between standard heavy (4.6 mW/g) and Label-type lines (2.7 mW/g) during incubation between 17.8 and 19.5 d for 60 g eggs. However in the study of Tona et al. (2004), eggs were incubated at a constant MT of 37.8ºC, resulting in higher EST

during the final stages of incubation, accelerating the timing of internal pipping in eggs from standard heavy breeders at an earlier age than in Label-type lines. This probably had a larger effect on HP than line per se.

Before d 9, CO_2 output is much higher than that of the embryo alone due to the output of free CO_2 which is evolved from the albumen (Romanoff, 1967). After d 9, the production of CO_2 and the consumption of O_2 are in balance, which resulted in a constant RQ. The RQ value in the present trial of 0.68 indicates the oxidation of fat with additional synthesis of glycogen (Beattie, 1964; Rahn, 1981). This RQ value is lower than reported by Decuypere (1984), who measured a RQ value of 0.72. Glycogen is stored in liver and muscles and is mobilised during the hatching process (Bell and Freeman, 1971). Wineland et al. (2000a,b) and Wineland and Christensen (2001) concluded that incubating eggs at higher MT (38.6 vs 37.5ºC) early or late in incubation resulted in less utilisation of the yolk and increased utilisation of glycogen as energy source. High temperatures therefore not only reduce the required energy available for the embryo to emerge from the eggshell, but also increase RQ. This may explain the differences in RQ observed between this experiment and the results of Decuypere (1984), who incubated at a constant MT and therefore increased EST at the end of the incubation process. When the embryo draws relatively more energy from the anaerobic system under high temperatures, HP and thus embryo development will be decreased.

Energy contents and changes

Total caloric values of eggs in the present study are comparable with data from Ar et al. (1987), who measured a caloric value of 368 kJ for an average sized hatching egg. They reported that YFB contained on average 137 kJ and the RY 132 kJ, so on average 99 kJ was lost, which is lower than the findings in the present study. Compared with the results of Ar et al. (1987), embryos in the present study retained less energy in RY and more in YFB, produced more heat, and were therefore less efficient in energy transfer between egg and YFB. Wiley (1950) reported that embryo's in small eggs find physical limitations beyond a certain size, which reduces growth and hence HP. In the present study, E_{yfb} was similar in small eggs and large eggs, which would suggest that embryos in small eggs used the available energy equally efficient as embryos in large eggs. Still, chicks that hatched from large eggs had a larger amount of energy left in the RY. Based on similar efficiency in energy transfer, we expect that embryos in large eggs had an surplus energy reserve that retained unused in the RY.

In order to incubate small and large eggs at an equal EST of 37.8ºC, MT profiles had to be adjusted for both size classes individually, to compensate for differences in HP from d 15

onwards. Embryos in small and large eggs incubated at both 37.8ºC were equally efficient in energy transfer from egg to YFB, and the surplus of nutrients in large eggs is likely to be responsible for the larger energy content of RY in chicks that hatched from large eggs. Relative energy density was the same in small and large eggs, but selective uptake of nutrients seemed to result in a higher fat content and lower protein content of RY in chicks that hatched from small eggs compared to large eggs. Relations between EST and nutrient uptake, growth and HP are largely unknown and need further investigation.

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Chapter 4

METABOLIC RESPONSES OF CHICK EMBRYOS TO SHORT TERM TEMPERATURE FLUCTUATIONS

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ABSTRACT

Two experiments were carried out to study embryonic metabolic responses to short term temperature fluctuations, to explore the possibilities to use embryonic metabolic responses as a tool to control the incubation process. In the first experiment, eggshell temperature (EST) in the control group was kept constant at 37.8°C and embryos in the experimental group were exposed to varying EST within the range of 36.8 – 38.8°C using EST steps of 0.2°C and time steps of 3 hrs. This was repeated in 3 periods between 6.5 - 9.5 d, 10.5 - 13.5 d and 14.5 – 17.5 d. In the studied EST range, heat production (HP) increased linearly with 4.9% per 1°C EST. In the second experiment, a standard machine temperature (MT) was used for the control group and eggs in the experimental group were exposed to low (MT-0.3°C) or high (MT+0.3°C) temperatures for 1 hr of time at d 8, 9, 11-16. When MT was decreased, CO_2 production initially increased with 0.5% and decreased thereafter. When MT was increased, CO_2 production initially decreased with 0.4% and increased thereafter.

It was concluded that embryonic HP responded linearly with short term EST changes in the studied EST range of 36.8 to 38.8°C. Changes in CO_2 concentration due to short term MT changes could not be explained by embryonic HP only. It can be speculated that blood flow through the chorio-allantoic membrane changes with MT, affecting heat transfer and diffusion of CO_2. A second, delayed response to MT changes was in accordance to the findings in experiment 1. Within the studied temperature range it will be difficult to use embryonic metabolic responses as a tool to control the incubation process. Since HP is linearly related with EST as in the studied temperature range, other factors as O_2 availability or CO_2 release may limit embryo development at higher EST. At this moment, research to the effects of gas exchange at different temperatures on embryo development and survival is lacking.

(*Key words*: eggshell temperature, metabolic responses, short term temperature variation, incubation)

INTRODUCTION

Embryo temperature is considered to be an important factor influencing embryo development, hatchability and post hatch performance (Lourens et al. 2005). Because of the difficulties to measure embryo temperature without damaging the eggs, in the study by Lourens et al. (2005), eggshell temperature (EST) was used as a reflection of embryo temperature. Best results were obtained when EST was kept constant at 37.8°C until internal pipping compared to 36.7°C between d 1-7 or 38.9°C between d 14 and internal pipping. It remained unclear however, if this constant EST of 37.8°C is optimal for embryo development for shorter periods at different stages of incubation; it may be better to monitor other, more direct embryonic metabolic responses as heat production (HP) or CO_2 production. Harun et al. (2001) observed decreased HP in eggs that failed to hatch. Unfortunately, Harun et al. (2001) did not measure EST, and it remained unclear if HP in unhatched eggs was decreased by overheating. Hulet (2001) and Hulet and Meijerhof (2001) maximized CO_2 production in commercial incubators by adjusting machine temperature (MT) settings to the CO_2 response of embryos between d 9 and d 18.

Hatchability was increased by 2 % and it was assumed that optimal embryo development would be found when CO_2 production was the highest. Their approach was primarily to increase CO_2 production and to avoid overheating, which seem to contradict to the findings by Nichelmann et al. (1998) and Janke et al. (2002), who observed that embryonic metabolic rate and thus HP increased with increasing incubation temperature; a poikilotherm reaction. Only when internal egg temperature was increased to over 40.0°C at d 20, the metabolic rate of chick embryos instantly decreased (Janke et al., 2002). However, it remains unclear how the development of embryonic HP is affected by moderate, short term EST variations, and if embryonic metabolic responses can be used as a tool to control the incubation process adequately. Therefore, two experiments were carried out. The objective in both experiments was to study embryonic metabolic responses to short term EST and MT fluctuations, in order to evaluate the possibilities to control the incubation process using metabolic responses.

MATERIALS AND METHODS

Laboratory experiment

Experimental set up

Hatching eggs were incubated at a constant EST of 37.8°C in an incubator, and at three different moments during incubation, eggs were transferred and placed in one of two identical climate respiration chambers (CRC). In one CRC EST was maintained constant at 37.8°C, whereas in the other CRC EST was increased or decreased in a range between 36.8 and 38.8°C. The laboratory experiment was executed twice: in the first series EST was adjusted from 37.8 - 38.8 - 36.8 - 37.8°C. In the second series EST was adjusted from 37.8 - 36.8 - 38.8 - 37.8°C. In each series, this procedure was repeated during three different periods. HP was calculated from oxygen consumption and carbon dioxide production according to Romijn and Lokhorst (1961).

Hatching eggs and incubation

In two series, a total of 1,200 first grade hatching eggs from one Hybro G grand parent stock were used in this trial. Eggs were incubated in a HT-combi1 incubator that has a maximum setting capacity of 4,800 eggs. Per series, 600 eggs were equally divided across 6 incubator trays. At each incubator tray, thermistors were attached at 3 different eggs to measure EST and to accordingly adjust MT daily in order to maintain EST at 37.8°C as described in Lourens et al. (2005). Per series, at d 6, 10 and 14, 200 eggs were transferred from the HT-combi to two identical small open circuit CRC's. A description of the CRC's and the respiratory gas measurements can be found in Lourens et al. (2006). In the CRC's, eggs were not turned to avoid any disturbing effect of positional changes with regard to differences in wind speed and hence EST. Turning is important for normal embryo development between d 2 – 6 of incubation and less thereafter (Deeming, 1989). Complete failure of turning affects the development of HP (Tazawa, 1980; Pearson et al. 1996), but it is believed that turning does not affect the relationship between EST and HP in short periods of time.

Eggshell temperature treatments

In each period per series, eggs remained 72 hrs in the CRC. Period 1 lasted from 6.5 - 9.5 d; period 2 from 10.5 - 13.5 d and period 3 from 14.5 to 17.5 d. At the start of each period, thermistors were attached at the eggshells of 5 individual, fertile eggs as described by Lourens et al. (2005). EST was measured every 30 s and MT was automatically adjusted every 5 min if

the median EST drifted away from the EST set point. The MT was adjusted using the median EST of 5 eggs per CRC to avoid that low EST of infertile eggs or eggs containing dead embryos affect the decision for the direction of the next MT step. Further, the eggs were spaced through the CRC to create the best uniform air speed across the eggs and hence uniform EST as possible. EST in the control group was remained constant at 37.8°C. In series 1, using time steps of 3 hrs, EST in the experimental group was increased with 0.2°C EST per time step from 37.8 °C to 38.8°C. Next, EST was decreased to 36.8°C, and increased again to 37.8°C. In series 2, also using time steps of 3 hrs, EST in the experimental group was decreased with 0.2°C EST per time step from 37.8°C to 36.8°C. Next, EST was increased to 38.8°C, and decreased again to 37.8°C. In both series, for the remaining 12 hrs of each period, EST was remained constant at 37.8°C to evaluate lasting effects of the EST treatments on HP.

Hatchery experiment

Experimental set up

Eggs were incubated in two commercial incubators. Eggs in one incubator followed the standard MT setting, whereas in the second incubator, eggs were subjected to 0.3 °C below (cold) or above (warm) the standard MT setting at different moments in incubation. CO_2 concentrations were adjusted for differences in fertile egg mass and ventilation rate, and the relation between changes in MT and changes in CO_2 concentrations were investigated.

Machine temperature and CO_2 release

A total of 115,200 first grade Ross 308 hatching eggs were equally split by parent stock age and storage time across two identical Hatchtech Microclimer incubators[1] at the Cobroed and Sloot hatchery[2]. Eggs in both incubators were incubated at standard MT settings. During 8 different days, (d 8, 9 and d 11-16), using time steps of 1 hr, MT in one incubator was set 0.3°C above or below standard MT. Each MT change lasted 1 hr, and CO_2 concentrations and ventilation rate were measured and monitored every 4 minutes.

[1] Hatchtech Incubation Technology, Veenendaal, The Netherlands
[2] Cobroed and Sloot Hatchery, Lievelde, The Netherlands

Ventilation measurements were performed to calculate the ventilation rate at different ventilation settings. At 10 different positions in the ventilation exhaust, wind speed was measured with a Testo 452 anemometer[3]. From the average wind speed and exhaust diameter, ventilation rate was calculated. Fertile egg mass was determined as transfer percentage of eggs after automatic candling at 18 d of incubation. CO_2 production was expressed as liters per day per embryo, according to calculations in O'Dea et al. (2004).

RESULTS

Laboratory experiments

Linear regression showed that on average, each temperature change of 1°C changed HP with 4.9 % (R^2 = 0.863; P<0.001), see Table 1. In Figure 1, the relative effects of EST changes (dEST) on HP in series 1 and series 2 are shown. At the end of period 1 in series 1, HP in the experimental group was increased with 7-8 % compared to the control group. At the end of period 1 in series 2, HP in the experimental group was decreased with 4-5 % compared to the control group. In periods 2 and 3, HP changes followed EST changes linearly (Table 1).

Table 1. Laboratory experiments: linear (dHP = a*dT + b) regression parameters between dT (°C) and dHP (%) in series 1 and series 2.

	Period (d)	a	b	R^2
Series 1	d 6.5 – 9.5	4.5	6.4	0.61
	d 10.5 – 13.5	4.7	-1.1	0.98
	d 14.5 – 17.5	5.0	-1.3	0.98
Series 2	d 6.5 – 9.5	5.5	-3.8	0.72
	d 10.5 – 13.5	5.3	-0.1	0.95
	d 14.5 – 17.5	4.3	-0.2	0.94
Average		4.9	0.0	0.86

[3] Testo GmbH, Lenzkirch, Germany

Hatchery experiment

The standard MT setting profile gradually decreased with incubation time, and the MT of the experimental group was increased or decreased with 0.3°C at eight different periods (d 8, 9, and d11-16), see Figure 2. In Figure 2, also the CO_2 production (L/d) per embryo is shown. In Figure 3, average CO_2 changes (dCO_2) in the period of 1 hr from the MT change are shown. The responses can be divided into an initial, quick response followed by a secondary, more delayed response. When MT was decreased, CO_2 production initially increased with 0.5% and decreased thereafter. When MT was increased, CO_2 production initially decreased with 1.4%; and increased thereafter.

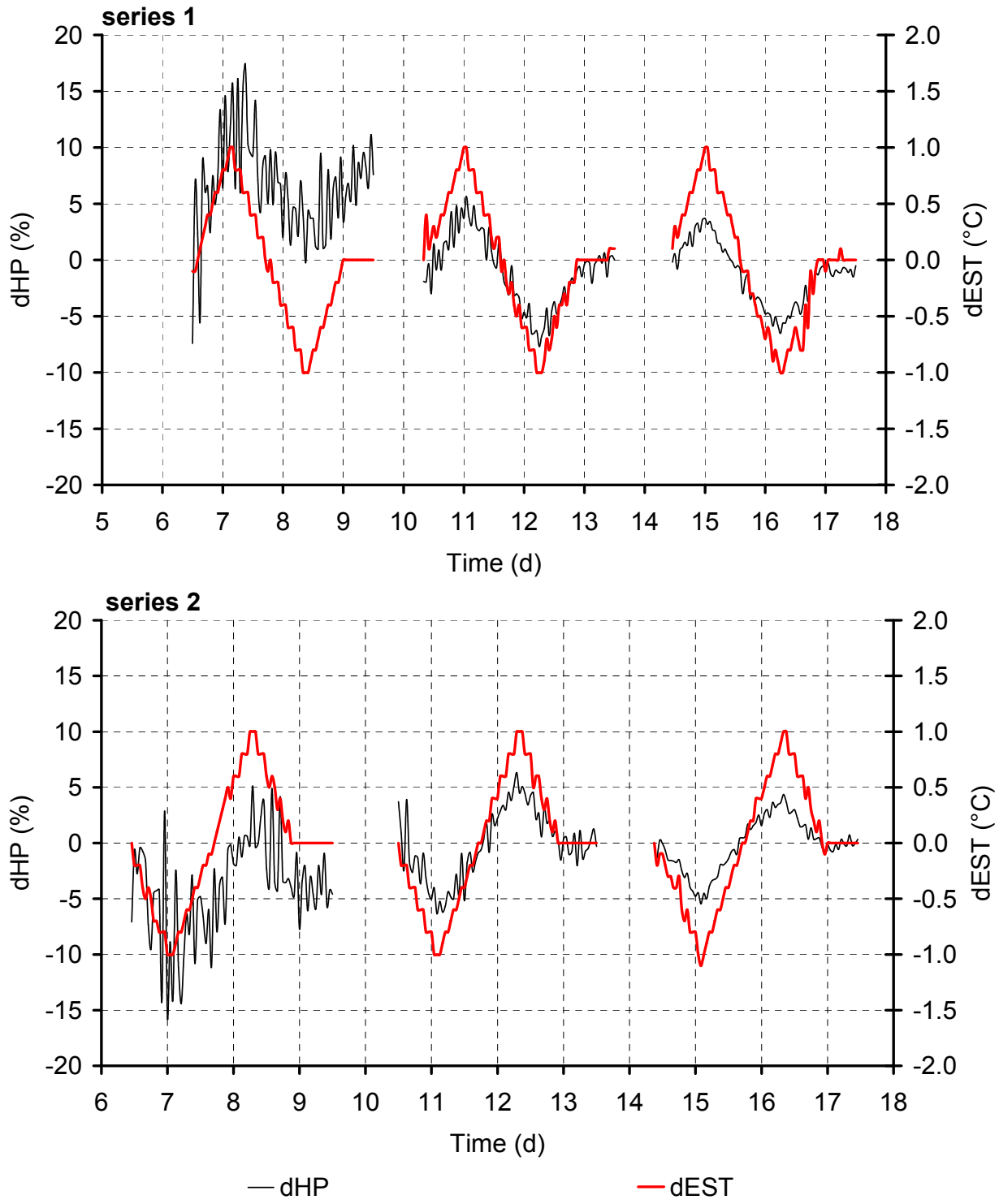

Figure 1. Relative effects of changes in eggshell temperature (dEST) away from 37.8°C on changes in heat production (dHP) in series 1 (top) and series 2 (bottom).

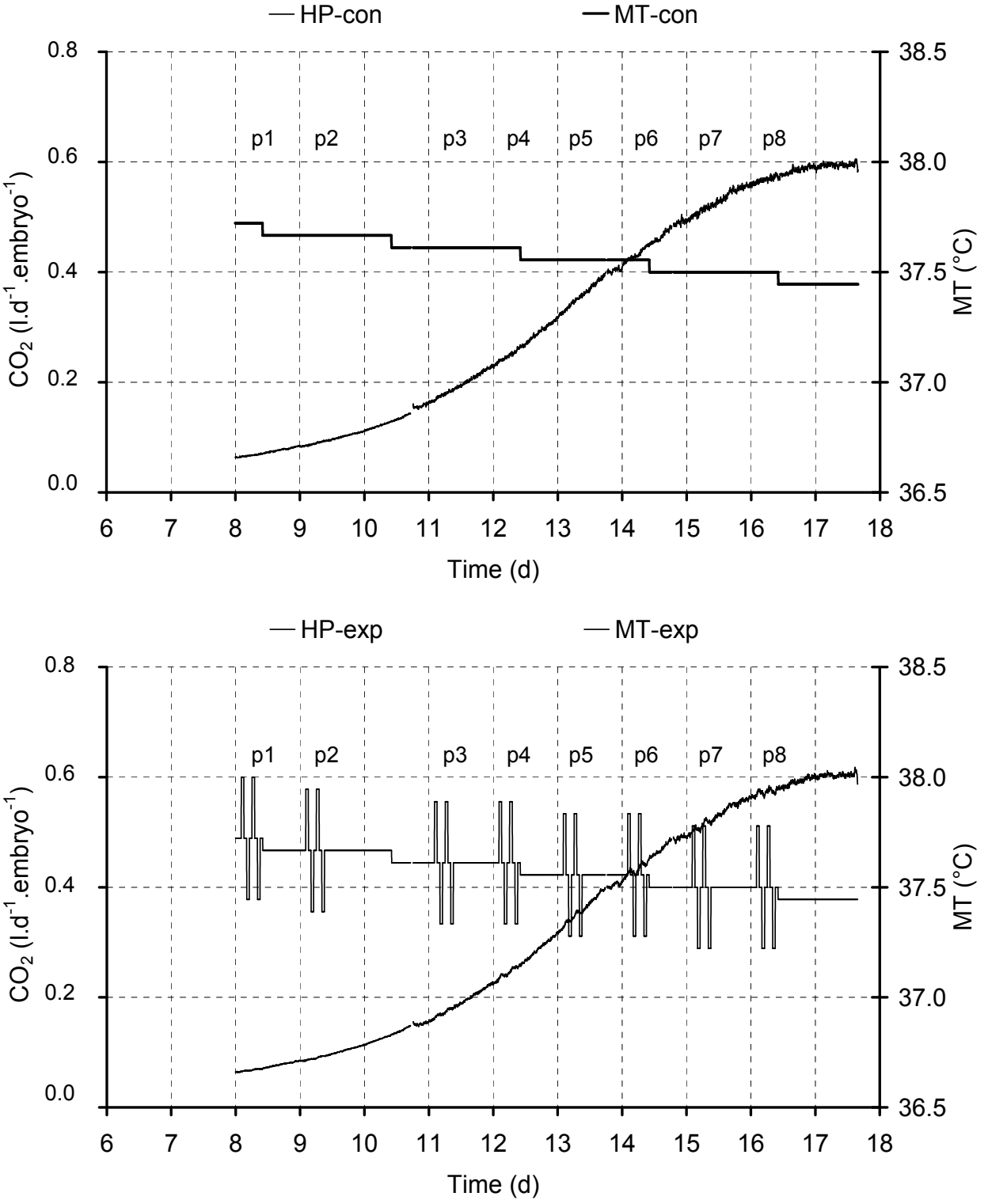

Figure 2. Hatchery experiment: machine temperature (MT) settings and CO_2 production (L/d/embryo) in the control group (top) and experimental group (bottom). In the control group, standard MT settings (MT-con) were applied. During 8 periods (p1-p8), MT-exp was increased or decreased 0.3°C above, at or below MT-con, using time steps of 1 hr.

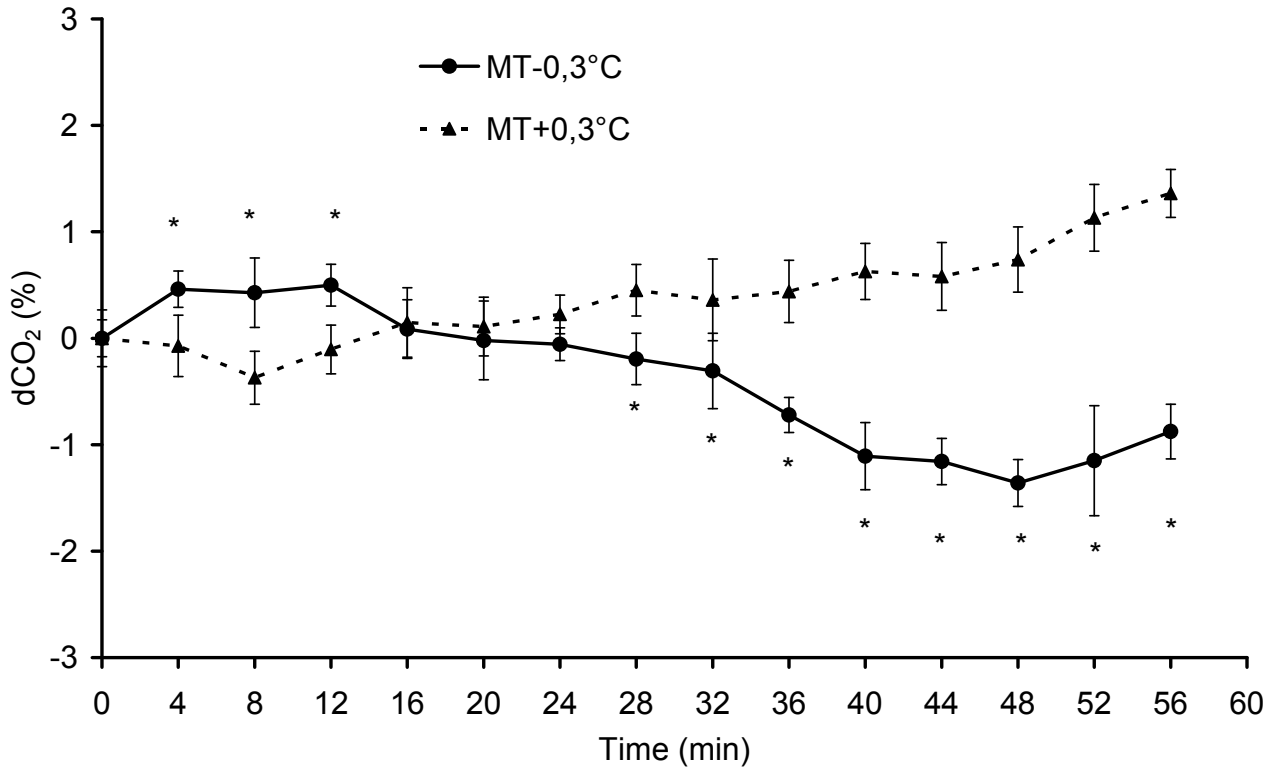

Figure 3. Hatchery experiment: effect of machine temperature (MT) changes on changes in CO_2 concentration (dCO_2) in the period of 1 hr after the MT change. Data points represent the average dCO_2 of 8 periods (d 8, 9, and d11-16) and error bars represent SEM (n=8). Significant differences ($P<0.05$) are indicated by *.

DISCUSSION

Heat production changes with temperature

Monitoring embryonic responses to external factors during incubation can provide important information about the current status and future direction of the incubation process (Bamelis et al., 2005). It was expected that under optimal conditions, embryonic growth is higher, hence the consumption of O_2 and yolk lipids and the production of waste products will be higher than under sub-optimal conditions (Meijerhof, 2002). In Hulet (2001) and Hulet and Meijerhof (2001), it was observed that HP can increase when MT was decreased. Lourens et al. (2005) observed decreased embryo development at low (36.7°C) and high (38.9°C) EST during respectively the first and last week of incubation compared to a constant EST of 37.8°C. When incubated at a constant EST of 37.8°C instead of at low EST during week 1 and high EST during week 3, the yolk free bodies of embryos in eggs from young and old parent stocks at 18 days of incubation weighed respectively 13.8% and 2.3% more and the embryos were respectively 0.5 cm and 0.4 cm longer. A maximum HP was expected to be found in a

EST zone of 1°C around 37.8°C. Thermal damage would occur when HP decrease at higher EST, coinciding with decreased embryo development and decreased hatchability. However, in the current experiments, in the EST range of 1°C below and above 37.8°C, HP was positively and linearly related with EST and no thermal damage due to short term EST variations with regard to HP was observed. Also in the hatchery experiment, when dCO_2 was evaluated 1 hr after a MT change, CO_2 production was increased when MT was increased and CO_2 production was linearly decreased when MT was decreased. However, during the first 16 minutes after a MT change, the reaction of the embryos was the reverse. An increased CO_2 production with decreased MT it is not likely a metabolic reaction of the embryo because it suggest homeothermy.

The incubator has to provide the basic environmental conditions as temperature for good embryo development for a large content, but the embryo is likely able to self regulate within a small temperature gradient. For example not by increasing or decreasing HP, but by increasing or decreasing the blood flow to the eggshell to increase or decrease the heat exchange (Nichelmann et al. 1997). Depending on the volume of blood that is transported through the chorio-allantoic membrane, more or less heat and CO_2 will be exchanged with the environment. This is not directly an homeotherm reaction as described in Nichelmann et al. (1997), but more an increased diffusion rate of CO_2 from the blood system to the environment outside the egg. This may explain the initial CO_2 response after a MT change. The success of Hulet (2001) and Hulet and Meijerhof (2001) may be explained by these initial, temporary CO_2 responses after a MT change because they also measured CO_2 response to MT changes after 20 min. It can be questioned however if these initial, temporary responses can be used for the purpose to control the incubation process. It can be speculated that this initial, temporary response will be observed only in a very restricted temperature zone. Down regulation of MT based on this initial response may save embryos from overheating, but the risk for cold injury may exist. However, when MT is adjusted based on delayed embryonic metabolic responses, EST may increase far above 37.8°C, leading to an increased percentage of overheated embryos.

EST, timing and exposure time

The combination of EST, timing, and exposure time determine the impact of EST treatments on embryo development (Yahav et al., 2004; Collin et al., 2005). For the development of an incubation control system, quick embryonic metabolic responses to mild temperature deviations are required. Timing of the temperature treatments seemed to play an important role with regard to the response of embryo's. From d 6.5 to 9.5, HP changed more

by EST treatment than later in incubation. Furthermore, in the first period, EST had a lasting effect on HP. These changes in (calculated) HP appeared to have different causes. In the first series where EST was increased first at d 6.5, CO_2 production at the end of period 1 was increased while O_2 consumption remained the same as in the control group. It can be hypothesized that increased EST stimulated the development of chorio-allantoic membranes, thereby increasing the heat transfer and O_2- and CO_2 diffusion rate. This will have a lasting effect, when future embryo development would be increased by a better developed cardio vascular support system. Research into that direction is unknown to the authors. In the second series, where EST was decreased first at d 6.5, the permanent decrease in HP at the end of period 1 may result from retarded embryo development, since both O_2 consumption and CO_2 production were decreased. Retarded embryo development at this stage decreased HP at later stages, which agrees to findings by Lourens et al. (2005) who observed lasting effects of low EST during the first week of incubation on embryo development, hatchability and post hatch thermoregulation.

How to control the incubation process in the future?

In the present laboratory and hatchery experiment, after a series of temperature increments or decrements, temperature was always reset to the standard temperature. The objective in both experiments was to study embryonic metabolic responses to short term temperature fluctuations, in order to evaluate the possibilities to control the incubation process better.

Based on the embryonic metabolic responses to short term temperature variations, it is not likely that optimal embryo development and highest hatchability will be reached when HP is maximized, because embryos will get overheated. The first, initial response may only be observed within a limited temperature range where the embryo has the capacity to increase or decrease the heat exchange with blood flow changes. As long as the embryo can react to MT changes, it may be saved from thermal damage. It can be questioned if this response can be used to support a control system for commercial incubation.

The second, delayed response with regard to CO_2 concentration was more in accordance to linear HP changes with EST, at least in the studied EST zone in the first experiment. It can be expected however that HP may decrease at higher and lower EST due to cold or heat damage. When overheated embryos are cooled down and saved from thermal damage, HP may increase due to temperature decrements. The temperature where thermal damage occurs with regard to HP is unknown, and needs further investigations. It is also unknown how embryo development, hatchability and chick quality is affected after thermal damage.

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Chapter 5

EFFECTS OF EGGSHELL TEMPERATURE AND OXYGEN CONCENTRATION ON EMBRYO GROWTH AND METABOLISM DURING INCUBATION

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ABSTRACT

Embryo development and heat production (HP) were studied in eggs of similar size (60-65g), that were incubated at normal (37.8°C) or high (38.9°C) eggshell temperature (EST) and exposed to low (17%), normal (21%) or high (25%) oxygen concentrations (O_2) from d 9 through d 19. High EST initially increased HP, but gradually O_2 became more important for HP than EST. Finally, HP was highest for the combination of high EST with high O_2 and lowest for the combination of high EST with low O_2. High EST decreased hatch time (HT), body weight (BW), yolk free body weight (YFB) and relative heart weight (HW). EST had no effect on residual yolk weight (RY), chick length (CL) and relative liver weight (LW). Increased O_2 increased YFB and CL and decreased RY at hatch. No interactions between EST and O_2 were observed with regard to embryo development and hatchling characteristics. If embryo development is reflected by HP, it can be concluded that high EST primarily increased embryonic development until the second week of incubation. During the third week of incubation, O_2 had a greater effect in determining embryo development than EST.

(*Key words*: eggshell temperature, oxygen concentration, embryo development, heat production.)

INTRODUCTION

Temperature determines the metabolic rate of yolk and albumen utilization and hence embryo development during incubation (Romanoff, 1972; Deeming and Ferguson, 1991). Temperature may have a divergent effect during the course of incubation, because a constant higher temperature initially accelerates embryonic growth and utilization of nutrients and energy from yolk and albumen, but decreases embryonic growth later in development (Romanoff, 1972). It was suggested by Rahn et al. (1974) that later in incubation, metabolic processes are limited by the insufficient exchange of oxygen, which would explain the plateau phase for gas exchange and heat production (HP). In the plateau phase, the maximum rate of oxygen diffusion into the egg is determined by the oxygen conductance and the partial pressure gradient of the gas between the ambient air and the inner side of the shell (Romijn and Roos, 1938; Romijn, 1950; Visschedijk, 1968; Visschedijk et al., 1985). Oxygen conductance is specific for breed (O'Dea, 2004; Janke et al, 2004), age or egg size (Visschedijk, 1980). However, the partial pressure gradient for oxygen during incubation depends on both metabolic rate and oxygen concentration (O2) of the ambient air.

Since oxygen consumption and HP increase rapidly from d 9-10 of incubation onwards (Lourens et al., 2006a), the demand for oxygen increases accordingly and gas exchange reaches the plateau phase some days later. During this plateau phase, large eggs incubated at the same eggshell temperature (EST) of 37.8ºC produce more heat than small eggs (Lourens et al., 2006a). EST would have increased more in large eggs than in small eggs when incubated at the same machine temperature (MT). Long term high EST during the plateau phase is detrimental for embryo development (Lourens et al., 2005). However, even during the plateau phase, HP can be further increased by moderate, short term increments of EST when eggs are incubated at normal O_2 (Lourens et al., 2006b). It is unknown how HP and embryo development are affected by long term high EST in eggs exposed to different O_2. Therefore, an experiment was conducted where eggs of one breed and of similar size were incubated at normal (37.8°C) or high (38.9°C) EST, and exposed to low (17%), normal (21%), or high (25%) O_2 from day 9 through day 19. It was hypothesized that high EST is not detrimental for embryonic development and metabolic rate, when oxygen is sufficiently available.

MATERIALS AND METHODS

Experimental design

In a 2x3 experimental design with eggshell temperature (EST) and oxygen concentration (O_2) as factors, heat production (HP), hatch time (HT) and chick development parameters were recorded. Each combination of ESTx O_2 was repeated twice.

Hatching eggs and incubation

In six trials, a total of 2,040 graded hatching eggs from Hybro G+ grand parent flocks were used. Eggs weighing between 60.0 and 65.0 g were selected at the Torsius Breeder Hatchery in Putten, The Netherlands and were stored between 3 and 7 days at 16 – 18ºC. Eggs were transported to the incubation and hatching facilities of Wageningen University, The Netherlands and incubated in a HT-combi incubator with a maximum setting capacity of 4,800 eggs (Hatchtech B.V., Veenendaal, The Netherlands). Each trial had 240 eggs equally divided across 4 incubator trays and turned every hr. On d 8, eggs were candled, clear eggs were removed and between 160 and 200 eggs containing living embryos were transferred and equally divided across two identical small open circuit CRC's (Lourens et al., 2006a,b). In the CRC's, eggs were turned every half hour. At d 8 and at d 19, individual eggs were weighed and weight loss (WL) was determined to explain possible variation in YFB and RY at hatch due to temperature treatment affecting water vapour conductance and to identify possible outliers due to for example eggshell cracks. Oxygen and carbon dioxide concentrations were measured every 9 minutes in both chambers and in fresh air. Carbon dioxide concentration was measured with a non dispersive infrared CO_2 analyser (type Uras 3G, Hartmann&Braun, Frankfurt, Germany). O_2 was measured with a paramagnetic oxygen analyser (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). The exact air volumes were measured with a Schlumberger G1.6 dry gas meter. HP was calculated from oxygen consumption and carbon dioxide production according to Romijn and Lokhorst (1961), and adjusted for fertility and embryo mortality, based on the description of Lourens et al. (2006a).

Eggshell temperature and oxygen concentration

Thermistors were attached to the eggshells of 5 individual, fertile eggs at the start of each trial in each CRC as described by Lourens et al. (2005). EST was measured every 30 s and machine temperature (MT) was automatically adjusted every 5 min when the median EST drifted away from the EST set point. The MT was adjusted using the median EST of 5 eggs per CRC to avoid the low EST of eggs containing dead embryos from affecting the decision for

the direction of the next MT step. EST in both CRC's remained constant at either 37.8ºC (normal) or 38.9ºC (high) in each trial. O_2 of the air entering the CRC's was adjusted by using a mixture of air with pure nitrogen (17% O_2) or of air with pure oxygen (25% O_2). Using a constant ventilation air flow, O_2 of the air entering the CRC's was set at about 17% (low = 17.2% ± 0.19 SD), about 21% (normal = 21.0% ± 0.03 SD) or about 25% (high = 25.1% ± 0.21 SD). Because ventilation of each CRC was not changed during the 11 day measurements, consequently O_2 of the outgoing air decreased from d 8 to d 19 in the low O_2 treatment from 17.1% to 16.5%; in the normal O_2 treatment from 20.9% to 20.2% and in the high O_2 treatment from 24.8% to 24.2%. Air pressure inside the chambers exceeded barometric pressure by +100 Pa.

Embryo mortality, hatch time and hatchling measurements

At d 8 and 19 of incubation and at the day of hatch, clear eggs and dead in shell were opened to determine true fertility and the timing of embryonic mortality by visual appraisal as described by Lourens et al. (2006a). HP calculations were corrected for the number of embryos that were alive at any day of incubation. At 19 d of incubation, per EST x O_2 treatment and per repetition, 60 randomly chosen eggs containing living embryos were transferred to identical hatching boxes. All hatching boxes were placed in one large CRC that allowed entrance of personnel. From 19 d onwards, only EST was set as factor at either 37.8ºC or 38.9ºC, and O_2 was no longer controlled. EST was measured from 8 individual eggs and MT was adjusted as described above. At 19.5 d of incubation, the thermistors were removed, MT remained constant and so EST was allowed to increase. Individual hatch time (HT) per chick was recorded using a video camera and recorder. At 21.5 d of incubation, all hatched chicks were sacrificed with a mixture of CO_2 and O_2. All chicks were weighed and individual chick length (CL) was measured by stretching the chick along a ruler and taking the length between the top of the beak and the tip of the middle toe of the right feet (Hill, 2001). Next, from all chicks RY was removed and weighed and from 15 at random chosen chicks per EST x O_2 repetition heart weights (HW) and liver weights (LW) were removed and weighed.

Statistical analyses

The non linear, sigmoid curves for HP and MT were analysed in Genstat 6.1 (2002) with a REML-procedure according to the following model for repeated measurements: $Y_{ijk} = \mu + EST_i + O_{2j} + D_k +$ interactions $+ \varepsilon_{ijk}$, where Y_{ijk} is HP or MT, μ is the overall mean, EST_i is eggshell temperature (i = normal, high), O_{2j} = oxygen concentration (j = low, normal, high), D_k = day

number (k = 9-19) and ε_{ijk} is the residual error term. CRC was used as experimental factor. HP and MT were calculated per day of incubation, so day of incubation was the repeated factor. Chick characteristics were analysed with the general linear model procedure of Genstat software (Genstat 6.1, 2002), where the average of one group of eggs in one CRC was used as experimental unit. The model was: $Y_{ij} = \mu + EST_i + O2_j + (EST \times O_2)_{ij} + \varepsilon_{ij}$, where Y_{ij} is the dependent variable, μ is the overall mean, EST_i is eggshell temperature (i = normal, high), O_{2j} is oxygen concentration (j = low, normal, high), and ε_{ij} is the residual error term.

RESULTS

Embryonic development

No interactions between EST and O_2 were observed with regard to embryo development and hatchling characteristics (Table 1). High EST compared to normal EST reduced HT, and decreased BW, YFB and relative HW. EST had no significant effect on CL, RY or relative LW. High O_2 decreased RY and increased YFB and CL at hatch. O_2 had no effect on HT, relative HW and relative LW.

Heat production and machine temperature

The effects of EST and O_2 on HP are summarized in Figure 1. From d 9 through d 15, high EST significantly increased HP compared to normal EST. From d 14 – 17, in both EST treatments, HP was lowest in the low O_2 treatment compared to the normal or high O_2 treatments. From d 16 onwards, HP in the normal O2 treatment was still significantly higher compared to the low O_2 treatment, but also significantly lower compared to the high O_2 treatment. At d 18 and d 19 of incubation, an interaction between EST and O_2 occurred. At low and normal O_2, HP was lower in the high EST treatment. At high O_2, HP remained high in the high EST treatment.

A consequence of differences in HP between treatments was that MT needed to be adapted to maintain EST constant at either 37.8ºC or 38.9ºC. At d 18 of incubation, eggs incubated at 37.8ºC EST and at O_2 of 17%, 21% or 25% required a MT of respectively 37.2, 37.0 and 36.7ºC (differences of 0.6, 0.8 and 1.1ºC). Likewise, eggs incubated at 38.9ºC EST and at O_2 of 17%, 21% or 25% required a MT of respectively 38.0, 37.9 and 37.9ºC (differences of 0.9, 1.0 and 1.0ºC).

Table 1. Characteristics of hatchlings incubated at two different EST profiles (37.8°C or 38.9°C) and three different oxygen concentrations (O_2 of 17%, 21% and 25%) from day 9 through day 19. CL = chick length; BW = body weight; YFB = yolk free body weight; HW = heart weight; LW = liver weight and HT = hatch time.

	CL (cm)	**BW (g)**	**YFB (g)**	**RY (g)**	**HW (%YFB)**	**LW (%YFB)**	**HT (d)**
EST							
37.8°C	19.7	41.5[a]	37.4[a]	4.0	1.1[a]	4.4	20.5[a]
38.9°C	19.8	39.8[b]	35.8[b]	4.0	0.9[b]	4.3	19.9[b]
O_2							
17%	19.0[c]	40.6	35.3[c]	5.3[a]	1.0	4.2	20.2
21%	19.9[b]	40.6	36.9[b]	3.7[b]	1.0	4.4	20.2
25%	20.4[a]	40.7	37.7[a]	3.0[c]	1.0	4.4	20.2
EST x O_2							
37.8°C x 17%	19.0	41.5	36.3	5.2	1.1	4.3	20.4
37.8°C x 21%	19.8	41.2	37.6	3.6	1.0	4.5	20.5
37.8°C x 25%	20.1	41.7	38.5	3.2	1.1	4.5	20.5
38.9°C x 17%	18.9	39.7	34.3	5.4	1.0	4.2	19.9
38.9°C x 21%	20.0	39.9	36.2	3.8	0.9	4.3	20.0
38.9°C x 25%	20.6	39.7	36.9	2.9	0.9	4.3	19.9
Overall SEM	0.08	0.20	0.15	0.06	0.01	0.05	0.04
Source of variation							
EST	n.s.	**	**	n.s.	**	n.s.	***
O2	***	n.s.	**	***	n.s.	n.s.	n.s.
EST x O2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant; * = P<0.05; ** = P<0.01 and *** = P<0.001; [a,b,c] refer to significant differences (P<0.05) between treatments

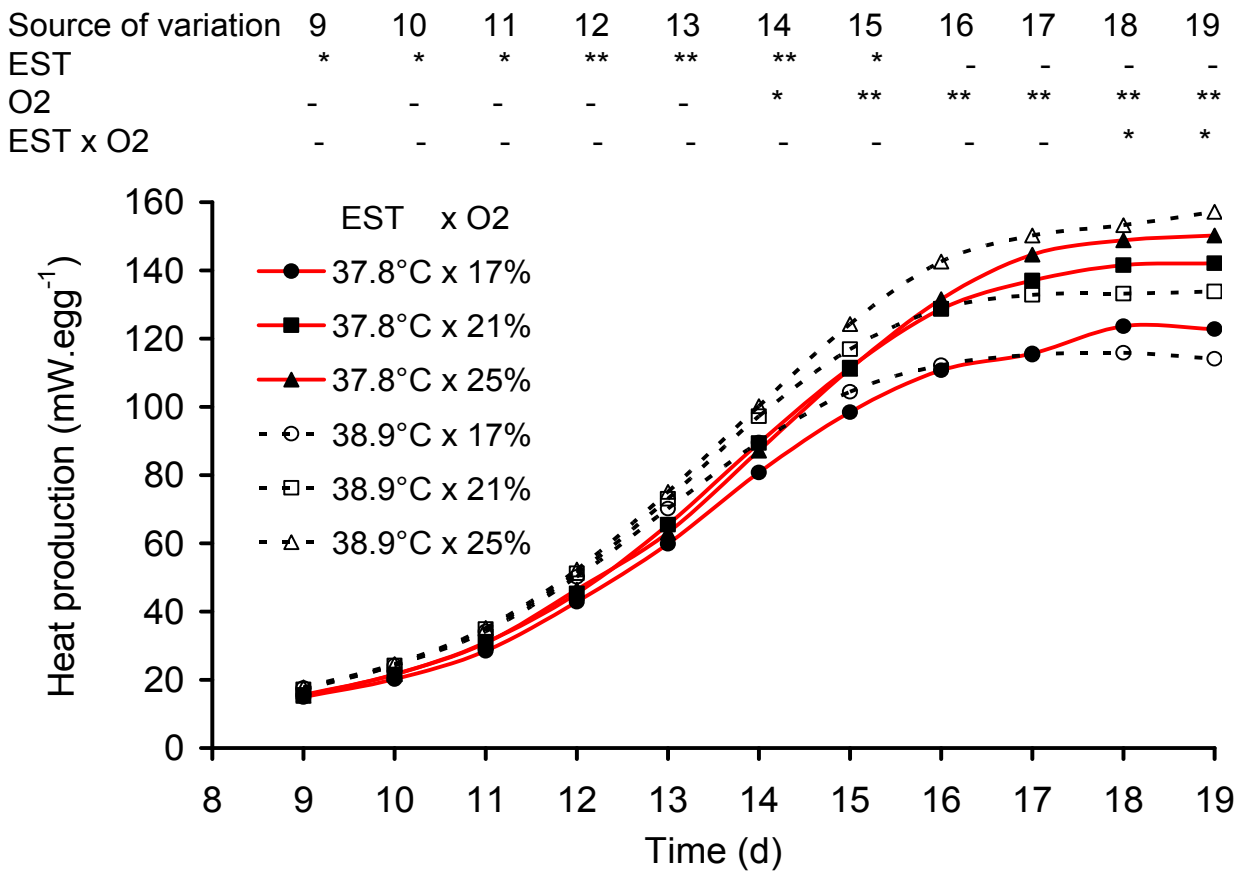

Figure 1. Heat production (mW.egg^{-1}) in eggs (60-65 g) incubated at two EST settings (37.8°C and 38.9°C) and three O_2 settings (17%, 21% and 25%) between d 9 through d 19, where * = P<0.05 and ** = P<0.01.

DISCUSSION

Embryo development and hatch time

It was hypothesized that high EST is not detrimental for embryonic development and metabolic rate when O2 is sufficiently available, but no interactions were observed for EST x O_2 concerning embryo development. In the present study it was clearly shown that high EST decreased embryo development in terms of BW, YFB and HW of hatchlings that were taken out of the hatching baskets at 21.5 d of incubation. EST however had no effect on RY, comparable to findings of Lourens et al. (2005). Differences in RY between breeds may be more related to genetics and eggshell conductance, as Christensen et al. (1999) showed that growth selected embryos did not respond to increased O_2 by increasing fat metabolism whereas embryos from their randombred controls did.

High EST decreased HT, and regression analysis showed that within each EST group, HT had no effect on YFB (P>0.05). Within each EST group, late hatching chicks had larger RY,

and as a consequence, also BW increased with HT. Also Hager and Beane (1983), Reis et al. (1997), Suarez et al. (1997) and Joseph and Moran (2005) concluded that heavier BW and RY at hatch were associated with late emergence. Nutrient utilization from the RY in the hatcher period is poorly understood. Newly hatched chicks may absorb the nutrients left in the RY for growth and maintenance of the YFB (Noy et al., 1996; Noy and Sklan, 1998; 2002). At the same time, dehydration occurs with extended holding time in the hatcher baskets (Hager and Beane, 1983; Reis et al., 1997). HT decreased as flock age increased (Smith and Bohren, 1975; Shanawany, 1984; Yannakopoulos, 1988; Christensen et al., 2001; Joseph and Moran, 2005). This may be related to higher EST due to the effect of increased HP with increased egg weight (Lourens et al., 2006a). However, when egg weight is held constant, HT will still decrease with increasing flock age (Hager and Beane, 1983; Fanguy et al. 1980; Burke, 1992, Christensen, et al., 2000). Older hens produce thin-shelled eggs (Roland, 1976; Britton, 1977; Peebles and Brake, 1987). Thin eggshells not only increase the rate of water loss (Tullett and Board, 1977), but also the eggshell oxygen conductance (Christensen et al., 1996) compared with thick eggshells. Oxygen conductance was observed to be inversely related to the incubation period (Rahn et al., 1974). Contrary to previous research, oxygen conductance was artificially increased and EST and not O_2 determined HT. Different from all other experiments is that in the recent experiment EST was maintained constant either at either 37.8ºC or at 38.9ºC, and EST was not allowed to increase with increased O_2. So the decrease in HT by increased oxygen conductance (Rahn et al., 1974) may be explained by increased more by EST than by oxygen conductance.

The partial pressure of oxygen can decrease by decreasing concentration, or by increasing altitude and hence by decreasing barometric air pressure. Because incubating embryos respire totally by diffusion, when barometric air pressures decrease, the diffusion rate and respiration decrease. Reduced diffusion rates at the same barometric pressure have been shown to affect organ maturation and body weight (Bagley and Christensen, 1989). In the present study, air pressure in the CRC exceeded outside barometric air pressure by +100 pa to avoid leakage, which relates to height difference of 9-10 m. Barometric pressure was the same in all treatments and so barometric pressure did not likely affect results between treatments.

At sea level, oxygen makes up 20.9% of the atmosphere and the equivalent percentage of oxygen drops approximately 1% for every 500 m rise in altitude (Julian, 2000). Lokhorst and Romijn (1965) observed decreased hatchability when eggs were incubated at low air pressures. Heat production was highest (151 $mW.egg^{-1}$) at sea level. Above a simulated altitude of 3.000 m (<15% O2), HP decreased to below 80 mW.egg-1 and embryos did not

survive. At high altitude of 2.000 m, embryos hatched earlier than those incubated at sea level (Hassanzadeh et al., 2004). The difference in partial pressures of 4% in the study by Hassenzadeh et al. (2004) was the same as in the present study, where it was concluded that HT was not affected by O_2 at all. In the study by Hassenzadeh et al. (2004), eggs were incubated at a constant MT of 37.8ºC at both altitudes. Since it was observed that eggs incubated at high altitude experience more difficulties to exchange heat (Meijerhof, 2002), these eggs are likely to be incubated at higher EST, which may have decreased HT as a result. Next to EST, incubation weight loss was also not measured in the study by Hassenzadeh et al. (2004), which could have explained the lower BW of chicks hatched at high altitude. Increased weight loss may also have reduced YFB of chicks incubated at high EST in the present study, because high EST increases the partial water vapour pressure between egg and air (Meijerhof and Van Beek, 1993). Weight loss (WL) between setting and transfer at 19 days of eggs that hatched was 11.5% in eggs incubated at 37.8ºC EST and 12.4% in eggs incubated at 38.9%. Regression analysis showed that BW and YFB decreased with WL at a significant higher rate in chicks that hatched after exposure to high EST compared to normal EST ($P<0.05$), and that RY was not affected at all by WL or EST. There were no significant correlations between WL and HT for BW, YFB or RY ($P>0.05$).

Heat production

Until d 15, eggs incubated at high EST produced more heat than eggs incubated at normal EST. Independently from EST, O2 determined HP at d 16 - 17. Between d 18 - 19 of incubation, HP was decreased by a combination of high EST with low or normal O_2. Golde et al. (1998) showed that already in the middle of the incubation period oxygen availability can be a limiting factor for growth, well before metabolism exceeds the oxygen diffusion capacity of the eggshell. Similar as in the present study, also McCutcheon et al. (1982); Stock et al. (1983); Stock and Metcalfe (1984) and Asson-Batres et al. (1989) observed increased embryonic development at increased oxygen availability only late in incubation. Rahn (1981) suggested that for normal embryo development, the total amount of oxygen consumed per gram of fresh egg weight averages about 100 ml. In the present study, O_2 and not EST affected total oxygen consumption per gram of fresh egg weight. Embryos incubated at O_2 of 17% consumed less than 100 $ml.g^{-1}$; eggs incubated at O_2 of 21 or 25 % consumed more than 100 $ml.g^{-1}$. High EST initially increased oxygen consumption, and gradually O_2 became more important. Therefore we believe that the level of oxygen consumption during the plateau phase is of more importance for embryonic growth, development and maturation than the total amount of oxygen consumed. Accordingly, embryo development in eggs incubated at high O_2

could not be increased by increased EST. It can be speculated that the total oxygen consumption in eggs incubated at low O_2 can be increased by decreasing EST, which would slow down development and decrease HP, but also increase HT which would allow the embryos more time to develop and mature.

Normal O_2 limited HP even in eggs incubated at normal EST, which was confirmed by the study of Visschedijk (1980), who showed that at increased O_2 above normal O_2, a small but significant increase in carbon dioxide production was found. Lokhorst and Romijn (1965) incubated eggs at 38.0ºC MT and at different O_2. Highest HP (143 $mW.egg^{-1}$) was found for eggs incubated at the highest O_2 of 20.9%. At 17% O_2, HP dropped to 107 $mW.egg^{-1}$, comparable to results of the present experiment. When eggs were incubated at low O_2 after setting, O_2 reached lethal levels below 15%. Bjønnes et al. (1987) exposed embryos between 18 – 21 d of incubation to reduced (6-7%) O_2. The effect of hypoxia on oxygen consumption was most pronounced in the 18-19d old embryos and less in older embryos after internal and external pipping when relatively more uptake occurs via the lungs and not through the chorio-allantois membrane by diffusion (Rahn et al., 1981).

From this study it was concluded that high EST initially increased HP, but gradually O_2 became more important for HP than EST. Finally, HP was highest for the combination of high EST with high O_2 and lowest for the combination of high EST with low O_2. If embryo development is reflected by the course of HP, it can be concluded that high EST increased embryonic development only until the second week of incubation. During the third week of incubation it was O_2 that determined embryo development more than EST. The hypothesis that high EST is not detrimental for embryonic development and metabolic rate when O_2 is sufficiently available was rejected.

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Chapter 6

ENERGY PARTITIONING DURING INCUBATION AND CONSEQUENCES FOR EMBRYO TEMPERATURE: A THEORETICAL APPROACH

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Submitted

ABSTRACT

In practice, many hatchability and chick quality problems in incubation are related to the control of embryonic temperature (ET). It is shown that within an incubator at a constant machine temperature (MT), ET at day 18 of incubation can largely vary. ET is the result of the balance between heat transfer to and from the embryo and the heat production (HP) of the embryo. Aim of this paper is to investigate which factors theoretically can account for the variation in ET within an incubator. First, effects of egg characteristics (egg size, breed) and incubation characteristics (MT and oxygen availability) on HP of embryos are quantified. Differences in HP can only be due to differences in the amount of energy utilized from the egg or to differences in the efficiency of this energy utilization (E_{YFB}). Results of these analyses show that differences in HP due to egg size, breed, or oxygen availability are mainly a result of the amount of energy used from the egg constituents and not of a change in E_{YFB}. However, at a given MT, this variation in HP could only count for a maximum increase in ET of 1.2ºC, suggesting that other factors also play a role. The most important factor is probably a difference in air velocity within an incubator, resulting in differences in heat transfer. Due to this variation, ET varies within an incubator and with rising ET, efficiency of energy utilization decreases, resulting in an even higher HP and consequently ET. We conclude that results obtained from this theoretical approach suggest that hatchability and chick quality problems can be due to variation in ET. This indicates that in incubation experiments it is of great importance to realize that any factor affecting HP or heat transfer influences ET. Changes in ET may have a greater effect on experimental results than the factor investigated itself, and therefore we strongly suggest to control ET (or indirectly as eggshell temperature) in any incubation experiment.

(*Key words*: embryo temperature, heat production, heat loss, energy utilization, efficiency of energy transfer)

INTRODUCTION

In practice, many hatchability and chick quality problems in incubation are related to the control of embryonic temperature (ET) (French, 1997). In studies by Lourens et al. (2005) and Joseph et al. (2006), eggshell temperature (EST) measurements were used as non-destructive parameter for ET and it was shown that even small deviations away from a constant EST of 37.8ºC can have a serious impact on embryonic development and hatch results. Earlier research (Lourens, 2001) already demonstrated that within an incubator EST largely varies. ET (or EST) is the result of the balance between 1) the transfer of heat to and from the embryo and 2) the heat production (HP) of the embryo. When either heat transfer or HP changes, ET changes from a given state to a new value (Meijerhof, 2002). Heat transfer is mainly determined by the temperature difference between egg and environment and air velocity across the egg, and to a lesser degree by air humidity (Kashkin, 1961, Meijerhof and Van Beek, 1993; Van Brecht et al., 2005). HP is determined by 1) the total amount of energy utilized from the egg and 2) the efficiency this energy utilization (Ar et al., 1987; Pearson et al., 1991).

Aim of this paper is to investigate which factors theoretically can account for the variation in ET within an incubator. To analyze that, effects of both egg (size, breed) and incubation (machine temperature (MT), oxygen availability) factors on maximum HP were taken into account. Additionally, the effect of maximum HP on ET at variable levels of heat transfer will be calculated using a theoretical model. Finally, the theoretical maximum levels of ET will be compared to EST found in practice and potential reasons for different values will be investigated.

FACTORS AFFECTING EMBRYONIC HEAT PRODUCTION

During incubation, the embryo builds up its body from the egg nutrients using the energy obtained mainly from oxidation of yolk fats (Romanoff, 1967; Noble and Cocchi, 1990), and to a lesser extent from oxidation of carbohydrates and proteins (Fiske and Boyden, 1926). Not all egg nutrients are utilized, but a certain amount of energy remains unused in the residual yolk. Furthermore, at hatch some of the energy is lost as extra embryonic membranes, meconium (Ar et al., 1987) and urates (Fiske and Bowden, 1926), but this amount is relatively limited (Romanoff, 1967) and therefore this will be neglected in this paper. Finally, part of the energy is lost as heat (HP) as a by-product of embryonic growth, maintenance and development (Ar et al., 1987; Pearson et al., 1991). HP is determined by 1) the amount of energy utilized from the

egg and 2) the efficiency of this energy utilization. The efficiency of energy utilization is indicated as E_{YFB}, and can be calculated according to Kleiber (1961) as:

$$E_{YFB} = \frac{YFB(kJ)}{Albumen(kJ) + Yolk(kJ) - RY(kJ)} x100\% \quad [1]$$

Variation in HP can be due to egg and incubation factors. In this paper, egg size and breed as egg factors and incubation temperature and oxygen availability as incubation factors will be discussed in their effect on HP.

Egg size

Hoyt (1987) and Vleck et al. (1980) demonstrated an increase in HP between 1.5 and 2.0 $mW.egg^{-1}$ per extra g egg weight. Large eggs not only produce more HP than small eggs, they also face more difficulties to transfer heat away from the egg, due to their smaller surface to volume ratio and larger layer of non-moving air surrounding the egg (Vogel, 1984; French, 1997). Consequently, at a constant MT and heat transfer rate, large eggs experience a higher ET than small eggs. So differences in HP due to differences in egg size may be confounded by differences in ET. Based on this suggestion, it seems reasonable to maintain ET or EST at a given level to compare effects of egg size on HP. When incubated at a constant EST of 37.8^{o}C, HP at d 18 of incubation in small (55.7 g) and large eggs (70.4 g) was 137 and 155 $mW.egg^{-1}$, respectively (Lourens et al., 2006; Table 1). Extrapolation showed that each extra g of egg increased HP at d 18 of incubation by 1.2 $mW.egg^{-1}$. This lower value supports the suggestion that the effect of egg size on HP was confounded with differences in ET in the studies of Hoyt (1987) and Vleck et al. (1980). Because HP is a result of energy utilization

from the egg and the E_{YFB}, Table 1 shows the energy partitioning between egg, hatchling and HP as found by Lourens et al. (2006). Embryos in large eggs utilized 58 kJ more energy than embryos in small eggs, and yolk free body (YFB) of hatchlings from large eggs contained 23 kJ more energy at hatch than from small eggs. E_{YFB} was comparable for both egg size classes, meaning that differences in HP due to differences in egg weight, are only due to the amount of energy utilized and not due to differences in efficiency of this energy utilization.

Table 1. Energy partitioning between egg, hatchling and heat production (HP) at d 18 of incubation in small (55.7 g) and large eggs (70.4 g). After Lourens et al. (2006).

	Small eggs	Large eggs
Albumen (kJ)	69[b]	90[a]
Yolk (kJ)	259[b]	320[a]
YFB (kJ)[1]	155[b]	178[a]
RY (kJ)[1]	27[b]	51[a]
Utilized (kJ)	301[b]	359[a]
E_{YFB} (%)[1]	51.6	49.6
HP at d 18 ($mW.egg^{-1}$)	137[b]	155[a]

[a, b] Values within a row lacking a common superscript differ ($P<0.05$); [1] YFB = yolk free body; RY = residual yolk; E_{YFB} = efficiency of energy utilization.

Breed

Broilers and layers are selected for different performance purposes, which may affect egg utilization and HP during incubation (Janke et al., 2004; Sato et al., 2006). Wolanski et al. (2006) showed a variation in RY among breeds from 0.8 and 10.6 g, as a reflection of differences in energy utilization. In the study of Sato et al. (2006) egg utilization was determined in broiler and layer hatching eggs, weighing 67.0 and 62.9 g, respectively. Yolks weighed 19.9 and 16.7 g in broiler and layer hatching eggs, respectively. We estimated albumen weights, using the relative shell weight of 9.0 and 9.3% of broiler hatching eggs and layer table eggs, respectively (Harms and Hussein, 1993). BW and RY weight at hatching was 43.4 and 8.2 g for broilers and 40.3 and 6.7 g in layers, respectively (M. Furuse, personal communication), resulting in a YFB at hatch of 35.2 and 33.6 g for broilers and layers, respectively. Based on these values, energy partitioning can be estimated. Because egg size affects energy partitioning (see 2.1.), results were corrected for egg weight and standardized at 65 g, assuming a constant yolk/albumen and YFB/RY ratio (Table 2).

Table 2. Energy partitioning between egg, hatchling and heat production (HP) at d 18 of incubation in broiler and layer hatching eggs. Based on Sato et al. (2006) and standardized for egg weight at 65.0 g.

	Broiler hatching eggs	Layer hatching eggs
Albumen (kJ)	84	88
Yolk (kJ)	288	257
YFB (kJ)[1]	151	154
RY (kJ)[1]	91	79
Utilized (kJ)	281	266
E_{YFB} (%)[1]	53.7	57.9
HP at d 18 ($mW.egg^{-1}$)	125	127

[1] YFB = yolk free body; RY = residual yolk; E_{YFB} = efficiency of energy utilization.

Using a standardized egg weight of 65 g, HP at d 18 was estimated at 125 and 127 $mW.egg^{-1}$, in broiler and layer embryos, respectively. Broiler embryos utilized 15 kJ (5.6%) more than layer embryos during incubation, whereas E_{YFB} was 4.2% lower in broiler embryos compared to layer embryos. Higher energy utilization together with a lower efficiency can be expected to result in higher HP. However, this was not expressed in the estimated HP (Table 2). This mismatching can possibly be due to our assumption for similar yolk/albumen and YFB/RY ratios for both the broiler and layer eggs, but possibly incubation conditions also play a role. In the study of Sato et al. (2006), both broiler and layer hatching eggs were incubated simultaneously in the same incubator at a MT of 37.6ºC. Janke et al. (2004) demonstrated at a MT of 37.8^{o}C a higher HP and an 0.3 – 0.5^{o}C higher ET during the plateau phase in broiler hatching eggs (Ross 508 and Ross 308) than in layer hatching eggs (White Leghorn). Probably the same occurred in the study of Sato et al. (2006). Because ET affects energy partitioning (see 2.3.), a confounding effect between breed and ET on energy partitioning and HP can be expected, possibly explaining the lack of difference in estimated HP between broiler and layer hatching eggs.

Incubation conditions

It has been estimated that more than 90% of the total energy requirement of the hatchling is derived from fatty acid oxidation of the yolk lipids (Noble and Cocchi, 1990). To be able to oxidize yolk nutrients, embryos require sufficient oxygen for aerobic metabolism. When

embryos turn to anaerobic metabolism, because oxygen is limiting (Bjønnes et al. 1987), they gradually decrease fat metabolism and increase glycogen metabolism (Bell and Freeman, 1971). Depressed oxygen concentrations or elevated hatcher temperatures are two factors that can induce a switch from aerobic to anaerobic metabolism, resulting in a decreased yolk utilization (Wineland and Christensen, 2001). In a 2x3 experimental design, Lourens et al. (2007) investigated the effects of both temperature and oxygen concentration on yolk utilization and embryonic development. From d 9 till d 18, EST was set at either 37.8ºC (normal) or 38.9ºC (high), in combination with oxygen concentrations of 17% (low), 21% (normal) or 25% (high). HP at d 18 ranged between 114 and 154 mW.egg^{-1}, depending on EST and oxygen concentration. Effects of EST and oxygen concentration on energy partitioning at d 18 of incubation are shown in Table 3.

High EST decreased YFB energy content, but did not affect energy utilization nor RY energy content. High EST decreased E_{YFB}, which explained the higher HP observed for eggs incubated at high EST compared to normal EST. The total amount of energy used increased with increased oxygen concentration, which was also reflected in the lower energy content of the RY at hatching. Oxygen concentration had no effect on E_{YFB}, but HP at d 18 increased with higher oxygen concentration due to a higher amount of energy utilized from the egg.

Table 3. Energy partitioning between egg, hatchling and heat production (HP) at d 18 of incubation in eggs incubated at 37.8 and 38.9^{o}C and at 17, 21 or 25% oxygen (O_2). Unpublished data based on Lourens et al. (2007).

	EST		O_2			P-values[2]		
	37.8^{o}C	38.9^{o}C	17%	21%	25%	EST	O_2	EST x O_2
Albumen (kJ)	73	76	73	75	76	ns	ns	ns
Yolk (kJ)	280	282	282	281	281	ns	ns	ns
YFB (kJ)[1]	170^{a}	158^{b}	150^{b}	172^{a}	171^{a}	*	*	ns
RY (kJ)[1]	48	46	68^{a}	43^{b}	31^{c}	ns	*	ns
Utilized (kJ)	305	311	286^{c}	313^{b}	326^{a}	ns	*	ns
E_{YFB} (%)[1]	55.7^{a}	50.8^{b}	52.5	54.9	52.4	*	ns	ns
HP at d 18 (mW.egg^{-1})	131^{b}	148^{a}	119^{c}	138^{b}	152^{a}	*	*	ns

[a, b] Values within a factor and within a row lacking a common superscript differ (P<0.05); [1] YFB = yolk free body; RY = residual yolk; E_{YFB} = efficiency of energy utilization; [2] Statistical significance: ns = not significant, * = P≤0.05.

Summary

HP can be affected by both the amount of energy utilized from the egg and the efficiency of this utilization (E_{YFB}). The amount of energy utilized can be affected by egg weight, breed and oxygen concentration. Eggshell temperature had no effect on energy utilization. E_{YFB} was affected by breed and eggshell temperature. In Table 4, the factors affecting HP through differences in energy utilization or efficiency are summarized.

Table 4. Factors affecting HP through amount of energy utilization and efficiency of energy utilization between egg and hatchling (E_{YFB})

	Energy utilization	E_{YFB}
Egg weight	Yes	No
Breed	Yes	Yes
Oxygen	Yes	No
Eggshell temperature	No	Yes

It can be concluded that in the case eggs of one breed are incubated at the same EST, differences in HP are determined mainly by differences in energy utilization. The effect of breed on E_{YFB} is possibly not a real breed effect, but the result of different EST, because in studies comparing layer and broiler hatching eggs MT was similar, although HP in broiler hatching eggs is higher.

EFFECTS OF INCREASED ENERGY UTILIZATION ON HP

In the previous paragraph, it was concluded that in eggs incubated at the same EST, HP is determined mainly by energy utilization. According to Romanoff (1967), energy utilizatoin substantially increases from d 13 of incubation onwards, reaching its maximum at d 16 of incubation, where after it remained more or less constant. Based on HP (Lourens et al., 2006) differences in energy utilization between small and large eggs mainly occurred from d 15 onwards. Consequently, changes in HP and ET are restricted to the last week of incubation. Theoretically, energy utilization will increase further when the embryo is also able to utilize the energy that remained in the RY at hatch. HP would reach an even higher level when this extra energy would be utilized within short periods of time before hatching.

Romanoff (1967) showed how energy from the albumen and yolk during incubation was

distributed between embryo and heat. From the total amount of energy available in an average 62 g Leghorn hatching egg (361 kJ), 248 kJ was deposited in the hatchling and 113 kJ of the initial energy was lost. The energy in the hatchling was divided between YFB (130 kJ) and RY (118 kJ). The total amount of energy utilized was 361 – 118 = 243 kJ and E_{YFB} was 53.5%. Romanoff (1967) also determined the relative energy utilization per day. At d 18 of incubation, 10.5% of the available energy (243 kJ) was utilized. Theoretically, when all energy that remained in the RY at hatch also would have been utilized with the same E_{YFB}, evenly distributed across the 21 d incubation period, energy utilization at d 18 would increase from 10.5% to 15.7% (Figure 1). When the 118 kJ extra available energy in the RY would be utilized in a shorter period of time, for example between 15-21 d or 18-21 d, relative energy utilization at d 18 would increase to 17.4% and 22.0%, respectively (Figure 1).

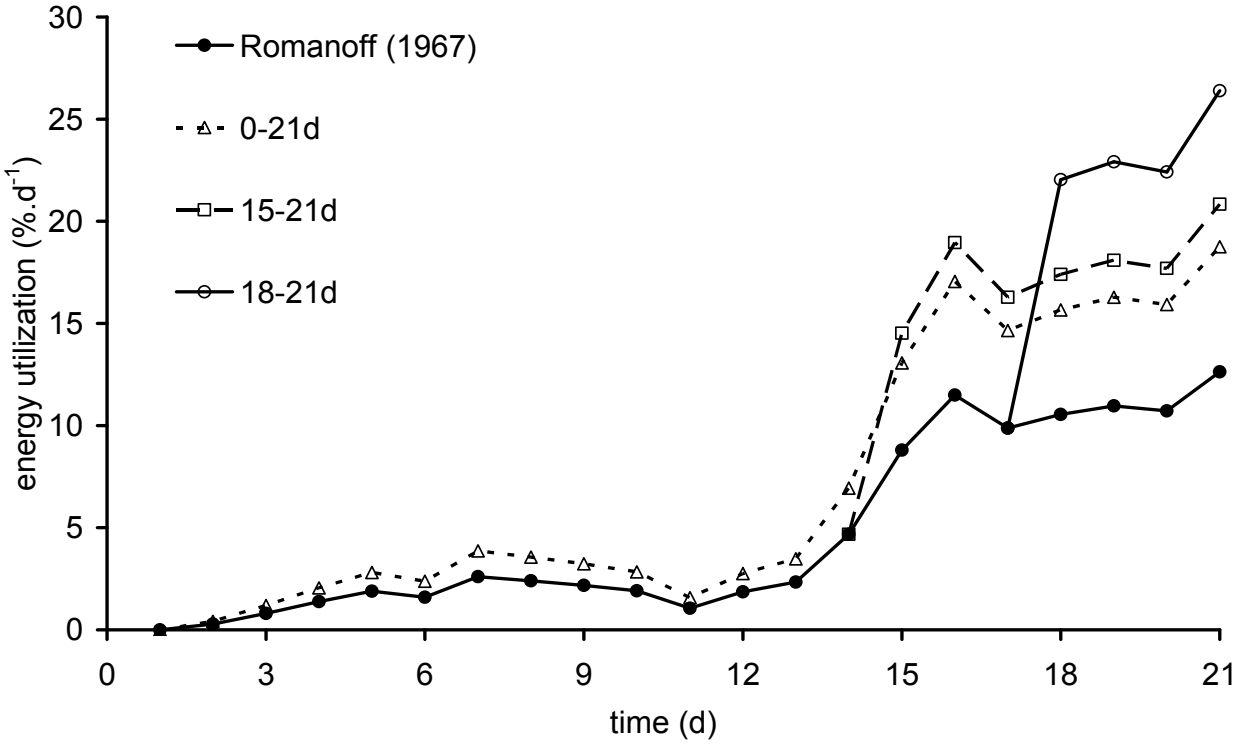

Figure 1. Relative energy utilization per incubation day according to Romanoff (1967), and in the theoretical situation where the energy remained in the RY at hatch also would have been utilized between 1-21 d, 15-21 d, or 18-21 d.

Embryos that utilized a total amount of energy of 243 kJ would reach a HP of 243 * 0.105 * (1-0.535) / (24*60*60) = 137 mW.egg^{-1} at d 18. When the remaining energy in the RY at hatch (118 kJ) would have been utilized evenly distributed across the 21 d incubation period, HP at d 18 would increase to 183 mW.egg^{-1}. When energy utilization would have been intensified

between 15-21 d or between 18-21 d, HP at d 18 would increase further to 188 and 202 mW.egg^{-1}, respectively. This means that from an egg of 62 g, with a given E_{YFB} of 53.5% theoretically HP can reach a maximum level of 202 mW.egg^{-1}.

Additionally, the effect of increased energy utilization on HP at d 18 was calculated for eggs in studies of Romanoff (1967), Sato et al. (2006), Lourens et al. (2006) and Lourens et al. (2007). Based on these results, HP at d 18 increased on average 3.2 mW.egg^{-1} with each 10 kJ increase in energy utilization (R^2=0.94). For each 10 kJ increase in energy utilization in the period of 15-21 d or 18-21 d of incubation, HP at d 18 would increase 4.2 and 7.1 mW.egg^{-1}, respectively.

CONSEQUENCES OF INCREASED HP FOR ET

To estimate the effect of HP on ET, we used the mathematical model of Meijerhof and Van Beek (1993). We verify this model using data of Lourens et al. (2006). Additionally, this model is used to estimate the effect of HP on ET, dependent on heat loss variables. Finally, we compare the theoretical results with observations of EST in practice.

Calculation of ET based on HP and heat loss

To determine the effect of HP on ET, the heat transfer coefficient from egg to environment needs to be quantified. Next, ET can be calculated based on mathematical relationships among egg characteristics, air temperature, heat transfer and thermal conductivity as described by Meijerhof and Van Beek (1993):

$$ET = T_{air} + \frac{Po}{6}.(1+\frac{2}{Bi}) \quad [2]$$

in which the Pomerantsev number (*Po*; dimensionless HP) can be defined as

$$Po = \frac{q.R^2}{\lambda(ET - T_{air})} \quad [3]$$

and the Biot-number (*Bi*) as

$$Bi = \frac{\alpha.R}{\lambda_{egg}} \quad [4]$$

so the temperature difference between embryo and air can be calculated as

$$ET - T_{air} = \frac{q.R^2}{6\lambda_{air}}.(1+\frac{2\lambda_{egg}}{\alpha.R}) \quad [5]$$

In these equations, *q* is the amount of heat produced by the embryo (W.m^{-3}), *R* = radius of a sphere (m) and λ_{air} is the thermal conductivity of air (W.m^{-1}.°C^{-1}). A thermal conductivity of air (λ_{air}) of 0.027 W.m^{-1}.K^{-1} can be used in commercial incubation (Van Brecht et al., 2005). The thermal conductivity of an egg was estimated to increase from 0.5 to 50 W.m^{-1}.K^{-1} with increasing HP, because of the increasing blood flow in a developing embryo (Meijerhof and Van Beek, 1993). Furthermore, for the calculation of heat transfer (α), the relation between the Nusselt number (dimensionless heat transfer) and the Reynolds number (dimensionless air velocity) can be used.

$$Nu = 2 + 1.3Pr^{0.15} + 0.66\sqrt{Re}.Pr^{0.33} \quad [6]$$

The dimensionless numbers used in equation [6] are defined as

$$Nu = \frac{2\alpha.R}{\lambda_{air}}$$

$$Pr = \frac{\nu}{a_{air}} \quad [7]$$

$$Re = \frac{2V.R}{\nu}$$

where ν = kinematic viscosity of air of 2.5.10^{-5} m^2.s^{-1}, a_{air} = thermal diffusity of 1.2.10^{-5} m^2.s^{-1} and V = air velocity (m.s^{-1}) across the eggs. The latent HP by moisture loss can be described by equation [8] (Meijerhof and Van Beek, 1993), and will result in a negative effect on ET.

$$ET = T_{air} + \frac{-k_a.dp.h}{\alpha} \quad [8]$$

In equation [8], the respiration coefficient k_a (based on surface of the egg) can be calculated from the transpiration coefficient based on mass (k_m) as explained by Meijerhof and Van Beek (1993). The weight loss of an egg depends on the water vapor pressure gradient between egg and environment. Therefore, weight loss of an egg is largely determined by the specific weight loss coefficient of an egg, the ambient temperature and relative humidity of air and, to a lesser extent, to the temperature difference between egg and environment in a situation where HP is high.

To validate the model described above, we used HP data of small and large eggs (Lourens et al., 2006), and the MT that was required to maintain EST constant at 37.8°C. An average air velocity across the eggs of 0.3 $m.s^{-1}$ was measured during the experiment. Based on total egg weight loss at d 18 of incubation and the calculated water vapor pressure gradient between egg and environment, we estimated the latent HP per day. Using the HP data and heat loss calculations [equation 8], the difference between machine temperature and ET (dT) was calculated [equation 5]. Calculated and observed values for dT fitted well (small eggs: R^2 = 0.99; large eggs R^2 = 0.98; Figure 2).

Because calculated and observed values for dT fitted well, the model was used to estimate the effect of increased HP on ET, based on energy utilization and E_{YFB} in eggs from different size, breed and incubated at different EST and oxygen concentrations.

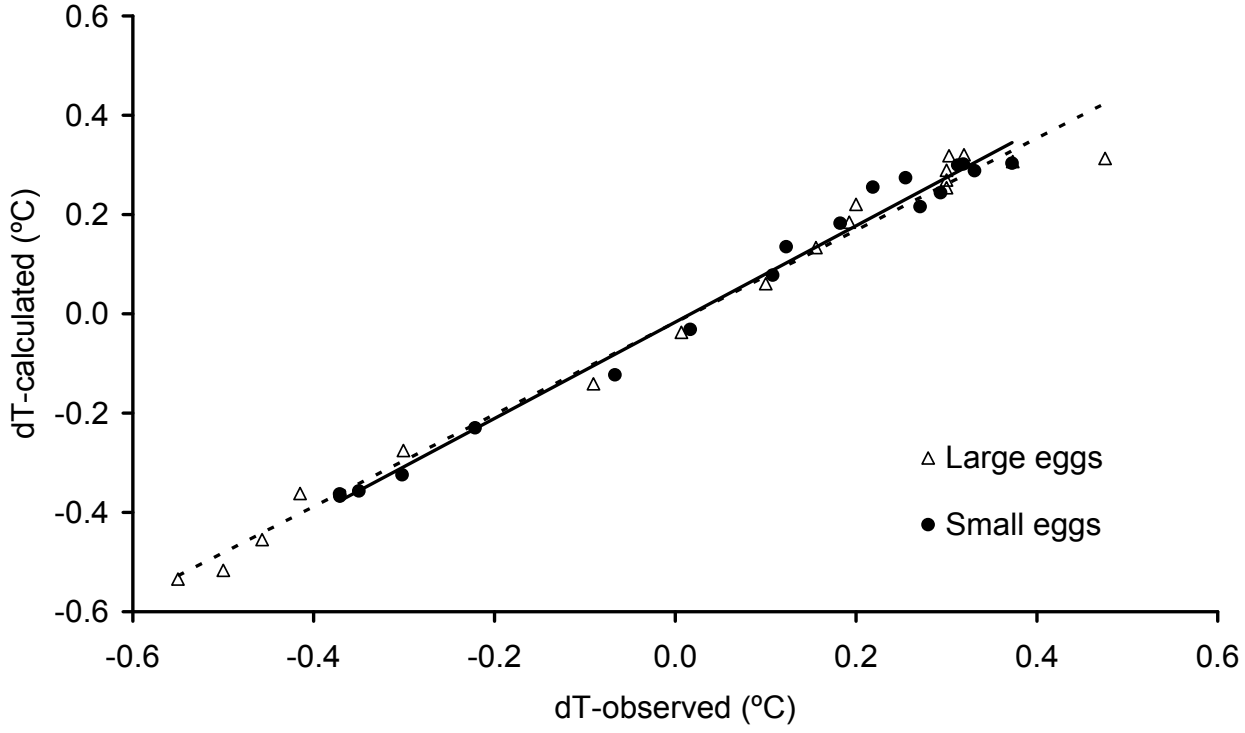

Figure 2. Relationship between observed difference (dT) between MT and EST and calculated difference (dT) between MT and ET in small and large eggs (based on Lourens et al., 2006).

Effect of HP on ET at variable air velocity

The effect of HP on ET is largely determined by air velocity (Meijerhof and Van Beek, 1993), so air velocity was taken into account calculating the effect of variation in HP on ET. The simulated effect of increased HP (dHP) on ET (dET) at d 18 of incubation at different air velocities is shown in Figure 3. In Figure 3, HP data from Romanoff (1967), Lourens et al. (2006; egg size), Sato et al. (2006; breed) and Lourens et al. (2007; incubation temperature and oxygen availability) are summarized. Effects of increased energy utilization between 0-21 d, 15-21 d, and 18-21 d of incubation on HP (see 3) and consequently on ET [equation 5] are also incorporated in Figure 3.

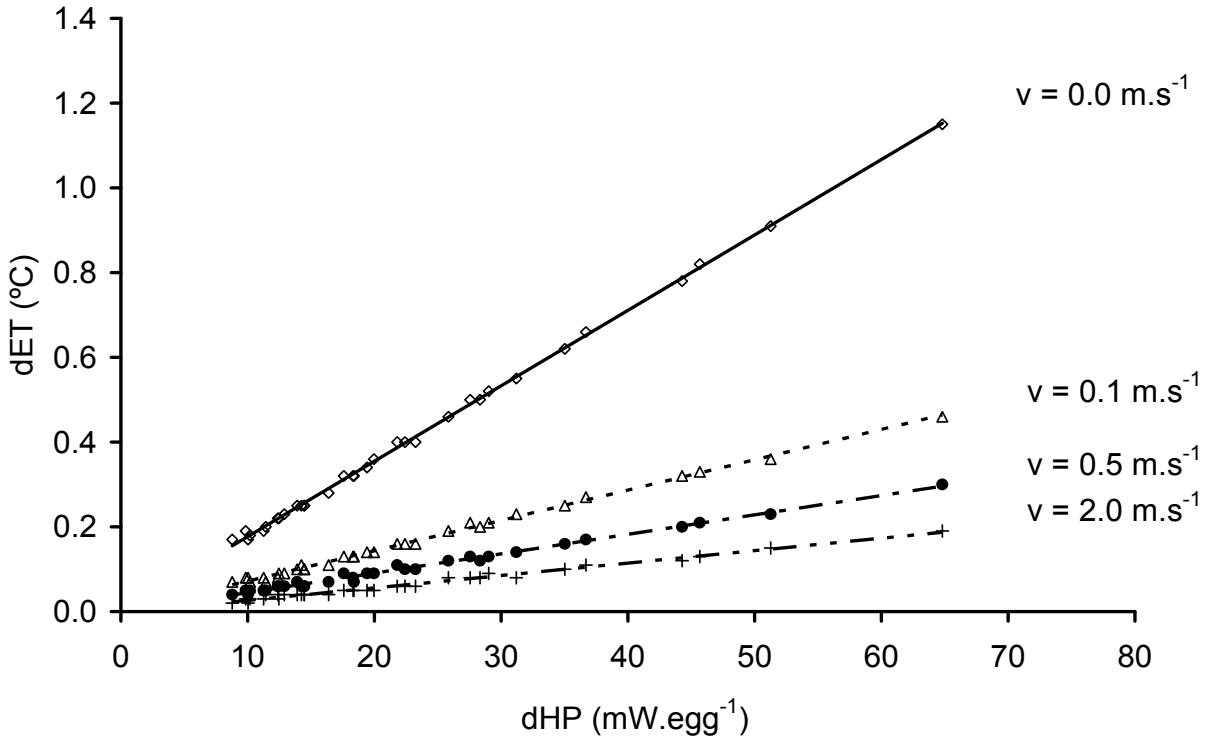

Figure 3. Calculated effects of increased heat production (dHP) on embryo temperature (dET) at d 18 of incubation as affected by air velocity.

At a constant air velocity, changes in HP are linearly related to changes in ET (R^2>0.99). The largest effect of increased HP on ET was observed in eggs incubated in still air. In still air, an increase in HP of 10 mW.egg^{-1} would increase ET by 0.18ºC. When air velocity was 0.1, 0.5 or 2.0 m.s^{-1}, an increase in HP of 10 mW.egg^{-1} would increase ET by 0.07, 0.05 and 0.03ºC, respectively. The theoretically maximum increase in HP was estimated at 65 mW.egg^{-1} (Romanoff, 1967; see 3). In still air, this would increase ET by 1.21ºC. When eggs were exposed to air velocities of 0.1, 0.5 or 2.0 m.s^{-1}, an increase in HP of 65 W.egg^{-1} would result

in an increase of ET of 0.47, 0.30 or 0.19ºC, respectively. Based on these calculations, it can be concluded that in the theoretical situation that all RY will be utilized in a short period (d 18-21) with a given E_{YFB} and an air velocity of 0 $m.s^{-1}$, ET of one single egg will increase with maximal 1.21^{o}C.

EST in practice

We calculated a maximal increase in ET of 1.21^{o}C above a given ET of 37.8ºC, resulting in a theoretically maximal ET of 39^{o}C. However, in practice, EST at d 18 of 40ºC or more are observed (Lourens, 2001). For example, at d18 of incubation, MT set point was 37.2ºC, but depending on the position of the eggs in the incubator EST varied between 37.6 and 40.4ºC. This variation largely depends on variation in air velocity within an incubator. In the corners of the incubator, air velocity was <0.2 $m.s^{-1}$, whereas close to the ventilator, air speeds of almost 2.0 $m.s^{-1}$ were observed (Figure 4). It can be seen in Figure 4 that at places where air velocity is low, both EST and air temperature were (substantially) increased.

So in practice, embryos in similar stage of development can experience ET that exceed the range of theoretical upper limits for ET. The discrepancy between the calculated maximal ET and the observed EST in practice can probably be explained with the following reasoning: The theoretical calculations are based on one single egg without interactions with other eggs. In practice an incubator is filled up to 100,000 eggs or more, each with their own heat production and heat transfer to each other. As a consequence, air temperature surrounding the egg is higher than with only one single egg, as expressed in Figure 4. This results in a reduced temperature gradient between egg and environment, resulting in an increased EST. As explained in chapter 2, a higher EST will result in lower E_{YFB} with the same amount of energy utilized, resulting again in a higher HP and consequently a higher ET. This will result in a vicious circle. When calculations as described in chapter 3 are performed using a MT of 38.8^{o}C (Figure 4) in still air, calculated ET will increase to 40.2^{o}C, which largely fits with observed EST in practice (Figure 4). In conclusion, higher EST as found in practice fits with the theoretical model described in chapter 3 and are due to reduced E_{YFB} and consequently higher HP.

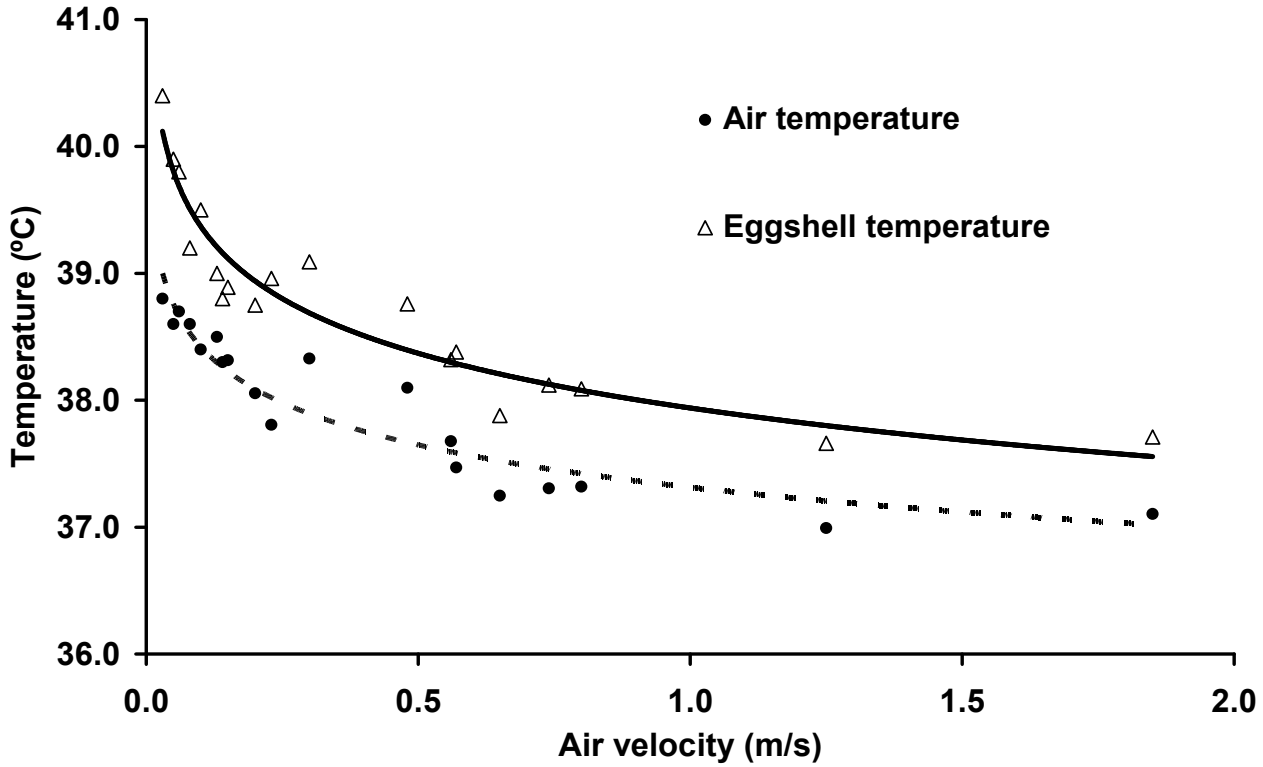

Figure 4. Effect of air velocity at a given MT of 37.2°C on air (5 cm distance from the egg) and eggshell temperature in a commercial incubator at d 18 of incubation (Lourens, 2001).

CONCLUSION

Factors affecting HP are the amount of egg energy utilized during incubation and the efficiency of this energy utilization. The amount of energy utilized depends on egg weight, breed and oxygen availability, but not on eggshell temperature. At the other hand efficiency of energy utilization depends eggshell temperature and possibly breed (may be confounded with EST), meaning that within a given breed and eggshell temperature HP only depends on the amount of energy utilized. In a theoretical situation that all egg energy (including the residual yolk) will be utilized in a relative short period at the end of incubation, the increased HP will result in an increase of ET of maximal 1.21°C for one single egg. At a set MT of 37.8°C this will result in an ET of 39.0°C. The higher values observed in practice are due to the fact that increasing the ET will result in lower efficiency of energy utilization and consequently higher HP, resulting in a higher ET and finally resulting in a vicious circle with ET above 40°C.

In incubation, it is of great importance to realize that any factor that affects either HP or heat transfer directly influences ET. Especially during incubation experiments, it should be realized that changes in ET may have a greater effect on results than the factor investigated

itself, and therefore we strongly suggest to control EST measurements in any incubation experiment.

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Abbreviation key

Symbol	Unit	Property
α_{air}	$m^2.s^{-1}$	Thermal diffusivity
α	$W.m^{-1}.K^{-1}$	Convective heat transfer coefficient
dp	Pa	Vapour pressure deficit
dT	ºC	Difference between ET and MT
EST	ºC	Eggshell temperature
ET	ºC	Embryo temperature
E_{YFB}	%	Efficiency of energy utilization
h	$J.kg^{-1}$	Latent heat of evaporation
HP	W	Heat production
k_a	$kg.kg^{-1}.m^{-2}.Pa^{-1}.s^{-1}$	Transpiration coefficient based on surface
k_m	$kg.kg^{-1}.Pa^{-1}.s^{-1}$	Transpiration coefficient based on mass
λ	$w.m^{-1}.K^{-1}$	Thermal conductivity
MT	ºC	Machine temperature
q	$W.m^{-3}$	Heat production
R	m	Radius of sphere
RY	g	Residual yolk
V	$m.s^{-1}$	Air velocity
v	$m^2.s^{-1}$	Kinematic viscosity of air
YFB	g	Yolk free body

Chapter 7

GENERAL DISCUSSION

INTRODUCTION

Many researchers have studied the influence of incubation conditions on embryonic development and hatchability. In most of these studies, machine temperature (MT) was used to control embryonic development. However, internal egg temperature or embryo temperature (ET) may be more relevant to control embryonic development than MT. MT is not necessarily the same as ET. Romijn and Lokhorst (1951, 1956, 1960) measured EST during incubation for eggs incubated at a constant MT of 37.5ºC throughout incubation in still air incubators. In these studies, EST was about 0.1ºC lower than MT during the first 9 days of incubation. After 9 days of incubation, EST rose gradually to 1.3 – 1.5ºC above the MT set point at day 18 of incubation; parallel to the course of heat production (HP). In large scale forced air incubators often step-down MT programs are used to prevent overheating of eggs during the final stages of incubation. However, EST can vary largely between different places within one incubator with maximum values for EST at day 18 of incubation of over 40ºC (Lourens, 2001; Van Brecht, 2003). Only during a short period of time, ET = MT (Chapter 2, 3, and 5). According to Briedis and Seagrave (1984) this period occurs at day 9 of incubation where evaporative heat loss equals embryonic HP. It was shown in Chapter 6 that the timing of this period depends largely on HP and total heat loss (including air velocity; see Chapter 6).

HP increased with embryonic age, even when EST was kept constant (Chapters 3 and 5), and the idea that ET and HP need to develop parallel with embryonic age was exchanged for the concept to measure and control ET regardless of HP. ET is the result of the balance between HP and total heat loss (Meijerhof and Van Beek, 1993). In this thesis, the influence of ET on embryonic development and hatchability is studied, with focus on how ET is affected by changes in the balance between HP and heat loss. In this general discussion, the results of experiments described in this thesis are reflected to experiments that used MT instead of ET to control incubation conditions. Next, factors are identified that affect HP and heat loss, and the causes and consequences of changes in the balance between HP and heat loss on ET are discussed.

THE IMPORTANCE TO CONTROL ET

Machine temperature (MT) is a very important factor affecting embryonic development (Romanoff, 1960), hatchability (Deeming and Fergusson, 1991; Wilson, 1991), and post hatch performance (Lundy, 1969; Wilson, 1991). In incubation trials often MT is used as treatment applied to the eggs (French, 1997), but only ET measurements will reveal the temperature

experienced by the embryo. Direct ET measurements are destructive and will influence embryonic development and hatchability. Using eggshell temperatures (EST) as a reflection of embryo temperature can overcome this problem, because EST will not deviate from ET by more than 0.1-0.2ºC (Meijerhof and Van Beek, 1993). Early in incubation, EST will be slightly higher than ET, whereas later in incubation EST will be slightly lower than ET (Meijerhof and Van Beek, 1993).

Measurements in a commercial single stage incubator set at a step-down MT program of 38.0^{o}C at d1 to 37.2^{o}C at d 18, showed that the overall average EST was close to 37.8^{o}C (Lourens, 2001). However, depending on the position in the incubator, EST ranged between 36.2 - 37.8ºC at d1, and between 37.8 - 40.2^{o}C at d18. These large EST fluctuations have a negative effect on hatchability and chick quality (Lourens, 2001), but most probably not too many eggs will be exposed to these extreme values (Van Brecht, 2003). It is therefore more interesting to study the effects of smaller deviations away from a constant EST of 37.8^{o}C that would occur more frequently. Therefore, in Chapter 2 effects of low EST (36.7ºC) during the first week and high EST (38.9ºC) during the last week of incubation on embryonic development, hatchability and post hatch performance were investigated. An EST of 37.8ºC throughout incubation was used as reference. During the first week post hatch, hatchlings were housed per EST treatment at either low (from 30ºC at placement to 25ºC at d7) or high (from 35ºC at placement to 30ºC at d7) housing temperatures.

Eggs incubated at low EST during the first week of incubation required a relatively higher MT during the second week of incubation and that effect lasted until hatching. Using a MT of 36.8ºC early in incubation, Geers et al. (1983) found comparable results as described in Chapter 2, showing that low MT early in incubation had a negative effect on embryonic growth and post hatch development. Although Geers et al. (1983) did not report EST values, it can be expected that the used MT settings will have resulted in more or less similar EST of 36.7ºC in the first few days of the incubation process as in the experiments described in Chapter 2. Geers et al. (1983) also found indications that low MT early in incubation reduced HP during the plateau phase. This is in accordance with the observations in Chapter 2 that during the plateau phase, MT needed to be increased for eggs incubated at low EST during the first week of incubation to achieve the same level of EST as the control group (37.8ºC). It was also concluded in Chapter 2 that relatively small deviations of 1.1ºC away from a constant EST of 37.8ºC decreased embryonic development and hatchability. Especially low EST during the first week of incubation decreased post hatch chick temperatures. Compared to a constant EST of 37.8ºC, the combination of low EST during the first week of incubation and high EST during the final week of incubation decreased hatchability and broiler performance; comparable to

findings of Lourens and Van Middelkoop (2000), Joseph et al. (2006), and Hulet et al. (2007). From the results in Chapter 2 it was concluded that chicks that hatched from different batches of eggs may require different housing temperatures, and that the EST experienced during incubation can affect the ability of young chicks to maintain sufficiently high body temperatures during cold stress. This expresses the importance of controlling EST during incubation, and it suggests that also housing temperatures should be adjusted to conditions during incubation.

Minne and Decuypere (1984), Nichelmann et al. (1994) and Tzschentke and Nichelmann (1997) observed that birds incubated at lower MT during the plateau phase were more tolerant to a cold environment after hatching. A possible explanation for this result can be that the eggs incubated at lower MT actually hatched more developed chicks, because the lower MT likely avoided higher ET. This might have resulted in a better thermoregulatory system with a higher metabolism with more HP and consequently a higher body temperature after hatch.

As ET is not equal to MT (Meijerhof and Van Beek, 1993) and can vary largely between different positions in an incubator (Lourens, 2001), results of experiments can be influenced by differences in ET that are not reflected in MT, which may lead to a misinterpretation of results. Therefore it is strongly advised to control EST measurements as reflection of ET in any incubation experiment.

FACTORS THAT AFFECT HEAT PRODUCTION

It can be concluded that relatively small deviations of 1.1^{o}C away from a constant EST of 37.8^{o}C decreased embryonic development, hatchability, post hatch performance and chick temperature, and that negative effects may last until slaughter age. It is therefore of great importance to identify the factors that affect ET. Because ET is the result of the balance between HP and heat loss (Meijerhof and Van Beek, 1993), factors that affect HP and heat loss are discussed in the following paragraphs. HP is determined by 1) the total amount of energy utilized from the egg and 2) the efficiency of this energy utilization (Ar et al., 1987; Pearson et al., 1991). ET changes when HP changes at a constant heat transfer (Chapter 6). The age of the embryo in the incubation process is an important factor that determines HP, but also physical, biological and environmental factors affect HP.

Embryonic age

Embryonic development proceeds with incubation time, and HP increases progressively during the incubation process; also when eggs are incubated at a constant EST (Chapters 3 and 5). Already after a few days, the embryo starts to produce a noticeable amount of heat.

After this initial stage, the weight of the embryo increases almost linearly and HP increases accordingly. HP reaches a plateau when about 70-80% of the incubation period is completed, but embryo weight still increases linearly (Romijn and Lokhorst, 1961; Romanoff, 1967; Dietz et al. 1998). Rahn et al. (1974) suggested that during the plateau phase, metabolic processes are limited by the insufficient exchange of oxygen. It can only be speculated how metabolic processes would run without such limitations, and if embryonic development would be improved when HP during the plateau phase is increased. In Chapter 5, eggs from similar size were incubated at different environmental conditions. See the paragraph on the effects of environmental conditions on HP for more details.

Under optimal conditions, HP in average sized broiler hatching eggs ranges between 140-150 $mW.egg^{-1}$ in the plateau phase (Chapter 5). When the embryo breaks through the inner shell membranes (internal pipping), it gradually changes from breathing through the chorio-allantois membrane to lung ventilation (Rahn et al., 1979) and increases oxygen uptake and HP. HP increases further when the embryo pips through the eggshell (external pipping). After external pipping, HP can be twice as high as in the plateau phase (Rahn et al., 1979), reaching 250 – 300 $mW.egg^{-1}$. When MT during the hatching phase is kept constant, the increased HP leads to higher ET that would gradually match the required temperature of young chicks of 40-41ºC as measured in Chapter 2.

Physical factors

Physical factors like storage time and egg turning can affect HP during incubation. Long storage time decreased embryonic growth rate, viability, and hatchability, especially in hatching eggs from older breeder flocks (Lapão et al., 1999, Fasenko et al., 2002) and extended the duration of incubation (MacLaury and Insko., 1968, Mather and Laughlin, 1976, Tona et al., 2003). These effects may be the indirect result of lower ET due to lower embryonic growth and hence decreased HP (Haque et al., 1996, Fasenko et al., 2003). However, no experiments have been described yet where eggs were stored for different periods of time, that accounted for differences in HP during the incubation process by incubating eggs at the same ET; a similar approach as described in Chapter 3 for egg size.

Egg turning during incubation is a precondition for normal embryonic development (Tona et al., 2003, 2005). According to Deeming et al. (1987), a critical period between 3 and 7 days of incubation exists, where the absence of turning eggs leads to increased mortality and decreased embryonic growth as a consequence. Due to decreased embryonic development in unturned eggs, HP in unturned eggs is lower than in eggs that are frequently turned (Tazawa, 1980, Pearson et al., 1996). Due to this lower HP in unturned eggs, ET will be lower as well,

which will decrease embryonic development even more. Turning will also influence air velocity over the eggs, therefore influencing heat loss which will influence ET especially later in incubation, when HP is increased. The effect of turning during critical periods may therefore be influenced by the additional effect that it has on HP or heat loss and therefore on ET. To examine the effect of turning on embryonic development without confounding effects of ET, the influence of turning should be investigated at a constant ET instead of at a constant MT.

Biological factors

Biological factors as egg size, breed and breeder age affect HP during incubation. Large eggs have a higher risk to get overheated than small eggs, due to differences in HP (Rahn et al., 1974; Hoyt, 1987; Vleck et al., 1980; Vleck and Vleck, 1987) and differences in heat loss (Meijerhof and Van Beek, 1993). Due to both differences in HP and heat loss, small and large eggs will reach different ET when incubated at the same MT. This is illustrated in Chapter 2, where two experiments were conducted with eggs from two different batches. In both experiments, eggs were incubated at identical EST profiles. Eggs in the second batch were larger, and required a lower MT from 8 days onwards to maintain EST at a constant level, compared to the smaller eggs in the first batch. If EST was not controlled, and when eggs would have been incubated at the same MT settings, eggs in the second batch may have reached higher ET during the plateau phase, which may have influenced the results. Using the calculations explained in Chapter 6, for eggs incubated at air velocity 0.3 $m.s^{-1}$, EST in large eggs would have been 0.4ºC higher than EST in small eggs.

A similar approach was chosen for Chapter 3, where an experiment is described where small and large eggs of the same breeder flock and same breeder age were incubated at the same constant EST of 37.8ºC to study the effects of egg size on HP, hatchability and energy transition from egg to hatchling, without disturbing effects of ET. In order to incubate small and large eggs at an equal EST of 37.8°C, MT profiles had to be adjusted for both size classes individually, to compensate for differences in HP from d15 onwards, more or less similar as in Chapter 2. Egg size had no effects on embryonic mortality and hatchability when EST was controlled at 37.8ºC. Embryos in small and large eggs incubated both at 37.8ºC EST were equally efficient in energy transfer from egg to embryo; large eggs likely produce more heat than small eggs, because embryos in large eggs utilize more energy (Chapter 6).

In studies where MT instead of EST was controlled, embryonic development in large eggs was retarded (Ricklefs, 1987) or late embryonic mortality in large eggs was increased (Reinhart and Moran, 1979; Hagger et al, 1986; Ogunshile and Sparks, 1995). These effects can likely be explained by the expected increase of ET in larger eggs compared to smaller

eggs. From the study described in Chapter 3, it can be calculated that each extra g of egg mass increased HP by 1.2 $mW.egg^{-1}$, when small and large eggs were incubated at the same EST of 37.8°C. In the studies by Hoyt (1987) and Vleck et al. (1987) HP increased with egg weight between 1.5 and 2.0 $mW.egg^{-1}$ per extra g egg weight. This may be the result of increased ET in larger eggs, because at higher ET, HP is further increased (Chapters 4, 5 and 6; Janke et al., 2002).

It was also observed that eggs from broiler breeds have a higher HP in the plateau phase than eggs from layer breeds (Janke et al., 2004, Sato et al., 2006). O'Dea et al. (2004) observed differences in HP in the plateau phase between breeds selected for broiler versus layer traits, but only for older flock ages. It was hypothesized in Chapter 6 that HP in broiler hatching eggs is higher than in layer hatching eggs, because the energy utilization in broiler eggs is higher. Also, effects of breed and breeder age on HP may be close related to differences in eggshell quality and oxygen availability to the embryo, because oxygen availability influences energy utilization and HP (Chapter 6). However, since cascading effects may occur because increased HP will increase ET, leading to increased metabolic rate and so further, factors that affect HP can only be studied under similar ET profiles.

Environmental factors

The environment surrounding the hatching eggs influences embryonic development and hatchability (Ognabesan et al., 2007). In general, the embryo consumes oxygen, and produces carbon dioxide, water and heat (Romanoff, 1967). According to Robertson (1923), the speed of the complex process of growth may be determined by the speed of the slowest component. So when transport of oxygen to the embryo, or transport of one of the waste products from the eggshell to the environment is limited, it will decrease embryonic growth, development and HP. Factors that affect gas exchange as eggshell conductance and partial pressure gradient are discussed in Chapter 4 of this thesis. The effects of temperature, oxygen concentration, carbon dioxide concentration and relative humidity on HP are discussed below.

Temperature

Adjusting MT avoided high EST during the last week of incubation on embryonic development (Chapter 2 and 3). It still remained unclear however, whether the constant EST of 37.8°C applied in Chapter 2 is optimal for embryo development for shorter periods of time and at different stages of incubation. Monitoring embryonic responses as for example vocalizations (Bamelis et al., 2005) or heart rate (Tazawa et al, 1992; Aubert et al., 2000) to external factors during incubation can provide important information about the current status

and following incubator settings. It was hypothesized in Chapter 4 that monitoring other, more direct embryonic metabolic responses as HP or CO_2 production, would provide a mechanism to control the incubation process better. It was expected that under optimal conditions, embryonic growth will be higher, hence the consumption of O_2 and yolk lipids and the production of waste products as H_2O, CO_2 and heat will be higher than under sub-optimal conditions (Meijerhof, 2002). This has already been tested in practice by Hulet (2001) and Hulet and Meijerhof (2001), who maximized CO_2 production in commercial incubators by adjusting MT settings to the CO_2 response of embryos between d9 and d18. Hatchability increased by 2 % and it was assumed that optimal embryo development would be found when CO_2 production was the highest. Their approach was primarily to increase CO_2 production and to avoid overheating, which seem to contradict to the findings by Nichelmann et al. (1998) and Janke et al. (2002) and the results from Chapter 3, where it was observed that embryonic metabolic rate and thus HP increased linearly with increasing incubation temperature. Only when internal egg temperature was increased to over 39.5°C at d 20, the metabolic rate of chick embryos instantly decreased (Janke et al., 2002). Younger embryos were also subjected to short term MT increments from 37.5 to 39.0ºC, but never reached higher ET than 39.5ºC due to their lower HP than older embryos. The determination of metabolic responses to temperature variations or Q_{10} factors (as described in Nichelmann et al., 1998 or Janke et al., 2002) therefore needs to be standardised in the same ET traject, for example as in procedure described in Chapter 4.

The results presented in Hulet (2001) and Hulet and Meijerhof (2001) were promising, but because CO_2 concentration increased with every MT step downwards, it did not become clear how embryonic development and HP would be affected when MT would be temporarily increased as well. Therefore, in Chapter 4, two experiments were carried out. One experiment was carried out under laboratory conditions, the other in commercial incubators in a broiler hatchery. The objective in both experiments was to monitor embryonic metabolic responses to short term EST and MT fluctuations. In the experiments described in Chapter 4, in the EST range of 1°C below and above 37.8°C, HP was positively and linearly related with EST, similarly as described in Janke et al. (2002). Within the studied EST zone, no maximum in HP was observed. Also in the hatchery experiment, when CO_2 responses were evaluated during 1 hr after a MT change, CO_2 production was linearly increased when MT was increased and CO_2 production was linearly decreased when MT was decreased. When the MT would be set according to increases in CO_2 production, EST would increase far above 37.8°C, reaching over 39ºC, leading to an increased percentage of overheated embryos. These findings are in contrast to the findings of Hulet (2001) and Hulet and Meijerhof (2001). The results described

in Hulet (2001) and Hulet and Meijerhof (2001) can probably be explained when their technique saved eggs from severe overheating, as it was shown by Janke et al. (2002) that HP decreases when ET reaches over 39.5ºC; avoiding eggs from severe overheating may therefore increase HP. Still, this explanation is not likely, since hatchability in both incubators were acceptable. Another explanation can originate from differences in ventilation rates between experimental and control incubator. After each temperature step, ventilation was adjusted to level CO_2 concentrations in both the experimental and control incubator. A temporal undershoot of the CO_2 concentration or temporal increase in O_2 concentration may have temporarily increased HP in the experimental incubator and hence may have affected the decision for the direction of the next temperature setting.

The fact that HP increased with higher EST does not automatically imply that embryonic growth is also increased. From Chapters 5 and 6 it appeared that HP was highest for the combination of high EST of 38.9ºC with high oxygen concentration of 25%. However, no interaction between EST and oxygen concentration for embryonic growth was observed, showing that HP but not embryonic growth can increase in overheated eggs when oxygen is sufficiently available. It was concluded from Chapters 4 and 5 that it will be difficult to use embryonic metabolic responses to short term EST fluctuations as a tool to control the incubation process.

Oxygen concentration

It has been shown that HP can be increased by higher oxygen concentration (Lokhorst and Romijn, 1965, Bjønnes et al., 1987, Van Golde et al., 1998), and be decreased by lower air pressures (Lokhorst and Romijn, 1965, Hassanzadeh et al., 2004). Next to oxygen concentration and air pressure, also other factors can affect the transport of oxygen through the eggshell to influence HP indirectly. For example, egg size and the quality of the eggshell may play an important role in gas exchange. Large eggs have a relatively smaller ratio between egg surface area and egg volume and face therefore more difficulties to acquire sufficient oxygen. This may be compensated by the decreased eggshell quality of eggs produced by older hens (Roland, 1976; Britton, 1977; Tullett and Board, 1977; Peebles and Brake, 1987), because eggs with poorer shell quality increase the eggshell oxygen conductance compared to eggs with good shell quality (Christensen et al., 1996).

A constant higher MT initially accelerated embryonic growth and utilization of nutrients and energy from yolk and albumen, but decreased embryonic growth later in development (Romanoff, 1972). The fact that embryonic growth can be accelerated when oxygen availability was increased suggests that oxygen was a limiting factor (Rahn et al., 1974; Metcalfe et al.,

1981). Limited oxygen availability may therefore explain the plateau phase for gas exchange to at least some extent and hence influence the maximum value for HP.

According to Dietz et al., (1998), the presence of a plateau phase for HP is unexpected, since the embryo continues to grow and maintenance costs must therefore increase. It was suggested by Dietz et al. (1998) that an increase of synthesis efficiency is expected to explain the plateau in energy expenditure, since small increases in synthesis efficiency can lead to great savings on synthesis costs. Reduction in growth rate or depressed formation of glycogen would play a minor role (Dietz et al., 1998). In Chapter 6 however, it was hypothesized that HP in the plateau phase can only be increased when more energy is utilized, or when the same amount of energy is utilized in a less efficient way. Efficiency of energy utilization as described in Dietz et al. (1998), however, decreases with increasing ET (Chapter 6). In other words, efficiency of energy utilization during the plateau phase may only increase when ET is decreased from high ET down to 37.8ºC. Therefore, if eggs are incubated at a constant ET, it may be more likely that HP during the plateau phase reached a maximum value because energy utilization during the plateau phase is decreased, for example due to limited oxygen availability as shown in Chapter 5. Decreased energy utilization may therefore explain the plateau phase for HP better than increased efficiency.

For eggs incubated at either a constant EST of 37.8 or 38.9ºC, higher O_2 concentrations increased HP. Therefore, when eggs are incubated at higher O_2 levels, MT need to be decreased to avoid an increase of ET, which may have confounded the results. For example, in study by Rahn et al. (1974) or in the review by Onagbesan et al. (2007) it was concluded that O_2 determined hatch time. Because in these studies EST was not controlled, it can be expected that EST increased with O_2 due to increased HP. In Chapter 5, where EST was maintained constant at higher O_2, it was shown that EST and not O2 determined hatch time.

Carbon dioxide concentration

The direct effects of CO_2 concentration on HP are unknown, but there is evidence that some indirect effects may exist. Onagbesan et al. (2007) for example, reviewed effects of elevated CO_2 levels on embryonic development and extra embryonic membranes. High CO_2 levels early or late in incubation seem to stimulate early hatching (Visschedijk, 1968; Buys et al., 1998; Hassanzadeh et al., 2002). High CO_2 levels are often achieved by reducing the ventilation, which increases not only CO_2 levels, but also humidity and ET. Therefore, it can be questioned whether early hatching is the effect of increased CO_2, increased relative humidity or increased ET. Injection of CO_2 in the incubator increases CO_2 concentration, but also decreases O_2 and N_2 concentration. If the initial O_2 before CO_2 injection was 21.0%, each

percentage of CO_2 injected will decrease O_2 concentration by 0.21%; injection of CO_2 to a concentration of 5% will therefore decrease O_2 by 1.05%. Lower oxygen availability will decrease HP, which will decrease ET, and thereby decrease embryonic development and hatching time (Chapter 5), if changes in ET are not accounted for. It is unknown whether CO_2 concentration affects hatching time when changes in ET are accounted for, but controlling ET would exclude the possible confounding effect of ET.

Oxygen availability to the embryo at high CO_2 concentrations is further affected by factors as blood oxygen capacity, haemoglobin and haemoglobin affinity for oxygen, that increase with development in association with an increase in erythrocyte count and hematocrit value (Tazawa, 1980). However, at high CO_2 concentrations, oxygen availability to the embryo decreases because increasing CO_2 concentration causes a decreased red cell pH, and under this condition the haemoglobin affinity for oxygen decreases (Bohr effect). For example, in order to maintain 50% hemogobin saturation rate O_2 pressure needs to increase with a factor of 2 when CO_2 pressure is increased with a factor of 4 (West, 2005). Additionally, CO_2 reacts covalently with hemoglobin to form carbamino hemoglobin which has a reduced O_2 affinity (West, 2005). The O_2 affinity is also decreased by an increased temperature (West, 2005). The combination of high temperature and high CO_2 levels will decrease oxygen availability to the embryo even more and hence decrease HP, which will have a lowering effect on ET. The role of CO_2 in incubation remains poorly understood. When O_2 availability to the embryo is reduced due to increased CO_2 levels in the incubator, the question rises to what extent HP and embryonic development are limited by “apparently normal” CO_2 concentrations of between 0.3-0.5% used in practice or during incubation experiments. It can be expected that during the second half of incubation, HP may increase and embryonic development may improve when eggs are incubated at lower CO_2 levels. This remains an interesting area for further research.

Humidity

Direct effects of egg weight loss during incubation on HP are unknown, but some indirect effects may exist. Weight loss during incubation is affected by the water vapour pressure deficit between egg and environment and is influenced by the shell conductance (Meijerhof and Van Beek, 1993). It has been suggested that for maximal hatchability, weight loss until pipping should range between 12-14% (Meir at al., 1984; Hulet et al., 1987). Embryos during the first week of incubation are more susceptible for high weight loss and osmotic changes than embryos during the last week of incubation (Snyder and Birchard, 1982; Ar, 1991). In general, sufficient weight loss is considered to be a prerequisite to form the air cell, to provide the embryo air after internal pipping (Rahn et al, 1979). Low weight loss may be associated

with a limited respiratory gas exchange, and low shell porosity have been shown to limit oxygen consumption (Tullett and Deeming, 1982; Burton and Tullet, 1983). Weight loss coincides with evaporative heat loss (see the next paragraph), and relative humidity affects heat transfer and hence ET as well (Chapter 6).

It can be concluded that several factors have direct or indirect influence on HP. To understand the relationships between HP and ET, the factors that influence heat loss need to be addressed as well.

FACTORS THAT AFFECT HEAT LOSS

Heat transfer is mainly determined by the temperature difference between egg and environment and air velocity across the egg, and to a lesser degree by air humidity and evaporative heat loss (Kashkin, 1961, Meijerhof and Van Beek, 1993; Van Brecht et al., 2005). Air velocity across the eggs in commercial incubators can range between 0 and 2 $m.s^{-1}$, depending on the position of the eggs within the incubator (Lourens, 2001, Van Brecht et al., 2003). The effect of air velocity on ET at different levels of HP is calculated in Chapter 6, and fit well with the observations done by Lourens (2001).

Relative humidity however in combination with temperature influence the water vapour pressure gradient between egg and environment. Depending on the water vapor pressure gradient between egg and environment and the conductance, a hatching egg loses a certain amount of its initial weight during incubation. High relative humidity of the air surrounding the eggs decrease the water pressure deficit, which decreases egg weight loss. Latent heat loss is low when egg weight loss is low (Meijerhof and Van Beek, 1993). Evaporative heat loss can cool down the eggs to a large extent. When for example 10,000 eggs of 60 g loose 0.6% of their initial weight per day, they produce 150 ml of water per hour. The evaporative heat loss will decrease the average ET by 0.2ºC (Meijerhof and Van Beek, 1993). Because all eggs lose more or less the same amount of water uniform across all positions in the incubator, in theory all eggs experience the same cooling effect. Humidifying discs or spraying nozzles deliver water more locally, and can have a larger, more local evaporative cooling effect. When for example humidifying discs or sprayers bring in 150 ml of water in the air per hour and reach only 10% of the eggs near the humidifiers, locally eggs can cool down as much as 2.0ºC.

Ventilation brings in air of low temperature that decreases relative humidity inside the incubator, so humidifiers are often used when ventilation is applied to remove excess of CO_2. There are only a few studies, where heat loss was adjusted for changes in HP in order to control ET. Apart from this thesis, only in studies by Lourens and Van Middelkoop (2000),

Joseph et al. (2006), Hulet et al. (2007) and Leksrisompong et al. (2007), ET was controlled by adjusting MT. At this moment, there are no studies known where heat loss was adjusted by changing evaporation, air velocity or humidity. When during experiments factors are studied that affect heat loss, the effect on ET should be taken in consideration.

EFFECTS OF UNCONTROLLED EMBRYO TEMPERATURE

If changes in HP are not balanced by changes in heat loss, ET will change. The most obvious factor that should be taken into consideration in incubation experiments is the time dependent HP. If changes in HP in time are not taken into consideration, and eggs are incubated at a constant MT, eggs have the risk of getting overheated, especially in situations of low air velocity (Chapter 6). The effects of low EST during the final week of incubation were not examined in this thesis, but the effect of 1ºC below 37.8ºC may be more harmful for embryonic development and hatchability than the effect of 1ºC above 37.8ºC. Especially in large commercial incubators, it will be more difficult to maintain uniform ET throughout the incubator than in small scale experimental incubators due to differences in heat transfer at different locations. Also, the optimum *average* EST for highest hatchability in large incubators may be different than the constant EST of 37.8ºC as applied in our experiments. Low EST will delay hatching time, and to prevent low EST it may be of practical use to have a higher average EST during the last week of the incubation process.

Using the mathematical model described in Meijerhof and Van Beek (1993), the effect of HP on ET was calculated for different air velocities. It can be seen from Figure 1 that at a constant MT and constant RH of 55%, ET can increase significantly, depending mainly on air velocity. It can also be seen in Figure 1 that the timing of the moment where ET = MT depends largely on air velocity. In still air, ET = MT at d4, whereas when air velocity is 2.0 $m.s^{-1}$, ET = MT at d12. The effects on ET in practice may be even greater, when the temperature of the air surrounding the eggs also increased by high ET (Chapter 6). Also the efficiency of energy transfer between egg and embryo is decreased in eggs incubated at higher ET, resulting in a even higher HP and ET (Chapter 6).

The effect of increased HP on ET was calculated in Chapter 6. Because air velocity has a major effect on heat transfer, the results were calculated for different air velocities ranging between 0.0 and 2.0 $m.s^{-1}$. For each 10% increase in HP above 150 $mW.egg^{-1}$, ET increased 0.27, 0.11, 0.06 and 0.05ºC for eggs incubated at 0.0, 0.1, 0.5 and 2.0 $m.s^{-1}$, respectively. Especially at low air velocities, changes in HP will affect ET. Because air temperature will also

increase more at places with low air velocities, which increases HP even more, ET can increase quickly above acceptable limits for optimum development (Chapter 6).

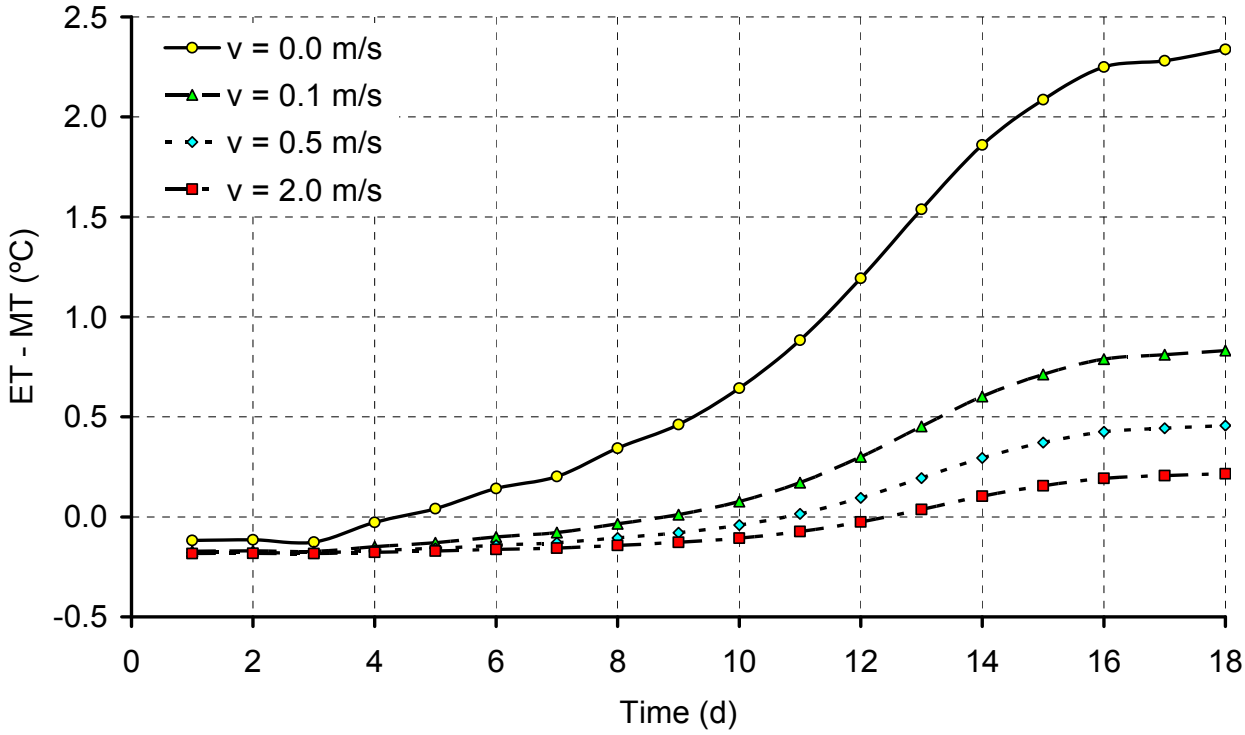

Figure 1. Development of ET during incubation, for eggs of 62g with a HP of 150 mW.egg^{-1}, incubated at a constant MT and at air velocities of 0.0, 0.1, 0.5 and 2.0 m.s^{-1} (based on calculations explained in Chapter 6).

CONCLUSION

During incubation, HP increased with embryonic age, even when ET was kept constant (Chapters 3 and 5), and the idea that ET and HP need to develop parallel with embryonic age was exchanged for the concept to control ET regardless of HP. To measure and control ET without disturbing the incubation conditions and embryonic development, EST measurements can be used as a reflection of ET. Embryos appear to be very sensitive for deviations away from a constant EST of 37.8ºC, and react with either decreased embryonic development or with instant metabolic changes to temperature fluctuations. Within the EST zone between 36.8 and 38.8ºC, HP increases linearly with EST. However, when EST is increased above 37.8ºC, the efficiency of energy utilization decreased and embryonic development decreased as well. ET is determined by the balance between HP and heat loss. Many factors affect HP and heat loss, and to study factors that affect either HP or heat loss, ET should be controlled to avoid compromising effects of ET on embryonic development and hatchability. Best embryonic

development occurred in eggs incubated at a constant EST of 37.8ºC until day 18 of incubation, but it is unknown whether this constant EST of 37.8ºC is optimal for all batches of eggs. Also, the optimum *average* ET in a large incubator may depend on the *uniformity* of ET. So ET is of great importance for embryonic development, but when ET is controlled, other factors become increasingly important. Embryonic development may be improved by any measure that increases gas exchange, energy utilization and HP, under the precondition that ET remains unaffected. This will be an interesting area for further incubation research.

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SUMMARY

Until recently, all incubator studies were performed using a constant machine temperature (MT). But it is embryo temperature (ET) that is of importance to the embryo, and not MT. MT is often measured at only one location within the incubator and variation in ET between different eggs within the same incubator occurs. ET is the result of the balance between heat production (HP) and heat loss, and if HP or heat loss is affected it will have consequences for ET. Attempts to improve embryonic development by adjusting incubation conditions may fail because changes in ET may confound the results. Aim of this dissertation was to identify the causes of variable ET and to describe the consequences of variable ET on embryonic development, hatchability, HP and chick quality. Directions for further improvement of embryonic development and hatchability are presented. Because the direct measurement of ET is destructive, it was chosen in this dissertation to use continuous, noninvasive eggshell temperature (EST) measurements as a reflection of ET.

In Chapter 2, a trial is described where the effects are evaluated of low EST of 36.7°C during the first week and high EST of 38.9°C during the last week of incubation on embryonic development, hatchability and post hatch performance. EST was measured, and MT was adjusted if EST drifted away from the set point. Eggs in the control group were incubated at a constant EST of 37.8°C. During the first week post hatch, per EST treatment, hatchlings were housed at different temperatures. It was concluded that relatively small deviations away from a constant EST of 37.8°C decreased embryonic development, hatchability, post hatch growth and the ability of young chicks to maintain a sufficient high body temperature, especially at low housing temperatures. Damage that occurred in the first week of incubation could not be repaired in later stages.

The trial in Chapter 2 was repeated twice, and eggs in the first batch were smaller and from a younger parent stock than eggs in the second batch. Eggs in the second batch required lower MT settings in order to maintain the similar EST profile. This was not unexpected, because HP and heat loss differ between small and large eggs, which will influence ET. To make a good comparison of hatching characteristics between small and large eggs, results should not be compromised by differences in ET. Therefore, in Chapter 3, a trial was described where small and large eggs of the same breed were incubated at the same constant EST of 37.8°C to study the effects of egg size on HP and energy transition from egg to hatchling. HP in large eggs was higher than in small eggs only from d 15 onwards. Accordingly, from d 15 onwards, MT needed to be decreased more in large eggs to maintain a

constant EST of 37.8°C. Egg size had no effect on hatchability, and embryos in small and large eggs were equally efficient in the transfer of energy between egg and embryo.

A constant EST of 37.8°C throughout incubation gave better hatching results than long term low or high EST during respectively the first or third week of incubation (Chapter 2). This does not automatically mean that the optimum EST is found at a constant EST of 37.8°C throughout incubation for any batch of eggs. Short term temperature variations may temporarily increase embryonic growth and development, so it will be very difficult to determine the exact optimum EST profile for each individual batch of eggs. A more practical approach to improve incubation conditions would be to monitor metabolic responses of embryos to changing incubation conditions, because it can be expected that embryonic development may be reflected by increased energy utilization, increased yolk free body weight, and hence increased HP. In Chapter 4, experiments were conducted to study the possibility to control the incubation process based on metabolic responses to short term temperature fluctuations. It was expected to find a maximum HP that would refer to an optimum ET for best embryonic development. However, in the EST range of 1°C below and above 37.8°C, HP and CO_2 production were positively and linearly related with EST and no maximum in HP or CO_2 production was observed. Young, mid term and late term embryos can increase HP or CO_2 production with increasing EST. It was concluded that it will be difficult to use embryonic metabolic responses to short term EST fluctuations as a tool to control the incubation process, because ET would reach values above the studied EST zone, leading to increased embryonic mortality and decreased hatchability.

High ET increased metabolism and the demand for O_2, and O_2 availability may be the limiting factor for embryonic development under higher ET. It was hypothesized in Chapter 5 that HP during the plateau phase can be increased with increased ET, as long as 0_2 availability would be increased as well and that high EST may not be detrimental for embryonic development and metabolic, rate, as long as 0_2 is sufficiently available. Therefore, in Chapter 5, it was studied how HP and embryonic development are affected by O_2 concentration (17, 21 and 25%) when eggs are incubated at different EST profiles of 37.8ºC (normal) or 38.9ºC (high). High EST decreased hatch time, body weight, yolk free body weight and relative heart weight. EST had no effect on residual yolk weight, chick length and relative liver weight. Increased 0_2 concentration increased yolk free body weight and chick length, and decreased residual yolk weight at hatch. 0_2 concentration had no effect on hatching time and organ weights. No interactions between EST and 0_2 concentration were observed with regard to embryonic development and hatchling characteristics. Until d15, eggs incubated at high EST produced more heat than eggs incubated at normal EST. High EST initially increased HP,

and gradually 0_2 concentration became more important. Independently from EST, 0_2 concentration determined HP at d 16 - 17. Between d 18 - 19 of incubation, HP was highest in eggs incubated at high EST and high 0_2 concentrations, but embryonic development was not highest in terms of yolk free body weight or chick length. The hypothesis that high EST is not detrimental for embryonic development and HP when 0_2 is sufficiently available was therefore rejected.

ET is the result of the balance between HP and heat loss. Aim of Chapter 6 was to investigate which factors theoretically can account for the variation in ET within an incubator. First, effects of egg characteristics (egg size, breed) and incubation characteristics (MT and oxygen availability) on HP of embryos are quantified. Differences in HP can only be due to differences in the amount of energy utilized from the egg or to differences in the efficiency of this energy utilization (E_{YFB}). Results of this analyses show that differences in HP due to egg size, breed, or oxygen availability are mainly a result of the amount of energy used from the egg constituents and not of a change in E_{YFB}; only EST influenced E_{YFB}. Decreased E_{YFB} with higher EST likely explains the results in Chapter 4, where HP but not embryonic development increased with higher EST.

Theoretically, HP will be highest when all available energy in the yolk is used during incubation within a limited amount of time during the final days of incubation. However, at a given MT, this variation in HP could only count for a maximum increase in ET of 1.2°C at d18 of incubation, suggesting that other factors also play a role. The most important factor is probably a difference in air velocity within an incubator, resulting in differences in heat transfer. Due to this variation, ET varies within an incubator and with rising ET, E_{YFB} decreases, resulting in an even higher HP and consequently higher ET. Results obtained from this theoretical approach suggest that hatchability and chick quality problems can be due to variation in ET, which fit the observations in practice closely. This indicates that it is of great importance to realize that any factor affecting HP or heat loss influences ET. Changes in ET may have a greater effect on experimental results than the factor investigated itself, and therefore we strongly suggest to control ET (or as a reflection of that EST) for any batch of hatching eggs.

In this thesis, the importance to measure and control ET during incubation was shown in Chapters 2, 3, 4, and 5. If eggs were incubated with MT as treatment applied to the eggs, results would have been compromised by differences in ET. Factors were identified that affect ET through changes in HP and heat loss (Chapters 5 and 6). Calculations of ET using a mathematical model showed remarkable good correlations with EST measured in practice (Chapter 6), so the factors that need to be controlled are well known and can be measured

and adjusted. It is unknown if a constant EST of 37.8ºC throughout incubation until internal pipping is the most optimal temperature for best embryonic development and hatchability for every batch of eggs, but the optimal temperature will not likely deviate far from this. Low ET during the last week of incubation was not studied and 1ºC below 37.8ºC ET may be more harmful to embryonic development and hatchability than 1ºC above 37.8ºC ET. When uniformity of ET in incubators is poor, it may be advised to allow higher average ET to prevent other eggs to be incubated at too low ET. When ET is controlled, embryonic development may be improved by measures that increase the gas exchange, which will increase HP. The consequence is that heat loss needs to be adapted to changes in HP, to maintain ET at a constant level. When the incubation process is viewed from the embryos point of view with regard to the control of ET, a much more comprehensible understanding of the incubation process will be gained.

SAMENVATTING

Tot voor kort werden alle broederij experimenten uitgevoerd met een constante machine temperatuur (MT). Maar het is de embryo temperatuur (ET) dat van belang is voor het embryo, en niet de MT. MT wordt vaak slechts op 1 plek in de broedmachine gemeten en zelfs in dezelfde broedmachine kan de ET tussen verschillende broedeieren aanzienlijk variëren. ET is het resultaat van de balans tussen warmte productie (HP) en warmte afgifte, en wanneer de HP of warmte afgifte wordt beïnvloed dan heeft dat consequenties voor ET. Pogingen om de embryonale ontwikkeling te verbeteren door de broedomstandigheden aan te passen kunnen hierdoor zelfs mislukken, omdat een veranderende ET de resultaten kan beïnvloeden. Het doel van deze dissertatie was om de oorzaken van variërende ET te identificeren en de gevolgen van variërende ET op embryonale ontwikkeling, broeduitkomst, HP en kuikenkwaliteit te omschrijven. Omdat het meten van de ET een destructieve methode is, werd er in deze dissertatie voor gekozen om continue, niet invasieve eischaaltemperatuur (EST) metingen te gebruiken als een afgeleide van ET.

In Hoofdstuk 2 wordt een proef beschreven waarbij de effecten worden geëvalueerd van een lage EST van 36,7ºC gedurende de eerste week van het broedproces en een hoge EST van 38,9ºC gedurende de laatste week van het broedproces op embryo ontwikkeling, broeduitkomst en kuikenprestatie gedurende de eerste week na uitkomst. Tijdens het broeden werd de EST gemeten, waarna de MT werd aangepast wanneer de EST van de gewenste waarde afweek. Eieren in de controlegroep werden gebroed op een constante EST van 37,8ºC. In de eerste week na uitkomst werden de kuikens per EST behandeling gehuisvest bij een verschillende staltemperaturen. Uit deze proef kon worden geconcludeerd dat, vergeleken met de constante EST van 37,8ºC, relatief kleine variaties in EST al een negatieve invloed kunnen hebben op embryonale ontwikkeling, broeduitkomst, groei na uitkomst en ook op het vermogen om de lichaamstemperatuur voldoende hoog te houden, vooral bij lagere staltemperaturen. Schade opgelopen in de eerste week van het broedproces kon niet zomaar worden gecompenseerd.

In de proef beschreven in Hoofdstuk 2 werden twee verschillende batches eieren uitgebroed, en de eieren in de eerste batch waren lichter en van jongere ouderdieren dan de eieren in de tweede batch. Eieren in de tweede batch hadden na een aantal dagen broeden een lagere MT instelling nodig om een gelijk EST profiel te volgen. Dit valt te verklaren uit het feit dat HP en warmte afgifte verschillen tussen lichte en zware eieren, en dat heeft invloed op ET. Om een goede vergelijking te kunnen maken tussen de broedtechnische eigenschappen van lichte en zware eieren, moeten de resultaten niet beïnvloed worden door verschillen in ET.

Daarom wordt er in Hoofdstuk 3 een proef beschreven waarbij lichte en zware broedeieren van dezelfde herkomst worden gebroed bij dezelfde EST van 37,8ºC. Hierbij wordt het effect van eigrootte op HP en energieoverdracht tussen ei en kuiken bestudeerd. Vanaf 15 dagen broeden was de HP in zware eieren hoger dan in lichte eieren. Als gevolg daarvan moest de MT ook vanaf 15 dagen broeden extra worden verlaagd voor de zware broedeieren. Eigrootte had geen effect op broeduitkomst, en de embryo's in lichte en zware eieren waren even efficiënt in de overdracht van nutriënten van broedei naar kuiken.

Een constante EST van 37.8ºC gedurende het hele broedproces gaf hogere broeduitkomsten dan langdurige lage of hoge EST gedurende respectievelijk de eerste of de derde week van het broedproces (Hoofdstuk 2). Dat betekent echter niet automatisch dat 37,8ºC altijd de meest optimale EST zou zijn voor iedere partij broedeieren. Kortstondige temperatuurveranderingen kunnen wellicht een tijdelijke verbetering geven van de embryonale groei en ontwikkeling, en dat maakt het moeilijk om voor iedere partij broedeieren al vooraf het meest optimale EST profiel te bepalen. Een meer praktische benadering zou kunnen zijn om metabole reacties van embryo's te monitoren als reactie op veranderende broedomstandigheden, omdat een verbetering van de embryonale groei en ontwikkeling wellicht gepaard gaat met een verhoogd energie verbruik, een verhoogd dooiervrij embryogewicht, en een hogere HP. In Hoofdstuk 4 worden daarom experimenten beschreven waarin getracht werd om het broedproces aan te sturen aan de hand van metabole reacties op kortstondige temperatuursveranderingen. Hierbij werd als uitgangspunt genomen dat een maximale HP overeen zou komen met de meest optimale ET en embryonale ontwikkeling. Echter, in de EST range van 1ºC boven en onder 37,8ºC waren HP en CO_2 productie positief lineair gerelateerd aan EST, en geen maximum in HP of CO_2 productie kon worden aangetoond. Embryo's bleken in staat om bij een temperatuurstoename hun HP of CO_2 productie te verhogen, zonder dat dit resulteerde in een verhoogde embryonale groei op de lange termijn. Uit deze experimenten bleek dat het niet eenvoudig zal zijn om embryonale metabole reacties op temperatuursveranderingen te gebruiken om het broedproces te controleren en te sturen. Wanneer zou worden getracht om met temperatuurvariaties de hoogste HP na te streven, dan zou dit leiden to EST boven de bestudeerde EST zone, die leiden tot verhoogde embryonale sterfte en verlaagde broeduitkomsten.

Hoge ET verhoogt het metabolisme en de vraag naar zuurstof, en de zuurstofbeschikbaarheid zou wel eens de beperkende factor kunnen zijn voor een goede ontwikkeling van embryo's gebroed onder hoge ET. Daarom werd in Hoofdstuk 5 de hypothese getoetst of in de plateau fase de embryonale ontwikkeling en de HP verder verhoogd zou kunnen worden door een te broeden bij hogere ET, mits de zuurstof

beschikbaarheid ook verhoogd wordt. Daarom werd in hoofdstuk 5 onderzocht hoe HP en embryonale ontwikkeling werden beïnvloed door O_2 concentratie (17, 21 en 25%) wanneer de broedeieren werden gebroed bij een EST profiel van 37,8ºC (normaal) of 38,9ºC (hoog). Hoge EST vervroegde het uitkomsttijdstip, en verlaagde het kuikengewicht, het dooiervrij kuikengewicht en het relatieve hartgewicht. EST had geen invloed op het gewicht van de dooierrest, de kuikenlengte en het relatieve levergewicht. Verhoogde O_2 concentratie had een positieve invloed op het dooiervrije kuikengewicht en de kuikenlengte, en verlaagde het gewicht van de dooierrest van de kuikens. O_2 concentratie had geen effect op uitkomsttijdstip en orgaangewichten bij uitkomst. Er konden geen interacties worden aangetoond tussen EST en O_2 concentratie voor wat betreft embryo-ontwikkeling en kuikenkarakteristieken. Tot dag 15 produceerden de broedeieren die bij hoge EST werden gebroed meer warmte dan de broedeieren die werden gebroed bij normale EST. In eerste instantie gaf een hogere EST een hogere HP, maar geleidelijk aan werd O_2 concentratie meer belangrijk. Onafhankelijk van EST bepaalde de O_2 concentratie de HP tussen d 16 – 17. Op dag 18 en 19 van het broedproces werd de hoogste HP gemeten in eieren die werden gebroed bij hoge EST en hoge O_2 concentratie, hetgeen echter niet gepaard ging met de hoogste embryonale groei en ontwikkeling in termen van dooiervrij gewicht en kuikenlengte. De hypothese dat hoge EST niet nadelig is voor embryo-ontwikkeling en HP mits zuurstof voldoende beschikbaar is kon hierom worden verworpen.

ET is het resultaat van de balans tussen HP en warmte afgifte. Het doel van Hoofdstuk 6 was om te onderzoeken welke factoren theoretisch gezien de variatie in ET in een broedmachine kunnen verklaren. Allereerst werden de effecten van broedeikarakteristieken (eigrootte, ras) en broedomstandigheden (MT en zuurstofbeschikbaarheid) op HP gekwantificeerd. Verschillen in HP kunnen alleen worden verklaard door verschillen in de hoeveelheid nutriënten aanwezig in het broedei en die door het embryo worden gebruikt, en door verschillen in efficiëntie van deze nutriëntenomzettingen (E_{YFB}). De resultaten van deze analyse gaven aan dat verschillen in HP door verschillen in eigrootte, ras en zuurstofbeschikbaarheid het gevolg zijn van een hogere nutriëntenomzetting en niet van een verandering in E_{YFB}; alleen EST had invloed op E_{YFB}; bij een hogere EST wordt E_{YFB} lager. Waarschijnlijk verklaarde een verlaagde E_{YFB} in broedeieren die werden gebroed bij een hogere EST de resultaten in Hoofdstuk 4, waarbij de HP maar niet de embryonale ontwikkeling toenam bij een hogere EST. Theoretisch gezien zal de HP het hoogst zijn wanneer alle beschikbare energie in het ei gedurende een beperkte hoeveelheid tijd wordt verbruikt in de laatste dagen van het broedproces. Echter, bij een gegeven MT zal deze variatie in HP op dag 18 van het broedproces slechts kunnen leiden tot een maximale

toename van de ET van 1,2ºC, hetgeen suggereert dat andere factoren ook een rol zullen spelen. De meest belangrijke factor is hoogst waarschijnlijk het verschil in luchtsnelheid op verschillende plaatsen in de broedmachine, als resultaat van de verschillen in warmteoverdracht. Door deze variatie in luchtsnelheid varieert de ET in een broedmachine en met toenemende ET neemt E_{YFB} af, waardoor de HP en dus ook de ET verder verhoogd worden. De resultaten van deze theoretische benadering laten zien dat het goed mogelijk is dat problemen met broeduitkomsten en kuikenkwaliteit verklaard kunnen worden uit een variatie in ET, hetgeen de waarnemingen in de praktijk onderschrijft. Dit laat ook zien dat het belangrijk is om te realiseren dat iedere factor die invloed heeft op HP of warmte afgifte, ook de ET direct beïnvloedt. Veranderingen in ET kunnen een groter effect op de uiteindelijke resultaten hebben dan de te onderzoeken factor zelf, en daarom wordt in de deze dissertatie benadrukt om de ET (of EST als een afgeleide daarvan) te controleren voor iedere partij broedeieren; zowel in proeven als in de praktijk.

In deze dissertatie werd het belang van het controleren van de ET tijdens het broedproces aangetoond in de Hoofdstukken 2, 3, 4 en 5. Wanneer broedeieren worden gebroed bij een constante MT, dan kunnen de resultaten beïnvloed zijn door verschillen in ET. Er werden factoren benoemd die invloed hebben op ET door verschillen in HP en warmte afgifte (Hoofdstukken 5 en 6). Berekeningen van ET met behulp van een rekenkundig model lieten opvallend goede overeenkomsten zien met de EST gemeten in de praktijk (Hoofdstuk 6), dus de factoren die gecontroleerd moeten worden zijn bekend en kunnen gemeten en aangepast worden. Het is niet precies bekend of een constante EST van 37,8ºC gedurende het hele broedproces tot aan het moment van intern aanpikken de meest optimale temperatuur voor de beste embryonale ontwikkeling en broeduitkomst is voor iedere partij eieren. Echter, de meest optimale temperatuur zal hier niet ver vandaan liggen. Het effect van lage EST gedurende de laatste week van het broedproces is niet onderzocht, maar het effect van 1ºC onder een constante EST van 37,8ºC kan weleens schadelijker zijn voor de embryonale ontwikkeling en broeduitkomsten dan het effect van 1ºC boven 37,8ºC. In broedmachines met een grote variatie in EST zou het daarom verstandig kunnen zijn om de *gemiddelde* ET op te laten lopen boven de 37.8ºC, om te lage EST zoveel mogelijk te vermijden. Wanneer de ET wordt gecontroleerd dan kan de embryonale ontwikkeling wellicht worden verbeterd door maatregelen die de gasuitwisseling verhogen, die hiermee ook de HP verhogen. Voorwaarde is dan wel dat de warmte afgifte wordt aangepast aan de veranderingen in HP, om de ET op een constant niveau te houden. Wanneer het broedproces wordt beschouwd vanuit het standpunt van het embryo en de controle van ET in plaats van MT, dan wordt een eenduidiger en beter begrijpbaar beeld verkregen van het gehele broedproces.

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CURRICULUM VITAE

Ik ben geboren op 9 november 1970 te Zevenaar en kreeg de naam Alexander Lourens. Ik ben vernoemd naar mijn opa, en mijn roepnaam was Sander. Ik groeide op in Wijhe, en mijn grote hobby's waren voetbal en vissen. Op het Florens Radewijns College in Raalte bracht ik mijn middelbare school door. In 1990 haalde ik hier mijn VWO diploma en in ditzelfde jaar ben ik begonnen met de studierichting Zoötechniek aan de Landbouwuniversiteit in Wageningen. In 1996 studeerde ik af in de afstudeerrichting Visteelt en Visserij. Op deze vakgroep heb ik vervolgens een aantal maanden gewerkt aan de ontwikkeling van een visstandsmonitoringsysteem gebaseerd op vangsten van hengelaars. Het jaar daarop kreeg ik een baan op het laboratorium van de Plukon in Wezep waar de koppeling van gezondheidsgegevens van vleespluimvee tussen de verschillende schakels centraal stond. De kuikenbroederij speelde hierbij een belangrijke rol in het monitoren en beheersen van besmettingen van *Salmonella* en *Campylobacter*. Collega en karpervisser Joop Kartouw wees me op de vacature van "onderzoeker broederij" bij het Praktijkonderzoek Pluimveehouderij op "Het Spelderholt" in Beekbergen en deed vervolgens een goed woordje voor mij bij mijn toekomstige chef Dr ir. Koos van Middelkoop. In Mei 1998 kon ik op "Het Spelderholt" aan de slag. Broederijexpert Dr Charles Deeming kwam uiteindelijk niet voor deze functie in aanmerking omdat hij geen Nederlands sprak, maar was gelukkig wel bereid om mij de eerste twee jaren in te werken en te adviseren bij proeven. In 2001 zijn de eerste plannen gemaakt om het broederijonderzoek te veredelen in een promotietraject. De verschillende praktijkonderzoekscentra fuseerden tot de Business Unit Veehouderij van de Animal Sciences Group van Wageningen UR en vanaf 2002 werd mijn standplaats Lelystad. Daarmee lieten we ook de prachtige broederij op "Het Spelderholt" achter, en werden steeds meer proeven uitgevoerd in de praktijk, of juist kleinschalig in de respiratiecellen in Wageningen. Het broederijpromotieonderzoek maakte steeds een belangrijk deel uit van mijn tijd; en ik werkte tegelijkertijd aan projecten over management van ouderdieren, *Salmonella* besmettingen van kuikens, broeden en opvang van eendagskuikens en kalkoenkuikens, het opwarmen van broedeieren op het vermeerderingsbedrijf, het effect van UMTS en GSM straling op de overleving van kippenembryo's, effecten van de voorschakels op uitval bij eendagskuikens, etc. Broederijonderzoek staat nooit op zichzelf; de schakels voor en na de broederij zijn minstens even belangrijk voor een goed resultaat. Maar de broederij blijft mijn inziens wel altijd de meest cruciale schakel, vooral wanneer het ketenrendement centraal staat.

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Pas Reform
Hatchery Technologies

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