

Metabolic Responses of Chick Embryos to Short-Term Temperature Fluctuations

A. Lourens,^{*1} H. van den Brand,[†] M. J. W. Heetkamp,[†] R. Meijerhof,[‡] and B. Kemp[†]

**Animal Sciences Group of Wageningen University and Research Centre, PO Box 65, 8200 AB Lelystad, The Netherlands; †Wageningen Institute of Animal Sciences, Adaptation Physiology Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands; and ‡HYBRO BV, Veerstraat 38, PO Box 30, 5830 AA, Boxmeer, The Netherlands*

ABSTRACT Two experiments were carried out to study embryonic metabolic responses to short-term temperature fluctuations in order to explore the possibilities of using embryonic metabolic responses as a tool to control the incubation process. In the first experiment, eggshell temperature (ET) in the control group was kept constant at 37.8°C, and embryos in the experimental group were exposed to varying ET within the range of 36.8 to 38.8°C using ET steps of 0.2°C and time steps of 3 h. This was repeated in 3 periods between 6.5 and 9.5 d, 10.5 and 13.5 d, and 14.5 and 17.5 d. In the studied ET range, heat production (HP) increased linearly at 4.9% per 1°C ET. 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 h of time at d 8, 9, and 11 to 16. When MT was decreased, CO₂ production initially increased at 0.5% and decreased thereafter. When

MT was increased, CO₂ production initially decreased at 0.4% and increased thereafter. It was concluded that embryonic HP responded linearly with short-term ET changes in the studied ET range of 36.8 to 38.8°C. Changes in CO₂ 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 CO₂. A second, delayed response to MT changes was in accordance with 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. Because HP is linearly related to ET as in the studied temperature range, other factors such as O₂ availability or CO₂ release may limit embryo development at higher ET. At this moment, research on the effects of gas exchange at different temperatures on embryo development and survival is lacking.

Key words: eggshell temperature, metabolic response, short-term temperature variation, incubation

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INTRODUCTION

Embryo temperature is considered an important factor influencing embryo development, hatchability, and post-hatch performance (Lourens et al., 2005a). Because of the difficulties of measuring embryo temperature without damaging the eggs, in the study by Lourens et al. (2005), eggshell temperature (ET) was used as a reflection of embryo temperature. Best results were obtained when ET was kept constant at 37.8°C until internal pipping compared with 36.7°C between d 1 to 7 or 38.9°C between d 14 and internal pipping. It remained unclear, however, if this constant ET of 37.8°C was 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 CO₂ pro-

duction. Harun et al. (2001) observed decreased HP in eggs that failed to hatch. Unfortunately, Harun et al. (2001) did not measure ET, and it remained unclear if overheating decreased HP in unhatched eggs. Hulet (2001) and Hulet and Meijerhof (2001) maximized CO₂ production in commercial incubators by adjusting machine temperature (MT) settings to the CO₂ response of embryos between d 9 and 18. Hatchability was increased by 2%, and it was assumed that optimal embryo development would be found when CO₂ production was the highest. Their approach was primarily to increase CO₂ production and to avoid overheating, which seems to contradict 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. When internal egg temperature was increased to >40.0°C at d 20, however, the metabolic rate of chick embryos instantly decreased (Janke et al., 2002). It remains unclear how the development of embryonic HP is affected by moderate, short-term ET variations, and if embryonic metabolic responses can be used as a tool to

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¹Corresponding author: sander.lourens@wur.nl

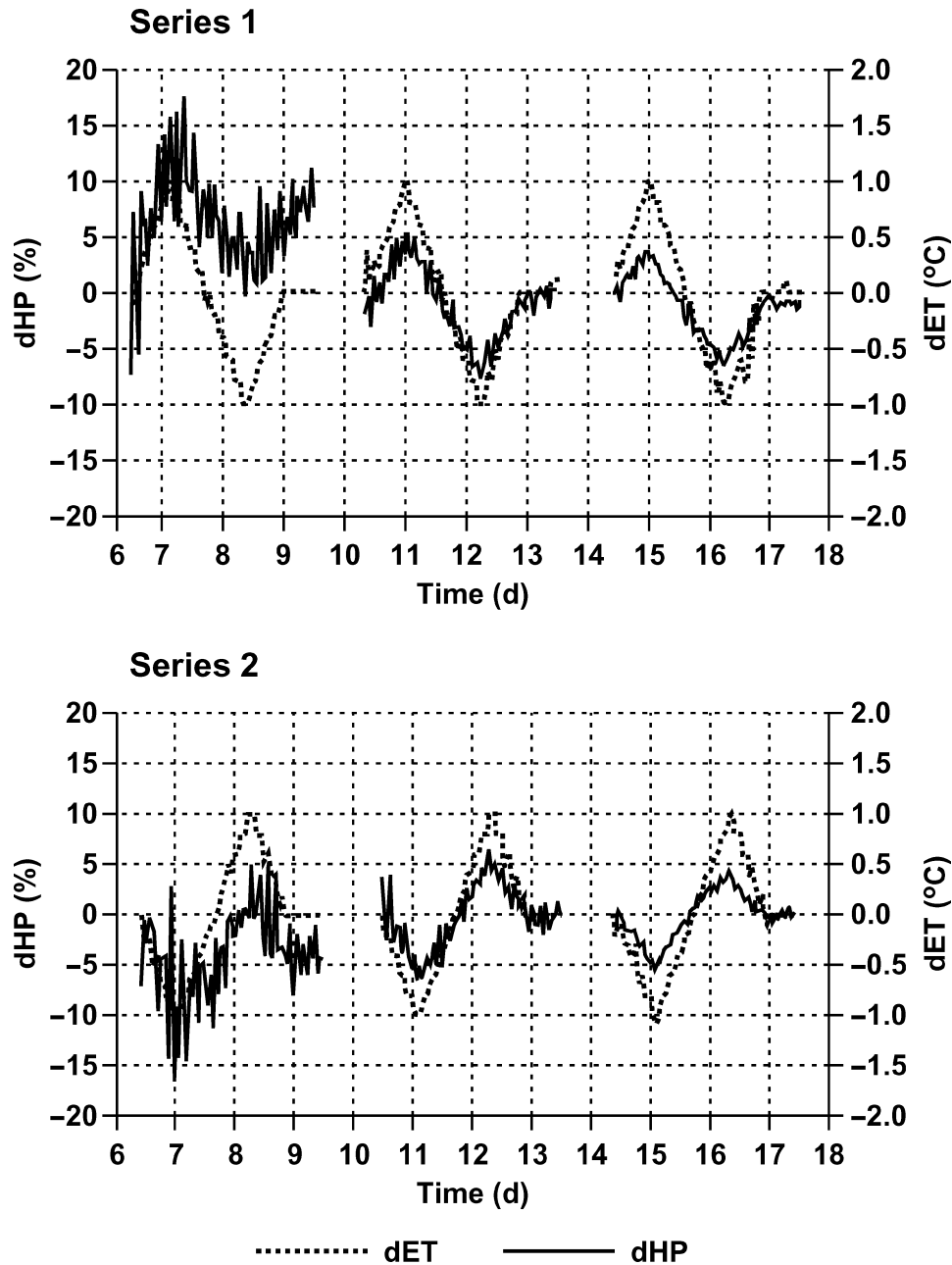


Figure 1. Relative effects of changes in eggshell temperature (dET) away from 37.8°C on heat production (dHP) in series 1 (top) and series 2 (bottom).

control the incubation process adequately. Therefore, 2 experiments were carried out. The objective in both experiments was to study embryonic metabolic responses to short-term ET and MT fluctuations in order to evaluate the possibilities of controlling the incubation process using metabolic responses.

MATERIALS AND METHODS

Laboratory Experiments

Experimental Set-Up. Hatching eggs were incubated at a constant ET of 37.8°C in an incubator, and at 3 different moments during incubation, eggs were transferred and placed in 1 of 2 identical climate respiration chambers

(CRC). In one CRC, ET was maintained constant at 37.8°C, whereas in the other CRC ET was increased or decreased in a range between 36.8 and 38.8°C. The laboratory experiment was executed twice: in the first series ET was adjusted from 37.8 to 38.8 to 36.8 to 37.8°C. In the second series, ET was adjusted from 37.8 to 36.8 to 38.8 to 37.8°C. In each series, this procedure was repeated during 3 different periods. The HP was calculated from oxygen consumption and carbon dioxide production according to Romijn and Lokhorst (1961).

Hatching Eggs and Incubation. In 2 series, a total of 1,200 first grade hatching eggs from one Hybro G grandparent stock were used in this trial. Eggs were incubated in a HT-combi (Hatchtech Incubation Technology, Veenendaal, The Netherlands) incubator that has a maximum

Table 1. Laboratory experiments: linear ($dHP = a \times dT + b$) regression parameters between dT ($^{\circ}C$) and dHP (%) in series 1 and 2

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

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 ET and to accordingly adjust MT daily to maintain ET at $37.8^{\circ}C$ as described in Lourens et al. (2005). Per series, at d 6, 10, and 14, 200 eggs were transferred from the HT-combi to 2 identical small open-circuit CRC. A description of the CRC and the respiratory gas measurements can be found in Lourens et al. (2006). In the CRC, eggs were not turned to avoid any disturbing effect of positional changes with regard to differences in wind speed and hence ET. Turning is important for normal embryo development between d 2 to 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

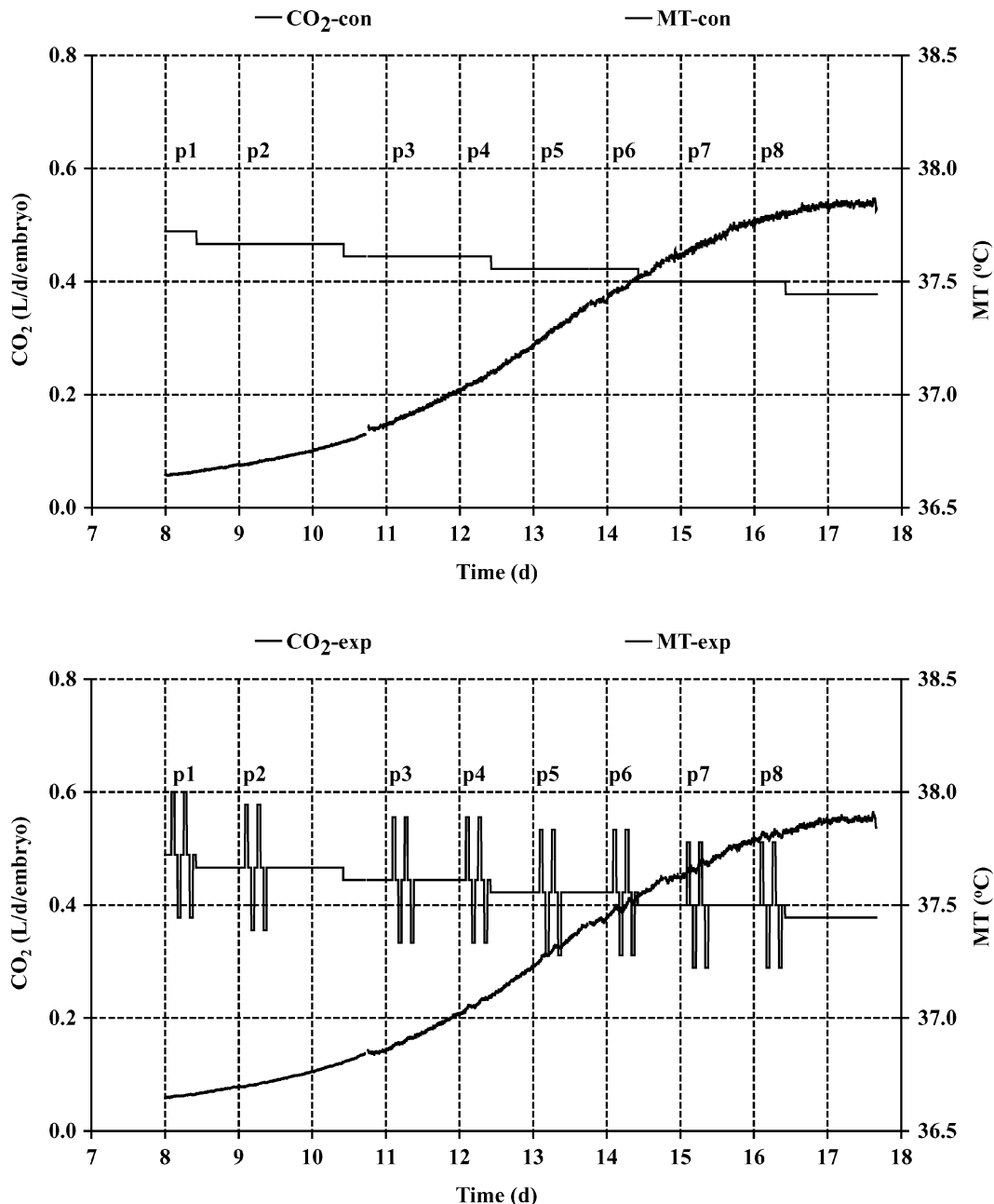


Figure 2. Hatchery experiment: machine temperature (MT) settings and CO_2 production (L/d/embryo) in the control group (-con; top) and experimental group (-exp; bottom). In the control group, standard MT settings (MT-con) were applied. During 8 periods (p1 to p8; p = period), MT-exp was increased or decreased $0.3^{\circ}C$ above, at, or below MT-con, using time steps of 1 h.

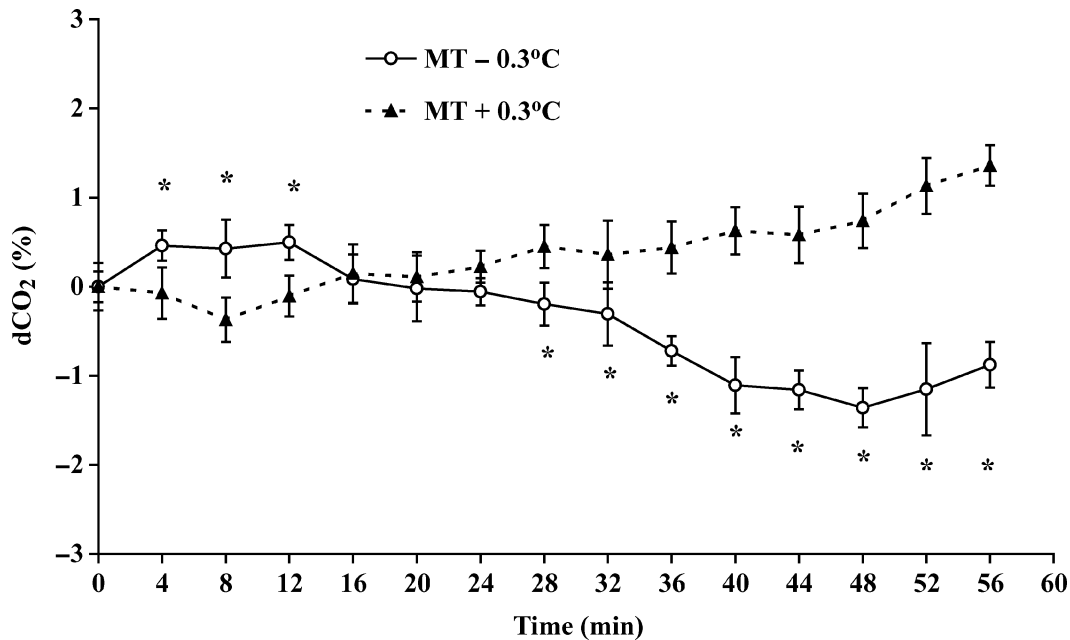


Figure 3. Hatchery experiment: effect of machine temperature (MT) changes on changes in CO₂ concentration (dCO₂) in the period of 1 h after the MT change. Data points represent the average dCO₂ of 8 periods (d 8, 9, and 11 to 16), and error bars represent SEM (n = 8). *Significant difference ($P < 0.05$).

that turning does not affect the relationship between ET and HP in short periods of time.

Eggshell Temperature Treatments. In each period per series, eggs remained 72 h in the CRC. Period 1 lasted from 6.5 to 9.5 d, period 2 from 10.5 to 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. (2005a). The ET was measured every 30 s, and MT was automatically adjusted every 5 min if the median ET drifted away from the ET setpoint. The MT was adjusted using the median ET of 5 eggs per CRC to avoid a situation in which low ET 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 ET as uniform as possible. The ET in the control group was kept constant at 37.8°C. In series 1, using time steps of 3 h, ET in the experimental group was increased with 0.2°C ET per time step from 37.8°C to 38.8°C. Next, ET was decreased to 36.8°C and increased again to 37.8°C. In series 2, also using time steps of 3 h, ET in the experimental group was decreased with 0.2°C ET per time step from 37.8 to 36.8°C. Next, ET was increased to 38.8°C and decreased again to 37.8°C. In both series, for the remaining 12 h of each period, ET was kept constant at 37.8°C to evaluate lasting effects of the ET treatments on HP.

Hatchery Experiment

Experimental Set-Up. Eggs were incubated in 2 commercial incubators. Eggs in 1 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. The CO₂ concentrations were adjusted for differences in fertile egg mass and ventilation rate, and the relationship between changes in MT and changes in CO₂ concentrations was investigated.

Machine Temperature and CO₂ Release. A total of 115,200 first grade Ross 308 hatching eggs were equally split by parent stock age and storage time across 2 identical Hatchtech (Hatchtech Incubation Technology) Microclimer incubators at the Cobroed and Sloot hatchery (Cobroed and Sloot Hatchery, Lielvelde, The Netherlands). Eggs in both incubators were incubated at standard MT settings. During 8 different days, (d 8, 9, and 11 to 16), using time steps of 1 h, MT in 1 incubator was set 0.3°C above or below standard MT. Each MT change lasted 1 h, and CO₂ concentrations and ventilation rate were measured and monitored every 4 min.

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 (Testo GmbH, Lenzkirch, Germany). 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. The CO₂ 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; Table 1). The relative effects of ET changes (dET) on HP in series 1 and 2 are shown in Figure 1. At the end of period 1 in series 1, HP in the experimental group was increased 7 to 8% compared with the control group. At the end of period 1 in series 2, HP in the experimental group was decreased 4 to 5% compared with the control group. In periods 2 and 3, HP changes followed ET changes linearly (Table 1).

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 8 different periods (d 8, 9, and 11 to 16); see Figure 2. In Figure 2, the CO_2 production (L/d) per embryo is also shown. In Figure 3, average CO_2 changes ($d\text{CO}_2$) in the period of 1 h 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 0.5% and decreased thereafter. When MT was increased, CO_2 production initially decreased 1.4% and increased thereafter.

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 suboptimal conditions (Meijerhof, 2002). In Hulet (2001) and Hulet and Meijerhof (2001), it was observed that HP could increase when MT was decreased. Lourens et al. (2005) observed decreased embryo development at low (36.7°C) and high (38.9°C) ET during the first and last week of incubation, respectively, compared with a constant ET of 37.8°C . When incubated at a constant ET of 37.8°C instead of at low ET during wk 1 and high ET during wk 3, the yolk-free bodies of embryos in eggs from young and old parent stocks at 18 d of incubation weighed, respectively, 13.8 and 2.3% more, and the embryos were, respectively, 0.5 and 0.4 cm longer. A maximum HP was expected to be found in an ET zone of 1°C around 37.8°C . Thermal damage would occur when HP decrease at higher ET, coinciding with decreased embryo development and decreased hatchability. However, in the current experiments, in the ET range of 1°C below and above 37.8°C , HP was positively and linearly related with ET, and no thermal damage due to short-term ET variations with regard to HP was observed. Also in the hatchery experiment, when $d\text{CO}_2$ was evaluated 1 h 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 min after a MT change, the reaction of the embryos was the reverse. An increased CO_2 production with decreased MT is likely not a metabolic reaction of the embryo because it suggests homeothermy. The incubator has to provide the basic environmental conditions such as temperature for good embryo development to a large content, but the embryo is likely able to self-regulate within a small temperature gradient. For example, this self-regulation could occur 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 to control the incubation process. It can be speculated that this initial, temporary response will be observed only in a very restricted temperature zone. Downregulation 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, ET may increase far above 37.8°C , leading to an increased percentage of overheated embryos.

ET, Timing, and Exposure Time

The combination of ET, timing, and exposure time determine the impact of ET 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 embryos. From d 6.5 to 9.5, HP changed more by ET treatment than later in incubation. Furthermore, in the first period, ET had a lasting effect on HP. These changes in (calculated) HP appeared to have different causes. In the first series where ET was increased first at d 6.5, CO_2 production at the end of period 1 was increased, whereas O_2 consumption remained the same as in the control group. It can be hypothesized that increased ET 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 cardiovascular support system. Research into that direction is unknown to the authors. In the second series, where ET was decreased first at d 6.5, the permanent decrease in HP at the end of period 1 may result from retarded embryo development because O_2 consumption and CO_2 produc-

tion were decreased. Retarded embryo development at this stage decreased HP at later stages, which agrees to findings by Lourens et al. (2005a) who observed lasting effects of low ET during the first week of incubation on embryo development, hatchability, and posthatch 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 to evaluate the possibilities of controlling 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 CO₂ concentration was more in accordance with linear HP changes to ET, at least in the studied ET zone in the first experiment. It can be expected, however, that HP may decrease at higher and lower ET 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 at which thermal damage occurs with regard to HP is unknown and needs further investigation. It is also unknown how embryo development, hatchability, and chick quality are affected after thermal damage.

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