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■ Keywords

Body weight, cloacal temperature, conductance, hatchability, loss of mass.

Effect of High Incubation Temperature on the Blood Parameters of Layer Chicks

ABSTRACT

Adequate environmental temperature control is essential for incubation efficiency. Layer breeder eggs (n=360) were weighed and distributed in a completely randomized experimental design with two treatments, consisting of two incubation temperatures (T1=37.5 °C, control; and T2=39.0 °C, hot), with two incubators per temperature, and 90 eggs per incubator, totaling 360 eggs. Hatchability, embryo mortality, and chick cloacal and body surface temperatures were not affected by incubation temperature. Eggs incubated at the hot temperature presented greater egg mass loss and higher eggshell conductance than those incubated at the control temperature. Layer chicks derived from eggs incubated at control temperature showed greater absolute weight, yolk-free egg mass, and heavier hearts than those from eggs submitted to heat stress during incubation. The control group presented lower base excess and ionized calcium blood levels. Incubating eggs at temperatures higher than those recommended compromises body and heart development of layer chicks and negatively affects blood ionized calcium availability, and therefore, bone mineralization during embryo development. Efficient temperature control during the incubation of fertile eggs is essential to obtain good quality layer chicks.

INTRODUCTION

Environmental temperature and humidity, airflow, and egg turning are some of the physical factors that influence incubation (Lourens *et al.*, 2005; King'ori, 2011). Environmental temperature is reported as the most critical physical factor affecting embryo development and hatchability (Yalçın & Siegel, 2003). Excessive heat inside incubator may reduce hatching efficiency (Willemsen *et al.*, 2011; Boleli & Queiroz, 2012) and yolk-free body mass (Lourens *et al.*, 2007; Hulet *et al.*, 2007; Leksrisonpong *et al.*, 2007), and may affect chick growth after hatching (Tona *et al.*, 2008).

High incubation temperature accelerates embryo metabolism, increases oxygen uptake and carbon dioxide production, which requires cardiovascular and/or hematological adaptations by the embryo. Respiratory, cardiovascular, and metabolic status can be evaluated using blood markers, which are particularly sensitive to temperature changes and are important indicators of the physiological responses of birds to stressors. Thermal stress can lead to changes in hematocrit values (blood erythrocyte levels) and hemoglobin levels (Borges *et al.*, 2003). In addition to metabolic changes, organ weight (particularly heart weight) is reduced when fertile eggs are exposed to high incubation temperatures (Leksrisonpong *et al.*, 2007; Lourens *et al.*, 2007). Chicks with low heart weight may present metabolic disorders related to cardiovascular development (Leksrisonpong *et al.*, 2007).



Moreover, high incubation temperatures lead to larger residual yolks, which result in worse chick quality and high mortality rates during the first week of life due to navel/yolk sac infections.

Although current literature provides relevant information on the effects of variations of incubation temperature in broilers (Kidd, 2004; Yahav *et al.*, 2004b; Yalçin *et al.*, 2005; Piastun *et al.*, 2008; Yalçin *et al.*, 2008; Ghonim *et al.*, 2009; Yahav, 2009; Leksrisompong *et al.*, 2007; Willemsen *et al.*, 2011, Druyan *et al.*, 2012; Selim *et al.*, 2012, Loyau *et al.*, 2013), few studies were carried out with layers (Salimet *et al.*, 2005; Walstra *et al.*, 2010). The present study aimed at evaluating the influence of high incubation temperature on the incubation and blood parameters, and organs weights of layer chicks.

MATERIALS AND METHODS

Three hundred and sixty fertile eggs laid by 47-week-old Hisex White layers breeders were weighed and randomly divided in two treatments: incubation at 37.5 °C (T1=control treatment) or at 39.0 °C (T2=hot incubation temperature treatment). Two horizontal incubators were used per treatment (130-egg capacity; Premium Ecológica, model IP-130, Brazil), with 90 eggs each. The incubators were equipped with automatic temperature control, and the eggs were turned every two hours. Relative humidity was maintained at 60% until the eggs were transferred to the hatcher (day 19 of incubation), where air relative humidity was increased to 70% and air temperature was reduced in 1 °C.

Hatchability, embryo mortality, egg mass loss, eggshell conductance, chick body surface temperature, chick cloacal temperature, chick relative weight, and absolute and relative weights of the liver, yolk sac, heart and gizzard, and blood parameters were analyzed.

The experiment was carried at the Laboratory of Avian Sciences of Jaboticabal Campus, at the State University of São Paulo, Brazil. All procedures were approved by the Ethics Committee of that university (Protocol number 7377/2010).

Hatchability and embryo mortality

Hatchability was defined as the number of chicks that hatched divided by the number of fertile eggs incubated. Embryo mortality was classified as early (1 to 7 days), intermediate (8 to 14 days), or late (15 to 21 days). Hatchability and embryo mortality were assessed in all the incubated eggs.

All studied variables below were measured 30 minutes after hatching in eight birds per treatment.

Body surface temperature, cloacal temperature, egg mass loss, and eggshell conductance

Wing, head, leg, and back surface temperatures were recorded using an infrared thermometer (Instrutemp, ITTI 380) to obtain the average body surface temperature (T). T was calculated using the following equation (1), according to Richard (1971).

$$T = (0.12 \times T_{\text{wing}}) + (0.03 \times T_{\text{head}}) + (0.15 \times T_{\text{leg}}) + (0.70 \times T_{\text{back}}) \quad \text{Eq. (1)}$$

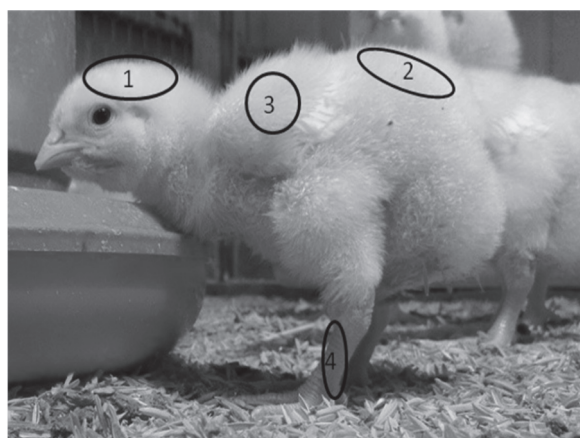


Figure 1 – Example of selection of specific areas of a pinto (1: head; 2: back; 3: wing; 4: leg).

The temperatures were measured at a distance of two mm from the skin of the chick. Cloacal temperature was measured using the sensor of a tele-thermometer (Max-Min Thermo Hygro) placed three cm inside the cloaca. Cloacal temperature was used for correcting blood pH, partial carbon dioxide pressure, and partial oxygen pressure blood values obtained using a portable blood analyzer (i-STAT® Co., Abbott Laboratories, USA; cartridge Cg8+®). Temperatures were measured immediately after chicks were removed from the hatcher.

Egg mass loss was calculated as the difference between egg weight at the onset of incubation and on day 19 of incubation, and expressed as a percentage. Eggshell conductance was calculated as egg mass loss (g) until the day of transference divided by steam saturation pressure (23.86 mm/Hg at 25 °C).

Organ weights and blood parameters

Chicks were individually weighed on a digital scale after the down dried, and their relative body weight (%) and relative yolk-free body mass were calculated (%) as a percentage of egg weight (g).

Absolute and relative weights of the liver, yolk sac, heart, and gizzard, were determined at hatching



in eight chicks per treatment. Chicks were sacrificed by cervical dislocation. Organ weight was calculated relative to individual chick body weight.

Arterial blood (1 mL) samples were collected directly from the aorta using a syringe containing heparin. Blood analyses were performed using a portable analyzer (i-STAT® Co., Abbott Laboratories, USA; cartridge Cg8+®). Hematocrit (HCT, % PCV), hemoglobin (HGB, g/dL), pH, partial carbon dioxide pressure (pCO₂, mmHg), partial oxygen pressure (pO₂, mmHg), base excess (BE_{ecf}, mmol/L), total carbon dioxide (TCO₂, mmol/L), oxygen saturation (sO₂, %), sodium concentration (Na⁺, mmol/L), potassium concentration (K⁺, mmol/L), ionized calcium concentration (Ca_i, mmol/L), bicarbonate (HCO₃⁻, mmol/L), and glucose (mg/dL) blood levels were analyzed. The analyzes were performed in eight chicks per treatment.

Statistical analysis

The effects of incubation temperature (37.5°C and 39.0°C) on all studied parameters were statistically analyzed using the model described in Eq. 1.

$$Y_{ij} = \mu + T_i + e_{ij} \quad \text{Eq. 1}$$

where Y = the studied parameters, μ = the mean value of the parameter, T = effect of the *i*th treatment (37.5°C or 39.0°C), and e_{ijk} = the residual error. Data were subjected to analysis of variance using the General Linear Model of SAS® program (SAS Institute, 2002). When significant effects at 5% probability level were detected, means were compared by the test of Tukey.

RESULTS

Hatchability and embryo mortality

Incubation temperature did not influence hatchability or early, intermediate, late, and total embryo mortality ($p > 0.05$; Table 1).

Cloacal temperature, body surface temperature, egg mass loss, and the eggshell conductance

The evaluated incubation temperatures did not affect chick cloacal or body surface temperature ($p > 0.05$). However, T2 eggs showed greater egg mass loss and higher eggshell conductance ($p < 0.05$; Table 1) than the control group.

Organ weight

Relative body weight was not different between treatments ($p > 0.05$). However, chicks of the control group (T1) presented heavier absolute and relative yolk-free body mass and heavier hearts ($p < 0.05$; Table 2) than T2 birds.

Blood parameters

Blood BE_{ecf} was lower in T2 chicks than in T1 chicks ($p < 0.05$; Table 3). However, this larger base loss did not result in HGB or HCT increase ($p > 0.05$; Table 4). Moreover, the treatments did not affect blood pH, pCO₂, pO₂, TCO₂, sO₂, or HCO₃⁻ ($p > 0.05$). Ionized calcium concentration was lower in the blood of the chicks incubated at T2 ($p < 0.05$; Table 5) than at T1. As for the other evaluated ions or blood glucose levels,

Table 1 – Effect of incubation temperature on embryo mortality, hatchability, loss of egg mass and the eggshell conductance.

Incubation temperature	Embryo mortality (day)* (%)			Total mortality* (%)	Hatchability (%)	Loss of mass (%)	Conductance
	0 - 7	8 - 14	15 - 21				
37.5 °C	4.18±2.82	0.50±0.99	5.43±2.85	10.11±5.36	89.89±5.36	10.11±2.01a	0.42±0.09a
39.0 °C	9.35±5.18	0.00±0.00	9.19±5.76	18.54±8.95	81.46±8.95	11.91±1.91b	0.50±0.08b
p-value	0.201	0.437	0.4094	0.253	0.177	<0.0001	<0.0001
CV (%)	35.23	62.15	25.94	22.82	8.14	17.80	17.77

± standard deviation; CV: Coefficient of variation. *ANOVA based on transformed data $y = \log(y + 1.5)$.

Table 2 – Effect of incubation temperature on an absolute and relative weight of post-hatch chicks organs.

Incubation temperature	BW	BW without the yolk sac	Yolk sac	Heart	Liver	BW without the yolk sac	Yolk sac	Heart	Liver
	(%)	(g)				(g)			
37.5 °C	69.68±2.40	33.67±1.35a	6.06±0.78	0.37±0.04a	0.89±0.06	58.78±1.64a	14.83±1.69	0.90±0.11a	2.17±0.20
39.0 °C	69.26±2.31	32.00±1.77b	5.95±1.10	0.28±0.04b	0.80±0.13	56.00±1.54b	14.96±2.54	0.71±0.07b	2.02±0.28
p-value	0.7492	0.0291	0.8206	0.0002	0.0918	0.0109	0.9243	0.0003	0.1596
CV (%)	3.37	4.79	15.84	12.22	12.34	2.77	14.48	11.68	11.49

BW: body weight; ± standard deviation; CV: Coefficient of variation; a-b: means followed by different letters in columns differ significantly at 5% probability (Tukey's test).



Table 3 – Effect of incubation temperature on cloacal temperature, body surface temperature and blood gases in post-hatch chicks.

Incubation temperature	Cloacal temperature (°C)	Body surface temperature (°C)	pH	pCO ₂ (mmHg)	pO ₂ (mmHg)	BE _{ecf} (mmol/l)	TCO ₂ (mmol/l)	sO ₂ (%)	HCO ₃ ⁻ (mmol/l)
37.5 °C	37.98±0.84	26.72±0.43	7.46±0.06	26.62±4.47	40.78±6.76	2.22±6.48 a	20.00±4.47	75.67±5.19	19.18±4.33
39.0 °C	38.23±0.78	27.88±1.47	7.43±0.04	25.11±4.09	42.78±5.78	-7.22±3.27 b	17.56±2.79	77.22±10.57	16.74±2.92
p-value	0.498	0.052	0.2978	0.4647	0.5096	0.0013	0.1831	0.2978	0.1810
CV (%)	2.11	3.83	0.71	16.55	15.06	-205.20	18.85	10.89	20.55

± standard deviation; CV: Coefficient of variation; a-b: means followed by different letters in columns differ significantly at 5% probability (Tukey's test).

Table 4 – Effect of incubation temperature on erythrocyte variables in chicks.

Incubation temperature	HCT (%PCV)	HGB x(g/dL)
37.5 °C	17.29±3.95	5.89±1.34
39.0 °C	15.88±3.04	5.40±1.05
p-value	0.4487	0.466
CV (%)	21.11	21.25

± standard deviation; CV: Coefficient of variation.

no difference was found between T1 and T2 (p>0.05; Table 5).

DISCUSSION

The present study aimed at investigating the effect of high incubation temperature on the blood parameters of layer. The use of thermal manipulation (TM) during incubation in broilers indicates that incubation temperature is a critical factor in the incubation process, affecting hatching and embryo quality (Yalçin & Siegel, 2003, Willemsen *et al.*, 2011; Boleli & Queiroz, 2012, Ferreira *et al.*, 2015). However, there are few literature reports on TM applied to layer chicks (Salimet *et al.*, 2005; Walstra *et al.*, 2010).

Eggshell conductance allows the gas exchange between the egg and the incubation environment, and allows embryo water and metabolic heat loss (Campos *et al.*, 2003; Hamidu *et al.*, 2007). High eggshell conductance leads to high water and heat loss through evaporation. In the present study, the higher mass loss of the eggs incubated at 39.0 °C was probably due to higher water evaporation from the embryos (Shafey, 2002). Fertile eggs absorb heat from the incubator

during the first half of the incubation period, when the embryo's temperature is lower than that of the incubator. However, embryos also lose heat during the second half of the incubation, when their metabolic rate and heat production increases (Meijerhof, 1999; Tazawa & Whittow, 2000). The greater egg mass loss of the eggs incubated in the hot environment in the current experiment was due to higher heat loss compared to those of the control treatment, as a result of their higher shell conductance.

Excessive embryo water loss (>14%) causes death by dehydration (Romanoff, 1930). In the present study, eggs incubated at 39.0 °C lost 11.91% of their egg mass, which value is very close to the rates reported for fertile broiler eggs incubated at 37.8 °C (Rosa *et al.*, 2002). Therefore, the egg mass loss obtained in the current study was within the acceptable limit.

Egg mass loss at the time of egg transference to the hatcher was used to determine the degree of embryo development, as it is associated with hatchling weight (Shafey, 2002). In the present study, the significant mass loss of the eggs incubated in the hot environment resulted in lower yolk-free body mass at hatch. This result is consistent with previous research reports on the effects of high incubation temperatures on broiler chicks (Lourens *et al.*, 2007; Molenaar *et al.*, 2007).

Yolk-free body mass is a better indicator of broiler development than body weight, because the latter included the weight of the residual, not yet metabolized yolk (Wolanski *et al.*, 2004). In the present study, the quality of the chicks derived from eggs incubated at high incubation temperature was worse compared with

Table 5 – Effect of incubation temperature on Na⁺, K⁺, ionized Ca and glucose in the blood of chicks.

Incubation temperature	Na ⁺ (mmol/l)	K ⁺ (mmol/l)	Ionized Ca (mmol/l)	Glucose (mg/dL)
37.5 °C	133.67±3.11	4.17±0.51	0.953±0.21 a	187.33±17.31
39.0 °C	134.79±2.96	3.94±0.62	0.699±0.21 b	184.89±25.79
p-value	0.4490	0.4161	0.0489	0.8163
CV (%)	2.26	13.92	25.35	11.80

± standard deviation; CV: Coefficient of variation; a-b: means followed by different letters in columns differ significantly at 5% probability (Tukey's test).



those incubated at standard temperature, as shown by their lower yolk-free body mass, consistent with the previous reports of Lourens *et al.* (2005, 2007), Joseph *et al.* (2006), Leksrisompong *et al.* (2007), Piestun *et al.* (2009), and Molenaar *et al.* (2011).

In the current study, layer chicks from eggs incubated at 39.0 °C showed lower absolute and relative heart weights than those incubated at standard temperature. According to Lilja & Olsson (1987), the ontogenetic development of birds after hatching is characterized by the preferential growth of the heart, intestines, and liver. In the present experiment, incubation temperature manipulation resulted heart hypoplasia, but did not affect the weight of the other evaluated organs, indicating that high incubation temperatures may affect individual organs differently. Heart hypoplasia reduces oxygen supply to the tissues, and may consequently impair layer body development and cause metabolic disorders. For instance, broilers derived from eggs incubated at high temperature presented higher incidence of ascites during the grow-out period compared with those incubated at standard temperature (Molenaar *et al.*, 2011).

Low hatching rates have been reported in broiler eggs exposed to long (Morita *et al.*, 2010) or short periods of hyperthermia (French, 2000). Although egg mass loss and eggshell conductance were higher in the eggs incubated at high temperature, in the present experiment, hatchability and embryo mortality were not affected. This may indicate that continuous incubation at 39.0 °C may not be a limiting thermal factor for embryo development. Such results are consistent with previous reports of Sgavioli *et al.* (2013) in fertile broiler eggs incubated at 39.0 °C, and of Walstra *et al.* (2010) in layer chicks. Studies on TM during broiler embryogenesis present hatching results that may be related to the temperature applied and its duration (Yahav *et al.*, 2004a, b.; Collin *et al.*, 2005). Laying hens embryos are probably more resistant to heat stress than broilers embryos due to their lower metabolic and body growth rates (Macari *et al.*, 2002).

Hyperventilation caused by heat stress results severe loss of carbon dioxide by the lungs, consequently reducing blood bicarbonate levels (Smith & Teeter, 1987; Balnave & Gornan, 1993). Therefore, reductions in BEecf may disturb the acid-base balance as a result of heat stress (Borges *et al.*, 2003). Blood BEecf values were lower in the layer chicks from eggs incubated at hot environmental temperature ($p < 0.05$). Embryos submitted to thermal challenge lost more bases than those at the control (Table 4). Values lower than 3 mEq/L indicate base loss due to primary metabolic disorder

(metabolic acidosis). They may also result from the renal excretion of HCO_3^- to compensate the pCO_2 reduction caused by respiratory alkalosis in order to reestablish normal blood pH values. Arterial blood pH, CO_2 partial pressure, and O_2 saturation pressure were not influenced by the treatments, and therefore, metabolic rate and respiratory physiology changes of embryos submitted to thermal manipulation was excluded in the present study. These results are different from the findings of Yalçın *et al.* (2008), Willemssen *et al.* (2011), and Walstra *et al.* (2010), who observed metabolic alkalosis during embryogenesis caused by TM.

In the current study, the high base loss values did not result in HGB or HCT increases which function as bases and contribute to the buffer system. These findings indicate that, despite the changes in the acid-base balance of layer hatchlings (low BEecf), there was no immediate metabolic acidosis, as detected by the erythrocyte parameters.

Birds submitted to heat stress commonly develop respiratory alkalosis, followed by metabolic acidosis as a compensatory response to the drop in pCO_2 (Borges *et al.*, 2003). This metabolism imbalance leads to bone mineral resorption and calcium excretion by the kidneys (Bushinsky, 1994). Calcium is present in three forms in the blood: calcium bound to protein, calcium complexed with organic acids, and free ionized calcium, which is used for bone mineralization. Heat stress results in blood electrolyte and mineral imbalances, which cause the calcium previously available for bone mineralization to form complexes with organic acids (Macari *et al.*, 2002). Therefore, the observed reduction in ionized Ca in the blood of chicks from eggs incubated at 39.0 °C demonstrates that less free calcium was available for bone development and mineralization, which could possibly compromise their subsequent growth. Moreover, there may be less calcium available in the bones for eggshell formation. Therefore, despite the lack of evidence of metabolic acidosis as detected by erythrocyte parameters, the high incubation temperature induced the formation of calcium complexes and reduced the blood concentration of ionized calcium.

Poultry exposed to heat stress need to increase body heat loss and reduce metabolic heat production. When body surface temperature increases, blood flow to the surface increases to allow heat dissipation (Macari *et al.*, 2002). The high incubation temperature did not influence chick body surface or cloacal rectal temperature at hatch, indicating no increase in heat loss at in chicks incubated at 39.0 °C.



The results of the present study may contribute to a better understanding of the effects of thermal manipulation during incubation on the development of layer chicks.

CONCLUSIONS

Incubating eggs at 39 °C compromises the body and heart development of layer chicks and reduces the availability of blood ionized calcium for bone mineralization during embryo development. Effective temperature control during the incubation of fertile layer eggs is essential to obtain good chick quality. Incubation temperatures above those recommended for a specific strain may compromise embryo development and consequently their post-hatch performance.

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