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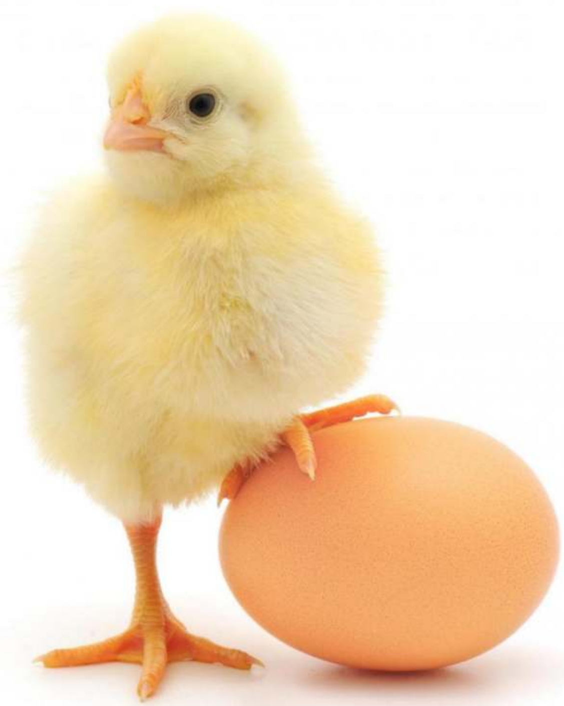

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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Relationship Between Incubation Temperature and Egg Size with Heart Hypoplasy in Broiler Chicks at Hatching

Viviane de Souza Morita[1], Isabel Cristina Boleli[1] and João Ademir Oliveira[2]
[1]Department of Animal Morphology and Physiology, [2]Department of Exact Sciences,
Faculty of Agricultural and Veterinary Sciences, São Paulo State University, UNESP, Brazil

Abstract: It was analyzed if the effects of continuous incubation temperature deviations during the second half on the development of body, organs and hematological respiratory and energetic parameters differ between male and female from 30- and 60-week-old breeder eggs. From day 13, Cobb eggs were exposed to 36°C, 37.5°C, or 39°C. At 3, 6, 12, 24, 48 and 72 h after this change in the temperature and at hatch, red cells count, hematocrit, hemoglobin, mean corpuscular volume, plasma glucose level and body, liver and heart weights were evaluated. Independent of incubation temperature, sexes and breeder ages, mean corpuscular volume decreased and the other variables increased during late incubation. In 30-week-old breeder eggs, body weights and erythrocytic parameters were not influenced by temperature but liver and heart weights decreased increasing incubation temperature and glucose level increased at 36 and 39°C. In 60-week-old breeder eggs, males were heavier at hatching with incubation at 36°C and females had smaller body weights with incubation at 39°C. In both sexes, liver weight decreased and glucose concentration was higher at 36 and 39°C and heart weights and erythrocytes parameters were not influenced by temperature. Independent of breeder age, hatchability was lower at 39°C. The data show that high temperature from day 13 of incubation reduced more intensively the hatching success and caused cardiac hypoplasia in chicks from 30-week-old breeder eggs only, revealing for the first time that the susceptibility for ascites syndrome, by reduced heart development at hatching, is associated to a relationship between incubation temperature and egg size.

Key words: Cardiac weight, erythrogram, glucose, incubation, thermal stress

INTRODUCTION

Temperature is a primary factor in avian embryonic development within eggs. During morphogenesis which occurs in the first third of the incubation period, embryonic metabolism is low and eggs require heat from an incubator; but, during the fetal period in the second half of the incubation period, metabolism increases and the eggs lose heat (French, 1997). From day 13 of incubation, eggshell temperature of eggs from 30- and 60-week-old broiler breeders is approximately 0.8°C higher than the temperature in the ambient incubator (Gualhanone, 2012), allowing heat dissipation from the eggs.

Independent of breeder age and egg weight, the intense growth in embryo body mass and organs during the second half of the incubation period requires markedly increased tissue metabolic activity and oxygen demand. This process necessitates optimization of blood gas exchanges (O_2 and CO_2) which in turn requires changes in erythrocytes parameters Red Blood Cell count (RBC), hematocrit (HCT), hemoglobin concentration (HGB) and Mean Corpuscular Volume (MCV) and in energy disposition. According to Morita *et al.* (2009) and Tazawa *et al.* (2011), developmental hematological changes from day 13 of incubation include marked increases in the RBC, HGB and HCT values, a decrease in MCV values and an increase in the heart mass. These changes in hematological respiratory variables show that fetuses respond to higher oxygen demand, resulting from their higher metabolic rate, by increasing cardiac output and increasing the number but reducing the volume, of erythrocytes to elevate oxygen transportation rate.

Older breeder eggs show higher eggshell conductance than do young breeder eggs which is related to decreased eggshell thickness, greater porosity and higher eggshell surface area of the older breeder eggs (Morita *et al.*, 2009). Eggshell conductance increases with egg size but is not proportional to increases in embryonic mass (Meijerhof and Van Beek, 1993), indicating that eggs from older breeders (heavier eggs) have greater difficulty dissipating heat than eggs from younger breeders (lighter eggs). Both eggshell conductance and embryonic developmental rate are also determined by incubation temperature and decrease and increase with low and high temperatures,

Corresponding Author: Isabel Cristina Boleli , Department of Animal Morphology and Physiology, Faculty of Agricultural and Veterinary Sciences, São Paulo State University, UNESP, Via de Acesso Prof. Paulo Donato Castellani s/n, 14884-900, Jaboticabal

respectively (Romanoff, 1960; Wilson, 1991; Leandro *et al.*, 2004; Nakage *et al.*, 2003; Morita *et al.*, 2010). Such alterations in egg development and eggshell conductance, promoted by incubation temperature, suggest the possible adaptability of the hematological respiratory parameters. Although literature exists on hematological parameters during embryonic development (e.g., Tazawa *et al.*, 1971a, b, 1991; 2011), whether incubation temperature deviations influence in a distinct form the development of hematological respiratory and energetic variables for both sexes in young and old breeders eggs remains unknown. Thus, the present study evaluated whether incubation temperatures differing by 1.5°C from the usual temperature from day 13 affect hematological respiratory and energetic parameters, as well as body, heart and liver growth of male and female fetuses and chicks from 30- and 60-week-old broiler breeders.

MATERIALS AND METHODS

Experimental design: Fertile eggs from 30- and 60-week-old broiler breeders (Cobb), weighing 61±2.61 g (light eggs) and 72±2.94 g (heavy eggs) (significant different means, $P \leq 0.05$) were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo). For each breeder age, eggs were weighed and distributed evenly by weight into six incubators (Premium Ecológica IP70) containing 70 eggs each and incubated for 12 days at 37.5°C dry bulb and 30°C wet bulb, with an automatic turning frequency of one per hour until day 18. From day 13 of incubation until hatching, two incubators were subjected to each of three temperatures: 36.5, 37.5 and 38.5°C. Relative Humidity (RH) was maintained at 60% during all incubation periods. Incubation temperature and RH were also controlled by a digital thermohygrometer (Hygrotherm, TFA, Germany).

Analyzed parameters: At 3, 6, 12, 24, 48 and 72 hours after incubation temperature change and at hatching, blood samples were collected and body and organ weights were measured for six fetuses and newly hatched chicks per sex per incubation temperature and breeder age. The following parameters were analyzed: body weight (g, percentage in relation to egg weight), heart and liver weights, somatic index as a percentage of body weight, hematological corpuscular volume (MCV, μm^3), RBC ($10^6/mm^3$), plasma glucose (GP, mg/dl) and hatching and mortality rates.

Body weights and blood samples were obtained prior to death by cervical dislocation for fetuses and chicks. The relative body weight was calculated as a percentage of egg weight and the relative weight of the organs was calculated as a percentage of body weight. Fetal blood samples were taken from the allantoic vessels, whereas hatchling blood samples were collected from the jugular vein. Blood samples were collected into tubes containing EDTA (Glistab, cat. 29; 15 µl/ml blood) and immediately analyzed. Blood samples were centrifuged at 3,500 rpm at 4°C for 15 minutes and plasma was collected and stored at 20°C for determination of glucose concentration using a commercially available kit (glucose PAP liquiform, 500 ml, cat. 84, Labtest Diagnóstica). All samples were prepared and analyzed following the manufacturer's recommendations. Two readings per sample were measured by spectrophotometry at a wavelength of 505 nm. Hatchability and mortality were calculated as percentages of incubated fertile eggs. Egg weight loss was calculated as the percentage of egg weight before incubation. For this determination, eggs were weighed prior to incubation and on day 18 of incubation. Eggshell conductance was calculated according to Christensen *et al.* (1994).

All animal handling protocols were approved by the Committee of Ethics in Animal Use (CEUA) of the Faculty of Agricultural and Veterinary Sciences (protocol number 020929/12), São Paulo State University.

Statistical analysis: For each broiler breeder age and sex group, the experiment was a 3x7 factorial arrangement of treatments, consisting of three incubation temperatures (36, 37.5 and 39°C) and seven ages (3, 6, 12, 24, 48 and 72 hours after incubation temperature manipulation and at hatching). All data were analyzed using the GLM procedure of SAS (SAS Institute, 2000), with different replicate numbers per treatment. Means were compared by F test and Tukey's test using significant difference at $P \leq 0.05$.

RESULTS

Body weights: As shown in Table 1, body weights of males and females from 30-week-old breeder eggs were influenced ($P \leq 0.05$) by age and increased from day 14 of incubation (24 hours after temperature change) to hatching. For males and females from 60-week-old breeder eggs, Absolute Body Weights (ABW) were affected by both age and temperature. Additionally, for males, a significant ($P \leq 0.05$) interaction between age and temperature occurred. The ABW of females increased from day 14 and were greater at 39°C than at 36 and 37.5°C. There was no significant difference between 36 and 37.5°C for this parameter. In males (Table 2), ABW increased from day 13 of incubation at 37.5°C and from day 14 at 36 and 39°C. At hatching, male chicks from eggs incubated at 37.5 and 39°C had similar ABW which was greater than ABW of chicks from eggs incubated at 36°C. Relative Body Weight (RBW) increased during late incubation in both sexes. In the female chicks, RBW were influenced by temperature and were lower ($P \leq 0.05$) when incubated at 36 and 39°C compared to 37.5°C.

Table 1: Effects of age and incubation temperature on body weight (g, %) of male and female fetuses and chicks from 30- and 60-weeks-old breeder eggs

	30 w. old				60 w. old			
	Absolute (g)		Relative (%)		Absolute (g)		Relative (%)	
Age (A)*	Male	Female	Male	Female	Male	Female	Male	Female
3 (13°)	9.30^{c}	9.10^{d}	15.0^{cd}	14.69^{d}	9.5	9.21^{d}	14.69^{d}	12.0^{d}
6 (13°)	9.03^{c}	9.12^{d}	14.64^{d}	15.02^{d}	9.72	9.47^{d}	15.02^{d}	11.54^{d}
12 (13°)	10.26^{c}	9.29^{d}	16.7^{cd}	15.12^{d}	9.93	10.04^{d}	15.12^{d}	13.92^{d}
24 (14°)	11.12^{c}	11.04^{d}	18.45^{c}	16.89^{d}	11.71	10.32^{d}	16.89^{d}	14.23^{d}
48 (15°)	15.06^{b}	13.67^{c}	24.85^{b}	22.54^{c}	14.56	14.86^{c}	22.54^{c}	20.20^{c}
72 (16°)	16.86^{b}	16.94^{b}	27.58^{b}	27.71^{b}	18.02	18.67^{b}	27.71^{b}	26.17^{b}
Hatching	45.69^{a}	44.84^{a}	75.23^{a}	73.16^{a}	53.82	50.59^{a}	73.16^{a}	72.80^{a}
Temperature (T)								
36°C	17.05	17.69	28.48	28.17	12.39	16.6^{a}	28.17	22.17^{b}
37.5°C	16.76	16.31	27.51	26.64	18.79	18.72^{a}	26.64	26.18^{a}
39°C	15.98	15.46	26.98	25.78	13.15	12.78^{b}	25.78	18.24^{b}
Probability								
A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T	0.6407	0.9587	0.5890	0.5630	0.0127	0.0003	0.5630	0.0003
AxT	0.8267	0.9208	0.7714	0.9596	0.0068	0.1998	0.9596	0.8059

$^{a-d}$: Values with different letters (columns) differ significantly by Tukey´s test (P<0.05).
*Age in hours after the change in the incubation temperature (days of incubation)

Table 2: Interaction between age and incubation temperature on body weight (g) of male fetuses and chicks from 60-weeks-old breeder eggs

	Temperature		
Age*	36°C	37.5°C	39°C
3 (13°)	8.67^{Ac}	9.93^{Ac}	9.89^{Ac}
6 (13°)	8.07^{Ac}	10.3^{Ac}	11.16^{Ac}
12 (13°)	9.22^{Ac}	9.66^{Ac}	11.25^{Ac}
24 (14°)	9.20^{Ac}	14.06^{Ab}	11.91^{Ac}
48 (15°)	12.47^{Ab}	16.29^{Ab}	15.04^{Ab}
72 (16°)	15.73^{Ab}	18.93^{Ab}	18.82^{Ab}
Hatching	59.72^{Aa}	52.34^{Ba}	49.98^{Ba}

$^{a-d}$,$^{A-B}$: Values with different letters (columns and lines, respectively) differ significantly by Tukey´s test (P≤0.05). *Age given in hours after the change in the incubation temperature (days of incubation)

Hepatic weights: The absolute and relative liver weights (ALW and RLW, respectively) of males and females from 30-week-old breeder eggs were clearly affected (P≤0.05) by age and temperature, with significant (P≤0.05) interactions between these two parameters (Table 3). In females, both ALW and RLW increased from day 13 of incubation (12 h after the incubation temperature change). Hepatic weight increased with incubation temperature but RLW remained unchanged at incubation temperatures of 36 and 37.5°C and increased at an incubation temperature of 39°C. In males, ALW increased from days 13, 15 and 14 at 36, 37.5 and 39°C, respectively (Table 4). At hatching, livers were heavier at 36°C than at 37.5 and 39°C which did not differ from each other. However, according to the interaction between age and temperature (Table 4), RLW increased from day 13 at 36°C and from day 14 at 37.5 and 39°C and was lower at hatching with higher incubation temperature. ALW of males and females from 60-week-old breeders were influenced significantly by age and incubation temperature and RLW was influenced only by age (P≤0.05). ALW increased from day 14 of incubation in females and from day 15 in males, whereas RLW increased from day 13 in the males and from day 14 in the females. ALW was lower at 36°C than at 37.5 and 39°C which did not differ from each other.

Heart weights: Absolute and relative heart weights of females and males from 30-week-old breeder eggs were significantly (P≤0.05) affected by breeder age, incubation temperature and the interaction between these two factors (Table 5). According to this interaction (Table 6), absolute heart weight increased from days 14, 15 and 16 of incubation at 36, 37.5 and 39°C, respectively. The effect of temperature on absolute heart weight occurred for all breeder ages. In males, this parameter was higher at an incubation temperature of 36°C than at 37.5 and 39°C, was similar between the two latter temperatures from day 14 until day 16 of incubation and diminished with increased temperature at hatching. In females, from day 16 of incubation until hatching, absolute heart weight was lower with higher incubation temperature. Relative heart weight was higher from day 16 of incubation compared with days 13, 14 and 15 and was lower at an incubation temperature of 39°C than at 36 and 37.5°C. In females, relative heart weight increased with age under incubation at 36°C and diminished at incubation temperatures of 37.5 and 39°C. From day 15, relative heart weights of females were higher at an incubation temperature of 36°C compared with the two other temperatures and

Table 3: Effects of age and incubation temperature on liver weight (g) and somatic index (%) of male and female fetuses and chicks from 30- and 60-weeks-old breeder eggs

	30 w. old				60 w. old			
	Weight (g)		Somatic Index (%)		Weight (g)		Somatic Index (%)	
Age (A)*	Male	Female	Male	Female	Male	Female	Male	Female
3 (13°)	0.16	0.16^{d}	0.25	0.25^{d}	0.17^{c}	0.17^{c}	0.23^{c}	0.22^{c}
6 (13°)	0.15	0.15^{d}	0.24	0.24^{d}	0.17^{c}	0.17^{c}	0.24^{c}	0.22^{c}
12 (13°)	0.17	0.14^{d}	0.27	0.25^{d}	0.18^{c}	0.18^{c}	0.25^{c}	0.24^{c}
24 (14°)	0.19	0.20^{cd}	0.32	0.33^{c}	0.19^{c}	0.18^{c}	0.33^{b}	0.25^{c}
48 (15°)	0.26	0.2^{c}	0.42	0.39^{c}	0.25^{c}	0.30^{b}	0.38^{b}	0.41^{b}
72 (16°)	0.32	0.33^{b}	0.52	0.51^{b}	0.33^{b}	0.35^{b}	0.45^{b}	0.49^{b}
Hatching	0.82	0.83^{a}	1.35	1.36^{a}	0.93^{a}	0.86^{a}	1.34^{a}	1.23^{a}
Temperature (T)								
36°C	0.33	0.31^{a}	0.50	0.49a	0.27^{b}	0.29^{b}	0.37	0.38
37.5°C	0.28	0.26^{b}	0.47	0.46a	0.33^{a}	0.36^{a}	0.36	0.34
39°C	0.19	0.20^{c}	0.30	0.33b	0.24^{b}	0.22^{b}	0.31	0.33
Probability								
A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T	0.0060	0.0051	0.0106	0.0108	0.0215	0.0464	0.2117	0.8271
AxT	0.0209	0.1135	0.0267	0.1790	0.8187	0.9090	0.8208	0.6881

$^{a-d}$: Values with different letters (columns) differ significantly by Tukey´s test ($P \le 0.05$). *Age given in hours after the change in the incubation temperature (days of incubation)

Table 4: Interaction between age and incubation temperature for liver weight and somatic index of male fetuses and chicks from 30-weeks-old breeder eggs

	Weight (g)			Somatic Index (%)		
Age*	36°C	37.5°C	39°C	36°C	37.5°C	39°C
3 (13°)	0.16^{Ad}	0.14^{Ac}	0.17^{Ac}	0.23^{Ad}	0.25^{Ad}	0.26^{Acd}
6 (13°)	0.15^{Ad}	0.15^{Ac}	0.14^{Ac}	0.25^{Ad}	0.24^{Ad}	0.22^{Ad}
12 (13°)	0.18^{Ad}	0.17^{Ac}	0.11^{Ac}	0.27^{Ad}	0.29^{Ad}	0.17^{Ad}
24 (14°)	0.20^{Acd}	0.19^{Ac}	0.18^{Ac}	0.31^{Ad}	0.33^{Acd}	0.30^{Ac}
48 (15°)	0.24^{Ac}	0.27^{Ac}	0.26^{Ab}	0.44^{Ac}	0.39^{Ac}	0.42^{Ab}
72 (16°)	0.38^{Ab}	0.31^{Ab}	0.28^{Ab}	0.53^{Ab}	0.61^{Ab}	0.46^{Ab}
Hatching	1.02^{Aa}	0.73^{Ba}	0.56^{Ca}	1.48^{Aa}	1.15^{Ba}	0.89^{Ca}

$^{a-d}$,$^{A-B}$: Values with different letters (columns and lines, respectively) differ significantly by Tukey´s test ($P \le 0.05$). *Age given in hours after the change in the incubation temperature (days of incubation)

Table 5: Effects of age and incubation temperature on heart weight and somatic index of male and female fetuses and chicks from 30- and 40-weeks-old breeder eggs

	30 w. old				60 w. old			
	Weight		Somatic Index		Weight		Somatic Index	
Age (A)*	Male	Female	Male	Female	Male	Female	Male	Female
3 (13°)	0.05	0.06	0.65^{ab}	0.67	0.08^{c}	0.09^{c}	0.11^{c}	0.12^{bc}
6 (13°)	0.07	0.06	0.64^{ab}	0.68	0.07^{c}	0.07^{c}	0.10^{c}	0.09^{c}
12 (13°)	0.06	0.05	0.55^{b}	0.56	0.07^{c}	0.07^{c}	0.10^{c}	0.09^{c}
24 (14°)	0.08	0.06	0.59^{b}	0.62	0.07^{c}	0.07^{c}	0.09^{c}	0.07^{c}
48 (15°)	0.09	0.07	0.58^{b}	0.57	0.12^{b}	0.11^{bc}	0.16^{b}	0.14^{b}
72 (162°)	0.11	0.11	0.74^{a}	0.72	0.10^{b}	0.12^{b}	0.14^{b}	0.16^{b}
Hatch	0.52	0.28	0.65^{a}	0.63	0.30^{a}	0.25^{a}	0.41^{a}	0.36^{a}
Temperature (T)								
36°C	0.12	0.11	0.66^{a}	0.67	0.10	0.10	0.16	0.16
37.5°C	0.09	0.09	0.62^{a}	0.65	0.11	0.11	0.15	0.15
39°C	0.06	0.06	0.55^{b}	0.57	0.10	0.10	0.16	0.16
Probability								
A	<0.0001	<0.0001	<0.0001	0.2742	<0.0001	<0.0001	<0.0001	0.0218
T	<0.0001	0.0077	<0.0001	0.0339	0.1465	0.6416	0.1749	0.1901
AxT	<0.0001	<0.0001	0.0508	<0.0001	0.0784	0.3423	0.0556	0.1574

$^{a-c}$: Values with different letters (columns) differ significantly by Tukey´s test ($P<0.05$). *Age given in hours after the change in the incubation temperature (days of incubation)

Table 6: Interaction between age and incubation temperature on heart weight and somatic index of male and female fetuses and chicks from 30-weeks-old breeder eggs

	Weight (g)						Somatic Index (%)		
	Male			Female			Female		
Age (A)*	36°C	37.5°C	39°C	36°C	37.5°C	39°C	36°C	37.5°C	39°C
3 (13°)	0.06^{Ac}	0.04^{Bb}	0.05^{Bb}	0.04^{Cc}	0.08^{Ac}	0.06^{Bb}	0.48^{Cd}	0.86^{Aa}	0.66^{Ba}
6 (13°)	0.07^{Ac}	0.05^{Bb}	0.07^{Ab}	0.06^{Ac}	0.06^{Ac}	0.06^{Ab}	0.66^{Ac}	0.70^{Ab}	0.69^{Aa}
12 (13°)	0.06^{Ac}	0.06^{Ab}	0.07^{Ab}	0.06^{Ac}	0.05^{ABc}	0.04^{Bb}	0.63^{Ac}	0.59^{Ac}	0.51^{Bb}
24 (14°)	0.08^{Ac}	0.07^{Bb}	0.07^{Bb}	0.07^{Ac}	0.07^{Ac}	0.06^{Ab}	0.63^{Bc}	0.74^{Ab}	0.56^{Cb}
48 (15°)	0.1^{Abc}	0.09^{Bb}	0.08^{Bb}	0.08^{Ac}	0.07^{Ac}	0.07^{Ab}	0.61^{Ac}	0.56^{Bc}	0.54^{Bb}
72 (16°)	0.16^{Ab}	0.11^{Bab}	0.08^{Bb}	0.15^{Ab}	0.11^{Bb}	0.08^{Cb}	0.91^{Aa}	0.63^{Bbc}	0.49^{Cc}
Hatch	0.37^{Aa}	0.25^{Ba}	0.17^{Ca}	0.34^{Aa}	0.22^{Ba}	0.17^{Ca}	0.78^{Ab}	0.51^{Bc}	0.37^{Cd}

$^{a-d}$,$^{A-C}$: Values with different letters (columns and lines, respectively) differ significantly by Tukey´s test ($P \leq 0.05$).
*Age given in hours after the change in the incubation temperature (days of incubation)

Table 7: Effects of age and incubation temperature on hematological parameters of male and female fetuses and chicks from 30-weeks-old breeder eggs

	Male				Female			
Age (A)*	HCT (%)	HGB (g/dl)	RBC (10^6/mm^3)	MCV (µm^3)	HCT (%)	HGB (g/dl)	RBC (10^6/mm^3)	MCV (µm^3)
3 (13°)	12.3^{b}	5.47^{b}	1.10^{b}	114.70^{a}	6.25^{b}	4.13^{b}	0.55^{b}	114.93^{a}
6 (13°)	8.91^{c}	5.18^{b}	0.81^{c}	111.29^{a}	8.24^{b}	4.99^{b}	0.76^{b}	112.96^{a}
12 (13°)	7.05^{c}	5.63^{b}	0.62^{c}	114.21^{a}	9.14^{b}	5.45^{b}	0.83^{b}	110.05^{a}
24 (14°)	9.0^{c}	5.47^{b}	0.81^{c}	107.56^{b}	6.08^{b}	4.30^{b}	0.58^{b}	104.69^{b}
48 (15°)	19.14^{b}	11.21^{a}	1.84^{b}	104.93^{b}	25.31^{a}	13.11^{a}	2.49^{a}	102.49^{b}
72 (16°)	19.56^{b}	10.82^{a}	1.96^{b}	100.77^{b}	26.12^{a}	13.24^{a}	2.69^{a}	102.09^{b}
Hatch	27.64^{a}	14.62^{a}	2.89^{a}	95.8^{c}	27.64^{a}	14.62^{a}	2.89^{a}	95.8^{c}
Temperature (T)								
36°C	13.56	8.89	1.33	109.85	15.63	9.11	1.89	104.63
37.5°C	14.78	8.65	1.29	106.58	15.89	8.59	1.63	107.85
39°C	15.88	7.66	1.67	104.56	17.88	7.59	1.74	103.86
Probability								
A	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0002
T	0.4588	0.6053	0.5083	0.3101	0.4339	0.3127	0.3422	0.8618
AxT	0.6944	0.5848	0.7821	0.7123	0.2144	0.0911	0.1989	0.1863

HCT: Hematocrit, HGB: Hemoglobin, RBC: Red body count, MCV: Mean cell volume.
$^{a-d}$: Values with different letters (columns) differ significantly by Tukey´s test ($P \leq 0.001$).
*Age given in hours after the change in the incubation temperature (days of incubation)

decreased with increasing temperature from day 16 of incubation. In males and females from 60-week-old breeder eggs (Table 5), both absolute and relative heart weights were influenced significantly ($P \leq 0.05$) only by age and increased from day 14 of incubation until hatch.

Blood parameters: Independent of broiler breeder age, in both sexes, all analyzed respiratory blood parameters were significantly ($P \leq 0.05$) influenced only by embryo age (Table 7 and 8). As shown in Table 7, in males from 30-week-old breeder eggs, both HCT and RBC values increased from 24 to 48 hours (days 14 and 15 of incubation) and from 72 hours to hatching. HGB values increased from 24 to 48 hours and then remained unchanged until hatching, whereas MCV values decreased from 12 to 24 hours and from 72 hours to hatching. In females from 30-week-old breeder eggs, HCT, HGB and RBC values increased from 24 to 48 hours and the values were maintained from 48 hours to hatching, whereas MCV values decreased from 12 to 24 hours and from 72 hours to hatching. Table 8 shows that in males from 60-week-old breeder eggs, HCT values increased from 12 to 24 hours and from 72 hours to hatching; HGB increased from 6 to 24 hours and from 72 hours to hatching and RBC values increased from 72 hours to hatching, whereas MCV values decreased from 72 hours to hatching. In females from 60-week-old breeder eggs, HCT values increased from 12 to 24 hours and from 72 hours to hatching; HGB increased from 72 hours to hatching; and RBC values increased from 12 to 24 hours and from 72 hours to hatching. MCV values decreased from 72 hours to hatching. Levels of blood glucose were significantly ($P \leq 0.05$) affected by age and incubation temperature in both sexes and broiler breeder ages. In males and females from 30- and 60-week-old breeder eggs, blood glucose levels increased from 72 hours to hatching and from 12 hours to hatching, respectively. Independent of broiler breeder age, males presented higher glucose levels at an incubation temperature of 39°C than at 37.5°C, whereas

Table 8: Effects of age and incubation temperature on hematological parameters of male and female fetuses and chicks from 60-weeks-old breeder eggs

	Male				Female			
Age (A)*	HCT (%)	HGB (g/dl)	RBC (10^6/mm^3)	MCV (µm^3)	HCT (%)	HGB (g/dl)	RBC (10^6/mm^3)	MCV (µm^3)
3 (13°)	6.83^c	0.68^c	3.02^b	98.60^a	4.44^c	7.74^b	0.46^c	97.47^a
6 (13°)	7.79^c	0.78^c	4.37^b	99.50^a	4.72^c	4.87^b	0.48^c	98.75^a
12 (13°)	4.16^c	0.44^d	4.83^b	92.16^b	5.27^c	4.88^b	0.56^c	93.89^b
24 (14°)	11.57^b	1.15^b	6.37^b	99.08^a	11.6^b	8.0^b	1.16^b	100.89^a
48 (15°)	10.75^b	1.18^b	5.73^b	97.58^a	11.2^b	6.92^b	1.14^b	98.59^a
72 (16°)	15.22^b	1.55^b	8.34^b	98.80^a	12.04^b	7.46^b	1.22^b	99.22^a
Hatch	35.48^a	3.80^a	17.82^a	93.70^b	38.45^a	18.8^a	4.15^a	92.52^b
Temperature (T)								
36°C	13.14	1.38	7.25	97.65	12.48	8.11	1.14	95.17
37.5°C	16.58	1.49	8.19	93.54	13.01	8.47	1.28	97.25
39°C	15.28	1.54	7.99	92.87	12.66	8.85	1.09	95.77
Probability								
A	<0.0001	<0.0001	<0.0001	0.0086	<0.0001	0.0096	<0.0001	<0.0001
T	0.2592	0.0733	0.0987	0.0818	0.4475	0.6944	0.3711	0.0901
AxT	0.3182	0.2268	0.6673	0.1282	0.9404	0.5840	0.9327	0.2614

HCT: Hematocrit, HGB: Hemoglobin, RBC: Red body count, MCV: Mean cell volume.
a-c: Values with different letters (columns) differ significantly by Tukey´s test (P<0.01).
*: Age given in hours after the change in the incubation temperature (days of incubation)

Table 9: Effects of age and incubation temperature on plasma glucose levels (µg/µL) of male and female fetuses and chicks from 30- and 40-weeks-old breeder eggs

	30 w. old		60 w. old	
Age (A)*	Male	Female	Male	Female
3 (13°)	0.21^b	0.20^b	0.18^c	0.17^c
6 (13°)	0.21^b	0.19^b	0.21^c	0.18^c
12 (13°)	0.23^b	0.24^b	0.21^c	0.22^c
24 (14°)	0.26^b	0.26^b	0.25^{bc}	0.27^{bc}
48 (15°)	0.26^b	0.26^b	0.26^{bc}	0.31^b
72 (16°)	0.29^b	0.30^b	0.31^b	0.36^b
Hatch	0.50^a	0.50^a	0.51^a	0.53^a
Temperature (T)				
36°C	0.27^{ab}	0.30^a	0.28^{ab}	0.32^a
37.5°C	0.23^b	0.23^b	0.23^b	0.23^b
39°C	0.33^a	0.30^a	0.32^a	0.32^a
Probability				
A	<0.0001	<0.0001	<0.0001	<0.0001
T	<0.0001	<0.0001	<0.0001	<0.0001
AxT	0.1598	0.2589	0.1353	0.2756

a-c: Values with different letters (columns) differ significantly ($P \leq 0.0001$). *Age given in hours after the change in the incubation temperature (days of incubation)

females had similar glucose levels at incubation temperatures of 36 and 39°C and lower levels at 37.5°C.

Hatching and mortality rates, eggshell conductance and egg mass loss: As shown in Table 10, hatching rate was highest and mortality rate was lowest for egg incubation at 37.5°C, followed by incubation at 36 and 39°C. Mortality rate at an incubation temperature of 39°C was 24.6% higher in 30-week-old breeder eggs than in 60-week-old breeder eggs. Both eggshell conductance and egg mass loss increased with incubation temperature.

DISCUSSION

The present study shows that continuously high temperature during the second half of incubation reduced the heart growth in males and females chicks from 30-weeks-old breeder eggs, causing them a cardiac hypoplasia at hatching, not observed for chicks from 60-weeks-old breeder eggs. Although others authors (Wineland *et al*., 2000; Leksrisompong *et al*., 2007) had reported smaller heart weight at high incubation temperature, this effect breeder age related of the high incubation temperature on this organ is being shown for the first time. Despite reduced heart weight and somatic index in 30-week-old breeder eggs (light eggs) had been reported after incubation at high temperature, blood respiratory parameters (RBC, HCT, HGB and MCV) remained unchanged, indicating that fetuses did not respond with increase in the hematological respiratory parameters to higher oxygen demand that result from the increase in metabolic rate promoted by higher incubation temperature. From the beginning of pulmonary respiration at internal pipping, the influx of air into the pulmonary system is a greater cardiac demand than previously experienced by the embryo during incubation. Cardiac deficits during this early period of pulmonary respiration can negatively affect the gas exchange and can have leaded the 30-week-old breeder eggs incubated at 39°C to present reduced hatching success which did not reach 10% in the present study. Cardiac deficits increase the susceptibility of the bird to metabolic diseases related to cardiovascular underdevelopment, such as ascites syndrome (Lubritz and MacPherson, 1994; Leksrisompong *et al*., 2007). Thus, our data show that the decreased heart development resulting from

Table 10: Effects of incubation temperature on hatching and mortality rates, eggshell conductance and egg mass loss in 30- and 40-weeks-old breeder eggs

	30 weeks old				60 weeks old			
Variables	36°C	37.5°C	39°C	P	36°C	37.5°C	39°C	P
Hatching rates (%)	48.5^{B}	56.3^{A}	8.7^{C}	<0.0001	50.0^{B}	61.0^{A}	33.3^{C}	<0.0001
Mortality rates (%)	51.5^{B}	43.7^{C}	91.3^{A}	<0.0001	50.0^{B}	39.0^{C}	66.7^{A}	<0.0001
Eggshell conductance	0.37^{C}	0.45^{B}	0.50^{A}	<0.0001	0.42^{C}	0.53^{B}	0.58^{A}	<0.0001
Mass loss (%)	15.6^{C}	19.1^{B}	21.4^{A}	<0.0001	13.4^{C}	16.7^{B}	18.4^{B}	<0.0001

$^{A-C}$: Values with different letters (columns) differ significantly (P $\leq$ 0.0001)

incubation at high temperature can predispose chicks from 30-weeks-breeder eggs but not chicks from old breeder eggs, to developing ascites.

We also encountered that temperatures continuously 1.5°C lower or higher than the standard incubation temperature had distinct effects on eggs body, liver and heart weights and indexes, plasma glucose levels of males and females from 30- and 60-week-old breeder eggs. The body weights of males and females from 30-week-old breeder eggs were not influenced by incubation temperature but their hepatic weights and indexes decreased increasing incubation temperature and females had higher glucose levels at 36 and 39°C and males had higher glucose levels at 39°C only. Differently, in 60-week-old breeder eggs, male weight was reduced by high and low incubation temperature, while the female weight was reduced by high incubation temperature only and, in both sexes, liver weight and hepatic index were lower at 36 and 39°C. At the same times, females had higher glucose levels at 36 and 39°C than at 37.5°C, whereas males had higher glucose levels only at 39°C. Considering that, with isometrical increases in the size, volume increases more rapidly than surface area (Woods, 1999) and that the increase in the eggshell conductance is not proportional to increase in fetal mass (Meijerhof and Van Beek, 1993), it is possible that the larger eggs have greater difficulty than smaller eggs to realize gas changes with the air of the incubator, mainly at high incubation temperature and that this had promoted the reduction in the chick weight from 60-weeks-old at hatching, not observed for chicks from 30-weeks old breeder eggs. Lower and higher temperature than the usual incubation temperature can slow or accelerate embryonic development, respectively (Romanoff, 1960; Lancaster and Jones, 1988; Wilson, 1991; Leandro *et al*., 2004; Nakage *et al*., 2003; Morita *et al*., 2010) by changing embryo growth, tissue metabolism and respiratory rate. Increased energy production and the subsequent increased metabolism occur from day 10 of eggs incubation in broiler and emu (Nichelman *et al*., 1998; Prizinger *et al*., 1997). Embryo exposure to high incubation temperature during the plateau phase of the oxygen requirement markedly stimulates growth and carbohydrate metabolism (Christensen *et al*., 1999). On the other hand, a longer incubator period also arise a longer period of energetic demand. Carbohydrate mobilization and hyperglycemia in response to stress can result from hepatic glycolysis (Barton *et al*., 1987) which can reduce hepatic growth and the hepatosomatic index (Davis *et al*., 1988; Barton *et al*., 1987). This indicates that the increased blood glucose levels and the reduced liver weights reported by the present study under incubation at temperatures 1.5°C lower or higher than usual at hatching are related. The results of the present study are in agreement with a previous study in which embryonic tolerance to higher temperature was shown to be less than tolerance to lower temperature (Wilson, 1991) and should be related to increased egg weight loss. Significant reductions in hatching success with increasing incubation temperature from day 13 of incubation were also observed for broiler breeder eggs by Givisiez *et al*. (2001) and for red-winged tinamou eggs by Nakage *et al*. (2003) and Boleli and Queiroz (2012).

Yet, according to our results, in all used incubation temperature, males and females from 30-w-old and 60-w-old maintained a fetal development characterized by increase in body and organ weights, plasma glucose levels and RBC, HCT and HGB values and by simultaneous decrease in MCV values. This reveals a preserved pattern of fetal ontogenetic development for broiler, independent of incubation temperature, sex and breeder age. These changes in the blood respiratory and energetic parameters and liver and heart weights are consistent with previous studies of Morita *et al*. (2009, 2010) and Tazawa *et al*. (2011) for broilers and emus, respectively, under usual incubation temperature and they appear to be necessary to supply the increased oxygen and energy demands promoted by metabolic increases during fetal growth in the second half of incubation (Nichelman *et al*., 1998; Prizinger *et al*., 1997).

Conclusion: High temperature during the second half of the incubation period predisposes chicks from light eggs but not chicks from heavy eggs, to ascites syndrome after hatching and has differential effects on plasma glucose-levels and body weight of male and female chicks from light and heavy eggs.

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