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

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Hematological and Incubation Parameters of Chicks from Young Breeders Eggs: Variation with Sex and Incubation Temperature

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

Abstract: This experiment analyzed the effect of sex and incubation temperature on daily mass loss and eggshell conductance, embryo mortality rates, incubation duration, hematological parameters and body, liver, heart and bursa weights of neonatal chicks from young breeders. The daily mass loss was higher at incubation temperature of 39°C. The eggshell conductance rate increased with the temperature. The total and partial duration of incubation were lower for eggs incubated at 39°C. The time taken by the chick to leave the eggshell did not differ below and above the thermoneutral temperature. The total and intermediate embryo mortality rates increased with the incubation temperature, whereas the early and late embryo mortality rates were higher at incubation temperature of 39°C. Sex did not influence the analyzed parameters, while the incubation temperature did not affect the body and bursa weight and the erythrocytes characteristics. The liver weight of chicks incubated at 36°C was higher than the incubated at 39°C, however there were no differences among the liver weight from chicks incubated at 36 and 39°C and those incubated at 37.5°C. The number of heterophils and the heterophil/lymphocyte ratio (H/L ratio) increased following the temperature, whereas the number of lymphocytes decreased at high temperatures. The other leukocyte parameters did not suffer influence of temperature. Males and females presented similar response to variation of incubation temperatures (36, 37.5 and 39°C) and demonstrated higher sensibility to temperatures above the thermoneutral. Moreover, temperatures below the thermoneutral demonstrated to be better for improvement of hatchability and development of chicks from light eggs.

Key words: Chick, erythrogram, leukogram, thermal stress

INTRODUCTION

The low hatchability rate of young breeder eggs associated with the low chick body weight at the moment of the hatch and at slaughter becomes these eggs less attractive and increases the possibility of discarding for incubation.

The appropriate temperature to maximize the hatchability rate and the neonatal chick quality is called optimum incubation temperature (Ar, 1995; French, 1997) and is primarily determined by hatchability rate and embryo malformations. This temperature for many domestic birds is 37-38°C and the ideal relative humidity between 50 and 60% (Boleli, 2003). The usual temperature to incubate *Gallus domesticus* eggs is from 37.5-37.8°C, independent of the egg weight.

Morita *et al*. (2009) described that young breeder eggs present lower eggshell conductance than the older breeder eggs, which is related with the higher values of eggshell thickness, the minor porosity and the lower eggshell surface of young breeder eggs. The eggshell conductance determine the gas exchange rate between the egg exterior and interior (Campos and Santos, 2003) and is the main factor responsible by egg heat loss together with the convection process (La Scala, 2003). Based on these observations, it is necessary to prove if the incubation temperatures more used currently (the usual incubation temperature) are the most appropriate to be used for light eggs.

Temperature above the thermoneutral increases the metabolic rate and alters the immune response, increasing the oxigen consumption (Von Bertalanffy, 1960; Woods, 1999) and modifying the blood characteristics (Barton *et al*., 1987; Maxwell e Robertson, 1995, 1998), respectively. Hematological values can be used to make diagnosis of respiratory, metabolic, nutritional and immune alterations resulted from handling problems.

This study was conducted to evaluate the effects of sex and incubation temperature (36, 37.5 and 39°C) on egg mass loss, eggshell conductance, embryo mortality

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

rates, incubation duration and blood parameters (erythrocytes and leukocytes), body, liver and bursa weights of newly-hatched chicks of eggs from young breeders.

MATERIALS AND METHODS

Incubation: Fertile eggs (Cobb^{TM}) were obtained at commercial incubatory weighing from 56-61 g. These eggs were originated from broiler breeders with 29 weeks of age. A total of 270 eggs were homogeneously distributed in six incubators, according to the weight (two incubators per temperature and 45 eggs per incubator) and separated in three incubation temperatures (36, 37.5 and 39°C). Incubators (Premium Ecológica) with temperature and turns controlled automatically (1 turn/2 h) were used. The relative humidity inside the incubator was maintained at 60% and controlled by digital thermohygrometer (Hygrotherm, TFA, Germany).
The eggs were stopped to be turned from 18th day of incubation.

Analyzed parameters: The daily and total loss of egg mass were determined for each incubation temperature and expressed in grams. A total of 10 eggs/incubator/incubation temperature were weighed daily during the first week of incubation, always in the same hour.
The eggshell conductance was calculated in agreement with Christensen and Nestor (1994) using the following formula: C = DLM/PVS, being C the conductance, DLM the daily loss of mass and PVS the pressure of vapor saturation = 23.86 mm Hg at 25°C.
The total duration of the incubation (TDI) was considered the period from the beginning of the egg incubation (zero hour) until the hatchability (moment that the chick left the eggshell). The Partial Duration of the Incubation (PDI) was considered the period between the beginning of the incubation and the moment of the external pipping; and the time taken by the chick to leave the eggshell (time to leave the eggshell-TLE) was the period between the egg breakage and the exit of the chicks from the eggshell. These parameters were expressed in hours.
The hatchability rate and embryo mortality rate were determined at the end of incubation period and were expressed in percentage in relation to the number of incubated fertile eggs. The total mortality rate and the early (from 1 to 7 days of incubation), intermediate (from 8 to 14 days of incubation) and late (from 15 days of incubation) embryo mortality rates/incubator/temperature were obtained.
The weight of the body, liver and bursa from chicks were measured after waiting the down drying of 10 chicks from each age and temperature. The weight was measured and the blood was collected before chick euthanasia that was done by using the method of cervical dislocation. The relative body weight was calculated as percentage of the egg weight and the relative organ weight as percentage of the weight of body and eggs.
The blood was collected in plastic tubes (1.5 ml) containing anticoagulant for analysis of the erythrocyte parameters: hematocrit (HCT, %), hemoglobin (HGB, g/dl), total count of red blood cells (RBC, $x10^6/mm^3$) and mean corpuscular volume (MCV, μ^3). For the total count of leukocytes, the blood samples were diluted (1:100) with Natt and Herrick solution (1952), in agreement with Sterzo (2007). After this procedure, the leukocytes count (n/µL) was done using Neubauer chamber. The differential count of leukocytes was done observing 100 white blood cells (Barton *et al*., 1987; Gonzáles *et al*., 2003), after colouring the glass slides containing the spread blood with the kit fast Panótipo.

Statistical analysis: The results of body and organ weights and blood parameters of the neonatal chicks were analyzed by completely randomized design with the following treatments: three temperatures (36, 37.5 and 39°C) and two sexes (male and female). The results of mean loss of egg mass, eggshell conductance, mortality rates and incubation duration were submitted to analysis of variance at completely randomized design to evaluate the incubation temperature effect. In both cases, the pair-wise comparisons of means were made using Tukey's test procedure at 5%, when F value was significant ($p \leq 0.05$). All the statistical results were obtained from SAS software (2004).

RESULTS

The data of daily mass loss, eggshell conductance, incubation duration and embryo mortality rates reported for the different incubation temperatures are presented in Table 1. The DML was higher ($p \leq 0.05$) at incubation temperature of 39°C than at 36 and 37.5°C. These two last cited temperatures did not differ from one another in this parameter. The eggshell conductance increased ($p \leq 0.05$) when the incubation temperature became higher. It was detected no significant difference ($p > 0.05$) in the values of total duration of the incubation and partial duration of the incubation between the temperatures of 36 and 37.5°C, but at these both temperatures these parameters were higher ($p \leq 0.05$) than at 39°C. The time to leave the eggshell was higher ($p \leq 0.05$) for the incubation temperature of 36°C than at 37.5 and 39°C. which did not differ from one another in relation to this time. The total and intermediate embryo mortality rates increased when the incubation temperature became higher. The early and late embryo mortality rates were similar at 36 and 37.5°C, but increased ($p \leq 0.05$) at the incubation temperature of 39°C. The hatchability of light eggs was 5 and 28% higher at incubation temperature of 36°C than at 37.5 and 39°C, respectively.

Table 1: Effects of incubation temperature on Daily Mass Loss (DML), Eggshell Conductance (ESC), Total (TDI) and Partial (PDI) duration of incubation, Time Between External Pipping and Hatching (TEPH) and Total (TEMR), Early (EEMR), Intermediate (IEMR) and Late (LEMR) embryonic mortality rates of eggs from 29 weeks old breeder

Temp. (°C)	DML (g)	ESC	TDI (h)	PDI (h)	TEPH (h)	TEMR (%)	EEMR (%)	IEMR (%)	LEMR (%)
36	0.14B	9.07C	489.6A	469.3A	20.3A	12.09C	3.22B	0.0C	9.67B
37.5	0.21B	11.6B	486.8A	476.3A	10.8B	18.18B	3.03B	3.03B	12.12B
39	0.30A	14.3A	470.6B	456.7B	13.9AB	40.0A	10.0A	6.66A	23.33A
p	0.030	0.016	0.001	0.004	0.002	0.001	0.025	0.001	0.012

A-C: Means in the same column followed by different letters differ significantly ($p<0.05$). Temp. = Temperature

Table 2: Effects of incubation temperature and sex on the absolute and relative body weight of newly-hatched chicks from eggs of 29 weeks old breeders

Probability	Absolute (g)	Relative (%)
Temperature (T)	0.575	0.413
Sex (S)	0.103	0.527
TxS	0.117	0.647
Temperature		
36°C	43.58±1.84A	74.04±2.02A
37.5°C	43.34±1.75A	73.07±2.32A
39°C	44.01±0.93A	74.30±1.73A
Sex		
Male	44.14±1.02A	74.04±1.98A
Female	43.22±1.62A	73.56±2.01A

A: Means in the same column followed by different letters differ significantly ($p<0.05$)

The incubation temperature, sex and the interaction between these both factors (Table 2) did not influence significantly ($p>0.05$) the chick body weight.

The absolute (g) and relative (%) weight of liver and bursa are presented at Table 3. The sex, incubation temperature and the interaction between sex and temperature did not present significant effect ($p>0.05$) on bursa weights. The absolute and relative weight of the liver decreased significantly ($p\leq0.05$) with the temperature increase.

The values of RBC, HCT, MCV and HGB (Table 4) did not suffer significant influence ($p>0.05$) of the incubation temperature, sex and interaction between temperature and sex.

The total and differential counts of leukocytes are presented in the Table 5. The sex, incubation temperature and the interaction between these both factors did not influence significantly ($p>0.05$) the total leukocytes counts and the percentage of monocytes, basophils and eosinofils. The percentage of heterophils and lymphocytes and the H/L ratio were influenced by incubation temperature, but the sex did not affect these parameters. The heterophils percentage was higher and the lymphocyte percentage was lower at incubation temperature of 39°C than at 36 and 37.5°C and these parameters did not differ between the last cited temperatures. The H/L ratio and the heterophil percentage presented similar response to temperature, being higher at incubation temperature of 39°C than 36 and 37.5°C. The temperatures of 36 and 37.5°C did not differ from one another for H/L ratio.

DISCUSSION

The results showed embryo development was faster for incubation at high temperatures and the mass loss and eggshell conductance increased at high incubation temperature. It is known that incubation temperature above the thermoneutral increases the metabolic rate and consequently, the oxigen consumption (Von Bertalanffy, 1960; Woods, 1999). Considering that the eggshell conductance corresponds to the capacity of gas exchange between the egg interior and exterior (Campos and Santos, 2003), the results obtained in this present experiment indicated that the embryos from eggs submitted to high temperature presented higher gas exchange rate, which is essential to supply the fast metabolism and guarantee the rapid development.

The thermoregulatory capacity of *G. domesticus* starts to develop only at the last days of incubation (Tazawa *et al*., 1989; Nair *et al*., 1983) and finishes to develop completely after the hatching (Dietz and Van Kampen, 1994). The embryo needs to receive heat for morphogenesis and maintenance during the first part of incubation, depending on the incubator heat source. The production of metabolic heat increases during the embryonic period (from 8 days of incubation) (Romojin e Lokhorst, 1960); thus, the maintenance of the embryo normothermia and homeostasis from 8 days of incubation is dependent of heat loss by the eggs. The eggs incubated at high temperatures need to lose more metabolic heat than the eggs incubated at low temperatures for the embryo did not suffer damage in its development. The eggs lose heat by conductance (water evaporation) and by emission of heat from eggshell surface (mainly by convection) (La Scala, 2003). In this present experiment, the eggshell conductance increased with the incubation temperature and the embryo mortality rate also increased, suggesting that the heat loss by the egg was insufficient to keep the normothermia inside the eggs and consequently the embryo homeostasis.

Ono *et al.* (1994) described that the embryos are more sensible to high temperatures at the end of the incubation period, because incubation temperatures above the optimum promote excessive water loss by the eggs, causing high late mortality rates by dehydration. In this experiment, the early, intermediate and late embryo mortality rates increased with the incubation temperature evidencing that the high incubation

Table 3: Effects of incubation temperature and sex on absolute and relative liver and bursa weights of newly-hatched chicks from eggs of 29 weeks old breeders

Probability	ALW (g)	RLW-EW (%)	RLW-BW (%)	ABW (g)	RBW-EW (%)	RBW-BW (%)
Temperature (T)	0.014	0.008	0.015	0.626	0.632	0.672
Sex (S)	0.124	0.057	0.054	0.924	0.825	0.890
TxS	0.122	0.187	0.263	0.640	0.712	0.807
Temperature						
36°C	0.73±0.05A	1.24±0.08A	1.68±0.12A	0.04±0.01A	0.06±0.02A	0.09±0.02A
37.5°C	0.68±0.06AB	1.14±0.11AB	1.57±0.17AB	0.04±0.01A	0.06±0.03A	0.09±0.04A
39°C	0.63±0.07B	1.07±0.12B	1.45±0.19B	0.03±0.01A	0.05±0.02A	0.08±0.03A
Sex						
Male	0.65±0.05A	1.10±0.09A	1.48±0.14A	0.04±0.02A	0.06±0.03A	0.09±0.04A
Female	0.70±0.07A	1.19±0.13A	1.62±0.15A	0.04±0.02A	0.07±0.03A	0.09±0.04A

ALW: Absolute Liver Weight, RLW-EW: Liver Weight Relative to Egg Weight, RLW-BW: Liver Weight Relative to Body Weight, ABW: Absolute Bursa Weight, RBW-EW: Bursa Weight Relative to Egg Weight, RBW-BW: Bursa Weight Relative to Body Weight. A-B: Means in the same column followed by different letters differ significantly ($p<0.05$)

temperature affected the embryo development of young breeders eggs during the whole period of incubation. The water loss of the egg increased with the incubation temperature, indicating that the high embryo mortality rate in high incubation temperatures can be due to excessive water loss by eggs and consequent dehydration. Morita *et al.* (2009) and Givisiez *et al.* (2000) registered a decrease of hatchability rate at temperatures higher than the usual for broiler chickens and Nakage *et al.* (2003) for partridges. The results of this experiment are in agreement with Wilson (1991), who concluded the chick tolerance zone for temperatures higher than the thermoneutral is lower than for temperatures lower than the thermoneutral.

In this present experiment the total embryo mortality rate reached aproximately 40, 20 and 10% of the eggs incubated at 39°C, 37.5 and 36°C, respectively. Moreover, as mentioned, the lower mortality rates occurred during the whole incubation period at 36°C. These results indicated that the incubation temperature lower than the usual should be used for young breeder eggs and therefore the incubation in multistage system is not the most appropriate.

The total and partial duration of the incubation were similar at 36 and 37.5°C and lower at 39°C, indicating that the duration of the embryo development of young breeder eggs does not present a direct correlation with the incubation temperature, as registered by French (1997) for turkeys and by Decuypere *et al.* (1979) and Wilson (1991) for broilers. Differently, the period between the eggshell breakage and the exit of the chicks from the eggshell was higher at 36°C, suggesting that when the incubation temperature is low the time spent by chick to finish the eggshell breakage and to have sufficient force to leave the eggshell is higher. This delay of the chicks to leave the eggshell can be related to the hypothermia caused by the incubation temperature.

In relation to the chick body weight at the moment of the hatchability in this present experiment, it was not verified influence of the incubation temperature, sex and interaction between these both factors. These results differ from that described by Henry and Burke (1997) for broilers, that registered higher weight for males than females. Significant differences between body weight of males and females were also observed by other authors for chicken and turkey embryos (Burke and Sharp, 1989; Burke *et al.*, 1990; Mitchell and Burke, 1995). However, Boleli and Moraes (2004) worked with different egg weights and did not find sex-specific differences in relation to body weight of neonatal chicks.

Neither between sex nor among temperatures the relative body weights differed. The relative weights were equivalent to approximately 75% of the initial egg weight, being this value inside the interval of relative weight percentage from 75-80% considered as normal to chicks by Henry and Burke (1997). Chicks obtain approximately 50% of the energy necessary to metabolic process from the fat reserve of the yolk sac (Murakami *et al.*, 1988) and for each gram of fat used, a quantity almost equal of water is produced (La Scala, 2003). Therefore, the absence of difference among the temperatures for chick body weight at hatchability can be related to higher production of metabolic water, in spite of the higher water loss presented by eggs incubated at high temperature.

The livers were weighed, presenting relative weight higher for incubation at 36°C than 39°C, evidencing high sensibility of this organ to high incubation temperature, that can have affected the liver organogenesis. The liver performs important function in energetic metabolism. Therefore, in this present experiment the low liver development probably prejudiced the energetic metabolism and consequently the development and survival of the embryo, increasing the mortality rate mainly in the end of the incubation period (late mortality).

The absolute and relative weights of bursa were not influenced significantly by incubation temperature and chick sex, indicating a low sensibility of this organ to deviation below or above 1.5°C from the thermoneutral temperature.

In relation to the erythrocyte parameters, it was observed that neither the temperature nor the sex influenced

Table 4: Effects of incubation temperature and sex on Red Blood Cells Counts (RBC, x10^6/mm^3), Hematocrit (HCT, %), Mean Corpuscle Volume (MCV, μ^3) and Hemoglobin (HGB, g/dl) of newly-hatched chicks from eggs of 29 weeks old breeders

Probability	RBC	HCT	MCV	HGB
Temperature (T)	0.502	0.331	0.575	0.645
Sex (S)	0.552	0.823	0.778	0.555
TxS	0.903	0.684	0.489	0.846
Temperature				
36°C	2.67±0.52A	22.76±4.33A	87.41±5.95A	14.13±3.27A
37.5°C	2.34±0.56A	19.75±3.38A	86.06±7.58A	12.55±3.33A
39°C	2.41±0.59A	20.14±4.49A	83.83±7.25A	13.26±3.47A
Sex				
Male	2.37±0.54A	20.47±3.79A	85.93±6.89A	12.76±3.38A
Female	2.54±0.57A	21.12±4.41A	85.40±7.24A	13.68±3.41A

A: Means in the same column followed by different letters differ significantly ($p<0.05$)

Table 5: Effects of incubation temperature and sex on total (cell number/uL of blood) and differential (%) leukocyte counts and H/L ratios in newly-hatched chicks from eggs of 29 weeks old breeders

Probability	Total counts	Heterophils	Lymphocytes	Monocytes	Basophils	Eosinophils	H/L
Temperature (T)	0.685	0.014	0.016	0.748	0.815	0.198	0.027
Sex (S)	0.391	0.361	0.184	0.477	0.123	0.803	0.780
TxS	0.606	0.085	0.078	0.082	0.196	0.593	0.023
Temperature							
36°C	9813±3846A	12.3±2.6B	80.9±6.0A	4.4±1.8A	2.0±0.3A	0.5±0.1A	0.15B
37.5°C	8450±3649A	13.0±3.0B	83.3±4.2A	2.6±0.9A	1.0±0.1A	0.1±0.1A	0.15B
39°C	10050±4369A	25.2±3.6A	70.1±6.2B	3.0±1.2A	1.6±0.4A	0.1±0.1A	0.36A
Sex							
Male	8538±3590A	16.0±2.8A	77.1±6.8A	3.3±0.9A	1.4±0.4A	0.2±0.1A	0.21A
Female	9883±3985A	17.8±3.2A	78.9±4.3A	3.3±1.1A	1.7±0.3A	0.2±0.1A	0.22A

A-B: Means in the same column followed by different letters differ significantly ($p<0.05$)

significantly the values of RBC, HCT, MCV and HGB. The absence of incubation temperature influence on the values of RBC, HCT and HGB was also observed by Yahav *et al.* (1997). However, these authors verified decrease of the MCV values in broilers submitted to high temperatures.

The leukocyte parameters (analysis of differential and total count of leukocytes) initially showed that the lymphocytes were the most frequent leukocyte type, followed by, heterophils, monocytes, basophils and eosinophils; independently of the incubation temperature and sex. These results are in agreement with Bounous *et al.* (2000) that described the limphocytes as the most numerous leukocyte in chickens and turkeys. However, the results of the present study differed from other domestic birds, such as ostrich that has similar number of lymphocytes and heterophils (Schmidt *et al.*, 2007). Lymphocytes and heterophils were the unique cell types influenced by incubation temperature. The heterophil values and the H/L ratio were higher and the lymphocyte values were lower when the incubation temperature increased. All these cell types are related with the natural cellular immunity of the birds, specially *in ovo* and immediately after the hatchability. Kogut *et al.* (1998) described that during these cited phases the birds did not develop acquired immunity yet. The heterophils are the cells present in higher quantity in the blood of adult birds (Brooks *et al.*, 1996; Kogut *et al.*, 1998). In the present work, the higher lymphocyte percentage in relation to the other leukocytes showed difference between the neonatal chick and the adult bird, indicating that the broiler immunological requirements suffer chronological alterations. The heterophilia has been considered the response to light and moderate stress in birds (Maxwell *et al.*, 1992), while the H/L ratio is a known index to measure stress in chickens (Gross and Siegel, 1983). The values of H/L ratio registered in this experiment indicated that the high incubation temperature (39°C) acted as stressor agent.

Bautista-Ortega *et al.* (1997) examined the relation between the H/L ratio values and the duration of eggshell breakage and the time taken by the chick to leave the eggshell and verified that the value of H/L ratio was higher for chicks that took more time to hatch. However, in the present study this relation was not observed. The H/L ratio value was higher for chicks from eggs incubated at 39°C, while the hatchability period was longer for incubation at 36°C. Brown (1979) described that chicks with higher time to leave the eggshell are exposed to an environment more hostile and stressing due to the damage caused by hypoxia and hypercapnia. This fact can explain the high late mortality rate registered for eggs incubated at high temperatures. In some domestic birds, the basopenia and the basophilia have been detected as response to the stress caused by intense cold and heat, respectively (Maxwell *et al.*, 1992; Spinu and Degen, 1993; Mitchell *et al.*, 1996). However, in this present experiment the chicks originated from eggs incubated at low and high

temperatures did not present significant basophilic responses.

Conclusion: The results showed that males and females from eggs of young breeders answered of similar form to variations in incubation temperature (36, 37.5 and 39°C) and that the temperature of 36°C demonstrated to be the most appropriate to improve the development and hatchability of chicks from light eggs.

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