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Early Female Hatching is Related to Sex Differences in Biochemical and Hematological Parameters

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Abstract: We assessed whether earlier hatching time in female compared to male broilers is related to differences in the duration of the actual embryonic development and/or hatching period, hematological parameters and hatchling quality. Four-hundred and fifty fertile eggs were incubated at 37.25-37.47°C and 37.19-37.91°C from day 1 to 13 and from day 14 of incubation, respectively, with eggshell temperature of 37.27±0.36°C until day 13 and 37.46±0.14°C from day 14 of incubation, as well as 60% relative humidity for the entire incubation period. To verified female tendency to earlier hatching than male, duration of the incubation and its phases was determined within a 34 h hatching window (470 to 504 h). All other variables were analyzed during periods of 478-489 and 493-504 h for females and males, respectively. Incubation length, hatching times between internal and external pipping and internal pipping and hatch were longer and the eggshell was thinner in males. There were no differences in the duration of embryonic plus fetal development, time between external pipping, hatch body, yolk sac, heart, or lung weights, or in blood concentrations of ions, gases, or glucose between the sexes. However, RBC, Hb and MCHC were higher in males, whereas MCV and MCH were higher in females. In addition, males had lower total cholesterol and triglyceride concentrations in the blood, but higher concentrations of urea, uric acid and total protein when compared to females. Altogether, these results suggest that early hatching time tendency in females is associated with lower energetic and gas exchange hematological potential as well as with a thicker eggshell.

Key words: Blood values, broiler chicks, embryonic development, gender difference, hatching time

INTRODUCTION

A growing demand for poultry meat continuously challenges farmers to increase production while maintaining product quality, minimizing costs and ensuring economic returns. Improvement in broiler performance depends, among other things, on increasing the quality and homogeneity of chick batches, which contribute to maximum growth and survival of broilers.

Asynchrony during hatching constitutes one of the factors that affect homogeneity of chick batch quality. Asynchronous hatching generates a hatch window between the first and last emergence ranging from 24 to 48 h within a single batch (Decuypere *et al*., 2001; Tong *et al*., 2013). This window of time deprives the first hatched chicks from food and water for a longer period than that experienced by late hatchlings (Decuypere *et al*., 2001). Delayed access to feed causes weight loss (Careghi *et al*., 2005; Lamot *et al*., 2014) and affects later growth (Noy and Sklan, 1999; Noy *et al*., 2001; Gonzales *et al*., 2008; Halevy *et al*., 2003; Uni *et al*., 2003). Hatching time also influences early chick behavior (Lotvedt and Jensen, 2014), gastrointestinal development, hormone levels and the immune system (Decuypere and Bruggeman, 2007).

The effects of breeding age (Ruiz and Lunam, 2002), egg size (Ulmer Franco *et al*., 2010) and egg storage duration (Tona *et al*., 2003a,b) on length of incubation have been documented. In addition, we have empirically observed that females tend to hatch earlier than males and a previous study has confirmed this view (Reis *et al*., 1997). Early female hatching results in females remaining feed-and water-deprived longer than males. This fact may increase heterogeneity and decrease quality of batches produced by hatcheries, although no difference in body weight at hatching between sexes has been observed (Reis *et al*., 1997).

Previous studies have usually approached asynchronous hatching as a general alteration in embryonic development time, with little attention to the lengths of different relevant periods such as those of embryonic and fetal development or hatching. Moreover, factors underlying early female hatching have yet to be elucidated. The hatching of chicks involves internal and external pipping and the breaking of the eggshell, whose times has been associated to high O_2 deficit and CO_2

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saturation (Khandoker *et al*., 2003; Tona *et al*., 2003b; Mortola, 2009) and high energy availability (Freeman, 1965; Dickson, 1983; Molenaar *et al*., 2010), respectively. Thus, it is reasonable to expected that earlier hatching time tendency in female broilers compared to males may be related to sex-dependent differences in hatching period duration, associated with blood gas transport and energy metabolism. To date, no studies have tested this hypothesis.

Here, we evaluated whether sex-related differences in the pattern of *in ovo* development as well as in hatchling morphological and physiological characteristics could explain early female hatching. To this end, we assessed the durations of incubation, *in ovo* development and hatching; measured egg mass loss and eggshell weight and thickness and assessed chick quality at hatching along with measurement of hematological values.

MATERIALS AND METHODS

Experimental conditions: The experimental protocol used in this study was approved by the Ethics Committee on Animal Use (CEUA, protocol number 022383/12), of the College of Agricultural and Veterinary Sciences, Sao Paulo State University (UNESP, Jaboticabal, Sao Paulo, Brazil).

Four-hundred and fifty fertile fresh eggs from 56-week old broiler breeders (Cobb®-500) obtained from a commercial hatchery (Globoaves, Itirapina, SP, Brazil), were individually weighted, numbered and distributed homogeneously by weight (65-70 g) in three incubators (150 eggs/incubator) (Premium Ecologica IP200, Belo Horizonte, MG, Brazil) with automatic control of temperature and egg turning every two hours. The air temperatures of the three incubators during periods of 1-13 and 14-19 days were 37.32±0.12 and 37.60±0.09, 37.33±0.16 and 37.52±0.16 and 37.38±0.13 and 37.67±0.08°C, whereas the average eggshell temperatures within these incubator were 36.68±0.18, 36.68±0.16, 36.71±0.25°C until day 13 and 37.57±0.15, 37.52±0.13 and 37.55±0.08°C from day 14. The incubator air and eggshell temperature measurements were carried out at every 30 min using thermosensers and the data stored in data loggers (Alutal Type T, Sao Paulo, Brazil) connected to a computer program (8 eggs per incubator). The relative humidity was maintained at 60% until hatching. Egg turning was stopped on day 18 of incubation. On 19th day of incubation, eggs were individually transfer to bags of tulli (10 x 18 cm), which were placed in the same incubators. This procedure was adopted to identify chicks and their correspondent eggshell. To verified female tendency to earlier hatching than male, duration of the incubation and its phases was determined within a 34 h hatching window (470 to 504 h of incubation). Based in previous study, all other variables were analyzed during periods of 478-489 and 493-504 h for females and males, respectively. Chicks were feather sexed after drying.

Duration of incubation, *in ovo* development and hatching: The durations of the following variables were analyzed: incubation time (from beginning of incubation until actual hatching), embryonic and fetal development time (from beginning of incubation until internal pipping), hatching time (from internal pipping until hatching), time between internal and external pipping and time between external pipping and hatching. All variables were expressed in hours. To determine the moment of internal pipping, eggs were subjected to ovoscopy every two hours from day 19 of incubation. The occurrence of external pipping was visually determined by observation every 15 min following internal pipping. The values of these variables were determined from 30 eggs per sex, which originated chicks hatched within the hatching window established to analysis.

Egg mass loss, eggshell percentage and shell thickness: Egg mass loss corresponded to mass lost from the beginning to day 19 of incubation and was expressed in grams and percentage relative to egg weight before incubation. Eggshell water vapor conductance was calculated according to Tullett (1981) by the following formula: C = ML/SVP, where C = conductance, ML = egg mass loss and SVP = saturated vapor pressure of 23.86 mm Hg at 25°C, utilizing total egg mass loss (g) from day 1 to 19 of incubation. These variables were determined based on analyses of 17 eggs per sex.

Eggshell percentage and thickness were determined at the end of incubation, after removal of the inner and outer shell membranes and cuticle, based on analysis of the 12 eggs per sex (Rahn *et al*., 1981). Eggshell percentage was calculated relative to egg weight at the beginning of incubation. For determination of eggshell thickness, eggshell fragments were obtained from the apical (opposite end of the air chamber), equatorial and basal (end of the air chamber) region of each egg. Shell membranes and cuticle were removed by boiling the fragments in 0.5% NaOH in aqueous solution, after which they were washed in distilled water and dried at ambient temperature for 72 h before analysis. Measurements of eggshell thickness was performed using a digital micrometer (Mitutoyo, Tokyo, Japan) with a resolution of 0.001 mm.

All eggs used in the analysis of mass loss and shell were selected based in the hatching window established for analysis.

Hatchability: Total hatchability and female and male hatchability were determined in percentage relative to the total number of fertile eggs (N = 3 incubators).

Chick quality: After drying, chick quality was evaluated based on direct observation and pontuation of physical characteristics (presence of dirt and humidity in the feathers, eye brightness and opening, permanence of chicks standing with or without difficulty and knee joints with inflammation and/or redness, closing, dirt and staining of the navel, persistence or not and length of the allantoic cord and incorporation degree of the yolk sac into the abdominal cavity) and activity (residence time in dorsal decubitus) (adapted from Tona *et al*., 2003a). According to the total score obtained, birds were classified into one of the following six quality classes: excellent (100 points), very good (81-99 points), good (61-80 points), low (41-60 points), bad (20-40 points) and very bad (0-19 points). Chick quality evaluation was based in analysis of 50 chicks per sex, which originated chicks hatched within the hatching window established for analysis.

Body and organ weight: After drying 24 birds with excellent quality per sex, hatched within the hatching window established for analysis, were weighed, submitted to blood collection (described below) and killed by cervical dislocation for yolk sac removal. Body weight with and without the yolk sac was determined using a digital balance with a precision of 0.001 g (Marte, Sao Paulo, Brazil) and expressed in grams and as a percentage of egg weight at the beginning of incubation. Yolk sac weight was expressed in grams and as a percentage of chick body weight with the yolk sac.

Hematological values: Blood was taken from the jugular vein of ten birds per sex for analysis of hematological indices and gas, mineral and biochemical parameters. Blood samples were collected in a commercial heparinized syringe and used immediately after collection. Red blood cell (RBC) counts were done in a Neubauer chamber using blood diluted 1:800 in Natt and Herrick's (1952) solution. Hematocrit (Hct), hemoglobin (Hb), partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), total carbon dioxide (TCO_2), rate of oxygen saturation (sO_2), base excess (BEefc), bicarbonate (HCO_3^-), pH, mineral ions (Ca^{2+}, K^+ and Na^+) and glucose values were determined using a portable clinical analyzer (I-STAT® cartridge Cg8+®, Abbott Laboratories, USA). Rectal temperature was obtained from each bird immediately prior to the blood test. The hematological indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formulae: MCV = Hct/RBC x 10, MCH = Hb/RBC x 10 and MCHC = Hb/Hct x 100. For blood biochemical analyses, blood samples of ten other chicks per sex were taken from the jugular vein without anticoagulant drugs, kept in an Eppendorf tube for one hour at ambient temperature for serum separation and then centrifuged at 3500 rpm and 4°C for 10 min. The serum (supernatant) was collected and kept frozen (-70°C) until analysis. Total concentrations of cholesterol, triglycerides, uric acid, urea and protein were determined using commercial kits (Labtest Diagnostica S.A., Belo Horizonte, MG, Brazil). Samples were prepared and analyzed according to the manufacturer's specifications. Three readings were taken on each sample using a spectrophotometer (Beckman Coulter DU-800, Brea, CA, USA). All chicks hatched within the hatching window established for analysis. They were free of physical abnormalities and were not submitted to analysis of activity (time in dorsal decubitus) to avoid influence on blood characteristics.

Statistical analyses: All data (i.e. mass loss, shell conductance and thickness, duration of the incubation and its phases, hatchability, blood characteristics, body and yolk sac weight, chick quality) were assessed with one-way analysis of Variance (ANOVA), using the General Linear Models procedure of SAS (SAS Institute, 2002), using the following model: $Y = \mu + S + e$, in which Y = dependent variable, μ = overall mean, S is the sex (male and female) and e = residual error. Results were reported as means±SD. In all cases, a difference was considered statistically significant at $p \leq 0.05$. For all variables the individual egg or chick was considered as the experimental unit.

RESULTS

Duration of incubation, *in ovo* development and hatching: Table 1 shows the effects of sex on the durations of: (1) the incubation period, (2) the period of embryonic and fetal development, (3) the hatching period, (4) the phase of hatching between internal and external pipping and (5) the phase of hatching between external pipping and hatch. No significant effect of sex was observed on the duration of embryonic and fetal development or on time between external pipping and hatch ($p>0.05$). However, time between internal and external pipping, total hatching time and total incubation time were shorter in females than in males ($p \leq 0.05$).

Egg mass loss and eggshell characteristics: Sex had no significant effect on egg mass loss ($p>0.05$). Eggshell water vapor conductance and weight were also not affected ($p>0.05$). However, sex did influence apical, equatorial and basal thickness of the eggshell, which was thicker for females than for males ($p \leq 0.05$) (Table 1).

Hatchability: Average total hatchability was 81.7±4.65%, of which 42.2±5.12 e 39.9±4.78% were female and males, respectively ($p = 0.2256$).

Chick quality: Both male and female neonates received a mean quality score of very good (Fig. 1). Physical abnormalities related to eyes, legs and yolk sac were

Table 1: Incubation duration and of its phases, egg mass loss and eggshell weight, conductance and thickness for male and female broiler hatchlings

	Sex			
Variables	Male	Female	p-values	SEM
Internal Pipping (h)	468.81±7.83	464.42±8.01	0.7893	1.17
Internal to External Pipping (h)	9.81±6.68[a]	7.43±4.04[b]	0.0193	0.81
External Pipping to Hatching (h)	16.01±7.22	14.86±7.39	0.9081	1.05
Internal Pipping to Hatching (h)	25.81±4.34[a]	21.35±8.02[b]	0.0046	0.96
Incubation Duration (h)	494.63±8.25[a]	486.72±7.02[b]	0.0056	1,24
Egg Mass Loss (g)	6.54±0.92	6.35±0.91	0.2917	0.82
Egg Mass Loss (%)[1]	9.54±1.32	9.22±1.30	0.2372	0.19
ES Weight (g)[2]	8.82±0.59	8.88±0.58	0.7555	0.09
ES Weight (%)[1]	12.84±0,99	12.89±0.79	0.8687	0.14
ES Condutance (g/mmHg)[2]	0.274±0.03	0.279±0.04	0.4053	0.01
ES Thickness-Apical (µm)[2]	0.34±0.03[b]	0.61±0.04[a]	<0.0001	0.02
ES Thickness-Equatorial (µm)[2]	0.34±0.02[b]	0.53±0.03[a]	<0.0001	0.01
ES Thickness-Basal (µm)[2]	0.36±0.02[b]	0.55±0.04[a]	<0.0001	0.02

[1]Weight calculated relative to egg weight before incubation. [2]ES: Eggshell.
[a-b]: Means fellowed by distinct letters (row) differ significantly (p<0.05).
(X±SD, N = 50 eggs/sex for incubation phases and 24 eggs for females and 17 eggs for males for mass loss and shell characteristics)

Table 2: Body, residual yolk sac, heart and lung weights and rectal temperature of male and female broiler hatchlings

		Sex			
Variables		Male	Female	p-values	SEM
Body weight	(g)	50.55±1.69	50.89±1.83	0.1835	0.25
	(%)[1]	73.38±1.77	73.35±1.92	0.0630	0.31
Yolk-free body weight	(g)	42.65±2.09	42.07±1.45	0.9496	0.26
	(%)[1]	62.16±2.91	61.09±1.83	0.6758	0.37
Residual yolk sac	(g)	7.71±1.49	8.45±0.99	0.6097	0.18
	(%)[2]	15.31±2.91	16.70±1.67	0.3268	0.34
Heart	(g)	0.37±0.06	0.38±0.05	0.4275	0.01
	(%)[2]	0.74±0.12	0.76±0.09	0.6616	0.01
Right lung	(g)	0.20±0.05	0.18±0.04	0.6426	0.01
	(%)[2]	0.41±0.09	0.36±0.09	0.9053	0.01
Left lung	(g)	0.20±0.06	0.18±0.04	0.3947	0.01
	(%)[2]	0.41±0.11	0.37±0.08	0.8212	0.01
Rectal temperature	(°C)	39.25±1.0064	39.21±1.01	0.9422	0.14

[1]: Relative to egg weight before incubation. [2]: Relative to body weight
No significant differences were found between male and female means (p>0.05). (X±SD, N = 24 birds with excellent quality/sex)

not observed in males or females. Feathering problems were reported only for males (18%). Yolk sac incorporation (57.5 and 48.5%) and navel abnormalities (52.5 and 30%) occurred for both sexes but with higher frequency for females than for males, respectively. On the other hand, low activity was observed for both sexes but was more frequent in males (67%) than in females (20%). In terms of the overall quality score, 3.04% of males and 5.13% of females were rated excellent and 96.96% of males and 94.87% of females were rated very good. The other quality ratings (good, fair, poor and very poor) did not occur.

Rectal temperature, body weight and organ weights: As shown in Table 2, rectal temperature and weights of body, yolk-free body, yolk sac, heart and right and left lungs were not influenced by sex (p>0.05).

Hematological parameters: Sex affected every erythrocyte value except for Hct (Table 3). Males had higher RBC counts, Hb and MCHC, whereas females had higher MCV and MCH. Table 4 shows blood pH and concentrations of gases, ions, glucose, cholesterol, urea, uric acid, triglycerides and proteins. No significant effect of sex was found for blood pH, TCO_2, PCO_2, $_SO_2$, HCO_3^-, Ca^{2+}, K^+, Na^+, BEecf and glucose values (p>0.05). However, concentrations of total cholesterol, triglycerides and total protein were higher in females than in males, whereas those of PO_2, urea and uric acid were higher in males than in females.

DISCUSSION

We tested whether early hatching in female compared to male broilers correlates with sex differences in duration of the embryo plus fetal phase, duration of the hatching phase, egg mass loss, eggshell characteristics, hatchling quality and hematological and biochemical indices.

Our results show that when compared to males, the shorter average incubation times of female birds resulted from a shorter time between internal and external pipping and between internal pipping and

Table 3: Venous blood pH, concentrations of gases, ions, glucose and total protein and lipid prolife in male and female broiler hatching

	Sex			
Variables	Male	Female	p-values	SEM
Hct (% PCV)[1]	10.50±1.93	11.04±1.35	0.1028	0.25
RBC (cells.10^6/mm^3)[2]	1.43±0.46[a]	0.97±0.69[b]	0.0100	0.09
Hb (g/dL)[3]	4.90±0.56	4.14±0.66	0.4828	0.11
MCV (fL)[4]	91.05±41.78[b]	153.50±38.93[a]	0.0010	6.16
MHC (pg)[5]	42.28±34.64[b]	55.73±23.95[a]	0.0010	4.35
MCHC (%)[6]	47.12±3.97[a]	37.96±7.05[b]	0.0100	1.06
pH	7.42±0.05	7.41±0.10	0.8712	0.01
TCO_2 (mmol/l)[7]	13.54±2.52	13.91±3.04	0.3783	0.40
sO_2 (%)[8]	69.29±15.47	63.62±12.54	0.3032	2.05
PCO_2 (mm/Hg)[9]	20.54±15.49	21.05±12.54	0.7398	0.61
PO_2 (mm/Hg)[10]	38.68±8.62[a]	35.96±7.48[b]	0.0182	1.57
HCO_3^- (mmol/l)	12.94±2.36	13.31±2.95	0.2950	0.38
Na^+ (mmol/l)	134.22±3.42	134.65±3.51	0.9924	0.63
K^+ (mmol/l)	5.67±1.29	5.62±1.27	0.9559	0.18
Cai (mmol/l)	0.30±0.02	0.29±0.04	0,1235	0.01
BEecf (mmol/l)[11]	10.79±3.11	10.70±4.03	0.2161	0.51
Glucose (mg/dL)	124.50±22.38	126.13±24.61	0.3075	3.62

[1]Hct: hematocrit. [2]RBC: red blood cell count. [3]Hb: hemoglobin. [4]MCV: mean corpuscular volume.
[5-6]MCH, MCHC: mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. [7]TCO_2: total carbon dioxide.
[8]sO_2: oxygen saturation. [9-10]PCO_2, PO_2: partial pressure of carbon dioxide and of oxygen. [11]BEecf: base excess.
[a-b]: Means followed by distinct letters (row) differ significantly ($p<0.05$). (X±SD, N= 10 birds with excellent quality/sex)

Table 4: Venous blood erythrocyte indices of male and female broiler hatching

	Sex			
Variables	Male	Female	p-values	SEM
Total cholesterol (mg/dL)	223.03±23.64[b]	299.00±56.81[a]	0.0063	15.04
Urea (mg/dL)	73.10±20.61[a]	28.71±4.41[b]	<0.0001	4.33
Uric acid (mg/dL)	6.41±3.92[a]	3.94±1.29[b]	0.0002	0.36
Triglycerides (mg/dL)	252.79±62.29[a]	103.80±46.03[b]	0.0011	12.21
Total protein (g/dl)	4.16±1.30[a]	2.01±0.46[b]	0.0004	0.26

[a-b]: Means followed by distinct letters (row) differ significantly ($p<0.05$). (X±SD, 10 birds with excellent quality/sex)

hatching. No sex differences were found in the duration of embryonic plus fetal development or in time between external pipping and hatch. These findings demonstrate that the female tendency for early hatching, observed also by Reis *et al.* (1997) and van de Ven *et al.* (2013), is related to a shorter hatching phase. Although previous studies have usually treated asynchronous hatching as a general alteration in the duration of embryonic development, we identified the interval between internal and external pipping as the critical period determining early hatching of female broilers.

The bird egg acts as an external incubation chamber. In this context, the eggshell plays important roles in the maintenance of embryonic and fetal development: protection, gas exchange (of O_2, CO_2 and H_2O vapor) and mineral supply. Here, we investigated possible relationships between early female hatching and egg characteristics. Previous work indicates that incubational egg weight loss and the associated eggshell water vapor conductance may influence broiler metabolism, growth and development *in ovo* (Peebles *et al.*, 2005; Hocking, 2009). In the present study, we found no sex-related differences in these two variables or in absolute and relative eggshell weight at the end of the incubation. However, eggshells were 34-43% thicker for females than males. The lack of difference in egg mass loss and eggshell conductance and weight between males and females, whereas at the same time the eggshell thickness differs, make interpretation difficult. The eggs were all originated from 56 weeks old broiler breeders from one flock and no difference occurred in egg weight between female and males pre-incubation (males: 68.73±1.36 g, females: 68.86±1.65, p = 0.7828) and at the end of the incubation (males: 62.17±1.49, females: 62.60±1.28 g, p = 0.3469). Thus, although it is known that eggshell thickness decreased as breeder age and egg weight (Peebles *et al.*, 2000; Morita *et al.*, 2009), the difference in eggshell thickness encountered between males and females can not be related to these two factors. This view is reinforced by no difference in the body weight between sexes, since that previous study showed chick weight at hatching differ between breeder ages and between egg weights (Morita *et al.*, 2009; Ulmer-Franco *et al.*, 2010). On the other hand, eggshell contributes with about 80% of the calcium requirements of the chick before hatch (Ruiz *et al.*, 1998) and any change in its thickness may have an effect on the amount of calcium available (Dieckert *et al.*, 1989; Nascimento *et al.*, 1992). Embryo-induced eggshell thinning occurs along the length of an egg from the

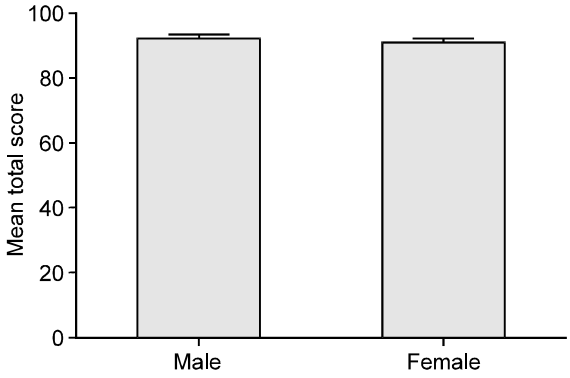

Fig. 1: Mean total quality scores (based on the methodology of Tona *et al*., 2003a) of broiler hatchlings, according to sex. No significant differences were found between male and female means (p>0.05). (X±SD, N = 50 birds/sex)

sharp pole to the blunt end, in which the reduction is least pronounced due to the presence of the egg air chamber (Castilla *et al*., 2010; Maurer *et al*., 2011). Therefore, it is reasonable to expect that the shorter female period between internal pipping and actual hatching had led to reduced mineral absorption from the eggshell, resulting in thicker shell. At the same time, moisture loss and gas exchange between the internal and external environments occurs through the eggshell pores and directly depends on eggshell thickness and pore number and area, among other factors (La Scala, 2003). For it, no difference in mass loss and shell conductance between eggs that had distinct shell thickness lead us also to consider eggshell porosity would be different between males and females.

Many authors have shown that chick exposure to hypoxic and hypercapnic environments can cause increased RBC and/or MCV, resulting in higher Hct and Hb (Ackerman, 1970; Burggren *et al*., 2012; Tazawa *et al*., 2012; Mueller *et al*., 2013; Andrewartha *et al*., 2014). These hematological changes have been interpreted as adaptive physiological responses that maintain an adequate supply of O_2 for the bird. In the current study, no differences in heart or lung weights were observed between sexes. However, female hatchlings exhibited lower RBC, Hb and MCHC and higher MCV and MCH values than did males, but did not differ in Hct. Thus, females did show alterations in face of an environment with slower gas exchange, but their lower RBC, MCHC and Hb values may reduce their oxygen carrying capacity. This deficit in association with a O_2 deficit and CO_2 saturation hypoxic and hypercapnic environment inside the egg air chamber at the end of incubation may induce the observed faster transition between internal and external pipping.

Some of the changes associated with hypoxia and hypercapnia include blood osmolality (Osm), PO_2, PCO_2 and pH, all of which affect hematological RBC, MCV, Hct and Hb values (Cossins and Gibson, 1997; Hoffmann *et al*., 2009). Lower eggshell porosity increases CO_2 retention within the egg and may promote increased blood PCO_2 and HCO_3^- (Tullett and Burton, 1985). Despite the thicker eggshells of females, we found no sex differences in blood concentrations of gases and minerals (PCO_2, TCO_2, sO_2, HCO_3^-, Na^+, K^+, Ca^{2+} and BEecf), as well as in blood pH. This lack of differences between males and females probably results from a rapid recovery in blood acid-base balance immediately after external pipping. However, females exhibited lower PO_2 than males, indicating that earlier female hatching may have resulted from faster O_2 depletion in the air chamber caused by lower O_2 availability.

The hatching process relies on carbohydrate energy sources from the liver or yolk sac (Freeman, 1965; Dickson, 1983; Molenaar *et al*., 2010). It has been assumed that all broiler chicks require similar amounts of energy to hatch (Molenaar *et al*., 2010). However, we might expect that female birds, compared to males, expend a higher amount of energy per unit of time because of their faster hatching. Although we found no differences in blood glucose concentration, this may have resulted from different rates of glycogen breakdown in the liver or glucose utilization from the yolk sac, which we did not investigate. In contrast to the results for glucose, we detected higher levels of cholesterol and lower levels of urea, uric acid, total protein and triglycerides in female hatchlings compared to males. In hatchlings, 98% of the cholesterol comes from the yolk (Connor *et al*., 1969). Yolk sac lipids constitute the major energy source for *in ovo* fetal development (Romanoff, 1960; Freeman and Vince, 1974; Ding and Lillburn, 1996). As lipids are transferred from the yolk sac to the fetus, hepatic lipids, mostly cholesterol, increase (Noble and Ogunyemi, 1989). In the last days of incubation, the internal and external pipping, disruption and emergence of the eggshell increase the energy demand of the chicks. Low oxygen availability in the period between the internal and external pipping induces birds to use energy from glucose anaerobic catabolism (Moran, 2007), whose output comes from carbon-rich substrates such as proteins of the amniotic fluid or muscle tissue, glycerol and other carbohydrates (Oliveira *et al*., 2008; Yadgary and Uni, 2012). Uric acid and urea constitute products of protein metabolism whose blood levels change with disturbances in production or excretion. Uric acid is the main pathway of nitrogen excretion in birds, while urea plays only a minor role (Sturkie, 1976), explaining thus the higher plasma concentration of uric acid than of urea observed in both sexes. Our results indicated differences in the sources of energy production between sexes, with males utilizing more protein and females more lipids, what may be related with the more long period between internal and external pipping and internal pipping and hatching in males.

Sub-optimal environmental conditions inside and outside the egg influence embryonic and fetal development and can cause physical and motor abnormalities. These deficits make chicks unsuitable for use in commercial production. In the present study, we evaluated early post-hatching chick quality based on physical analysis of the occurrence of abnormalities, degree of activity and body and yolk sac weight. Chicks had very good quality scores and similar body and yolk sac weights regardless of sex, indicating that early female hatching did not interfere with quality.

Conclusion: We demonstrated that early female hatching is associated with metabolic and hematological changes as well as with a thicker eggshell. Together, these alterations, suggest that females have fewer resources for adapting to a hypoxic and hypercapnic air chamber and thus accelerate the rate of hatching in the period between internal and external pipping.

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