



Egg Traits and Physiological Neonatal Chick Parameters from Broiler Breeder at Different Ages

■ Author(s)

Luquetti BC¹
Gonzales E¹
Bruno LDG¹
Furlan RL¹
Macari M¹

¹ Centro Virtual de Pesquisa em Ciência Avícola
Departamento de Morfologia e Fisiologia
Animal, Faculdade de Ciências Agrárias e
Veterinárias – Campus de Jaboticabal, Unesp,
Jaboticabal, SP – Brazil.

■ Mail Address

Marcos Macari
Departamento de Morfologia e Fisiologia Animal.
Faculdade de Ciências Agrárias e Veterinárias
Campus de Jaboticabal – Unesp.
14.884-900 - Jaboticabal, SP, Brazil.
Telephone: 55 16 3209 2656
Fax: 55 16 3202 4275

E-mail: macari@fcav.unesp.br

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ABSTRACT

This investigation was carried out to study egg traits (weight, specific gravity and eggshell thickness), and neonatal chick parameters (weight, blood, cardiac and lung parameters) from breeder at different ages. After hatching, neonatal chicks, male and female, from broiler breeder at three different ages (30, 45 and 60 weeks) were sacrificed and blood (red blood cell number, hematocrit, mean cell volume, blood viscosity, and haemoglobin), cardiac (right ventricle and total ventricle weights, cardiac index) and lung (mean pulmonary pressure, fresh relative lung weight) parameters were measured. No significant differences in eggshell thickness or specific gravity were observed in eggs from the three different breeder age. The incubated eggs and neonatal chicks showed heavier absolute weights with increasing breeder age. Broiler breeder age did not affect blood parameters or cardiac index, but affected right ventricle and total ventricle absolute weights. Red blood cell number and hematocrit were higher and lung weight and lung weight:chick weight index were lower for female neonatal chicks. The findings of this study revealed that breeder age affects neonatal chick parameters such as body weight, heart and lung absolute weights, but not blood parameters.

INTRODUCTION

Several factors can affect the poultry egg characteristics, including hen age, environment, nutrition and genetics. It is well known that egg weight increases with hen age, reaching a plateau by the end of the laying cycle (Weatherup & Foster, 1980). Hen age also affects the proportion of yolk, albumen and eggshell (Fletcher *et al.*, 1981; Akbar *et al.*, 1983; Danilov, 2000). The egg specific gravity and eggshell thickness decrease with advancing breeder age. This occurs basically because the egg size increases more quickly than the shell weight (Butcher *et al.*, 1991) and because there is an increase in egg size rather than calcium deposition in the eggshell (Curtis *et al.*, 1985).

There are many reports in the literature showing the influence of broiler breeder age on eggshell quality. However, the relationship between eggshell quality and the physiological parameters of embryos, especially those potentially involved in triggering metabolic diseases such as ascites or sudden death syndrome, has been poorly studied.

Several studies have attempted to relate broiler chicken susceptibility to ascites after hatching to anatomical or physiological parameters like right ventricle:total ventricle weight, hematocrit, thyroid hormones, and blood viscosity (Maxwell *et al.*, 1986; Julian, 1987; Silversides *et al.*, 1997; Gonzales *et al.*, 1998; Nir, 1998); however, there is no report in the literature showing if in neonatal chicks these parameters are related to breeder age. Thus, the objective of this study was to investigate how



breeder age (30, 45 and 60 weeks) affects eggshell characteristics and blood, cardiac and lung parameters of neonatal chicks.

MATERIAL AND METHODS

Six-hundred fertile eggs (200 per age) from three different breeder flocks (Cobb500®) aged 30, 45 and 60 weeks were obtained from a commercial hatchery. The eggs were individually weighed, identified, and incubated in a machine at 37.8 °C and 60 % relative humidity, with the egg tray being turned once an hour through an angle of 90° (45° to each side). One day before hatching each egg was wrapped in an air-permeable sac (berloc). Approximately 2 h after hatching the chicks were separated by sex (feather sexed), and egg, blood, cardiac and lung parameters measured. This study was conducted in accordance with the Faculdade de Ciências Agrárias e Veterinárias Institutional Animal Care Committee.

Egg specific gravity and shell thickness were measured in 30 randomly chosen eggs per breeder age (30, 45 and 60 weeks). Egg specific gravity was determined by the method of Moreng & Avens (1990), which involved placing the eggs in a series of saline solutions with increment of 0.005 units. For each egg, the shell thickness was determined at three different points in the middle part of the egg using a Mitutoyo caliper 7313 micrometer with 0.01 mm (0.01-10 mm) precision.

Blood samples (1.5 mL) of all hatched chicks of each breeder age were collected by cardiac puncture using a sterile heparinized syringe after previous sedation of newly hatched chicks using sodium thiopental (10 mg/kg BW). After sampling, the chicks were sacrificed with a lethal dose of anesthetic (sodium pentobarbital, 50 mg/kg, i.v.), and the heart was collected and the atrium removed. Total ventricle (TV) weight was obtained, and the right ventricle (RV) was dissected and weighed using a precision balance (\pm 0.001 g). The cardiac index (CI) was calculated according to the procedure described by Julian (1987) and Maxwell *et al.* (1992). Hematocrit (Ht, %), red blood cell number (RBC, 10^6 cells/mm³), mean cell volume (MCV, μ^3) and hemoglobin (Hb, g/dL) were determined using an automatic blood analyzer (Celm DA 500®). Blood viscosity was assessed with a Brookfield DV III+® viscosimeter using plane geometry, with spindle S42 at 41 °C, and speed of 250 rpm.

Mean pulmonary pressure (MPP) was calculated according to the formula proposed by Cueva *et al.* (1974), $MPP = - 20.16 + 1.85 RV/TV$, where RV is right

ventricle weight and TV is total ventricle weight, in grams, and MPP is expressed as mmHg. The lungs were also collected, dried on Whatman filter paper and weighed to obtain the lung:chick weight ratio.

The experiment was performed in a 3 (breeders age – 30, 45 and 60 week) x 2 (sex) factorial arrangement according to a completely randomised design. Data were analysed statistically using the GLM procedure of the SAS program (SAS, 2000). Differences between means were determined by the Tukey test. The egg specific gravity and shell thickness data were subjected to an analysis of variance using the GLM procedure considering only the breeder age as effect.

RESULTS

Egg parameters and neonatal chick weights

The influence of broiler breeder age on eggshell thickness and egg specific gravity are summarized in Table 1. No significant differences in eggshell thickness or specific gravity were observed in eggs from the three different breeder age groups.

Table 1 - Means and analysis of variance for the effect of broiler breeder age on eggshell thickness and specific gravity. Each value represents the mean \pm SEM.

	N ¹	Eggshell thickness (mm)	Specific gravity
Breeder age (week)			
30	30	0.44 \pm 0.008	1.085 \pm 0.001
45	30	0.44 \pm 0.007	1.082 \pm 0.001
60	30	0.43 \pm 0.006	1.082 \pm 0.001
F value		1.69ns	1.75ns

1 - Number of observations.

The data concerning egg weight, eggshell weight (absolute and relative), and chick neonatal weight (absolute and relative) are presented in Table 2. No interaction ($p < 0.05$) was observed between breeder age and sex for the data analysed. The incubated eggs increased in weight with increasing breeder age. The difference between 30-week *vs* 45-week, and 30-week *vs* 60-week groups for egg weight was more evident than between 45-week *vs* 60-week groups. The same results were observed for the chick weights, with the relative weight being greater with younger hens. Thus, older breeders produced heavier chicks; however, the relative proportion of chick body weight was inversely related to breeder age.

Eggshell weight increased as the breeders became older. Nevertheless, the test of the means revealed that



only the absolute eggshell weight of eggs from 30-week-old-breeders was lighter than eggs from 45-week and 60-week-old breeders. Interestingly, the eggshell weight:egg weight ratio decreased in value with increasing breeder age. Thus, the youngest hens (30-weeks) laid lighter eggs with lighter eggshells that produced lighter chicks. For egg and eggshell weights no sex effect was observed (Table 2).

Blood, cardiac and lung parameters

The blood parameters of neonatal chicks are shown in Table 3. Red blood cell number, hematocrit, mean cell volume, hemoglobin and blood viscosity of neonatal chicks were not affected ($p>0.05$) by broiler breeder age. However, a significant effect of sex was observed, with male chicks having a lower ($p<0.05$) number of red blood cells and a lower hematocrit than females.

The effects of breeder age and sex on cardiac and lung parameters are presented in Table 4. Right ventricle

weight (RV) and total ventricle (TV) plus septum weight were affected by breeder age, but not by sex ($p>0.05$) or the interaction between age and sex. Chicks coming from eggs laid by 30-week-old breeders presented lighter RV and TV than those from 60-week-old ones, but no difference in chick cardiac index was observed between the three breeder groups. As a consequence, the mean pulmonary pressure of chicks from broiler breeders of different age was similar.

The calculated mean pulmonary pressure did not reveal pulmonary arterial hypertension in the chicks irrespective of broiler breeder age; however, absolute lung weight was dependent on breeder age, with chicks from older breeders having a heavier lung weight (Table 4). The data also revealed that the lung weight was heavy for male chicks from older hens. Although no significant ($p>0.05$) differences in lung weight:chick weight ratio among breeders groups of different ages were observed, male chicks presented a higher ratio than females (Table 4).

Table 2 - Means and analysis of variance for the effects of broiler breeder age (A) and chick sex (S) on egg weight before incubation, and eggshell and chicks absolute and relative weights after hatching. Each value represents the mean \pm SEM.

	N ¹	Egg Weight, g	Eggshell Weight, g	Eggshell Relative Weight, % ²	Chicks Weight, g	Chicks Relative Weight, % ³
Breeder age (week)						
35	103	58.3 \pm 0.25c	5.82 \pm 0.07b	9.97 \pm 0.12a	42.2 \pm 0.24c	72.35 \pm 0.24
45	71	64.6 \pm 0.44b	6.14 \pm 0.11a	9.49 \pm 0.14b	46.0 \pm 0.44b	71.21 \pm 0.44
60	76	68.2 \pm 0.49a	6.07 \pm 0.09a	8.91 \pm 0.12c	48.6 \pm 0.48a	71.33 \pm 0.48
Sex						
Male	122	63.5 \pm 0.50	6.02 \pm 0.07	9.50 \pm 0.10	45.7 \pm 0.39	71.97 \pm 0.39
Female	128	62.7 \pm 0.48	5.96 \pm 0.08	9.51 \pm 0.12	44.8 \pm 0.39	71.47 \pm 0.39
F value						
Age (A)		195.40*	3.36*	19.10*	76.56*	4.28*
Sex (S)		0.12ns	0.24ns	0.20ns	0.93ns	1.31ns
A x S		0.05ns	0.03ns	0.07ns	0.15ns	0.24ns

1 - Number of observations. 2 - Eggshell Relative Weight = (eggshell weight/egg weight) x 100. 3 - Chicks Relative Weight = (chick weight/egg weight) x 100. a-c - Means within a column for each variable with no common superscript differ significantly by Tukey's test. * - $p<0.05$; ns - non-significant.

Table 3 - Means and analysis of variance for the effects of breeder age (A) and chick sex (S) on blood parameters of neonatal chicks. Each value represents the mean \pm SEM.

	N ¹	Blood Parameters				N ¹	Viscosity (cP)
		Red Blood Cells (x 10 ⁶ cells/mm ³)	Hematocrit (%)	Mean Cell Volume (μ m ³)	Hemoglobin (g/dL)		
Breeder age (week)							
30	102	2.48 \pm 0.03	34.7 \pm 0.57	140.4 \pm 1.39	8.78 \pm 0.10	38	0.26 \pm 0.009
45	70	2.49 \pm 0.04	36.0 \pm 0.65	145.3 \pm 1.87	8.96 \pm 0.11	35	0.25 \pm 0.008
60	73	2.44 \pm 0.05	35.0 \pm 0.71	143.8 \pm 1.85	8.83 \pm 0.13	33	0.27 \pm 0.006
Sex							
Male	120	2.42 \pm 0.03b	34.5 \pm 0.49b	143.7 \pm 1.44	8.73 \pm 0.10	52	0.25 \pm 0.007
Female	125	2.52 \pm 0.03a	35.8 \pm 0.54a	142.0 \pm 1.30	8.96 \pm 0.10	54	0.26 \pm 0.006
F value							
Age (A)		0.28ns	0.10 ns	1.64 ns	0.39 ns		1.27ns
Sex (S)		6.05*	5.04*	1.23 ns	3.48 ns		1.20 ns
A x S		0.39 ns	0.85 ns	1.25 ns	0.50 ns		0.34 ns

1 - Number of observations. a-b - Means within a columns for each variable with no common superscript differ significantly by Tukey's test. * - $p<0.05$; ns - non-significant.



Table 4 - Means and analysis of variance for the effects of breeder age (A) and chick sex (S) on cardiac and lung parameters. Each value represents the mean \pm SEM.

	N ¹	Right Ventricle (g)	Total Ventricle (g)	Cardiac index ² (%)	N ¹	MPP ³ (mmHg)	Lung Weight (g)	LW:CW ⁴ (%)
Breeder age (week)								
30	95	0.046 \pm 0.001b	0.210 \pm 0.004b	22.06 \pm 0.40	101	-19.74 \pm 0.01	0.280 \pm 0.004b	0.666 \pm 0.010
45	54	0.048 \pm 0.001ab	0.215 \pm 0.004b	22.30 \pm 0.52	70	-19.72 \pm 0.01	0.291 \pm 0.007ab	0.636 \pm 0.015
60	62	0.052 \pm 0.001a	0.232 \pm 0.006a	22.65 \pm 0.49	75	-19.71 \pm 0.01	0.303 \pm 0.007a	0.628 \pm 0.016
Sex								
Male	105	0.048 \pm 0.001	0.218 \pm 0.004	22.10 \pm 0.36	121	-19.73 \pm 0.01	0.302 \pm 0.005a	0.664 \pm 0.01a
Female	106	0.048 \pm 0.001	0.217 \pm 0.004	22.49 \pm 0.39	125	-19.72 \pm 0.01	0.279 \pm 0.006b	0.627 \pm 0.01b
F value								
Age (A)		6.72*	6.29*	0.64 ns		2.25ns	3.64*	2.53 ns
Sex (S)		0.66 ns	0.019ns	0.92 ns		1.36 ns	11.86*	7.30*
A x S		0.52 ns	0.18 ns	0.54 ns		0.05 ns	1.62 ns	1.26 ns

1 - Number of observations. 2 - Cardiac index = (right ventricle/total ventricle) \times 100. 3 - Mean Pulmonary Pressure = (-20.16 + 1.85 right ventricle/total ventricle). 4 - Lung chick weight ratio = (lung weight/chick weight) \times 100. a-b - Means within a columns for each variable with no common superscript differ significantly by Tukey's test. * - $p < 0.05$; ns - non-significant.

DISCUSSION

The eggshell quality is an important factor to poultry industry due to reproductive and economic implications. However, eggshell quality is related with bird age (Peebles & Brake, 1987). McDaniel *et al.* (1979) observed a decline in specific gravity in older breeders and Garlich *et al.* (1984) verified a decrease in shell thickness during the course of the first laying year. In the present study, although the egg weight increased and proportional eggshell deposition decreased significantly with breeder age, no significant differences were observed in specific gravity of eggs or eggshell thickness from older breeders. The differences found between results may be partially attributable to differences in lines, nutrition and sample period, which are also related to shell quality.

The difference in chick weight at hatching is due to egg size, since Wiley (1950) proposed that egg volume is a limiting factor to embryo development. As shown by Hassan & Nordskog (1971), the most striking relationship between egg weight and embryo growth was seen at 16 days of incubation and at hatching, irrespective of breeder age. Our findings showed that neonatal chick weight was dependent on broiler breeder age, supporting the hypothesis of Wiley (1950), since the older the breeder, the bigger the egg.

The blood parameters of neonatal chicks were not affected by broiler breeder age, but red blood cell number and hematocrit were lower in males than in females. These findings suggest that, irrespective of breeder age, the blood capability to transport oxygen and related metabolic substrates was similar in all chicks

despite the difference in body weight. A sex difference had been reported during embryo development in broiler chicks (Mitchell & Burke, 1995) and turkey chicks (Burke *et al.*, 1994), especially related to hormone concentration; however, no data were found in the literature regarding blood parameters in the embryo due to breeder age.

Other blood parameter studied in broiler chickens was blood viscosity. High hematocrit values have been recognised as one of the conditions that increase blood viscosity (Maxwell *et al.*, 1992). Our results showed that red blood cell number and hematocrit value were affected by broiler chick sex, but did not affect blood viscosity. These findings may indicate that hematocrit value cannot be used to support the total change in blood viscosity. The results provide no evidence that breeder age affects blood viscosity.

Hypertrophy of the right ventricular wall can be directly related to increased pulmonary arterial pressure, and the ratio of the right ventricle to the total ventricle mass can be used as a measurement of the increased pressure load on the right ventricle (Burton *et al.*, 1968; Julian *et al.*, 1986). In this study, absolute right ventricle and total ventricle weights, as expected, were affected by breeder age but, the cardiac index was not, and sex had no effect on these heart parameters. Thus, these findings revealed that ventricle development follows a normal growth during embryogenesis, and that breeder age and sex are not determining factors that could influence right ventricle or total ventricle weights until hatching. These results suggest that chick susceptibility to metabolic diseases after hatching seems not to be related to breeder age.



In conclusion, the findings of this study revealed that broiler breeder age affects the characteristics of neonatal chicks, such as live body weight and absolute heart and lung weight, but blood parameters (viscosity, red blood cell number, hematocrit, mean cell volume and hemoglobin) and the eggshell characteristics are not dependent on breeder age.

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