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ABSTRACT

Two experiments were carried out to study the effect of breeder age on incubation parameters (hatchability, eggshell thickness, egg surface temperature and chick weight). In Exp. 1, fertile eggs (30- and 60-wk-old breeders) were incubated at three different temperatures (36.8, 37.8 and 38.8 °C). Eggshell surface temperature was measured by attaching a thermocouple to the shell and data were collected in a datalogger every ten minutes. This study was conducted according to a 3 x 2 factorial design (three temperatures and two breeder ages). Data revealed that eggshell surface temperature changed according to incubation temperature, with the main increase occurring between 10 and 13 days of incubation, and that the maximum increase in eggshell surface temperature was not higher than +0.6 °C, irrespective of incubator temperature. The incubator temperature affected total incubation period and hatchability (%) at 38.8 °C, independent of breeder age. Heavier eggs resulted in heavier chicks, irrespective of incubator temperature. In Exp 2, the eggs (30- and 60-wk-old breeders) were incubated at 37.8 °C and eggs characteristics (weight, specific gravity, total hatchability and chicks weight) were evaluated according to a randomized experimental design. The data showed that breeder age affected eggshell thickness and chick weight (heavier eggs resulted in heavier chicks), but not specific gravity, eggshell surface temperature or hatchability. The findings of this study revealed that hatchability can be influenced by incubation temperature, but not by the breeder age. Breeder age can affect eggshell thickness, egg weight and eggshell surface temperature, but not specific gravity.

INTRODUCTION

Optimal incubation temperature is described as the temperature in which hatchability reaches its maximum and chick quality is the best (French, 1997; Decuypere & Michels, 1992). Newly-hatched chicks can have their thermoregulatory capability affected by incubation temperature (Tzschentke & Rumpf, 2011), which can also affect their blood hormones levels (Christensen *et al.*, 2001) and development after hatching (Molenaar *et al.*, 2011).

Incubator temperature in hatcheries is set between 37.5 to 37.8 °C (Tullet, 1990), at which the embryo presents adequate development, but French (1997) reported that hatching may occur at incubator temperatures ranging from 35.0 to 40.5 °C. Sotherland *et al.* (1987) reported that the main increase in eggshell surface temperature is observed after D12 to D13 of incubation, mainly due to the increase in metabolic embryo heat production. At this stage internal egg temperature can increase by 1.5 °C above incubator air temperature.

Internal egg temperature measurement implies invasive procedures

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that can affect embryo development (Holland *et al.*, 1998). Since egg thermal conductivity is high compared with air conductivity, it is expected that internal egg temperature is slightly different from eggshell surface temperature (Lourens *et al.*, 2005), and therefore, during embryo development, eggshell surface temperature measurements have been used to estimate embryo temperature.

A good correlation between breeder age and egg size was previously demonstrated by Raju et al. (1997). It was shown that heat exchange in heavier eggs is higher since eggshell thermal conductivity increases in larger eggs (Sotherland et al., 1987; Meijerhof & van Beek, 1993). Breeder age had been also investigated in relation to embryo gut development (Maiorka et al., 2002) or embryo heat acclimation (Yalçin et al., 2008; Givisiez et al., 2003).

This study was carried out to investigate the effect of incubator temperature (36.8, 37.8 and 38.8 °C) and breeder age (30 and 60 weeks) on hatchability, eggshell temperature and chick development (Experiment 1), and if the characteristics of eggs produced by 30-or 60-wk-old breeders interfere on hatchability and chick weight when eggs are incubated at 37.8 °C (Experiment 2).

MATERIALS AND METHODS

Incubator

Both experiments were carried out using a Petersime® incubator, Labo model, containing six blocks with 90° turning every hour and with a capacity of 900 eggs. The incubator was provided with hatching space. The incubator temperature was adjusted according to dry bulb thermometer and kept constant until the end of the incubation period. The relative humidity inside the incubator machine was of approximately $55 \pm 2\%$.

Fertile eggs

Fertile eggs (360 in Exp 1 and 900 in Exp 2) produced by Cobb® 500 broiler breeders were obtained from a commercial hatchery, being half produced by 30-week-old breeders, and the other half by 60-week-old breeders.

Eggshell surface temperature

Eggshell surface temperature was measured by attaching a thermocouple (cooper-constant) to the shell with an adhesive tape. The thermocouple was connected to a datalogger CR10X (Campbell Sci. Inc., USA) and the temperature was recorded during the

entire experimental period. Data were collected at every 10 seconds and the mean temperature at every 10 minutes was recorded.

The thermocouple had a precision of 0.05 °C and it was calibrated by using standard mercury thermometer immersed in water.

Experimental Design

Experiment 1 was performed at three different incubation temperatures, using a total of 360 fertile eggs, with 180 eggs from each breeder age group, and 10 for each temperature, being 60 eggs from each breeder age.

Egg weight, total hatchability and chick weight were evaluated in Exp 1 according to a 3 x 2 factorial design (three incubation temperatures: 36.8, 37.8 and 38.80 C, and two breeder ages: 30 and 60 wk). Eggshell surface temperature data were analyzed according to a 3 x 2 x 19 factorial design (three temperatures, two breeder age and 19 days of incubation). Both analyses were performed using the General Linear Models (GLM®, SAS, 2000) with different number of replicates. Means were compared by "F" test and Tukey's test (p<0.05).

In Exp 2 an incubation temperature of 37.8 °C was applied and 900 fertile eggs were used (450 eggs from each breeder age). In this experiment, before incubation, 30 eggs per breeder age were used to determine egg specific gravity according to the method described by Moreng & Avens (1990). The same egg samples were used to determine the eggshell thickness using a micrometer. The same procedure to determine eggshell thickness was used on D13 and D21 of incubationt. The experiment was conducted according to a randomized experimental design. Eggshell thickness data were analyzed according a 2 x 3 factorial arrangement (two breeder ages and three incubation times – D0, D13 and D21), and eggshell surface temperature data were analyzed according to a 2 x 20 factorial design (two breeder ages and 20 days of incubation).

The analyses were performed using the General Linear Models (GLM®, SAS, 2000) with different number of replicates, and means were compared by "F" test and Tukey's tests (p<0.05).

RESULTS

Experiment 1

Total hatchability of eggs produced by breeders of different ages and incubated 36.8 or 37.8 °C were



not statistically different (p>0.05), but incubation temperature of 38.8 °C significantly reduced (p<0.05) hatchability, irrespective of breeder age (Table 1).

Table 1 – Hatchability (%) of eggs from breeders of different ages and incubated at different incubation temperatures. Number of eggs per temperature and age = 50.

Breeder age	Incubation temperature - °C		
	36.8 °C	37.8 °C	38.8 °C
	Hatchability (%)*		
30 weeks	78Aa*	76Aa	66Ba
60 weeks	72Aa	72Aa	60Ba

^{*}Means followed by the same capital letters in the same row are not statistically different (p>0.05) and by different small letters in the same column are different (p<0.05). Initial mean egg weight (g) according to breeder age (BA) and incubation temperature (IT) were: IT - 36.8 °C: 56.8 g for 30-wks and 68.4 g for 60 wks; IT - 37.8 °C: 63.0 for 30 wks and 70.9 g for 60 wks; IT - 38.8 °C: 59.8 g for 30 wks and 71.5 g for 60 wks.

Chick weight was affected both by incubation temperature and breeder age (Table 2).

Table 2 – Chick weight (g) of eggs produced by breeders of different ages (30 or 60 wks) and incubated at different incubation temperature. Each value represents mean±SEM. Number of birds per temperature: 30.

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Incubation temperature (IT)	Chick weight at hatch (g)*	
Cool (36.8 °C)	47.7±0.6c*	
Neutral (37.8 °C)	49.5±0.6a	
Warm (38.8 °C)	48.7±0.6b	
Breeder age (BA)		
30 weeks	44.5±0.3b	
60 weeks	52.8±0.2a	
	P values	
p (IT x BA)	< 0.05	

^{*}Means in the same column followed by different small letters within each factor are different at 5% probability level (Tukey's test).

The effect of the interaction between breeder age and incubation temperature on chick weight is shown in Table 3. Eggs from 30-wk-old breeders incubated at neutral temperature (37.8 °C) resulted in heavier chicks compared with the other temperatures; however, no effect (p>0.05) of incubation temperature was observed on the weight of the chicks derived from the eggs of 60-wk-old breeders. As expected, irrespective of incubation temperature, chicks derived from the eggs of 60-wk-old breeders were heavier than those derived from 30-wk-old breeders (p<0.01).

Figure 1 (a, b and c) shows the time-course response of eggshell surface temperature as influenced by breeder age and incubation temperature. Interactions

Table 3 – Effect of the interaction between incubation temperature and breeder age on chick weight (g).

Breeder age	Incubation temperature - °C		
	36.8	37.8	38.8
	Chick weight at hatching (g)*		
30 week	43.0Bb*	45.8Ab	44.5Bb
60 week	52.4Aa	53.2Aa	52.9Aa

* Means followed by capital letter in the same row and by small letters in the same column are different by Tukey's test (p<0.05 and p<0.01, respectively).

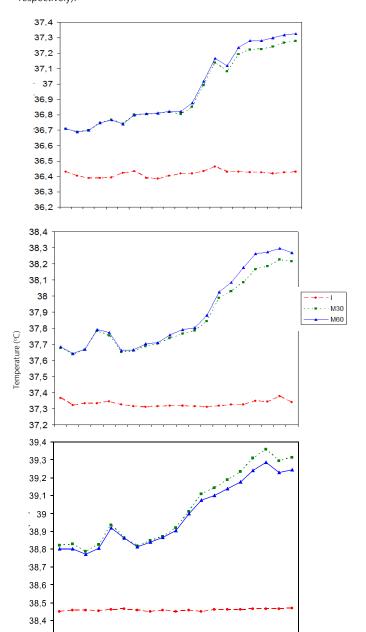


Figure 1 – Incubator (I) and eggshell temperatures of eggs from breeders of 30 (M30) and 60 (M60) weeks and incubated at 36.8 (A), 37.8 (B) and $38.8 \,^{\circ}\text{C}$ (C).

Days of incubation

9 10 11 12 13 14 15 16 17 18 19

38,3

38 2



between incubation temperature and breeder age (Table 4) and incubation temperature and period of incubation (Table 5) were also observed.

Table 4 – Effect of the interaction between incubation temperature and breeder age on mean eggshell surface temperature during 19 days of incubation. Each value represents mean±SEM.

Breeder age	Incubation temperature - °C			
	36.8	37.8	38.8	
30 wk	36.91±0.02bC*	37.84±0.02bB	39.02±0.02aA	
60 wk	36.93±0.02aC	37.88±0.02aB	38.99±0.02bA	

^{*}Means followed by different capital letters in the same row and different small letters in the same column are different by Tukey's test (p<0.05).

Table 5 – Effect of the interaction between incubation temperature and days of incubation. Each value represents the daily mean eggshell surface temperature of eggs produced by 30- and 60-wk-old breeders. Coefficient of variation = 0.16%

Days of	Incubation temperature (°C)			
Incubation	36.8	37.8	38.8	
01	36.71cHIJ*	37.68bHI	38.81aHl	
02	36.69cJ	37.64bl	38.81aHI	
03	36.70cIJ	37.67bl	38.78al	
04	36.75cFGHIJ	37.79bE	38.81aHI	
05	36.77cFGHI	37.77bEFG	38.93aF	
06	36.74cGHIJ	37.66bl	38.86aFGH	
07	36.80cEFG	37.66bl	38.82aHl	
08	36.81cEFG	37.70bFGHI	38.84aGHI	
09	36.81cEFG	37.71bFGHI	38.87aFGH	
10	36.82cEF	37.75bEFGH	38.91aFG	
11	36.81cEF	37.78bEF	39.01aE	
12	36.86cE	37.79bD	39.09aD	
13	37.01cD	37.86bD	39.12aD	
14	37.15cBC	38.01bC	39.17aCD	
15	37.10cC	38.06bC	39.21aBC	
16	37.21cAB	38.13bB	39.28aAB	
17	37.25cA	38.22bA	39.32aA	
18	37.25cA	38.23bA	39.26aAB	
19	37.27cA	38.26bA	39.28aAB	

^{*} Means followed by different capital letters in the same row and different small letters in the same column are different Tukey's test (p<0.05).

The results revealed that mean eggshell surface temperature was slightly higher in the eggs from 60-wk-old breeders when incubated at 36.8 and 37.8 °C, but when incubation temperature was 38.8 °C, eggshell temperature was higher in the eggs of 30-wk-old breeders.

A sharp increase in eggshell temperature on D12 in eggs incubated at 36.8 and 37.8 °C and on D10 for eggs incubated at 38.8 °C was also observed.

Experiment 2

In this experiment, eggs were incubated at the same temperature (37.8 °C) and the results are shown in Table 6. The findings revealed that there was no significant statistical difference (p>0.05) in hatchability or egg specific gravity between breeder ages, and, as expected, heavier eggs (60-wk-old breeders) resulted heavier chicks (p<0.01).

Figure 2 shows the time course response of eggshell surface temperature during the incubation period of eggs produced by breeders of both ages at the same incubation temperature (37.8 °C). Breeder age did not affect eggshell temperature (p>0.05), but, as expected, it changed according to the incubation period. A sharp increase in eggshell temperature was again observed after D12, irrespective of breeder age.

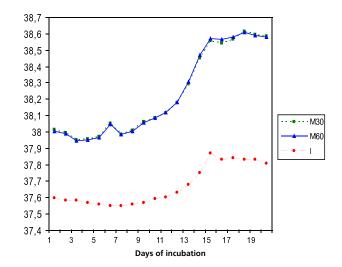


Figure 2 – Incubator (I) and eggshell temperatures of eggs from breeder of 30 (M30) and 60 (M60) weeks of age which were incubated at 37.8 °C.

Table 6 – Egg weight (g), specific gravity, total hatchability (%) and chick weight (g). Each value represents mean±SEM. Number of eggs per breeder age = 450.

Breeder age (wk)	Egg weight (g)	Specific gravity	Total hatchability (%)	Chicks weight (g)
30 wk	59.48±0.57b*	1.084±0.006a	74a	44.45±2.70a
60 wk	70.39±0.46a	1.082±0.004a	71a	52.12±3.66b

^{*} Means followed with the same small letters in the column are not different by Tukey's test (p>0.05).



Eggshell thickness was influenced by breeder age, with young breeders producing eggs with thicker eggshells (0.376 vs. 0.365 mm; p <0.01). On the other hand, during incubation, shell thickness was reduced irrespective of breeder age (mean value at D0=0.392 mm, D13=0.376 mm and D21=0.343 mm; p<0.01).

DISCUSSION

The findings of this study showed that heavier eggs resulted in heavier chicks, as expected, corroborating several authors that reported a positive correlation between egg weight and chick weight (Shanawany, 1984; Raju et al., 1997). On the other hand, egg weight increases with the breeder age (Suarez et al., 1997; Novo et al., 1997; Lourenço da Silva, 1994; North & Bell, 1990; Sooncharenying & Edwards, 1989); thus, older breeders with heavier eggs produced heavier chicks, irrespective of incubation temperature. Chick weight is an important factor in broiler growth, since it was reported that there is a positive and strong correlation between chick weight at hatch and broiler market weight (42 to 45 days of age) (Raju et al., 1997; Proudfoot & Hulan, 1981).

Hatchability was significantly lower in the eggs incubated at high ambient temperature (38.8 °C), but there was no influence of breeder age on hatchability. Heier & Jarp (2001) reported that mean hatchability increased in eggs from breeders in their first production cycle (28 to 36 wk), decreasing afterwards. The lack of differences in hatchability observed in present study may be related to correct egg management, the short interval between lay and incubation (Roque & Soares, 1994) and eggshell quality (Berrang et al., 1998). Bennett (1992) reported reduced hatchability in eggs from old breeders; however, in that study, the specific gravity of about 90% of the eggs of 55-wk-old breeders waslower than 1.080 (low eggshell quality). In our study, specific gravity was not statistically different between breeder ages; however, it was higher than 1.080, an indication of good eggshell quality (Berrang et al., 1998; Lourenço da Silva, 1994; Bennett, 1992). It must be noted that aging does not necessarily impair eggshell quality; in fact, hatchability may still be high in eggs from old breeders (Berrang et al., 1998; North & Bell, 1990). Hamidu et al. (2007), studying eggshell conductance, reported that neither breeder strain or parent flock age affected this parameter.

Eggshell thickness was reduced during the incubation period, independently of breeder age. Lourenço da Silva (1994) stated that the embryo can use about 47%

of the eggshell mass during the incubation period, since it provides approximately 80% of the calcium for skeleton formation after D10 (Peebles & Brake, 1985). In the present study, the most striking difference in eggshell thickness was observed on D13, which is consistent with the findings of Peebles *et al.* (2001), who reported a decrease in eggshell weight of eggs produced by 27- and 36-wk-old breeders after D12.

Eggshell surface temperature changed as a function of incubation temperature and breeder age. The effect of breeder age on eggshell temperature can be partly explained by the fact that embryos from larger eggs have higher metabolic heat production, but their relative surface for heat exchange is lower (Meijerhof & van Beek, 1993; Hamidu et al., 2007). On the other hand, due their size, heat exchange may be reduced among eggs in the incubator. At all incubation temperatures, eggshell temperature increased the most between 10 and 13 days of incubation, which is consistent with the findings of Nichelmann et al. (1998) and Decuypere et al. (1979) that embryo heat production increases during the second period of incubation (after D12). Romijin & Lokhorst (1960) also reported that at the beginning of incubation period evaporative heat loss is higher than the metabolic heat production, and therefore, the egg gains heat; however, during the second incubation period (D12 onwards) embryo metabolic heat production is much higher than its evaporative heat loss, thereby increasing eggshell surface temperature.

In conclusion, the findings of the present study showed that hatchability can be influenced by incubation temperature, but not by breeder age. Breeder age can also affects eggshell thickness, egg weight, and eggshell surface temperature, but not specific gravity.

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