



## EFFECT OF BROILER BREEDERS' AGE ON EGGSHELL TEMPERATURE, EMBRYO VIABILITY AND HATCHABILITY PARAMETERS\*

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### Abstract

The aim of the study was to analyze eggshell temperature, embryo viability, and hatchability parameters of broiler breeders at different ages (26–30, 31–35, 36–40, 41–45, and 46–64 weeks). A total of 33,150 eggs from Ross 308 broiler breeders collected from commercial flocks were used to analyze the following: egg weight and egg weight loss during 18 days of incubation; eggshell temperature controlled on days 3, 14, and 18 of incubation; number of infertile eggs including eggs with dead embryos at early stages of development, that is, in the oviduct or after oviposition; the total percentage of embryonic mortality and the percentage of embryonic mortality at different times of incubation; unhatched, dead after hatching, and culled chicks as well as hatchability percentage from fertilized eggs. The hatchability results of Ross 308 broiler breeders were high from the beginning of reproductive season till the 40th week. However, the study failed to reveal any relationships between hatchability and the egg weight, average temperature of the shell, and egg weight loss during incubation period. The oldest hens, over 45 weeks of age, had the highest mortality of chicks and the greatest share of unhatched chicks.

**Key words:** broiler breeders, egg incubation, eggshell temperature, hatchability

High production of broiler meat depends on various factors, such as genetics, environmental conditions, nutrition, incubation process, etc. As a result, adequate keeping of broiler breeders and frequent analysis of hatchability results seem to be key factors for efficient production of high quality chicks.

A significant impact of females' age on morphology and quality of eggs on reproductive performance were proved and described in many scientific publications

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(Lapao et al., 1999; Tona et al., 2004; Adamski, 2008; Pirsaraei et al., 2011). Significant deterioration in the characteristics of the eggshell with the age of the hens was also found (Yilmaz and Bozkurt, 2009). A positive correlation between the thickness and strength of the eggshell and the number of hatched chicks can be explained by the fact that a thicker eggshell has lower water vapor conductance (Christensen, 1983; Bennett, 1992).

Changes in the quality of eggs, especially of their shells, with the passage of reproductive season can affect the embryonic development during the incubation period and, finally, the hatchability results. For example, Lourens et al. (2006) showed that the heavier eggs, obtained usually from older broiler breeder hens, had a greater weight loss during incubation period than lighter ones. However, Alsobayel et al. (2013) reported that eggs from heavier hens, 40–45 weeks of age, were characterized by significantly lower weight loss during incubation process than those obtained from younger (lighter) birds. Diversity of porosity and permeability of the eggshell may further result in changes in egg temperature during hatching, an important indicator of proper embryogenesis process. In addition, a relationship was shown between the abovementioned parameters and the body weight of broiler chickens on days 1, 21, 35, and 44 and feed conversion ratios (Hulet et al., 2007). On the one hand, differences in eggshell temperatures in terms of female age were found in broiler breeders Cobb 500 (Gualhanone et al., 2012) after day 10 of incubation. Moreover, it was also observed that age did not have an impact on the hatchability parameters. On the other hand, Islam et al. (2008) observed the best egg fertilization and hatchability in hens aged between 41 and 60 weeks. Also, Al-Bashan and Al-Harbi (2010) and Othman et al. (2014) reported the effect of age of females on fertility, hatchability parameters, and embryonic mortality during incubation period.

Only few researchers attempted to analyze the development of embryos in eggs derived from hens at different ages. Alsobayel and Albadry (2012) reported some results of embryonic mortality in early and final stages of incubation of eggs obtained from laying hens aged 24–52 weeks. However, they did not observe any influence of female age on these traits.

The aim of the research was to study the impact of broiler breeders' age on eggshell temperature, the embryo viability, and hatchability parameters.

### **Material and methods**

The experiments were conducted in a commercial hatchery belonging to the DanHatch company located in Poland. The hatchery was equipped with Smart Set Pro 115 setters (Pas Reform, Zeddum, The Netherlands) with a capacity of 115,200 chicken eggs in each. There were 24 trolleys with 32 setting trays in each setter; each tray could accommodate 150 eggs.

The experimental material comprised 33,150 eggs from broiler breeders Ross 308 collected from commercial flocks situated in western Poland. All stocks were kept on litter with standard environmental conditions (Aviagen, 2009). All breeder

houses were equipped with mechanical nests, egg collecting system, chain feeders (with grill profiles) for feeding females and pan feeders for feeding males. Males and females were fed different diets according to the recommendations of Aviagen (2009). During the production period, birds were fed using layer I or layer II diets according to their production level. Diet nutritional values (crude protein, Ca, Na, starch fat content), from each investigated flock, were analyzed using AOAC (2005) methods four times per production period. Appropriate formula was used to calculate the metabolizable energy level. All investigated parameters were in line with Aviagen (2009) recommendations. All diets were standard commercial maize–wheat–soybean based, in a mash form delivered from one feed plant.

During the research, data from a total of 74 incubations were analyzed. Eggs were placed on 3 setting trays at the bottom, middle, and top level of each trolley. In addition, on each setter, eggs were positioned in 3 different places: front, center, and back.

Before incubation, the eggs were stored (for 7 days) at 14°C and 40% relative air humidity. After the eggs were set in the trays, they were sprayed with formaldehyde as a disinfectant. To avoid steam condensation of shell surface, the eggs were gradually tempered for approximately 4–6 hours at 25–27°C and 50–55% relative humidity before the start of the incubation process. In the early stages of incubation (1–3 days), the temperature inside the setting compartment varied between 37.8°C and 38.0°C. Thereafter, it gradually decreased (0.1–0.2°C daily), and on the 18th day of incubation, the temperature had already reached 36.7°C. The relative humidity ranged from 57% to 64% on the first day of incubation. Then, relative humidity was maintained at approximately 54% (up to the 8th day) and was reduced to 44% (from the 14th to the 18th day). Trays were turned at an angle of 45° once every 30 minutes. On the last 3 days of incubation, the hatching temperature was maintained at 36.7–36.3°C and relative humidity at 50–60%.

While transferring eggs to the hatching compartment (18th day of incubation), the eggs were not candled because all the breakouts were opened only after the hatching of chicks. The experimental eggs were placed as close as possible in the hatching compartment to minimize the effect of this compartment on the analyzed traits.

The evaluation of embryo viability and hatchability indices were based on the analysis of breakouts. The following were assessed: number of infertile eggs, including eggs with dead embryo at early stage of development, that is, in oviduct or after oviposition, and the number of dead embryos in successive stages of incubation (days 2, 3–4, 5–6, 7–8, 9–10, 11–12, 13–14, 15–16, 17–18, and 19–20, respectively). Based on the data obtained, the subsequent values were calculated: total percentage of embryo mortality and percentage of embryo mortality at different stages of incubation, unhatched chicks (*i.e.*, chicks that were either dead or alive during external pipping and unable to leave the egg), chicks that were dead after hatching together with culled chicks that were unfit for further production cycle, and also hatchability from fertilized eggs (%). All of these traits were examined in terms of broiler breeder hens' age, which were as follows: 26–30, 31–35, 36–40, 41–45, and 46–64 weeks of life. The results from 7,650, 7,950, 6,300, 5,850, and 5,400 eggs were analyzed in each stage of hens' life, respectively.

The setting trays were weighed on the first and on the 18th day of incubation using a WPW/T15HR2/FH RADWAG type balance ( $\pm 5$  g) to determine the average egg weight loss (%) in this period. Using a Braun ThermoScan IRT 3020 thermometer ( $\pm 0.1^\circ\text{C}$ ), eggshell temperature during each hatching was controlled on days 3, 14, and 18 of incubation (10 eggs on each tray at random, 30 eggs per trolley). Temperature measurements were performed in the equatorial part of each eggshell.

Statistical analysis was performed using SAS<sup>®</sup> v.9.2 statistical package (SAS, 2011). Mean values ( $\bar{x}$ ) and standard error of the mean (SEM) were estimated for all of these traits.

In order to determine the impact of the hens' age on egg weight, egg weight loss, eggshell temperature in different days of incubation, egg fertilization, embryonic mortality, and hatchability results, a three-way linear model of ANOVA was used:

$$Y_{ijkl} = \mu + a_i + v_j + h_k + e_{ijkl}$$

where:

$y_{ijkl}$  – phenotypic value of the trait for the  $l$ th group of eggs, from  $i$ th hens' age, and the  $j$ th vertical and the  $k$ th horizontal location in the setter,

$\mu$  – mean value of the trait for a given population,

$a_i$  – effect of  $i$ th age ( $i = 1, 2, 3, 4, 5$ ),

$v_j$  – effect of  $j$ th location—vertical ( $j = 1, 2, 3$ ),

$h_k$  – effect of  $k$ th location—horizontal ( $k = 1, 2, 3$ ), and

$e_{ijkl}$  – effect of experimental error.

The significance of differences between the means of each age group of hens was estimated at the level  $P \leq 0.05$  and verified by Duncan's test.

## Results

Changes in eggshell temperature during incubation process are presented in Table 1. The overall eggshell temperature was significantly different; only eggs from breeders at 36–40 weeks of age and others. It is worth noting that this group was also characterized by the highest value of the eggshell temperature. The lowest temperature of shells was recorded in eggs from the oldest hens, over 45 weeks of age. On day 3 of incubation, eggs laid by hens aged 26–40 and >45 weeks had essentially the same shell temperature ( $37.8^\circ\text{C}$ ). Minimum value ( $P \leq 0.05$ ) of the trait was recorded in birds in the period between 41 and 45 weeks of age. However, the highest shell temperature ( $\bar{x} = 37.9^\circ\text{C}$ ) on the 14th and 18th day of incubation was observed in the eggs from hens aged 36–45 weeks.

The difference between the average weight of the eggs from youngest (26–30 weeks of age) and oldest hens (46–64 weeks of age) was as high as 11.4 g (Table 2). As far as egg weight loss percentage during incubation was concerned (Table 2), the lowest value was found in hens at the age of 26–30 weeks and the high-

est in 4 consecutive weeks of birds' life. In other age groups, this value was similar and on average amounted to 10.7%.

Table 1. Effect of broiler breeders' age on eggshell temperature during incubation period

Traits	Age (weeks)					Model P	Pooled SEM
	26–30	31–35	36–40	41–45	46–64		
Eggshell temperature (°C)							
3rd day of incubation	37.81 a	37.81 a	37.81 a	37.77 b	37.81 a	0.046	0.012
14th day of incubation	37.85 ab	37.84 ab	37.89 a	37.88 a	37.81 b	0.050	0.034
18th day of incubation	37.92 b	37.93 b	38.03 a	37.97 ab	37.90 b	0.022	0.048
Mean	37.86 ab	37.86 b	37.91 a	37.87 ab	37.84 b	0.048	0.022

a, b – mean values in rows with different letter differ significantly at  $P \leq 0.05$ .

Table 2. Effect of broiler breeders' age on egg weight and hatchability results

Traits	Age (weeks)					Model P	Pooled SEM
	26–30	31–35	36–40	41–45	46–64		
Egg weight (g)	55.12 e	58.13 d	62.22 c	63.78 b	66.49 a	< 0.0001	0.276
Fertile eggs (%)	92.6 b	95.5 a	95.1 ab	93.7 ab	83.9 c	< 0.0001	0.770
Embryonic mortality (%)*							
1–2 days	51.5	50.2	52.2	50.1	46.6	0.700	2.714
3–4 days	6.8	6.7	7.3	7.4	6.2	0.985	1.428
5–6 days	2.3	1.5	1.9	4.0	2.4	0.394	0.942
7–8 days	1.3	2.3	1.1	0.7	2.0	0.499	0.626
9–10 days	2.9	3.8	3.0	1.6	2.5	0.381	0.798
11–12 days	0.8	1.0	0.4	0.4	0.6	0.782	0.388
13–14 days	1.8	1.5	1.2	2.3	1.7	0.843	0.686
15–16 days	0.5 b	3.1 b	2.8 b	1.9 b	5.9 a	0.001	0.806
17–18 days	5.6	5.2	6.3	4.7	6.2	0.894	1.188
19–20 days	23.0 a	20.9 ab	16.2 b	21.9 ab	18.2 ab	0.047	2.041
Overall mortality (%)	8.9 b	8.1 b	8.4 b	8.2 b	11.2 a	0.001	0.500
Egg weight loss up to 18th day (g)	10.3 c	11.1 a	10.7 b	10.6 bc	10.9 ab	< 0.0001	0.112
Dead** and culled chicks (%)	3.5 b	3.8 ab	7.6 a	5.0 ab	7.7 a	0.049	1.302
Hatchability from fertilized eggs (%)	90.2 a	90.6 a	90.1 a	89.8 a	85.8 b	< 0.0001	0.588
Unhatched chicks (%)	1.0 c	1.3 c	1.5b c	2.0 b	3.0 a	< 0.0001	0.224

a, b, c, d, e – mean values in rows with different letter differ significantly at  $P \leq 0.05$ .

\* Mortality at each incubation period + dead and culled chicks = 100%.

\*\* After hatching.

The best egg fertilization was observed in hens from 31 to 35 weeks of age. The oldest females were characterized by the lowest value of the egg fertilization. The difference between this group and others was statistically confirmed and amounted to 10.3 percentage points. While examining the characteristics of the hatchability results (Table 2), it was found that hatchability from fertilized eggs was very similar in all groups up to the 45th week ( $\bar{x} = 90.2\%$ ). The worse value ( $P \leq 0.05$ ) of the hatchability and the highest share of dead embryos during incubation were recorded

in the oldest hens. Moreover, in the case of unhatched chicks percentage (Table 2), it was explicitly stated that this value significantly increased with the age of the hens. The difference ( $P \leq 0.05$ ) between birds aged over 45 weeks and younger ones was approximately 1.6 percentage points.

No significant difference was found in the analyzed groups of hens in terms of embryonic mortality up to the 14th day of incubation (Table 2). Only on days 15–16, significantly higher embryonic mortality was found in older birds in comparison with other groups. However, birds aged 36–40 weeks were characterized by the lowest embryonic mortality during the final stage of the incubation process (19–20 days). In this period, the highest value of the said feature was observed in eggs obtained from the youngest females. Conversely, younger hens (up to 35 weeks of age) were characterized by the smallest share of chicks which died after hatching and those which were not suitable for further production ( $\bar{x} = 3.7\%$ ). In other age groups, the mean value of embryonic mortality was approximately 6.8%. The ratio of the dead to culled chicks was approximately 1:3 in each group.

## Discussion

As far as egg temperature during the incubation is concerned, the hens aged 36–40 weeks were characterized by the highest value of this trait. However, the obtained results are difficult to interpret because of the insufficient literature data in this area. The average temperature of eggs from young hens (average egg weight 55.1 g) was similar to the heaviest (66.5 g), which were laid by the oldest hens aged over 45 weeks. On the one hand, Elibol and Brake (2008) found that eggs of broiler breeders with different weight, incubated close to the fan inside setter, were characterized by similar eggshell temperature on the 18th day of embryogenesis. However, when the authors compared eggs located far from the fan, the eggs with the greatest weight were characterized by significantly higher eggshell temperature. On the other hand, results published by Gualhanone et al. (2012) proved that eggs of older hens (60 weeks old) incubated at the temperature of 36.8°C and 37.8°C were characterized by higher eggshell temperature (after day 10 of incubation) than those obtained from younger hens aged 30 weeks. When eggs were incubated at a higher temperature (38.8°C), the authors observed a reverse situation. For the above reason, it is difficult to explain clearly the results of our own study. It should be noted that the experiments were conducted with a lot of repetitions in different setters with identical incubation parameters.

The optimal total egg weight loss during incubation is 11%–12% of its initial weight (Van Brecht et al., 2005; Boerjan, 2012). A slightly lower value was found in this study ( $\bar{x} = 10.71\%$ ). Nevertheless, the egg weight loss percentage was within the range reported by Tona et al. (2001). In fact, no significant changes in terms of the egg weight loss percentage were observed up to the 35th week. Therefore, it cannot be proved that with the passage of reproduction period, a significant egg weight increase is reflected in a higher egg weight loss during the incubation. Lourens et al.

(2006) and Nowaczewski et al. (2010) observed such a relationship in broiler breeders and Japanese quails. However, the authors compared eggs with different weights from birds of the same age group.

Egg fertilization was very good in comparison with other studies. Hristakieva et al. (2014) and Tarasewicz and Aniško (2015) observed this value at 84.2–90.7% level in Ross 308 parent stock. In this experiment, the average hatchability percentage from fertilized eggs ranged from 85.8% to 90.6%. This result can also be considered suitable in terms of the declared value hatchability (approx. 85%) by Aviagen (2011). The worst hatchability, the greatest share of dead embryos, unhatched and dead chicks after hatching as well as the highest percentage of chicks, which were not suitable for further production were recorded in the oldest hens aged over 45 weeks. It is worth noting that the lowest temperature of eggshells during incubation was also demonstrated in this group. In other groups as well, these values were very similar. Joseph et al. (2006) and Lourens (2008) obtained the best hatchability in broiler breeders from eggs incubated at a higher temperature. Therefore, it appears that hatchability results should consider egg temperature during embryogenesis. The experiment conducted by Gualhanone et al. (2012) seems to confirm this statement. These authors observed the effect of broiler breeders' age on hatchability results only for eggs incubated at 36.8°C. When incubation temperature ranged from 37.8°C to 38.8°C, differences were also found (better hatchability was recorded in younger hens) but were not confirmed statistically. Al-Bashan and Al-Harbi (2010) analyzed reproductive results of broiler breeders (Arbor Acres × Lohmann Brown hybrid) in the period from 24 to 65 weeks of age and observed the best hatchability from fertilized eggs between 35 and 50 weeks of age ( $\bar{x}$  = 90.7%). In the case of our own research, high hatchability value persisted from the beginning of laying season till the 45th week. In other studies, Elibol and Brake (2006) indicated better hatchability results from fertilized eggs in Ross 308 broiler breeders aged 34–37 weeks in comparison with older birds (59–61 weeks of age). Differences in hatchability over production period could be attributed to the quality of eggshell deterioration. The main reason for shell quality decline over the laying period is due to a gradual loss in Ca deposition efficiency and withdrawal of the medullary bone (Farmer et al., 1986). However, El Sabry et al. (2013) reported that birds' age did not influence the thickness of eggshell.

Tona et al. (2001) analyzed the process of embryo development in Cobb hens at different ages (27–60 weeks) and also found a rapid increase in the total embryonic mortality during incubation of eggs laid by older hens (46–64 weeks of age). The lowest value of this feature was demonstrated in hens aged between 35 and 40 weeks. Our research confirmed this to some extent. Abudabos (2010) compared embryo mortality at different stages of embryogenesis in 26- and 44-week-old broiler breeders. The only difference between the abovementioned groups of birds were noticed for dead embryos percentage on the 14th day of incubation. Higher embryonic mortality was observed in older hens (8.5% vs. 0.7%). Similar results were obtained in the present study, where the highest embryo mortality between the 15th and 16th day of incubation was recorded in eggs obtained from the oldest hens ( $\bar{x}$  = 5.9%).

On the basis of the results obtained, it can be concluded that lower levels of hatchability parameters were recorded in Ross 308 broiler breeders over 40th weeks of age. The oldest hens, more than 45 weeks of age, were also characterized by the highest mortality of chicks and the highest share of unhatched chicks. However, the results do not warrant the conclusion that the hatchability results were associated with egg temperature changes during the incubation process.

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