



## EFFECT OF HEN AGE AND STORAGE TIME ON EGG WEIGHT LOSS AND HATCHABILITY RESULTS IN TURKEYS

Monika Stepińska<sup>1</sup>, Emilia Mróz<sup>1\*</sup>, Magdalena Krawczyk<sup>1</sup>, Kamil Otowski<sup>1</sup>, Alina Górską<sup>2</sup>

<sup>1</sup>Department of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland

<sup>2</sup>Faculty of Natural Sciences, University of Natural Sciences and Humanities, Konarskiego 2, 08-110 Siedlce, Poland

\*Corresponding author: emilia.mroz@uwm.edu.pl

### Abstract

The aim of this study was to determine the effect of egg water loss during storage and incubation on hatch rates in heavy-type broad-breasted white BUT Big 6 turkeys. Turkey hens started laying eggs at 30 weeks of age. In weeks 2, 8, 16 and 21 of the laying season, 1512 eggs were selected randomly and divided into 4 groups of 378 eggs each. The groups of eggs were stored for 7, 10, 13 or 17 days before incubation. At the beginning and end of the storage period and on days 9, 15, 21 and 24 of incubation, eggs were weighed to determine the percent water loss relative to the egg's weight. Four incubation cycles of 378 eggs each were performed for each storage period. A total of 16 incubation cycles were carried out (4 weeks of the laying season × 4 egg storage periods) and the following parameters were determined (%): egg fertilization, dead embryos, unhatched eggs and hatchability results from fertilized eggs. The percentages of dead embryos and unhatched poults with physical defects and abnormal position were determined in hatchery waste from each incubation cycle. Egg water loss varied throughout storage and reached 0.57% of total egg weight after 7 days, 0.79% after 10 days, 0.87% after 13 days and 1.28% after 17 days ( $P \leq 0.05$ ). After 7 days of storage, egg water loss during a 15-day and 21-day incubation period reached 5.76% and 8.72%, and lower values were noted after 17 days of storage ( $P \leq 0.05$ ). Egg water loss of 1.28% during storage resulted in a high rate of early embryonic mortality (14.81%) and a low hatch rate (72.12%) ( $P \leq 0.05$ ). High water loss in stored eggs contributed to a higher percentage of congested embryos. During storage, egg water loss reached 0.72% in week 2 of the laying season, 0.78% in week 8 and  $\geq 1\%$  in weeks 16 and 21 of the laying season ( $P \leq 0.05$ ). Egg water loss during a 21-day incubation period was similar in weeks 2, 8 and 16 of the laying season, and lower in week 21 ( $P \leq 0.05$ ). The hatchability of turkey eggs was lowest in weeks 16 and 21 of the laying season ( $P \leq 0.05$ ). Low water loss during incubation contributed to a high rate of late embryonic mortality (13.2%). High water loss during egg storage is accompanied by lower water loss during incubation. Water loss should be monitored after storage and on days 15 and 21 of incubation to evaluate water metabolism in hatching eggs.

**Key words:** turkey, egg storage, breeder age, water loss, hatchability

The yolk of turkey eggs contains 43–49% of water, and water content varies over the laying season (Applegate and Lilburn, 1996; Faruga et al., 1996). According to some studies, the water content of the egg yolk is constant in wild birds and turkeys (Tazawa and Whittow, 2000; Mróz et al., 2014). The albumen of turkey eggs contains 87.3–88.2% of water, and water content increases with the progress of the laying season (Faruga et al., 1996; Mróz et al., 2014). An identical trend is also observed in other poultry species (Lourens et al., 2006; Willems et al., 2014).

Water evaporates from eggs during storage. The evaporation process is also determined by environmental factors and egg characteristics (Garip and Dere, 2011; Hristakieva et al., 2011). Water vapor conductance of the eggshell varies across poultry species and utility types (Mróz, 1996; Mróz et al., 2007; Demirel and Kırıkçı, 2009; Alsobayel et al., 2013). Prolonged storage of turkey eggs, from 4 to 14 days, increased water loss by 1.04% (Hristakieva et al., 2011) whereas prolonged storage of chicken eggs, from 7 to 14 days, increased water loss by 1.14% (Alsobayel et al., 2013). In other studies, water loss from chicken eggs was lower, ranging from 0.30% after 4 days of storage to 0.83% after 14 days of storage (Reijrink et al., 2010). During 24–25 days of incubation, total water loss from turkey eggs is 9.5% to 14% (Christensen et al., 1996; Mróz and Pudyszak, 1997; Hristakieva et al., 2011). Egg water loss during 24 days incubation is significantly influenced by incubation technology and the time of first lay and length of lay (Christensen et al., 1996; Mróz and Pudyszak, 1997; Hristakieva et al., 2011). According to the cited sources, turkey egg water loss is higher and stable at the beginning and in the middle of the laying season, reaching 11–12%, whereas towards the end of the laying season water loss is lower and varies widely from 9.5% to 11.9% relative to the egg's weight before incubation. Similar trends can also be observed in laying hens (Meir and Ar, 2008; Ulmer-Franco et al., 2010). Water loss from turkey eggs during incubation results from changes in egg quality (Christensen et al., 2003; Mróz et al., 2004) and incubation technology (Boerjan, 2006; Lopez and Hergott, 2014).

Water evaporation from the egg is also determined by the developing embryo's metabolism (Christensen et al., 1996). A higher metabolic rate accelerates water evaporation, thus maintaining an adequate hygienic status of the egg (Tazawa and Whittow, 2000). Water permeates through the eggshell, hydrolyzes  $\text{CaCO}_2$  and carries  $\text{Ca}^{2+}$  ions into the embryo's bloodstream. Carbon dioxide, a product of hydrolysis, acidifies the eggshell and protects the embryo against infections (Solomon, 1996; Tazawa and Whittow, 2000).

Excessive water evaporation from the egg during incubation reduces nutrient availability to the embryo, increases embryonic mortality and decreases hatch rates (Christensen et al., 1996; Abudabos, 2010; Madeddu et al., 2013). In turkeys, embryonic mortality rates were lower at egg water loss of 12.85%, compared with 14.29% (Hristakieva et al., 2011).

Insufficient water evaporation from the egg during incubation increases the volume of amniotic fluid and tissue hydration, hinders free movement of the embryo and transition from allantoic to lung respiration, and delays yolk sac resorption (Christensen et al., 1996, 2003; Stepińska, 2013).

Breeding progress and advances in turkey reproduction research followed by changes in egg quality (Nestor et al., 2008), have promoted studies into the water metabolism of hatching eggs. The objective of this study was to determine the effect of egg water loss during storage and incubation on hatchability results in heavy-type broad-breasted white turkeys.

## **Material and methods**

### **Birds and nutritional regimen**

The experimental materials comprised eggs of heavy-type broad-breasted white BUT Big 6 turkeys. A total of 1800 turkey hens and 180 turkey toms were raised in Poland, under intensive management conditions (Faruga and Jankowski, 1996). The birds were fed diets formulated to meet their nutrient requirements, in accordance with the relevant standards (Jankowski, 2005). Turkey hens received complete diets containing 16–19% of crude protein and 11.7–12.1 MJ/kg of metabolizable energy. Diets for turkey toms contained 10% of crude protein and 13.4 MJ of metabolizable energy. Turkey hens started laying eggs at 30 weeks of age. In weeks 2, 8, 16 and 21 of the laying seasons, 1512 eggs were selected randomly, on the same day in weeks 2 and 8, and on two consecutive days in weeks 16 and 21 (due to decreased laying performance). The eggs were marked individually and divided into 4 groups of 378 eggs each. The groups of eggs were stored for 7, 10, 13 or 17 days, beginning in each of the analyzed weeks of the laying season. The storage periods were determined based on the Polish Standard for Hatching Eggs (1998). Turkey hatching eggs should be stored for 7 days, it is acceptable to store them for 10–13 days, whereas a 17-day storage period is unacceptable. The unacceptable, prolonged storage period (17 days) was included into the experimental design because eggs are stored for such a period in case of overproduction and when the poult market is unstable.

### **Analyses before incubation. Eggshell defects**

In each week of the laying season, eggs were weighed individually within an accuracy of  $\pm 0.1$  g. The surface of each eggshell was evaluated visually, using the method of Mróz and Faruga (2000), to determine the percentage of eggs with normal shell structure, rough-shelled eggs and eggs without shell pigmentation relative to all analyzed eggs, based on the following criteria:

- normal eggshell – a smooth outer surface, darker pigment spots distributed over a lighter-colored surface,
- rough eggshell – deposits of eggshell mass of various size,
- eggshell without pigmentation – a smooth, white outer surface without spots.

### **Analysis of egg water loss during storage**

Eggs of each group were stored in an egg storehouse at a commercial hatchery, at ambient temperature of 15°C and relative humidity of 75 to 80%. At the completion of the storage period, eggs were weighed individually and percent water loss was determined using the following formula:

$$\text{relative weight loss, \%} = 100 \times (W_o - W_{sp})/W_o$$

where:

$W_o$  = egg weight on day 0 of the storage period,

$W_{sp}$  = egg weight after 7, 10, 13 and 17 days of storage.

#### **Analyses during incubation. Analysis of egg water loss**

Prior to incubation, eggs were transported from the storehouse to a hatching room where they were held for 8 hours at an ambient temperature of 18°C and relative humidity of 60% to warm them up. After storage, eggs were incubated in the Peter-sime P13 incubator, with a setting compartment and a hatching compartment. Four incubation cycles of 378 eggs each were performed for each storage period. Incubation was carried out in line with the relevant technological standards for turkey eggs. Temperature and relative humidity were as follows: setting compartment – 37.6°C and 58%, respectively; hatching compartment – 37.2°C and 62%, 85% and 70%, respectively, on days 26–28. In the setting compartment, eggs were turned automatically by 90° every hour. Eggs were candled on days 11 and 25, and they were transferred to hatching trays on day 25 of incubation. Eggs containing live embryos were weighed during intensive embryo growth, i.e. on days 9, 15, 21 and 24 of incubation, always at 8:00 p.m. Egg weight determined within an accuracy of ± 0.1 g during incubation provided a basis for calculating water loss based on the following formula:

$$\text{relative weight loss, \%} = 100 \times (W1 - Wx)/W1$$

where:

$W1$  – egg weight at setting,

$Wx$  – egg weight on days 9, 15, 21 and 24 of incubation.

#### **Analysis of egg hatchability**

After candling, discarded eggs were cracked, and the actual numbers of fertile eggs and eggs containing dead embryos were determined using the key developed by Dziaczkowska and Faruga (1981). The number of embryos that died after 25 days of incubation and the number of punctured eggs containing live unhatched chicks were determined at the end of incubation. The percentage of fertile eggs relative to set eggs, the percentage of embryos that died between days 1 to 11 and 12 to 28 of incubation, and the percentage of unhatched chicks and hatchability from fertilized eggs were also calculated.

#### **Analysis of breakouts**

Dead embryos and unhatched poults were evaluated based on body conformation. The percentage of embryos with the following defects was determined:

– congestion,

– abnormal accumulation of fluid in the head and neck area and in the abdominal cavity,

- abnormal position of the embryo in the egg,
- congenital disorders (monsters),
- abnormal/irregular yolk sac.

The body structure of embryos was analyzed based on the authors' expertise and experience, with the use of the key proposed by Dziaczkowska and Faruga (1981). The percentage of embryos with the above defects relative to all dead embryos was calculated using the following formula:

$$\text{Embryos with a given defect, \%} = \frac{\text{number of embryos with a given defect} \times 100\%}{\text{number of dead embryos}}$$

The study was carried out in accordance with the protocols approved by the Ethical Committee of University Warmia and Mazury in Olsztyn (No. 16/BŻ/2009).

### Statistical analysis

The values of egg weight before storage and eggshell disorders were analyzed by one-way analysis of variance and expressed as arithmetic means (g) and SEM. A random effect model was used. The values of egg water loss during storage and incubation hatchability and embryonic defects were analyzed by two-way analysis of variance. The significance of differences between means was estimated by Duncan's test. All calculations were performed using STATISTICA 10.0 software (StatSoft Inc., 2011). All statements of significance were based on  $P \leq 0.05$ . A split-plot design was used to analyze the data, as the effects of storage period and week of the laying season. The effects of the interaction between the experimental factors (interaction = egg storage period, day  $\times$  laying season, week) on the analyzed parameters were presented in graphical form.

## Results

Egg water loss was significantly affected by the length of the storage period ( $P \leq 0.05$ ) (Table 1). After 17 days of storage, water loss was 0.71% higher than after 7 days of storage. The storage period had no influence on water loss during a 9-day incubation period. During 15 and 21 days of incubation, the lowest water loss was noted in eggs stored for 17 days. Eggs stored for 10 and 13 days were characterized by similar water loss during 15 and 21 days incubation, which was lower than that noted in eggs stored for 7 days by 0.16 to 0.29 percentage points (Table 1).

Egg water loss was also related to the progress of the laying cycle (Table 1). The amount of water loss during storage was 0.22 and 0.30 percentage points higher in weeks 16 and 21 of the laying season than in weeks 2 and 8, respectively ( $P \leq 0.05$ ). During 9, 15, 21 and 24 days of incubation, the loss of water by evaporation was much lower in week 21 of the laying season than in the remaining weeks. On day 24 of incubation, egg water loss was highest in week 8 of the laying season (Table 1). Week 21 of the laying season was characterized by the lowest (1.08 to 1.86%) water loss during 24 days of incubation ( $P \leq 0.05$ ).

Table 1. Egg water loss during storage time and incubation (%)

Item	During storage <sup>1</sup>	During incubation <sup>2</sup>			
		9 days	15 days	21 days	24 days
Egg storage period (days)					
7	0.57 d	3.11	5.76 a	8.72 a	10.22
10	0.70 bc	3.05	5.47 b	8.56 ab	10.19
13	0.87 b	3.10	5.53 b	8.47 b	10.11
17	1.28 a	3.02	5.17 c	8.22 c	9.96
Laying season (weeks)					
2	0.72 c	2.99 b	5.63 a	8.70 b	10.29 b
8	0.78 b	3.17 a	5.66 a	8.92 a	10.94 a
16	1.02 a	3.18 a	5.55 a	8.66 b	10.16 b
21	1.00 a	2.93 b	5.09 b	7.70 c	9.08 c
SEM	0.007	0.024	0.037	0.039	0.039
P-value					
Storage period	0.001	0.490	0.001	0.001	0.074
Laying season	0.001	0.001	0.001	0.001	0.001
Storage period (day) × laying season (week)	0.001	0.001	0.001	0.001	0.001

<sup>1</sup>Water loss relative to egg weight before storage, <sup>2</sup>Water loss relative to egg weight after storage.  
a, b, c, d – values in columns followed by different letters are significantly different at  $P \leq 0.05$ .

The interaction between the experimental factors for egg water loss is presented in Figure 1. In the analyzed weeks of the laying season, the time of egg storage had varied effects on water loss during incubation. In week 2 of the laying season, water loss depended on the storage period, which had no influence on the amount of water lost by evaporation during incubation. In week 8 of the laying season, only a 17-day storage period significantly ( $P \leq 0.05$ ) decreased water loss during incubation. In week 16 of the laying season, the highest water loss during incubation was noted in longest-stored eggs, although they lost the largest amount of water over storage. In week 21 of the laying season, water loss during storage exceeded 1% of total egg weight, excluding eggs stored for 7 days, and differences in water loss were noted already on day 9 of incubation ( $P \leq 0.05$ ). In previous weeks of the laying season, egg water loss remained at a similar level until day 9 of incubation (Figure 1).

The hatchability results of turkey eggs are shown in Table 2. Egg fertilization was comparable in groups of eggs selected for storage. Early embryonic mortality (days 1–11) was 9.10, 5.15 and 5.21 percentage points higher in eggs stored for 17 days than in those stored for 7, 10 and 13 days, respectively ( $P \leq 0.05$ ). Eggs stored for 10 days were characterized by the lowest rate of late embryonic mortality (days 12–28 of incubation,  $P \leq 0.05$ ) and by the highest percentage of unhatched poults ( $P \leq 0.05$ ). Egg hatchability was 5.03, 2.67 and 9.51 percentage points higher in eggs stored for 7 days than in those stored for 10, 13 and 17 days ( $P \leq 0.05$ ) (Table 2).

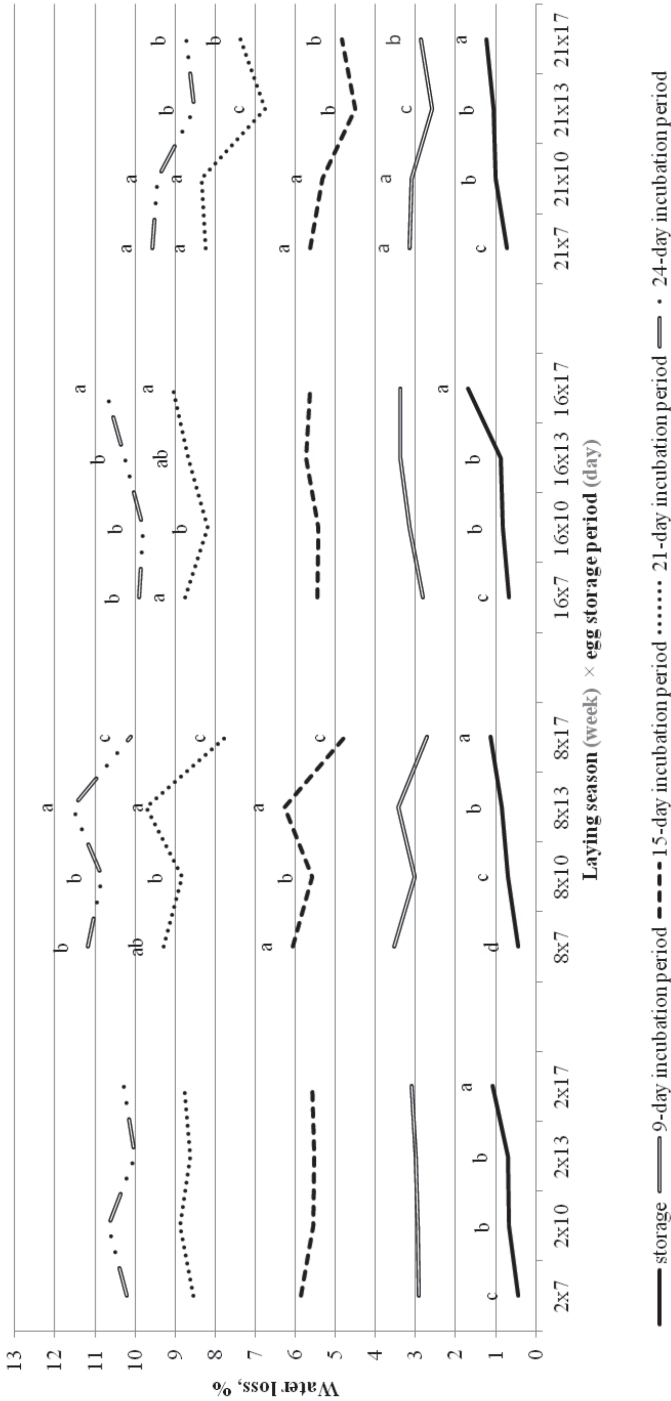


Figure 1. Egg water loss [interaction = laying season (week) x egg storage period (day)], significant differences  $P \leq 0.05$  a, b, c

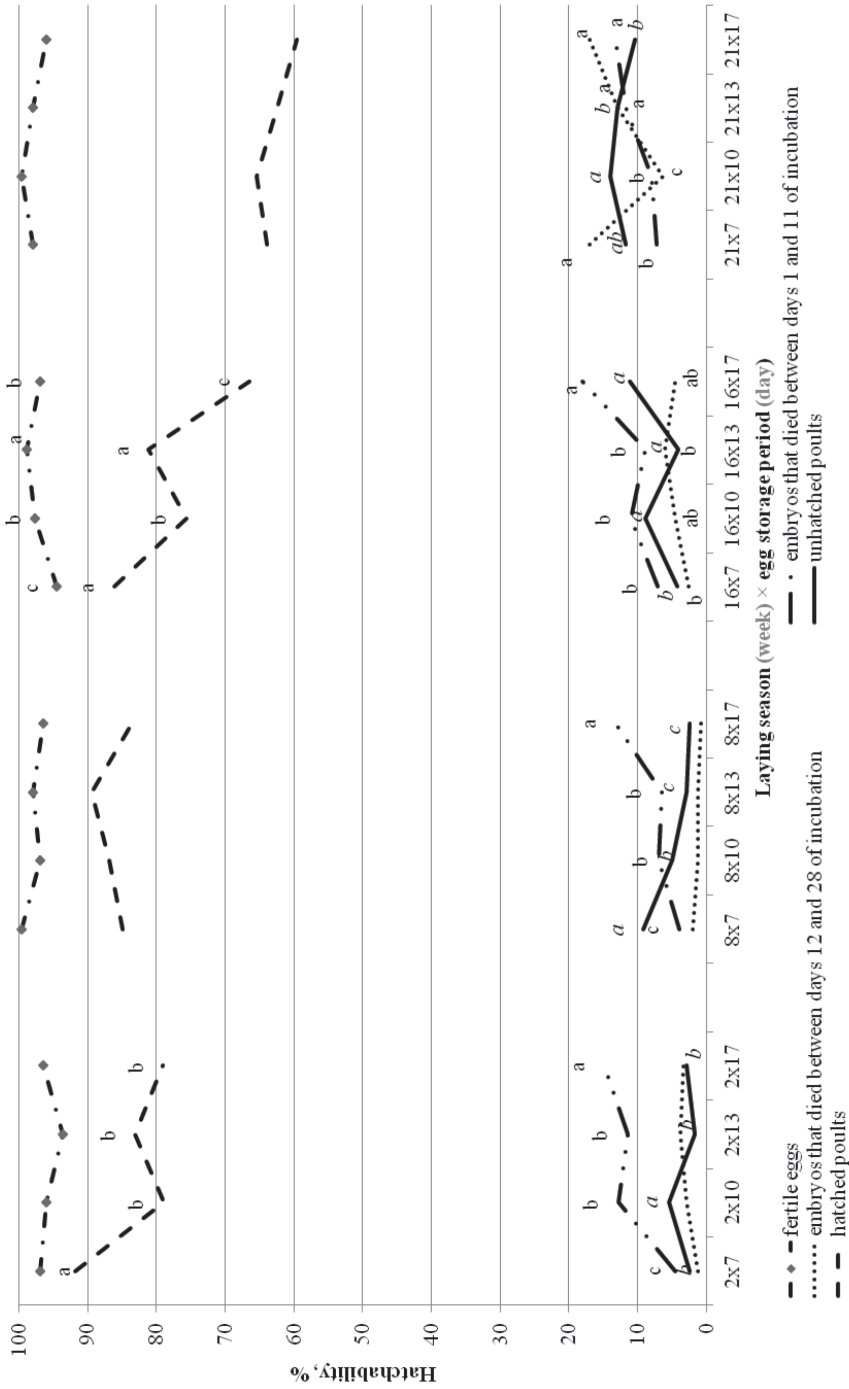


Figure 2. Hatchability [interaction = laying season (week) × egg storage period (day)], significant differences P ≤ 0.05 a, b, c



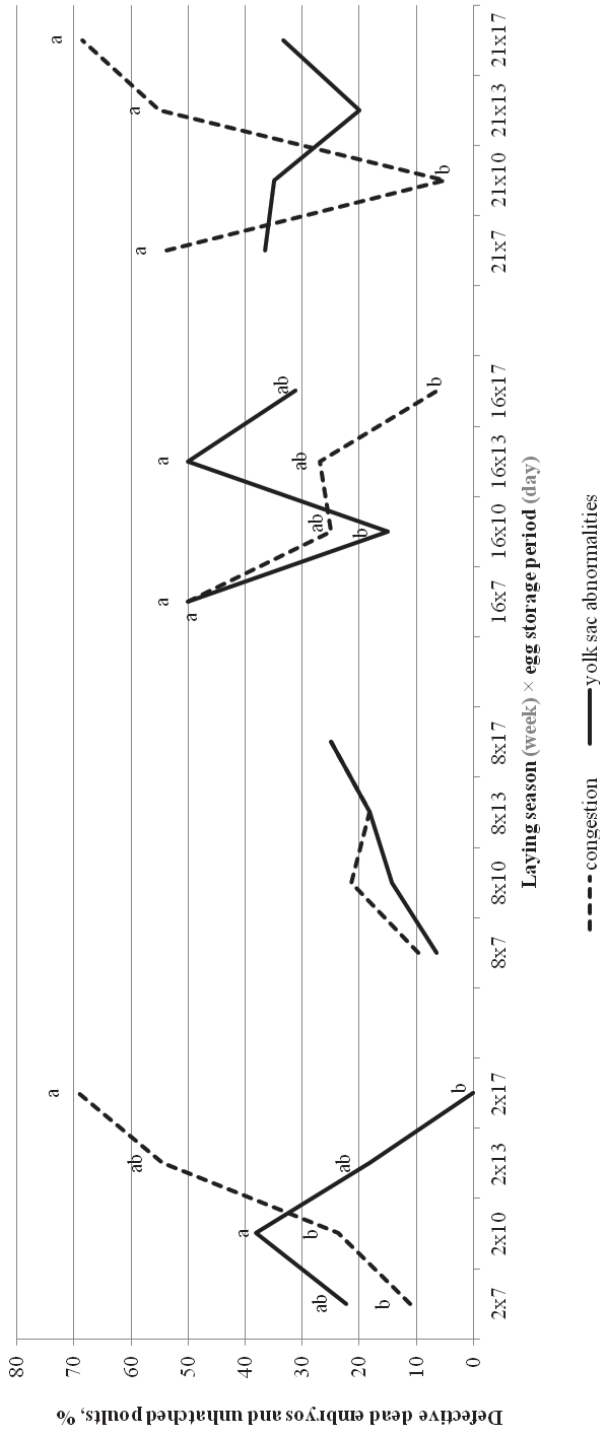


Figure 3. Defective dead embryos and unhatched poults [interaction = laying season (week) × egg storage period (day)], % relative to total hatchery waste, significant differences  $P \leq 0.05$  a, b

Table 2. Hatchability results (%)

Item	Egg fertilization <sup>1</sup>	Dead embryos <sup>2</sup>		Hatchability	
		days 1–11 of incubation	days 12–28 of incubation	unhatched chicks <sup>2</sup>	from fertilized eggs
Egg storage period (days)					
7	97.22	5.71 b	5.71 a	6.94 b	81.63 a
10	97.52	9.66 b	3.76 b	9.90 a	76.60 b
13	97.12	9.60 b	6.03 a	5.41 b	78.96 ab
17	96.43	14.81 a	6.38 a	6.69 b	72.12 c
Laying season (weeks)					
2	95.73 b	10.88 a	2.80 b	3.11 d	83.21 a
8	97.72 a	7.61 b	1.32 c	4.87 c	86.19 a
16	96.92 ab	11.26 a	4.40 b	7.06 b	77.28 b
21	97.92 a	10.03 a	13.27 a	13.88 a	62.82 b
SEM	0.40	0.95	1.30	1.02	2.55
P-value					
Storage period	0.073	0.001	0.041	0.001	0.001
Laying season	0.032	0.046	0.001	0.001	0.001
Storage period × laying season	0.037	0.042	0.039	0.041	0.001

<sup>1</sup> – egg fertilization relative to eggs set, <sup>2</sup> – dead embryos and hatchability relative to fertile eggs.  
a, b, c, d – values in columns followed by different letters are significantly different at  $P \leq 0.05$ .

Table 3. Characteristics of defective dead embryos and unhatched poult ( % of all dead embryos and unhatched poult)

Item	Embryo defects				Abnormal/irregular yolk sac
	congestion	abnormal accumulation of fluid	abnormal position	congenital disorders (monsters)	
Egg storage period (days)					
7	35.72 b	4.08	6.12	4.08	26.53
10	18.67 c	4.00	8.00	1.33	26.67
13	42.05 ab	3.41	11.36	5.68	28.41
17	53.41 a	0.00	27.27	1.14	27.27
Laying season (weeks)					
2	38.89 ab	3.70	3.70	7.41	22.22 ab
8	15.63 c	1.56	4.69	3.13	12.50 b
16	23.56 c	0.00	13.24	1.47	35.29 a
21	52.76 a	4.29	9.20	2.45	31.29 a
SEM	5.37	0.88	1.71	3.46	1.84
P-value					
Storage period	0.002	0.328	0.333	0.350	0.429
Laying season	0.001	0.740	0.150	0.350	0.040
Storage period × laying season	0.001	0.060	0.110	0.238	0.036

a, b, c – values in columns followed by different letters are significantly different at  $P \leq 0.05$ .

Hatchability results varied throughout the laying season (Table 2). The lowest egg fertilization rate was noted in week 2 of the laying season ( $P \leq 0.05$ ). Week 8 of the laying season was characterized by the lowest rates of early and late embryonic mortality ( $P \leq 0.05$ ). The number of unhatched poults increased in successive weeks of the laying season ( $P \leq 0.05$ ). The highest hatchability results were observed in weeks 2 and 8 of the laying season ( $P \leq 0.05$ ) (Table 2). Egg hatchability decreased by 20.39 to 23.37 percentage points between weeks 2 and 8 and 21 of the laying season.

The egg storage period exerted differential effects on hatch rates in the analyzed weeks of the laying season. The interaction between the experimental factors revealed that fertility rates varied only in week 16 of the laying season, due to random selection of eggs for the study ( $P \leq 0.05$ ) (Figure 2). Until day 11 of incubation, embryonic mortality rates were similar in eggs stored for 10 and 13 days ( $P \leq 0.05$ ) (Figure 2). In each week of the laying season, eggs stored for 17 days were characterized by the highest early embryonic mortality. Late embryonic mortality (days 12–28 of incubation) was not affected by the egg storage period only in weeks 2 and 8 of the laying season (Figure 2). The highest percentage ( $>10\%$ ) of unhatched eggs was noted in the last week of the experiment (Figure 2). Hatch rates decreased significantly with extended storage in weeks 2 and 16 of the laying season (interaction:  $P \leq 0.05$ ) (Figure 2). The final hatchability was influenced by the progress of laying cycle rather than by the egg storage period (Figure 2).

Abnormalities of dead embryos and unhatched poults are characterized in Table 3. The most common defect was congestion. The percentage of congested embryos was 6.33 to 23.38 percentage points higher in eggs stored for 13 days, and 17.69 to 34.74 percentage points higher in eggs stored for 17 days than in those stored for 10 and 7 days ( $P \leq 0.05$ ). The storage period had no influence on the number of embryos with other defects. The percentages of congested embryos and embryos with abnormal yolk sacs varied throughout the laying season. The percentage of congested embryos was 13.87 to 37.13 percentage points higher in week 21 of the laying season than in the remaining weeks. Yolk sac abnormalities were encountered more frequently in weeks 16 and 21 of the laying season than in the previous weeks ( $P \leq 0.05$ ) (Table 3).

Table 4. Egg quality characteristics

Laying season (weeks)	Number of analyzed eggs	Average egg weight (g)	Eggshell defects (%) <sup>1</sup>		
			normal	rough	without pigmentation
2	1512	87.40 d	85.32 a	10.52 b	4.17
8	1512	94.22 c	83.14 a	12.60 b	4.27
16	1512	97.45 b	84.43 a	11.41 b	4.17
21	1512	98.05 a	74.50 b	20.33 a	5.16
SEM		0.099	2.16	1.94	0.21
P-value		0.001	0.001	0.001	0.081

<sup>1</sup> – relative to all examined eggs.a, b, c, d – values in columns followed by different letters are significantly different at  $P \leq 0.05$ .

The interaction between the experimental factors revealed that the percentage of defective embryos was lowest in week 8 of the laying season, and it was not affected by the storage period (Figure 3). In week 21 of the laying season, the storage period had no influence on the percentage of embryos with yolk sac abnormalities. In weeks 16 and 21 of the laying season, the percentage of congested embryos increased even after 7 days of storage.

Egg weight increased by 10.65 g between weeks 2 and 21 of the laying season ( $P \leq 0.05$ ) (Table 4). The percentage of rough-shelled eggs increased by 9.81% in week 21. The percentage of eggs with shell color abnormalities remained low at 4.17–5.16% in the analyzed weeks of the laying season (Table 4). Significant changes in egg weight and, towards the end of the laying cycle, also in shell quality, point to changes in egg quality throughout the laying season.

## Discussion

Prolonged storage increases water loss in turkey eggs. In the present study, a 17-day storage period led to lower water loss (1.28%) than that observed by Hristakieva et al. (2011) over 14 days (1.55%). The differences between the results of our study and the findings of Hristakieva et al. (2011) and Christensen et al. (1996) are due to the different methodological approaches used (laying season, average egg weight, egg storage and humidity level in the storehouse). Microclimate conditions in the egg storehouse were typical of Polish hatcheries.

Most studies conducted to date have investigated water loss from chicken hatching eggs during storage (Petek and Dikmen, 2006; Reijrink et al., 2010). Our results confirm the previously formulated conclusions that turkey eggs should not be stored for more than 7 days.

Evaporative water loss during storage was higher in eggs from older hens, which is consistent with the findings of Brake et al. (1997). Eggs from older turkeys were also characterized by higher weight, lower shell quality and higher moisture content of the albumen, which increases the rate of water evaporation (Mróz et al., 2004, 2014), although not every study observed such a relationship in the hatching eggs of broiler breeders (Zakaria et al., 2009).

Water loss from incubated eggs varied during the period of rapid embryonic growth. Long-stored eggs were characterized by the lowest water loss during incubation. The highest water loss was noted in week 8 of the laying season. The amount of water lost by evaporation during a 24-day incubation period was lower than that reported by Christensen et al. (1996). During 24 days of incubation, the highest water loss was determined in week 8 of the laying season, which confirmed the results of earlier studies (Applegate and Lilburn, 1996; Mróz and Pudyszak, 1997; Mróz et al., 2004). During the incubation of chicken eggs, the highest rate of water evaporation was also observed at the peak-of-lay (Tona et al., 2001; Zakaria et al., 2009; Ulmer-Franco et al., 2010; Alsobayel et al., 2013), but not in all studies (Peebles et al., 2001; Meir and Ar, 2008). High water loss during incubation noted in week

16 of the laying season, despite considerable loss over storage, is hard to explain, but it was not associated with any methodological errors.

The egg storage period had no influence on water evaporation during 9 days of incubation. During that period, water contained in the albumen permeates the yolk, and the rate of evaporation is low. In eggs collected in week 21 of the laying season, water loss during the first 9 days of incubation was lowest, most probably due to a slower rate of embryo growth. Water loss during 24 days of incubation was lower than 10%, which indicates that there is a relationship between a late laying period, a long storage time and water evaporation in incubated eggs (Mróz and Pudyszak, 1997; Hristakieva et al., 2011). The results of our study confirm the observations of other authors that higher water loss during storage is followed by lower water loss during incubation (Ruiz and Lunam, 2002; Yildirim, 2005; Romao et al., 2008).

The hatchability results of eggs stored for 7 days were higher than the values obtained in previous studies that used similar experimental materials (Orłowska and Mróz, 2006). A 7-day storage period is considered optimal for turkey hatching eggs (Polish Standard PN-R-78564, 1998; Mróz et al., 2004; Orłowska and Mróz, 2006), which was also confirmed in the present study. The rates of late embryonic mortality suggest that prolonged storage of turkey eggs prior to incubation (10 days) delays embryonic development and contributes to increased mortality during the time of hatching. A high rate of late embryonic mortality in eggs stored for 7 and 17 days in week 21 of the laying season is hard to explain, and could result from high egg weight and shell quality deterioration.

Higher egg fertilization was noted in the first weeks of the laying season, which was also reported by Grimes et al. (2004). In turkeys, hatchability decreases after the peak-of-lay due to increased embryonic mortality and a higher percentage of unhatched poults, which was also demonstrated by Faruga et al. (1996), Grimes et al. (2004) and Hristakieva et al. (2011). The embryonic mortality rates noted in our study are comparable with those reported by Christensen et al. (2003) and Orłowska and Mróz (2006). A higher number of dead embryos is related to lower water loss during incubation, which was also observed by Christensen et al. (1996). Congested embryos and embryos with abnormal yolk sacs had the highest share of hatchery waste, which is consistent with previous studies of turkeys (Mróz et al., 2002, 2004; Stępińska et al., 2012) and laying hens (Malec et al., 2002; Sharaf et al., 2010). The percentage of embryos with abnormal yolk sacs was similar to that reported by Stępińska et al. (2012) at 14–34%, whereas the cited authors noted a lower percentage (13–22%) of congested embryos. The number of defective embryos determined in our study differed from those reported previously due to differences in egg quality and experimental period. Mróz et al. (2002) noted only 7% of embryos with physical defects in highest quality eggs.

The results of this study and previous research show that water loss is influenced by egg weight and shell quality (Mróz and Faruga, 2000; Mróz et al., 2002, 2004, 2007). A high percentage of rough-shelled eggs could affect average water loss over a 24-day incubation period, and hatchability in week 21 of the laying season. A similar relationship was also observed in other meat-type poultry species (Tona et al., 2001; Ulmer-Franco et al., 2010).

Our findings suggest that the amount of water lost by evaporation from hatching eggs was affected by both the length of the storage period and laying season. Water loss accounting for 0.57–0.87% of total egg weight was typical of optimal and permissible egg storage periods in turkeys. Water loss accounting for 1.00–1.28% of total egg weight was characteristic of a long storage period and a late laying period. During rapid embryonic development, water loss was also influenced by the length of the storage and laying periods. Higher water loss (8.47–8.72%) was noted in eggs stored for 7, 10 and 13 days than in those stored for 17 days. Higher water loss (8.66–8.92%) was also observed in weeks 2, 8 and 16 of the laying season than in week 21. Only in week 2 of the laying season, egg water loss during incubation was not determined by the storage period.

Egg hatchability decreased significantly by 8.81% to 13.27% when water loss during storage increased to 1.00–1.28%, reaching 7.70, 8.22 and 8.66% during rapid embryo growth.

At the beginning of incubation, embryonic mortality caused by cardiovascular anomalies was highest in eggs stored for 13 and 17 days. Late embryonic mortality and the percentage of unhatched eggs were influenced by the progress of the laying cycle rather than by the egg storage period. A high percentage of abnormal/irregular yolk sacs was noted in weeks 16 and 21 of the laying season. Week 8 of the laying season was characterized by the highest egg hatchability of 86.19%, water loss during storage of 0.78%, and the highest water loss during rapid embryo growth (8.92%) and over a 24-day incubation period (10.94%). The results of this study indicate that eggs have mechanisms responsible for regulating evaporative water loss. Lower percent water loss during storage contributes to higher water loss during rapid embryo growth and higher hatch rates. Further research investigating egg water loss during storage and incubation should account for various egg storage conditions (e.g. relative humidity) and eggshell quality characteristics.

The results of this study indicate that water loss should be monitored during storage to control water evaporation from turkey hatching eggs and to determine the causes of any abnormalities in water metabolism during embryonic development. Evaporative water loss during embryogenesis should be controlled on days 15 and 21 of incubation.

## References

- Abudabos A. (2010). The effect of broiler breeder strain and parent flock age on hatchability and fertile hatchability. *Int. J. Poult. Sci.*, 9: 231–235.
- Alsobayel A.A., Almarshade M.A., Albaldry M.A. (2013). Effect of breed, age and storage period on egg weight, egg weight loss and chick weight of commercial broiler breeders raised in Saudi Arabia. *J. King Saudi Univ. Agric. Sci.*, 12: 53–57.
- Applegate T.J., Lilburn M.S. (1996). Independent effects of hen age and egg size on incubation and poul characteristics in commercial turkeys. *Poultry Sci.*, 75: 1210–1216.
- Boerian M.L. (2006). Incubation for uniformity. *Aust. Poult. Sci. Symp.*, 18: 174–181.
- Brake J., Walsh T.J., Benton JR C.E., Petite J.N., Meijerhof R., Peñalva G. (1997). Egg handling and storage. *Poultry Sci.*, 76: 144–151.

- Christensen V.L., Donaldson W.E., McMurry J.P. (1996). Physiological differences in late embryos from turkey breeders at different ages. *Poultry Sci.*, 75: 172–178.
- Christensen V.L., Grimes J.L., Wineland M.J., Davis G.S. (2003). Accelerating embryonic growth during incubation following prolonged egg storage. I. Embryonic livability. *Poultry Sci.*, 82: 1869–1878.
- Demirel S., Kırıkçı K. (2009). Effect of different egg storage times on some egg quality characteristics and hatchability of pheasants (*Phasianus colchicus*). *Poultry Sci.*, 88: 440–444.
- Dziaczkowska L., Faruga A. (1981). The guidebook for recognizing the age of embryonic development of turkey (in Polish). COBRD (ed.), Poznań.
- Faruga A., Jankowski J. (1996). Turkeys. Breeding and use (in Polish). PWRiL, Poznań.
- Faruga A., Pudyszak K., Puchajda H., Jankowski J., Kozłowski K. (1996). Characteristics of eggs quality depending on the laying period and origin of turkeys (in Polish). *Zesz. Nauk. Prz. Hod.*, 24: 91–99.
- Garip M., Dere S. (2011). The effect of storage period and temperature on weight loss in quail eggs and the hatching weight on quail chicks. *J. Anim. Vet. Adv.*, 10: 2363–2367.
- Grimes J.L., Noll S., Brannon J., Godwin J.L., Smith J.C., Rowland R.D. (2004). Effect of a chelated calcium proteinate dietary supplement on the reproductive performance of Large White turkey breeder hens. *J. Appl. Poultry Res.*, 13: 639–649.
- Hristakieva P., Lalev M., Oblakova M., Mincheva N., Ivanova I. (2011). Effect of storage duration on the quality of hatching turkey eggs. *Arch. Zootech.*, 14: 57–65.
- Jankowski J. (2005). Dietary recommendations for turkeys (in Polish). In: Dietary recommendations and the nutritional value of fodder, Smulikowska S., Rutkowski A. (eds). IFiZZ, PAN, Jabłonna, pp. 48–63.
- Lopez J., Hergott T. (2014). Monitoring hatchery performance to ensure turkey poult quality. *Int. Hatch. Pract.*, 28: 7–10.
- Lourens A., Molenaar R., Van Den Brand H., Heetkamp M.J., Meijerhof R., Kemp B. (2006). Effect of egg size on heat production and the transition of energy from egg to hatchling. *Poultry Sci.*, 85: 770–776.
- Madeddu M., Zaniboni L., Mangiagalli M.G., Cassinelli C., Cerolini S. (2013). Egg related parameters affecting fertility and hatchability in the Italian bantam breed Mericanel della Brianza. *Anim. Reprod. Sci.*, 137: 214–219.
- Malec H., Borzemska W., Niedziółka J., Pijarska I. (2002). Hatchability value of eggs with eggshell pimpling (in Polish). *Med. Weter.*, 58: 49–51.
- Meir M., Ar A. (2008). Changes in eggshell conductance, water loss and hatchability of layer hens with flock age and moulting. *Brit. Poultry Sci.*, 49: 677–684.
- Mróz E. (1996). Some morphological characters of the turkey egg shell surface and their relationship with egg hatchability (in Polish). *Zesz. Nauk. Prz. Hod.*, 24: 77–84.
- Mróz E., Faruga A. (2000). Correlations between incubation indices in egg groups with different egg shell and turkey origins. *Nat. Sci.*, 7: 101–113.
- Mróz E., Pudyszak K. (1997). An analysis of egg incubation and turkey poult quality results depending on the age of turkey hens (in Polish). *Zesz. Nauk. Prz. Hod.*, 32: 97–102.
- Mróz E., Puchajda K., Pudyszak K. (2002). Structure and pigmentation of egg shell and biological value of turkey hatching eggs. *Pol. J. Nat. Sci.*, 10: 141–152.
- Mróz E., Orłowska A., Reiter A. (2004). Effect of turkey egg storage period on hatching rates and poult quality (in Polish). In: Towaroznawstwo żywności i przedmiotów użytku, K.A. Skibniewska (ed). UWM, Olsztyn.
- Mróz E., Michałak K., Orłowska A. (2007). Hatchability of turkey eggs as dependent on shell structure. *Pol. J. Nat. Sci.*, 22: 31–42.
- Mróz E., Stępińska M., Krawczyk M. (2014). Morphology and chemical composition of turkey eggs. *J. Appl. Poultry Res.*, 23: 196–203.
- Nestor K.E., Anderson J.W., Patterson R.A., Velleman S.G. (2008). Genetics of growth and reproduction in turkey. 17. Changes in genetic parameters over forty generations of selection for increased sixteen-week body weight. *Poultry Sci.*, 87: 1971–1979.
- Orłowska A., Mróz E. (2006). Effect of age of turkey-hens and egg storage period on embryo mortality and poults quality. *Proc. XVIII International Poultry Symposium PB WPSA, Rogów, Poland, 4–6.09.2006*, pp: 49–54.

- Peebles E.D., Doyle S.M., Zumwalt C.D., Gerard P.D., Latour M.A., Boyle C.R., Smith T.W. (2001). Breeder age influences embryogenesis in broiler hatching eggs. *Poultry Sci.*, 80: 272–277.
- Petek M., Dikmen S. (2006). The effects of prestorage incubation and length of storage of broiler breeder eggs on hatchability and subsequent growth performance of progeny. *Czech. J. Anim. Sci.*, 51: 73–77.
- Reijrink I.A.M., Berghmans D., Meijerhof R., Kemp B., van den Brand H. (2010). Influence of egg storage time and preincubation warming profile on embryonic development, hatchability, and chick quality. *Poultry Sci.*, 89: 1225–1238.
- Romao J.M., Moraes T.G.V., Teixeira R.S.C., Cardoso W.M., Buxade C.C. (2008). Effect of egg storage length on hatchability and weight loss in incubation of egg and meat type Japanese quails. *Brazil. J. Poultry Sci.*, 10: 143–147.
- Ruiz J., Lunam C.A. (2002). Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. *Brit. Poultry Sci.*, 43: 374–383.
- Sharaf M.M., Taha A.E., El-Sayed Ahmed N.A. (2010). Deformities and abnormalities of Egyptian chicken embryos as reasons of poor hatchability percentages. *Egypt. Poultry Sci.*, 30: 317–337.
- Solomon S.E. (1996). The eggshell – a barrier? *Turkeys*, 44: 8–10.
- Stępińska M. (2013). Influence of hens age and egg storage time on embryonic development and hatchability of turkey (in Polish). PhD Thesis, University of Warmia and Mazury, Olsztyn.
- Stępińska M., Mróz E., Jankowski J. (2012). The effect of dietary selenium source on embryonic development in turkeys. *Folia Biol. (Kraków)*, 60: 235–241.
- Tazawa H., Whittow G.C. (2000). Incubation physiology. In: *Sturkie's avian physiology*. Academic Press San Diego, 5th ed., pp. 617–634.
- Tona K., Bamelis F., Coucke W., Bruggeman V., Decuyper E. (2001). Relationship between broiler breeder's age and egg weight loss and embryonic mortality during incubation in large-scale conditions. *J. Appl. Poultry Res.*, 10: 221–227.
- Ulmer-Franco A.M., Fassenko G.M., O'Dea Christopher E.E. (2010). Hatching egg characteristics, chick quality and broiler performance at 2 breeder flock ages and from 3 egg weights. *Poultry Sci.*, 89: 2735–2742.
- Willems E., Decuyper E., Buyse J., Everaert N. (2014). Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. *World Poultry Sci. J.*, 70: 503–517.
- Yildirim I. (2005). Effects of breeder age and pre-incubation storage of eggs on hatchability, time of hatch and relative organ weight of quail chicks at hatch. *S. Afr. J. Anim. Sci.*, 35: 135–142.
- Zakaria A.H., Plumstead P.W., Romero-Sanchez H., Leksrisonpong N., Brake J. (2009). The effects of oviposition time on egg weight loss during storage and incubation, fertility, and hatchability of broiler hatching eggs. *Poultry Sci.*, 88: 2712–2717.

Received: 5 IV 2016

Accepted: 21 IX 2016