PRE-INCUBATION AND TURNING DURING LONG STORAGE AS A METHOD OF IMPROVING HATCHABILITY AND CHICK QUALITY OF JAPANESE QUAIL EGGS*

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Abstract

In this study, we investigated the effect of modification of 12 d storage conditions of quail eggs from two flocks: at 17–20 weeks of life (A) and 36–39 weeks of life (B) on the incubation time, hatch window (HW), hatching results and chicks quality. The eggs were divided into experimental groups: COI = no pre-incubation (PI); SPIDES = were treated with 4 h PI, delivered at 4, 6, 8 and 10 d of storage; EG SPIDES = were treated with variable time of PI according to the scheme 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h. In addition, in each group, half of the eggs were turned (T) every 12 h, which is indicated as COI T, SPIDES T and EG SPIDES T. Pre-incubation was conducted at 37.8°C and 50–55% RH. It has been demonstrated that both storage profiles contribute to shortened incubation time, yet the HW is deteriorated if T is not applied. The benefits resulting from the EG SPIDES profile is an increase of 2.3 points in the “average score of all chicks” index compared to the COI group. In summary, the use of PI and T, and in particular the EG SPIDES profile with the concomitant use of T during long storage of quail eggs is recommended to obtain a considerable improvement of hatching results and chick quality.

Key words: hatching eggs, hatch window, young and old flock

The growing demand for Japanese quail eggs requires an increase in the populations of these birds. The mass production of quail eggs strictly depends on artificial incubation of eggs. The hatching results are influenced by both their biological quality as well as handling during storage and incubation (Anders et al., 2018; Batkowska et al., 2018; Onbaşilar et al., 2018). Intensification of laying quail husbandry results

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in the need to also obtain a high number of chicks, which requires longer egg harvest and storage time. The time from laying to incubation should be as short as possible, as it is negatively correlated with the hatching results and chick quality. The accepted optimum egg storage time is 7 d under strictly determined conditions, i.e. temperature below physiological zero (21°C) and RH within the 55–75% range (Edwards, 1902). According to Reijrink et al. (2008), longer storage results in increased necrotic cell indicators, which contribute to an early mortality of the embryos. To counteract the negative results of prolonged hatching egg storage, several modifications to storage conditions have been developed. Pre-incubation (PI) and egg turning (T) emerged as the most efficient. Pre-incubation is a short period of incubation, which, even after single use, enables the completion of hypoblast formation and improving hatching results (Fasenko et al., 2001 a, b). A series of subsequent PI performed at intervals of several days contributes to further improvement of hatching results and it improves chick quality (Dymond et al., 2013; Damaziak et al., 2018). On the other hand, Elibol and Brake (2008) demonstrated that T in the egg storage period further counteracts embryo mortality.

The positive effect of PI and T of hatching eggs during storage was proven based on the example of chicken (Fasenko et al., 2001 b; Reijrink et al., 2009) and turkey eggs (Bakst and Gupta, 1997; Fasenko et al., 2001 a). The scarce studies on quail eggs, in which PI (Usman et al., 2008; Yildirim et al., 2015) and T (Roriz et al., 2016) were applied during storage have not provided comprehensive results.

The aim of the study was to investigate the impact of PI and T of Japanese quail eggs in prolonged storage period on the hatching results, hatching synchrony and chicks quality.

Material and methods

All procedures were approved by the II Local Ethical Committee for Animal Experiments in Poland (application 23 January 2017, Warsaw, PL).

Experimental flock

Hatching eggs were obtained from two reproduction flocks of Japanese quail: young flock (A) at 17–20 weeks of age and old flock (B) at 36–39 weeks of age. Both flocks were maintained as a closed experimental laying line of Japanese quail at the Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Poland (53°05'25,8"N 20°44'16,8"E). The quails were kept in aviaries. A single aviary containing 20 females and 5 males measured 230×230 cm, height 170 cm and 13×25 mm mesh size. The quails had *ad libitum* access to fresh water and were fed a corn-wheat-soybean complete feed with the 2750 ME (kcal/kg) energy content and 18.50% crude protein.

Eggs storage conditions, profile of pre-incubation and turning

Hatching eggs were obtained from flock A at 18 weeks of life and at the same time from flock B at 36 weeks of life. With each flock, 2160 eggs from a daily

harvest were obtained. The total number of eggs covered by the experiment was 4 320 (540 eggs $\times$ 4 replications $\times$ 2 flocks). Directly after collection, the eggs were placed on cardboard trays with the large end up and were stored in the storehouse for 12 h (17°C and RH 65–75%). Subsequently, they were transported to the laboratory of the Poultry Breeding Division, Warsaw University of Life Sciences, Poland (52°09'49,680"N 21°02'52,872"E). The transport lasted for about 45 min. (30 km) and was carried out on paved roads at a temperature below 19°C. Weight of each egg was determined (± 0.1 g) directly after transport and the eggs were placed on hatching trays. A single hatching tray contained 90 eggs. Numbers of individual eggs were written with a pencil on the edge of the hatching tray. Eggs were stored for 12 d in a laboratory incubator (Binder, series KB, Model KB 240, Tuttlingen, Germany) with a set temperature of $17 \pm 1^\circ\text{C}$ at $70 \pm 5\%$ RH.

Short periods of incubation during egg storage (SPIDES) and gradual extension short periods of incubation during egg storage (EG SPIDES) were conducted in a Brinsea setter (OvaEasy 380 Setter, Brinsea, Weston, UK). In both profiles, equal heating conditions (37.8°C and 50–55% RH) and equal heating time (16 h) were applied but in the case of SPIDES this was an equal setup of 4×4 , whereas for EG SPIDES the heating time was systematically increased (Figure 1). Egg turning every 12 h consisted in the change of the position of an entire hatching tray by 90° (alternating sides 45° from the horizontal position).

Figure 1. Storage treatments. COI = eggs storage 12 d, no pre-incubation (PI) and no turning (T); COI T = eggs storage 12 d, no pre-incubation (PI) and turning (T) every 12 h; SPIDES = 4 h pre-incubation (PI), delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h pre-incubation (PI), delivered at 4, 6, 8 and 10 d over 12 d of storage and turning (T) every 12 h; EG SPIDES = 4 h pre-incubation (PI), delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = 4 h pre-incubation (PI), delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and turning (T) every 12 h

Before their placement in a Binder incubator, individual hatching eggs were assigned to one of 6 experimental groups, according to the following scheme. Thus, each experimental group included 360 eggs (90 eggs $\times$ 4 replications) separately for flock A and B.

COI = eggs storage 12 d, no PI and no T

COI T = eggs storage 12 d, no PI, T every 12 h

SPIDES = 16 h PI, delivered: 4 d – 4 h, 6 d – 4 h, 8 d – 4 h and 10 d – 4 h during 12 d of storage

SPIDES T = 16 h PI, delivered: 4 d – 4 h, 6 d – 4 h, 8 d – 4 h and 10 d – 4 h during 12 d of storage and T every 12 h

EG SPIDES = 16 h PI, delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h during 12 d of storage

EG SPIDES T = 16 h PI, delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h during 12 d of storage and T every 12 h

Incubation conditions

After 12 d of storage, the egg weight was determined again (± 0.1 g). Subsequently, all eggs were placed in laboratory setters, which were equipped with humidity pumps (OvaEasy 380 Setter, Brinsea, Weston, UK). Incubation was carried out at 37.6–38.0°C and 60 – 65% RH. The CO₂ level measured on 5 and 10 d of incubation using a mobile meter (GM8805, Benetech, Palo Alto, California) remained in the range between 3.500 and 4.500 ppm. Egg turning (by 90°) was performed automatically every 12 h.

Hatching results and chicks quality

On 10 d all remaining eggs were manually candled (OvaScope Brinsea, Weston, UK). After candling, all unfertilized eggs or eggs containing dead embryos were discarded. The removed eggs were cracked to verify based on the appearance of the embryonic disc whether they were indeed unfertilized or contained dead embryos. Based on the obtained results, the fertilization (%) and early mortality of embryos (%) were calculated according to the formula:

$$\text{Fertility} = \frac{\text{number of set eggs} - \text{number of unfertile eggs during candling at 10 d of incubation}}{\text{number of set eggs}} \times 100$$

$$\text{Early embryonic mortality of fertile eggs} = \frac{\text{number of eggs with dead embryos during candling at 10 d of incubation}}{\text{number of fertile eggs}} \times 100$$

On 15 d all remaining eggs were moved to hatcher (OvaEasy 380 Hatcher, Brinsea, Weston, UK) but separated from the other eggs and each other by a metallic mesh grid measuring 80 × 80 × 80 mm to allow individual monitoring (Romanini et al., 2013). Hatching was carried out at 37.6°C and 65% RH. From the moment the first chick hatched, each hatcher was checked every 2 h and the hatched chicks were systematically removed. A hatched chick was considered to be one that left the shell completely independently. After the removal, chicks were marked with individual

chick labels and their number, time of hatching was recorded, and they were moved to a separate collective hatcher (R COM Maru, Bandury, UK). The control of hatching time enabled calculation of the percentage share of hatched chicks at individual time intervals and the total incubation time that is the hatch window (HW = time between the hatching of the first and the last chick). Ten hours was assumed as the optimum time necessary for chicks to dry. After this time, portions of chicks (analogously to those removed directly after hatching and selected based on the individual chick labels) were removed from the collective hatcher, their body weight (± 0.1 g) and quality was determined and the chicks hatching weight as a percentage of eggs weight was calculated. The chicks quality was determined following the methodology described by Tona et al. (2003), including such traits as an activity, the appearance of the down feathers, retracted yolk, eyes, legs, navel area, remaining membrane, and remaining yolk. The maximum score to be granted to a chick was 100 points. This number was a total sum of points assigned for all traits. Based on the assessment, for each of the examined groups the share (%) of chicks assessed at 100, mean of points granted to chicks assessed at <100 and the mean for all chicks within the group were calculated. After hatching was completed, the amount of unhatched eggs was recorded (all remaining eggs), and then chicks that did not hatch from fertile eggs (%) and hatchability of set and fertile eggs (%) were calculated according to the formulas:

$$\text{Hatchability of set eggs} = \frac{\text{number of hatched chicks}}{\text{number of set eggs}} \times 100$$

$$\text{Hatchability of fertile eggs} = \frac{\text{number of hatched chicks}}{\text{number of fertile eggs}} \times 100$$

Statistical analysis

Comparison of means between various treatments of quail eggs and flock age was conducted based on three-way analysis of variance (ANOVA) and Duncan's multiple range test at 0.05 significance level. The following major effects were taken into account: flock age with two levels (A and B), pre-incubation with three levels (COI, SPIDES and EG SPIDES) and turning with two levels (no, yes). Analysis of variance was performed according to the linear model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

Where: Y_{ijkl} is the observed value of the trait, α_i is the effect of i^{th} quail flock age, β_j is the effect of j^{th} treatment, γ_k is the effect of k^{th} egg turning, $(\alpha\beta)_{ij}$ is the interaction between quail flock age and treatment, $(\alpha\gamma)_{ik}$ is the interaction of quail flock age and eggs turning, $(\beta\gamma)_{jk}$ is the interaction of treatment and eggs turning, $(\alpha\beta\gamma)_{ijk}$ is the interaction of quail flock age, treatment and eggs turning, ε_{ijkl} is the error term.

Effects of quail flock age, treatments and eggs turning were treated as fixed and independent (not nest). All variables were characterized by normal distribution; parametric statistical procedures were applied and the means and standard deviations

were presented. All results for one quail flock collected on four dates (replications) were presented as mean values. Statistical analyses were carried out with the use of Statistica 12.0 software (Statsoft, INC, 2014).

Results

Fertility and hatchability results

Results of apparent fertility and hatchability of Japanese quail eggs including early and other embryonic mortality were presented in Table 1. The mentioned index was referred to as apparent fertility, because its percentages refer to the sum of eggs that are actually unfertilized and embryos dead up to 10 d of incubation. Flock A was characterized by higher apparent fertility, better hatchability and fewer unhatched eggs ($P<0.05$). Early embryonic mortality was higher in flock B but the difference of 0.8% has not been confirmed ($P=0.068$). The greatest impact of flock age was observed in COI (T) groups, where the negative effects of 12 d egg storage were not counteracted by the use of PI. In these groups flock A was characterized by 10.5% higher hatchability of apparently fertile eggs ($P=0.001$), 3.3% lower early embryonic mortality ($P=0.028$) and 7.8% less unhatched chicks ($P=0.001$).

Table 1. Results (%) (means and $\pm$ SD) of the effect of Japanese quail's age, pre-incubation and eggs turning during storage time on apparent fertility, hatchability and early embryonic mortality¹

Treatment	Apparent fertility	Hatchability of set eggs	Hatchability of apparently fertile eggs	Early ² embryonic mortality of fertile eggs	Unhatched chicks from apparently fertile eggs
1	2	3	4	5	6
Effect of PI treatment					
COI	82.4 $\pm$ 2.9ND	66.4 $\pm$ 1.9 c	80.5 $\pm$ 2.2 c	4.6 $\pm$ 0.5 a	14.9 $\pm$ 2.0 a
SPIDES	89.0 $\pm$ 2.8ND	77.0 $\pm$ 1.9 b	86.4 $\pm$ 1.0 b	3.1 $\pm$ 0.4b	10.5 $\pm$ 1.8 b
EG SPIDES	84.5 $\pm$ 2.0ND	86.9 $\pm$ 1.7 a	91.6 $\pm$ 1.3 a	3.6 $\pm$ 0.4 b	4.8 $\pm$ 1.1 c
Effect of turning					
No	88.6 $\pm$ 2.5ND	76.1 $\pm$ 2.1 b	85.7 $\pm$ 2.1 b	3.2 $\pm$ 0.4 a	11.1 $\pm$ 1.7 a
Yes	89.2 $\pm$ 3.1ND	77.4 $\pm$ 1.8 a	86.8 $\pm$ 1.9 a	4.2 $\pm$ 0.7 a	9.0 $\pm$ 1.5 b
Effect of flock age					
A	89.3 $\pm$ 2.3a	78.1 $\pm$ 1.7 a	87.5 $\pm$ 1.7 a	3.5 $\pm$ 0.4 b	9.0 $\pm$ 1.8 b
B	88.4 $\pm$ 2.9b	75.4 $\pm$ 2.1 b	84.9 $\pm$ 2.1 b	4.0 $\pm$ 0.7 a	11.1 $\pm$ 2.0 a
Effect of PI treatment $\times$ turning					
COI $\times$ No	81.8 $\pm$ 2.5ND	62.4 $\pm$ 2.7 f	76.3 $\pm$ 2.1 d	4.6 $\pm$ 0.9 b	19.1 $\pm$ 2.9 a
COI $\times$ Yes	83.0 $\pm$ 3.9ND	70.5 $\pm$ 1.1 e	84.9 $\pm$ 1.5 c	4.2 $\pm$ 0.5 c	10.9 $\pm$ 1.4 bc
SPIDES $\times$ No	89.2 $\pm$ 2.2ND	80.1 $\pm$ 2.4 c	89.9 $\pm$ 1.2 b	1.0 $\pm$ 0.0 d	9.1 $\pm$ 1.0 c
SPIDES $\times$ Yes	88.8 $\pm$ 3.0ND	73.8 $\pm$ 1.5 d	83.0 $\pm$ 1.1 c	5.3 $\pm$ 0.0 a	11.7 $\pm$ 2.7 b
EG SPIDES $\times$ No	94.5 $\pm$ 1.6ND	85.6 $\pm$ 2.0 b	90.9 $\pm$ 2.8 ab	4.0 $\pm$ 0.2 c	5.1 $\pm$ 1.5 d
EG SPIDES $\times$ Yes	95.9 $\pm$ 2.4ND	88.1 $\pm$ 1.7 a	92.4 $\pm$ 2.1 a	3.0 $\pm$ 1.3 cd	4.6 $\pm$ 1.4 d

Table 1 – contd.

1	2	3	4	5	6
Effect of PI treatment × flock age					
COI × A	82.8±2.9ND	71.1±2.3 c	85.8±2.0 b	2.9±0.4 c	11.3±2.5 b
COI × B	82.0±2.3ND	72.7±2.1 c	75.3±2.1 c	6.0±1.2 a	18.7±1.8 a
SPIDES × A	89.5±3.2ND	76.7±1.9 b	85.5±1.4 b	3.9±0.6 b	10.6±1.7 b
SPIDES × B	88.5±2.5ND	77.2±1.9 b	87.4±1.2 b	2.4±0.6 c	10.2±2.3 b
EG SPIDES × A	95.7±1.1ND	86.6±1.8 a	90.9±2.5 a	3.4±0.7 bc	5.7±1.2 c
EG SPIDES × B	94.6±3.1ND	87.2±2.1 a	92.2±2.8 a	3.7±0.6 b	4.1±2.0 c
Effect of turning × flock age					
No × A	89.2±1.9ND	77.7±1.3 a	86.9±1.1 a	2.1±0.3 b	11.0±1.6 a
No × B	87.7±2.7ND	74.4±2.4 b	84.4±2.9 b	4.3±0.6 a	11.3±2.3 a
Yes × A	89.4±3.2ND	78.5±1.4 a	87.9±2.3 a	4.6±0.9 a	7.5±1.7 b
Yes × B	89.0±3.0ND	76.3±1.7 ab	85.4±1.5 ab	3.7±0.7 a	10.9±1.9 a
Effects of PI treatment × turning × flock age					
COI × No × A	81.1±2.2ND	65.5±3.1 f	80.8±1.2 de	2.7±0.6 d	16.5±3.7 b
COI × No × B	82.5±3.1ND	59.2±2.3 g	71.7±3.0 f	6.4±1.8 ab	21.9±2.1 a
COI × Yes × A	84.5±3.8ND	76.7±1.0 d	91.3±2.4 a	2.9±0.1 d	5.8±1.3 f
COI × Yes × B	81.4±3.7ND	64.2±2.0 f	78.8±2.0 e	5.5±1.1 b	15.7±1.4 b
SPIDES × No × A	93.3±2.3ND	84.4±1.4 bc	90.5±0.9 ab	0.0±0.0 e	9.5±0.4 d
SPIDES × No × B	85.0±2.2ND	75.8±3.1 d	89.2±1.4 b	2.0±0.4 d	8.8±1.9 de
SPIDES × Yes × A	85.6±3.7ND	68.9±1.6 e	80.5±1.5 de	7.8±1.0 a	11.7±3.4 c
SPIDES × Yes × B	92.0±2.3ND	78.6±1.2 d	85.5±0.8 c	2.7±0.8 d	11.8±2.7 c
EG SPIDES × No × A	93.3±0.1ND	83.2±1.9 c	89.3±1.8 b	3.6±0.2 c	7.1±0.2 e
EG SPIDES × No × B	95.6±3.2ND	88.3±2.2 ab	92.4±4.2 a	4.4±0.2 c	3.2±2.7 g
EG SPIDES × Yes × A	98.1±2.2ND	90.0±1.8 a	92.8±2.9 a	3.0±1.2 d	4.2±2.2 g
EG SPIDES × Yes × B	93.6±3.0ND	86.1±1.9 b	92.0±1.3 a	3.0±1.0 cd	5.0±1.2 fg
Source of variation					
PI treatment	ND	0.020	0.029	0.084	0.001
Turning	ND	<0.001	0.011	0.130	<0.001
Flock age	<0.001	<0.001	<0.001	<0.001	0.021
PI treatment × Turning	ND	0.001	<0.001	0.016	0.018
PI treatment × Flock age	ND	<0.001	<0.001	<0.001	<0.001
Turning × Flock age	ND	<0.001	0.135	0.484	0.040
PI treatment × Turning × Flock age	ND	0.020	0.780	0.405	0.515

¹Values of 360 eggs in one group in four trials.

²Embryos died up to 10 d of incubation.

ND – Not determined.

A = 17, 18, 19, 20 week of quail hens life; B = 36, 37, 38, 39 week of quail hens life

COI = eggs storage 12 d, no PI and no T; COI T = eggs storage 12 d, no PI and T every 12 h; SPIDES = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage and T every 12 h; EG SPIDES = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and T every 12 h.

a-g – significant effect in ANOVA at 0.05 probability level.

The use of SPIDES and EG SPIDES contributed to improving the studied indicators but mainly in flock B. Compared to COI, the use of SPIDES in flock B caused a significant ($P < 0.05$) increase of the hatchability of set eggs (by 4.5%), an increase of the hatchability of apparently fertile eggs (by 12.1%), a decrease of early embryonic mortality (by 3.6%) and a decrease of unhatched chicks (by 8.5%). In flock A after the application of SPIDES, only an increase of hatchability of set eggs (by 5.6%) and a decrease of early embryonic mortality (by 1.2%) was confirmed ($P < 0.05$). Application of EG SPIDES compared to COI in flock B yielded even greater benefits than SPIDES. Higher hatchability of set and apparently fertile eggs by 14.5% and 16.9% respectively were observed (both $P = 0.001$). EG SPIDES were also characterized by a reduced number of early embryonic mortality by 2.3% ($P = 0.016$) and a reduced number of unhatched chicks by 14.7% ($P = 0.001$). When EG SPIDES has been applied in flock A, it improved the hatchability of set and apparently fertile eggs (15.5% and 4.8% respectively) ($P < 0.05$). Decreases of unhatched chicks were also observed in those groups ($P = 0.025$) but no impact on early embryonic mortality was noticed ($P = 0.084$). Comparing different PI profiles, the results show that EG SPIDES has a greater impact on improving hatching results and contributes to the reduction in the number of unhatched chicks ($P < 0.05$), followed by SPIDES, regardless of the flock age. No significance was observed between profiles in early embryonic mortality ($P < 0.05$). When analyzing the treatment $\times$ flock age interaction, it can be seen that compared to the one-way analysis, the differences for these indicators between flocks A and B due to the different impact of PI are reduced (Table 1). This is particularly evident in EG SPIDES where none of the studied parameters were different between flocks A and B ($P > 0.05$). In SPIDES, the difference was confirmed only for early embryonic mortality ($P = 0.032$).

The use of T during egg storage resulted in a 2.3% reduction of unhatched chicks ($P = 0.042$) (Table 1). In interaction with flock age, it was shown that the use of T resulted in a 2.2% increase in early embryonic mortality and a 3.0% reduction in the number of non-hatched chicks in flock A ($P < 0.05$). In flock B, there was no effect of T on the analyzed parameters ($P > 0.05$). In the treatment $\times$ T interaction, a significant improvement in all analyzed parameters was observed in the COI vs COI T groups ($P > 0.05$) and their deterioration in the SPIDES vs SPIDES T groups ($P > 0.05$). The interaction of T with the EG SPIDES profile was significant ($P = 0.035$) only for the hatchability of set eggs and this parameter improved by 2.5%.

The interaction of all three factors (flock age $\times$ treatment $\times$ T) was significant only for the number of unhatched chicks ($P = 0.015$). In all other studied parameters, T did not interact. The best hatchability of set eggs was observed for flock A, EG SPIDES profile and turning (T) (90%). The highest improvement was observed when the EG SPIDES profile without T was used in flock B. The difference between the COI and EG SPIDES group was 29.1% for hatchability of set eggs and 21.7% for the hatchability of apparently fertile eggs. The highest hatchability of apparently fertile eggs ($> 90\%$) was confirmed in all EG SPIDES groups, except for flock A, the EG SPIDES without T, and in the COI T and SPIDES groups of flock A. The SPIDES group in flock A was characterized by lack of early embryonic mortality of fertile eggs. In the case of unhatched chicks, the smallest percentage was found in

the EG SPIDES group in flock B without T and in flock A with turning T. The biggest difference in unhatched chicks was found between flock B EG SPIDES without T and flock B COI, mortality was reduced by 18.7%.

Hatch window

Table 2 and Figure 2 present the results of incubation duration and hatch window (HW). Eggs incubation duration in flock B was longer for about 11 h ($P<0.001$) and HW for about 5 h ($P=0.020$) compared to flock A. Use of SPIDES for eggs from flock B caused a reduction of incubation duration by 11 h ($P=0.028$) but had no impact on HW ($P>0.05$). In case of flock A, the use of SPIDES did not influence incubation duration but prolonged HW by 4 h ($P=0.045$). In flock B, the use of EG SPIDES contributed to reducing the duration of incubation by 16 h ($P=0.015$) and had no impact on HW. In flock A, the use of EG SPIDES reduced the duration of incubation by 9 h ($P=0.036$) and prolonged HW by 5 h ($P=0.040$). Comparing PI profiles, the use of EG SPIDES vs SPIDES resulted in a reduction in the duration of incubation by 5 h and 6 h respectively in flock B and A ($P<0.05$), but in both flocks there was no difference between the HW ($P>0.05$).

Table 2. Results (means and $\pm$ SD) of the effect of Japanese quail's age, eggs' pre-incubation and eggs' turning during storage time on start of hatching, duration of incubation and hatch windows

Treatment	N	Start of hatching (h of incubation)	Duration of incubation (h)	Hatch windows (h)
1	2	3	4	5
Effect of PI treatment				
COI	1099	381 $\pm$ 6.7 a	405 $\pm$ 5.8 ab	24 $\pm$ 5.7 c
SPIDES	1068	372 $\pm$ 6.6 b	398 $\pm$ 5.0 bc	26 $\pm$ 3.5 bc
EG SPIDES	1184	365 $\pm$ 3.6 c	393 $\pm$ 4.6 c	28 $\pm$ 2.6 ab
Effect of turning				
No	1644	371 $\pm$ 6.5 a	399 $\pm$ 5.2 a	28 $\pm$ 4.5 a
Yes	1707	374 $\pm$ 5.8 a	398 $\pm$ 4.6 a	24 $\pm$ 3.4 b
Effect of flock age				
A	1723	369 $\pm$ 6.1 b	393 $\pm$ 4.8 b	24 $\pm$ 3.0 b
B	1628	375 $\pm$ 6.4 a	404 $\pm$ 5.6 a	29 $\pm$ 4.9 a
Effect of PI treatment $\times$ turning				
COI $\times$ No	449	382 $\pm$ 7.1 a	404 $\pm$ 6.5 ab	22 $\pm$ 7.3 c
COI $\times$ Yes	507	379 $\pm$ 6.3 a	406 $\pm$ 5.4 a	27 $\pm$ 4.3 b
SPIDES $\times$ No	577	365 $\pm$ 6.8 b	395 $\pm$ 7.0 c	30 $\pm$ 4.1 ab
SPIDES $\times$ Yes	566	378 $\pm$ 6.4 a	401 $\pm$ 3.1 b	23 $\pm$ 3.0 c
EG SPIDES $\times$ No	618	365 $\pm$ 5.7 b	398 $\pm$ 3.9 bc	33 $\pm$ 2.4 a
EG SPIDES $\times$ Yes	634	364 $\pm$ 5.1 b	387 $\pm$ 5.7 d	23 $\pm$ 2.9 c

Table 2 – contd.

1	2	3	4	5
Effect of PI treatment × flock age				
COI × A	512	376±7.7 b	397±7.1 c	21±4.2 c
COI × B	444	385±5.7 a	413±4.7 a	28±7.1 a
SPIDES × A	587	369±5.8 cd	394±3.5 c	25±3.9 b
SPIDES × B	556	374±7.3 b	402±6.6 b	28±3.3 a
EG SPIDES × A	624	362±4.6 d	388±3.9 d	26±1.0 ab
EG SPIDES × B	628	367±6.2 c	397±5.7 c	30±4.2 a
Effect of turning × flock age				
No × A	840	367±6.5 c	393±5.5 b	26±3.0 b
No × B	804	375±6.6 a	405±6.1 a	30±6.1 a
Yes × A	883	371±5.7 b	393±4.1 b	22±3.1 c
Yes × B	824	376±6.2 a	403±5.3 a	27±3.5 ab
Effects of PI treatment × turning × flock age				
COI × No × A	236	376±7.3 c	396±8.8 cd	20±5.2 d
COI × No × B	276	388±6.9 a	412±4.2 a	24±9.1 c
COI × Yes × A	304	376±8.1 c	398±5.3 cd	22±3.3 cd
COI × Yes × B	283	382±4.5 b	414±5.2 a	32±5.3 a
SPIDES × No × A	300	362±6.6 e	390±4.1 e	28±2.8 b
SPIDES × No × B	324	368±6.9 d	400±9.9 bc	32±5.4 a
SPIDES × Yes × A	213	376±5.1 c	398±2.9 cd	22±4.9 cd
SPIDES × Yes × B	231	380±7.8 bc	404±3.3 b	24±1.1 c
EG SPIDES × No × A	273	362±5.5 e	394±3.6 d	32±0.9 a
EG SPIDES × No × B	283	368±6.0 d	402±4.2 b	34±4.0 a
EG SPIDES × Yes × A	318	362±3.8 e	382±4.1 f	20±1.2 d
EG SPIDES × Yes × B	310	366±6.4 de	392±7.3 de	26±4.4 bc
Source of variation				
PI treatment		<0.001	0.135	0.018
Turning		0.265	<0.001	<0.001
Flock age		0.045	<0.001	0.020
PI treatment × Turning	3351	0.743	0.048	0.001
PI treatment × Flock age		0.380	0.015	0.069
Turning × Flock age		0.047	<0.001	0.130
PI treatment × Turning × Flock age		0.360	0.025	0.088

A = 17, 18, 19, 20 week of quail hens life; B = 36, 37, 38, 39 week of quail hens life.

COI = eggs storage 12 d, no PI and no T; COI T = eggs storage 12 d, no PI and T every 12 h; SPIDES = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage and T every 12 h; EG SPIDES = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and T every 12 h.

a-f – significant effect in ANOVA at 0.05 probability level.

Figure 2. Distribution of hatching in relation to the quail eggs treatments, where: A = 18, 19 week of Japanese quail hens life; B = 36 week of Japanese quail hens life; COI = eggs storage 12 d, no PI and no T; COI T = eggs storage 12 d, no PI and T every 12 h; SPIDES = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage and T every 12 h; EG SPIDES = 4 h PI, delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = 4 h PI, delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and T every 12 h

Number of quail chicks for which the charts figure is the same as presented in Table 2 for the treatment group.

a-d – significant effect in ANOVA at 0.05 probability level.

The use of T did not affect the duration of incubation in flocks B and A ($P > 0.05$). The effect of T was observed for flock A in the reduction of HW time by 4 h ($P = 0.045$). In interaction treatment $\times$ T in COI groups, there was no effect on the incubation duration but a prolongation of HW of 5 h was observed ($P = 0.089$). In

the interaction SPIDES $\times$ T, incubation duration was prolonged by 6 h ($P=0.036$) but HW was reduced by 7 h ($P=0.018$). In the interaction EG SPIDES $\times$ T, the duration of incubation and HW was reduced by 11 h ($P=0.001$) and 10 h ($P=0.016$) respectively.

The interaction of all three factors showed no significant change in the duration of incubation ($P=0.225$) and HW ($P=0.088$). The shortest duration of incubation was observed in flock A EG SPIDES T and the longest in flock B in both COI and COI T. The shortest HW was observed in flock A in COI and COI T groups and in the SPIDES T and EG SPIDES T groups. In both flock A and B the use of EG SPIDES prolonged HW. The same trend was observed for flock B SPIDES.

Eggs and chicks weight

Table 3 shows the weight of eggs used in the study, eggs' weight loss during 12 d storage (%), chicks' weight and chicks' weight as a percentage of egg weight (%). Flock age did not affect any studied parameters ($P>0.05$). The use of PI profiles had an impact on increased egg weight loss during storage in both flocks in comparison to COI ($P<0.05$). The use of SPIDES results in decreased hatching chicks weight by about 0.40 g in flock B compared to the COI ($P=0.048$).

Table 3. Results (means and $\pm$ SD) of Japanese quail eggs' weight loss, chicks hatching and chicks ratio

Treatment	Egg weight (g ¹)	Egg weight loss during storage time (%)	Chicks hatching weight (g ²)	Chicks hatching weight, % of eggs weight (% ^{2,3})
1	2	3	4	5
Effect of PI treatment				
COI	11.5 $\pm$ 0.8 ND	1.21 $\pm$ 0.6 b	9.43 $\pm$ 0.8 a	81.9 $\pm$ 6.5 a
SPIDES	11.6 $\pm$ 0.9 ND	1.47 $\pm$ 1.0 a	9.23 $\pm$ 0.7 b	81.4 $\pm$ 5.2 a
EG SPIDES	11.6 $\pm$ 1.0 ND	1.43 $\pm$ 1.0 a	9.33 $\pm$ 0.8 ab	82.2 $\pm$ 8.3 a
Effect of turning				
No	11.6 $\pm$ 0.9 ND	1.41 $\pm$ 0.9 a	9.30 $\pm$ 0.6 a	81.6 $\pm$ 8.0 a
Yes	11.6 $\pm$ 0.9 ND	1.33 $\pm$ 1.0 b	9.35 $\pm$ 0.9 a	82.1 $\pm$ 5.3 a
Effect of flock age				
A	11.6 $\pm$ 0.8 a	1.41 $\pm$ 1.1 a	9.45 $\pm$ 0.9 a	82.2 $\pm$ 5.5 a
B	11.6 $\pm$ 1.0 a	1.34 $\pm$ 0.8 b	9.20 $\pm$ 0.8 a	81.4 $\pm$ 6.9 a
Effect of PI treatment $\times$ turning				
COI $\times$ No	11.5 $\pm$ 0.8 ND	1.31 $\pm$ 0.8 b	9.30 $\pm$ 0.8 b	81.3 $\pm$ 9.1 a
COI $\times$ Yes	11.6 $\pm$ 0.9 ND	1.12 $\pm$ 0.7 c	9.55 $\pm$ 0.8 a	82.6 $\pm$ 3.5 a
SPIDES $\times$ No	11.7 $\pm$ 0.9 ND	1.57 $\pm$ 1.2 a	9.25 $\pm$ 0.9 b	81.1 $\pm$ 6.7 a
SPIDES $\times$ Yes	11.6 $\pm$ 0.8 ND	1.38 $\pm$ 1.0 b	9.20 $\pm$ 0.7 b	81.7 $\pm$ 3.7 a
EG SPIDES $\times$ No	11.6 $\pm$ 0.8 ND	1.36 $\pm$ 0.7 b	9.35 $\pm$ 0.7 b	82.4 $\pm$ 7.9 a
EG SPIDES $\times$ Yes	11.6 $\pm$ 1.1 ND	1.51 $\pm$ 1.3 a	9.30 $\pm$ 0.9 b	82.0 $\pm$ 8.4 a

Table 3 – contd.

1	2	3	4	5
Effect of PI treatment × flock age				
COI × A	11.6±0.9 ND	1.14±0.8 d	9.50±0.8 a	82.0±6.2 a
COI × B	11.5±0.8 ND	1.29±0.5 cd	9.35±0.8 a	81.9±6.7 a
SPIDES × A	11.6±0.7 ND	1.60±1.1 a	9.50±0.9 a	82.2±4.9 a
SPIDES × B	11.7±1.0 ND	1.34±0.9 bc	8.95±0.8 b	80.6±5.7 a
EG SPIDES × A	11.5±0.8 ND	1.48±1.2 b	9.35±0.9 a	82.4±8.6 a
EG SPIDES × B	11.7±1.1 ND	1.38±0.6 b	9.30±0.7 a	81.9±6.6 a
Effect of turning × flock age				
No × A	11.6±0.8 ND	1.43±1.1 a	9.43±0.9 a	82.0±8.6 a
No × B	11.6±0.9 ND	1.39±0.6 a	9.17±0.7 b	81.1±7.5 a
Yes × A	11.5±0.8 ND	1.39±1.0 a	9.47±0.8 a	82.4±4.6 a
Yes × B	11.6±1.0 ND	1.28±0.8 b	9.23±0.8 ab	81.8±9.1 a
Effects of PI treatment × turning × flock age				
COI × No × A	11.5±0.9 ND	1.25±1.3 d	9.42±1.0 ab	80.8±10 a
COI × No × B	11.4±0.7 ND	1.37±0.4 c	9.20±0.7 b	81.7±8.8 a
COI × Yes × A	11.6±0.9 ND	1.03±0.5 e	9.60±0.8 a	83.2±2.7 a
COI × Yes × B	11.6±0.9 ND	1.20±0.9 d	9.55±0.9 a	82.0±4.7 a
SPIDES × No × A	11.6±0.8 ND	1.54±1.5 a	9.51±0.9 a	82.1±6.7 a
SPIDES × No × B	11.7±1.1 ND	1.59±0.9 a	9.08±0.9 b	80.1±7.0 a
SPIDES × Yes × A	11.6±0.7 ND	1.66±0.9 a	9.55±0.8 a	82.3±3.1 a
SPIDES × Yes × B	11.6±0.9 ND	1.09±1.1 e	8.94±0.7 b	81.0±4.4 a
EG SPIDES × No × A	11.6±0.8 ND	1.49±0.7 b	9.41±0.9 ab	83.2±9.2 a
EG SPIDES × No × B	11.6±0.8 ND	1.22±0.7 d	9.30±0.6 ab	81.5±6.8 a
EG SPIDES × Yes × A	11.4±0.9 ND	1.47±1.9 bc	9.30±0.9 ab	81.6±8.0 a
EG SPIDES × Yes × B	11.7±1.3 ND	1.54±0.9 ab	9.37±0.9 b	82.3±9.3 a
Source of variation				
PI treatment	ND	0.040	0.047	0.635
Turning	ND	0.028	0.660	0.540
Flock age	0.890	0.010	0.058	0.690
PI treatment × Turning	ND	<0.001	0.140	0.632
PI treatment × Flock age	ND	0.115	0.185	0.654
Turning × Flock age	ND	0.096	0.092	0.125
PI treatment × Turning × Flock age	ND	0.035	0.200	0.087

¹Values of 360 eggs in one group in four trials.

²The same number of chicks is presented in Table 4.

³Chicks : eggs (d after laying) weight ratio at hatching.

ND – Not determined.

A = 17, 18, 19, 20 week of quail hens life; B = 36, 37, 38, 39 week of quail hens life.

COI = eggs storage 12 d, no PI and no T; COI T = eggs storage 12 d, no PI and T every 12 h; SPIDES = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage and T every 12 h; EG SPIDES = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and T every 12 h.

a-e – significant effect in ANOVA at 0.05 probability level.

The use of T during egg storage resulted in a decrease of egg weight loss in both flocks and in increasing the weight of the chicks in flock B ($P>0.05$). In interaction with PI, the use of T resulted in a decrease of egg weight loss ($P>0.05$) and did not affect other parameters.

The interaction of all three factors (flock age $\times$ treatment $\times$ T) has not been confirmed ($P>0.05$).

Chicks quality

Table 4 presents the results of chicks quality analysis. In flock B, 6% fewer chicks with a score of 100 ($P<0.001$), 2.2 point of the lower average score of all chicks ($P=0.046$) and 19.6 point of the lower average score of chicks with a score below 100 ($P<0.001$) were found. The use of SPIDES in flock B did not affect the quality of the chicks, while in flock A the average score of all chicks was improved by 1.8 points ($P=0.025$). The use of EG SPIDES for flock B contributed to decreasing the average score of all chicks by 1.9 point ($P=0.042$), but increased the average score of chicks with a score below 100 by 6.0 points ($P=0.018$). Use of EG SPIDES in flock A resulted in an increased average score of all chicks by 6.7 points ($P=0.036$) and a decrease in the average score of chicks with a score below 100 by 9.9 points ($P=0.004$). In summary, the use of PI profiles compared to the COI group did not affect the number of chicks with the score of 100 ($P=0.380$), nor the average score of chicks with the score below 100 points ($P=0.175$). As regards the average score of all chicks, only the EG SPIDES profile resulted in a 2.3 point increase compared to the COI group ($P=0.022$).

Table 4. Results (means and $\pm$ SD) of the effect of Japanese quail's age, eggs pre-incubation and eggs turning on chicks quality¹

Treatment	N	Chicks with score 100 (%)	Average score of all chicks	Average score of chicks with score <100
1	2	3	4	5
Effect of PI treatment				
COI	1099	72.7 $\pm$ 7.7 a	91.0 $\pm$ 10 b	84.4 $\pm$ 11 a
SPIDES	1068	73.2 $\pm$ 5.8 a	91.5 $\pm$ 6.8 ab	84.0 $\pm$ 6.6 a
EG SPIDES	1184	73.1 $\pm$ 4.1 a	93.3 $\pm$ 6.0 a	82.4 $\pm$ 8.9 a
Effect of turning				
No	1644	71.5 $\pm$ 5.3 b	90.5 $\pm$ 9.1 a	84.2 $\pm$ 10 a
Yes	1707	74.4 $\pm$ 6.2 a	93.3 $\pm$ 8.2 a	83.0 $\pm$ 7.4 a
Effect of flock age				
A	1723	76.0 $\pm$ 2.8 a	93.0 $\pm$ 3.2 a	93.4 $\pm$ 5.0 a
B	1628	70.0 $\pm$ 8.8 b	90.8 $\pm$ 12 b	73.8 $\pm$ 12 b
Effect of PI treatment $\times$ turning				
COI $\times$ No	449	72.2 $\pm$ 7.3 b	91.5 $\pm$ 11 ab	85.8 $\pm$ 14 a
COI $\times$ Yes	507	73.2 $\pm$ 7.9 ab	90.4 $\pm$ 10 b	83.0 $\pm$ 7.8 ab
SPIDES $\times$ No	577	72.1 $\pm$ 6.3 b	88.5 $\pm$ 6.3 b	85.4 $\pm$ 7.0 a
SPIDES $\times$ Yes	566	74.4 $\pm$ 5.0 a	94.5 $\pm$ 7.3 a	82.7 $\pm$ 6.3 ab
EG SPIDES $\times$ No	618	70.4 $\pm$ 2.4 b	91.6 $\pm$ 4.6 ab	81.5 $\pm$ 9.6 b
EG SPIDES $\times$ Yes	634	75.8 $\pm$ 5.8 a	95.1 $\pm$ 7.5 a	83.4 $\pm$ 8.1 ab

Table 4 – contd.

1	2	3	4	5
Effect of PI treatment × flock age				
COI × A	512	75.6±2.2 a	90.2±2.9 c	97.6±4.1 a
COI × B	444	70.0±11 c	91.7±18 b	71.2±17 d
SPIDES × A	587	77.4±3.4 a	92.0±3.1 b	95.1±2.0 a
SPIDES × B	556	69.1±7.6 c	91.0±10 b	73.0±11 d
EG SPIDES × A	624	75.2±2.8 a	96.9±3.4 a	87.7±9.1 b
EG SPIDES × B	628	71.1±5.3 b	89.8±8.5 c	77.2±8.6 c
Effect of turning × flock age				
No × A	840	74.6±2.0 a	90.7±2.6 b	93.0±5.1 a
No × B	804	68.5±8.7 c	90.3±12 b	75.4±15 b
Yes × A	883	77.5±3.6 a	95.3±3.8 a	93.9±5.5 a
Yes × B	824	71.4±8.8 b	91.3±13 b	72.1±9.4 b
Effects of PI treatment × turning × flock age				
COI × No × A	236	75.8±2.7 ab	91.3±3.8 c	97.9±4.3 a
COI × No × B	276	68.5±12 c	91.7±18 c	73.7±23 de
COI × Yes × A	304	75.3±1.8 ab	89.1±2.0 c	97.3±3.7 a
COI × Yes × B	283	71.0±14 c	91.7±18 c	68.6±12 e
SPIDES × No × A	300	76.3±2.9 a	85.3±2.7 d	96.5±1.1 a
SPIDES × No × B	324	67.8±9.7 c	91.7±10 c	74.2±13 d
SPIDES × Yes × A	213	78.4±4.1 a	98.6±3.6 a	93.6±2.9 ab
SPIDES × Yes × B	231	70.3±5.9 c	90.3±11 c	71.8±9.8 e
EG SPIDES × No × A	273	71.6±0.4 b	95.6±1.2 b	84.6±8.2 c
EG SPIDES × No × B	283	69.2±4.4 c	87.6±7.9 cd	78.3±11 d
EG SPIDES × Yes × A	318	78.7±5.0 a	98.2±5.9 a	90.7±10 b
EG SPIDES × Yes × B	310	72.9±6.6 b	91.9±9.0 c	76.0±6.3 d
Source of variation				
PI treatment		0.380	0.036	0.175
Turning		0.002	0.147	0.142
Flock age		<0.001	<0.001	<0.001
PI treatment × Turning		0.180	0.870	0.040
PI treatment × Flock age	3351	0.044	0.035	0.001
Turning × Flock age		0.010	0.058	0.047
PI treatment × Turning × Flock age		0.078	0.055	0.025

¹According to Tona et al. (2003).

A = 17, 18, 19, 20 week of quail hens life; B = 36, 37, 38, 39 week of quail hens life.

COI = eggs storage 12 d, no PI and no T; COI T = eggs storage 12 d, no PI and T every 12 h; SPIDES = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage; SPIDES T = 4 h PI, delivered at 4, 6, 8 and 10 d over 12 d of storage and T every 12 h; EG SPIDES = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage; EG SPIDES T = PI delivered: 5 d – 2 h, 7 d – 3 h, 9 d – 5 h and 11 d – 6 h over 12 d of storage and T every 12 h.

a-e – significant effect in ANOVA at 0.05 probability level.

The use of T in flock B caused an increase in the number of chicks with the score of 100 by 2.9% ($P=0.025$) and in flock A an increase in the average score of all chicks by 4.6 points ($P=0.029$). There was no effect of T in the COI groups ($P>0.05$). In the interaction SPIDES $\times$ T, an increase in the number of chicks with the score of 100 by 2.3 points ($P=0.047$) and an increase in the average score of all chicks by 6.0 points ($P=0.001$) was observed. In the interaction EG SPIDES $\times$ T, an increase in the number of chicks with the score of 100 by 5.4 points ($P=0.038$) was noticed.

The interaction of all three factors (flock age $\times$ treatment $\times$ T) has not been confirmed ($P>0.05$). Generally, it should be stated that most chicks with the score of 100 were observed in flock A regardless of the use of PI and T. Only the use of EG SPIDES without turning (T) in flock A resulted in a decrease in the mentioned parameter. A similar situation was observed for the average score of chicks with the score below 100. For the average score of all chicks, the best results were obtained in flock A after using SPIDES with T and EG SPIDES without T.

Discussion

The ideal result of incubation is to get one healthy chick from one hatching egg. Both the aging of reproductive birds and the prolonged storage time of eggs reduce the hatching results and quality of the obtained chicks (Fasenko et al., 2001 a, b; Reijrink et al., 2009). The PI in 12 d storage quail eggs used in this study contributed to a significant counteraction of this phenomenon by increasing the hatching of apparently fertile eggs and reducing early and late embryonic mortality. Research conducted on a reproductive flock of meat type hens at the end of the reproduction period also showed a beneficial effect of PI after 11 days of egg storage (Yassin et al., 2008; Reijrink et al., 2010; Gucblimez et al., 2013). Similar observations were previously documented by Fasenko et al. (2001 a) for turkey eggs.

According to Reijrink et al. (2010), the loss of membrane integrity is one of the early steps of the apoptotic cascade and leads to the translocation of a phosphatidylserine from the cytoplasmic side of the cell to the outer membrane. Disrupted integrity of the cell membrane causes an increased loss of CO_2 and, as a consequence, a rapid increase in egg albumen pH from 7.8 to 9.5 after 6 days of storage (Marzec et al., 2019). Reijrink et al. (2010) hypothesize that the beneficial effect of PI comes from the fact that it allows to finish the formation of embryonic hypoblast and makes the embryo less sensitive to changes in albumen pH. Van Roover-Reijrink et al. (2018) also hypothesized that the use of PI affects the expression of specific genes associated with apoptosis. Gómez-de-Travededo et al. (2014) presented results which are in contradiction to the mentioned research. Use of pre-incubation in red-legged partridge did not influence hatchability of the fertile eggs stored for 7 d, and in case of 42 d of storage even worsened it. The above result indicates the need to adapt the PI to the tested poultry species.

The most important observation of this study is that the EG SPIDES profile had a more beneficial effect on embryo livability and hatching results compared

to SPIDES. Until now, studies on the effects of PI have been limited mainly to two schemes: single several hour PI (Fasenko et al., 2001 a, b), or a series of several equal hourly PI (SPIDES) (Dymond et al., 2013; Damaziak et al., 2018). The first study using SPIDES as the method of prolonging the hatchability of long-stored eggs was motivated by the fact that birds act similarly in their natural habitat (Conway and Martin, 2000; Deeming, 2002). Meijer and Siemers (1993) were first to discover that heating of older eggs in the nest always occurs when new ones are laid. However, it was not noticed whether the time spent by the birds in the nest by laying new eggs is equal as it is proposed in the SPIDES profile. Based on own observations on the behavior of precocial avian species, in which a clutch consists of at least over a dozen eggs, it was found that birds spend an increasing amount of time with each subsequent egg laid. This is a kind of successive training before the commencement of the actual incubation. Until now, only van Roover-Reijrink et al. (2018) presented a two-stage PI scheme for chicken eggs and obtained a significant improvement in hatchability. The aim of the EG SPIDES profile was to replicate the process, which is a component of reproductive success for natural bird reproduction. The details of the mechanism of action of EG SPIDES, leading to increased hatchability of healthy chicks, are unknown and require further research. However, the issue of longer HW in this system remains controversial, the more so that synchronization in natural clutches is almost perfect (Vince, 1964; Eichholz and Towery, 2010). However, one should note that a combination of two modifications, that is EG SPIDES and T during storage have largely improved the HW. Both modifications are used by birds in nature because during the laying of subsequent eggs, the older eggs are being heated with increasing intensity as well as turned and their positions are being changed by the parents. It was also proven that almost simultaneous hatching of chicks in the nest is also controlled by a series of other factors, such as cooling (Hepp and Kenamer, 2018), vocal (Brua, 2002) or chemosensory communication (Webster et al., 2015). Therefore, it is possible that the application of a suitable PI profile (and based on the present study, EG SPIDES T can be considered as such a profile) can greatly improve the hatchability indicators for long-stored eggs. However, at the same time, obtaining a suitable hatch window requires application of additional environmental synchronizations acting on the embryos in the final stage of incubation.

Although both SPIDES and EG SPIDES affected the HW negatively, in both cases they contributed to a reduction of the entire incubation time. Previously, no significant reduction in incubation time was observed after using SPIDES for chicken eggs (Damaziak et al., 2018) and turkey eggs (Fasenko et al., 2001 a). It can be assumed that the embryonic development of the quail is naturally much faster compared to the development of chicken and turkey embryos as evidenced by the shorter time of the entire incubation. In addition, the difference in egg weight and shell thickness can be of crucial importance. Several times lower weight of quail eggs and a very thin shell can contribute to faster attainment of the right temperature and earlier activation of embryogenesis. In the author's opinion, these factors could be a sufficient reason for the different reactions of chicken, turkey and quail embryos to the PI used.

Reduction of the incubation time may be desirable in mass hatching but only with simultaneous strong synchronization. Otherwise, it may result in a reduction in the

quality of the obtained chicks. In general, the quality of chicks obtained from eggs of the older quail flock was lower, which confirms previous studies in chickens (Tona et al., 2003, 2004; Pokhrel et al., 2018). As a consequence of the SPIDES study used in this study, the quality of the chicks did not change compared to the COI groups. A similar result was previously obtained by Dymond et al. (2013) using four four-hour SPIDES during 21 d storage of chicken eggs. Similarly, the use of EG SPIDES did not affect the quality of the chicks. The best quality of chicks was obtained in EG SPIDES T group. There is no information in the literature on the impact of EG SPIDES and T during egg storage on the quality of chicks, which makes discussion difficult. It can be assumed that reduction of the incubation time to a dozen hours and extending the HW by a few hours do not yet have negative effects in the form of deterioration in the quality of the chicks. Especially that the synchronization disorder usually results in a disorder during resorption of the yolk sac and this defect was not observed in this study.

In conclusion, to obtain a higher number of high quality quail chicks from eggs stored for 12 d, it is recommended to use PI, particularly in line with the scheme of the discussed EG SPIDES profile with the concomitant T every 12 h. Application of the EG SPIDES profile improves hatchability but it contributes to prolongation of the HW duration, which may impede clutch organization in hatcheries. It seems appropriate to extend the future research with the use of EG SPIDES T and a selected environmental factor in the final incubation stage contributing to the improvement of the hatch window.

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