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Short Periods of Incubation During 10-Day Egg Storage (SPIDES): Effects on Hatchability, Hatchling Traits, and Productive Performance of Japanese Quail

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A B S T R A C T

This study evaluated short-period incubation during egg storage (SPIDES) in fertile Japanese quail eggs stored for 10 days. A total of 600 hatching eggs were randomly assigned to four treatments (150 eggs/treatment; three replicates of 50 eggs): T1, control; T2, 2-h SPIDES; T3, 3-h SPIDES; and T4, 4-h SPIDES. SPIDES was applied twice, on storage days 4 and 8, at 37.7°C and 55–60% relative humidity. T3 produced the highest hatchability of fertile eggs; hatchability was higher than in T2 and T4 but did not differ significantly from T1. T3 also showed lower total and early embryonic mortality, whereas apparent fertility and mid- and late-embryonic mortality were not significantly affected. T3 produced the shortest incubation duration and hatch window and the greatest chick length, whereas T1 and T2 had higher chick hatch weights than T3 and T4. For post-hatch evaluation, we reared 45 healthy chicks from each treatment to 35 days of age. T3 showed the highest final body weight and total weight gain, the lowest feed intake, and the best feed conversion ratio, while total chick mortality did not differ among treatments. Under the conditions of this study, applying 3-h SPIDES on days 4 and 8 of a 10-day storage period produced the most favorable overall response among the tested durations. These findings suggest that an appropriately timed SPIDES program may help mitigate the adverse effects of prolonged egg storage on hatchability and subsequent productive performance in Japanese quail.

Keywords: *Pre-incubation; Japanese Quail Eggs; Hatchability; Hatchling Traits; Productive Performance.*

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1 Introduction

Improving hatching egg storage conditions is essential to increase hatchability efficiency and rates and enhance chick quality (Próchniak et al., 2025). Fluctuations in chick demand and economic considerations often require extending the storage period before incubation to reduce the costs of daily egg collection or to accumulate enough eggs to operate hatcheries at full capacity. Additionally, extending storage may improve hatch synchronization, thereby facilitating egg collection management when collection schedules are irregular (Ansah et al., 2023). In chicken eggs, a storage period of up to 7 days is considered optimal for maintaining hatchability (Mafruchat & Makuwira, 2024). However, no specific recommendations exist for the optimal storage duration of Japanese quail eggs, despite the assumption that their physiological characteristics differ from those of chicken eggs (Oliveira et al., 2023). Taha et al. (2019) reported a decline in hatchability percentage when quail eggs were stored for more than 4 days (Taha et al., 2019). In contrast, Seker et al. (2005) recommended that the storage period not exceed 9 days to achieve optimal hatchability (Seker et al., 2005).

Furthermore, Hassan & Alsattar (2015) indicated that the appropriate storage duration for Japanese quail eggs should not exceed 7 days to maintain hatch quality. These variations among previous studies highlight the need for further research to determine the optimal storage conditions for Japanese quail eggs (Hassan & Alsattar, 2015). Despite the economic importance of hatchability traits, most poultry genetic improvement programs have focused on enhancing productive traits, whereas hatchability traits have received less attention. This may be because of their lower heritability, which limits the potential for improvement through genetic selection. Therefore, improving environmental and managerial factors affecting hatching eggs has become one of the most important approaches for increasing hatchability efficiency and enhancing the quality of produced chicks (Dowden, 2009). Storage duration is one of the most important factors affecting hatchability, as prolonged storage induces physical and chemical changes that affect egg quality. These changes include water and carbon dioxide loss, an increase in albumen pH, degradation of ovomucin and chalaza structures, and the movement of water from the albumen to the yolk, resulting in weakening of the vitelline membrane and an increase in yolk size. These alterations negatively affect embryonic development by increasing dehydration and embryonic mortality rates, particularly during the early stages of incubation. Moreover, oxidation of yolk lipids may form harmful compounds that impair embryonic development (Lacin et al., 2008). Therefore, hatching eggs should not be stored for more than 7–10 days, and storage should be at physiological temperature (21°C) to maintain embryo viability and achieve optimal hatchability (Redondo et al., 2023). To mitigate the negative effects of prolonged storage, researchers developed the pre-incubation-during-storage technique. This technique mimics wild-bird behavior before incubation, in which parents expose the first eggs laid to gradual warming, with the duration of warming increasing as subsequent eggs are laid. This process aims to stimulate embryonic development and maintain embryo viability (Damaziak et al., 2021). The efficiency of this technique depends on the timing of its application, the number of warming cycles, and their duration. Dhotre et al. (2023) reported that the best hatch performance was achieved when the pre-incubation (SPIDES) treatment was applied for 2 hours on the fifth and tenth days of storage in broiler breeder eggs stored for 15 days (Dhotre et al., 2023). This procedure offers a practical approach to improve hatchability traits and maintain egg quality, particularly under conditions that require extended storage periods. Numerous studies have demonstrated the effectiveness of the pre-incubation technique in improving incubation outcomes. This technique has contributed to enhancing hatchability percentage (Dymond et al., 2013; Fasenko et al., 2001; Reijrink et al., 2010), improving hatchling quality (Ebeid et al., 2017; Elmenawey, 2019), reducing incubation duration (Dymond et al., 2013), and decreasing embryonic mortality rates, particularly during the early stages of embryonic development (Abdel-Halim et al., 2015; Dhotre et al., 2023). However, studies on applying this technique to Japanese quail eggs stored for extended periods remain limited, particularly for storage periods exceeding one week.

Accordingly, this study aimed to evaluate the effects of different SPIDES durations during storage on Japanese quail hatching eggs for 10 days. We investigated the effects of SPIDES treatments on hatchability traits, hatchling quality, embryonic mortality rates, incubation duration, hatch window, and subsequent productive performance of hatched chicks to evaluate incubation efficiency.

2 Materials and Methods

The study was conducted during March and April 2026 using 600 Japanese quail hatching eggs. Eggs were

randomly allocated to four experimental treatments, with 150 eggs per treatment (three replicates); each replicate consisted of a separate tray containing 50 eggs. The average initial egg weight was 12.2 g. Tray positions within the incubator were randomized and rotated during incubation. Eggs were collected from a 25-week-old Japanese quail breeder flock housed in cages measuring 40×35×35 cm. We maintained a male-to-female ratio of 1:3 in each cage, and birds had free access to feed and water. The breeders were fed a complete diet containing approximately 2,900 kcal of metabolizable energy/kg, 20% crude protein, and 2.5% calcium (Table 1), formulated according to NRC (1994). A photoperiod of 16 h light/day was provided. Eggs were selected based on weight, size, and shell quality. Eggs that were excessively small, cracked, broken, or had soft shells were excluded. The selected eggs were then placed in incubator trays with the broad end facing upward. The eggs were then stored for 10 days in a refrigerated, air-conditioned room maintained at 15°C and 70% relative humidity. We monitored room temperature and relative humidity daily using a digital thermo-hygrometer. During storage, the eggs were turned once every 24 h at a 45° angle to the left and right. Before the SPIDES treatment, the incubation temperature was gradually increased. The SPIDES procedure was then carried out in an incubator at 37.7°C and 55–60% relative humidity. The SPIDES treatment was simultaneously applied to all replicate trays within each treatment. However, each replicate tray remained a separate experimental unit for data collection and statistical analysis. Different SPIDES durations were applied among treatments according to the experimental design. To minimize thermal fluctuations, eggs were gradually equilibrated in a room maintained at approximately 30°C before and after each SPIDES treatment until the average eggshell temperature reached 28°C. Eggshell temperature was monitored in 15 eggs per replicate every 15–20 min using an infrared thermometer, and the equilibration period lasted approximately 50 min under our experimental conditions. After each SPIDES treatment, we returned the eggs to the designated storage conditions to minimize thermal shock to embryos and prevent egg condensation (Seker et al., 2005). All treatment groups were subsequently incubated simultaneously in the same incubator. The 2-, 3-, and 4-hour SPIDES durations referred only to the incubation period and excluded the prewarming and cooling periods. Eggshell temperature was measured using an infrared thermometer (Benetech GM320, China). Incubation was carried out in a Cimuka T3200 SH (Cimuka Inc., Turkey) incubator with a capacity of 3,000 quail eggs. Before incubation, the eggs were disinfected by formaldehyde fumigation for 20 min using 35 mL of 40% formalin and 17.5 g of potassium permanganate (Özdemir et al., 2025). The fumigation chamber had a volume of 54.4 m³ (4 m×4 m×3.40 m), and fumigation was conducted at 24–25°C and 75% relative humidity, with appropriate safety precautions. Eggs from all treatments were incubated simultaneously under identical conditions. During the first 15 days of incubation, the incubator was maintained at 37.7°C with 55–60% relative humidity. The eggs were automatically turned 45° to either side once every 2 hours, while incubation temperature and air circulation were automatically controlled. On day 6 of incubation, the eggs were candled to identify fertile eggs, which were recognized by the presence of distinct blood vessels or embryonic shadows. Infertile eggs, as well as eggs showing no embryonic development or only minimal embryonic development that could not be distinguished from infertile eggs, were removed (Abdel-Azeem, 2009). After 15 days of incubation, the eggs were transferred to the hatcher. The incubation temperature was reduced to 37.5°C, relative humidity was increased to 75–80%, and egg turning was discontinued. At the end of the hatching period, chicks were removed from the hatcher after approximately 95% had dried, and the number of hatched chicks was recorded. Hatchability (%) was calculated as follows: Hatchability (%) = (Number of hatched chicks÷Number of fertile eggs)×100. Unhatched eggs were counted and subsequently broken out to determine the stage of embryonic mortality. Embryonic mortality (%) was calculated as the number of dead embryos divided by the total number of fertile eggs in each treatment. Embryonic mortality was classified as early (up to 6 days), mid (7–14 days), and late (15–18 days). The hatched chicks were weighed collectively within each replicate using a precision digital balance, and chick length was measured from the tip of the beak to the tip of the middle toe using a ruler. The incubation period was calculated in hours from when the eggs were set in the incubator until hatching was complete. The hatch window was determined as the time interval between the hatching of the first and last chick within each replicate (Damaziak et al., 2021a). We recorded chick weight, chick length, incubation duration, and hatch window separately for each replicate. The same three replicate groups were separately maintained throughout candling, egg transfer, hatching, and the subsequent rearing period.

2.1 Experimental Treatments

The experiment consisted of four experimental treatments. During the 10-day egg storage period, eggs assigned to the SPIDES treatments were exposed twice to 37.7°C and 55-60% relative humidity, on the 4th and 8th days of storage. The experimental treatments were as follows: T1 (control), no pre-incubation (0 h); T2, 2 h of pre-incubation; T3, 3 h of pre-incubation; and T4, 4 h of pre-incubation, with each pre-incubation treatment applied on both occasions.

After hatching, 76, 72, 86, and 62 chicks were obtained from T1, T2, T3, and T4, respectively. Chicks with normal physical appearance, no visible abnormalities, and normal activity were selected from each replicate. Because only 45 suitable chicks were available in T4, we used 45 chicks across all treatments. They were equally distributed among three replicates (15 chicks per replicate), while maintaining their original treatment and replicate identities. Unsexed chicks were reared for 35 days under identical management and environmental conditions, with ad libitum access to feed and water. The diet contained approximately 2,938 kcal/kg metabolizable energy, 22% crude protein, and 0.9% calcium, formulated according to NRC (1994) (Table 1). We evaluated post-hatch performance only for the selected chicks and measured body weight using a sensitive digital scale (C400-SF, China), recording readings to the nearest 0.01 g. Body-weight gain was calculated for each pen as the difference between the mean final and initial body weight for each week (final body weight−initial body weight). Daily feed intake was separately measured for each pen and calculated as the difference between the amount of feed offered and the amount of feed remaining after 24 h. Feed intake and feed conversion ratio (FCR) were calculated with mortality taken into consideration, including the number of days during which deceased birds had access to feed before death. Weekly FCR was calculated by dividing weekly feed intake by weekly body-weight gain.

In contrast, total FCR was calculated from total feed intake and total body weight gain over the experimental period. Mortality was recorded daily whenever it occurred. Each pen was considered the experimental unit, and the mean values for each pen were used for statistical analysis. Individual chicks were not considered independent experimental observations.

2.2 Statistical analysis

Each replicate was considered the experimental unit. Data were analyzed using one-way analysis of variance (ANOVA) in SAS/STAT 15.1 (SAS Institute Inc., 2018). Normality testing indicated that the continuous quantitative data were approximately normally distributed, and homogeneity of variance was also confirmed. Duncan's (1955) multiple range test was used to compare means. Differences were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$.

Table 1. Calculated chemical composition of the diets used in the study.

Ingredients	Starter diet	Production diet
	Percentage (%)	Percentage (%)
Yellow corn	62	56.1
Soybean meal	31	31.1
Protein concentrate*	5	5
Fat		2
Limestonx	0.5	4.9
Dicalcium phosphate	1.2	0.6
Sodium chloride (NaCl)	0.3	0.3
Total	100%	100%
Calculated chemical composition		
Metabolizable energy (kcal/kg)	2938.4	2903
Crude protein (%)	22.1	20
Lysine (%)	1.11	1.11
Methionine + cystine (%)	1.50	0.77
Calcium (%)	0.90	2.54
Available phosphorus (%)	0.40	0.35

*The protein concentrate used in the diet contained, per kg: 40% crude protein, 7.5% fat, 2.5% crude fiber, 8% calcium, 2,100 kcal metabolizable energy, 2.30% phosphorus, 2.60% salt, 2.4% lysine, 1.70% methionine, 2.20% methionine + cystine, 2,500 IU vitamin D_3, 300 mg vitamin B, 10 mg vitamin B_6, 200 mg vitamin E, 200 mg niacin, 500 mg iron, 10 mg cobalt, 600 mg zinc, 100,000 IU vitamin A, 10 mg vitamin B1, 100 mg vitamin B_{12}, 20 mg vitamin K_3, 0.5 mg biotin, 80 mg pantothenic acid, 50 mg copper, 700 mg manganese, 10 mg iodine, 2 mg selenium, and 5 mg folic acid.

3 Results and Discussion

3.1 Effect of the SPIDES Technique on Apparent Fertility, Hatchability Rates, and Embryonic Mortality of Japanese Quail Hatching Eggs

Table 2 presents the effects of short periods of incubation during egg storage (SPIDES) on apparent fertility, hatchability, and embryonic mortality of Japanese quail eggs. No significant differences (p>0.05) were observed among the experimental treatments in apparent fertility percentage. This finding may be because apparent fertility is determined before egg storage and incubation and is primarily influenced by breeder flock age, nutrition, health status, male-to-female ratio, environmental conditions, and season. In contrast, the SPIDES technique affects only embryonic development after fertilization; therefore, it is not expected to affect apparent fertility percentage (Abdel-Azeem, 2009) directly. This finding is consistent with Zhang et al. (2025) (Zhang et al., 2025), who observed no significant differences in apparent fertility percentage between SPIDES-treated and untreated eggs, despite applying SPIDES at 35$^{\circ C}$ for 1 h during egg storage periods of 7, 14, or 21 days. Kgwatalala et al. (2013) also suggested that estimating apparent fertility based on egg candling may not be entirely accurate because it cannot distinguish unfertilized eggs from very early embryonic mortality or embryos that die during egg storage or SPIDES treatment (Kgwatalala et al., 2013), which may affect the actual estimation of apparent fertility percentage. In such cases, the germinal disc may have been fertilized, but very early embryonic death may cause the egg to be incorrectly classified as infertile (Fasenko et al., 2001).

Experimental treatments significantly affected the hatchability of fertile eggs (p≤0.01). Treatment T3 recorded the highest hatchability (64.73%), differing significantly from T2 and T4, but not from the control treatment (T1), whereas T4 showed the lowest hatchability (47.09%). These findings are consistent with those reported by Okasha et al. (2023), who reported that applying the SPIDES technique for 3.5 h improved the hatchability of fertile broiler breeder eggs stored for 5, 10, and 15 days compared with the control treatment (Okasha et al., 2023).

Experimental treatments significantly affected total embryonic mortality (p=0.0078). Treatment T3 recorded the lowest value (35.25%) and differed significantly from T2 and T4, but not from T1. T4 showed the highest value (52.89%) and did not differ significantly from T2, while T1 and T2 recorded 42.85% and 46.94%, respectively. It was also observed that T2, in which eggs were exposed to pre-incubation for 2 h, did not differ significantly from the control treatment (T1) in hatchability and embryonic mortality percentages, suggesting that short-term pre-incubation of quail hatching eggs does not have a detrimental effect on hatchability traits.

When embryonic mortality was classified into early, mid, and late categories, T3 significantly reduced early embryonic mortality (8.98%) compared with T1, T2, and T4 (19.54%, 23.55%, and 25.04%, respectively). The highest early embryonic mortality was observed in the T4 treatment. Mid-embryonic mortality did not differ significantly among treatments (p=0.9343), with values of 12.79%, 13.84%, 13.50%, and 14.33% in T1, T2, T3, and T4, respectively. No significant differences were observed among treatments for late embryonic mortality (p = 0.1681). The values were 10.52%, 9.54%, 12.76%, and 13.50% for T1, T2, T3, and T4, respectively; T4 had the highest numerical value, but the treatment effect was not significant. These results were consistent with the findings of Kowsalya et al. (2025), who indicated that applying pre-incubation for 3 hours during the storage of Japanese quail eggs for 21 days reduced embryonic mortality rates and improved hatchability and the quality of hatched chicks.

In contrast, the present findings disagreed with those reported by Gucbilmez et al. (2013), who found no significant differences in early and late embryonic mortality rates when applying 6 hours of pre-incubation to broiler breeder eggs stored for 11 days compared with the control treatment. The higher hatchability of fertile eggs observed in T3 may reflect the beneficial effects of the 3-h SPIDES treatment reported in previous studies. Fasenko (2007), Reijrink et al. (2010), and Pennings et al. (2025) suggested that the beneficial effects of SPIDES on hatchability may be associated with improved embryonic viability during egg storage. However, the present study did not evaluate the specific developmental mechanisms underlying this response; therefore, these mechanisms are considered possible explanations rather than direct findings. The corresponding reduction in total embryonic mortality in T3 is consistent with the higher hatchability and is therefore considered a complementary outcome rather than an independent effect. Similarly, Dymond et al. (2013) reported that SPIDES could reduce embryonic cell damage and improve hatchability.

In contrast, the lower hatchability observed in the control treatment (T1), accompanied by a corresponding increase in

total embryonic mortality, may be associated with prolonged egg storage without pre-incubation. Previous studies have suggested that prolonged storage may adversely affect blastoderm viability, embryonic metabolic activity, eggshell permeability, and water loss (Yassin et al., 2008; Gómez-de-Travecedo et al., 2014). Likewise, reduced hatchability in T4, accompanied by a corresponding increase in embryonic mortality, may relate to embryos advancing toward the more storage-sensitive primitive streak stage following the 4-h SPIDES treatment. This increased sensitivity at this stage has been associated with higher metabolic activity. At the same time, depletion of glycogen reserves and ATP has been proposed as a possible factor contributing to reduced tolerance of embryos to storage stress (Reijrink, 2010). However, the present study did not evaluate embryonic developmental stage, metabolic activity, glycogen reserves, or ATP levels; therefore, these mechanisms should be considered possible explanations rather than demonstrated effects.

Table 2. Effect of pre-incubation (SPIDES) of Japanese quail hatching eggs on apparent fertility, hatchability, and embryonic mortality (Mean ± SE).

Treatment	Mean ± SE (actual counts in parentheses)					
	Apparent fertility (%)	Hatchability of fertile eggs (%)	Total embryonic mortality (%)	Early embryonic mortality (%)	Mid-embryonic mortality (%)	Late embryonic mortality (%)
T1	88.67 ± 0.66 (133/150)	57.13 ± 2.64 (76/133) ab	42.85 ± 2.64 (57/133) bc	19.54 ± 0.69 (26/133) b	12.79 ± 1.56 (17/133)	10.52 ± 0.72 (14/133)
T2	90.67 ± 2.40 (136/150)	53.04 ± 2.98 (72/136) bc	46.94 ± 2.98 (64/136) ab	23.55 ± 2.63 (32/136) ab	13.84 ± 2.33 (19/136)	9.54 ± 0.57 (13/136)
T3	88.67 ± 1.33 (133/150)	64.73 ± 2.51 (86/133) a	35.25 ± 2.51 (47/133) c	8.98 ± 1.19 (12/133) c	13.50 ± 1.14 (18/133)	12.76 ± 1.40 (17/133)
T4	88.00 ± 3.05 (132/150)	47.09 ± 2.08 (62/132) c	52.89 ± 2.08 (70/132) a	25.04 ± 1.43 (33/132) a	14.33 ± 1.73 (19/132)	13.50 ± 1.88 (18/132)
Level of Sig.	NS	**	**	**	NS	NS

Means with different letters within the same column differ significantly. * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$). Treatments: T1 = Control (0 h); T2 = SPIDES (2 h); T3 = SPIDES (3 h); and T4 = SPIDES (4 h). SPIDES was applied on storage days 4 and 8. Exact treatment *P*-values from replicate-level one-way ANOVA: apparent fertility $P = 0.8194$; hatchability $P = 0.0078$; total embryonic mortality $P = 0.0078$; early embryonic mortality $P = 0.0005$; mid-embryonic mortality $P = 0.9343$; late embryonic mortality $P = 0.1681$.

3.2 Effect of the SPIDES technique on chick weight and length, incubation duration, and hatch window of Japanese quail hatching eggs.

Table 3 presents the effects of pre-incubation (SPIDES) on chick weight and length at hatch, as well as incubation duration and hatch window. All studied traits were significantly affected ($p \leq 0.01$).

Pre-incubation treatments significantly influenced chick weight at hatch. The control treatment (T1) recorded the highest mean value (8.20 g), which did not differ significantly from T2 (8.19 g), whereas chick weight significantly decreased in T3 and T4 treatments, reaching 8.09 and 8.11 g, respectively. These findings are consistent with those reported by Elkhaiat et al. (2024) in Fayoumi and Avian-34 chickens, who found the highest chick weight at hatch in eggs not exposed to SPIDES (Elkhaiat et al., 2024), whereas pre-incubation application reduced chick weight compared with the control treatment. However, these results disagree with those of Reijrink (2010), who reported no significant effect of the interaction between pre-incubation (6 and 9 hours) and egg storage duration (3–12 days) on the relative weight of hatched broiler chicks (Reijrink, 2010). The lower chick weight in T3 may be attributed to earlier hatching than in the other treatments, resulting in a longer holding period in the incubator and increased utilization of yolk reserves due to accelerated embryonic development and elevated metabolic activity (Biesek et al., 2026). In T4, the reduced chick weight may be attributed to increased water loss from the egg as a consequence of the prolonged pre-incubation duration, since chick weight at hatch is closely associated with egg weight (Oliveira et al., 2023).

Regarding incubation duration, treatment T3 had the shortest incubation period (403.33 h), whereas treatment T4 had the longest (414.67 h), compared with treatments T1 and T2, which recorded 410.33 and 411.67 h, respectively. These findings suggest that applying the SPIDES technique for 3 h twice during the egg storage period reduced the total incubation duration by approximately 1.7% compared with the control treatment (T1). This result is consistent with

Biesek et al. (2026), who reported that applying the SPIDES program to Grey Partridge eggs stored for 15 days reduced total incubation duration (Biesek et al., 2026), advanced hatch time, and improved hatch synchrony compared with the control group. This effect may be explained by the SPIDES technique's ability to preserve embryonic viability by stimulating blastoderm development and reducing embryonic cell death during egg storage, allowing embryonic development to resume more rapidly and uniformly after the onset of incubation and ultimately shortening the total incubation duration (Duncan, 1955; Dymond et al., 2013).

For the hatch window, treatment T3 recorded the shortest hatch window (24.38 h), compared with treatments T1, T2, and T4, which recorded 26.37, 26.40, and 28.21 h, respectively. This finding is consistent with the results reported by Wlaźlak et al. (2026) in Common pheasant hatching eggs, where the application of the SPIDES technique improved hatch synchrony and reduced hatch dispersion (Wlaźlak et al., 2026). In contrast, the present findings partially differed from those of Damaziak et al. (2021a), who demonstrated that the effect of the SPIDES technique on the hatch window depends on breeder flock age and the type of the pre-incubation program (Damaziak et al., 2021). Specifically, the conventional SPIDES program did not affect the hatch window of eggs produced by older breeder flocks but prolonged it in eggs from younger breeder flocks. Likewise, the gradual pre-incubation program (EG SPIDES) increased the hatch window in eggs from younger breeder flocks, while having no significant effect on eggs from older breeder flocks. The shorter hatch window observed in treatment T3 may be attributed to applying the SPIDES technique for 3 h twice during the egg storage period, which reduced variation in embryonic developmental stages among embryos and allowed embryonic development to resume more synchronously at the onset of incubation. Consequently, hatch timing became more synchronized, resulting in a narrower hatch window than in the other treatments. Conversely, the absence of SPIDES treatment or its application for an unsuitable duration may have increased variation in embryonic development rates among embryos, thereby widening the hatch window (Nicholson et al., 2013; Taha et al., 2019).

Table 3. Effect of different SPIDES durations on chick weight, chick length, total incubation duration, and hatch window of Japanese quail hatching eggs (Mean±SE).

Treatment	Mean±SE			
	Chick weight (g)	Chick length (cm)	Time of incubation (hours)	Hatch window (hours)
T1	8.20±0.01^{a}	11.12±0.01^{b}	410.33±0.33^{b}	26.37±0.07^{a}
T2	8.19± 0.01^{a}	11.12±0.01^{b}	411.67±0.88^{ab}	26.40±0.08^{a}
T3	8.09±0.01^{b}	11.19±0.01^{a}	403.33±1.67^{c}	24.38±0.07^{b}
T4	8.11±0.01^{b}	11.10±0.01^{b}	414.67±0.33^{a}	28.21±0.06^{a}
Level of Sig.	**	**	**	**

Means with heterogeneous letters within the same column differ significantly. **=Highly significant (p≤0.01). Treatments: T1=Control (0 h); T2=SPIDES (2 h); T3=SPIDES (3 h); and T4=SPIDES (4 h). SPIDES was applied on storage days 4 and 8.

3.3 Effect of the SPIDES technique on subsequent productive performance of Japanese Quail chicks

Table 4 shows the effects of short periods of incubation during egg storage (SPIDES) on the productive performance traits of Japanese quail chicks, including final body weight, feed intake, body weight gain, feed conversion ratio, and total mortality rate. Treatment T3 exhibited significant superiority (p≤ 0.01) in most productive performance traits, recording the highest final body weight of 187.33 g. Treatment T3 also achieved the highest body weight gain (179.24 g), the lowest feed intake (438.33 g/bird), and the best feed conversion ratio (2.44) compared with all other treatments. In contrast, treatment T4 recorded the lowest final body weight (152.33 g), the lowest body weight gain (144.22 g), and the poorest feed conversion ratio (3.23), while feed intake reached 466.67 g/bird. No significant differences (p>0.05) were observed between treatments T1 and T2 for any productive performance trait, as their values were comparable. Likewise, the total mortality rate was not significantly affected by the SPIDES treatments (p>0.05), despite ranging from 4.44% to 6.66%. The findings of the present study were partially consistent with those reported by Elkhaiat et al. (2024), suggesting that improvements in chick productive performance depend on the SPIDES protocol applied during egg storage (Elkhaiat et al., 2024).

In the present study, treatment T3 achieved the best results in final body weight, body weight gain, and feed conversion ratio. In contrast, the referenced study reported the best productive performance when SPIDES was applied for 5 h every 3 days during egg storage. The findings of the present study were also consistent with those reported by Abdel-Fattah et al. (2024), who found that exposing broiler breeder eggs stored for 20 days to 37.8°C for 5 h on days 5, 10, and 15 of the storage period, in combination with embryonic thermal conditioning at 39.5°C for 6 h daily from days 14 to 18 of incubation, improved the subsequent productive performance of the chicks (Abdel-Fattah et al., 2024). In contrast, the findings of the present study differed from those reported by Oliveira et al. (2023), who observed no significant effect of applying short periods of incubation during egg storage (SPIDES) for 4 h at 37.5°C on final body weight, body weight gain, feed intake, or feed conversion ratio of Japanese quail chicks up to 35 days of age (Oliveira et al., 2023). In contrast, treatment T3 in the present study showed significantly superior performance in all of these traits compared with the other experimental treatments. This discrepancy may be attributed to differences in the SPIDES protocol between the two studies. In the previous study, SPIDES was applied once for 4 h on the third day of egg storage. In the present study, we evaluated different SPIDES protocols and observed improved productive performance only in treatment T3. These findings suggest that the effectiveness of SPIDES depends on its application protocol, particularly its duration and timing during egg storage. The superior productive performance of treatment T3 may also be associated with the greater chick length at hatch, which has been positively associated with gastrointestinal development and subsequent productive performance (El Sabry & Yalcin, 2023; Molenaar et al., 2008). This interpretation is consistent with Abdel-Fattah et al. (2024), who reported improved subsequent productive performance in chicks after applying an appropriate SPIDES protocol (Abdel-Fattah et al., 2024). Furthermore, the total mortality rate was not significantly affected by the SPIDES treatments (p>0.05). A limitation of the post-hatch evaluation is that productive performance was assessed in selected healthy chicks rather than in all chicks produced by each treatment; therefore, these findings should be interpreted as the performance of chicks considered suitable for rearing.

Table 4. Effects of short periods of incubation during egg storage (SPIDES) on productive performance traits of Japanese quail chicks at 35 days of age (Mean±SE).

Treatment	Mean±SE				
	Final body weight (g)	Feed intake (g/bird)	Body weight gain (g)	Feed conversion ratio (g/g)	Total mortality (% , number)
T1	166.33±0.33^{b}	465.00±2.89^{a}	158.13±0.34^{b}	2.93±0.01^{b}	4.44±2.22 (2/45)
T2	164.33±1.20^{b}	468.33±1.67^{a}	156.14±1.20^{b}	2.99±0.03^{b}	6.66±3.85 (3/45)
T3	187.33±1.45^{a}	438.33±1.67^{b}	179.24±1.45^{a}	2.44±0.01^{c}	4.44±2.22 (2/45)
T4	152.33±1.20^{c}	466.67±1.67^{a}	144.22±1.20^{c}	3.23±0.02^{a}	6.66±3.85 (3/45)
Level of Sig.	**	**	**	**	NS

Means with heterogenous letters within the same column differ significantly. **=Highly significant (p≤0.01). Treatments: T1=Control (0 h); T2=SPIDES (2 h); T3=SPIDES (3 h); and T4=SPIDES (4 h). SPIDES was applied on storage days 4 and 8.

4 Conclusion

The results indicated that applying the short periods of incubation during egg storage (SPIDES) technique to Japanese quail hatching eggs stored for 10 days influenced hatchability traits, chick quality characteristics, and subsequent productive performance, depending on the duration of SPIDES application. The 3-hour SPIDES treatment (T3) produced the highest hatchability of fertile eggs, significantly higher than T2 and T4 but not significantly different from the control treatment (T1). T3 also showed lower total and early embryonic mortality and improved final body weight, body weight gain, feed conversion ratio, and feed intake. In contrast, the 2-hour SPIDES treatment (T2) showed no significant negative effects on the studied traits; however, it did not achieve improvements comparable to those observed in T3. Extending the SPIDES duration to 4 hours (T4) reduced hatchability traits and subsequent productive performance in the chicks. The SPIDES technique did not significantly affect apparent fertility percentage, mid- or late-embryonic mortality, or total chick mortality. Under the conditions of the present study, the 3-hour SPIDES treatment applied twice on days 4 and 8 during the 10-day egg storage period produced the most favorable overall results among the durations tested, suggesting its potential usefulness for

improving hatchability and subsequent productive performance of Japanese quail chicks.

Use of Artificial Intelligence

ChatGPT (OpenAI) was used solely for language editing and to improve the manuscript's clarity and readability. The authors take full responsibility for the content, scientific accuracy, and interpretation of the results.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Authors equally contributed to this study.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

All experimental procedures involving animals were conducted in accordance with internationally recognized ethical guidelines for the care and use of animals in research and were approved by the Animal Ethics Committee of the College of Agriculture, University of Al-Qadisiyah, Iraq, on 10 February 2026 (Approval No. 15).

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