International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 174-177
www.biochemjournal.com
Received: 04-08-2025
Accepted: 07-09-2025

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Impact of short periods of incubation during egg storage on hatchability and chick quality of long-term stored Japanese quail hatching eggs

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10c.5939

Abstract
This research examined how applying Short Periods of Incubation During Egg Storage (SPIDES) influences the embryonic mortality, hatchability, and chick quality of Japanese quail eggs subjected to long-term storage. A total of 360 eggs collected from 26-week-old breeder quails were allocated into four experimental groups: T_1 (stored for 21 days without SPIDES, incubated on day 22), T_2 (stored for 3 days without SPIDES, incubated on day 4), T_3 (stored for 21 days with two SPIDES treatments, incubated on day 22), and T_4 (stored for 21 days with three SPIDES treatments, incubated on day 22). All eggs were kept under 17 °C and 75% relative humidity, while SPIDES-treated groups were intermittently warmed to 37.7 °C for 3 hours at 5-day intervals. The findings revealed significant treatment effects on parameters such as storage weight loss, moisture loss, hatchability (of both total and fertile eggs), chick quality, and embryonic mortality at early and mid-stages, as well as dead-in-shell rates. The T_2 group achieved the highest hatchability and lowest embryonic mortality, whereas T_3 and T_4 also showed notable improvements in hatchability and chick quality compared with T_1. Conversely, T_1 exhibited a markedly ($p<0.01$) higher rate of early and mid-embryonic mortality than the other groups. Overall, SPIDES treatment successfully alleviated the adverse effects of prolonged egg storage, indicating its potential to enhance hatchability and chick viability in Japanese quail eggs. In summary, exposing eggs to 3-hour SPIDES cycles every 5 days during a 21-day storage period improved hatchability and chick quality while lowering embryonic mortality.

Keywords: SPIDES, Japanese quail eggs, hatchability, chick quality

1. Introduction

The global quail meat market was valued at approximately USD 11.71 billion in 2023 and is projected to grow from USD 12.76 billion in 2024 to USD 25.4 billion by 2032, reflecting a compound annual growth rate (CAGR) of about 8.98% during the forecast period (2024-2032) (Wise Guy Reports, 2024) [20]. Similarly, the global quail egg market was estimated at USD 1,782.9 million in 2021 and is expected to reach USD 2,365.6 million by 2028, registering a CAGR of 4.1% (Business Research Insights, 2024) [3].

In commercial hatcheries, hatching eggs are often stored for more than seven days, especially under conditions such as market demand fluctuations, disease outbreaks, or the need to synchronize incubation for pedigreed breeding programs. However, extended storage durations negatively affect egg quality, leading to reduced hatchability (Fasenko*et al*., 2001) [11], delayed hatching times (Dymond *et al*., 2013) [6] and lower chick quality (Fasenko *et al*., 2001; Tona *et al*., 2003) [11, 19]. Prolonged storage can also result in higher early and late embryonic mortality rates (Elibol *et al*., 2003; Tona *et al*., 2004) [8, 19].

To counter these effects, researchers have developed a technique known as Short Periods of Incubation During Egg Storage (SPIDES). This method involves intermittently warming stored hatching eggs, which helps stimulate embryonic development and maintain viability during long-term storage. After each warming phase, the eggs are returned to cool storage to retain freshness and improve hatching success. The timing and frequency of SPIDES application are critical for achieving optimal results. Studies suggest that the best outcomes occur when eggs are warmed weekly, with a cumulative pre-incubation time not exceeding 15 hours at temperatures above 32 °C (Nicholson *et al*., 2013) [14]. Research has demonstrated that SPIDES can enhance hatchability (Fasenko*et al*., 2001; Dymond *et al*., 2013; Reijrink *et al*., 2010 [12]) [11, 6, 12], improve chick quality (El-Menawewy, 2019; Ebeid *et al*., 2017) [10, 7], shorten hatching time (Dymond *et al*., 2013) [6], and reduce early embryonic mortality (Dhotre *et al*., 2023; Abdel-Halim *et al*., 2015) [4, 1].

Therefore, the present study aimed to evaluate the effect of SPIDES treatment on Japanese quail eggs stored for extended periods, focusing on embryonic mortality, hatchability, and chick quality.

2. Materials and Methods

A total of 360 hatching eggs obtained from 26-week-old Japanese quail breeders were utilized to evaluate the effect of Short Periods of Incubation During Egg Storage (SPIDES) on hatchability and chick quality. The eggs were randomly assigned to four treatment groups of 90 eggs each, as follows: T_1-eggs stored for 21 days without SPIDES and incubated on day 22; T_2-eggs stored for 3 days without SPIDES and incubated on day 4; T_3-eggs stored for 21 days with two SPIDES treatments and incubated on day 22; and T_4-eggs stored for 21 days with three SPIDES treatments and incubated on day 22. Each treatment was further divided into three replicates of 30 eggs. During storage, all eggs were maintained at 17 °C and 75% relative humidity, and those in the SPIDES groups were periodically warmed and rotated every five days. For each SPIDES treatment, the eggs were exposed to 37.7 °C and 55% relative humidity for 3 hours, with hourly turning at a 45° angle in both directions. Following the storage period, eggs were transferred to a forced-draft incubator maintained at 99.3°F and 56% relative humidity from day 1 to day 17. On day 15, eggs were weighed and moved to the hatcher, which was set at 98.9°F and 76% relative humidity. Standard temperature and humidity were maintained throughout the 17-day incubation period, and chicks were removed from the hatcher once 95% had dried. Data on storage weight loss, moisture loss, hatchability, and chick quality were recorded throughout the experiment.

The data collected on various parameters were subjected to statistical analysis in Completely Randomized Design (CRD) as per the methods suggested by Snedecor and Cochran (1989) [18] and the means of different treatment groups were tested for statistical significance by Duncan's multiple range test (Duncan, 1955) [5].

3. Results and Discussion

3.1 Storage and moisture loss

Table 1 illustrates the impact of short incubation periods during egg storage on both storage and moisture loss percentages. The findings revealed a highly significant difference (p<0.01) in storage loss across treatment groups during the first, second, and third weeks. In the first week, no significant variation was found between the SPIDES-treated groups (T_3 and T_4). However, eggs stored for three days without SPIDES (T_2) showed a significant (p<0.01) difference compared to eggs stored for 21 days with SPIDES (T_3 and T_4) and those stored for 21 days without SPIDES (Control). During the second and third weeks, storage loss differed significantly (p<0.01) between the SPIDES-treated (T_3 and T_4) and non-SPIDES (Control) groups, though no significant difference was detected between T_3 and T_4. Eggs stored for only three days without SPIDES exhibited significantly (p<0.01) lower moisture loss than those stored for 21 days, regardless of SPIDES treatment. No significant difference was found between T_1 and T_3, whereas T_4 showed significantly (p<0.01) higher moisture loss (15.17±0.50%) compared with other treatment groups.

The present result is in accordance with Abdel-Halim *et al*., (2015) [1], Ebeid *et al*., (2017) [7], Fasenko*et al*., (2001) [11] as they found that eggs storage loss increased with longer storage period and SPIDES treatment over the non-SPIDES treatment. Gharib *et al*. (2013) [12], Elkhaiat*et al*. (2024) [9] and Silva *et al*., (2008) [17] also stated that longer storage period and SPIDES treatment increase the both storage and moisture loss. However, Abdel-Halim *et al*., (2015) [1], Ebeid *et al*., (2017) [7] and Fasenko *et al*., (2001) [11] were not in accordance with this present result and theyfound no significant difference in moisture loss with longer storage period and SPIDES treatment. The findings of Dhotre *et al*., (2023) [4] and Ansah *et al*., (2023) [2] resulted that SPIDES treatment did not significantly impact on both storage and moisture loss.

The Short period of incubation during egg storage on 21 days of stored eggs have recorded higher storage and moisture loss percent than non-SPIDES (control). These shows prolonged storage and heat treatment increase the water evaporation from the eggs.

Table 1: Illustrates the impact of short incubation periods during egg storage on both storage and moisture loss percentages.

Treatment	Egg weight (g)	Storage loss (%)			Moisture loss (%)
		1st week	2nd week	3rd week	
T_1 (Eggs stored for 21 days without SPIDES)	11.53±0.04	1.34^{b}±0.12	3.65^{a}±0.24	4.81^{a}±0.14	12.53^{b}±0.38
T_2 (Eggs stored for 3 days without SPIDES)	11.49±0.02	0.60^{a}±0.00	-	-	7.10^{a}±0.66
T_3 (Eggs stored 21 days with 2 SPIDES)	11.50±0.03	2.50^{c}±0.12	4.43^{b}±0.12	5.97^{b}±0.25	12.90^{b}±0.42
T_4 (Eggs stored for 21 days with 3 SPIDES)	11.46±0.02	2.51^{c}±0.12	4.42^{b}±0.24	6.56^{b}±0.33	15.17^{c}±0.50

Mean within a column bearing different superscripts differ significantly (p<0.01)

3.2 Hatchability

Table 2 presents the effect of short incubation periods during egg storage on hatchability percentage. The results indicated a highly significant difference (p<0.01) in hatchability, both based on total eggs set and fertile eggs set, among the treatment groups. Hatchability percentages for total and fertile egg sets were highest in T_2, followed by T_4 and T_3. In contrast, T_1 exhibited significantly (p<0.01) lower hatchability for both total and fertile egg sets compared to the other treatment groups.

Some of the authors namely Abdel-Halim *et al*., (2015) [1], Ebeid *et al*., (2017) [7], Gharib (2013) [12], Okasha *et al*., (2023) [15] and Reijrink *et al*., (2010) [16] were agreed with our results and they concluded that egg stored for longer days and exposed with SPIDES had higher hatchability of total egg set and fertile egg set than non-SPIDES treatment.

Table 2: Presents the effect of short incubation periods during egg storage on hatchability percentage.

Treatment	Hatchability of set eggs (%)	Hatchability of fertile eggs (%)
T_1 (Eggs stored for 21 days without SPIDES)	$38.88^{d}\pm0.70$	$40.22^{d}\pm0.72$
T_2 (Eggs stored for 3 days without SPIDES)	$71.11^{a}\pm0.70$	$74.42^{a}\pm0.65$
T_3 (Eggs stored 21 days with 2 SPIDES)	$56.66^{c}\pm1.21$	$59.97^{c}\pm0.92$
T_4 (Eggs stored for 21 days with 3 SPIDES)	$61.11^{b}\pm0.70$	$66.34^{b}\pm0.93$

Mean within a column bearing different superscripts differ significantly ($p<0.01$)

3.3 Chick quality

The effect of short period of incubation during egg storage on chick quality percent was presented in Table 3. The result showed highly significant ($p<0.01$) difference between treatments in chick weight (g), chick yield (%), chick length (cm) and healthy chicks (%). There was no significant difference noticed between SPIDES groups (T_3 and T_4) and 3 days stored egg without SPIDES group (T_2). All the treatment groups (T_2, $T_3$and T_4)had significantly higher chick weight (g), chick yield (%), chick length(cm), healthy chicks (%) than control group (T_1) and hadsignificant ($p<0.05$) difference between treatments in weak chicks percent. The T_4, T_3 and T_2had no significant difference between the treatments but T_1 had significantly higher weak chicks percent than the other treatment groups. There was no significant (P>0.05) difference observed in chick mortality during incubation(%).The numerical difference was noticed between SPIDES groups (T_4 and T_3) 2.22% and 1.11% respectively and other treatment groups (T_1 and T_2)0% each.

Ebeid *et al*., (2017) [7], Elmenawey (2019) [10] and Gharib *et al*., (2013) [12] were in accordance with our results, as they found chicks hatched from SPIDES treatment had a highest chick weight compared to the non-heated eggs. The findings of Elkhaiat *et al*., (2024) [9] were not agreed with our result because SPIDES treatment had the lowest chick weight than non-SPIDES.

Inagreement with our result, Okasha *et al*., (2023) [15] reported that chicks from SPIDES treatment had higher chick yield percent than non-SPIDES treatment. Dhotre*et al*. (2023) [4] and Gharib (2013) [12] findings are in accordance with our result and they found SPIDES had a significantly higher chick length than non-SPIDES treatment. Elmenawey (2019) [10] and Gharib *et al*., (2013) [12] they findings agreed with our results and found that egg stored for more than 4 days had a lowest percentage of healthy chicks and those chicks from SPIDES treatment for long term stored eggs had higher percentage of healthy chicks than non-SPIDES treatment.

Table 3: The effect of short period of incubation during egg storage on chick quality percent was presented

Treatment	Chick weight (g)	Chick yield (%)	Chick length (cm)	Weak chicks (%)	Healthy chicks (%)	Chick mortality (%)
T_1 (Eggs stored for 21 days without SPIDES)	$7.90^{b}\pm0.12$	$68.56^{b}\pm1.10$	$11.05^{b}\pm0.02$	$3.33^{b}\pm1.21$	$28.88^{d}\pm0.70$	0.00 ± 0.00
T_2 (Eggs stored for 3 days without SPIDES)	$8.42^{a}\pm0.02$	$73.30^{a}\pm0.03$	$11.32^{a}\pm0.05$	$2.22^{ab}\pm0.70$	$61.11^{a}\pm1.40$	0.00 ± 0.00
T_3 (Eggs stored 21 days with 2 SPIDES)	$8.19^{a}\pm0.01$	$71.24^{a}\pm0.24$	$11.20^{a}\pm0.00$	$1.11^{ab}\pm0.70$	$42.22^{c}\pm1.40$	1.11 ± 0.70
T_4 (Eggs stored for 21 days with 3 SPIDES)	$8.20^{a}\pm0.00$	$71.51^{a}\pm0.18$	$11.20^{a}\pm0.01$	$0.00^{a}\pm0.00$	$52.22^{b}\pm0.70$	2.22 ± 1.40
Significance	**	**	**	*	**	NS

Mean within a column bearing different superscripts differ significantly NS-Non significant, *-significant ($p<0.05$), **-Highly significant ($p<0.01$)

3.4 Break open study

The effect of short period of incubation during egg storage on embryonic mortality is depicted Table 4. The result showed highly significant ($p<0.01$) difference between treatments in early embryonic mortality, mid embryonic mortality and dead in shell percent. The early embryonic mortality percent is significantly lower in T_2 followed by T_4 than T_3 and T_1 and there was no significant difference noticed between SPIDES treatment groups but significant difference was observed between SPIDES groups, T_2 and T_3. T_2, $T_3$and T_4 had no significant difference in mid embryonic mortality but T_1 had significantly higher mid embryonic mortality (14.44±0.70%). T_4 had significantly lower dead in shell than other treatment groups. pipped and live-in shell percent between treatments. There was no significant ($p>0.05$) difference in late embryonic mortality, Live, Pipped percent between treatment. Numerically T_2, T_3 and T_4 had lowest late embryonic mortality than control group.

The result of Abdel-Halim *et al*. (2015) [1], Ebeid *et al*. (2017) [7] and Gharib (2013) [12] were agreed with our results because they found SPIDES treatment reduce early and mid-embryonic mortality in egg stored for longer days than non-SPIDES treatment. The result disagreement with Dhotre *et al*. (2023) [4] and Dymond *et al*. (2013) [5] who found that egg stored for longer days with SPIDES treatment had significantly ($p\leq0.05$) lower late embryonic mortality. The result was in accordance with Gucbimez *et al*. (2013) [13] stated that eggs stored for longer day with SPIDES treatment had numerical decrease in late embryonic mortality compare to control groups.

The short periods of incubation during egg storage might be increased the stage of embryonic development, increased metabolic rate and higher weight of embryos during incubation which are resulted in lowering the embryonic mortality, increased hatchability and improved chick quality.

Table 4: The effect of short period of incubation during egg storage on embryonic mortality is depicted

Treatment	Infertile (%)	Early embryonic mortality (%)	Mid embryonic mortality (%)	Late embryonic mortality (%)	Dead in shell (%)	Live (%)	Pipped (%)
T_1 (Eggs stored for 21 days without SPIDES)	$3.33^{a}\pm0.00$	$24.44^{c}\pm1.40$	$14.44^{b}\pm0.70$	8.88±0.70	$8.88^{c}\pm0.70$	0.00±0.00	0.00±0.00
T_2 (Eggs stored for 3 days without SPIDES)	$4.44^{a}\pm0.70$	$5.55^{a}\pm0.70$	$6.66^{a}\pm0.00$	6.66±0.00	$3.33^{ab}\pm1.21$	1.11±0.70	0.00±0.00
T_3 (Eggs stored 21 days with 2 SPIDES)	$5.55^{ab}\pm0.70$	$15.55^{b}\pm0.70$	$8.88^{a}\pm0.70$	6.66±1.21	$6.66^{bc}\pm1.21$	0.00±0.00	0.00±0.00
T_4 (Eggs stored for 21 days with 3 SPIDES)	$7.77^{b}\pm1.85$	$12.22^{b}\pm0.70$	$8.88^{a}\pm1.40$	6.66±1.21	$0.00^{a}\pm0.00$	0.00±0.00	1.11±0.70
SIGNIFICANCE	*	**	**	NS	**	NS	NS

Mean within a column bearing different superscripts differ significantly NS-Non significant, *-significant ($p<0.05$), **-Highly significant ($p<0.01$)

4. Conclusion

In conclusion, this study showed that applying 3 hours every 5 days during storage at 37.7 °C over a 21 days egg storage period was an effective method for reducing embryonic mortality, increasing hatchability percentages, and improving chick quality. This method helps to minimize the harmful effects of long-term storage of hatching eggs from Japanese quail.

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