

# Evaluating The Impact of Prolonged Egg Storage on Hatchability, Moisture Loss and Quality Parameters in Japanese Quails

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**Abstract.** Storing hatching eggs is a common practice in the poultry industry, but data on the effects of storage under tropical ambient temperatures are limited. This study evaluated the impact of prolonged egg storage on the hatchability, moisture loss, incubation period, and embryonic mortality of Japanese quail (*Coturnix japonica*) eggs, alongside their effects on hatchling weight. The experiment was carried out at the Quail Rearing Facility and Hatchery Production Site at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan. A total of 495 Japanese quail eggs were divided into 11 storage groups (0 to 10 consecutive days), with three replicates of 15 eggs in each experimental group. The eggs were stored at room temperature ( $28 \pm 1$  °C, 60–65% relative humidity) before incubation under controlled conditions at 37.5 °C and 60% relative humidity. The results indicated that hatchability significantly decreased with increasing storage period ( $p < 0.05$ ), from a peak of 82.22% in eggs stored for two days to only 4.44% in eggs stored for 10 days. The total moisture loss increased significantly with increasing storage period ( $p < 0.05$ ), with notable differences between the storage and incubation phases. However, only storage moisture loss significantly differed across all the storage periods ( $p < 0.05$ ). The incubation moisture loss did not significantly differ across the different storage periods ( $p > 0.05$ ). Prolonged storage also delayed the incubation period ( $p < 0.05$ ) and increased embryonic mortality rates ( $p < 0.05$ ). However, both hatchling weight and relative chick weight did not differ significantly across all the storage periods ( $p > 0.05$ ). These findings demonstrate that storing Japanese quail eggs at tropical ambient temperatures for up to seven days resulted in a progressive decrease in the hatchability of the eggs with excessive moisture loss. Hence, for producers without access to refrigeration, storing eggs for no more than three days is recommended to maintain economically viable hatch rates.

**Keywords:** embryonic mortality, hatchability, japanese quail, moisture loss, storage period

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## 1 Introduction

The Japanese quail (*Coturnix japonica*) industry has become an important and expanding component of poultry production in tropical regions such as Malaysia, because of its rapid growth rate, high reproductive potential, efficient feed conversion, and minimal space requirements [1]. The success of quail farming, particularly for small and medium-scale producers, is heavily dependent on maximizing the hatchability of eggs, which is a key economic driver in the poultry sector. Furthermore, quail eggs are gaining popularity among consumers because of their rich nutritional profile, which includes high-quality proteins, vitamins, and minerals, as well as their hypoallergenic properties [2].

A standard and necessary practice within the poultry industry involves collecting and storing of hatching eggs for a period to accumulate a sufficient number to fill an incubator [3], which ensures a more synchronized hatch, simplifies management, and optimizes operational efficiency. The duration of this storage period, the time between egg laying and the start of incubation, is a critical management factor that profoundly influences embryonic viability.

Extensive research has firmly established that prolonged egg storage negatively impacts hatchability by causing detrimental changes within the internal egg environment. As the storage time increased, the thick albumen degraded and thinned, whereas carbon dioxide loss through the shell pores caused a sharp increase in the albumen pH [4-5]. Concurrently, the vitelline membrane that encases the yolk weakens, compromising the structural integrity necessary for embryonic development [6]. These cumulative degradations create a suboptimal, and eventually hostile, environment for the blastoderm, leading to well-documented consequences such as increased rates of early and late embryonic mortality, a widened and less predictable hatch window, and reduced post-hatch chick quality.

However, many existing studies on egg storage are based on studies conducted in temperate climates, where eggs are typically stored under controlled refrigeration at temperatures between 10 °C and 15 °C. These conditions are designed to keep the egg's internal temperature below the "physiological zero" the threshold, generally considered to be between 20 °C and 24 °C, at which embryonic development is effectively arrested [7]. In many tropical countries, small-scale producers often lack access to refrigeration and store eggs at ambient temperatures, which can be significantly greater. There is a critical knowledge gap regarding the effects of storing eggs under these tropical ambient conditions (e.g., 28 °C), which are well above the physiological zero and may induce abnormal embryo development rather than simply arresting it.

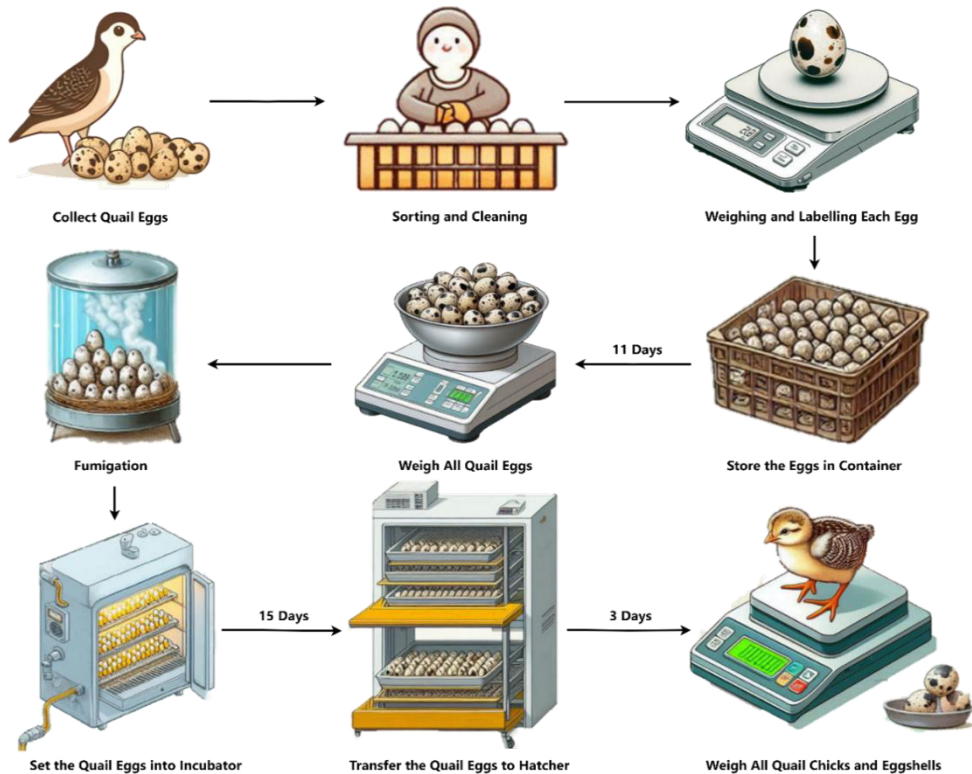
Therefore, this study was conducted to evaluate the impact of consecutive daily storage periods (0 to 10 days) at a tropical ambient temperature on the hatchability, moisture loss, incubation period, embryonic mortality, and hatchling weight of Japanese quail eggs.

## 2 Materials and methods

### 2.1 Experiment location

This experiment was conducted at the Quail Rearing Facility, for the collection of Japanese quail eggs, and at the Hatchery Production Site, for the incubation of Japanese quail eggs. Both facilities are located at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan.

## 2.2 Experimental procedure



**Fig. 1.** A comprehensive flowchart of the experimental procedure.

According to Figure 1, the experiment began with the collection of a total of 45 Japanese quail eggs per day. The collected eggs were then sorted and cleaned after which the defecated eggs were removed. Each egg was subsequently weighed and labelled for identification before being stored in a container for up to 11 days. After the designated storage period, all eggs were reweighed according to their experimental groups to obtain the egg weight before incubation. Then, the eggs were fumigated for 20 minutes and set into an incubator at 37.5 °C and 60% relative humidity. After 15 days in the incubator, the eggs were transferred from the setter to the hatcher at the same temperature and relative humidity, where they remained for three days until the chicks hatched. Finally, the newly hatched Japanese quail chicks and their eggshells were weighed to assess incubation success.

## 2.3 Experimental design, birds, and management

A breeder flock of 150 Japanese quail (*Coturnix japonica*), approximately 17 months old, was used for egg collection. The birds were housed in experimental cages in an open-house system with a male-to-female ratio of 1:3. They were provided with commercial layer pellet feed (17% crude protein) and water *ad libitum*.

A total of 495 freshly laid eggs were collected over 11 consecutive days (45 eggs per day). The experiment followed a complete randomized design (CRD). The eggs were divided into 11 treatment groups corresponding to storage periods of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Each treatment group consisted of 45 eggs, which were divided into three replicates of 15 eggs each.

## 2.4 Egg collection, storage, and incubation

On each of the 11 days, 45 Japanese quail eggs were collected. Eggs with defects such as cracks or excessive dirt were excluded (as shown in Figure 2). Each selected egg was individually weighed and labelled with its treatment group and replicate number by using a permanent marker. The eggs were then stored in containers within the facility at an ambient room temperature of  $28 \pm 1$  °C and a relative humidity of 60–65% after the daily egg collection.



**Fig. 2.** Eggs that should be collected is (a) normal; Eggs with defects that should be removed from collection are (b) small; (c) large; (d) cracked; (e) blood stained; (f) dirty; (g) rounded; and (h) elongated.

At the end of the 11-day collection period, all 495 eggs were reweighed. The eggs were then fumigated with formaldehyde gas that was produced from a 1:2 ratio of potassium permanganate and formalin, for 20 minutes in a closed chamber. Following fumigation, all eggs were set simultaneously in an incubator (Ecobator 1320s model) at a temperature of 37.5 °C and 60% relative humidity. The eggs were turned automatically every 90 minutes at a turning angle of  $\pm 45^\circ$  to either side in a vertical position. On the 15th day of incubation, turning was stopped, and the eggs were transferred to hatcher trays within the same incubator, where they remained until hatching. In order to clearly identify which quail chicks hatched from each experimental group, the eggs were sorted in separate partitions within the hatcher trays during the transfer.

## 2.5 Data collection

The following parameters were measured:

- **Hatchability:** Hatchability was calculated as the percentage of total number of eggs that successfully hatched in each treatment group [8].

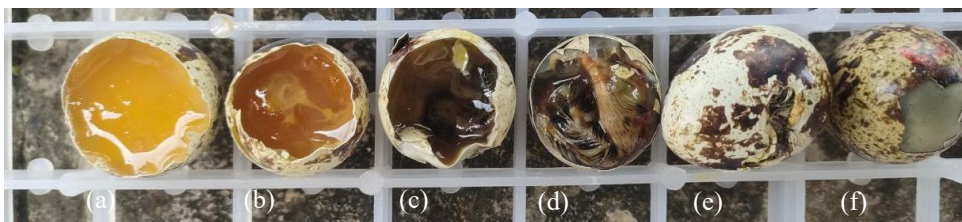
$$\text{Hatchability of incubated eggs (\%)} = \frac{\text{Total number of quail chicks hatched}}{\text{Total number of quail eggs set}} \times 100 \quad (1)$$

- **Moisture loss:** Eggs were weighed individually at three points: upon collection (initial weight), before incubation (after storage), and on day 15 of incubation. The storage moisture loss, incubation moisture loss, and total moisture loss were calculated as percentages of the relevant initial weight [8].

$$= \frac{\text{Storage moisture loss (\%)}}{\text{Initial weight of eggs prior to storage} - \text{Weight of eggs after 11 storage days}} \times 100 \quad (2)$$

$$\text{Incubation moisture loss (\%)} = \frac{\text{Initial weight of eggs prior to incubation} - \text{Weight of eggs at day 15}}{\text{Initial weight of eggs prior to incubation}} \times 100 \quad (3)$$

- **Incubation period:** After transfer to the hatcher, the eggs were monitored every six hours, and the hatching time of each chick was recorded.
- **Embryonic mortality:** All unhatched eggs were subjected to egg breakout analysis (EBA). The embryos were then classified as infertile or categorized by the time of death: early (1–7 days), intermediate (8–15 days), or late (16–21 days) [5]. Pipped but unhatched eggs and contaminated eggs were also recorded, as shown in Figure 3.



**Fig. 3.** Embryonic mortality based on the stages of embryonic death (a) infertile; (b) early death; (c) intermediate death; (d) late death; (e) pipped egg; and (f) contaminated egg.

- **Hatchling weight:** All newly hatched chicks were weighed individually. Relative chick weight was calculated as a percentage of hatchling weight over egg weight before incubation [6].

$$\text{Relative chick weight (\%)} = \frac{\text{Weight of newly hatched chicks}}{\text{Egg weight before incubation}} \times 100 \quad (4)$$

## 2.6 Statistical analysis

The data obtained from hatchability, moisture loss, incubation period, embryonic mortality and hatchling weight were subjected to the Shapiro-Wilk test to check the normality of the data. The data were considered normal if the significance level was greater than 0.05. In addition, Levene’s test was used to check the homogeneity of the data. The data were then subjected to one-way analysis of variance (ANOVA) with a subsequent Duncan’s test for the comparison of mean values. The mean values and standard errors of the means (SEMs) were obtained from the analysis, and significant differences were indicated by  $p < 0.05$ . All analyses were carried out via SPSS (Version 29.0) software.

## 3 Results

### 3.1 Hatchability

The duration of storage had a highly significant effect on the total hatchability of Japanese quail eggs ( $p < 0.05$ ). As shown in Table 1, hatchability was highest in eggs stored for two days (82.22%), followed closely by those stored for one or three days (80.00%). A

progressive decline was observed after three days of storage. A sharp and significant drop occurred after seven days, with hatchability decreasing from 62.22% (7 days) to 15.56% (8 days). The lowest hatchability was recorded in eggs stored for 10 days (4.44%).

**Table 1.** Percentages of total hatchability of Japanese quail eggs according to different storage days.

Storage Period (Days)	Number of Eggs		Total Hatchability (%) Mean ± SEM
	Incubated	Hatched	
0	45	31	68.89 ± 2.22 <sup>bc</sup>
1	45	36	80.00 ± 10.18 <sup>c</sup>
2	45	37	82.22 ± 8.01 <sup>c</sup>
3	45	36	80.00 ± 0.00 <sup>c</sup>
4	45	32	71.11 ± 4.44 <sup>bc</sup>
5	45	31	68.89 ± 4.44 <sup>bc</sup>
6	45	28	62.22 ± 2.22 <sup>b</sup>
7	45	28	62.22 ± 5.88 <sup>b</sup>
8	45	7	15.56 ± 2.22 <sup>a</sup>
9	45	4	8.89 ± 2.22 <sup>a</sup>
10	45	2	4.44 ± 2.22 <sup>a</sup>
Total	495	272	54.95 ± 5.20
<i>p</i> -value			< 0.001

<sup>a,b,c</sup> Means within the same column with different superscripts differ significantly ( $p < 0.05$ ).

### 3.2 Moisture loss

The storage moisture loss increased significantly and progressively with increasing length of the storage period ( $p < 0.05$ ), increasing from 0.73% for one day of storage to 4.76% for 10 days of storage (Table 2). In contrast, incubation moisture loss (measured from setting to day 15) was not significantly affected by the pre-incubation storage period ( $p > 0.05$ ), with values remaining relatively stable across all groups. Consequently, the total moisture loss (from collection to day 15 of incubation) significantly increased with longer storage periods ( $p < 0.05$ ), ranging from 10.73% for unstored eggs to 16.43% for eggs stored for 10 days.

**Table 2.** Egg weight and moisture loss during storage and incubation of Japanese quail eggs according to different storage days.

Storage Period (Days)	Number of Eggs	Egg Weight Before Storage (g)	Egg Weight After Storage (g)	Storage Moisture Loss (%)	Egg Weight at 15 Days of Incubation (g)	Incubation Moisture Loss (%)	Total Moisture Loss (%)
		Mean ± SEM					
0	45	12.34 ± 0.24 <sup>bc</sup>	12.34 ± 0.24 <sup>c</sup>	0.00 ± 0.00 <sup>a</sup>	11.01 ± 0.26 <sup>b</sup>	10.73 ± 0.43	10.73 ± 0.43 <sup>a</sup>
1	45	12.15 ± 0.05 <sup>abc</sup>	12.06 ± 0.05 <sup>bc</sup>	0.73 ± 0.02 <sup>b</sup>	10.80 ± 0.08 <sup>b</sup>	10.42 ± 0.32	11.15 ± 0.34 <sup>a</sup>
2	45	12.10 ± 0.03 <sup>abc</sup>	11.94 ± 0.02 <sup>bc</sup>	1.28 ± 0.04 <sup>c</sup>	10.68 ± 0.02 <sup>b</sup>	10.58 ± 0.30	11.86 ± 0.33 <sup>ab</sup>
3	45	12.17 ± 0.09 <sup>abc</sup>	11.97 ± 0.09 <sup>bc</sup>	1.60 ± 0.05 <sup>cd</sup>	10.72 ± 0.07 <sup>b</sup>	10.46 ± 0.11	12.05 ± 0.10 <sup>abc</sup>
4	45	12.07 ± 0.04 <sup>abc</sup>	11.83 ± 0.05 <sup>b</sup>	2.00 ± 0.06 <sup>d</sup>	10.58 ± 0.07 <sup>b</sup>	10.55 ± 0.22	12.56 ± 0.27 <sup>abcd</sup>
5	45	12.44 ± 0.09 <sup>c</sup>	12.12 ± 0.08 <sup>bc</sup>	2.56 ± 0.05 <sup>e</sup>	10.84 ± 0.05 <sup>b</sup>	10.53 ± 0.43	13.09 ± 0.48 <sup>bcd</sup>
6	45	12.19 ± 0.13 <sup>abc</sup>	11.83 ± 0.15 <sup>b</sup>	2.96 ± 0.23 <sup>ef</sup>	10.55 ± 0.20 <sup>b</sup>	10.84 ± 0.62	13.80 ± 0.84 <sup>cd</sup>
7	45	12.40 ± 0.09 <sup>c</sup>	11.99 ± 0.08 <sup>bc</sup>	3.26 ± 0.03 <sup>f</sup>	10.69 ± 0.05 <sup>b</sup>	10.85 ± 0.21	14.12 ± 0.22 <sup>de</sup>
8	45	11.80 ± 0.15 <sup>a</sup>	11.29 ± 0.15 <sup>a</sup>	4.34 ± 0.11 <sup>g</sup>	10.00 ± 0.15 <sup>a</sup>	11.40 ± 0.29	15.74 ± 0.39 <sup>ef</sup>
9	45	11.80 ± 0.09 <sup>a</sup>	11.24 ± 0.11 <sup>a</sup>	4.71 ± 0.33 <sup>g</sup>	9.94 ± 0.21 <sup>a</sup>	11.60 ± 1.00	16.32 ± 1.33 <sup>f</sup>
10	45	11.95 ± 0.14 <sup>ab</sup>	11.39 ± 0.15 <sup>a</sup>	4.76 ± 0.19 <sup>g</sup>	10.06 ± 0.18 <sup>a</sup>	11.67 ± 0.46	16.43 ± 0.64 <sup>f</sup>
Total	495	12.13 ± 0.05	11.82 ± 0.07	2.56 ± 0.27	10.53 ± 0.07	10.88 ± 0.14	13.44 ± 0.37
<i>p</i> -value		0.008	< 0.001	< 0.001	< 0.001	0.467	< 0.001

<sup>a-g</sup> Means within the same column with different superscripts differ significantly (*p*<0.05).

### 3.3 Incubation period

The average incubation period per egg was significantly affected by the storage duration ( $p < 0.05$ ). As shown in Table 3, the incubation period progressively increased with increasing storage time. The shortest average incubation period was observed in eggs stored for one day (17.03 days), whereas the longest was in eggs stored for 10 days (19.00 days).

**Table 3.** Total incubation period and average incubation period of Japanese quail eggs according to different storage days.

Storage Period (Days)	Number of Hatched Eggs	Total Incubation Period (Hours)	Average Incubation Period per Egg (Hours)	Average Incubation Period per Egg (Days)
		Mean $\pm$ SEM		
0	31	4258.00 $\pm$ 154.62 <sup>bc</sup>	411.96 $\pm$ 2.09 <sup>a</sup>	17.17 $\pm$ 0.09 <sup>a</sup>
1	36	4908.00 $\pm$ 637.03 <sup>bc</sup>	408.71 $\pm$ 1.26 <sup>a</sup>	17.03 $\pm$ 0.05 <sup>a</sup>
2	37	5114.00 $\pm$ 506.81 <sup>c</sup>	414.53 $\pm$ 1.55 <sup>a</sup>	17.27 $\pm$ 0.06 <sup>a</sup>
3	36	5000.00 $\pm$ 17.09 <sup>bc</sup>	416.67 $\pm$ 1.42 <sup>a</sup>	17.36 $\pm$ 0.06 <sup>a</sup>
4	32	4474.00 $\pm$ 262.37 <sup>bc</sup>	419.63 $\pm$ 2.09 <sup>ab</sup>	17.48 $\pm$ 0.09 <sup>ab</sup>
5	31	4444.00 $\pm$ 296.02 <sup>bc</sup>	429.94 $\pm$ 1.02 <sup>bc</sup>	17.91 $\pm$ 0.04 <sup>bc</sup>
6	28	4050.00 $\pm$ 136.34 <sup>b</sup>	434.00 $\pm$ 2.34 <sup>cd</sup>	18.08 $\pm$ 0.10 <sup>cd</sup>
7	28	4034.00 $\pm$ 395.35 <sup>b</sup>	431.94 $\pm$ 1.47 <sup>bc</sup>	18.00 $\pm$ 0.06 <sup>bc</sup>
8	7	1016.00 $\pm$ 146.37 <sup>a</sup>	435.33 $\pm$ 5.21 <sup>cd</sup>	18.14 $\pm$ 0.22 <sup>cd</sup>
9	4	592.00 $\pm$ 142.17 <sup>a</sup>	446.00 $\pm$ 8.00 <sup>de</sup>	18.58 $\pm$ 0.33 <sup>de</sup>
10	2	304.00 $\pm$ 152.16 <sup>a</sup>	456.00 $\pm$ 12.00 <sup>e</sup>	19.00 $\pm$ 0.50 <sup>e</sup>
Total	272	3472.18 $\pm$ 323.50	426.82 $\pm$ 2.62	17.78 $\pm$ 0.11
<i>p</i> -value		< 0.001	< 0.001	< 0.001

<sup>a-c</sup> Means within the same column with different superscripts differ significantly ( $p < 0.05$ ).

### 3.4 Embryonic mortality

The total embryonic mortality increased significantly as the storage period increased ( $p < 0.05$ ) (Table 4). The lowest mortality rate was observed in eggs stored for two and three days (17.78% and 20.00%, respectively), whereas the highest mortality rate was observed in eggs stored for 10 days (95.56%). Analysis of the different stages of mortality revealed that early embryonic death was the primary contributor to this increase, with a significant increase after seven days of storage ( $p < 0.05$ ). The percentage of contaminated eggs also increased significantly in eggs stored for eight days or longer ( $p < 0.05$ ). In contrast, the rates of infertility, intermediate mortality, late mortality, and pipped egg mortality were not significantly affected by the storage period ( $p > 0.05$ ).

**Table 4.** Percentages of embryonic mortality of Japanese quail eggs at various stages according to different storage days.

Storage Period (Days)	Infertile (%)	Early Death (%)	Intermediate Death (%)	Late Death (%)	Pipped Egg (%)	Contaminated Egg (%)	Total Embryonic Mortality (%)
	Mean ± SEM						
0	4.44 ± 2.22	6.67 ± 3.85 <sup>a</sup>	4.44 ± 2.22	8.89 ± 2.22	4.44 ± 4.44	2.22 ± 2.22 <sup>a</sup>	31.11 ± 2.22 <sup>ab</sup>
1	4.44 ± 2.22	0.00 ± 0.00 <sup>a</sup>	2.22 ± 2.22	8.89 ± 5.88	4.44 ± 2.22	0.00 ± 0.00 <sup>a</sup>	20.00 ± 10.18 <sup>a</sup>
2	0.00 ± 0.00	6.67 ± 0.00 <sup>a</sup>	4.44 ± 2.22	4.44 ± 4.44	2.22 ± 2.22	0.00 ± 0.00 <sup>a</sup>	17.78 ± 8.01 <sup>a</sup>
3	0.00 ± 0.00	6.67 ± 0.00 <sup>a</sup>	2.22 ± 2.22	8.89 ± 2.22	2.22 ± 2.22	0.00 ± 0.00 <sup>a</sup>	20.00 ± 0.00 <sup>a</sup>
4	2.22 ± 2.22	2.22 ± 2.22 <sup>a</sup>	6.67 ± 3.85	13.33 ± 6.67	2.22 ± 2.22	2.22 ± 2.22 <sup>a</sup>	28.89 ± 4.44 <sup>ab</sup>
5	4.44 ± 2.22	8.89 ± 2.22 <sup>a</sup>	4.44 ± 2.22	13.33 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 <sup>a</sup>	31.11 ± 4.44 <sup>ab</sup>
6	0.00 ± 0.00	6.67 ± 3.85 <sup>a</sup>	4.44 ± 2.22	15.56 ± 4.44	8.89 ± 2.22	2.22 ± 2.22 <sup>a</sup>	37.78 ± 2.22 <sup>b</sup>
7	2.22 ± 2.22	4.44 ± 2.22 <sup>a</sup>	8.89 ± 2.22	17.78 ± 5.88	2.22 ± 2.22	2.22 ± 2.22 <sup>a</sup>	37.78 ± 5.88 <sup>b</sup>
8	17.78 ± 11.76	20.00 ± 6.67 <sup>b</sup>	11.11 ± 11.11	17.78 ± 2.22	4.44 ± 4.44	13.33 ± 3.85 <sup>b</sup>	84.44 ± 2.22 <sup>c</sup>
9	6.67 ± 3.85	44.44 ± 4.44 <sup>c</sup>	15.56 ± 4.44	6.67 ± 0.00	0.00 ± 0.00	17.78 ± 4.44 <sup>b</sup>	91.11 ± 2.22 <sup>c</sup>
10	13.33 ± 3.85	37.78 ± 2.22 <sup>c</sup>	13.33 ± 0.00	13.33 ± 3.85	4.44 ± 2.22	13.33 ± 6.67 <sup>b</sup>	95.56 ± 2.22 <sup>c</sup>
Total	5.05 ± 1.42	13.13 ± 2.62	7.07 ± 1.30	11.72 ± 1.27	3.23 ± 0.77	4.85 ± 1.34	45.05 ± 5.20
<i>p</i> -value	0.107	< 0.001	0.358	0.346	0.523	0.001	< 0.001

<sup>a,b,c</sup> Means within the same column with different superscripts differ significantly (*p*<0.05).

### 3.5 Hatchling weight

The storage period had no significant effect on either the absolute weight of the newly hatched chicks ( $p>0.05$ ) or their relative weight as a percentage of the initial egg weight ( $p>0.05$ ). The average hatchling weight across all groups was 8.55 g.

**Table 5.** Newly hatched Japanese quail chicks' weight and relative chick weight according to different storage days.

Storage Period (Days)	Number of Chicks	Weight of Newly Hatched Chicks (g)	Relative Chick Weight (%)
		Mean $\pm$ SEM	
0	31	8.79 $\pm$ 0.08	71.69 $\pm$ 1.92
1	36	8.56 $\pm$ 0.12	70.71 $\pm$ 0.84
2	37	8.61 $\pm$ 0.01	71.69 $\pm$ 0.38
3	36	8.59 $\pm$ 0.07	71.85 $\pm$ 0.80
4	32	8.63 $\pm$ 0.04	72.82 $\pm$ 0.03
5	31	8.80 $\pm$ 0.93	72.64 $\pm$ 8.27
6	28	8.39 $\pm$ 0.09	70.43 $\pm$ 1.19
7	28	8.68 $\pm$ 1.03	72.08 $\pm$ 7.69
8	7	8.16 $\pm$ 0.37	70.34 $\pm$ 0.06
9	4	8.53 $\pm$ 0.15	71.46 $\pm$ 0.96
10	2	8.16 $\pm$ 0.63	72.84 $\pm$ 2.14
Total	272	8.55 $\pm$ 0.12	71.65 $\pm$ 0.92
<i>p</i> -value		0.994	1.000

This study reveals that storing Japanese quail eggs at 28 °C progressively reduces embryonic viability as the storage duration increases, underscoring the major importance of temperature control for hatchability.

The total hatchability of Japanese quail eggs was significantly influenced by the storage period, where the peak hatchability of 82.22% for eggs stored for two days aligns remarkably well with the findings of Romao *et al.* (2010), who reported 80.00% hatchability under similar storage conditions (28 °C) [9]. This suggests that a short storage period at this temperature may be beneficial. However, the subsequent decline and catastrophic drop after seven days highlight the critical nature of the storage temperature. In contrast, Roriz *et al.* (2016) reported a higher peak hatchability of 84.62% when eggs were stored at a lower temperature of 23 °C, demonstrating that even a few degrees of difference can substantially impact embryonic survival [5]. The storage temperature of 28 °C used in this study is above the widely accepted physiological zero (20–24 °C), the point at which embryonic

development is arrested. Storing eggs above this threshold does not halt development but rather induces abnormal and uncoordinated cellular activity, leading to irreparable damage and apoptosis [10].

Moisture loss is a critical factor during egg storage and incubation. This study revealed a significant increase in moisture loss during the storage period, which is an expected consequence of storage at high ambient temperatures that accelerate water evaporation through the eggshell pores [11]. The highest storage moisture loss of 4.76% in eggs stored for 10 days was considerably greater than the 3.26% reported by Roriz *et al.* (2016) for eggs stored at 23 °C [5], further emphasizing the impact of temperature. This excessive dehydration can lead to deterioration of the protective qualities of albumen and increase its pH, creating a hostile environment for the embryo [4, 9]. Interestingly, a finding that concurs with El-kazaz and Abo-Samaha (2018) was the moisture loss during the incubation phase was not significantly affected by the prior storage period [12]. This suggests that the highly controlled and humid environment of the incubator likely standardized the rate of water loss once the eggs were set, overriding the initial differences caused by storage [3]. However, this does not imply that the initial damage was reversed; the negative effects of pre-incubation dehydration had already compromised embryonic viability.

Moreover, the present study also confirmed that prolonged storage significantly delays the incubation period. The average hatching time increased from approximately 17 days for eggs stored for a short period to 19 days for those stored for 10 days. This finding is consistent with previous research by Yildirim (2005) and is attributed to the fact that embryos from long-stored eggs must first repair cellular damage before they can resume normal development [13], thus extending the total time required to hatch [10]. This delay is commercially undesirable as it widens the hatch window, leading to greater variability in chick size and quality.

A key finding of this study was the significant increase in total embryonic mortality with extended storage, reaching 95.56% in eggs stored for 10 days. Crucially, the breakout analysis attributed this mainly to early embryonic death, which increased significantly after seven days. This trend agrees with studies by González-Redondo *et al.* (2023) and Romao *et al.* (2010), who also reported increasing embryonic mortality with longer storage [6, 9]. The underlying mechanism involves the degradation of albumen and an increase in its pH, which negatively impacts the initiation of embryonic growth [4-5]. In contrast, some studies have reported higher rates of late embryonic mortality, which has been attributed to insufficient moisture loss in shorter storage periods, leading to excess water in the egg that prevents the embryo from inflating its lungs just before hatching [14]. The prevalence of early death indicates that the primary damage resulted from the high storage temperature.

Despite the dramatic effects on viability, the storage period had no significant effect on the weight of the chicks that hatched successfully. The average hatchling weight of 8.55 g was consistent across all groups and is in line with the findings of several other studies [1]. This suggests that while prolonged storage at high temperatures acts as a potent selective pressure that eliminates a large proportion of embryos, those that are robust enough to survive can undergo compensatory development and achieve a normal hatch weight. Genetic and maternal factors, rather than storage conditions, are likely the primary determinants of hatchling weight, provided that the embryo survives to term [15].

A limitation of this study was the potential for incubator temperature fluctuations due to power outages, which may have influenced the results. However, given the clear and dramatic dose-dependent effect of storage duration, this is unlikely to have altered the main conclusions.

## 4 Conclusion

In conclusion, this study demonstrated that storing Japanese quail eggs at a tropical ambient temperature of 28 °C for more than seven days results in a catastrophic loss of viability, primarily due to a significant increase in early embryonic mortality. Therefore, for small-scale quail producers in tropical regions without access to refrigeration, hatching eggs should be stored for a maximum of three days to maintain economically viable hatch rates. Future research should use larger sample sizes, different temperatures and humidity levels, and evaluating the long-term post-hatch performance of the chicks.

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