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■Author(s)

Roriz BC^I
Sgavioli S^{II}
Garcia RG^I
Nääs IA^I
Domingues CHF^I
Caldara FR^I
Rombola LG^{III}
Ayla CM^I
Bernneck K^{II}

^I Federal University of Grande Dourados –
College of Agricultural Sciences, 79.804-
970, Dourados-MS, Brazil.

^{II} Brazil University, 13.690-970, Descalvado-
SP, Brazil.

^{III} Animal Scientist, 13.690-000, Descalvado-
-SP, Brazil.

■Mail Address

Corresponding author e-mail address
Sarah Sgavioli
Brazil University, Av. Hilário da Silva Passos,
950, Descalvado, SP, Brazil.
Zip code: 13690-790
Tel: +55 16 98116-4120
Email: sarahsgavioli@yahoo.com.br

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Embryo, hatchability, incubation, laying,
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Storage Period Affects Weight Loss of Japanese Quail Eggs

ABSTRACT

Long storage periods may increase embryo mortality. The objective of this study was to evaluate the effects of storage period on the weight loss, eggshell surface temperature, hatchability, and embryonic mortality of Japanese quail eggs. Two hundred fertile eggs were collected from a flock of 30-week-old Japanese quails (*Coturnix coturnix japonica*). The eggs were collected for 10 consecutive days after lay, and immediately incubated. A completely randomized experimental design with 10 treatments, corresponding to the number of days eggs were stored between egg collection and setting, with 20 replicates each, was applied. Egg weight loss increased with storage period duration, starting on day 6 (2.1%, on average) and reached 3.26%, on average, in eggs stored for 10 days. The highest hatchability ($p>0.05$) was obtained in eggs stored for two days, which also lost the least weight (1.20%). Storage period did not influence eggshell surface temperature ($p>0.05$) during incubation, but higher temperatures ($p<0.05$) were measured on days 10 and 15 of incubation compared with day 5. Eggs stored for ten days presented the highest weight loss, and therefore, a storage period of up to five days is recommended to maintain the quality of incubated Japanese quail eggs. Furthermore, egg surface temperature increases during the second half of the incubation period as a result of increasing embryonic metabolic rate.

INTRODUCTION

Egg storage is essential in modern poultry production to supply the constant market demands and hatchery production flow (Christensen, 2001). Egg storage is a routine step in commercial hatcheries. It aims at reducing the cost of egg transportation to the hatchery, obtaining a sufficient number of eggs to fully load the setters, and synchronizing hatching (Fasenko *et al.*, 2001a).

The success of incubation requires optimal management practices that take into account environmental conditions, biological factors (stress level, electrolyte balance, thermoregulation, and post-hatch livability), and physical factors (temperature, humidity, and egg turning) (Sarcinelli, 2012). Temperature and humidity are the main factors that affect embryo livability during incubation (Boleli, 2013).

The storage period can influence egg quality. Long storage periods may increase embryonic mortality by inducing cell death via necrosis and apoptosis, and delay embryonic growth recovery, even when optimal incubation conditions are provided, resulting in slow embryonic growth rates (Fasenko, 2007). Embryonic mortality rates are highest during the first three days of incubation and pre-hatch phase, and are usually due to embryo malformation (Murakami & Ariki, 1998).



Fertile Japanese quail eggs producers often do not have adequate equipment and technology that allow minimal egg quality losses during storage (Pedroso *et al.*, 2006). Considering the high demand of eggs and the factors that directly influence incubation, the gradual effect of storage on egg quality should be taken into account.

Therefore, the objective of this study was to evaluate the effects of storage period on weight loss, eggshell surface temperature during incubation, hatchability, and embryonic mortality of eggs laid by 30-week-old Japanese quails.

MATERIALS AND METHODS

The experiment was conducted in the experimental hatchery of the School of Agricultural Sciences, Federal University of Grande Dourados, state of Mato Grosso do Sul, Brazil. Two hundred fertile eggs were collected from a flock of 30-week-old Japanese quails (*Coturnix coturnix japonica*) for 10 consecutive days before incubation.

Immediately after collection, eggs were individually weighed, labeled, packed in cardboard boxes, and stored in a room at 23°C and 60% humidity. Egg weight loss during storage was determined as weight at the beginning of incubation minus weight at the time of collection. Eggs were individually placed in tulle bags and randomly distributed in a setter at 37.5°C average temperature and 65% relative humidity. The eggs were kept in a horizontal position and automatically turned at a 45° angle to either side every hour. On day 16 of incubation, the eggs were transferred to a hatcher at 37.5° and 60% humidity.

Egg surface temperature

Egg surface temperature in the setter was measured using infrared thermography (IR Text Software Testo 880 V1.4, 2009®) with $\pm 0.1^\circ\text{C}$ accuracy and at 7.5 to 13 μm spectra on incubation days 5, 10, and 15, recording three thermographic images per treatment. For image analyses, a point was marked on each egg, using 0.95 emissivity ratio. The software program used to translate the spectrum of color temperature was IRSoft.

Relative weight, hatchability, and embryonic mortality

The chicks were weighed at hatch. Hatchability was calculated as the number of hatched chicks relative to the number of fertile eggs (determined by embryo

diagnosis) set. Unhatched eggs were submitted to embryo diagnosis and classified as infertile (INF), early mortality (0-7 days; EM), intermediate mortality (8-14 days; IM), and late mortality (15-21 days; LM).

Experimental design and statistical analyses

A completely randomized experimental design (CRED) with 10 treatments, corresponding to the number of days were stored between egg collection and setting (days of storage: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10), with 20 replicates each, was applied. Eggshell surface temperature was analyzed according to a CRED in 10 x 3 factorial arrangement (10 days of storage vs. 3 days of incubation). Data were submitted to analysis of variance, and in case of significance ($p < 0.05$), means were compared by Tukey's test using SAS statistical package (SAS, 2002). Hatchability and embryonic mortality data were submitted to the frequency test, and means were compared by the Chi-square test at 5% probability level.

RESULTS

Storage duration influenced egg weight loss ($p < 0.05$; Table 1). Egg weight loss increased with storage period duration, starting on day 6 (2.1%, on average) and reaching 3.26%, on average, in eggs stored for 10 days. Hatchability rates ranged between 40-85%. The highest hatchability ($p > 0.05$) was obtained in eggs stored for two days, which weight loss was low (1.20%) (Table 2). Although not statistically significant, it was observed that early embryonic mortality was higher ($p > 0.05$) when eggs lost more weight during storage, particularly in eggs stored for eight days.

Table 1 – Parameters of incubated quail eggs stored during 10 days.

Storage period (days)	Initial weight (g)	Final weight (g)	Weight loss (%)	Relative weight (%)
1	9.95	9.85	0.97c	68.21
2	9.91	9.79	1.20c	69.92
3	9.88	9.74	1.45bc	68.18
4	10.19	10.03	1.53bc	68.33
5	9.89	9.70	1.99b	66.55
6	9.94	9.59	2.44a	66.81
7	10.01	9.54	2.80a	68.27
8	9.90	9.49	3.03a	69.10
9	10.01	9.69	2.76a	69.40
10	10.07	9.74	3.26a	68.43
P	0.4840	0.0864	<0.0001	0.3699
EP	0.103	0.129	0.290	0.927

P: probabilidade. EP: erro padrão. a-b means followed by different letters differ significantly.



Table 2 – Embryonary hatching and mortality of quail fertile eggs during different periods of storage.

Storage period (days)	Hatching (%)	Embryo mortality (%)			
		INC	INT	TAR	TT
1	50.00	12.50	25.00	12.50	50.00
2	84.62	0.00	7.69	7.69	15.38
3	70.60	11.75	0.00	17.65	29.40
4	79.17	4.17	8.33	8.33	20.83
5	70.59	23.53	5.88	0.00	29.41
6	70.00	15.00	10.00	5.00	30.00
7	65.22	13.04	8.70	13.04	37.78
8	50.00	35.00	10.00	5.00	50.00
9	40.00	15.00	25.00	20.00	40.00
10	52.17	21.74	8.70	17.39	47.83
P-QQ	0.2424	0.2673	0.2424	0.2424	0.2424
EP	14.46	9.89	7.96	6.52	12.11

INC:initial. INT:intermediate.P-QQ:probability using Chi-Square test. EP:standard error.

Egg surface temperature was not influenced ($p>0.05$) by storage period (Table 3). However, egg surface temperature was lower on day 5 of incubation ($p<0.05$) compared with days 10 and 15.

Table 3 – Fertile quail egg mean surface temperature at the 5th, 10th, and 15th day of incubation.

Storage period (PA)	Average
1	34.72
2	34.85
3	34.81
4	34.67
5	34.02
6	34.92
7	34.84
8	34.84
9	34.96
10	35.03
Days of incubation (DI)	
5	34.20b
10	35.15a
15	35.15a
Probability	
DA	0.207
DI	0.000
DA x DI	0.799
EP	0.50

a-b means followed by different letters differ significantly

DISCUSSION

The aim of this study was to evaluate the influence of storage duration on egg weight loss, hatchability, embryo mortality, and surface temperature of Japanese quail eggs stored for 1-10 days at 23°C before incubation.

Egg weight loss increased up to 2.1% as storage period increased. Eggs lost, on average, 0.2% of their weight per day, and up to 0.5% when stored for 10 days. Pedrosa *et al.* (2006) also reported 0.2% average weight loss per day of storage at room temperature in Japanese quail eggs, and losses greater than 0.6% on 3 and 6 days of storage. Moura *et al.* (2008) observed higher weight loss in Japanese quail eggs stored at room temperature compared with those stored under refrigeration, and increasing egg weight loss with storage period, which reached 2.67% in eggs stored for 10 days.

Egg weight loss is the result of the release of water caused by albumen breakdown, and its subsequent passage through the eggshell. Albumen quality is influenced by storage time and conditions, as well as breeder age (Brake *et al.*, 1997; Tona *et al.*, 2003). High egg weight losses can cause dehydration and high early embryonic mortality, whereas insufficient losses result in late embryo mortality, because the embryos are not able to inflate their lungs immediately before hatch due to the presence of excessive water in the egg (Sarcinelli, 2012).

The higher egg weight loss from the fifth day of storage may have caused worse hatchability. Although the results were not significant, eggs stored for more than five days presented 55.49% hatchability, while for eggs stored for two days hatchability was 84.62% on average.

According to Brake *et al.* (1997), external factors that influence the successful hatching of fertile eggs also affect albumen characteristics. Eggs lose weight due to dehydration, which also affects albumen quality.



Albumen quality losses are caused by egg pH reduction and high temperature and humidity fluctuations in the setter of the incubator, which, combined with the effects of storage duration, result in egg weight loss.

Fasenko *et al.* (2001a) observed 87.5% and 70.5% hatchability in chicken eggs stored at 11.5°C for four and 14 days, respectively, and 70.42% and 65.6% hatchability in turkeys eggs stored at 17.4°C for the same periods (Fasenko *et al.*, 2001b). Seker *et al.* (2005) stored Japanese quail eggs between 9 to 12 °C and 70-75% relative humidity for 15 days and obtained 90.00, 88.74, 67.96, 72.45, and 50.31% in eggs stored up to three days, 4-6 days, 7-9 days, 10-12 days, and 13-15 days, respectively. Moraes *et al.* (2009) determined 90% hatchability in meat-type Japanese quail eggs stored for four days and 75% in those stored for 14 days.

Eggshell surface temperature is used as an indicator of *in-ovo* metabolic heat production (Lourens *et al.*, 2007). According to Murakami & Araki (1998), Japanese quail eggs should be set at 37.5°C and 60% relative humidity. During the first ten days of incubation, corresponding to the first half of incubation, embryonic metabolic rate is low, and the egg needs to absorb heat from the setter environment, whereas during the fetal stage, metabolic heat production increases and consequently, eggshell surface temperature (French, 1997).

In the present study, higher eggshell temperatures were recorded on days 10 and 15 of incubation, independently of the storage time, indicating that embryonic metabolic rate was not affected by storage period. These results are consistent with the reported by Sgavioli *et al.* (2015), who observed higher eggshell surface temperature in the second half of the incubation period of broiler eggs submitted or not heat stress during incubation and injected or not with vitamin C.

Eggshell surface temperature measurements during incubation indicated an uneven distribution of temperature inside the setter, which was lower than that recommended. This suggests that the use of the thermographic camera to measure eggshell temperature may not have been efficient, because of the need to open the setter to make the measurements. Therefore, it is recommended to place thermocouples on the eggs and/or a fixed infrared thermometer in the setter.

CONCLUSIONS

Eggs stored for ten days presented the highest weight loss, and therefore, a storage period of up to five days is recommended to maintain the quality of incubated Japanese quail eggs. Furthermore, egg

surface temperature increases during the second half of the incubation period as a result of increasing embryonic metabolic rate.

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