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Influence of Storage Period on Egg Quality Characteristics, Hatching Results, Productive Performance and Behaviour of Hatched Japanese Quail Chicks

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ABSTRACT

The objective of this study was evaluating the effect of storage duration of Japanese quail eggs for two, seven and fourteen days before incubation on egg quality traits, egg weight loss, hatchability, growth performance and behaviour (exploratory behaviour, fearfulness, learning ability and memory) of birds as those behaviours were measured by performing open field test and spatial learning test. A total of 740 fertile eggs stored at 23°C and 65% humidity and divided into three treatments (Each treatment represented with four replicates), treatment 1 (T1) = egg stored for two days, treatment 2 (T2) = eggs were stored for seven days and treatment 3 (T3) = eggs were stored for 14 days. A significant effect of egg storage period on Haugh unit, yolk index and albumen Percentage were recorded. Weight loss and embryonic mortality rates increased when egg storage prolonged. Hatch weight and body weight at 5th week of age were significantly decreased in T3 birds. Also prolonged egg storage negatively affected the exploratory behaviour, fearfulness, learning ability and memory of birds. Birds of T1 exhibit more exploratory behaviour due to decreased fearfulness and higher learning ability and stronger memory than birds of T2 and T3 that considered as second grade chicks.

1. INTRODUCTION

Maintaining a high quality fertile egg is the key chain in poultry production cycle. Egg storage is an important step before incubation of eggs. Temperature, relative humidity and storage period all influence embryo development during storage. However, even with the correct incubation environment, prolonged egg storage could increase embryonic mortalities by causing necrosis, apoptosis, embryo malformation (Fasenko, 2007) and degradation of the albumen viscosity (Petek and Dikmen, 2006). Also, long storage duration affect hatchability as well as egg quality traits due to physicochemical changes of egg content (Samli et al., 2005). Most of studies investigating the effect of different storage durations of eggs have focused on post-hatch growth reporting differences at later ages (Tona et al., 2003). However, little is known about the influence of storage period on the exploratory behaviour, fearfulness, emotional reactivity, learning ability of birds. Open field test is commonly used to asses fear and anxiety reactions from animal to a novel environment (Kembro, et al., 2008), while Spatial learning is performed for measuring learning and memory in birds (Forkman, et al., 2007) and social recognition memory that depend upon the visual processing of information that appear to be a lateralized brain function in chicks (Vallortigara and Andrew, 1994). Therefore, the objective of this study was to evaluate the effect of storage duration on egg quality traits, egg weight loss, hatchability, embryonic mortality, subsequent growth performances and behavioural traits of Japanese quail progeny.

2. MATERIALS AND METHODS

Experiment was carried out at Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Egypt. In this experiment, a total of 740 fertile eggs were collected from a breeder flock of 15 weeks old Japanese quails (*Coturnix coturnix japonica*). Eggs stored in a room at 23°C and 65% humidity. Eggs were allotted to three treatments (Each treatment represented with four replicates) according to the storage period treatment 1 (T1) = egg stored for 2 days, treatment 2 (T2) = eggs were stored for 7 days and treatment 3 (T3) = eggs were stored for 14 days. Eggs were Fumigated and incubated vertically with broad end up in the setting trays in Automatic incubator at 37.5°C (dry bulb temperature), with 65% relative humidity. Eggs were turned automatically with turning angle ± 45 degree from vertical position eight times daily using automatic timer. On day 15 of incubation, eggs were transferred to the hatcher. Data for growth performance and behaviour on progeny were recorded. A total of 138 chicks / treatment were wing banded and weighed at hatch and every week till five weeks of age. Chicks were fed commercial starter diets *ad libitum* containing 22% crude protein and 3100 kcal of Metabolizable energy meeting NRC (1994) requirements. Chicks were allowed for free access to water as well.

2.1. Egg quality Analysis:

12 eggs were used from each treatment (three eggs/ replicate) before egg incubation. Egg shape index was calculated according to equation:

I = [width/Length] x 100 by using digital caliper.

While for internal egg quality, eggs were broken on a flat glass plate and each component was individually weighed to determine their weight relative to total egg weight. In addition, yolk height and diameter was measured with the aid of a caliper to allow the calculation of the yolk index, Yolk index = (height of yolk)/ (average diameter of yolk). Haugh units were calculated from the records of albumen height and egg weight using the formula: Haugh unit = 100 × log (albumen height + 7.57 − 1.7 × egg weight$^{0.37}$) (Nesheim *et al*., 1979).

2.2. Egg Weight loss:

Eggs (60 eggs/ treatment) were weighed before setting in the incubator and every 5 days during incubation. The differences between two successive weights of incubated eggs were estimated in grams and percentage from egg weight.

2.3. Hatchability percentage:

Commercial Hatchability % = (Number of hatched chicks)/ (Total number of set eggs) × 100

Scientific Hatchability % = (Number of hatched chicks)/ (Number of fertile eggs) × 100

2.4. Embryonic mortalities: Unhatched eggs were submitted to embryo diagnosis and the infertile eggs were excluded. Then only the number of fertile unhatched eggs were used to determine the percent of early mortality (EM, 0-7 days), intermediate mortality (IM, 8-14 days), and late mortality (LM, 15-18 days)

2.5. Behavioural tests:

2.5.1.Open field test: At the age of three weeks hatched birds were exposed to an open field test (OF), which performed to evaluate anxiety, exploratory behaviour, fearfulness and emotional reactivity and spontaneous behaviour is measured, as it is based on subjecting birds to unknown environment whose escape is prevented by surrounding walls Buitenhuis, *et al.,* (2004). Birds placed in an arena which they have not been before measured (120 cm × 120 cm× 80 cm). Test started when light is turned on and the movement of birds was monitored using camera for recording behaviour. The arena was divided into 5 rows and 5 columns with the same size. The variables recorded were latency to start walking, total distance moved, latency to enter center and time spent in center. Sixteen random birds' sample were chosen from each treatment and tested with total number of 48 birds. Each bird was tested individually and time of test was 5 min. for each one.

2.5.2. Spatial learning test: At the age of four weeks, learning ability and memory of birds was tested. They were exposed to simple (T-maze).It consisted of isolation room or start point measuring 25 cm × 30 cm (length × width) opening with a sliding door to a 50 cm long × 30 cm wide corridor link to two open-ended perpendicular arms. At the end of left arm there was a plate of food while empty plate on the other arm of the maze with a total length of this corridor about 190 cm length and 30 cm width covered with wire mesh. 16 random birds from each treatment were tested with total number 48 birds. Time of test was 10 min., birds were tested individually. At the start of each trail, bird was placed in the start point for about 20 sec. then the sliding door was opened, allowing bird to start walking to arm with food which considered a right trail or empty arm which considered a failed trial. Test terminated when bird make no contact within 7 min. or not leave isolation room for 3 min. Birds were trained once every day for five consequent days then they allowed to be rested for five days followed by a single trail as retrieval test. Before test is operated the birds were depraved from feed for about 7 hr. Test was monitored using camera for recording behaviour. Variables recorded were latency to reach right arm, frequency of visit right arm, frequency to visit wrong arm. In the data analysis latency to start walking was subtracted from latency to reach the goal.

2.6. Statistical Analysis:

Data were analysed by one way ANOVA, and in case of significance ($P<0.05$), means were compared by Duncan's test using SAS statistical package (SAS, 2014). Statistical model: $X_{ij} = \mu + T_i + e_{ij}$ Where: Xij = Value of ith observation of the ith treatment, μ = Overall mean, Ti = Effect of ith treatment, eij=Random error

3. RESULTS AND DISCUSSION

4.1. Egg quality results:

In Table 1 albumen percentage was statistically different among treatments (P = 0.03), where eggs of T1 had higher albumen percentage and the lower percentage was at T3. Similar results showed that albumen quality is influenced by prolonged storage duration and conditions of egg as albumen breakdown as a result of water evaporation as a weight loss (Tona *et al*., 2003). Moreover, there was a significant effect of storage period on yolk percentage (P < 0.05) with yolk weight increasing as storage time prolonged.

Haugh unit values decreased significantly (P <.0001) with prolonged egg storage period. This could be a result of weight loss and breakdown of albumen that influence Haugh unit values.

Yolk index values showed a significant decrease with increased egg storage period (P = 0.01), most likely due to the flattening of the yolk caused by the weakening of the vitelline membrane. Similar results on Fayoumi chicken eggs were obtained by Khan *et al*., 2013. Other studies reported that prolonged storage influence egg quality due to physicochemical changes resulted in flattening of the yolk caused by the weakening of the vitelline membrane (Samli *et al*., 2005).

4.2. Egg Weight loss:

In **Table 2** significant effect on egg weight loss was recorded after the 5th day of incubation. Eggs stored for 7 and 14 days lost weight more than eggs stored for 2 days (P value = 0.0006). For the entire period from egg setting till 15 day of incubation, eggs lost significantly more weight when the storage duration was longer as eggs stored for 14 days lost 9.42% of weight while, eggs stored for 7 or 2 days lost 8.85% and 8.28%, respectively. This result is similar to previous observations by Roriz, *et al.,* (2016). High egg weight losses could be a reason for incidence of high early embryonic mortalities because of dehydration, while, insufficient weight losses causes presence of excessive water in the egg that so embryos are not able to inflate their lungs immediately before hatch and that leads to late embryonic mortalities (Sarcinelli, 2012).

4.3. Hatchability percentage and embryonic mortalities:

The main effects of storage period on commercial, scientific hatchability and embryonic mortalities are presented in Table 3. Hatchability, either commercial or scientific were negatively affected by increasing storage of eggs, where hatchability dropped significantly in T2 and T3 than in T1 (P<0.0001). Embryonic mortalities showed significant differences among different treatments for EM where the higher percentage were recorded for T3 (P = 0.03) followed by T2 then T1. However, IM was higher in T1 with significant differences from the other two treatments (0.005), however, LM was not statistically different among three treatments. Prolonged storage of eggs caused high incidence of early and late mortalities could be reason for reduction of hatchability. Similar results were obtained by Schmidt, *et al.,* (2009) and Fasenko *et al*., (2001).

4.4. Growth performance of hatched chicks:

Hatch weight was significantly decreased in chicks hatched from eggs of T3 (P = 0.002) than other treatments (Table, 4), this is probably due to higher percentage of egg weight loss during incubation and storage. Also, storage period had significant effect on body weight at two weeks (p <0.05), body weight was lower in T2 and T3 than T1. Eggs storage for 14 days significantly depressed the average body weight of quail at five weeks of age and this could be a due to an increase in second grade chicks at hatch after prolonged storage duration. This result was in agreement with (Reis et al., 1997) who reported that the body weight of chicks hatched from eggs stored for a short duration was higher than that those stored for longer duration.

Table 1. Effect of storage time of eggs on egg quality

Egg Parameter	Treatment			p value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean± SD	Mean ± SD	Mean ± SD	
Egg weight (g)	12.85 ± 1.00	12.86 ± 1.18	12.81 ± 1.11	0.99
Albumen %	53.83 ± 2.41^{a}	52.40 ± 3.45^{ab}	50.38 ± 3.43^{b}	0.03
Yolk %	31.31 ± 2.06^{b}	30.96 ± 1.66^{b}	34.69 ± 3.14^{a}	0.0007
Egg shell %	14.86 ± 2.96	16.65 ± 2.55	14.93 ± 2.55	0.19
Egg shape index	77.73 ± 3.03	76.64 ± 2.02	77.82 ± 2.75	0.48
Haugh unit	94.71 ± 2.49^{a}	89.99 ± 2.10^{b}	85.76 ± 0.84^{c}	0.0001
Yolk index	0.56 ± 0.07^{a}	0.51 ± 0.06^{ab}	0.48 ± 0.06^{b}	0.01

Means bearing different litters within the same raw are significantly different (P<0.05)

Table 2: Effect of storage Period of eggs on egg weight loss:

Parameter	Treatment			p value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Egg weight*	12.99 ± 1.06	12.90 ± 1.20	12.92 ± 1.12	0.89
0-5 (g)	0.39 ± 0.07	0.37 ± 0.17	0.41 ± 0.16	0.34
0-5 (%)	3.05 ± 0.60	2.82 ± 1.17	3.17 ± 1.29	0.18
5-10 (g)	0.31 ± 0.16^{b}	0.40 ± 0.13^{a}	0.41 ± 0.14^{a}	0.0005
5-10 (%)	2.43 ± 1.26^{b}	3.13 ± 1.09^{a}	3.17 ± 1.13^{a}	0.0006
10-15 (g)	0.35 ± 0.19^{b}	0.37 ± 0.13 ^{ab}	0.42 ± 0.13^{a}	0.03
10-15 (%)	2.67 ± 1.36^{b}	2.86 ± 0.94^{ab}	3.24 ± 1.04^{a}	0.02
Overall 0-15 (g)	1.07 ± 0.28^{b}	1.15 ± 0.33^{ab}	1.21 ± 0.23^{a}	0.01
Overall 0-15 (%)	8.28 ± 2.03^{b}	8.85 ± 2.26^{ab}	9.42 ± 1.96^{a}	0.01

Means bearing different litters within the same raw are significantly different (P<0.05)
*0-5: Before egg setting till 5th day of incubation, 5-10: from 5th day till 10th day of incubation, 10-15: from 10th till 15th day of incubation, Overall 0-15: before egg setting till 15th day of incubation.

Table 3. Effect of storage time of eggs on hatchability and embryonic mortalities:

parameter	Treatment			P value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Commercial hatchability (%)	82.79 ± 1.32^{a}	64.15± 7.78^{b}	55.02± 5.05^{c}	0.0001
Scientific hatchability (%)	88.14± 1.35^{a}	68.59± 4.59^{b}	59.11± 4.35^{c}	0.0001
Early mortalities (EM)	34.39 ± 5.93^{b}	41.28± 4.39^{ab}	48.53~~5~~± 7.79^{a}	0.03
Intermediate mortalities (IM)	23.89 ± 5.68^{a}	11.96± 5.40^{b}	9.17~~25~~± 3.52^{b}	0.005
Late mortalities (LM)	41.71± 4.57	46.75±6.49	42.29 ±7.73	0.50

Means bearing different litters within the same raw are significantly different (P<0.05)

Table 4. Effect of storage time of eggs on chicks' weight:

Chicks weight (g)	Treatment			p value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Hatch weight	9.84 ± 0.78^{a}	9.92 ± 0.76^{a}	9.52 ± 0.77^{b}	0.002
Week 1	21.38 ± 2.71	21.34 ± 3.68	21.31 ± 2.56	0.98
Week 2	46.38 ± 5.77^{a}	43.84 ± 6.60^{b}	43.34 ± 6.73^{b}	0.007
Week 3	91.59 ± 11.67	90.23 ± 13.07	89.59 ± 13.21	0.14
Week 4	141.78 ± 17.77	139.32 ± 15.08	139.93 ± 17.55	0.54
Week 5	181.91 ± 35.67^{a}	179.17 ± 19.19^{ab}	173.85 ± 18.81^{b}	0.01

Means bearing different litters within the same raw are significantly different (P<0.05)

Table 5. Effect of storage time of eggs on open field parameters (OF):

parameter	Treatment			P value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Time in center (s)	86.79 ± 9.32^{a}	82.75± 11.18^{ab}	76.02± 4.22^{b}	0.01
Latency to enter center (s)	79.25± 8.22^{b}	99.55± 5.59^{ab}	105.11± 10.35^{a}	0.01
Latency to start walking (s)	49.52 ± 7.23^{c}	63.5± 10.39^{b}	83.87± 9.58^{a}	0.001
Total distance moved (cm)	395.21± 18.52^{a}	353.25± 30.40^{b}	220.23± 20.68^{c}	0.005

Means bearing different litters within the same raw are significantly different (P<0.05)

Table 6. Effect of storage time of eggs on spatial learning test parameters:

parameter	Treatment			P value
	T1 (2 days)	T2 (7 days)	T3 (14 days)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Time to reach right arm (s)	229.58 ± 23.25^{c}	296.59± 30.18^{b}	399.23± 29.56^{a}	0.01
Frequency to reach right arm (%)	55.25± 9.22^{a}	47.21± 5.29^{ab}	20.17± 3.35^{b}	0.01
Frequency to reach wrong arm (%)	23.26 ± 5036	29.23 ± 6.32	65.98 ± 9.31	0.01

Means bearing different litters within the same raw are significantly different (P<0.05)

4.5. Behavioural tests results:

4.5.1. Open field test:

Table 5 show values of behavioural variables of open field test (OF) for each storage time of eggs to estimate fearfulness and exploratory behaviour of birds. There was a slight difference between birds hatched from eggs stored for 2 days and those hatched from eggs stored for 7 days in time elapsed in center but it was significantly the lowest in birds produced from eggs of T3. On other hand latency to enter center and latency to start walking was significantly higher in T3. However, Total distance moved was highest in T1 and the lowest distance in T3. These results showed that increasing storage time increase fearfulness of birds as exposure to a novel environment promotes inactivity. Therefore birds that remain inactive in this new environment which here in this test explained as increase in latency to start walking or latency to enter to center or total distance moved are considered to be more frightened than those showed explorative behaviour Jones, *et al.*, (2002) and Uitdehaage, *et al.*, (2008). This is may be due to eggs were stored beyond 7 d have an increase in cell death. It is possible that the increase in cell death overtime leads to stored embryos that have less viable cells left at onset of incubation than embryos of fresh eggs Reijrink *et al.*, (2010). Also these results can be explained due to increasing storage time lead to Yolk lipid oxidation increased significantly with storage time, because storage of egg for longer periods at different temperatures may reduce the antioxidant activity. This result agrees with Lakins *et al*., (2009). All these negative effects of increasing storage time may cause decreasing exploratory behaviour and birds became more fearful in T3.

4.5.2. Spatial learning test (T-maze):

In Table 6 which demonstrates the effect of storage time of egg on learning behaviour in T-maze test. It was observed that storage time had significant effect on time elapsed to reach to right arm as it was highest in T1 and lowest in T3. Frequency to reach right arm was lowest in T1 and highest in T3. This result can be explained as total egg weight loss increased when storage duration increased. Weight loss occurs due to loss of solvents from the egg content through the shell by evaporation Fasenko *et al*., (2001). Tilki and Inal (2003). This lead to decrease in body weight of the chick Tona, *et al.*, (2003). So T1 chicks at 5^{th} week of age was the highest in body weight therefor it was the fastest group reached to right arm and have the highest frequency to reach right arm. These results are in agreement with Marin, *et al*., (1997) and Marin, *et al*., (2003) which performed that rapidity of T-maze was associated with higher growth rate birds.

5. CONCLUSION:

Storage of fertile Japanese quail eggs at room temperature even less than seven days caused increased embryonic mortalities and negatively affect egg quality, hatchability percentage and progeny performance. Interestingly, prolonged egg storage increases fearfulness and decrease exploratory behaviour and learning ability for the birds. So we recommend using special techniques for storage of egg especially more than seven days to improve hatching results, hatched chicks performance and behaviour.

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