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The effect of storage time on egg quality and hatchability characteristics of Rhode Island Red (RIR) hens

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ABSTRACT

In this research study, hatchability results and some internal egg quality characteristics of 0, 2, 3, 5, 7 and 9 d stored eggs in RIR were examined. It was determined that the effect of storage time on hatchability, hatchability of fertile eggs, embryonic mortality, chick weight, albumen weight, yolk weight, albumen index, yolk index and Haugh unit was significant ($P < 0.05$). There was no positive or negative effect of storage time on the fertility rates, but there was a negative effect of storage time on egg weight, hatchability, embryonic development and chick weight on d 3 ($P < 0.05$). It was determined that prolonged storage time caused a decrease in the albumen weight, yolk weight, albumen index, yolk index and Haugh unit value of Rhode Island Red eggs. Rhode Island Red eggs should not be stored more than 3 d.

Key words: Rhode Island Red hen, egg quality, egg weight loss, hatchability

Introduction

Fertility and hatchability are the major determinant of profitability in a hatchery enterprise (PETERS et al., 2008). Hatching eggs are frequently stored on breeder farms and at hatcheries to reduce transportation costs or to provide for sufficient eggs available to fill large incubators. However, the storage of eggs for more than a week is known to increase embryonic abnormalities and mortality due to the degradation of the viscosity of egg albumen (PETEK and DIKMEN, 2006). The elongated storage of eggs also shows reduced hatchability and an increase in the amount of incubation time required to hatch. In fact, a rule-of-thumb in the hatchery business is that for every day after 10-days (d)

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of storage, hatchability will decrease by 1% (BAKST and AKUFFO, 2002). It has been reported that temperature, relative humidity (RH), storage time and egg positioning all influence embryo development during egg storage and incubation (BUTLER, 1991). The storage time of eggs, which is only one of these factors, is the most important factor in obtaining chicks of suitable number and quality. NARAHARI et al. (1988) stored Japanese quail's eggs for a period of 1-7 d and determined that the highest rates of fertility and hatchability of eggs were observed in eggs stored for 1-3 d.

However, ROMAO et al. (2008) reported that quail eggs showed better hatchability until 10 d of storage and that eggs offered for storage have a reduced weight loss during incubation. For chickens, it was recommended that pre-storage incubation has no effect on hatchability, when storage time is shorter than 8 d. The detrimental or beneficial effect of pre-storage incubation was noted when storage time was prolonged (REIJRINK et al., 2009). Some negative changes in egg quality of all poultry species have been reported due to prolonged storage time. For example, water loss from eggs was related to hatchability results in pheasants (KOZUSZEK et al. 2009). On the other hand, TILKI and SAATÇI (2004) reported that storage time influences egg white quality (index, Haugh unit). RAJI et al. (2009) reported that lower egg quality in the Bovans brown strain was recorded with increased storage time. The eggs stored at a high temperature were already spoilt and not fit for human consumption after 2 weeks of storage. Similarly, JIN et al. (2011) reported that egg weight loss, albumen pH, and HU were greatly influenced by the storage temperature and time, of eggs from Lohmann Light-Brown hens.

Most studies associated with the effect of egg storage period on internal egg quality and hatchability characteristics are focused on Japanese quail and broiler breeders. However, these parameters have not been fully examined in Rhode Island Red, which is a rural poultry breed. Thus, the aim of the current study was to inspect the effect of the length of egg storage time on egg weight, internal egg quality characteristics, as well as hatchability results and day-old-chick weight in the RIR breed.

Materials and methods

Hatching eggs. Rhode Island Red fertile eggs were collected three times (Table 1) in the 32nd, 34th and 36th week of the birds' age. A flock of RIR breeding stocks was maintained at the Government Poultry Farm, Multan, Pakistan, and housed at a density of 0.22 m² per bird. The hens were reared in floor pens and kept during laying under standard management conditions (FASS, 2010). At bird placement (20 wk of age) the male:female ratio was 1:10. All birds received the same complete diet (16.50% CP; 2,800 kcal ME/kg, 3.10% calcium, 0.35% available phosphorus), formulated to meet or exceed NRC (1994) requirements. Water was available for ad libitum consumption and natural daylight was supplemented with artificial light to give a 17-h photoperiod. Temperature recordings

showed that low and high in-house air temperatures at egg collection day ranged from 16 to 28 °C at ages 32 to 36 wk. All flocks laid eggs at an 85% laying rate.

Table 1. Outline of experiment

Batches	Egg Collection Time		Number of eggs used at different storage periods					
	Flock age	Date	0d	2d	3d	5d	7d	9d
1	32 weeks	10-04-2012	330	330	330	330	330	330
2	34 weeks	25-04-2012	330	330	330	330	330	330
3	36 weeks	09-05-2012	330	330	330	330	330	330

Eggs were collected at 32, 34 and 36 wk of age. Eggs were collected on a single day, as outlined in Table 1. Eggs laid before 8:00 h were removed from the nest boxes and discarded from the experiment. Eggs were collected from nests between 08:30 and 12:30 h, placed in setter trays (150 eggs per tray), and transported to the hatchery located near the poultry houses. A total of 5940 hatching eggs (1980 hatching eggs / batch) were used in this study (Table 1). At approximately 14:00 h, the eggs were sorted to exclude from the experiments those that were cracked, visibly dirty or misshapen. After that eggs from each flock were randomized into six groups (Table 1) and placed on incubator trolleys, to allow air circulation around the eggs. Thereafter, eggs were fumigated for 20 min with formaldehyde gas, and one group was set in the incubator on the same day (collection day), whereas the other five groups were stored for 2, 3, 5, 7 or 9 d in the store room at 16 °C and 78% RH.

Incubation and hatching. This experiment was conducted using electronically controlled, single-stage incubators (Model 2007; Chick Master, France). The eggs were pre-warmed in the incubator for 8:00 h at around 24 °C and 65% RH, just before the incubation period, and fumigated in the incubator on the day of setting. The eggs were turned hourly through 90° and incubated according to conventional temperature and humidity conditions (REIS et al., 1997), which were automatically monitored. On the 18th d of incubation, eggs were individually candled in the transfer-room (around 24 °C and 60% RH). Eggs which did not exhibit signs of embryo development were removed and broken out for macroscopic examination, in order to determine early-dead embryo (<7 d) and those that were infertile, as outlined in BRAKE (1996). Unhatched eggs were opened, examined macroscopically, and assigned to one of the following categories: mid-dead (8 to 18 d) and late-dead (after 19 d).

The remaining eggs, with apparently living embryos, were transferred to hatching baskets and randomly distributed in the front part of the same trolley. The hatcher operated under conventional conditions (REIS et al., 1997). All chicks were removed at 21d of incubation.

Data recording. All eggs were weighed before and after storage. Weight losses during the storage period were calculated for each individual egg, as a percentage of the initial weight. After the 21-d incubation period, the number of hatched and unhatched chicks was recorded. To determine egg fertilisation, unhatched eggs were analysed via breakout examination.

From the data, hatchability (the number of saleable chicks hatched per all eggs set ' 100) was calculated. In fact, some very early dead are likely to be classified as "infertile" using macroscopic examination (NOVO et al., 1997).

Internal egg quality analysis. To determine the quality characteristics of the eggs, 20 eggs were used from each group. The eggs were broken out individually onto a flat surface and allowed to sit for 5 min. The heights of the yolk and albumen, the long and short diameters of the albumen, and the diameter of the yolk were measured using a calliper with sensitivity of 0.001 mm. The yolks were separated from the albumen, and both were weighed. On the basis of the obtained data, the following traits were calculated using the formulas shown below (YANNAKOPOULOS and TSERVENI-GOUSHI, 1986):

Yolk index = (yolk height/yolk diameter) × 100;

Albumen index = [albumen height/(long diameter of albumen + short diameter of albumen/2)] × 100

The egg weight and Haugh unit were measured automatically by an Egg Analyzer™ manufactured by Orka Food Technology Limited. The weight of day old chicks was also recorded.

Statistical methods. When the differences between the storage periods were significant, means were separated using Duncan's multiple range tests at the 0.05 level of significance (STEEL and TORRIE 1984). The analyses were conducted using SPSS 15.0 software (SPSS Inc., 2006).

Results

The initial and final egg weights during the storage period and the weight loss of fertile eggs during the storage period are presented in Table 2. No differences were found in the initial weight of the fertile eggs before their storage ($P < 0.804$). Significant differences were found in egg weight loss during the storage period, as a function of its length ($P < 0.05$). The fertile egg weight loss gradually increased with storage time before incubation, the eggs stored for 9 d showing the greatest weight loss.

The internal quality characteristics of RIR eggs during different storage periods are shown in Table 3. There was a significant effect of storage time on yolk weight ($P < 0.05$), with yolk weight increasing as storage time increased. Albumen index values decreased significantly with increased storage period ($P < 0.05$). The albumen index values decreased after 5 d of the storage period ($P < 0.05$).

Table 2. Egg weight and egg weight losses during storage periods in Rhode Island Red fertile eggs according to the length of the storage period¹ (Mean \pm SEM)

Storage period (d)	Number of eggs	Egg weight before storage (g)	Egg weight after storage (g)	Egg weight loss during storage (%)
0	990	44.73 \pm 2.41	44.73 \pm 2.41	0.00 \pm 0.00 ^d
2	990	44.83 \pm 2.22	44.66 \pm 2.19	0.38 \pm 0.05 ^d
3	990	45.07 \pm 2.39	44.64 \pm 2.21	0.95 \pm 0.11 ^c
5	990	45.06 \pm 2.40	44.45 \pm 2.09	1.35 \pm 0.20 ^c
7	990	44.94 \pm 2.26	44.06 \pm 2.07	1.96 \pm 0.51 ^b
9	990	44.77 \pm 2.09	43.64 \pm 1.65	2.52 \pm 0.74 ^a
Total	5940	44.90 \pm 2.20	44.36 \pm 2.08	1.20 \pm 0.24

a - d Values in the same column with different superscripts are significantly different ($P < 0.05$).¹Values are expressed as a percentage of egg weight at the beginning of storage period.

Table 3. Internal quality characteristics of Rhode Island Red eggs according to storage time (Mean \pm SE)

Storage period (d)	Yolk weight (g)	Albumen weight (g)	Albumen Index	Yolk Index	Haugh unit
0	15.90 \pm 0.25 ^c	32.00 \pm 1.50	8.50 \pm 0.34 ^a	45.00 \pm 1.08 ^a	88.25 \pm 1.60 ^a
2	16.16 \pm 0.26 ^{bc}	32.05 \pm 1.35	8.30 \pm 0.39 ^a	44.80 \pm 0.66 ^a	86.20 \pm 1.41 ^a
3	16.23 \pm 0.28 ^{bc}	31.59 \pm 1.25	8.27 \pm 0.33 ^a	44.60 \pm 0.67 ^a	87.15 \pm 1.22 ^a
5	16.34 \pm 0.29 ^{bc}	31.55 \pm 1.20	8.20 \pm 0.27 ^a	40.80 \pm 0.58 ^b	87.66 \pm 1.34 ^a
7	17.09 \pm 0.32 ^{ab}	31.44 \pm 1.31	8.09 \pm 0.12 ^b	40.53 \pm 0.60 ^b	80.96 \pm 1.23 ^b
9	17.27 \pm 0.26 ^a	31.33 \pm 1.22	8.00 \pm 0.16 ^b	40.08 \pm 0.73 ^b	81.00 \pm 1.30 ^b

a-c: the differences between values with different superscript letters in the same column are significant ($P < 0.05$).

Haugh unit values decreased significantly ($P < 0.05$) with increased egg storage time (Table 3). Haugh unit values decreased after 5 d of the storage period ($P < 0.05$).

No differences were found in the eggs' fertility among the experimental groups ($P = 0.808$). No significant effect of storage time was found on the hatchability of set eggs stored up to 3 d before incubation, but eggs stored for 5 to 9 d showed a significant decrease in hatchability (Table 4).

Differences in embryonic mortality were found to be significant due to the main effect of egg storage duration ($P < 0.01$, Table 4). Most of the deaths were in eggs stored for 9 d. The embryos of eggs stored for 9 d showed evidently lower hatchability and higher mortality during incubation. Significant differences were found in chick weight at different egg storage times ($P = 0.036$; Table 4). The chick weight decreased with the increase in the egg storage period.

Table 4. Fertility, hatchability, embryonic mortality and chick weight of Rhode Island Red eggs according to the length of the storage period

Storage period (d)	Number of eggs			Fertility ¹ (%)	Hatchability ² (%)	Hatchability of fertile eggs ³ (%)	Embryonic mortality (%)			Chick weight (g)
	Incubated	Fertile	Hatched				1 st wk	2 nd wk	3 rd wk	
0	990	780	728	78.78	73.53 ^a	93.33 ^a	3.0 ^c	3.2 ^c	4.6 ^c	30.46 ^a ± 1.20
2	990	777	725	78.48	73.23 ^a	93.30 ^a	3.6 ^c	3.7 ^c	4.8 ^{bc}	28.92 ^b ± 1.16
3	990	778	700	78.58	70.70 ^a	89.97 ^a	4.1 ^c	6.0 ^{bc}	8.4 ^{abc}	28.66 ^b ± 1.10
5	990	777	582	78.48	58.78 ^b	74.90 ^b	11.8 ^c	11.4 ^{abc}	10.8 ^{abc}	28.33 ^b ± 1.08
7	990	776	263	78.38	26.56 ^c	33.89 ^c	37.2 ^b	14.8 ^{ab}	10.7 ^{abc}	25.50 ^c ± 0.99
9	990	761	56	76.86	5.65 ^d	7.35 ^d	48.3 ^a	17.5 ^a	17.6 ^a	23.89 ^d ± 0.88
Total	5940	4649	3054	78.26	51.41	65.69	18.00	10.02	9.48	

a-d Values in the same column with different superscripts are significantly different ($P < 0.05$). ¹Percentage of incubated eggs that were fertile. ²Percentage of incubated eggs that hatched. ³Percentage of fertile eggs that hatched.

Discussion

The egg weight loss increased with the length of storage, as reported by many researchers for various poultry species. The egg weight loss observed during storage in the present research followed the expected pattern and was higher than that found by REIJRINK et al. (2009) for broiler breeder eggs when stored at 16 to 18 °C and unspecified relative humidity, and by GONZÁLEZ-REDONDO (2010) for red-legged partridge eggs when stored at room temperature (15 °C) and 80% RH. Weight losses, which occur during the storage of eggs, are related to the temperature and humidity of the environment in which the eggs are stored, and to the length of the storage period. GARIP and DERE (2011) reported that egg weight losses in quail eggs stored for 10 d were determined to be 1.3% at 11 °C, 3.1% at 21 °C and 3.7% at 27 °C. Our observations following the logic that long-term stored eggs may lose weight due to moisture loss, which may affect the viability of the eggs, corroborate the findings of other authors (DEMIREL and KIRIKÇI, 2009; KOZUSZEK et al., 2009) for pheasants. When the storage period is extended, the internal quality of pheasants' eggs progressively declines due to loss of moisture from the egg.

The yolk weights of RIR eggs recorded in the present study are very similar to those of other studies (GUPTA et al., 2007; AKHTAR et al., 2007) in the same breed. There was no significant effect of storage time on albumen weight. The albumen weights of RIR eggs

in the current study are very similar to the values reported by GUPTA et al. (2007) and KHAWAJA et al. (2012) in the same breed.

In this study, albumen index values decreased with a longer period of egg storage. Water loss from the egg, or movement of water from the albumen to the yolk is the most likely cause of this result. Albumen index values in the present study were approximately similar to reported values (AKHTAR et al., 2007) in RIR eggs. Yolk index values showed a significant decrease with increased egg storage time ($P < 0.05$), most likely due to water loss from the egg. The yolk index values were similar to the findings of GUPTA et al. (2007) in RIR eggs.

Similarly, Haugh unit values also decreased with egg storage time in the current study. These results are consistent with the findings of MONIRA et al. (2003), who reported that Haugh unit values of RIR fertile eggs decreased from 1 d (88.20) to 7 d (57.79) of storage time. The Haugh unit values of fresh eggs in this study were almost the same as RIR hen, partridge, geese, pheasant and quail eggs (MONIRA et al., 2003; TILKI and SAATCI, 2004; TILKI and INAL, 2004; DEMIREL and KIRIKÇI, 2009; NOWACZEWSKI et al., 2010). The present results relating to the effect of storage time on egg internal quality are similar with the findings of TILKI and SAATCI (2004) and DEMIREL and KIRIKÇI, (2009), who reported that the Haugh unit, albumen and yolk index values of partridge and pheasants eggs significantly decreased with increasing days of storage, due to loss of water from the eggs.

The average fertility of the eggs recorded in this study (78.26%) was higher than in a previous report by ZELLEKE et al. (1965) on RIR (76.67%). However, ISLAM et al. (2002) and MALAGO and BAITILWAKE (2009) reported higher fertility (88.29% and 91.10%, respectively) in RIR chickens than the present findings. The eggs' fertility depends on various factors, such as: breed, season, lighting, level of nutrition and time of mating (MIAZI et al., 2012). The average hatchability of the fertile eggs we recorded (65.69%) showed a value lower to those (80.60 to 88.37%) reported for these hens by FAROOQ et al. (2001), ISLAM et al. (2002), MALAGO and BAITILWAKE (2009). This may be due to inbreeding of RIR stock over a long period of time. Variations in hatchability may be accounted for by various factors, such as: storage duration, care of hatching eggs, age of broody birds, quality of eggs, seasons and nutrition (MIAZI et al., 2012). Our results coincide with the findings of MAHMUD et al. (2011) in broiler breeders, whose eggs had no appreciable loss in hatchability when held up to 3 d under similar conditions to our experiment. The highest hatchability (82.5%) was recorded for eggs stored for 3 d and the lowest (22.5%) in those eggs stored for 12 d before incubation. Our findings also partly coincide with other studies. PETEK and DIKMEN (2006) reported that the hatchability results of broiler breeder eggs stored for 5 d were significantly better compared to eggs stored for 15 d at 14 °C and 65 RH. Similarly, REIJRINK et al. (2009) found that the

hatchability of fertile eggs in broiler breeders stored for 3, 5 and 8 d were higher than the hatchability of fertile eggs stored for 12 d ($P = 0.005$) at 16 to 18 °C. KARABAYIR (2010) reported that better hatchability in quail was observed for up to 7 d of egg storage time than after 10 d at 15 °C and 70-80% RH. MOREKI and DITSHUPO (2012) also noted that the best hatchability of guinea fowl eggs was recorded at 0 d storage time (88%) followed by 4 d (76%) at 20 °C, which indicated that storing guinea fowl eggs beyond 4 d contributes to a decline in hatchability. These hatchability values are very close to the results of the present study. Some embryos of eggs stored for a long period could not begin developing immediately after normal incubation temperatures were provided (TONA et al., 2001). Another view is that the development of embryos from eggs stored for a long time proceeds at a slower rate in the first period of incubation (KHAN et al., 2013).

In this study, more embryonic deaths occurred with a longer period of egg storage. Similar findings were found by PETEK and DIKMEN (2006), who reported that most embryonic deaths were observed in broiler breeder eggs stored for 15 d, as compared to a 5 d storage period. Some studies (SCOTT and MACKENZIE, 1993; ELIBOL et al., 2002; PETEK et al., 2005) showed that early and late embryonic death was increased with an increased egg storage period. SCHMIDT et al. (2009) reported that storage time linearly influenced hatchability and embryo mortality, with an estimated 1.17% reduction and a 1.15% increase, respectively, for each single day of storage. They further reported that hatchability was reduced by 21% between 2 and 7 d storage time, resulting from a 62% increase in embryo mortality. The current study also showed that the hatchability of fertile and all eggs decreased, and early-mid-late embryonic deaths increased from eggs stored for 9d, due to water loss and albumen degradation during storage. Longer periods of storage will increase the spread of time over which hatching takes place, and this may influence the total hatchability and overall quality of chicks (HASSAN et al., 2005). Studies have explained that embryos at the pre-gastrula stage at oviposition are less able to survive prolonged storage than those at the gastrula stage (WILSON, 1991; DECUYPERE and MICHELS, 1992). As heat treatment for one or a few hours daily, before and during egg storage, may promote hatchability in lines of chickens which normally show a rather low hatching percentage, it may be considered that these lines lay their stage of blastoderm development. It has been suggested that the decrease in viability of the embryo may be caused by changes in the embryo or by changes in certain physical aspects of the egg, namely albumen pH (LAPAO et al., 1999). After oviposition, carbon dioxide is released from the egg, resulting in an increase in albumen pH from about 7.6 to 9.5 within a short period of time, whereas the yolk remains slightly acid, with a pH around 6.5 (STERN, 1991). Therefore, a 1,000-fold hydrogen ion concentration gradient (3 pH units) may exist across the blastoderm (STERN, 1991), in its intermediary position between the albumen and the yolk. Excess carbon dioxide loss causes the albumen to have an excessively

high pH and this negatively affects the initiation of embryo development (LAPAO et al., 1999). If the loss of carbon dioxide is too low, the pH of the albumen will also be too low, resulting in eggs which are “too fresh” and which do not hatch as well as those stored for 3-4 d. Literature showed that the rise in albumen pH with storage time is related to a decrease in albumen index. Albumen liquefaction probably facilitates the movement of nutrients from the albumen to the blastoderm and may reduce resistance to gaseous diffusion (LAPAO et al., 1999). The above cited effect of storage upon albumen viscosity would also explain why short-term storage may have beneficial effects on hatchability, as these flocks generally lay eggs that have albumens of good quality and that are quite resistant to degradation. However, extended periods of egg storage allow the albumen to degrade excessively. This degradation causes the blastoderm to move into close proximity to the eggshell, so that early embryonic mortality results from dehydration during the early stages of incubation (BRAKE et al., 1993).

In the present study, lower chick weight (30.46 g) was recorded, in relation to the findings of KHAWAJA et al. (2012), who reported that chick weight in the RIR breed was 31.30 g. However, MALAGO and BAITILWAKE (2009) found 30.12 g RIR chick weight, which is very close to this study. The results of the current study are in line with the findings of GARIP and DERE (2011), who reported that chick weight of quail decreased from 1d (9.47 g) to 15 d (9.13 g) of egg storage periods at 11 °C. Our findings are in contrast to the results of other workers (PETEK et al., 2003; GARIP and DERE, 2006; GARIP et al., 2005), who reported that chick weight was not affected by storage period. This may be due to a short storage period, different breeds, different storage temperatures and the weight of the eggs used in these studies.

Conclusions

In conclusion, after d 3 of storage period there is a decrease in hatchability results. Some changes in interior quality (Haugh unit, albumen and yolk indices), with the effect of storage time might indirectly affect the hatchability index. Storage time of RIR eggs may be extended until 3 d, but longer storage may negatively influence hatchability.

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SAŽETAK

U radu su istraženi valivost i karakteristike jaja pasmine Rhode Island Red u uvjetima različite duljine skladištenja od 0, 2, 3, 5, 7 i 9 dana. Duljina skladištenja statistički je značajno ($P < 0,05$) utjecala na valivost, valivost oplođenih jaja, smrtnost embrija, masu pilića, masu bjelanjka, masu žumanjka, indeks bjelanjka, indeks žumanjka te na vrijednost Haughove jedinice. Nije utvrđen ni pozitivan ni negativan učinak duljine skladištenja na stopu plodnosti, ali je duljina skladištenja od 3 dana značajno utjecala ($P < 0,05$) na masu jaja, valivost, razvoj embrija i masu pilića. Ustanovljeno je da u jaja Rhode Island Red kokoši produženo vrijeme skladištenja dovodi do smanjenja mase bjelanjka, mase žumanjka, indeksa bjelanjka, indeksa žumanjka i vrijednost Haughove jedinice. Zaključeno je da jaja kokoši Rhode Island Red ne bi trebalo skladištiti dulje od 3 dana.

Gljučne riječi: kokoš, Rhode Island Red, kvaliteta jaja, gubitak mase jaja, valivost
