

Research Note: Storage period and prewarming temperature effects on synchronous egg hatching from broiler breeder flocks during the early laying period

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ABSTRACT The effects of the storage period and prewarming temperature on embryonic mortality, hatchability, and synchronous hatching of broiler eggs were investigated. Eggs were obtained from commercial flocks of Ross 308 broiler breeders at 27 and 28 wk of age for trials 1 and 2, respectively. In both trials, 2,400 eggs were stored for 4 d (short) or 11 d (long) at 18°C (64.4°F) and 75% RH and were randomly assigned to 2 groups at either a prewarming temperature of 26.1°C (79°F, low) or 29.4°C (85°F, high) for 8 h before setting. The eggs were transferred from setters to hatching baskets at 444 h (18.5 d) of incubation. The hatched chicks were counted at 6-h intervals between 468 h and 516 h of incubation and categorized as early, middle, or late hatching. The eggs stored for 4 d hatched earlier than the eggs stored for 11 d ($P < 0.05$). An increased prewarming temperature (29.4°C) resulted in a 1.0-h shorter incubation duration, but this difference was not significant ($P = 0.064$). An interaction between the storage period and prewarming temperature was observed for middle- and late-hatched chicks ($P < 0.05$). No interactions between the storage

period and prewarming temperature were observed for hatchability of fertile eggs or embryonic mortality; however, a significant interaction was found between the storage period and prewarming temperature on the second-quality chick percentage ($P < 0.05$). The eggs stored for 11 d had a significantly reduced hatchability of fertile eggs owing to increased embryonic mortality than short-stored eggs ($P < 0.05$). The interaction effect indicated that eggs held for 8 h with prewarming at 29.4°C after 11 d of storage had more middle- and fewer late-hatched chicks and improved chick quality than those that received the 26.1°C prewarming treatment ($P < 0.05$), but no significant difference was found among the prewarming treatments for eggs stored for 4 d. This study demonstrated that prolonged egg storage resulted in reduced hatchability, increased incubation duration, and an asynchronous hatching time. Moreover, increasing the prewarming temperature could be used to promote uniformity among embryos through synchronous hatching, thus improving broiler flock uniformity and performance of the prolonged stored eggs.

Key words: prewarming temperature, egg storage, synchronous hatching, embryonic mortality, early laying period

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INTRODUCTION

Broiler hatching eggs can be stored at temperatures lower than ambient temperature before incubation, which is a common practice in commercial breeding farms and hatcheries. As per the variable market demand for 1-day-old parent stock chicks and the hatchery capacity, the duration of egg storage varies. Normally, commercial hatcheries set their eggs after 3 to 5 d of

storage to minimize the negative effects of prolonged storage on hatchability and chick quality. Hatching eggs stored longer than 7 d had decreased hatchability (Elibol and Brake, 2008; Nasri et al., 2020), decreased chick quality (Tona et al., 2003a; Reijrink et al., 2009), and increased hatching time (Mather and Laughlin, 1976; Shiranjang et al., 2018). Hatching eggs from younger flocks have also been most often subjected to extended storage on a routine basis owing to the combined effects of low egg production and less-than-minimum hatching egg weight, such that extra time was required to accumulate sufficient numbers for setting (Gucbilmez et al., 2013). Moreover, eggs from flocks during the early laying phase have a reduced surface area relative to egg volume, which results in an increased duration of incubation (Brake, 1996).

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Similarly, Hudson et al. (2004) reported that the mean incubation length was greatest among eggs from breeders aged 29 wk and decreased as the flocks grew older.

Temperature is one of the most important environmental factors before and during the incubation of hatching eggs (Decuyper and Michels, 1992). Reduced temperature during egg storage enables embryo survival until incubation begins (Fasenko, 2007). At the onset of incubation, eggs need to be gradually warmed from the storage temperature to the incubation temperature (Molenaar et al., 2010). Therefore, the effects of prewarming before incubation on embryonic mortality and hatchability after prolonged egg storage have been investigated, but the optimum prewarming temperature or duration after different storage periods for improved hatchability is largely unknown. Renema et al. (2006) reported that prewarming has been used to promote uniformity among embryos before setting. In addition, gradual prewarming prevents condensation on eggs before incubation (Reijrink et al., 2010) and temperature shock to embryos (Hodgetts, 1999). Moreover, Reijrink et al. (2010) and van Rooyert-Reijrink et al. (2018) reported that gradual prewarming is beneficial for hatchability during prolonged storage times and that a prewarming profile higher than 29.4°C (84.92°F) reduces early embryonic mortality in prime breeder flock eggs; however, some authors have suggested that eggs from younger breeder flocks should be rapidly warmed to the desired prewarming temperature (Elibol et al., 2009) or incubation temperature (Wilson, 1990; Renema et al., 2006) because a prolonged time at temperatures lower than 35°C may increase embryonic mortality or abnormal embryonic development (Wilson, 1990; Renema et al., 2006). In general, prewarming of hatching eggs before setting is assumed to be beneficial when the storage time is extended (Mayes and Takeballi, 1984). For example, eggs stored for 21 or 28 d were more responsive to prewarming treatments known to improve hatchability than eggs stored for shorter periods (Becker and Bearnse, 1958).

The prewarming temperature is reported to be between the storage temperature (12°C–18°C) and the incubation temperature (37.8°C). The duration of the prewarming stage varies from 3 h to 24 h as per previous reports, which determined the effects of prewarming after different storage periods when compared with directly setting eggs in the incubator after lay (Elibol et al., 2002; Piestun et al., 2013). In addition, the prewarming duration (Elibol et al., 2009; Kamanli et al., 2009; Yousaf et al., 2017) and temperature have been investigated (Meijerhof et al., 1994; Kamanli et al., 2009; Lin et al., 2017). However, a lack of information on the effect of prewarming after extended egg storage on the hatching time and synchrony remains. The objective of the present study was to determine the effects of both the storage period and the prewarming temperature before incubation on embryonic mortality, fertile hatchability, and hatching synchronization. Because young flocks produce eggs with low hatchability and extended incubation

duration (Bruzual et al., 2000), this study investigated the effects of prewarming and storage on eggs laid during the early laying period (27–28 wk).

MATERIALS AND METHODS

The experimental procedures used in this study were approved by the Ankara University Institutional Animal Care and Use Committee (Ankara, Turkey) and were in compliance with recommended guidelines.

Broiler Breeder Flocks and Egg Collection

Eggs from 2 commercial Ross 308 broiler breeder flocks fed with the same diet, subjected to identical management schemes, and reared in the same geographic area were used. Hatching eggs were collected manually from the flocks at ages of 27 and 28 wk for trials 1 and 2, respectively. For each trial, 4,800 eggs laid within 1 h were collected and transferred in a climate-controlled vehicle to a hatchery storage room (Erpiliç, Bolu, Turkey) within 2 h after collection.

Egg Storage and Prewarming Treatments

Each trial was a 2 × 2 factorial design consisting of 2 storage periods (4 d, short and 11 d, long) and 2 prewarming temperatures: low (LO; 26.1°C [79°F]) and high (HI; 29.4°C [85°F]). For each storage period, 2,400 eggs were stored for the appropriate number of days at 18°C and 75% RH in the hatchery. The stored eggs were randomly assigned to 2 identical Petersime Model 576 setters (Petersime, Zulte, Belgium) that were prewarmed at LO or HI temperature for 8 h and 70–72% RH before setting. After the prewarming period, both incubators were operated at an air set temperature of 38.1°C for the first 10 h of the incubation period. All eggs were held horizontal to the floor and not turned during the storage or prewarming period. The air temperature and RH were verified using 174H Dataloggers (Testo, Lenzkirch, Germany) during the storage and prewarming periods.

Incubation

For each trial, the eggs were assigned to randomized trays holding 150 eggs each and transferred to a single incubator (model 576; Petersime, Zulte, Belgium) at 10 h of the incubation period. Moreover, extra egg trolleys collected from same flock were placed with the experimental eggs to ensure uniform airflow in the machine. A single-stage incubation program was used with a machine set-point temperature gradually decreasing from 38.1 ± 0.2°C on embryonic d 1 (E1) of incubation to 37.5°C ± 0.2°C on E18.5. The eggs were turned through 90° on an hourly basis until E18.5 of incubation, and the RH was maintained at 53 ± 2% from E1 to E18.5. The eggs were then transferred to a single hatcher (model 192; Petersime, Zulte, Belgium), which had a dry bulb temperature of 37.2°C at E18.5 that was gradually decreased to 36.4°C ± 0.2°C at E21. Each tray of 150

eggs served as a replicate, and 8 replicate trays were used for each prewarming treatment after each storage period. The machines were monitored 4 times daily for proper operation.

Hatch Time Determination

From 486 h of incubation onward, hatched chicks were counted and recorded individually every 6 h to calculate the incubation duration and hatch synchronization for each treatment. The hatching process was divided into 3 time periods: early (468–480 h), middle (486–498 h), and late (504–516 h). These periods were calculated as a percentage of total hatched chicks for each group. All chicks that had completed the hatching process were removed from the hatching baskets at 516 h of incubation.

Embryonic Mortality, Hatchability, and Second-Grade Chicks

Infertile eggs and early embryonic mortality (0–6 d) were identified by candling eggs at E10, followed by removal and macroscopic identification of the opened eggs. After the chicks were removed from the hatcher at 516 h of incubation, all the remaining unhatched eggs were opened and examined macroscopically by a single experienced blinded individual to determine the remaining embryonic mortality (middle [7–18 d], late [19–21 d plus pipped]) according to Özlü et al. (2019). Second-grade chicks were determined by experienced hatchery staff for each group upon removal of the chicks from the hatcher. The chicks were separated into 2 distinct groups according to Tona et al. (2004); good-quality chicks were clean, dry, and free from defects, whereas chicks with unhealed navels, leg deformities, or other defects that would hinder sell were denoted as poor quality. Hatchability was calculated as the number of good-quality chicks (as determined previously) hatched based on the number of fertile eggs in each tray.

Statistical Analysis

The study consisted of a 2×2 factorial design with 2 storage periods and 2 prewarming temperatures. The data of both trials were analyzed together using the GLM procedure in SAS (SAS version 9.1; SAS Institute, Cary, NC). The model used to analyze the effects of hatch time, fertility, hatchability of the fertile eggs, embryonic mortality, and second-quality chick percentage was as follows: $Y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + e_{ijk}$, where Y_{ijk} is the dependent variable, μ is the overall mean, S_i is the storage period ($i = 4$ d, short or 11 d, long), P_j is the prewarming temperature ($j = 26.1^\circ\text{C}$ [79°F] or 29.4°C [85°F]), $(SP)_{ij}$ is the interaction between the storage period and the prewarming temperature, and e_{ijk} is the error term. Differences in the percentages of hatched chicks were determined using the Minitab 14.0 (Minitab Inc., State College, PA) packet program incorporating the 2P application. The Z-Test function was used to determine the existence of differences between 2

proportional values for the time intervals (early, middle, and late). Statements of statistical significance were based on $P \leq 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

Incubation Period and Hatching Synchrony

The eggs subjected to an extended storage period exhibited significantly longer incubation duration (Table 1; $P < 0.05$). Tona et al. (2003b) reported that a longer storage period prolonged the incubation period by at least 15 h in eggs stored for 18 d compared with eggs stored for 3 d, but the hatching time distribution was unaffected by the storage time (Tona et al., 2003a). Similarly, Mather and Laughlin (1976) found that storing eggs for 14 d increased the mean incubation period by 13.4 h (0.96 h/d of storage) but had no effect on hatch synchrony. In the present study, the eggs stored for 11 d had an increased incubation duration by approximately 4 h (497.6 vs. 493.4 h; $P < 0.05$). Moreover, an increased prewarming temperature reduced the incubation duration by 1.0 h compared with a reduced prewarming temperature, but this difference was not significant ($P = 0.064$). The percentage of early- and middle-hatched chicks was significantly greater in eggs stored for 4 d, and the percentage of late-hatched chicks was significantly greater in eggs stored for 11 d (Figure 1; $P < 0.05$). These results are consistent with those recently reported by Shiranjang et al. (2018), which indicated that eggs stored for 4–5 d hatched earlier than those stored for 11–12 d, and the percentage of late (492–514 h of incubation)-hatched chicks was significantly greater in eggs stored for 12 d than for 5 d (54.8 vs. 14.6%, respectively).

Increasing the prewarming temperature improved hatching synchronization. The percentage of chicks hatched in the middle time period was significantly greater in the HI prewarming treatment ($P < 0.05$). Furthermore, a significant interaction was found between the storage period and the prewarming temperature on the percentages of middle- and late-hatched chicks observed in the present study (Figure 1). This interaction indicated that eggs held at the HI prewarming temperature after 11 d of storage had a greater percentage of middle- and a reduced percentage of late-hatched chicks than eggs held at the LO prewarming treatment, but no significant difference was found among the prewarming treatments after the 4-d storage period. The greatest percentage of late-hatched chicks was observed from the eggs held at the LO (26.1°C) prewarming temperature after a 11-d storage period ($P < 0.05$). Similarly, Elibol et al. (2002) showed that prewarming eggs for 10 or 18 h in a setter kept at 26°C after a 14-d storage period resulted in a lower percentage of late-hatched chicks than no prewarming treatment. Furthermore, no difference was found in the percentage of late-hatched chicks owing to the prewarming treatment for eggs stored for 1 d. The present study demonstrates that an elevated prewarming temperature for

Table 1. Effects of the storage period and prewarming temperature on hatchability of fertile broiler eggs; early, middle, and late embryonic mortality; cull chicks; and average broiler hatching time in 2 trials.

Item	n ¹	Hatchability of fertile eggs	Embryonic mortality			Cull ²	Hatch time
			Early	Middle	Late		
Storage period			(%)				(h)
Short (4 d)	32	87.3 ^a	6.7 ^b	0.8 ^b	4.0 ^b	1.2 ^b	493.4 ^b
Long (11 d)	32	77.8 ^b	11.8 ^a	1.7 ^a	6.9 ^a	1.8 ^a	497.6 ^a
SEM		0.45	0.38	0.15	0.33	0.09	0.40
Prewarming temperature							
26.1°C (79°F)	32	82.3	9.6	1.2	5.2	1.7 ^a	496.0 ^x
29.4°C (85°F)	32	82.8	9.0	1.3	5.6	1.4 ^b	495.0 ^y
SEM		0.45	0.38	0.15	0.33	0.09	0.40
Storage period/prewarming temperature							
Short/26.1°C	16	86.8	7.3	0.8	3.9	1.2 ^b	493.8
Short/29.4°C	16	87.7	6.2	0.8	4.0	1.3 ^b	493.1
Long/26.1°C	16	77.8	11.8	1.6	6.5	2.2 ^a	498.3
Long/29.4°C	16	77.8	11.8	1.8	7.2	1.5 ^b	496.9
SEM		0.65	0.53	0.21	0.46	0.10	0.56
P value							
Storage period		<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Prewarming temperature		0.487	0.280	0.723	0.392	0.014	0.064
Storage period/prewarming temperature		0.458	0.363	0.581	0.524	0.002	0.600

^{a,b}The means in a column with different superscripts differ significantly ($P < 0.05$).

^{x,y}The means in the average hatch time column with different superscripts differ significantly ($P = 0.064$).

¹Each group contained 8 replicate trays of 150 eggs in each trial (a total of 16 trays or 2,400 eggs).

²The cull indicated the percentage of second-quality chicks.

eggs stored as long as 11 d can improve hatch synchronization by reducing the number of late-hatched chicks.

Hatchability and Embryonic Mortality

No interaction between the storage period and the prewarming temperature was observed for hatchability of fertile eggs or embryonic mortality (Table 1). Long-stored eggs had a significantly reduced hatchability of fertile eggs owing to increased embryonic mortality compared with short-stored eggs ($P < 0.05$). The effect of the storage period on hatchability and embryonic mortalities may have been confounded by the 1-wk difference in the breeder flock age. The effect of pullet

maturity on fertility and hatchability of eggs, in addition to diminished fertility, and more early embryonic deaths during the first week of laying were observed (Sunde and Bird, 1959). Similarly, early dead percentages were significantly different between 4-d and 11-d storage periods in the present study (6.7 vs. 11.8%, respectively); however, the prewarming temperature did not affect the hatch results ($P > 0.05$). Likewise, Kamanli et al. (2009) reported no significant difference among the hatch results owing to the prewarming temperature treatments (24°C vs. 28°C) after an 8-d storage period of eggs from a 44-wk-old layer breeder flock. In another study, Lin et al. (2017) observed minimum effects of the prewarming temperature (23.9°C vs. 29.4°C) on

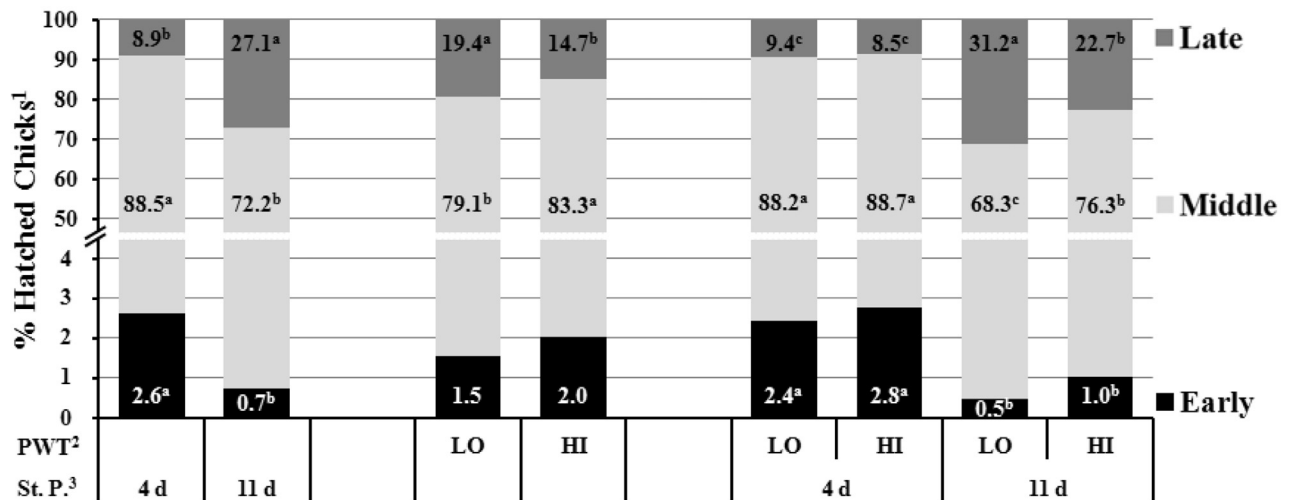


Figure 1. Effects of the storage period and prewarming temperature on the hatched chick percentage. ¹The early hatch time was 468–480 h, the middle hatch time was 486–498 h, and the late hatch time was 504–516 h. ²Prewarming temperature: LO = low (79°F; 26.1°C), HI = high (85°F; 29.4°C). ³Storage period: 4 d = short, 11 d = long. ^{a-c}The percentages in a main factor (storage period or prewarming temperature) or interaction groups (4 groups) with different superscripts differ significantly ($P < 0.05$).

broiler embryo development, live performance, and broiler carcass characteristics after using eggs stored for 1 d. However, Meijerhof et al. (1994) collected eggs from flocks of 2 ages (37 wk and 59 wk) and prewarmed the eggs at 27°C for 16 h compared with prewarming at 20°C for the same period. The author suggested that egg hatchability from prime breeder flocks was not significantly influenced by prewarming but was significantly reduced by prewarming at 27°C from older flocks.

Elibol et al. (2009) suggested a rapid increase to the prewarming temperature, whereas Reijrink et al. (2010) and van Roover-Reijrink et al. (2018) conversely suggested a prolonged prewarming profile. The latter suggestion was based on the observed reduction of early embryonic mortality and improved fertile hatchability for young flocks. Nevertheless, previous investigators have hypothesized that a longer prewarming period could be beneficial for young and prime flocks because the early stages of embryonic development would be observed more frequently in younger breeder flocks (Özlü et al., 2018a; Pokhrel et al., 2018). Heating eggs during extended storage significantly increased fertile hatchability in eggs from younger breeder flocks but not in those from older broiler breeder flocks (Gucbilmez et al., 2013).

Second-Quality Chick Percentage

A significant interaction of the storage period and the prewarming treatment was found for the percentage of poor-quality chicks ($P < 0.05$). In a study conducted by Ebeid et al. (2017), the percentage of good-quality chicks was greater for eggs stored for 4 d than for those stored for 14 d, and Elibol et al. (2002) reported that eggs subjected to extended storage (14 d vs. 1 d) resulted in a greater number of poor-quality chicks. In the present study, the interaction effect indicated that the eggs that were prewarmed at 29.4°C (HI) after a 11-d storage had fewer poor-quality chicks than eggs subjected to the 26.1°C (LO) prewarming treatment ($P < 0.05$); however, no significant differences were found among the prewarming treatments for eggs stored for 4 d ($P > 0.05$). These data indicate that eggs stored for longer periods may be more responsive to prewarming practices.

Previous studies reported that prolonged storage resulted in an increase in the incubation time (Elibol et al., 2002; Reijrink et al., 2010; Dymond et al., 2013) and that egg storage caused some live chicks to be rejected only because they hatched later (Nicholson, 2012). In addition, chick quality and broiler live performance were reduced in late-hatched chicks compared with early- and middle-hatched chicks (Özlü et al., 2018b). Therefore, the overall results of the present study indicate that higher prewarming temperatures could be used as a method to decrease late-hatching chicks and improve overall chick quality in eggs stored for a long period.

One-day-old chicks are the ultimate product of hatcheries and supply broilers for live production. Hatcheries therefore strive to obtain maximum hatchability of

salable chicks within a narrow hatching interval (Willemsen et al., 2010). As the hatching interval increases, so does the number of chicks that are deprived of feed and water for a prolonged period of time (Careghi et al., 2005), which can result in lasting performance deficits that diminish broiler production. The present study demonstrated that prolonged egg storage, which is a necessity for high-volume hatcheries, resulted in reduced fertile hatchability, longer incubation duration, and asynchronous hatching time compared with short storage; however, increasing prewarming temperatures for 8 h after a 11-d storage period mitigated some of these effects. Specifically, fewer late-hatched and poor-quality chicks were found despite no difference in fertile hatchability of eggs set from young flocks. Increased prewarming temperatures could be used to promote uniformity among embryos by synchronizing the hatching time in eggs collected from young flocks and stored for long periods. Further research should investigate eggs from older flocks stored for longer than 11 d as these are likely affected similarly by increased prewarming temperatures.

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DISCLOSURES

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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