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# **Effects of Egg Storage Duration on Hatching Traits of Commercial Layers and Crossbreds of Indonesian Local Chickens**

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#### ABSTRACT

Egg storage duration is highly critical to hatching performance and chick development. The objective of the present study was to assess the impact of different egg storage durations on the hatching traits of crossbred chickens. In total, 128 hatching eggs were used in this experiment. The eggs were randomly distributed into four treatments and four replications, with each replication consisting of eight eggs. The treatments were based on the storage duration of the hatching eggs, including one day (T1), four days (T2), seven days (T3), and ten days (T4). Hatching performance was evaluated by fertility, the hatchability of fertile eggs, the hatchability of set eggs, and embryonic mortality. Day-old chickens were evaluated by chicken length, chicken weight, hatch yield, pasgar score, and saleable chickens. The present study's findings indicated that egg storage duration up to ten days did not significantly influence the hatchability of fertile eggs or embryonic mortality. However, egg storage duration significantly affected the fertility and hatchability of set eggs. Eggs stored for four and seven days tended to have improved fertility and hatchability. Additionally, hatching eggs stored for ten days had the lowest fertility and hatchability of set eggs. Body weight, body length, hatch yield, and pasgar score of chickens were not significantly different among all treatments. No significant differences among T1, T2, and T3 were observed in saleable chickens. However, T4 had significantly fewer saleable chickens compared to T1, T2, and T3. It can be concluded that the hatching eggs of crossbred chickens can be safely stored for up to seven days without adverse effects on hatching traits. Extended storage beyond this period, specifically up to 10 days, could worsen key hatching performance traits of crossbred chickens.

Keywords: Crossbred chicken, Hatchability, Pasgar score, Saleable chick, Storage duration

# INTRODUCTION

The crossbreeding of Indonesian local chickens with commercial laying hens has recently attracted significant interest among Indonesian chicken meat consumers. Local chickens are widely known for their superior meat texture, distinctive flavor, and adaptability to local environmental conditions, which makes them highly valued in traditional markets (Tamzil et al., 2013). However, their slow growth rate and low productivity have limited their commercial viability (Sumantri et al., 2020). By incorporating genetic material from commercial layers into local chicken lines, poultry producers aim to develop a hybrid that preserves the desirable meat traits of indigenous breeds while enhancing growth performance and production efficiency (Ukhro et al., 2021). This unique crossbreeding program created a more flavorful and satisfying product than modern broilers, which many consumers appreciate, generating specific demand in the local market.

A reliable supply of day-old chickens is essential to support the rising demand for crossbred chickens. Hatcheries play a critical role in ensuring the steady production of high-quality chickens, but one of the significant challenges they face is the management of hatching eggs before incubation. Maintaining a consistent supply of hatching eggs often requires short-term storage, as eggs cannot always be incubated immediately after being laid. Logistically, balancing egg production with market demand necessitates appropriate storage protocols to preserve egg quality before incubation (Andri et al., 2023). The egg storage duration is critical because it significantly affects hatching performance and chick development. Long storage periods can lead to deteriorated egg quality, reduced hatchability, and altered characteristics of hatched chicks (Edi et al., 2018; Narinç and Aydemir, 2021). Therefore, managing egg storage duration is crucial in ensuring crossbred chickens' overall performance.

Although considerable studies exist on the effects of egg storage for broilers and layer breeds (Reijrink et al., 2010; Terčič and Pestotnik, 2016; Pokhrel et al., 2018; Martínez et al., 2021), the impacts of egg storage on crossbred chickens

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remain underexplored. Given the increasing interest in crossbred chickens in the Indonesian market, there is a need for more insights on optimizing hatching practices for crossbreeding poultry. Therefore, the objective of the present study was to assess the impacts of different egg storage durations on the hatching traits of crossbred chickens and commercial layers in Indonesia.

# MATERIALS AND METHODS

## **Ethical approval**

This study complied with the standard protocols for animal use in research as established by the Faculty of Animal Science, Universitas Brawijaya, Indonesia. Additionally, all procedures adhered to the regulations outlined in Law of the Republic of Indonesia Number 41 of 2014 on Livestock and Animal Health.

## Hatching eggs

The present study utilized hatching eggs from crossbreeding male Indonesian local chickens and female Lohmann laying hens. In total, 128 hatching eggs were used, with an average weight of  $60.19 \pm 4.68$  g and a coefficient of variation of 7.78%. The eggs were clean, free from any shell cracks, and dark brown in color. The eggs were disinfected using a 70% alcohol solution before being stored.

#### **Experimental design**

The present study employed an experimental approach featuring a fully randomized design. It included four treatments, each group replicated four times, with every replication consisting of eight hatching eggs. The treatments focused on the storage durations, specifically one day (T1), four days (T2), seven days (T3), and ten days (T4) for the hatching eggs.

#### Egg storage and incubation

The hatching eggs were arranged in setter trays with the blunt end oriented upwards. Eggs were stored at a room temperature of  $27.91 \pm 0.55$ °C and a relative humidity of  $55.39 \pm 4.88\%$  for the duration specified by each treatment. All eggs were incubated at a temperature of 37.8°C and a relative humidity of 55% for 516 hours. Eggs were rotated  $45^{\circ}$  every 60 minutes from days one to 18. Subsequently, eggs containing viable embryos, as indicated by candling, were positioned in hatcher trays without any rotation until the end of the incubation period.

## Hatching performance

The evaluation of hatching performance was made according to fertility (%), hatchability of fertile eggs (%), hatchability of set eggs (%), and embryonic mortality (%). Fertility evaluation was performed by candling after seven days of incubation. Fertility was determined by Formula 1 (Yulianti et al., 2023).

Fertility (%) = (number of fertile eggs/numbers of set eggs) x 100% (Formula 1) A hatchability assessment was conducted following 21.5 days of incubation. The hatchability of fertile eggs was determined using Formula 2 (Ayeni et al., 2020).

Hatchability of fertile eggs (%) = (number of natched eggs/numbers of fertile eggs) $\times$ 100% (Form	11a Z)
The hatchability of set eggs was determined using Formula 3 (Goliomytis et al., 2015).	
Hatchability of set eggs (%) = (number of hatched eggs / total number of incubated eggs) x 100% (Form	ula 3)
Embryonic mortality was determined using Formula 4 (Andri et al., 2024).	

Embryonic mortality (%) = (number of dead embryos/numbers of fertile eggs) x 100% (Formula 4)

#### Day-old chicken development

The evaluation of day-old chicken development was made according to chicken length (cm), chicken weight (g), hatch yield (%), pasgar score, and saleable chickens (%). Chicken length (cm) was measured with a ruler from the beak tip to the toe tip (Goliomytis et al., 2015). Chicken weight (g) was assessed by weighing chickens with a digital scale following their removal from the incubator (Ayeni et al., 2020). Hatch yield (%) was determined using Formula 5 (Andri et al., 2024).

Hatch yield = (chick weight / initial egg weight) x 100%

(Formula 5)

The pasgar score was determined by evaluating the criteria of activity, navel condition, leg structure, and beak condition of the chickens (Boerjan, 2006). Saleable chickens were considered to exhibit clean and dry feathers and were devoid of deformities. The calculation of saleable chickens was determined by Formula 6 (Yulianti et al., 2023).

Saleable chickens = (number of saleable chicks/numbers of fertile eggs) x 100% (Formula 6)

## Data analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) with a significance level of p < 0.05. Duncan's multiple range test was implemented as a post hoc assessment. The data for each parameter were displayed as means with standard deviations. All statistical analyses were conducted using IBM SPSS version 25 (2017).

# **RESULTS AND DISCUSSION**

## Hatching performance

The effect of egg storage duration on the hatching performance of crossbred chickens was presented in Table 1. The findings indicated that an egg storage duration of up to 10 days did not significantly influence the hatchability of fertile eggs or embryonic mortality (p > 0.05). Egg storage duration significantly affected the fertility and hatchability of set eggs (p < 0.05). Eggs stored for four and seven days (T2 and T3) showed a tendency of improved fertility and hatchability compared to those stored for one day (T1). Additionally, storage of hatching eggs for 10 days (T4) resulted in a significant reduction in both fertility and hatchability of set eggs when compared to T2 and T3 (p < 0.05). In a previous study, Abioja et al. (2021) revealed that the length of storage had a notable impact on the fertility and hatchability of set eggs from FUNAAB-alpha chickens. Specifically, decreased fertility and hatchability were observed when storage exceeded eight days. Similarly, Terčič and Pestotnik (2016) observed that hatching eggs stored for nine days or more could reduce the hatchability of set eggs. Moreover, Pokhrel et al. (2018) reported a significant decrease in hatchability when eggs were stored beyond seven days.

The current study indicated decreased fertility and hatchability of set eggs when stored for 10 days. These findings might be related to the structural changes in the vitelline membrane, chalazae, and storage duration. The vitelline membrane was essential for maintaining yolk integrity, while the chalazae stabilize the yolk in the central position of an egg (Hasan and Khaliduzzaman, 2022). Prolonged storage may weaken the vitelline membrane, leading to yolk rupture, which could subsequently put the embryo at risk of physical harm (Adriaensen et al., 2022). Likewise, long-term storage also diminishes the effectiveness of the chalazae in stabilizing the yolk, potentially resulting in yolk displacement and reduced internal egg stability (Nasri et al., 2020a). The deterioration of the vitelline membrane and chalazae was assumed to create a suboptimal environment for early embryonic development. The observed reduction in the fertility and hatchability of set eggs in the present study could be attributable to alterations in albumen pH. The diffusion of carbon dioxide from the egg increases albumen pH (Martínez et al., 2021). Since the blastoderm was located next to the albumen, the embryo's viability in the earliest stages of development could be affected by changes in albumen pH.

Variables	T1	T2	Т3	T4	p value
Fertility (%)	$81.25\pm7.22^{ab}$	$96.88\pm6.25^{b}$	$96.88\pm6.25^{b}$	$62.50\pm20.41^{\text{a}}$	0.004
Hatchability of fertile eggs (%)	$72.02\pm17.21$	$80.80 \pm 12.23$	$75.00\pm17.68$	$54.05\pm20.52$	0.200
Hatchability of set eggs (%)	$59.38\pm18.75^{ab}$	$78.13 \pm 11.97^{b} \\$	$71.88 \pm 11.97^{b}$	$34.38\pm15.73^{a}$	0.006
Embryonic mortality (%)	$27.98 \pm 17.21$	$19.20\pm12.23$	$25.00\pm17.68$	$45.95\pm20.52$	0.200

Table 1. Effect of egg storage duration on the hatching performance of crossbred chickens

<sup>ab</sup> Mean values with different superscript letters indicate significant differences (p < 0.05) in a row. T1: Eggs stored for one day, T2: Eggs stored for four days, T3: Eggs stored for seven days, T4: Eggs stored for 10 days.

The key finding in the current study was that optimal hatching performance was not achieved after one day of storage; instead, it was attained after four days of storage. The optimal hatching performance after four days of storage was likely linked to the physiological conditions of the egg contents. The albumen exhibited a dense consistency on the initial days of storage (Abioja et al., 2021). The dense consistency of the albumen may impede oxygen diffusion, adversely affecting hatching performance (Nasri et al., 2020b; Tainika et al., 2024). Subsequent days of storage led to a transformation in the albumen, transitioning from a viscous to a liquid state (Abioja et al., 2021). The liquefaction of the albumen was anticipated to remove barriers to oxygen diffusion, thus fostering an environment conducive to embryonic development and further improving fertility and hatchability (Nasri et al., 2020b; Tainika et al., 2024).

# Day-old chicken development

The effect of egg storage duration on the development of day-old chickens was presented in Table 2. The findings indicated that an egg storage duration of up to 10 days did not significantly influence body weight, body length, hatch

yield, or pasgar score for all groups (p > 0.05). No significant differences were observed in saleable chickens among T1, T2, and T3 (p > 0.05). However, T4 had significantly fewer saleable chickens than T1, T2, and T3 (p < 0.05). The decrease in saleable chickens in T4 was attributed to the prolonged storage of eggs, which can delay hatching and adversely affect the physical condition of chickens at hatch. Long-term egg storage can indeed delay chickens' hatching period (Reijrink et al., 2010; Bergoug et al., 2013; Dymond et al., 2013). The delayed hatching period results from slowed metabolic activity during storage, which could pause the initial stages of embryonic development (Nasri et al., 2020a). Consequently, embryos from eggs stored for extended periods usually need extra time to develop when incubated, leading to an extended hatching duration.

The delayed hatching period impacted the number of saleable chickens due to the tight timing between hatching and chicken pulling. This delay caused some chickens to miss the drying phase or lack the necessary vitality for the scheduled pulling time, as late-hatched chickens were more prone to having wet feathers and showing signs of weakness. These low-quality chickens could decrease the number of saleable chickens. Additionally, Andri et al. (2024) noted that a longer storage duration of hatching eggs could diminish the number of saleable chickens. In another study, Reijrink et al. (2010) found an increase in second-grade broiler chickens due to the prolonged storage for 13 days.

Variables	T1	T2	Т3	T4	p value
Body weight (g)	$39.81\pm2.02$	$40.60\pm1.79$	$39.70 \pm 0.90$	$39.67 \pm 1.23$	0.831
Body length (cm)	$16.12\pm0.48$	$16.59\pm0.27$	$16.08\pm0.06$	$15.81\pm1.21$	0.452
Hatch yield (%)	$68.45 \pm 1.25$	$66.37\pm2.06$	$66.38\pm2.58$	$65.57\pm0.79$	0.190
Saleable chicks (%)	$72.02\pm17.21^{a}$	$77.23\pm12.40^a$	$71.88\pm21.35^{a}$	$40.48\pm13.83^{b}$	0.023
Pasgar score	$9.31\pm0.32$	$9.54\pm0.25$	$9.42\pm0.10$	$9.54\pm0.42$	0.661

Table 2. Effect of egg storage duration on the development of crossbred chicken

<sup>ab</sup> Mean values with different superscript letters indicate significant differences (p < 0.05) in a row. T1: Eggs stored for one day, T2: Eggs stored for four days, T3: Eggs stored for seven days, T4: Eggs stored for 10 days.

# CONCLUSION

Crossbred chicken eggs can be stored for a maximum of seven days without negatively affecting hatching characteristics. Prolonged storage beyond this duration, particularly up to 10 days, was inadvisable as it diminishes essential hatching traits in crossbred chickens. Further research was needed to evaluate the long-term impact of egg storage duration on post-hatch chick development, growth performance, and overall productivity.

## DECLARATIONS

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#### Authors' contributions

Faizal Andri provided the study design, laboratory work, data analysis, manuscript review, and editing. Terisa Meta Nuraini conducted the laboratory work, data collection, and manuscript drafting. Muhammad Alfan Maslihan conducted the laboratory work, data collection, and manuscript drafting. Filoza Marwi composed and executed the study design, laboratory work, data analysis, and manuscript drafting. Eka Nurwahyuni provided the study design, laboratory work, data analysis, and manuscript drafting. Dyah Lestari Yulianti composed and executed the study design, data analysis, and manuscript drafting. Heni Setyo Prayogi provided the study design, data analysis, manuscript review, and editing. All the authors read and approved the latest edition of the manuscript.

## **Competing interests**

There are no competing interests to disclose.

#### **Ethical considerations**

The authors have reviewed the manuscript for ethical issues such as plagiarism, publication consent, misconduct, forgery, and/or data falsification, re-publication and/or submission, and redundancy.

## Availability of data and materials

The datasets in the present study are available from the corresponding author upon reasonable request.

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