

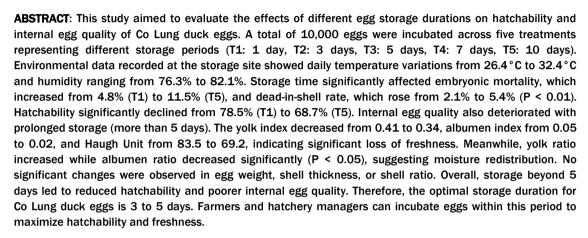
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# EFFECT OF EGG STORAGE DURATION ON HATCHABILITY AND EGG QUALITY OF CO LUNG DUCKS

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## INTRODUCTION

Among Vietnam's many indigenous poultry breeds, the Co Lung duck stands out for its adaptability and reproductive potential. This breed originated from Ba Thuoc District, Thanh Hoa Province, and has become regionally recognized for its quality meat and egg production (Ha and Mui, 2018). In addition to the farming of local duck breeds, many high-yielding poultry breeds, including exotic duck varieties, have been introduced and crossbred in various regions. This trend has led to genetic dilution and degradation of indigenous duck breeds (Cuc, 2010; Pham et al., 2021). Moreover, uncontrolled crossbreeding has contributed to the emergence and spread of infectious diseases. The Co Lung duck, in particular, is at risk of genetic erosion due to a lack of systematic conservation and investment at the local level. Without a clear and effective strategy for conserving, developing, and utilizing this genetic resource, the purebred Co Lung duck may eventually disappear as a distinct indigenous breed (Ha et al., 2020).

Duck eggs are an affordable and nutrient-rich food that play a significant role in the diet of many Asian populations. They contribute approximately 10% to 30% of the world's total egg consumption (Quan and Benjakul, 2019). While duck eggs are traditionally consumed in processed forms such as salted eggs, pidan, and balut, there has been a growing preference for consuming them fresh in recent years (Huang et al., 2007; Quan and Benjakul, 2019). However, studies focusing on the storage-related quality changes in duck eggs remain limited (Lokaewmanee, 2017; Quan and Benjakul, 2018). In contrast, numerous researches have focused on the quality deterioration of chicken eggs during storage (Liu et al., 2016; Brodacki et al., 2019; Yamak et al., 2021). Egg storage is an essential procedure in hatchery operations, allowing synchronization of incubation and flexibility in production scheduling. Effective incubation and hatchery management are critical for achieving high hatchability and ducklings' quality, while recent innovations in incubation systems have created new technological opportunities and raised broader ethical concerns regarding poultry breeding practices (Kasielke, 2020; Adame and Ameha, 2023; Underwood et al., 2021). However, prolonged storage can negatively affect internal egg quality, increase embryonic mortality, and reduce hatchability rates. Egg quality is assessed through several indicators, including egg weight, shape index, Haugh unit, albumen weight, yolk weight, and shell weight (Robert, 2004; Hisasaga et al., 2020; Nasri et al., 2020). According to Curtis et al. (1985), poultry breeds and lines selected for different production purposes exhibit variations in egg quality, which are correlated with both egg yield and weight. Therefore, selecting for egg quality traits may influence other production-related characteristics (Falconer and Mackey, 1996). Despite its importance, limited research has been conducted on how different durations of egg storage affect hatchability and egg quality in indigenous duck breeds under smallholder and non-industrial farm conditions. Therefore,

this study was designed to investigate the effect of various egg storage durations on hatchability, embryonic mortality, and selected egg quality traits in Co Lung ducks.

## MATERIALS AND METHODS

## Time and place of study

The experiment was conducted on 10,000 eggs of Co Lung ducks, collected from a duck farm located in Phong My commune, Dong Thap province of Vietnam, during the period from January to April 2024.

## Animals and experimental design

Co Lung ducks were raised in open-sided housing with corrugated metal roofs. The floor was covered with a 10 cm-thick layer of sand to enhance drainage and ventilation. The sides of the shed were enclosed with nylon mesh to block wind and insects. The ducks were fed a commercial layer diet containing 18% crude protein (CP) and 2,800 kcal/kg metabolizable energy (ME). Eggs were incubated using a fully automatic Mactech 5000 incubator (Mactech Technology Co., Ltd., Hanoi, Vietnam) with a capacity of 5,000 eggs per batch. The incubation conditions followed standard duck egg protocols: temperature ranged from 37.2 to 37.7°C and relative humidity from 75% to 80%. Eggs were automatically turned six times per day. From day 1 to 14, eggs were only turned; from day 15 to 32, turning was combined with cooling. The incubator was equipped with digital sensors for temperature and humidity to ensure consistent environmental control during the incubation period. The experiment was arranged in a completely randomized design (CRD) with five treatments corresponding to different egg storage durations, including T1 (1 day), T2 (3 days), T3 (5 days), T4 (7 days), and T5 (10 days). Each treatment was replicated five times, and each replicate represented an independent experimental unit. In each unit, 400 eggs were incubated to monitor hatchability parameters, and 50 eggs were sampled to assess egg quality. A total of 10,000 eggs were used for incubation, and 1,250 eggs were used for quality evaluation.

#### **Data collection**

Eggs were collected daily at 7:00 AM and 3:00 PM, and marked by date and treatment. During incubation, candling was conducted on day 6 (stage 1) to identify infertile and early dead embryos, and again on day 18 (stage 2) to record late embryonic mortality. After hatching was completed, the number of successfully hatched and dead-in-shell eggs was recorded for each replicate. Temperature and relative humidity at the egg storage site were measured using a Fluke 971 Temperature-Humidity Meter at five fixed time points: 6:00 AM, 9:00 AM, 2:00 PM, 6:00 PM, and 10:00 PM.

**Infertility rate (%):** Infertility rate = 
$$\frac{\sum infertile\ eggs}{\sum\ incubated\ eggs} \ x\ 100$$

**Embryonic mortality rate (%):** Embryonic mortality rate = 
$$\frac{\sum early\ dead\ embryos\ (day\ 6) + \sum late\ dead\ embryos\ (day\ 18)}{\sum fertile\ eggs}$$
  $x\ 100$ 

**Dead-in-shell rate (%):** Dead-in-shell rate = 
$$\frac{\sum dead \ in \ shell \ eggs}{\sum fertile \ eggs} \ x \ 100$$

**Hatchability rate (%):** Hatchability rate = 
$$\frac{\sum hatched\ eggs}{\sum\ fertile\ eggs} \ x\ 100$$

Egg shape Index (%): Shape index = 
$$\frac{egg \ width}{eggs \ length} \ x \ 100$$

**Yolk index:** Yolk index = 
$$\frac{Yolk\ height}{Yolk\ diameter} \times 100$$

**Albumen index:** Albumen index = 
$$\frac{Albumen\ height}{Albumen\ diameter} \ x\ 100$$

**Haugh unit (HU):** 
$$HU=100 \times log(H-1.7 \times W^{0.37}+7.57)$$

where: H: Albumen height (mm); W: Egg weight (g)

Yolk color: Determined using the Roche color fan (scale 1 to 15)

**Shell thickness (mm):** Measured at three locations (blunt end, equator, pointed end) using a micrometer; the final value was the average of the three measurements.

## Statistical analysis

The experimental data were initially processed using Microsoft Excel 2016 and then analyzed by analysis of variance (ANOVA) based on the general linear model (GLM) using Minitab version 16.0. Differences among treatment means were compared using Tukey's test at a 95% confidence level.

#### **RESULTS AND DISCUSSION**

## **Environmental conditions during egg storage**

Environmental conditions at the storage site are presented in Table 1. The recorded temperature showed a typical daily variation pattern, ranging from 26.4°C at 22:00 to a peak of 32.4°C at 14:00. Humidity fluctuated between 76.3% and 82.1%, with the lowest value also observed at 14:00. Excessively high humidity can inhibit proper water loss from the egg, while overly low humidity may lead to excessive evaporation, both of which can negatively impact embryo survival (Ibrahim et al., 2012). Embryonic development may be hindered when relative humidity levels are either too high or too low. Optimal growth is typically achieved when the surrounding humidity approaches a maximum level within the recommended range. These fluctuations in ambient conditions may influence the rate of egg quality deterioration and embryo viability, especially during prolonged storage periods. The range of temperature and humidity observed in this study was within tolerable limits for egg storage, although sustained exposure to temperatures above 30°C during the day might have accelerated water loss and albumen thinning, which can compromise hatchability and internal egg quality.

Table 1 - Environmental Conditions	During Egg Storage	
Time	Temperature (°C)	Humidity (%)
6:00	26.8	82.1
9:00	29.6	79.1
14:00	32.4	76.3
18:00	29.5	79.6
22:00	26.4	81.9

## Effect of storage time on hatching performance

The results presented in Table 2 indicate that while egg weight remained unaffected by storage time (P = 0.22), the duration of storage exerted a substantial influence on hatching performance and embryo viability in Co Lung ducks. Hatchability decreased significantly from 78.5% in T1 to 68.7% in T5 (P = 0.002), with the highest rates observed in eggs stored for 1 to 3 days (T1 and T2), and a marked decline evident from T3 onward. This reduction of nearly 10 percentage points underscores the negative impact of prolonged storage. Embryonic mortality followed a similar trend, increasing from 4.8% in T1 to 11.5% in T5 (P = 0.004), suggesting that the viability of developing embryos diminishes with longer storage periods. Dead-in-shell rates also rose significantly with time, from 2.1% in T1 to 5.4% in T5 (P = 0.009), possibly due to impaired gas exchange or shell membrane alterations. These results are consistent with previous findings that linked extended storage to declining hatchability and increased embryo loss (Pokhrel et al., 2018). Although the infertile egg rate varied from 9.8% to 15.3%, the difference was not statistically significant (P = 0.36), indicating that infertility may depend more on breeder performance than on storage duration. The observed trends in mortality and hatchability are supported by research showing that prolonged storage alters embryonic morphology and leads to blastodermal degeneration (Arora and Kosin, 1966; Reijrink et al., 2008). Additional physiological mechanisms may include elevated lipid peroxidation, which compromises embryonic development (Cherian et al., 2007), and degradation of the internal albumen environment. Studies have also reported that storing eggs beyond 7 to 10 days increases the risk of early and late embryonic death (Ombansılar et al., 2007; Onbaşılar, 2007), and even short-term storage of more than 3 days may negatively affect certain avian species such as golden pheasants (Kustra et al., 2020). On a cellular level, extensive investigations have identified apoptosis and necrosis as key contributors to reduced embryo survival during storage (Bloom et al., 1998; Fasenko, 2007; Hamidu et al., 2011), though some evidence suggests that these forms of cell death may arise from intrinsic embryonic mechanisms rather than storage duration or temperature. Furthermore, microbial contamination, particularly Salmonella, can affect egg safety and viability, as highlighted by Saitanu et al. (1994), who found that 12.4% of duck eggs in Thai markets carried Salmonella on the shell surface. Preventive measures such as egg washing, refrigeration, and thorough cooking have been recommended to mitigate such risks (Messens et al., 2011). Collectively, these findings reinforce the conclusion that limiting egg storage to less than one week is essential to maintain high hatchability and minimize embryonic loss in Co Lung ducks.

Table 2 - Effect of storage time on ha	tching perf	ormance					
Indicator	T1	T2	Т3	T4	T5	SEM	P value
Egg weight (g)	80.6	81.2	83.7	82.4	82.5	0.26	0.22
Infertile egg rate (%)	15.3	12.4	12.5	9.8	11.3	0.21	0.36
Embryonic mortality (%)	4.8c	6.1 <sup>bc</sup>	7.9 <sup>b</sup>	9.4ª	11.5a	0.52	0.004
Dead-in-shell rate (%)	2.1°	2.7bc	3.9 <sup>b</sup>	4.8a	5.4a	0.35	0.009
Hatchability (%)	78.5ª	77.1ª	73.4b	70.6bc	68.7°	0.61	0.002

a.b.c. Means within a column with different superscripts differ significantly (P<0.05). Storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days)

## Effect of storage time on internal egg quality

Internal egg quality characteristics were significantly influenced by the duration of storage, as indicated in Table 3. Egg weight ranged from 80.4 g in T1 to 83.8 g in T3 and did not show statistically significant differences among treatments (P = 0.41), suggesting that initial egg mass was consistent regardless of storage time. Similarly, the shape index remained unaffected (P = 0.52), with values fluctuating narrowly between 72.3% and 73.1%, reflecting uniformity in egg dimensions. However, several key quality traits declined with longer storage. The yolk index decreased significantly from 0.41 in T1 to 0.34 in T5 (P = 0.008), indicating weakening of the vitelline membrane, likely due to water migration from the albumen into the yolk during storage. This is consistent with observations by Onbaşilar et al. (2007), who found that dehydration during storage negatively affects albumen consistency and yolk integrity in Pekin ducks. The albumen index followed a similar downward trend, dropping significantly from 0.05 in T1 to 0.02 in T5 (P = 0.001), which suggests structural degradation and thinning of the albumen. The deterioration of albumen is closely related to the increase in pH over time.

Table 3 - Effect of storage time on internal egg quality							
Indicator	T1	T2	Т3	T4	T5	SEM	P value
Egg weight (g)	80.4	81.6	83.8	80.5	82.2	0.35	0.41
Shape index (%)	72.8	73.1	72.4	72.3	72.9	0.45	0.52
Yolk index	0.41 <sup>a</sup>	0.39a	0.36 <sup>b</sup>	0.35 <sup>b</sup>	0.34°	0.01	0.008
Albumen index	0.05ª	0.04a	0.03b	0.02c	0.02°	0.004	0.001
Albumen pH	8.1°	8.4bc	8.7b	8.9a	9.1a	0.06	0.001
Haugh Unit	83.5ª	80.4b	76.3 <sup>b</sup>	73.1 <sup>bc</sup>	69.2°	1.76	0.001
Yolk color (Roche scale)	11.4	11.2	10.9	10.6	10.5	0.29	0.09
Yolk ratio (%)	31.9	32.2	33.1	34.4	35.1	0.52	0.07
Albumen ratio (%)	57.3ª	56.5a	55.4⁵	54.1 <sup>b</sup>	52.8°	0.61	0.005
Shell ratio (%)	10.8	11.3	11.6	11.5	12.1	0.41	0.11
Shell thickness (mm)	0.41	0.39	0.40	0.41	0.40	0.004	0.06

a.b.c. Means within a column with different superscripts differ significantly (P<0.05). Storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days)

In this study, albumen pH rose from 8.1 on day 1 to 9.1 by day 10, consistent with the findings of Pereira et al. (2022), who reported a negative correlation between albumen pH and egg freshness. This pH increase is mainly attributed to the escape of carbon dioxide through eggshell pores, which disrupts the carbonic acid-bicarbonate buffering system within the albumen, making the environment more alkaline (Samli et al., 2005; Ragni et al., 2007; Shin et al., 2012). Yuceer and Caner (2014) explained that this alkalization leads to depolymerization of proteolytic enzymes, destabilizing the ovomucin-lysozyme complex, which causes the thick albumen to lose its gel-like consistency and become thinner. Brake et al. (1997) further emphasized that elevated storage temperatures can accelerate protein denaturation and moisture transfer from the albumen to the yolk, contributing to faster deterioration. These biochemical changes were reflected in the Haugh Unit (HU), a primary indicator of egg freshness, which declined sharply and significantly from 83.5 in T1 to 69.2 in T5 (P = 0.001). This substantial reduction of over 14 points supports the findings of Dassidi et al. (2022), who noted that eggs stored for 14 days exhibited significantly lower HU values, indicating compromised internal quality. Although yolk color, measured on the Roche scale, decreased slightly from 11.4 in T1 to 10.5 in T5, the change was not statistically significant (P = 0.09). Nonetheless, this trend may suggest pigment fading due to oxidative degradation or breakdown of carotenoids. Notably, the yolk color of Co Lung duck eggs in this study was markedly higher than the 5.1 reported for Beijing ducks by Denley et al. (2005), likely due to differences in dietary pigment intake between breeds. In terms of component proportions, the yolk ratio increased from 31.9% in T1 to 35.1% in T5, while the albumen ratio declined from 57.3% to 52.8%, with both showing significant differences (P = 0.005), indicating a redistribution of internal contents likely driven by moisture loss from the albumen and swelling of the yolk. The shell ratio varied between 10.8% and 12.1% but did not show a significant effect from storage time (P = 0.11), and shell thickness remained relatively stable between 0.39 and 0.41 mm (P = 0.06), indicating that external shell traits were not altered. The presence of a calcified eggshell serves as a protective barrier, shielding the egg from mechanical injury and reducing the risk of microbial infiltration (Hincke et al., 2011). Collectively, these findings confirm that internal egg quality progressively deteriorates with longer storage duration, especially under ambient or elevated temperatures. Similar observations were reported in multiple studies, which found that prolonged storage and higher temperatures significantly affect egg integrity (Huang and Lin, 2011; Pandian et al., 2012; Lokaewmanee, 2017; Quan and Benjakul, 2018, and 2019). Interestingly, Jones et al. (2018) demonstrated that refrigeration was more effective in preserving egg quality than washing or applying oil coatings. These patterns emphasize the importance of controlled storage conditions to maintain internal egg quality and extend shelf life.

#### CONCLUSION

This study demonstrated that prolonged storage of Co Lung duck eggs adversely affects hatching performance and internal egg quality. When eggs were stored for more than 5 days, embryonic mortality increased significantly from 4.8% (T1) to 11.5% (T5), and hatchability declined sharply from 78.5% to 68.7%. In terms of internal quality, the yolk index dropped from 0.41 to 0.34, and Haugh Unit decreased from 83.5 to 69.2 with longer storage duration. These results highlight that storage beyond 5 days leads to notable declines in egg viability and freshness. Therefore, to maintain optimal hatchability and internal quality, Co Lung duck eggs should be incubated within 3 to 5 days after laying. This finding provides practical guidelines for duck farmers and hatchery managers in Vietnam to improve productivity and conserve the genetic value of indigenous Co Lung ducks.

## **DECLARATIONS**

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#### **Data availability**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## **Ethical regulations**

Formal ethical approval was not required for this study because no invasive procedures were performed. All animal care, handling, and sample collection complied with the Law on Animal Husbandry (No. 32/2018/QH14) of the National Assembly of the Socialist Republic of Vietnam. Animal welfare was carefully monitored and maintained throughout the experimental period. In addition, the authors confirm that the study was conducted in accordance with the ARRIVE guidelines and the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education of the New York Academy of Sciences, Ad Hoc Animal Research Committee.

## **Authors' contribution**

Phan Nhan was solely responsible for the conceptualization and design of the study, experimental execution, data collection and analysis, as well as drafting and revising the manuscript. All aspects of this work were conducted independently by the author.

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## **Competing interests**

The authors have not declared any competing interests.

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