



Impact of Eggs Storage Time and Duration of Fumigation on Hatchability, Embryonic Mortality and Characteristics of Day Old Chicks

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ABSTRACT

This study aimed to investigate the effects of egg storage time and fumigation duration on chick quality, hatchability, and breakout analysis in broilers. A total of 600 eggs from each storage time (24 and 48 h) were divided into three fumigation groups (15, 20, and 25 min), with each group further split into four replicates of 50 eggs, resulting in six experimental groups: S24F15, S24F20, S24F25, S48F15, S48F20, and S48F25. The results indicated that egg storage times of 24 or 48 h and fumigation durations of 15, 20, and 25 min did not significantly affect most chick quality parameters, including egg weight, A-grade chicks, B-grade chicks, contamination, and water loss ($P>0.05$). However, chick weight was significantly higher ($P<0.05$) in the F15 group (44.4 ± 0.73 g) compared to the F20 (43.3 ± 0.47 g) and F25 (43.2 ± 0.58 g) groups. Similarly, chick yield was significantly higher ($P<0.05$) in the F15 group (68.8 ± 0.62 %) than in the F20 (67.9 ± 0.33 %) and F25 (67.5 ± 0.39 %) groups. Interaction effects showed significantly higher chick weight (44.8 ± 0.68 g) and chick yield (69.3 ± 0.38 %) in the S48F15 group compared to other groups. The breakout analysis revealed no significant effect ($P>0.05$) of egg storage time or fumigation duration on dead chicks at different weeks, dry chicks, contaminated chicks, and total unhatched eggs. Similarly, hatchability and the percentage of hatch of fertile eggs were not significantly affected ($P>0.05$) by egg storage time or fumigation duration, nor by their interaction. In conclusion, while most parameters remained unaffected, a fumigation duration of 15 min, especially with 48 h of egg storage, resulted in higher chick weight and yield, suggesting potential benefits for broiler production efficiency.

INTRODUCTION

Hatching eggs are stored in breeder farms and hatcheries to ensure an adequate supply for large incubators. The quality of day-old chicks is crucial for hatcheries and broiler producers (Aydin and Sozcu, 2013). High hatchability is important, but chick quality is equally significant

(Goliomytis *et al.*, 2015). Storing eggs for more than a week increases embryonic abnormalities and mortality due to the degradation of egg albumen viscosity (Petek and Dikmen, 2006). Hatchability decreases by 1% for each day of storage beyond 10 days (Tainika *et al.*, 2024). Fumigation is critical before transferring eggs to the incubator as microorganisms can penetrate the eggshell, potentially killing the developing embryo and reducing hatchability (Cadirci, 2009). An eggshell may initially contain 300 to 500 bacteria (Aygun, 2017), increasing to 20,000 to 30,000 bacteria within an h (Aygun *et al.*, 2012). Dirty eggs can harbor up to 80,000 bacteria (Mauldin, 1999). Formaldehyde is commonly used in poultry houses and for fumigating eggs and poultry litter (Saleh *et al.*, 2022).

Formaldehyde is the most common fumigant for eggs in commercial hatcheries (Bekele and Leta, 2016). Reduced

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early incubation ventilation improves hatchability, uniformity, and post-hatch performance but can cause formaldehyde to remain on the eggs' surface, potentially entering the egg and adversely affecting hatchability. The blastoderm, where the embryo develops, is located on the upper surface of the yolk (Biesek *et al.*, 2023). About three days before hatching, eggs are transferred from setters to hatchers. During this transfer, infections can start on the egg surface, penetrate the eggshell and membrane, hinder gaseous exchange, and become a source of infection, deteriorating chick quality. Fumigating eggs immediately after transferring to hatchers minimizes infection risk as the embryo becomes a direct-breathing animal (Cadirsi, 2009). Chick quality is influenced by factors such as storage time before incubation and the breeder's age (Tona *et al.*, 2005). Egg storage time and fumigation duration significantly impact hatchability. This study aims to investigate the impact of storage time and fumigation duration on egg hatchability, determine their effect on embryonic mortality during incubation, and evaluate their impact on the characteristics of day-old chicks.

MATERIALS AND METHODS

Experimental eggs

A total of 1200 hatching eggs (A grade, from Ross-308) used for experiment were obtained from broiler breeder farm, a flock on artificial insemination from jadeed farm (JF)-108. Out of 1200 total eggs, 600 eggs were stored for 24 h and 600 eggs were stored for 48 h before incubation. Out of 600 eggs from each egg storage time (24 and 48 h), 200 eggs were fumigated for 15 min, other 200 eggs were fumigated for 20 min, and the rest of 200 eggs were fumigated for 25 min. Each group was further divided into four replicates with 50 eggs per replicate (n=4; 50 egg/replicate). Thereby, eggs were distributed into 6 experimental groups. The groups were designated as S24F15, S24F20 and S24F25, for egg storage time of 24 h with fumigation duration of 15, 20 and 25 min, respectively. Similarly, groups S48F15, S48F20 and S48F25, were named for egg storage time of 48 h with fumigation duration of 15, 20 and 25 min, respectively. Main interaction effects were denoted as F15, F20 and F25 for fumigation duration at 15, 20 and 25 min, respectively, and S24 and S48 represented storage time of 24 and 48 h.

Eggs fumigation

All experimental eggs were fumigated through automatic system of fumigation, provided by EMTech. Eggs were fumigated while using 20g KMnO₄ with 40 ml (37% aqueous) formalin and 40 ml water for 100ft³.

To avoid the carcinogenic effects of formalin, a

computer-based technical fumigation system provided by EMTech was used. The fumigation process included several steps: Initially, Damper-1's room air inlet position was closed to bring fresh air into the fumigation room, and Damper-2's room air exhaust was closed to exhaust gas after fumigation. The fumigation room doors remained open, and the circulation fans (12 fans set up on the walls) and the extraction fan were kept off. During the next step, the fumigation room doors closed automatically to prevent any entrance. The fumigation process began once the doors were locked, with the circulating fans turning on a few seconds later. This step lasted 20 min, during which Damper-1 and Damper-2 remained closed, the fumigation room doors were locked, 12 circulation fans were on, the exhaust fan was off, and the heating room was maintained at 26°C. After fumigation, air extraction was required for 15 min. During this time, Damper-1 and Damper-2 were opened, the fumigation room doors were closed, circulation fans remained on, and the exhaust fan was turned on.

Eggs transfer and incubation

After fumigation, the eggs were shifted into a single-stage incubator for 458 h, including 8 h for pre-warming, divided into 11 stages. The temperature was maintained at 99.56°F with 55.5% humidity, and in the setter hall, it was 24°C with 45-50% humidity. Eggs were turned every h at a 45° angle. After 458 h, eggs were transferred to the hatcher. Before this, candling was performed to calculate fertility percentage. The total duration in the hatcher was 57 h, divided into 10 stages, with a temperature of 98.07°F and 79.83% humidity. After 507 h, chicks were pulled and graded.

Chick quality

Chick quality was determined by considering egg weight and chick weight.

For chicks grading A-grade chicks were recognized for having activity, shinning leg, shinning nose, bright eye, properly healed navel, and proper beak with no physical abnormalities. The B-grade chicks were graded for having any type of physical abnormality, mainly unhealed naval and any other physical abnormalities. Chick yield were obtained through the following formula.

$$\text{Chick yield (\%)} = \frac{\text{Average weight of chicks}}{\text{Average weight of fresh eggs}} \times 100$$

Contamination of eggs after fumigation was estimated using the following formula:

$$\text{Contamination \%} = \frac{\text{No. of eggs contaminated}}{\text{Total No. of eggs}} \times 100$$

For determining egg water loss (%) eggs were weighted before setting in the setter. At day 19, the eggs

before transferring from setter to hatcher were weighted once more. Egg water loss percentage were calculated.

$$\text{Egg water loss (\%)} = \frac{\text{Setting initial weight} - \text{Transfer egg weight}}{\text{Setting egg initial weight}} \times 100$$

Breakout analysis was performed of unhatched eggs and embryonic mortality. Data for dead chicks in shell at week 1, week 2 and week 3 were recorded and percentage of total eggs set was calculated. Similarly, data for dry chicks were recorded and percentage was calculated. No. of contaminated as well as infertile eggs and total un-hatched eggs were counted and percentages were determined of total eggs set in incubator.

Fertility was calculated by using following formula:

$$\text{Fertility (\%)} = (\text{Total fertile eggs} / \text{total eggs set}) \times 100$$

Hatchability was obtained by dividing total number of chicks hatched by total number of eggs set, multiplied by 100.

$$\text{Hatchability (\%)} = (\text{Total no of chicks hatched} / \text{total number of eggs set}) \times 100$$

$$\text{Hatch of fertile (\%)} = (\text{No. of chicks hatched} / \text{total fertile eggs}) \times 100$$

Statistical analysis

Data were collected on routine bases and were recorded on excel spreadsheets. For statistical analysis, a 3 × 2 factorial arrangement was used with 3 levels of fumigation duration (15, 20 and 25 min) and 2 levels of egg storage time (24 and 48 h). Different parameters were analyzed through ANOVA using the GLM procedure. The statistical package SPSS 20.0 (SPSS Inc., Chicago, IL) was used to analyze the data. Treatment means were ranked by Tukey as post-hoc test at $P \leq 0.05$. All parameter for chick quality, breakout analysis, as well as fertility and hatchability, were measured on the basis of pen as experimental unit.

RESULTS

Table I represents the effect of egg storage time and

fumigation duration on chick quality. Egg weight (g), A-grade chicks (%), B-grade chicks (%), contamination (%) and water loss (%) was not affected ($P < 0.05$) by egg storage time of 24 and 48 h, as well as fumigation duration for 15, 20 and 25 min. Chick weight (g) was not affected ($P > 0.05$) by egg storage time at 24 and 48 h; Chick weight (g) was significantly higher ($P < 0.05$) for F15 group (44.4±0.73) than F20 group (43.3±0.47) and F25 group (43.2±0.58). Similarly, chick yield (%) was not affected ($P > 0.05$) by egg storage time at 24 and 48 h; However, chick yield (%) was significantly higher ($P < 0.05$) for F15 group (68.8±0.62) in comparison with F20 group (67.9±0.33) and F25 group (67.5±0.39).

Interaction effect (EST x FD) of egg storage time (EST) and fumigation duration (FD) on chick quality has been shown in Table II. No significant differences ($P > 0.05$) were observed for interaction of egg storage time and fumigation duration on egg weight (g), A-grade chicks (%), B-grade chicks (%), contamination (%) and water loss (%). Chick weight (g) was significantly higher ($P < 0.05$) for S48F15 group (44.8±0.68) as compared to S24F20 group (43.4±0.54), S24F25 group (43.2±0.69), S48F20 group (43.3±0.47) and S48F25 group (43.2±0.55), with no differences for S24F15 group (44.0±0.56). Similarly, chick yield (%) was significantly higher ($P < 0.05$) for S48F15 group (69.3±0.38) in comparison with S24F15 group (68.3±0.36), S24F20 group (67.9±0.36), S24F25 group (67.5±0.50), S48F20 group (67.9±0.35) and S48F25 group (67.5±0.32).

Table III illustrates the effect of egg storage time and fumigation duration on breakout analysis. Egg storage time at 24 and 48 h and fumigation duration at 15, 20 and 25 min had no significant effect ($P > 0.05$) for various parameters of breakout analysis including dead chicks (%) at Wk-1, Wk-2, and Wk-3, dry chicks (%), contaminated chicks (%) and total un-hatched eggs (%).

Table I. Effect of egg storage time (EST) and fumigation duration (FD) on chick quality.

Variables	Egg storage time		Fumigation duration			SEM	P - value		
	24 H	48 H	15 Min	20 Min	25 Min		EST	FD	EST x FD
Group designation	S24	S48	F15	F20	F25				
Egg weight (g)	64.5	64.1	64.5	64.2	64.1	0.207	0.392	0.744	0.368
Chick weight (g)	43.5	43.8	44.4 ^a	43.3 ^b	43.2 ^b	0.162	0.342	0.001	0.238
% A-grade chicks	99.5	99.7	99.2	99.8	99.7	0.173	0.612	0.423	0.440
% B-grade chicks	0.52	0.34	0.77	0.26	0.26	0.175	0.622	0.425	0.425
% Chick yield	67.9	68.2	68.8 ^a	67.9 ^b	67.5 ^b	0.145	0.059	<0.001	0.027
% Contamination	0.17	0.17	0.25	0.25	0.00	0.115	1.000	0.615	0.250
% Water loss	11.7	11.7	11.6	11.7	11.8	0.066	0.737	0.510	0.212

The same row having different superscripts show significant difference at $\alpha=0.05$.

Table II. Interaction effects (EST x FD) of egg storage time (EST) and fumigation duration (FD) on chick quality.

Groups	S24F15	S24F20	S24F25	S48F15	S48F20	S48F25	SEM	P-value
Egg weight (g)	64.4	64.8	64.3	64.7	63.6	64.0	0.207	0.633
Chick weight (g)	44.0 ^{ab}	43.4 ^b	43.2 ^b	44.8 ^a	43.3 ^b	43.2 ^b	0.162	0.006
% A-grade chicks	99.5	99.5	99.5	99.0	100	100	0.173	0.591
% B-grade chicks	0.52	0.52	0.52	1.02	0.00	0.00	0.175	0.584
% Chick yield	68.3 ^b	67.9 ^b	67.5 ^b	69.3 ^a	67.9 ^b	67.5 ^b	0.145	<0.001
% Contamination	0.00	0.50	0.00	0.50	0.00	0.00	0.115	0.564
% Water loss	11.6	11.9	11.7	11.6	11.5	11.9	0.066	0.457

The same row having different superscripts show significant difference at $\alpha=0.05$.

Table III. Effect of egg storage time (EST) and fumigation duration (FD) on breakout analysis.

Variables	Egg storage time		Fumigation duration			SEM	P - value		
	24 H	48 H	15 Min	20 Min	25 Min		EST	FD	EST x FD
Groups	S24	S48	F15	F20	F25				
% Dead Wk-1	0.67	0.17	0.75	0.00	0.50	0.169	0.100	0.126	0.075
% Dead Wk-2	0.17	0.17	0.00	0.50	0.00	0.115	1.000	0.164	1.000
% Dead Wk-3	1.17	1.00	0.75	1.50	1.00	0.318	0.759	0.622	0.144
% Dry chicks	0.00	0.00	0.00	0.00	0.00	0.000	.	.	.
% Contaminated chicks	0.17	0.33	0.50	0.25	0.00	0.138	0.521	0.301	0.075
% Total un-hatched	2.17	1.67	2.00	2.25	1.50	0.390	0.534	0.753	0.203

Table IV. Interaction effects (EST x FD) of egg storage time (EST) and fumigation duration (FD) on breakout analysis.

Groups	S24F15	S24F20	S24F25	S48F15	S48F20	S48F25	SEM	P-value
% Dead Wk-1	1.50	0.00	0.50	0.00	0.00	0.50	0.169	0.052
% Dead Wk-2	0.00	0.50	0.00	0.00	0.50	0.00	0.115	0.564
% Dead Wk-3	0.50	2.50	0.50	1.00	0.50	1.50	0.318	0.407
% Dry chicks	0.00	0.00	0.00	0.00	0.00	0.00	0.000	.
% Contaminated chicks	0.00	0.50	0.00	1.00	0.00	0.00	0.138	0.164
% Total un-hatched	2.00	3.50	1.00	2.00	1.00	2.00	0.390	0.500

Table V. Effect of egg storage time (EST) and fumigation duration (FD) on hatchability (%).

Variables	Egg storage time		Fumigation duration			SEM	P - value		
	24 H	48 H	15 Min	20 Min	25 Min		EST	FD	EST x FD
Groups	S24	S48	F15	F20	F25				
% Hatchability	96.5	95.3	96.3	95.8	95.8	0.531	0.292	0.909	0.242
% Hatch of fertile	98.3	98.3	98.0	98.4	98.5	0.402	0.990	0.874	0.654

Table IV displays interaction effect (EST × FD) of egg storage time and fumigation duration on breakout analysis. Non-significant effect ($P>0.05$) was noted for interaction of egg storage time at 24 and 48 h with fumigation duration of 15, 20 and 25 min for different parameters of breakout analysis including dead chicks (%) at Wk-1, Wk-2, and Wk-3, dry chicks (%), contaminated chicks (%) and total

un-hatched eggs (%).

Table V reveals the effect of egg storage time and fumigation duration on hatchability (%) of eggs. The results revealed that egg storage time at 24 and 48 h and fumigation duration at 15, 20 and 25 min had no significant ($P>0.05$) effect on, hatchability (%) and % hatch of fertile in eggs.

Table VI. Interaction effect (EST x FD) of egg storage time (EST) and fumigation duration (FD) on hatchability (%).

Groups	S24F15	S24F20	S24F25	S48F15	S48F20	S48F25	SEM	P-value
% Hatchability	95.5	97.0	97.0	97.0	94.5	94.5	0.531	0.509
% Hatch of fertile	98.0	98.0	99.0	98.0	98.9	97.9	0.402	0.945

The same row having different superscripts show significant difference at $\alpha=0.05$.

The interaction effect (EST x FD) of egg storage time and fumigation duration on hatchability (%) has been demonstrated in Table VI. The % hatchability and % hatch of fertile was not affected ($P>0.05$) by interaction of egg storage time and fumigation duration.

DISCUSSION

The study explored optimal egg storage time and fumigation duration in hatcheries, noting limited comparative literature. Egg storage for 24 and 48 h, and fumigation durations of 15, 20, and 25 min, didn't significantly affect egg quality parameters. However, a 15-min fumigation duration yielded higher chick weight and yield, especially at 48-h storage. Interaction effects were observed only in chick weight and yield, indicating nuanced impacts of storage and fumigation.

The literature on egg storage and fumigation's impact on chick quality is varied. Some studies suggest prolonged storage leads to higher moisture loss and reduced egg viability (Demirel and Kirikci, 2009; Kozuszek *et al.*, 2009). Conversely, others find no influence on chick weight, potentially due to factors like temperature and breed (Petek *et al.*, 2003; Garip and Dere, 2005). Additionally, shorter storage periods may yield chicks with higher body weights (Sachdev *et al.*, 1988; Reis *et al.*, 1997). It's intriguing to observe that the current study recorded higher chick weight and yield with a short fumigation duration (15 min), especially when combined with a longer storage period (48 h), despite similar egg weights. It's widely recognized that egg weight influences chick weight post-incubation. For instance, Farooq *et al.* (2001) demonstrated that larger eggs tend to yield larger offspring, and vice versa. Hence, the precise reasons for these findings remain uncertain and warrant further investigation and confirmation.

The current study found that various egg breakout analysis parameters, such as dead chicks, dry chicks, contaminated chicks, and total unhatched eggs, remained unaffected by different egg storage times and fumigation durations. Interaction effects were also negligible. In contrast, literature suggests longer egg storage periods correlate with increased chick mortalities and decreased hatchability (El-Kazaz and Abo-Samaha, 2018; Schmidt *et al.*, 2009). Extended egg storage durations can lead

to increased embryonic mortality, with studies showing higher rates in eggs stored for longer periods (Elibol *et al.*, 2002; Petek *et al.*, 2005; Petek and Dikmen, 2006). Factors like storage time, hen age, breed, and environmental conditions influence embryonic viability, potentially causing morphological abnormalities and cell necrosis (Brake *et al.*, 1997). Longer storage also reduces albumen viscosity and height, impacting nutrient transfer and gaseous diffusion, which can decrease hatchability (Brake *et al.*, 1997). Shorter storage periods are associated with increased hatchability due to reduced albumen degradation and blastoderm proximity to the eggshell (Brake, 1996a).

The conflicting results in the current study regarding egg breakout analysis, including chick mortality, may be attributed to the comparatively shorter storage duration (up to 48 h) utilized, whereas most literature reporting opposing findings often examines much longer storage periods (up to 15 days). However, the current study's crucial findings indicating no significant effect of egg storage time (24 and 48 h) are essential for informing future investigations and guiding prevailing hatchery operations.

The outcome of present experiment displayed that egg storage time (24 and 48 h) and fumigation duration (15, 20 and 25 min) had no significant effect on hatchability (%) and % hatch of fertile in eggs. Similarly, interaction of egg storage time and fumigation duration did not show any changes for hatchability (%) and % hatch of fertile in eggs. The literature provides limited yet intriguing insights into the impact of egg storage duration on hatchability. While some studies, such as Petek *et al.* (2003) and Elibol *et al.* (2002), reported reduced fertility after storing eggs for over a day, others, like Nester and Nable (2000) and Smith (2000) found no significant effect on hatchability. Similarly, Mahmud *et al.* (2011) found no impact on hatchability when storing eggs for three days. Contrarily, Oluyemi and George (1972) noted increased hatchability after four to six days of storage. Additionally, Khan *et al.* (2014) observed decreased hatchability after storing eggs for over three days. Some studies, including Petek and Dikmen (2006), and Moreki and Ditshupo (2012), found higher hatchability rates with shorter storage durations. Longer storage periods may hinder embryo development or slow it significantly (Tona *et al.*, 2001; Khan *et al.*, 2013). It needs to be considered that fertility of egg may be

influenced by various factors including nutrition, mating time, breed, lighting and season, whereas hatchability of egg depends on storage duration, egg quality, age of hens, care of hatching eggs, nutrition and season (Miazi et al., 2012). As current study did not focus all of the mentioned variables, no concrete logic could be presented for differences in fertility recorded in some groups during current study.

CONCLUSION

Based on the results, egg storage times of 24 or 48 h and fumigation durations of 15, 20, and 25 min did not significantly affect most chick quality parameters, hatchability, or egg breakout analysis. However, chick weight and yield were higher with a 15-min fumigation duration, especially when combined with 48 h of egg storage.

DECLARATIONS

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IRB approval

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Statement of conflict of interest

The authors have declared no conflict of interest.

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