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## Research Article

# Impact of Egg Storage Duration and Temperature on Egg Quality, Fertility, Hatchability and Chick Quality in Naked Neck Chickens

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### Abstract

**Background and Objective:** It is well a known fact that storing hatching eggs over a longer period of time affects egg quality, fertility and hatchability. The current study evaluated the impact of five egg storage durations and two temperature conditions on egg quality, fertility, hatchability and chick quality in a 6th generation heterozygous naked neck chickens. **Methodology:** A total of 900 hatching eggs were initially obtained from 45 weeks old flocks in five batches and weighed. Eggs (n=180) were obtained each time and divided into 2 groups. Each group was stored at one of two egg storage temperature conditions: cold room temperature (18°C) or ambient temperature (25-30°C). In each temperature condition the eggs were subjected to five pre-incubation egg storage duration of 1, 3, 7, 10 and 14 days. Thus, the experimental design used was a 2×5 factorial design consisting of two temperature conditions (cold room and ambient) and 5 pre-incubation egg storage durations (1, 3, 7, 10 and 14 days). After storage 15 eggs per treatment were weighed and broken to determine egg quality parameters including blastoderm diameter, eggshell, yolk and albumen weights. The remaining eggs were incubated for 21 days at 37.5°C and 60% relative humidity. The parameters measured were fertility, total hatchability, embryonic mortality and chick quality. The fertility, hatchability and embryo mortality were expressed as percentages. Data were analyzed using the Proc. mixed Model procedure of SAS at p<0.05. **Results:** The results showed no impact of experimental treatments on initial egg weight before and after eggs storage. However, the blastoderm quality reduced as the egg storage duration increased. Storing eggs in ambient temperature compared to cold room temperature resulted in increased blastoderm diameter and advanced embryo development. This resulted in higher embryo mortality during incubation, lowered fertility and reduced hatchability. The albumen weight and dry yolk weight significantly reduced after longer storage. Chick weight and chick shank length were increased in eggs stored at cold room temperature compared to eggs stored at ambient temperature but reduced as storage duration increased. **Conclusion:** Improperly storing eggs can greatly reduce fertility, hatchability and chick quality as a result of poor embryogenesis and overall chick development.

**Key words:** Storage temperature, storage duration, egg quality, blastoderm quality, chick quality

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Poultry production is an area of animal production with significant contributions to human food security. Poultry, particularly chicken provides products such as eggs and meat or protein of high biological value<sup>1</sup>. Therefore, chickens need proper management for higher performance. In hot climatic areas such as the Sub-Saharan Africa region, the production performance of exotic breeds is challenging<sup>2</sup>. However, efforts are being continually made to find local breeds that can survive in the hot regions of the world as well as produce a high number of eggs for human consumption<sup>3</sup>. Naked neck, a mutant bird known for its high adaptability under high environmental temperature conditions has high post-embryonic vitality and high carcass yield<sup>4-6</sup>. It is previously demonstrated that a cross between the indigenous naked neck male chickens and a Lohmann commercial female chickens produced offspring with higher body weight, increased body weight gain, increased number of eggs per clutch, increased hen-house and an increased hen-day rate of egg production. In addition, the cross has higher egg size, Haugh unit, eggshell thickness, embryo survivability and increased carcass yield<sup>7</sup>. While it seems that the naked neck breed has the potential as a commercial chicken breed in the hot climate, it has certain limitations with respect to hatchability and chick quality and this could be a setback for commercial production<sup>8-10</sup>.

The reproductive fitness of naked neck chickens developed in Ghana is under studied. Peters<sup>11</sup> discovered very high numbers of dead-in-shells in naked neck chickens following incubation. Dunga *et al.*<sup>9</sup> also recorded low numbers of chicks hatched in naked necks compared to the frizzle chicken. Postmortem examination conducted on the dead embryos showed that the high embryonic mortality observed in the above study<sup>9</sup> was mainly due to malposition. It occurred during the last stage of embryonic development before hatching, resulting in chicks that had externally pipped but could not hatch<sup>4,6,9</sup>. It is, however, suggested that the high embryonic mortality associated with naked neck chickens is a genetic problem<sup>9,12</sup>. Since the high embryonic mortality has also been linked to inability of naked neck embryos to place their heads under the left-wing, which is a major requirement for chicks to hatch<sup>13</sup>, the abnormal position could not be linked to genetics. Many factors including genetic selection or background of the parent stock may contribute to the increased late embryonic malposition and mortality. In addition, handling of eggs before incubation could be one of the factors limiting hatchability.

We hypothesized that due to the small collection of eggs from naked neck breeder layers for incubation, this could trigger unintentionally storing eggs until the numbers of eggs could fill the incubator. In addition, farmers and hatcheries in Ghana pay little attention to proper storage of eggs which, may affect the development of the blastoderm (or a day zero embryo)<sup>14,15</sup>. Meijerhof<sup>16</sup> reported that hatching is influenced by storage duration, storage temperature, the position of the eggs in the incubator, humidity and other environmental factors. Therefore, the objectives of this study were to store naked neck eggs under different temperature conditions over varying number of days and assess egg quality, fertility, blastoderm quality, chick quality and hatchability and to offer some background understanding on some of the possible causes of the increased late embryonic mortality and lower hatchability in naked neck embryos during incubation.

## MATERIALS AND METHODS

### Experimental design

**Experimental site:** The current study was carried out at Akate Farms and Trading Company Limited, Bosore in Kumasi and also the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The study areas reside between latitude 06°43' N and longitude 01°36' W with an altitude of 261.4 m above sea level<sup>17</sup>. It has a tropical climate with prevailing mean ambient daily temperature ranging from 23-35°C and annual rainfall of 30-200 mm in January and July, respectively. In the absence of Animal Care Committee at the Kwame Nkrumah University of Science and Technology at the time of this research, the research was supervised by the team leader following previous knowledge in experimental procedures approved by the University of Alberta Animal Care and Use Committee, where he worked previously, according to the Canadian Council on Animal Care guidelines<sup>18</sup>.

**Egg collection:** A total of 180 hatching eggs with no visible signs of unclean shells, without cracks and no discoloration, were initially collected from 45 weeks old heterozygote naked neck chickens developed after six generations of crosses. The cross was between Lohmann tradition commercial females and indigenous naked neck males through a series of backcrossing. The new breed, therefore, carried the naked neck gene which, enables the bird to withstand high environmental temperatures. All the eggs collected were divided into two groups and each stored at one of two temperature treatments: ambient temperature (25-30°C) and

cold room temperature (18°C and 65% RH). The eggs were stored for 14 days in the two temperature treatments. Subsequently, 180 eggs were collected, divided into two groups each time and stored at the two temperature treatments for 1, 3, 7 and 10 days. The treatments set up 2×5 factorial design with 2 egg storage temperature treatments (cold room and ambient) and 5 egg storage duration treatments (1, 3, 7, 10 and 14 days). All eggs were labeled individually using a non-harmful permanent marker and weighed to a precision of 0.01g using Pro-scout balance (model Scout Pro SPU 402, Ohaus Corp. USA) before storage. At the end of storage, all the eggs were weighed again to calculate weight loss.

**Egg quality characteristics and blastoderm diameter:** A total of 15 eggs were selected from each treatment for egg and blastoderm quality analysis. Ten out of the 15 eggs were weighed and broken out. The yolk and the albumen were separated using an egg separator. The egg components comprising the wet eggshell and wet yolk were weighed. The eggshell thickness was determined using a micrometer screw gauge (DONGRUN-8A4001, Ningbo-Dongrum, China). The wet yolk and wet eggshell were placed in a drying oven (model GR 50555-2, Wagtech International Ltd, UK) at 70°C for 4 days to obtain the dry weights. The wet albumen was calculated by subtracting the sum of the yolk weight and eggshell weight from the egg weight after egg storage. The weights of the wet and dry egg components were expressed as a percentage of the initial egg weight after storage. The remaining 5 eggs were individually broken open and the yolk, albumen and eggshell separated as described above. Using a filter paper the yolk was gently rotated on the egg separator until the blastoderm was visible on the surface of the yolk. The fertility of each egg was determined using the clear observation technique of an intact blastoderm with clearly displayed area opaca and area pellucida<sup>11,12</sup>. When observed, the blastoderm of a fertile egg could be seen as two white rings like 'doughnut', about 4 mm in diameter. A vernier caliper was used to measure the diameter of the blastoderm on the surface of the yolk and transferred to a ruler (Fig. 1).

**Incubation, candling and hatching:** After the period of egg storage, the 750 eggs remaining after egg breakout were fumigated and placed in a Petersime incubator. The incubator was operated as a multistage machine at a temperature of 37.5°C and relative humidity of 60-65% for the first 18 days. On the 18th day of incubation, eggs were candled in a dark

room to sort out infertile eggs and eggs with early embryonic mortality. The candled out eggs were broken to confirm infertility and early embryonic dead embryos. The fertile eggs were transferred into hatching baskets and then into a Petersime hatcher unit for 3 days. On the 21st day, the numbers of hatched chicks and dead-in-shell embryos were recorded. The eggs with dead-in-shell embryos (eggs with embryos that died in the process but could not hatch) were broken out to ascertain reasons for their inability to hatch. Fertility and total hatchability were calculated. Fertility was defined as the number of fertile eggs divided by total number of eggs set and multiplied by 100, hatchability of total eggs was also defined as number of chicks that hatch divided by total number of eggs set and multiplied by 100, while percentage of early (1-7 days), mid (8-14 days) and late (15-21 days) term embryo mortalities were determined.

**Chick quality:** All chicks hatched were weighed with pro-scout balance scale when chicks appeared 90% dried. The chick length was measured by laying the chick over and stretching it to its comfortable length on a 30 cm ruler from the tip of the beak to the longest toe. The shank length of each chick was measured using a caliper and ruler and recorded as the distance between the hock to the posterior end of the middle toe.

**Statistical analysis:** Data were analyzed using the Proc. mixed model procedure of SAS at  $p < 0.05$ <sup>19</sup>. The statistical model used was defined as below:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad (1)$$

Where:

$Y_{ij}$  = Response recorded on a measured parameter

$\mu$  = Overall mean

$\alpha_i$  = Main effect of storage duration

$\beta_j$  = Main effect of egg storage temperature

$(\alpha\beta)_{ij}$  = Interaction effect of egg storage duration and storage temperature

$\varepsilon_{ij}$  = Residual error term

The random error used for analysis was defined as the interaction of storage temperature and storage duration and nested in the number of eggs used. Each egg served as the experimental unit. Where significant differences were observed between ls-means they were separated using the probability difference option (PDIFF) of SAS. The hatchability and fertility were expressed as percentages.

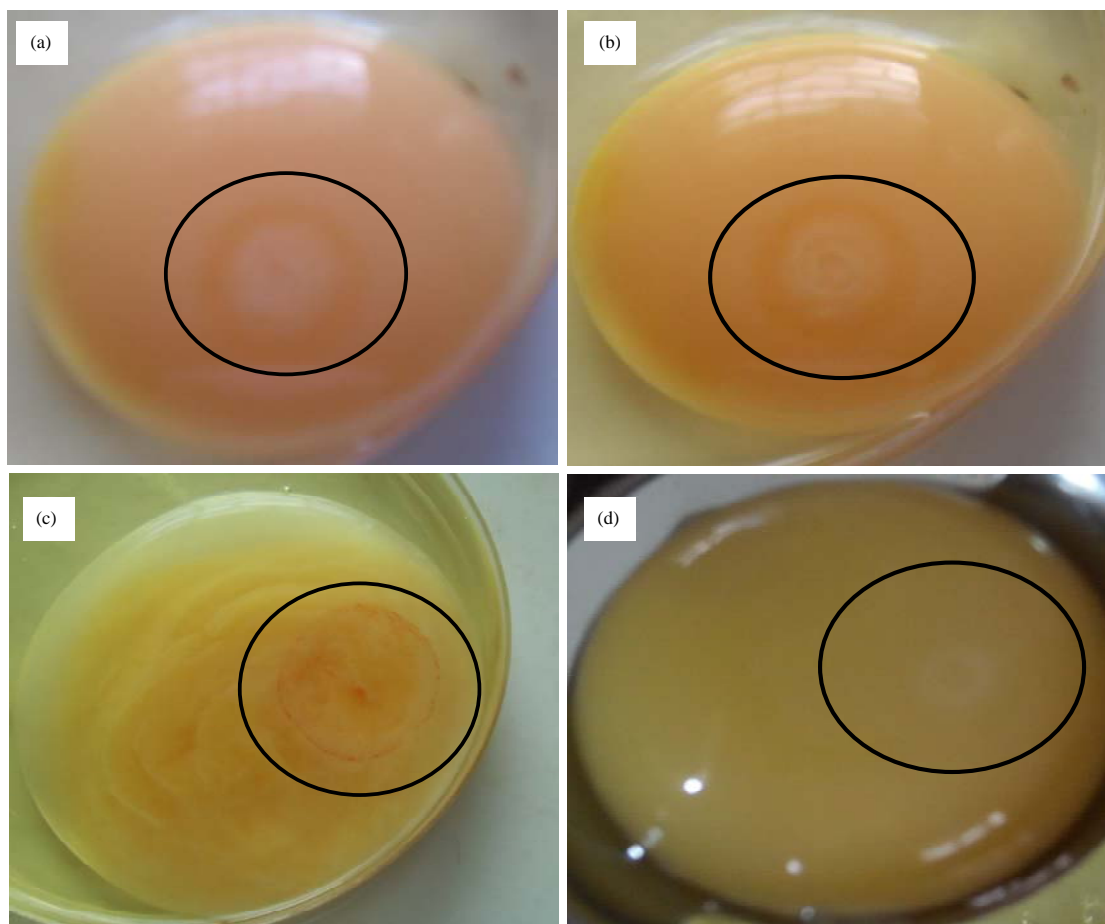


Fig. 1(a-d): Effect of storage temperature and storage duration on blastoderm size and symmetry for eggs stored under ambient temperature: (a) Blastoderm of egg stored for 3 days, (b) Blastoderm of egg stored for 7 days and (c) Blastoderm of eggs stored for 10 days with increased yolk mottling as increased concentric circles from blastoderm; d) Blastoderm of egg stored for 1 day

## RESULTS AND DISCUSSION

**External egg quality characteristics of naked neck eggs:** The weight of eggs before storage were not significantly different between the two storage temperature treatments (cold room versus ambient). The egg weights were not different between the egg storage durations and between the interaction of storage temperature and egg storage duration (Table 1). A similar trend was noticed in weight of eggs after storage. While storage temperature and interaction of temperature and storage duration did not affect moisture loss, the fixed effect of egg storage duration significantly affected egg moisture loss. Eggs stored for 10 days had higher moisture loss followed by those of 14 days storage duration. The moisture loss increased with increasing storage duration from 1 day until 14 days of storage and was associated with decreasing egg weight (Table 1). The egg

weight loss can be attributed to a loss of water, ammonia, carbon dioxide and nitrogen as well as hydrogen sulfide from the eggs<sup>20-23</sup>.

The eggshell thickness and dry eggshell weight were not different between temperature treatments (Table 1). However, the percentage of wet eggshell weight was significantly higher in cold room temperature treatment compared to ambient temperature treatment. The eggs stored for 7 days had higher wet eggshell weights compared to eggs stored for 1, 3, 10 and 14 days before incubation. There were however no specific trends in the eggshell weights. The interaction of egg storage temperature and egg storage duration did have effect on eggshell thickness and dry eggshell weight.

**Internal egg quality characteristics of naked neck egg:** Blastoderm diameter was greater at ambient temperature

Table 1: Effect of ambient and cold room storage temperature on external egg quality of naked neck eggs stored for five different storage durations

Source	Egg weight before storage (g)	Egg weight after storage (g)	Egg weight loss (%)	Shell thickness (mm)	Wet shell weight (%)	Dry shell weight (%)
<b>Storage temperature</b>						
Ambient (23-31 °C)	55.39 (125) <sup>2</sup>	54.36 (125)	1.82 (125)	0.046 (75)	9.32 <sup>b</sup> (75)	9.04 (75)
Cold room (18 °C)	55.31 (132)	54.47 (132)	1.51 (132)	0.048 (75)	9.66 <sup>a</sup> (75)	9.39 (75)
SEM	00.897	0.9116	0.1404	0.0013	0.2137	0.2259
<b>Days of storage</b>						
1	56.78 (50)	56.52 (50)	0.42 <sup>d</sup> (50)	0.039 (30)	8.89 <sup>d</sup> (30)	8.53 (30)
3	53.22 (52)	52.77 (52)	0.81 <sup>d</sup> (52)	0.044 (30)	9.86 <sup>b</sup> (30)	9.56 (30)
7	54.26 (50)	53.52 (50)	1.35 <sup>c</sup> (50)	0.052 (30)	10.38 <sup>a</sup> (30)	10.10 (30)
10	55.98 (50)	54.36 (50)	2.91 <sup>a</sup> (50)	0.053 (30)	9.30 <sup>c</sup> (30)	9.11 (30)
14	56.52 (55)	54.91 (55)	2.83 <sup>b</sup> (55)	0.049 (30)	8.99 <sup>d</sup> (30)	8.78 (30)
SEM <sup>1</sup>	1.176	1.1814	0.3446	0.0022	0.3234	0.3068
<b>p-values</b>						
Storage temperature	0.9364	0.9063	0.0621	0.6150	0.2134	0.1409
Storage duration	0.0642	0.0937	<0.0001	0.0623	0.0044	0.0008
Interaction	0.1391	0.1321	0.6536	0.1006	0.8662	0.7665

<sup>a-d</sup>Significant differences among means in the same column (p<0.05), <sup>1</sup>SEM: Standard error of mean, <sup>2</sup>Number of experiment unit

Table 2: Effect of ambient and cold room storage temperature on internal egg quality for eggs stored for five different durations

Source	Blastoderm diameter (mm)	Albumen weight (%)	Wet yolk weight (%)	Dry yolk weight (%)
<b>Storage temperature</b>				
Ambient (23- 31 °C)	6.96 <sup>a</sup> (25) <sup>2</sup>	59.99 (41)	55.45 (41)	18.44 (41)
Cold room (18 °C)	5.41 <sup>b</sup> (25)	61.97 (47)	54.61 (47)	17.89 (47)
SEM <sup>1</sup>	0.935	1.0869	0.9269	0.5016
<b>Days of storage</b>				
1	4.40 <sup>c</sup> (10)	64.06 <sup>a</sup> (17)	52.10 (17)	15.20 <sup>d</sup> (17)
3	4.16 <sup>c</sup> (10)	63.7 <sup>a</sup> (16)	55.71 (16)	17.16 <sup>d</sup> (16)
7	4.66 <sup>c</sup> (10)	63.36 <sup>a</sup> (17)	56.12 (17)	20.04 <sup>a</sup> (17)
10	6.92 <sup>b</sup> (10)	58.57 <sup>b</sup> (19)	56.73 (19)	19.38 <sup>b</sup> (19)
14	10.79 <sup>a</sup> (10)	59.28 <sup>b</sup> (19)	54.49 (19)	19.04 <sup>c</sup> (20)
SEM	0.2851	1.6147	1.3989	0.7885
<b>Interaction</b>				
Ambient × 1 day	4.17 <sup>c</sup> (5)	64.06 <sup>a</sup> (8)	50.22 (8)	14.63 (8)
Ambient × 3 days	3.81 <sup>c</sup> (5)	62.48 <sup>b</sup> (8)	56.58 (8)	16.46 (8)
Ambient × 7 days	4.61 <sup>a</sup> (5)	58.31 <sup>c</sup> (7)	57.06 (7)	21.38 (7)
Ambient × 10 days	8.02 <sup>b</sup> (5)	56.76 <sup>d</sup> (9)	58.87 (9)	19.56 (9)
Ambient × 14 days	14.19 <sup>a</sup> (5)	58.31 <sup>c</sup> (9)	54.50 (9)	20.16 (9)
Cold room × 1 day	4.63 <sup>c</sup> (5)	64.06 <sup>a</sup> (9)	53.97 (9)	15.78 (9)
Cold room × 3 days	4.51 <sup>c</sup> (5)	64.91 <sup>a</sup> (8)	54.84 (8)	17.85 (8)
Cold room × 7 days	4.71 <sup>c</sup> (5)	63.12 <sup>a</sup> (10)	55.17 (10)	18.69 (10)
Cold room × 10 days	5.81 <sup>b</sup> (5)	60.2 <sup>b</sup> (10)	54.59 (10)	19.21 (10)
Cold room × 14 days	7.38 <sup>b</sup> (5)	60.25 <sup>b</sup> (10)	54.48 (10)	17.92 (10)
SEM	0.3929	2.1395	1.9538	1.0143
<b>p-values</b>				
Storage temperature	<0.0001	0.2573	0.4849	0.4391
Storage duration	<0.0001	0.0302	0.1194	0.0003
Interaction	<0.0001	0.0288	0.2892	0.2415

<sup>a-d</sup>Different letters indicate significant (p<0.05) differences among means in the same column. <sup>1</sup>SEM: Standard error of mean, <sup>2</sup>Number of experiment unit

(6.96 mm) than in cold room temperature (5.41 mm). Blastoderm diameter significantly increased as storage duration increased. It increased from 3 days of storage to 14 days of storage with 14 days recording the longest blastoderm. The eggs stored for 3 days recorded the lowest blastoderm diameter. The blastoderm diameter recorded at 3 days of storage was not different from 1 and 7 days of storage (Table 2). An increase in blastoderm diameter may

suggest embryonic development as storage duration increases. The embryonic development appeared greater when storage duration exceeded a week. Similar results were reported by Van Schalkwyk *et al.*<sup>24</sup>. Although the later was measured in ostrich the blastoderm diameter increased after 7 days of storage. Interaction of storage duration and storage temperature affected blastoderm diameter significantly (p<0.0001).

Blastoderm diameter increased irrespective of the storage temperature as storage duration increased. With increasing storage duration, storing eggs in ambient temperature resulted in higher blastoderm diameter than cold room temperature (Table 2). Compared to previous studies, it was surprising that the blastoderm diameter increased with increasing storage duration under cold room temperature<sup>14,15,25</sup>. The results could be due to fluctuating cold room temperature conditions in the commercial hatchery where the eggs were stored. This is a common problem in Ghana where hatcheries sometimes have sub-optimal conditions for egg storage, especially during very hot days. The results may contribute to early embryonic mortality when eggs are incubated as a result of advanced embryo development especially where eggs are kept for longer periods. This is because when eggs are stored at a temperature closer to incubation temperature and above physiological zero (ambient temperature) embryonic development occurs.

The internal egg quality characteristics during storage are presented in Table 2. There was a significant effect of storage duration on percent albumen weight ( $p=0.0302$ ). The percent albumen weight was higher from 1 day of storage to 7 days and decreased in 10 days and 14 days of storage treatments. This could be due to reduced albumen quality in longer stored eggs as a result of the loss of CO<sub>2</sub> or moisture into the yolk<sup>26,27</sup>. No significant effect of storage temperature on percent albumen weight was recorded. But the interaction of different storage duration and different storage temperature significantly affected albumen weight. The albumen appeared to absorb water from the yolk when storage duration exceeded 10 days, whether the eggs were stored in a cold room or under ambient temperature<sup>28-30</sup> (Table 2).

Egg storage duration did not affect percentage wet yolk weight but affected percentage dry yolk weight significantly (Table 2). Percentage dry yolk weight increased from 1 day of storage to 7 days of storage. Eggs stored for 7 days had the highest dry yolk weight, followed by 10 days and the 14 days stored eggs. There was no significant effect of the interaction of storage temperature and storage duration on wet or dry yolk weights. Storage duration did not affect percentage wet yolk weight but affected percentage dry yolk weight significantly (Table 2). Percentage dry yolk weight increased from 1 day of storage to 7 days of storage. Eggs stored for 7 days had the highest dry yolk weight, followed by 10 days and the 14 days stored eggs. There was no significant effect of the interaction of storage temperature and storage duration on wet or dry yolk weights.

**Egg fertility, hatchability and chick quality:** The percentage of fertile eggs in ambient temperature was lower (58.7%) compared to the eggs kept at cold room temperature (92.0%) (Table 3). The total hatchability was also lower in ambient temperature storage (42%) compared to cold room temperature storage (71.9%). The fertility and hatchability declined with increasing storage duration. The hatchability decreased with increasing egg storage duration starting from 3 days until 14 days (79.8, 71.7, 38.3 and 25.0%) (Table 3). This was due to increasing level of early embryonic mortality. It is a normal practice for commercial hatcheries to classify breakout eggs into infertile eggs, clear eggs, early embryo mortality, late embryo mortality and pipping mortality. More often hatchery managers lacking training get confused with clear eggs and add them to the infertile eggs. These clear eggs are as a result of mortalities resulting from advanced blastoderm development. The causes for advanced blastoderm development include high ambient temperature in breeder houses, high transit temperature and first in sequence eggs<sup>27,31</sup>. Study confirms that embryos of eggs that remained longer (3.5-6.5 h) in nest boxes at an environmental temperature of 28°C were advanced in development than eggs collected immediately after oviposition and stored at 18.9-20.7°C for 10 h (with embryos at stage 11.67 and 10.38, respectively)<sup>31-33</sup>. Blastoderms that have developed pass the Eyal-Giladi and Kochav stage X level of blastodermal development have high embryonic mortality, reduces hatchability and reduced egg quality<sup>27,31</sup>.

The weight of chicks hatched in the current study was not different between egg storage durations. Eggs stored under cold room temperature had significantly higher chick weight than eggs stored under ambient temperature. The chick weights were significantly different between the interaction of storage duration and storage temperature but with no apparent trend (Table 3). Chick length was not affected by storage duration and storage temperature. The chick length was also not affected by the interaction between storage temperature and storage duration (Table 3). However, chick shank length was longer in eggs stored at cold room temperature compared to ambient temperature storage. The shank length reduced with increasing egg storage duration. The development of proper frame size during embryogenesis is linked to longer shank length. Chicks with longer shank length tend to have good frame size that supports broiler growth and egg production<sup>31,32,34</sup>.

In summary, storing eggs of naked neck chickens for extended periods and at ambient temperature appeared to negatively affect the external and internal egg quality

Table 3: Effect of ambient and cold room storage temperature on fertility, hatchability and chick quality of eggs stored under five different storage durations

Storage duration	Fertility (%)	Total hatchability (%)	Chick weight (g)	Chick length (cm)	Chick shank length (cm)
<b>Storage temperature</b>					
Ambient (23-31 °C)	58.67	42.00	35.01 <sup>b</sup> (63) <sup>2</sup>	15.81 (63)	1.59 <sup>a</sup> (63)
Cold room( 18 °C)	92.00	71.93	36.13 <sup>a</sup> (108)	15.50 (108)	1.63 <sup>b</sup> (108)
SEM <sup>1</sup>	-	-	0.3228	0.1670	0.0086
<b>Days of storage</b>					
1	96.67	70.00	35.21 (42)	15.74 (42)	1.66 <sup>a</sup> (42)
3	91.67	79.83	36.47 (48)	15.34 (48)	1.60 <sup>b</sup> (48)
7	86.67	71.67	35.3 (43)	15.99 (43)	1.61 <sup>b</sup> (43)
10	61.67	38.34	35.32 (23)	15.43 (23)	1.59 <sup>b</sup> (23)
14	40.00	25.00	36.59 (15)	15.34 (15)	1.56 <sup>b</sup> (15)
SEM	-	-	0.4798	0.249	0.0136
<b>Interaction</b>					
Ambient × 1 day	93.33	64.29	33.10 <sup>c</sup> (18)	16.01 (18)	1.59 <sup>a</sup> (18)
Ambient × 3 days	90.00	85.18	37.06 <sup>a</sup> (23)	15.02 (23)	1.58 <sup>a</sup> (23)
Ambient × 7 days	86.67	84.61	34.44 <sup>b</sup> (22)	16.39 (22)	1.60 <sup>a</sup> (22)
Ambient × 10 days	23.33	0.00	-	-	-
Ambient × 14 days	0.00	0.00	-	-	-
Cold room × 1 day	100.00	80.00	36.79 <sup>a</sup> (24)	15.46 (24)	1.72 <sup>a</sup> (24)
Cold room × 3 days	93.33	89.29	35.93 <sup>b</sup> (25)	15.64 (25)	1.62 <sup>b</sup> (25)
Cold room × 7 days	86.67	80.77	36.19 <sup>b</sup> (21)	15.58 (21)	1.61 <sup>c</sup> (21)
Cold room × 10 days	100.00	76.67	35.32 <sup>b</sup> (23)	15.430 (23)	1.59 <sup>d</sup> (23)
Cold room × 14 days	80.00	66.67	36.59 <sup>a</sup> (15)	15.36 (15)	1.56 <sup>c</sup> (15)
SEM	-	-	0.6625	0.3563	0.0202
<b>p-values</b>					
Storage temperature	0.3894	0.2457	0.0086	0.3106	0.0001
Storage duration	0.1444	0.1122	0.0825	0.3724	<0.0001
Interaction	-	-	0.0013	0.0680	0.0062

<sup>a-c</sup>Different letters indicate significant ( $p < 0.05$ ) differences among means in the same column, <sup>1</sup>SEM: Standard error of mean, <sup>2</sup>Number of experiment unit

parameters. Therefore, the hypothesis that eggs collected in small quantities may lead to an unwanted egg storage in poor environmental setting, resulting in lower hatchability in naked neck chicken appears to be true. Parameters that were greatly affected include the albumen (important for establishing proper pH for embryo development) and blastoderm. It is recommended that due to the greater impact of ambient temperature eggs collected in breeder farms should be placed in cold storage as soon as possible. Additionally, where prolonged storage of eggs is inevitable mechanisms of reducing the impact of egg storage should be adopted. These may include the practice of short periods of incubation during egg storage (SPIDES)<sup>31</sup>, especially where proper egg storage facilities do not exist. Due to limited resources in the current study (eggs), the study needs to be tested in a commercial setting with a large number of eggs to reveal statistical difference in the hatchability.

### CONCLUSION AND FUTURE RECOMMENDATIONS

It is concluded that under the conditions of this experiment, prolonged storage of naked neck chicken eggs beyond 7 days and storage in ambient temperature were

detrimental to eggs, blastoderm and chick quality as well as hatchability. Further research using a large number of eggs is needed. The practice of egg storage in ambient temperature needs to be well-managed in tropical hatcheries to maximize hatchability.

### SIGNIFICANCE STATEMENT

This study discovered that prolonged storage of eggs and at ambient temperature were more detrimental to developing chicken embryos. This is a critical aspect of layer breeder production and should be managed well to maximize hatchability and chick quality. This study will help researchers, poultry producers and hatchery operators to develop proper egg storage protocols and reduce the number of embryo mortality that reduces hatchability and post-hatch chick quality.

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