Nig. J. Anim. Prod. 2024, 51(1):41 - 51 https://doi.org/10.51791/njap.v51i1.7637 *Nigerian Journal of Animal Production*

Effects of egg storage on hatchability, hormonal changes and duration of hatching in fertilized eggs of Lohmann Brown hens

Nwosu, E. U.*[1], Ojeleke, O. O.[1]., Oyekemi, P.[1]

[1] *Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria,*

Corresponding author: emmanuelanwosu0164@gmail.com

Abstract

*Fertile eggs are stored at low temperature at the breeder farm and also at the hatchery with the purpose to stockpile enough eggs to fill the incubator. The embryos diapause at the low temperature, thereafter, resumes normal development at optimal temperature and humidity of the incubator. It will be difficult to avert egg storage since it is a necessary poultry practice. However, it still poses adverse effects on hatchability, chick quality and post hatch performance. In this study, an investigation was carried out to identify the effect of duration of egg storage on fertilized eggs of Lohmann Brown chickens. The trial involved a total of fertilized 1200 eggs of Lohmann brown. The eggs were divided into three treatment groups representing storage duration (T4: 4 days, T7: 7 days and T10: 10 days) and were stored at a temperature of 18°C and relative humidity of 75%. The fertilized eggs were incubated using a forced-draft incubator that was set at 37.7°C temperature and 55% relative humidity for 21 days. The variables analyzed were: changes in egg weight during storage and incubation, percentage mortality and hatchability, levels of cortisol, triiodothyronine, thyroxine, and hatching events. The data obtained were analyzed using one way ANOVA model in SAS statistical software package (SAS Version 6.124). The results showed that relative egg weight loss during storage increased with extended duration of storage. Seven days egg storage had the highest percentage egg weight loss during incubation followed by 10 days storage and lowest was 4 days egg storage. Storage day 10 recorded higher (P < 0.05) percentage (47. 41%) of dead embryos compared to (25.91% and 10.78%) percentages of dead embryos from 7 and 4 day storage respectively. Also, average percentage hatchability decreased with extended storage. Storage day 4 recorded higher (P < 0.05) percentage (86.42%) hatchability followed by 7 and then 10 day storage with (71.87%) and (52.59%) percentage hatchability respectively. The cortisol levels where comparable at IP, EP and at hatch for all storage groups (Table 3). Triiodothyronine (T3) and thyroxine (T4) concentrations of eggs were similar at IP and EP for all storage groups. At hatch, the levels of thyroxine (T4) increased with storage duration (P <0.05) with 10 day having the highest levels (0.75 pmol/L) but the levels were comparable for day 4 and 7 (0.66 and 0.67) storage duration respectively. Also, Triiodothyronine (T3) levels at hatch increased with storage duration (P <0.05) with 10 day having the highest levels (1.33 pmol/L) but the levels were similar for day 4 and 7 (1.21 and 1.18) storage duration respectively. Eggs from 4 day storage recorded internal pipping (**IP**), and hatching (**HH**) events in fewer hours (P < 0.05) compared to eggs from 7 and 10 storage duration but had similar **EP** time with day 7 stored eggs. It was concluded from the experiment that, storing of fertilized Lohmann Brown eggs beyond 7 days had negative effects on the variables considered.*

Keywords: fertilized eggs, Egg weight loss, Mortality, Hatchability, hatching hormones and hatching events.

Running Title: Egg storage impacts on hatchability, hormonal changes and duration of hatching in fertilized eggs of Lohmann Brown hens

Effets du stockage des œufs sur l'éclosabilité, les changements hormonaux et la durée de l'éclosion des œufs fécondés de poules Lohmann Brown

Résumé

Les œufs fertiles sont stockés à basse température à la ferme d'élevage et également à l'incubateur afin de constituer un stock suffisant pour remplir l'incubateur. Les embryons entrent en diapause à basse température, puis reprennent un développement normal à la température et à l'humidité optimales de

l'incubateur. Il sera difficile d'éviter le stockage des œufs, car c'est une pratique nécessaire en aviculture. Cependant, cela présente encore des effets néfastes sur l'éclosabilité, la qualité des poussins et la performance post-éclosion. Dans cette étude, une enquête a été réalisée pour identifier l'effet de la durée de stockage des œufs sur les œufs fécondés de poules Lohmann Brown. L'essai a impliqué un total de 1200 œufs fécondés de Lohmann Brown. Les œufs ont été divisés en trois groupes de traitement représentant la durée de stockage (T4 : 4 jours, T7 : 7 jours et T10 : 10 jours) et ont été stockés à une température de 18°C et une humidité relative de 75 %. Les œufs fécondés ont été incubés à l'aide d'un incubateur à air forcé réglé à une température de 37,7°C et une humidité relative de 55 % pendant 21 jours. Les variables analysées étaient : les changements de poids des œufs pendant le stockage et l'incubation, le pourcentage de mortalité et d'éclosabilité, les niveaux de cortisol, de triiodothyronine, de thyroxine, et les événements d'éclosion. Les données obtenues ont été analysées à l'aide d'un modèle ANOVA à un facteur dans le logiciel statistique SAS (SAS Version 6.124). Les résultats ont montré que la perte de poids relative des œufs pendant le stockage augmentait avec la durée de stockage prolongée. Le stockage des œufs pendant sept jours avait le pourcentage de perte de poids le plus élevé pendant l'incubation, suivi du stockage de dix jours, tandis que le stockage de quatre jours avait le pourcentage le plus bas. Le dixième jour de stockage a enregistré un pourcentage plus élevé (P < 0,05) d'embryons morts (47,41 %) comparé aux pourcentages d'embryons morts de 25,91 % et 10,78 % respectivement pour les jours de stockage de 7 et 4 jours. De plus, le pourcentage moyen d'éclosabilité a diminué avec un stockage prolongé. Le quatrième jour de stockage a enregistré un pourcentage d'éclosabilité plus élevé (P < 0,05) de 86,42 %, suivi des 7 et 10 jours de stockage avec des pourcentages respectifs de 71,87 % et 52,59 %. Les niveaux de cortisol étaient comparables à IP, EP et à l'éclosion pour tous les groupes de stockage (Tableau 3). Les concentrations de triiodothyronine (T3) et de thyroxine (T4) des œufs étaient similaires à IP et EP pour tous les groupes de stockage. À l'éclosion, les niveaux de thyroxine (T4) augmentaient avec la durée de stockage (P < 0,05), le stockage de 10 jours ayant les niveaux les plus élevés (0,75 pmol/L), mais les niveaux étaient comparables pour les jours 4 et 7 (0,66 et 0,67) respectivement. De plus, les niveaux de triiodothyronine (T3) à l'éclosion augmentaient avec la durée de stockage (P < 0,05), le stockage de 10 jours ayant les niveaux les plus élevés (1,33 pmol/L), mais les niveaux étaient similaires pour les jours 4 et 7 (1,21 et 1,18) respectivement. Les œufs du stockage de 4 jours ont enregistré des événements de perçage interne (IP) et d'éclosion (HH) en moins d'heures (P < 0,05) par rapport aux œufs des jours de stockage de 7 et 10, mais avaient un temps EP similaire avec les œufs stockés pendant 7 jours. Il a été conclu de l'expérience que le stockage des œufs fécondés de Lohmann Brown au-delà de 7 jours avait des effets négatifs sur les variables considérées.

Mots-clés : œufs fécondés, perte de poids des œufs, mortalité, éclosabilité, hormones d'éclosion et événements d'éclosion.

Introduction

Fertilized eggs to be incubated are always stored until enough eggs are available to fill large incubator racks or stockpiled with the view of dwindling of egg production or demand during the production year (Fasenko *et al*., 2001a). It is true that pre-incubation cannot be prevented as it is a necessary poultry practice; it still poses adverse effects on hatchability, chick quality and post hatch performance (Tona *et al*., 2003; Rejrink *et al*., 2008; Pawlowska and Sosnowka-Czajka, 2019). Studies (Bakst and Akuffo 1999; Lapão *et al*., 1999; Fasenko, 2007) showed that storage of fertile eggs for long duration results in decrease in albumen, perivitellin membrane, yolk, blastoderm quality, gas exchange and embryonic metabolism. Fasenko *et al.* (1992) reported that embryonic mortality increases as storage time lengthens. Egg stored for 14 days had more embryonic mortality at early and late stages of incubation compared to 4 days storage (Fasenko *et al*., 2001b). However, some researchers have recommended that storage duration should not exceed 3 to 5 days (Khan *et*

al. 2014; Senbeta 2016). King-Ori (2011) reported that the length of storage should not exceed 10-14 days in order to maintain good hatchability. Furthermore, Fasenko *et al.* (1992) and Whitehead *et al.* (2002) showed that optimum storage duration for fertile chicken egg should not be more than 7 days and that any extension beyond this duration decreases egg quality, increases embryonic mortality, and extends incubation time. This is because the embryonic metabolism of embryos during longer egg storage proceeds at a slower rate than embryos from shorter stored eggs (Christensen *et al*., 2001).

Prolong incubation time has been shown to affect concentration of hatching hormones; tri-iodothyronine (T3), thyroxine (T4), and corticosterone concentrations (Tona *et al*., 2003 and Morita *et al*., 2016). Thyroid hormones (T_3 and T_4) are involved in numerous physiological processes such as regulating heat production of chick embryos during the incubation (McNabb, 2000), transition from allantoic to lung respiration (McNabb, 2000; Reyns *et al*., 2003) and preparation for pipping and hatching process (De Smit *et al*., 2008) as well as they are important for regulating metabolic rate during the post-hatch period (Decuypere *et al*., 2000). According to De Groef *et al*. (2008), thyroid activity increases towards hatching time due tohigher release of thyroid-stimulating hormone (TSH) by the pituitary gland. Thyroid hormones (TH) enhance growth by modulating the metabolism of lipid, protein and carbohydrate (Lee and Laycock, 1978). This TH and glucocorticoid (CORT) are the most important hormones that act as stimulants for the hatching process.

Previous studies have shown that prolonged egg storage may induce embryonic stress in form of blastodermal cell apoptosis and necrosis (Bloom *et al*., 1998; Hamidu *et al*., 2011; Dymond *et al*., 2013) and may lead to increased embryonic mortality, depressed embryonic metabolism and developmental delays (Hamidu *et al.*, 2010). Stress is closely linked to increased activity of hypothalamic-pituitary-adrenal (HPA)-axis, resulting in increased levels of glucocorticoids (Moberg and Mench, 2000). The Glucocorticoids (cortisol and corticosterone) are stress hormones and levels reflect the activity of adrenal glands (Mostl and Palme, 2002). The priority of the breeder farmers and hatcheries are to have chicks with good hatchability, viability, and post-hatch performance at the end of incubation. Therefore, it becomes necessary to evaluate one necessary poultry practice, pre-incubation as well as the consequences of it towards having healthy embryo development and hatching chick. The objective of the present study was to estimate the effects of egg storage durations on hatchability, hormones of hatching and hatching events of Lohmann brown fertile eggs.

Materials and methods

Experimental site

One thousand and two hundred fertile eggs of Lohmann Brown chickens for this experiment were imported from Incubel n.v. Hoogstraten, Belgium. Storage and 21 days incubation of eggs were carried out in *Center D'excellence regional sur les science avaries*, University de Lome, Togo. The hormonal assays were carried out at Anatomy and Physiology laboratory, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta.

Ethical approval

All experimental protocol and procedures used in this study were reviewed and approved by the Research Supervisory Committee of the Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Management of eggs

A total of 1200 fertilized eggs of Lohmann Brown were used for the experiment. The eggs were divided into 3 treatment groups representing storage duration (T4: 4 days, T7: 7 days and T10: 10 days). Each treatment group was marked, numbered, weighed and stored at a temperature of 18°C and relative humidity of 75% RH. Before setting, eggs from each treatment were reweighed, set for incubation in forced-draft incubator at a dry-bulb temperature of 37.6°C and wet-bulb temperature of 29°C, relative humidity of 55%, and turning once an hour until day 18. On day 18 of incubation, the eggs were candled, and those with evidence of living embryos were transferred from turning trays to hatching baskets. During the last 3 days of incubation, eggs were monitored every 2 h for evidence of

hatching events: internal piping (IP), external piping (EP), and hatch. At IP, EP stage and at hatch, samples of 24 eggs and 24 chicks per storage time were used to collect blood sample for the determination of levels of T3, T4, and cortisol.

Egg weight loss

Egg weights were recorded before storage, end of storage, at setting and at day 18 of incubation. The weights were used to determine relative egg weight loss during storage and incubation as:

Egg weight loss during storage = 100 × (initial weight before storage – weight at the end of storage)/ initial weight before storage.

Egg weight loss during incubation = 100 × (egg weight at setting – egg weight at day 18 of incubation)/egg weight at setting.

Percentage mortality and hatchability

At the end of 21 D of incubation, all unhatched eggs were opened to identify dead embryos. The numbers of dead embryos and hatched chicks were used to calculate embryo mortality and hatchability:

$$\text{Mortality} = 100 \times \frac{\text{Number of dead embryos}}{\text{Number of fertile eggs}}$$

$$\text{Hatchability} = 100 \times \frac{\text{Number of hatched chicks}}{\text{Number of fertile eggs}}$$

Triiodothyronine (T3), Thyroxine (T4) and cortisol levels determination

Blood samples were collected from 24 embryos from experimental groups at IP, EP and hatchlings for determination of levels of T3, T4, and cortisol. Blood was collected by cardiac aspiration from embryos and from jugular vein of the hatchlings. The blood samples were centrifuged at 3,000 rpm for 15 min, and the plasma obtained were emptied into Eppendorf tubes stored in a freezer at –20ºC. A volume of 100 µL of plasma was used for cortisol, T4, and T3 concentrations determination by using automated BioTek systems, which is an enzyme-linked immunosorbent assay (ELISA) technique. The ELISA kits used for the hormones concentrations were manufactured by Bio-Inteco, UK. The determinations of the hormones were carried out according to manufacturer's protocols.

Hatching events

At the end of 18 days of incubation, the eggs were candled and those with the evidence of living embryos were transferred into the hatching basket. From 456 to 528 hrs of incubation, eggs were checked individually every 2 hr for evidence of pipping and hatching events. Timing of internal pipping (IP), external pipping (EP), and hatching were determined during the last days of incubation of eggs stored for 4, 7 and 10 d before incubation. Time of IP, EP, and hatch of eggs from treatments groups were recorded. These data were used to calculate: hours of IP, EP and hatch starting from first day of setting. Also data generated were used to determine duration of IP

(time of EP – time of IP), duration of EP (hatching time – EP time), and duration of hatch (hatching time to IP). Total incubation duration was also calculated as the time between setting and hatching.

Statistical analysis

SAS statistical software package (SAS Version 6.124) was used to analyze the data obtained. One way ANOVA model was used to analyze: egg weight loss, percentage mortality and hatchability, cortisol, T3, T4, hatching events duration and incubation time,

The model was as follows: $Yi = \mu + \alpha i + \varepsilon i$

Where, Yi = egg weight loss, mortality, hatchability, cortisol, T3, T4, IP and EP duration or hatching time of egg from storage time i,

μ = overall mean,

αi = main effect of storage time i,

εi = random error term from storage.

Results and discussion

Table 1 shows the mean egg weights of stored eggs and percentage egg weight losses during storage and incubation. The average initial weights of egg (g) between the storage groups were comparable. This result agrees with the work of Onbaşılar *et al*. (2007) who reported no significant differences in initial egg weight of Pekin ducks stored for 0, 3, 7 and 11 days. The percentage egg weight loss increased with increasing days of storage, with day 10 egg storage having the highest percentage (1.45±0.113) weight loss. There was also significant ($P<0.05$) difference in percentage weight loss during incubation. Eggs stored for seven days had the highest percentage weight loss during incubation followed by day 10 stored eggs

and the least were eggs stored for four days. The result of this experiment is in line with the work of Lacin *et al*. (2008), who reported that eggs stored from 1-3 days had a total percentage weight loss of 1.44% and 1.99% for eggs stored for 6-8 days. The work of Ioniţă *et a*l. (2013) agrees with the result of this present work. The authors
observed a 0.47% average percentage of weight loss per day and a 2.82% total weight loss during a 1- 6 days storage of quails eggs and 12.79% average weight loss during incubation. Onbaşılar *et al*. (2007) reported a similar significant effect with storage period. According to their observations, Pekin ducks egg stored for 11 days had the highest weight loss compared to 0, 3 and 7 storage days. According to Scott and Mackenzie (1993) percentage egg weight loss of fertile eggs during storage is determined by temperature and humidity levels within the environment of storage. It has been noted that at normal temperature and humidity for incubation conditions, hatchability tends to decrease approximately by 1 - 1.4% each day of storage (Popescu-Miclosanu Elena (2007). Rahn (1974) stated that extremely low or high percentage of egg weight loss at storage time could negatively impact embryo's development and the number of chicks that will be hatched (Meir *et al*., 1984).

Table 1: Effect of storage time on egg weight loss during storage and incubation

[a,b,c] Means ± SEM within columns with different superscripts differ significantly (P<0.05).

Parameters Factors			
Storage days	**Egg weight (g)**	**% Weight loss during storage**	**% Weight loss during incubation**
4	55.22±0.55	0.00±0.00[c]	9.28±0.41[c]
7	55.06±0.54	0.45±0.11[b]	12.10±0.34[a]
10	54.43±0.67	1.45±0.11[a]	11.07±0.34[b]

Table 2 shows average percentages of mortality of embryos and hatchability of chicks among the storage days. The average percentage mortality was observed to increase with increasing storage days, as storage day 10 had more percentage of dead embryos compared to 7 and 4 days storage. According to previous researches, embryonic mortality increases with increasing pre-storage days (Sittman *et al*., 1971; Mather and Laughlin, 1976). The work of Fasenko *et al.* (1992) using Single Comb White Leghorn hens and stored their egg for a period of 0, 4, 7, 14, and 21days showed that embryonic mortality increased with storage days. Using broiler embryos, Fasenko *et al.* (2001a) observed an increase in overall embryo mortality from 10.7% to 27.7% in eggs stored for 4-d versus 14-d. Fasenko *et al.* (2001b) observed an increase of embryonic mortality in turkey eggs stored for 4 (22.9%) vs. 14 d (29.28%). The average percentages hatchability for 4 and 7 day storage were comparable and were higher than the 10 day storage. The result of this study in which hatchability declined after 7 days storage, agrees with the work of several researchers (Becker, 1963; Merritt, 1964; Sittman *et al.* 1971; Whitehead *et al*., 1985), that pre-storage incubation of more than seven days reduced hatchability. The research of Fasenko *et al.* (2001b) showed that the hatchability of turkey eggs stored for 4 and 14 decreased from 70.9 to 64.4%, respectively. Fasenko *et al*. (2001b) also reported a decrease in hatchability in broiler breeder eggs stored for 4 versus 14 days from 89.7 to 72.2%, respectively. This result also, corroborated the outcome of the experiment carried out in chickens (Sittmann *et al*., 1971; Fasenko *et al*., 1992) and in quails and turkeys (Sittmann *et al*., 1971). According to (Fasenko and Robinson, 1999) hatchability reduced significantly after storage for 8, 12, and 16 d.

Table 2: Effects of storage time on embryonic mortality and hatchability

Parameters		
Factors		
Storage days	**% Mortality**	**% Hatchability**
4	10.78±0.70[b]	86.42±1.18[a]
7	25.91±5.91[ab]	71.87±3.69[a]
10	47.41±2.59[a]	52.59±2.59[b]

[a,b,c] Means ± SEM within columns with different superscripts differ significantly (P<0.05).

The plasma levels of cortisol at IP, EP and in newly hatched chicks (day old) are shown in Table 3. For all of the hatching event time, IP, EP and at hatch the cortisol levels were the same for embryos of eggs stored for 4, 7 and 10 durations. Stress hormones: corticosterone, cortisol, and cortisone are synthesized and secreted into the plasma of embryos, starting at least from 9 days of incubation (Kalliecharan and Hall, 1974). The authors noted that the concentrations of these hormones are almost the same throughout embryonic development but their concentrations in plasma do not follow a common pattern. The pattern of circulating levels of corticosterone, cortisol, and cortisone during the embryonic stage showed a partially developed hypothalamus-pituitary-adrenal axis (Ericsson and Jensen, 2016). As a result, newly hatched chicks have a fully reactive, yet developing HPA-axis (Ericsson and Jensen, 2016). Therefore, chicks would be expected to show a weak stress-hyporesponsive period, since the HPA-axis is immature and environmental stressors do not cause release of cortisol/corticosterone (Ericsson, and Jensen, 2016). The work of (Sindtand Tönhardt, 2005) is in line with the present work, who indicated that brief lowering of the temperature (to 21°C) had no effect on the cortisol concentration in the plasma chicken embryo. Falahatkar*et al.* (2013) observation with Persian sturgeon showed that the exposure of developing eggs to an acute stress (10 min out of water) had no effect on whole-body cortisol concentration in both stressed (2 and 6 h post-stress) and unstressed experimental groups. Caipang*et al.* (2015) also reported that cortisol content of the embryos of Atlantic cod was not significantly affected upon exposure of the eggs to air as a stressor. McCormick and Nechaev (2002) concluded that it is likely that the hypothalamus-pituitary-interrenal axis (HPI) that produces and regulates cortisol in fish is not fully functional during the embryonic stage.

Table 3: Effects of egg storage duration on cortisol's level at internal pipping, external pipping and hatch

Parameter	**Hatching events**	**Egg storage duration (days)**		
		4	7	10
Cortisol (ng\ml)	Internal pipping	0.32±0.07	0.33±0.09	1.07±0.49
	External pipping	0.63±0.25	0.34±0.04	0.41±0.13
	Hatch	1.94±0.04	1.66±0.17	1.36±0.52

[ab]*Means within same rows having different superscript differs significantly (p<0.05)*

Table 4 presents the effect of egg storage periods on plasma Triiodothyronine (T3) and Thyroxine (T4) in chick embryos and newly hatched chicks during internal pipping, external pipping and at hatch. Egg storage duration influenced (p<0.05) plasma levels of T3 and T4 at hatch but not at internal or external pipping. Triiodothyronine level in chicks stored for 10 days was highest but comparable to the concentrations in chicks of 7 and 4 days storage. Also, thyroxine level in chicks stored for 10 days was highest (p<0.05) compared to the concentration observed in chicks from 7 and 4 days storage. Chicks of 7 days storage had the least concentrations of T4. This research is in agreement with work of Lu *et al.* (2007) that plasma T_3 and T_4 concentrations were significantly elevated before hatching and their levels increased with age after hatching.

This means that thyroid hormones are important for successful hatching and also for stimulating a variety of developmental and metabolic processes after hatching (Reyns *et al.*, 2003; Lu *et al.*, 2007). The present work also corroborated the research of Christensen *et al.*(2002) in turkey embryos where elevation in TH during the final stages of incubation affected the maturation and survival functions thus, affecting hatching time. The present study is in agreement with work Tona *et al.*(2003) that chicks from eggs stored for 18 d had higher T3 at hatch. Reyns *et al.* (2003) and Lu *et al.* (2007) reported that after internal pipping, T3 reaches a peak during hatch, then after hatch, it returns to low levels. The higher T3 level in embryos of eggs stored for 10 d may indicate that embryos were weaker and required more T3 for hatching to occur because this will enable embryos to pierce the egg membranes and shell. Decuypere *et al.* (1990) stated that the concentrations of thyroid hormones T3 and T4 at a given time during incubation is dependent on embryo variability as well as an interaction between its chronological age and developmental stage. In chicken, hatching is typically associated with peak values in circulating thyroxine (De Groef *et al*., 2013). Ockleford *et al.* (1983) showed that advancing hatching time increased T3 concentrations in chicks but found no differences in T4 hormone concentrations between stimulated and non-stimulated chicks at same physiological stages of development. Thyroxine accelerates hatching in chum salmon (Dales and Hoar, 1954) by stimulating embryonic development and/ or the hatching mechanism. This suggests that the thyroid hormones play an important role in the hatching process between the start of pulmonary respiration and hatching. At IP and EP, T3 concentrations were same for all storage periods. This study differed from earlier submission of Tona *et al.* (2003) that at IP, T3 concentration was lower for eggs stored for 18 d. Also, Decuypere *et al*. (1979) observed that T3 and T4 levels were highest on the day of IP.

Table 4: Effects of egg storage duration on triiodothyronine and thyroxine levels at internal pipping, external pipping and hatch

Parameters	**Hatching events**	**Egg storage duration (days)**		
		4	7	10
T3 (pmol/L)	Internal l pipping	0.53±0.05	0.51±0.03	0.52±0.04
	External pipping	0.57±0.10	0.54±0.04	0.61±0.04
	Hatch	0.66±0.03^{b}	0.67±0.02^{b}	0.75±0.02^{a}
T4 (pmol/L)	Internal pipping	1.34±0.04	1.12±0.08	1.23±0.07
	External pipping	1.30±0.10	1.16±0.06	1.30±0.18
	Hatch	1.21±0.04^{ab}	1.18±0.02^{b}	1.33±0.03^{a}

^{ab} *Means within same rows having different superscript differs significantly (p<0.05)*

Table 5 shows the hour of start of hatching events from setting to emergence and duration of specific incubation hatching activity during embryonic development. Storage duration significantly (p<0.05) affected hours of embryos entering IP, EP and hatching but did not influence duration of time embryos spent on specific incubation event. The embryos of eggs stored for 4 days started IP earlier (465.00 ± 1.34) than embryos of eggs stored for 7 and 10 days (476.50 ± 4.61 and 476.50 ± 1.63) respectively. There were similarities in the hours of start of IP among the embryos of eggs stored for 7 and 10 days. The hours to exhibit EP was highest (488.83 ± 2.30) in embryos stored for 7 days compared to embryos of 4 and 10 days (477.50± 3.04 and 483.50 ± 1.69) respectively. Similarities were noticed in hours of entering EP among embryos of eggs stored for 4 and 10 days. The hours it took eggs stored for 4 days to hatch was lesser (486.00± 1.55) compared to hours it took eggs stored for 7 and 10 days to hatch. Eggs of 7 days storage spent more hours (497.50 ± 1.80) before emergence followed by 10 days egg storage

(491.50 ± 1.43). The present study showed that pre-storage period extended period of hatching events and time of hatch. This study is in line with the work of Mather and Laughlin (1976) who observed extended incubation time with pre-stored eggs of 14 days. Tona *et al*. (2003) reported a longer incidence of incubation duration of about 15 h in eggs pre-stored for 18 d compared to eggs stored for 3 days. We observed that eggs stored for 4 days had lesser hours of incubation compared to longer storage of 7 and 10 days. This earlier hatch is the result of a lesser time to start of IP.

Table 5: Effects of egg storage on hours of hatching events

Storage duration (days)			
Hatching events	**4**	**7**	**10**
IP	465.00± 1.342[b]	476.50± 4.610[a]	476.50± 1.628[a]
EP	477.50± 3.044[b]	488.83± 2.301[a]	483.50± 1.688a[b]
HH	486.00± 1.549[c]	497.50± 1.803[a]	491.50± 1.432[b]
EP-IP	12.50± 2.837	12.33± 4.208	7.00± 1.265
HH-EP	8.50± 2.247	8.67± 1.308	8.00± 1.483
HH-IP	21.00± 1.342	21.00± 3.950	15.00± 1.342

[ab] *Means within same rows having different superscript differs significantly (p<0.05)*

Conclusion

It can be concluded that Lohmann brown fertile eggs stored for 4 days before incubation had least weight loss during storage and incubation, least percentage embryo mortality and highest hatchability. The embryos from 4 days storage also were able to start IP and hatching event earlier compared to 7 and 10 days storage. The Stored eggs of 10 days yielded lowest hatchability and took longer time to initiate hatching. These experiments so far showed that the pre-storing of Lohmann brown eggs beyond 7 days is detrimental to the embryos.

Recommendation

For a higher number of chicks at hatch and less embryo dead, Lohmann brown egg storage should be between 4 - 7 days.

References

Bakst, M. R. and Akuffo, V. 1999. Impact of egg storage on embryonic development. *Avian and Poultry Reviews* 13: 125-131.

Becker, W. A. 1963. Length of preincubation storage of turkey eggs and its effects on body weight. *Poultry Science*, 42, pp. 1356-1359

Bloom, S. E. and Muscarella, D. E. 1998. Stress responses in the avian early embryo: Regulation by pro- and anti-apoptotic cell death genes. *Poultry Avian Biology*, **9**(2), 43–55.

Caipang, C. M. A., Fagutao, F. F. and Fatira, E. 2015. Cortisol levels and expression of selected stress- and apoptosis-related genes in the embryos of Atlantic cod, *Gadus morhua* following short-term exposure to air. *International Aquatic Research.* **7,** 75–84

Christensen, V. L., Wineland, M. J., Fasenko, G. M. and Donaldson, W. E. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. *Poultry Science*, 80, 1729–1735

Christensen V. L., Davis, G. S. and Nestor, K. 2002. Environmental incubation factors influence embryonic thyroid hormones. *Poultry Science*, 81, pp. 442-450

Dales, S. and Hoar, W. S. 1954. Effects of thyroxine and thiourea on the early development of chum salmon (*Oncorhynchus keta*) *Canadian Journal of Zoology*.32, pp.244-251

De Groef, B., Grommen, S. V. H. and Darras, V. M. 2008. The chicken embryo as a modelfor developmental endocrinology: development of the thyrotropic, corticotropic and somatotropic axes. *Molecular and Cellular Endocrinology*. 293, 17–24.

De Groef, B., Grommen, S. V. H., and Darras, V. M. 2013. Hatching the Cleidoic Egg:

The Role of Thyroid Hormones. *Frontiers in Endocrinology*, 4. 63.

De Smit, L., Bruggeman, V., Debonne, M., Tona, J. K., Kamers, B., Everaert, N., Witters, A., Onagbesan, O., Arckens, L., De Baerdemaeker, J. and Decuypere. E. 2008. The Effect of Nonventilation During Early Incubation on the Embryonic Development of Chicks of Two Commercial Broiler Strains Differing in Ascites Susceptibility *Poultry Science*, 87, pp. 551-560

Decuypere, E., Dewil, E. and Kühn, E. R. 1990. The hatching process and the role of hormones. *British Poultry Symposium*, Butterworths, London, pp. 239-256

Decuypere, E., Nouwen, E. J., Ku¨hn, E. R. and Michels, G. H. 1979. Iodohormones in the serum of chick embryos and posthatching chickens as influenced by incubation temperature. Relationship with the hatching process and thermogenesis. *Ann. Biol. Anim. Bioch. Biophys*. 19:1713–1723

Decuypere, E., Buyse J. and Buys, N. 2000. Ascites in broiler chickens: Exogenous and endogenous structural and functional causal factors *World's Poultry Science*. J., 56, pp. 367-377

Dymond, J., Vinyard, B., Nicholson, A. D., French, N. A. and Bakst, M. R. (2013). Short periods of incubation during egg storage increase hatchability and chick quality in long-stored broiler eggs. *Poultry Science*. 92:2977–2987

Ericsson, M. and Jensen, P. (**2016)**. Domestication and ontogeny effects on the stress response in young chickens (*Gallus gallus*). *Scientific reports.* 6**,** 35818.

Falahatkar, B., Akhavan, S. R. and Ghaedi, G. (2013). Egg cortisol response to stress at early stages of development in Persian sturgeon *Acipenser persicus*. *Aquaculture International*, 22(1), 215–223.

Fasenko, G. M. 2007. "Egg Storage and the Embryo." *Poultry Science*. 86(5): 1020-1024.

Fasenko, G. M., Robinson, F. E., Hardin, R. T. and Wilson, J. L. 1992. Variability in preincubation embryonic development in domestic fowl. 2. Effects of duration of egg storage period. *Poultry Science*. 71, pp. 2129-2132

Fasenko, G. M. and Robinson, F. E. 1999. Profiling egg storage: The effects on egg weight loss, egg characteristics, and hatchability. *Poultry Science*. 78 (Suppl. 1), p. 9

Fasenko, G. M., Robinson, F. E., Whelan, A. I., Kremeniuk, K. M. and Walker, J. A. 2001a. Prestorage incubation of long-term stored broiler breeder eggs. 1. Effects on hatchability. *Poultry Science*. 80, pp. 1406-1411

Fasenko, G. M., Christensen, V. L., Wineland, M. J. and Petitte, J. N. 2001b. Examining the effects of prestorage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. *Poultry Science*. 80, pp. 132-138

Hamidu, J. A., Feddes, J. J. R., Ouellette, C. A., Fasenko, G. M. 2010. The respiration of an avian embryo measured by an indirect calorimetric system Can. Biosys. Eng., 52, pp. 4-9

Hamidu, J. A., Uddin, Z., Li, M., Fasenko, G. M., Guan, L. L., and Barreda, D. R. 2011. Broiler egg storage induces cell death and influences embryo quality. *Developmental Biology*, **90**, 1749–1757.

Ioniţă L., Popescu-Micloşanu Elena, Oprea D. and Ioniţă M. T. 2013: Study on the influence of storage duration of eggs under conditions of high temperature on quail population "Baloteşti"performance hatching, International Scientific Symposium"Modern Animal Husbandry -Strategies, Opportunities and Performance","Ion Ionescu de la Brad"University of Agricultural Sciences and Veterinary Medicine of Iaşi, Faculty of Animal Sciences, Iaşi, April, Scientific Papers, *Animal Science Series*. 59 (18), pp. 133 – 138

Kalliecharan, R. and Hall, B. K. 1974. A Developmental Study of the Levels of

Progesterone, Corticosterone, Cortisol, and Cortisone Circulating in Plasma of Chick Embryos. *General And Comparative Endocrinology*, 24, 364-372.

Khan, M. J. A., Khan, S. H., Bukhsh, A. and Amin, M. 2014. "The Effect of Storage Time on Egg Quality and Hatchability Characteristics of Rhode Island Red (RIR) Hens." *Journal Veterinarski arhiv*. 84(3): 291-303.

King'ori, A. M. 2011. "Review of the Factors that Influence Egg Fertility and Hatchability in Poultry." *International Journal of Poultry Science.* 10(6): 483-492.

Lacin E., Coban, O. and Sabuncuoglu, N. 2008: Effects of egg storage material and storage period on hatchability in Japanese quail, *Asian-Australasian Journal of Animal Sciences.* 21, No. 8: pp. 1183-1188.

Lapao, C., Gama, L. T. and Soares, M. C. 1999. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability, *Poultry Science*, 78(5), 640-645

Lee, J. and Laycock, J. 1978. Essential Endocrinology. *Oxford Medical Publications*. New York. 202 p.

Lu, J. W., McMurtry, J. P. and Coon, C. N. 2007. Developmental changes of plasma insulin, glucagon, insulin-like growth factors, thyroid hormones, and glucose concentrations in chick embryos and hatched chicks. *Poultry Science*. 86, 673-683.

Mather, M. C., and Laughlin, K. F. 1976. Storage of hatching eggs: The effect on total incubation period. *British Poultry Science*. 17:471–479.

McCormick, M. I. and Nechaev, I. V. 2002. Influence of cortisol on developmental rhythms during embryogenesis in a tropical damselfish. *Journal of Experimental Zoology* 293:456–466

McNabb F. M. A. 2000. Thyroids. Sturkie's Avian Physiology, 5th ed., G. C. Whittow. *Academic Press,* New York; 461–471

Meir, M., Nir, A. and Ar, A. 1984. Increasing hatchability of turkey eggs by matching incubator humidity to shell conductance of individual eggs; *Poultry Science*. 63:1489-1496.

Merritt. E. S. 1964. Pre-incubation storage effects on subsequent performance of chickens. *British Poultry Science*, 5, pp. 67-73

Moberg, G. P. and Mench, J. A. 2000. The Biology of Animal Stress: *Basic Principles and Implications for Animal Welfare.* CABI Publishing: Wallingford. 384pp Wallingford, Oxon OX10 8DE, UK; or for North American orders from 10 E 40th St, Suite 203, New York.

Moberg, G. P. and Mench, J. A. 2000. The biology of animal stress*: basic principles and implications for animal welfare.* (CABI, 2000).

Morita, V. DeS., Almeida, V. R., Matos, J. B. J., Vicentini, T. I., Van den Brand, H. and Cristina, B. L. 2016. Incubation temperature during fetal development influences morphophysiological characteristics and preferred ambient temperature of chicken hatchlings. *Plos One* 11:1–16.

Möstl, E. and Palme, R. 2002. Hormones as indicators of stress, *Domestic Animal Endocrinology*, 23(1–2): 67-74.

Ockleford, E. M., Davison, T. F. and Vince, M. A. 1983. Changes in plasma iodohormone concentrations during the day before hatching in Gallus domesticus. *Comparative. Biochemistry and Physiology*., 75A, pp. 139-140

Onbaşılar, E. E., Poyraz, Ö and Erdem, E. 2007. Effects of egg storage period on hatching egg quality, hatchability, chick quality and relative growth in Pekin ducks.*Archiv fur Geflugelkunde*., **71** (4). S. 187- 191

Pawlowska, J. and Sosnówka-Czajka, E. 2019. "Factors Affecting Chick Quality in Poland." *World's Poultry Science.* 75(4): 621-632.

Popescu-Miclosanu Elena, 2007: "Poultry for egg production", *Publisher Printech, Bucharest*. Pages 80-87,

Rahn H. and Ar A. 1974: The avian egg: Incubation time and water loss; *Condor* 76:147-152.

Reijrink, I. A. M., Miejerhof, R., Kemp, B. and Van den Brand, H. 2008. The Chickens Embryo and its Micro Environment during Egg Storage and Early Incubation. *World'sPoultry Science*. 64(4): 581-598.

Reyns, G. E., Venken, K., Morreale de Escobar, G., Kühn, E. R. and Darras, V. M. 2003. Dynamics and regulation of intracellular thyroid hormone concentrations in embryonic chicken liver, kidney, brain, and blood. *General and Comparative Endocrinology*. 15;134(1):80-7

Scott T. A. and Mackenzie C. J. 1993. Incidence and classification of early embryonic mortality in broiler breeder chickens; *British Poultry Science* 34:459–470.

Senbeta, E. K. 2016. "Effect of Egg Storage Periods on Egg Weight Loss, Hatchabilityand Growth Performances of Brooder and Grower Leghorn Chickens." IOSR *Journal of Agriculture and Veterinary Science*. 9(11): 75-79

Sindt, M and Tönhardt, H. 2005. Cortisol in Allantois Liquid and in the Plasma of Chickens` Embryos at Common Temperature and After Cooling. The 2nd Combined Workshop of Fundamental Physiology of the European Working Group of Physiology, Berlin, 23. – 25.09.2005. *Institut für Veterinär-Physiologie*.

Sittman, K., Abplanalp, H. and Myerdick, C. F. 1971. Extended storage of quail, chicken and turkey eggs. 1. Hatchability and embryonic mortality. *Poultry Science*. 50, pp. 681-688

Tona, K., Malheiros, R. D., Bamelis, F., Careghi, C., Moraes, V. M. B., Onagbesan, O., Decuypere, E. and Bruggeman, V. 2003. Effects of Storage Time on Incubating Egg Gas Pressure, Thyroid Hormones, and Corticosterone Levels in Embryos and on their Hatching Parameters. *Poultry Science*. 82:840–845

Whitehead, C. C., Maxwell, M. H., Pearson, R. A. and Herron, K. M. 2002. Influence of egg storage on hatchability, embryonic development and vitamin status in hatching broiler chicks. *Brazilian Journal of Poultry Science*. 26:221–228.

Whitehead, C. C., Maxwell, M. H., Pearson, R. A. and Herron, K. M. 1985. Influence of egg storage on hatchability, embryonic development, and vitamin status in hatching broiler chicks. *British Poultry Science*.26, pp. 221-228

Date received : 31st July, 2024

Date accepted : 4th September, 2024