

EFFECT OF STRAIN AND PRE-INCUBATION STORAGE ON EGG PARAMETERS OF BROILER BREEDERS

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ABSTRACT

This study was conducted to evaluate the effect of breed, pre-incubation storage days and their interaction on hatching parameters of Arbor acre and Ross breeder hens. A total number of 1200 eggs were collected in three (3) batches and stored for 4 days, 7 days and 10 days before incubation. The experiment was designed as a completely randomized design (CRD) with 2 x 3 factorial arrangement. Data collected on fertility and hatching parameters were subjected to the General Linear Model of SAS®, while significant differences were separated using Tukey's Honestly Significant Difference. Fertility and hatchability of eggs set were significantly influenced by breed with Arbor acre showing better performance than Ross ($P < 0.05$). Pre-incubation storage days had a significant effect on the hatchability on eggs set (HES), hatchability on fertile eggs (HFE) and embryonic mortality (EMF) ($P < 0.05$). The interaction of breed and pre-incubation storage revealed that both breeds produced the best HFE on 7-days pre-incubation egg storage interval ($P < 0.05$) with least embryonic mortality (3.5, 7.5 %). In the future, the practice of pre-storage incubation of eggs for 6–12 hours can be applied with the 7-day pre-incubation storage period to improve overall egg hatchability and chick quality.

Key words: Arbor acre; embryonic mortality; fertility; hatchability; Ross-308; pre-incubation storage-days

INTRODUCTION

Every fertile broiler breeder egg should produce a healthy chick. Many factors can cause the failure of a fertile egg to hatch. These include lethal genes, insufficient nutrients in the egg and exposure to conditions that do not meet the needs of the developing embryo either on-farm or in the hatchery (King'ori, 2011). Egg batching and storage on the farm, pre-hatchery, is a common practice. It occurs mostly due to an imbalance in the hatching egg supply, low quantity of eggs laid in the breeding farm and low hatchery capacity in the face of high demand for day-old chicks (Goliomytis *et al.*, 2015).

Several studies report that hatchability is directly affected by storage days (King'ori, 2011; Goliomytis *et al.*, 2015; Ayeni *et al.*, 2020 and Abioja *et al.*, 2022). Hatching

eggs are often stored for some periods on broiler breeder farms for 1–4 days to minimize transportation costs. Storage continues in the hatchery cold room till setting time, to gather a large number of fertile eggs to fill the large setting compartment of incubators. Storage of eggs for more than 7 days is known to increase embryonic abnormality and mortality due to degradation of the viscosity of egg albumen (Petek *et al.*, 2003; Van de Ven, 2004). Goliomytis *et al.* (2015) reported that hatchability was not affected by egg storage but declined a little after four (4) days of storage. Ayeni *et al.* (2020) also reported maximum hatchability at days 1–4; but a decline of up to 78 % in the hatchability on storage extension to day 13.

The information on pre-hatching storage provided, can help farmers in decision-making when considering the appropriate egg storage period to use. Hatchability

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could be lower than fertility by up to 8 % because the embryo is usually lost during incubation. Several studies on pre-incubation storage have been conducted on many breeds such as Cobb, Ross 308 and Hubbard Classic (Tona *et al.*, 2003; Goliomytis *et al.*, 2015 and Gharib, 2013) but research is still needed on breed response to the length of pre-incubation egg storage especially in the humid tropics highly fluctuating weather conditions. This can help farmers in decision-making, when considering the appropriate breed-specific storage days to adopt on-farm before setting. The objective of this research was to determine the effect of breed and pre-incubation storage days on the hatching performance of broiler eggs.

MATERIALS AND METHODS

Experimental site

The hatched chicks were reared at the Teaching and Research Farm of the Department of Animal Production and Health, Federal University, Oye–Ekiti, Ikole-Ekiti campus, Ekiti State. Ikole Ekiti is in the Rainforest Zone of South-west Nigeria. It is located at latitude 7.783333° North and longitude 5.516667° East (Bolarinwa *et al.*, 2017). Hatchery operation was carried out at Nothing Pass God (NPG) Hatchery, Oluyole Estate, Sharp Corner, Ibadan, Oyo State, Nigeria.

Experimental procedure

Collection, setting and hatching of eggs

A total of 1200 eggs of both Ross 308 and Arbor acre strains were sourced from a reputable farm (Repute

Table 1. Experimental treatments with replication

Treatment	Number of eggs per batch	
Egg storage days	Strains	
	Ross 308 eggs	Arbor Acre eggs
10	R _{3,4} (50, 50, 50, 50)	A _{3,4} (50, 50, 50, 50)
7	R _{2,4} (50, 50, 50, 50)	A _{2,4} (50, 50, 50, 50)
4	R _{1,4} (50, 50, 50, 50)	A _{1,4} (50, 50, 50, 50)

R – Ross, A – Arbore acre, first subscript represent storage day, second subscript represent number of replications within each storage day = 4

Agro and Allied Matters) at Ibadan. Both breeds of broiler breeder birds were reared on the same farm under the same management practices. Hatchable eggs (400) were collected in three batches. Each batch of eggs collected consisted of 200 eggs of Ross 308 and 200 eggs of Arbor Acre. The first batch of eggs was collected on day 1 and stored for 10 days; the second batch of eggs was collected on day 4 and stored for 7 days and the last batch of 400 eggs was collected on day 7 and stored for 4 days. Each treatment (storage days: 10, 7, 4) had four replicates with 50 eggs in each replicate. Hatchable eggs for each treatment and replicate were labelled for proper identification with different letters and colours. All eggs were stored in the hatchery at a cold-room temperature of 18°C and 75 % of relative humidity (RH) before setting into the incubator on day 11. The eggs were distributed into treatments and replicates. Due to reductions from cracked eggs, only 1116 hatching eggs were set into the incubator out of the 1200 eggs collected (93 %). On day 11, the eggs were placed into the setter at 37°C

Table 2. Number of eggs collected from the farm for hatching in batch and breed, by replicate

Egg storage days	Strains	Rep 1	Rep 2	Rep 3	Rp 4	Total collection
Batch 1 (10 days)	Ross	46	46	47	47	186
	AA	46	46	47	47	186
Batch 2 (7 days)	Ross	46	46	46	47	185
	AA	46	46	46	47	185
Batch 3 (4 days)	Ross	46	47	47	47	187
	AA	46	47	47	47	187
Overall Total		276	278	280	282	1116

AA – Arbor Acres, Rep – Replicates, Batch – pre-incubation holding days

and 75 % RH. Egg candling was done on the 18th day to transfer fertile eggs from the setter to the hatcher compartment of the incubator.

Data collection

The eggs (n = 116) were collected from REPUTE AGRO and ALLIED MATTERS farms for Breed, Batch and Replicate, as presented on Table 2.

The following traits were counted and recorded: number of good eggs set into the setter per breed, batch and replicate, number of fertile eggs on candling at day 18 per breed, batch and replicate, number of eggs hatched at day 21 per breed, batch and replicate, number of eggs with no food in the shell at day 21 per breed, batch and replicate and number of deformed and unhealed navels out of hatched chicks per breed, batch and replicate.

The above primary data were used to generate secondary trait values using the formulae below:

1. Percent fertility on eggs set =

$$\frac{\text{No. of fertile eggs} \times 100}{\text{No. of the egg set}}$$

2. Percent hatchability on eggs set =

$$\frac{\text{Total number of chicks hatched} \times 100}{\text{No. of the egg set}}$$

3. Percent hatchability on fertile eggs =

$$\frac{\text{Total number of chicks hatched} \times 100}{\text{No. of fertile eggs}}$$

4. Percent embryonic mortality on fertile eggs =

$$\frac{\text{Total number of chicks unhatched} \times 100}{\text{No. of fertile eggs}}$$

Experimental design, model and statistical analysis

This was a completely randomized design (CRD) with a 2 × 3 factorial treatment arrangement, while the statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk},$$

where:

Y_{ijk} – general observation

μ – universal mean (constant)

α_i – i^{th} effect of breed (i = Ross, Arbor Acre)

β_j – j^{th} effect of pre-hatching storage days (j = 10, 7, 4)

$\alpha\beta_{ij}$ – interaction effects of the i^{th} breed and j^{th} storage days

ϵ_{ijk} – random error term.

Data generated were subjected to ANOVA ($\alpha = 0.05$) using the General Linear Model of SAS version v 9.4 (2013), and means were separated by Tukey's HSD procedure.

RESULTS

Effect of breed on hatching parameters of broiler breeder eggs

Table 3 shows the effect of breed on the fertility on eggs set (FES), hatchability on eggs set (HES), hatchability on fertile eggs (HFE) and embryonic mortality on fertile eggs (EMF). Arbor Acre eggs recorded better ($P < 0.05$) fertility than Ross eggs (90.3, 78.0 %). Although breed had no significant effect on HFE and EMF, however, it significantly ($P < 0.05$) influenced the FES (90.3, 82.2, %) and HES (78.0, 69.1 %) with Arbor Acre recording higher values than Ross.

Effect of storage days on hatching parameters of broiler breeder eggs

Table 4 shows the effect of storage days on the hatching parameters of broiler breeder eggs. Eggs stored for 7 days had the best HFE (94.5 %) followed by

Table 3. Effect of breed on hatching parameters of broiler breeder eggs

Breed	Fertility (%)	Hatchability on fertile eggs (%)	Hatchability on egg set (%)	Embryonic mortality (%)
Arbor Acre	90.3 ^a	90.9	82.2 ^a	9.0
Ross	78.0 ^b	88.5	69.1 ^b	11.5
SEM	1.72	1.47	2.16	1.47
P-values	0.0001	0.0810	0.0001	0.0850

SEM – standard error of the mean, AA – Arbor Acre, P – level of significance, ^{ab} means with different superscripts in a column are significantly different at $p < 0.05$

Table 4. Effect of storage days on hatching parameters of broiler breeder eggs

Storage days	Fertility on (%) eggs set (%)	Hatchability of fertile eggs (%)	Hatchability on egg set (%)	Embryonic mortality (%)
4	81.2	84.1 ^b	68.3 ^b	15.9 ^a
7	84.1	94.5 ^a	79.5 ^a	5.5 ^b
10	87.2	90.6 ^a	79.2 ^a	9.4 ^b
SEM	2.91	1.23	3.01	1.23
P-values	0.1720	0.0001	0.0001	0.0001

SEM – standard error of the mean, AA – Arbor Acre, P – level of significance, ^{ab} means with different superscripts in a column are significantly different at $p < 0.05$

eggs stored for 10 days (90.6 %), while 4 days storage resulted in the least HFE (84.1 %). Significant differences ($p < 0.05$) were observed in HES and EMF, where eggs stored for 4 days showed the least HES (68.3 %) and the highest EMF (15.9 %). The best and least EMF was revealed on setting egg for 7 days pre-incubation.

Effect of interaction of breed and storage days on hatching parameters of broiler breeder eggs

The effect of interaction of breed and storage days (Table 5) were significant on hatching parameters ($P < 0.05$). Arbor acre (AA) eggs stored for 10, 7 and 4 days revealed the best fertility on eggs set (92.5, 91.4 and 87.1 %) respectively, followed by Ross (RS) breeder eggs stored for 10 days (76.8 %). The AA breeder eggs for 7, 10 and 4 days exposed the best HES (88.1, 86.2 and 72.6 %), while RS breeder eggs stored for 7 days reported best HES at 7 days storage (70.8 %). The AA eggs stored for 7 and 10 days revealed best HFE (96.5 and 93.0 %); while RS breeder eggs had the best HFE at 7 days pre-incubation

storage interval. The embryonic mortality on fertile eggs, EMF in AA eggs stored for 7 and 10 days (3.5, 6.9 %) were least, followed by Ross eggs stored for 7 days (7.5 %). The RS and AA eggs stored for 4 days had the highest embryonic mortalities of 15.2 and 16.6 % respectively.

DISCUSSION

The success of a hatchery procedure often is dependent on either pre-hatchery or within hatchery conditions. Pre-hatchery conditions, such as the breed of parent stock, nutrition, environmental conditions, management style, hen age and egg storage conditions among other factors are important considerations that determine the success of a breeder farm operation. Any of these factors or their combinations may undermine the fertility and hatchability of breeder eggs. The fertility of an egg is achieved by a fusion between the male sperm cell and the female ovum, while hatchability measures

Table 5. Effect of interaction of breed and storage days on hatching parameters of broiler breeder eggs

Interaction	Fertility on (%)	Hatchability of fertile eggs (%)	Hatchability on egg set (%)	Embryonic mortality (%)
AAX 4 days	87.1 ^c	83.4 ^e	72.6 ^b	16.6 ^a
AAX 7 days	91.4 ^a	96.5 ^a	88.1 ^a	3.5 ^d
AAX 10 days	92.5 ^a	93.0 ^b	86.2 ^a	6.9 ^c
ROSSX 4 days	75.3 ^d	86.4 ^d	64.0 ^c	15.2 ^a
ROSSX 7 days	76.8 ^d	92.6 ^b	70.8 ^b	7.5 ^c
ROSSX 10 days	81.9 ^c	88.3 ^c	72.3 ^b	11.8 ^b
SEM	2.87	1.60	2.69	1.60
P-value	0.0450	0.0360	0.0340	0.0360

SEM – standard error of the mean, AA – Arbor Acre, P – level of significance, ^{abcde} means with different superscripts in a column are significantly different at $p < 0.05$

how frequently incubated or fertile eggs hatch into live chicks after a 21-day incubation period (Ulmer-Franco *et al.*, 2010). The probability of an embryo survival is a function of its genotype and the quality of the environment provided by the egg, whereas fertility depends on genetic and environmental factors coming from the female and her mate (Wolc *et al.*, 2009).

The above information corroborates the observations from present study, where fertility rate in the two breeds differed, whereas hatchability on fertile eggs and embryonic survival rates were similar. Arbor acre (AA) breed with higher FES and HES than Ross (RS) breed exhibits superior genetic attributes for egg hatching than the Ross breed by 12.30 and 13.10 % respectively. The values obtained presently were higher than FES, HES and HFE of 74.80–84.00, 67.20–74.40 and 86.67–88.42 % reported on three genotypes of ducks in Bangladesh (Mia *et al.*, 2019). In an interaction study of two breeds (Cobb MV and Ross 308AP) and three storage times of fertile eggs (4, 7 and 9 days), breeder strain was also found to influence hatching parameters of fertile eggs and infertile eggs (Volpe *et al.*, 2024). Same authors also reported the effect of breed on phase 1 (at days 1–4; 6.07–3.83 %) and phase 2 (days 5–8; 0.56–1.45 %) embryo mortality, discarded chicks (1.45–1.10 %) and infertile eggs (2.76–10.25 %) in egg embryos between Ross-308 and Cob broiler breeders respectively. Alsobayel *et al.* (2012) reported a better HFE for Cobb, than Ross and Arbor Acre breeds (82.4, 78.0 and 71.2 %) respectively. This result was explained by the evolution or genetic improvement between the period of experiments. Taemchuay (2023) showed that the number of infertile eggs, rejected eggs and dead eggs of Arbor Acre Plus S was slightly but not significantly higher than that of Ross 308. In present study, hatchability of fertile eggs (HFE) and embryonic mortality (EMF) were not affected statistically by breed differences. Joseph and Moran (2005) reported no differences in embryonic mortality among three commercial broiler breeder strains.

Egg storage time is important for adequate incubation, (hatching) and production indices (Volpe *et al.*, 2024). The act of incubating eggs immediately after laying reduces the hatching rate, and thus eggs require at least a short storage interval of 24 hours to improve hatchability (Macari *et al.*, 2013, Volpe *et al.*, 2024). However, only short pre-incubation storage periods (3–7 days) help incubation, as this period is necessary for gelatinization, for an adequate pH to be reached, and for

the formation of the air sac, essential for embryonic development (Nasri *et al.*, 2020). The storage days examined in present study were within 7–10 days recommended for optimum hatchability (King'ori, 2011). Contrary to expectation, fertility on eggs set (FES) was unaffected by storage days; but while HFE and HES improved with storage, embryonic mortality decreased with storage to till the 7th day. Thus, FES was numerically best at 10 days storage, while HES and HFE were highest at 7-days storage. The EMF was lowest and best at 7 days. The similarity in the FES of breeds irrespective of storage days further emphasized the opinion that the egg fertility has more genetic implications and may not necessarily be altered by external factors.

Previous authors (Tona *et al.*, 2003; Fassenko *et al.*, 2001; Tona *et al.*, 2004; Yassin *et al.*, 2008) had posited that storage of eggs beyond 7 days increased incubation time, affected hatchability negatively and reduced chick quality. Petek and Dikmen (2006) observed better hatchability in eggs pre-incubated for 5 days than 15 days. This position may explain the observed decline in percent fertility on eggs set (FES) and hatchability on eggs set (HES) obtained in present study.

Embryonic mortality was however affected by storage days. Percent EMF from eggs stored for 4 days was higher, decreased to 7 days but increased slightly at again 10 days. This behaviour may be due to the short duration of days of acclimatization to the pre-hatching conditions before exposure to incubation, thus, causing shock to the egg embryos. Contrary to this finding, Goliomytis *et al.* (2015) did not observe an influence of egg storage days on embryonic mortality. Uyanga *et al.* (2020) reported that extended storage of eggs up till 10 days from 65-wk-old breeders resulted in significant deterioration of egg quality traits and decreased egg weight, whereas egg weight loss increased with storage duration for both breeder ages of 43 and 65 weeks. Nasri *et al.* (2020) reported thickened albumen diameter and increased egg pH, decreased yolk dry-matter between 2 and 19 days of storage; while prolonged egg storage resulted in a lower HES and HFE due to a higher percentage of embryonic mortality in Arbor acre breeder eggs. Volpe *et al.*, (2024) reported that egg pre-incubation storage (4, 7 and 9-day) affects phase 1 mortality in broilers. This is the first-phase mortality in which egg embryos die-out between day 1 and day 4 of incubation, with mortality range of 4.01–5.62 %. The storage of fertile eggs for more than

4 days thus influenced the initial embryonic mortality. Alsobayel *et al.* (2012) observed lower rates of early and late embryonic mortality in eggs with zero day of storage, compared to eggs with 7 and 14 days of storage (9.6, 17.7, 31.5 %) respectively. The increase in embryonic mortality with longer storage period beyond 7 days storage observed could be associated with reduction in egg quality such as poorer albumen quality—due to albumen pH changes that affect the quality of the chalaza and the vitelline membrane (Rocha *et al.*, 2013). The fragility of the chalaza and the vitelline membrane make the embryo more exposed in the first days of incubation, increasing mortality rates. It has also been reported that a handling-time interval—of about 5 hours between oviposition and cold room storage—for fertile eggs have led to best hatching rates (Fiuza *et al.*, 2006).

Information on effects of interaction of both factors studied could inform us on the best breeding and hatchery management practices for specific breeds. This interaction can also help to optimize egg storage interval (days) for breeds, leading to breed-specific storage recommendations, reduced embryo mortality, improved chick quality and enhance hatchery efficiency. The AA eggs stored for 10 and 7 days with the best FES, HES, HFE and least EMF values provide the best storage intervals for optimization of hatching traits in the breed. But the 7-day storage interval seem to be the better option with improvement in 3 other parameters than the 10-day storage period. On the other hand, Ross eggs stored for 7 days pre-hatching produced the best FES and HES with lowest EMF, although higher than in AA. The 7 days storage had a better HFE than the 10 days storage by 4.3 % in Ross breed. Findings from present study on the effect of breed and storage interval (of hatching eggs) on hatching parameters suggest the use of breed-specific storage interval to optimize hatchability in breeder birds. Mia *et al.* (2024) reported significant effect of breeder strain and storage time on the body weight of chicks at 7 days of age, weight gain of chicks between day 1 to day 7 of life (first 7-days of life) and on multiplication of the initial weight of chicks at 7 days of age. Their results show that the number of infertile eggs, rejected eggs and dead eggs of Arbor Acres Plus S was only numerically higher than that of Ross 308.

CONCLUSION

Study showed that Arbor Acre breed had higher hatching parameter values at 7 days than Ross breed. Eggs stored for 7 days showed the best hatching parameters with drastic reduction of embryonic mortality. Interaction study revealed an improvement in hatchability and reduction in embryonic mortality in both breeds when eggs were set on 7-days cold storage. More studies are needed on the interaction of breed, storage days above 10 and seasons to give better year-round information in the region.

STATEMENT OF ANIMAL RIGHTS AND ETHICAL APPROVAL

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The experimental procedure was reviewed and approved by the FUYOE Institutional Animal Care and Use Committee (IACUC) concerning animal ethics and welfare.

AUTHOR'S CONTRIBUTIONS

Conceptualization: ADESINA, H. O., JESUYON, O. M. A., ORUNMUYI, M.

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All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data for this work are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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