Effect of Pre-Incubation Storage Periods on Weight Loss, Embryonic Development, and Hatchability of Pullet Eggs.

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ABSTRACT

This study was carried out to determine the effect of pre-incubation storage periods on egg weight loss, embryonic development and hatchability of hatching pullet eggs. Three hundred eggs were used for this experiment. The eggs were sourced on five different days (sixty eggs per day) at three days interval (day 0, 3, 6, 9, and 12) and weighed. The sixty (60) eggs collected on each day were subdivided into three replicates of twenty eggs each. Data obtained were subjected to one-way analysis of variance. Results showed that egg weight loss during incubation was inversely ($P<0.05$) influenced by the pre-incubation storage periods. Pre-incubation storage periods did not significantly ($P>0.05$) affect yolk weight, albumen height and Haugh unit value. The relative embryonic development showed a uniform and continuous increment in the rate of embryonic development in the different storage periods. Eggs stored for a few days had higher ($P<0.05$) hatchability than those set in the incubator on the day of oviposition. Hatchability was adversely ($P<0.05$) affected by prolong egg storage. It could be concluded that hatching eggs should neither be set in incubator on the day of oviposition nor subjected to pre-incubation storage period more than 6 days at 18ºC for higher hatchability.

(Keywords: hatchability, eggs, embryonic development)

INTRODUCTION

Egg possesses excellent nutritive value and constitutes a food use in many basic and formulated preparations. Besides its important reserves of high digestible proteins, lipids, vitamins, and minerals, the egg contains molecules with numerous health promoting and biotechnological properties (Froning, 1991). Its original role as an embryonic chamber implies that it contains many components essential for life. Eggs are fragile commodities and are subject to quality loss with age. Planned production method and efficient quality control procedures help to reduce this variation and are fundamental to the successful marketing of eggs (De ketelaere *et al*., 2004). The avian egg is a biological system intended to ensure the well-being of the embryo and its successful hatching into a fully developed chick (Narushin and Romanov, 2002).

Egg characteristics are affected by various factors including the genetic structure of the flock, feeding, health, age of the flock, housing, storage conditions, and duration. The internal and external characteristics of the egg change significantly by age. While egg shell quality deteriorates, egg weight, yolk weight, and albumen weight increase as the age increases in chickens.

Although numerous studies have shown that there is strong positive correlation between pre-incubation egg weight, length of storage periods, hatching weight and growth performance of different species of poultry such as guinea fowl (Ayorinde *et al*., 1994; Farooq *et al*., 2001), Emu (Danczak and Majewska, 1999) broiler (McLoughlin and Gous, 1999) and Ostriches (Nahm, 2001), but the effect of egg storage periods on embryonic development and hatchability of hatching pullet eggs have not been fully investigated; hence, the need for this study.

MATERIALS AND METHODS

Location of the Experiment

This experiment was carried out at the hatchery unit of the Federal University of Agriculture,

Abeokuta (FUNAAB)-Leventis Agro Industrial Limited, Kotopo, Abeokuta, Ogun State, Nigeria.

Egg Management

A total number of three hundred (300) hatching eggs of Dominant Black strain of pullet were used for this experiment. The eggs were sourced from a reputable breeder farm on five different days (sixty eggs per day) at three days interval (day 0, 3, 6, 9, and 12) and then weighed. The sixty (60) eggs collected on each day were subdivided into three replicates of twenty eggs each. They were stored at a temperature of 18°C in the cold room of the hatchery.

On the last day of storage, the eggs were removed from the cold room and allowed to adjust to ambient (room) temperature for 6 hours, so as to allow the eggs temperature to increase gradually and to prevent early embryonic mortality as a result of exposure to high temperature in the incubator. Potassium tetraoxomanganate VII ($KMnO_4$) and formaldehyde (HCHO) at ratio 1:2 were used as fumigant for the eggs. The treatment lasted for 20 minutes in a closed chamber. The eggs were set in egg trays with broad ends upward to prevent rupture of air cell. Egg turning was automatic which was on hourly basis. The egg turning involves rotating the axis of the eggs. This was to prevent developing embryos from adhering to the shell and to ensure uniform distribution of nutrients. Candling was done on the 18th day to test for fertility of eggs. All infertile eggs were discarded while the fertile eggs were transferred into the hatcher where they spent three days to complete the incubation period.

Data Collection

Measurements taken include:

Pre-Incubation Storage Periods Weight Loss: The eggs were weighed before storing in the cold room and on the last day of storage, pre-incubation weight loss was determined using the formula below:

Pre-incubation weight loss (g) = Initial weight – Final weight

Pre-incubation weight loss (%) =

$$\frac{\text{Weight loss (g)}}{\text{Initial weight (g)}} \times \frac{100}{1}$$

Egg Quality Evaluation: Both external and internal egg qualities were carried out at the end of the pre-incubation storage period. Egg and yolk weight were measured using sensitive scale. Egg length and width were measured with Vernier calipers while shell thickness was measured with micrometer crew gauge. Haugh units (HU) were calculated according to formula (Eisen *et al*., 1962) based on the height of albumen as determined using spherometer.

Determination of Embryonic Development: A total number of ninety (90) hatching eggs (18 eggs from each treatment) were used for this experiment. At days 0, 6, 9, 12, 15 and 18 of incubation, three (3) eggs from each treatment were gently broken to determine weight of growing embryos and the amount of moisture loss. After breaking, each embryo was carefully removed and separated from all attachments such as yolk sac and chorioallantoic membrane and then weighed on sensitive scale.

Determination of Incubation Weight Loss fertility and hatchabiilty: The eggs were weighed before setting and on the 18th day, incubation weight loss was determined using the formula below:

Weight loss (g) = Initial weight – Final weight

$$\text{Weight loss (\%)} = \frac{\text{Weight loss (g)}}{\text{Initial weight (g)}} \times \frac{100}{1}$$

Determination of Fertility and Hatchability: Fertility was determined using the formula stated below:

$$\text{Fertility (\%)} = \frac{\text{No of fertile eggs}}{\text{No of eggs set}} \times \frac{100}{1}$$

Hatchability was determined using the formula stated below:

$$\text{Hatchability (\%)} = \frac{\text{No of chicks hatched}}{\text{No of fertile eggs}} \times \frac{100}{1}$$

Determination of Embryonic Mortality: This was carried out in the laboratory. The unhatched incubated eggs were broken gently to observe the stage of embryonic mortality. This was categorized into two: Dead-in-germ (early embryonic mortality) and Dead-in-shell (late embryonic mortality).

Statistical Analysis

The experimental design was a completely randomized design (CRD). Data obtained were subjected to one-way analysis of variance using SAS (1999). Significant (P<0.05) means among variables were separated using Duncan Multiple Range Test of the statistical package.

RESULTS AND DISCUSSION

Egg Weight Loss during Pre-Incubation Storage

Table 1 showed the result obtained for egg weight loss during pre-incubation storage periods. Generally, results obtained for all the treatments were significantly (P<0.05) different. The results obtained for the pre-incubation storage period showed that storage of eggs leads to increased water loss during storage. Egg weight loss during storage increased regularly with a rate around 0.77grams/per week, in chicken eggs (Silversides and Villeneuve, 1994). Samli *et al.* (2005) stored chickens eggs at 5ºC for 2, 5 and 10 days and observed 0.27%, 0.51% and 0.66% of egg weight loss during storage respectively. Egg weight loss is an important parameter for incubation. Schmidt *et al*. (2009) reported that long storage of eggs decreases egg weight

The results on the external quality parameters of eggs stored at different periods before setting into the incubator showed that egg length, width and shell weight were significantly (P<0.05) influenced (Table 2). This result could not be attributed to the storage periods since egg storage has no or little effect on external parameters of egg. Table 3 showed the internal quality parameters of eggs stored at different periods before setting into the incubator. The results showed that all the parameters measured (albumen weight, albumen height, yolk weight and Haugh unit) were not significant (P>0.05). Haugh unit and yolk indices are generally considered as good indicators to evaluate egg quality (Ayorinde, 1987). The HU result obtained in this study contradict the findings of Lapao *et al*. (1990), Benton and Brake (1996), Silversides and Scott (2001) who reported decrease in the values of HU with prolong pre-incubation storage, the variation in the result could be as a result of variation in the environmental condition such as temperature and relative humidity of the storage room.

Both Lapao, *et al*. (1990) and Benton and Brake (1996) showed that although the changes in pH occur mostly in the first 4 days of storage, albumen height and embryo viability continues to diminish with each additional day of storage. Haugh (1937) also indicated that egg yolk size increased with storage time due to movement of water from the albumen to the yolk as a result of osmotic pressure differences. Brake *et al*. (1993) stated that water loss from the albumen may have a negative effect on the viscosity of the albumen, although later Benton and Brake (1996) were unable to find a direct relationship between water loss and albumen pH and height.

Table 1: Effect of Pre-Incubation Storage Periods on Pullet Egg Weight Loss during Storage.

Parameter	Day of Pre-incubation Storage				
	Control	3	6	9	12
Initial weight (g)	55.95± 0.86	56.89±0.47	56.39±1.26	55.97±0.58	55.89±0.48
Final weight (g)	55.95±0.86^{ab}	56.59±0.77^{a}	55.90±1.27^{ab}	54.87±0.51^{b}	54.79±0.50^{b}
Weight loss (g)	0.00±0.00^{c}	0.30±0.32^{bc}	0.49±0.26^{b}	1.09±0.12^{a}	1.10±0.16^{a}
Weight loss (%)	0.00±0.00^{c}	0.52±0.56^{bc}	0.87±0.46^{b}	1.95±0.18^{a}	1.97±0.29^{a}

a, b, c means in the same row with different superscripts differ significantly (P<0.05)

Table 2: External Egg Quality Parameters after Pre-Incubation Storage.

Parameter	Day of Pre-incubation Storage				
	Control	3	6	9	12
Egg weight (g)	56 98±0.14	57.35±0.11	57.03±0.05	57.17±0.93	57.31±0.42
Egg length (cm)	4.23±0.02^{b}	4.33±0.04^{a}	4.19±0.04^{b}	4.15±0.1^{b}	4.02±0.03^{c}
Egg width (cm)	2.61±0.03^{b}	2.66±0.01^{ab}	2.69±0.05^{ab}	2.71±0.07^{a}	2.69±0.06^{ab}
Shell weight (g)	5.19±0.05^{bc}	5.45±0.02^{ab}	5.73±0.31^{a}	5.41±0.21^{ab}	5.01±0.11^{c}
Shell weight (%)	9.15±0.07^{b}	9.44±0.05^{b}	10.05±0.55^{a}	9.46±0.21^{b}	8.92±0.26^{b}
Shell thickness (mm)	0.37±0.01^{ab}	0.35±0.00^{b}	0.37±0.02^{ab}	0.36±0.01^{b}	0.39±0.01^{a}
Egg shape index	0.62±0.01^{b}	0.62±0.01^{b}	0.64±0.02^{ab}	0.65±0.03^{ab}	0.67±0.02^{a}

a, b, c means in the same row with different superscripts differ significantly (P<0.05)

Table 3: Internal Egg Quality Parameters after Pre-Incubation Storage.

Parameter	Day of Pre-Incubation Storage				
	Control	3	6	9	12
Yolk weight (g)	14.84±0.26	15.73±0.29	16.65±0.71	14.57±2.85	16.01±1.13
Yolk weight (%)	26.16±0.39	27.24±0.45	29.19±1.26	25.41±4.57	28.51±2.22
Albumen height(mm)	7.86±0.23	7.61±0.14	7.34±0.36	7.20±0.69	7.04±0.46
Albumen weight (g)	36.69±0.17	36.57±0.16	34.66±1.07	37.20±2.13	35.16±1.65
Albumen weight (%)	64.70±0.46	63.33±0.39	60.77±1.82	65.14±4.78	62.58±2.47
Haugh unit	89.14±1.31	87.42±0.00	84.62±0.00	84.99±4.52	84.39±2.70

Table 4: Effect of Storage Periods on Incubation Weight Loss.

Parameter	Day of Pre-Incubation Storage				
	Control	3	6	9	12
Incubation weight (g)	55.88±0.03^{c}	56.01±0.01^{b}	56.07±0.03^{a}	55.78±0.02^{d}	56.03±0.04^{ab}
Weight at candling (g)	46.02±0.03^{e}	47.02±0.01^{d}	47.47±0.03^{c}	47.69±0.02^{b}	47.99±0.04^{a}
Incubation weight loss (g)	9.86±0.00^{a}	8.99±0.00^{b}	8.60±0.00^{c}	8.09±0.00^{d}	8.04±0.00^{e}
Incubation weight loss (%)	17.64±0.00^{a}	16.05±0.00^{b}	15.34±0.00^{c}	14.50±0.00^{d}	14.35±0.00^{e}

a, b, c, d, e means in the same row with different superscripts differ significantly (P<0.05)

Incubation Weight Loss

The result obtained during incubation on egg weight loss of the pullet eggs with different storage periods is presented in Table 4**.** The results were significantly (P<0.05) influenced by the treatment.

Egg weight loss decreased as the pre-incubation storage period increased. Reduction in incubation weight loss for eggs stored for long duration could be attributed to the increase in weight loss as the pre-incubation storage increased. Weight loss during incubation confirms with the study of Romao *et al.* (2008) who stated that eggs stored for longer periods presented lower levels of weight loss during incubation as compared to fresh incubated eggs or those that were subjected to a few days of storage. Most of the water of the egg is initially in the albumen which declines continuously during incubation as a result of water loss to the ambient air and movement to the compartments (Romanoff, 1967).

Embryonic Development

Embryonic development in the various egg storage periods is presented in Figure 1. A uniform and continuous increment in the rate of embryonic development in relation to the incubation days was observed among the groups, only a very slight difference could be observed prior to incubation day 9. Starting from incubation day 9, there was observable difference seen in the increased rate of embryonic development in egg storage period day 6 while at 18th day of incubation there were distinctively clear differences in the treatment groups, with storage period day 6 eggs having the best and highest rate of embryonic development.

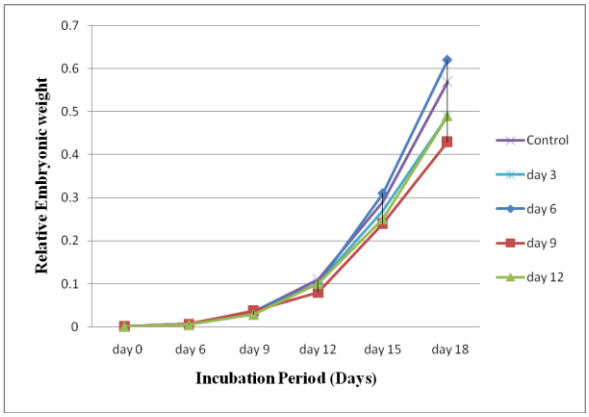

Figure 1: Effect of Storage Periods on Relative Embryonic Development during Incubation.

The relative embryonic development showed a uniform and continuous increment in the rate of embryonic development in the different storage periods, which was evidenced between day 9 and day 18 of incubation. This may be as a result of albumen utilization by the growing embryo influenced by turning treatment. Turning treatments showed advantages in enhancing albumen use and growth reflecting advanced development of embryos from turned eggs compared with unturned ones (Tona *et al.,* 2005). Some of the benefits of egg turning during incubation include reduction in malpositioning of the embryo (Robertson, 1961), prevention of abnormal adhesion of the embryo or embryonic membranes to the shell membrane (New, 1957). These benefits have been related to have influence on embryo physiology such as protein accumulation in amniotic fluid, growth rate of the area vasculosa, gas exchange (Deeming, 1989a,b; Wilson, 1991; Tona *et al*, 2003), and thyroid hormone levels (Tona *et al.,* 2003).

Regular turning of eggs during incubation is important to ensure the nutrition and fluid balance of the embryo and improve hatchability. High content of yolk observed at the 18th day of incubation could be as a result of the need for energy and protein by the embryo as hatch approaches and to support immediate post-hatch nutritional requirement (Yadgary *et al*., 2010).

Hatchability

The effects of different storage periods of pullet eggs on hatchability of chicks are presented in Table 5. The values obtained on fertility ranged from 66.67 to 88.10%. The highest value was obtained in eggs stored for 3 days while the least value was recorded for eggs stored for 12 days. The work of Elibol *et al.* (2002) and Petek *et al.* (2003) suggested that long egg storage, except for one day prior to incubation, decreased ($P<0.05$) apparent fertility.

Schmidt *et al*. (2009) reported that long storage of eggs decreases egg fertility. The hatchability percentage values ranged from 58.73 to 85.71% while chick hatching weight ranged from 30.70 to 31.95g. The highest value for hatchability percentage (85.71%) was recorded in eggs stored for 3 days while the lowest value (58.73 %) was obtained in eggs stored for 12 days. This study demonstrated a detrimental effect of prolonged pre-incubation storage on hatchability of pullet hatching eggs which fall off sharply after 9 days of storage. It was reported that egg storage prior to incubation can have both detrimental and beneficial effects (Brake *et al*., 1993).

Higher hatchability obtained for eggs stored for 3 days is consistent with Schmidt *et al*. (2009) who reported that storing eggs from 5 to 10 days reduces hatchability. Also, Petek and Dikmen (2006), Romao *et al*. (2008) and Schmidt *et al*. (2009) reported that long storage of eggs reduces hatchability**.** The poor hatchability manifested itself as higher proportion of unhatched eggs whose embryos apparently failed to develop at all. Prolonged pre-incubation storage therefore most likely makes the environment of the embryo in the egg increasingly unsuitable for survival and development.

Table 5: Effect of Storage Periods on Hatchability of Eggs.

Parameter	Day of Pre-Incubation Storage				
	0	3	6	9	12
Ave. Egg weight(g)	55.88±0.03^{c}	56.01±0.01^{b}	56.07±0.03^{a}	55.78±0.02^{d}	56.03±0.04^{ab}
Fertility (%)	76.19±2.38^{b}	88.10±2.38^{a}	71.43±2.38^{c}	71.43±2.38^{c}	66.67±2.38^{d}
Hatchability (%)	62.70±11.25^{ab}	85.71±0.00^{a}	83.81±3.30^{ab}	69.79±14.61^{ab}	58.73±22.00^{b}
Chick Hatching weight (g)	30.70±0.11^{c}	31.90±0.10^{a}	31.81±0.08^{a}	31.11±0.29^{b}	31.95±0.29^{a}
Male (%)	58.89±8.39^{ab}	44.44±9.62^{bc}	66.67±0.00^{a}	35.89±9.62^{c}	50.00±10.00^{bc}
Female (%)	41.11±8.39^{bc}	55.56±9.62^{ab}	33.33±0.00^{c}	61.11±9.62^{a}	50.00±10.00^{ab}
Total embryonic mortality (%)	37.30±11.25^{ab}	14.29±0.00^{b}	16.19±3.30^{ab}	30.21±14.61^{ab}	41.27±22.00^{a}
%DIG	27.78±5.46	44.44±9.62	27.78±5.46	19.05±6.50	9.53±8.25
%DIS	72.22±5.46	55.56±9.62	72.22±5.46	80.95±6.50	90.47±8.25

a, b, c, d means in the same row with different superscripts differ significantly (P<0.05)
DIS –Dead in shell
DIG- Dead in germ

The low weight loss during incubation in batches of eggs stored for longer periods may be responsible for the decrease in hatchability that was observed and partly coincides with that observed for the rock partridge, a species showing low hatchability for eggs with extreme percentage mass loss during incubation (Kırıkcı *et al*., 2004). Some researchers have reported the decrease in hatchability due to storage in turkey eggs, Japanese quail eggs (Seker *et al*., 2005) and ostrich eggs (Deeming, 1996a).

The highest chick hatching weight of 31.95g was obtained in eggs stored for 12 days followed by eggs stored for 3 (31.90g) while control recorded the lowest chick hatching weight of 30.70g. Embryonic mortality ranged from 14.29 to 41.27%. The least value of 14.29% was obtained in 3 days pre-incubation storage period while the highest value of 41.27 % was obtained in egg stored for 12 days. The values obtained for early embryonic mortality (Dead in Germ, DIG) % ranged from 9.53 to 44.44 % with eggs stored for 3 days having the highest value, eggs stored for 12 days with 9.53% early embryonic mortality. The values obtained for late embryonic mortality (Dead in Shell, DIS) were statistically significant (P<0.05) and ranged from 1.33 to 5.00. DIS % ranged from 55.56 to 90.47%, the highest value of 90.47 was obtained in eggs stored for 12 days. The least value of 55.56% was obtained in eggs stored for 3 days.

The higher embryonic mortality could be as a result of cell death during pre-incubation storage period at 18^{o}C. Bloom *et al.* (1998) reported that eggs stored at a temperature below 20^{o}C declines embryo viability due to cell death.

CONCLUSION

It could be concluded that hatching Pullet eggs should neither be set in incubator on the day of oviposition nor subjected to pre-incubation storage period more than 6 days at 18ºC for higher hatchability.

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