

Effect of storage duration on egg quality, embryo mortality and hatching traits in Transylvanian naked neck chickens under humid tropical conditions

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Running title: Storage length and chicken egg quality

Abstract

Storage of hatching eggs for a period prior to incubation is unavoidable. Extended storage periods do affects egg quality, chick development and hatchability. There are variations in responses of eggs from different strains of chickens. Transylvanian naked neck chicken eggs were stored at $16 \pm 1.5^\circ\text{C}$ for either 0, 3, 6, 9, 12, 15 or 18d. Forty-four eggs in each batch were set after egg quality determination with 6 eggs per batch. Egg weight and surface area ($P < 0.001$) linearly decreased with increased storage length ($R^2 = 0.9756$ and 0.9469 respectively). Eggshell in the 0d group was ($P < 0.01$) heavier than those of 18d. Eggs in 0 and 3d storage groups had lower ($P < 0.001$) yolk% than eggs in 15 and 18d storage. Yolk diameter was higher in 9 to 18d of storage than in fresh eggs ($P < 0.01$). Higher yolk height and index ($P < 0.001$) were observed in 0, 3 or 6d eggs than in prolonged storage. Albumen weight was reduced ($P < 0.001$) in stored eggs. Egg stored for 15d recorded smaller albumen% ($P < 0.01$) than 0 and 3d storage. Albumen diameter, height, index, Haugh, and internal quality unit ($P < 0.01$) were higher in fresh eggs than in stored eggs. Hatchability declined ($P < 0.001$) with the extension in storage duration. Storage beyond 6d caused a reduction in quality and hatching traits. Therefore, other mitigating interventions are required.

Key words: albumen; cold room; egg shell; embryo; incubation; yolk



Effet de la durée de stockage sur la qualité des œufs, la mortalité embryonnaire et les traits d'éclosion chez des poulets à cou nu de Transylvanie dans des conditions tropicales humides

Résumé

Le stockage des œufs à couvrir pendant une période précédant l'incubation est inévitable. Les périodes de stockage prolongées affectent la qualité des œufs, le développement des poussins et l'éclosion. Il existe des variations dans les réponses des œufs de différentes souches de poulets. Les œufs de poulet à cou nu de Transylvanie ont été stockés à $16 \pm 1,5^\circ\text{C}$ pendant 0, 3, 6, 9, 12, 15 ou 18 jours. Quarante-quatre œufs de chaque lot ont été pondus après détermination de la qualité des œufs avec 6 œufs par lot. Le poids et la surface des œufs ($P < 0,001$) ont diminué de manière linéaire avec l'augmentation de la durée de stockage ($R^2 = 0,9756$ et $0,9469$ respectivement). La coquille d'œuf du groupe 0 jour était ($P < 0,01$) plus lourde que celle du groupe 18 jours. Les œufs des groupes de stockage 0 et 3 jours avaient un pourcentage de jaune inférieur ($P < 0,001$) aux œufs des groupes de stockage 15 et 18 jours. Le diamètre du jaune était plus élevé dans les 9 à 18 jours de stockage que dans les œufs frais ($P < 0,01$). Une hauteur et un indice de jaune plus élevés ($P < 0,001$) ont été observés dans les œufs de 0, 3 ou 6 jours par rapport à un stockage prolongé. Le poids de l'albumen a été réduit ($P < 0,001$) dans les œufs stockés. L'œuf stocké pendant 15 jours a enregistré un pourcentage

d'albumen plus petit ($P < 0,01$) que le stockage 0 et 3 jours. Le diamètre, la hauteur, l'indice Haugh et l'unité de qualité interne de l'albumen ($P < 0,01$) étaient plus élevés dans les œufs frais que dans les œufs stockés. L'éclosion a diminué ($P < 0,001$) avec l'allongement de la durée de stockage. Le stockage au-delà de 6 jours a entraîné une réduction de la qualité et des traits d'éclosion. Par conséquent, d'autres interventions d'atténuation sont nécessaires.

Mots-clés : albumine ; chambre froide; coquille d'œuf; embryon; incubation; Jaune d'œuf

Introduction

Extended storage of chicken eggs negatively affects internal and external quality (Reijrink *et al.*, 2008; Mwesiwa *et al.*, 2015; Feddern *et al.*, 2017), chick development (Hamidu *et al.*, 2010) and hatchability (Abioja *et al.*, 2020a; 2021). Traditionally, eggs are accumulated in storage for 7d before being set in an incubator. However, the storage may be prolonged beyond 7d for several reasons. This adversely affects the development and survival of the embryo *in ovo*, and hatchability (Abioja *et al.*, 2021). Storing for a long time causes changes in chemistry, function, nutrition and hygiene in the eggs (Wang *et al.*, 2019). Loss in egg quality has been implicated as the reason for reduced hatchability (Reijrink *et al.*, 2008). In a previous study, storing FUNAAB-alpha chicken eggs for more than 8d exhibited higher dead in germ and had lower hatchability (Abioja *et al.*, 2021).

Loss of too much water, carbon dioxide and albumen quality in aged eggs had been implicated in the reduced hatchability (Onagbesan *et al.*, 2007; Garcia *et al.*, 2010; Khan *et al.*, 2014; Abioja *et al.*, 2020a). Water is lost through shell pores on the surface of eggs. Eggs lose water beginning immediately after oviposition, and the rate is dependent on ambient temperature, relative humidity and speed of airflow around the eggs. Reduction in egg weight as the duration extends also contributes to the loss in viability of the eggs (Addo *et al.*, 2018). Other authors reported that prolonged egg storage had no significant effect on egg length, width, shape index and shell weight but resulted in weight loss and shrinkage in surface area (Raji *et al.*, 2009). Egg weight loss linearly increased with length of storage

(Samli *et al.*, 2005; Hasan *et al.*, 2009; Jin *et al.*, 2011; Alsobayel *et al.*, 2013; Goliomytis *et al.*, 2015; Addo *et al.*, 2018). Albumen height, Haugh unit and internal quality unit diminish as the duration of storage extends. Yolk diameter gradually increases as length of storage increases (Feddern *et al.*, 2017). This occurs as a result of loss of yolk membrane integrity, making yolk assume watery conformity, with a conspicuous decline in yolk height. Prolonged egg storage leads to a decrease in yolk index in stored eggs (Demirel *et al.*, 2009; Khan *et al.*, 2014).

There are variations in results obtained by different authors on the effects of storage length on embryonic mortality and egg hatchability. Storage length did not affect embryonic mortality and hatchability in eggs stored for 4, 12 or 16d (Goliomytis *et al.*, 2015). In contrast, egg storage beyond 7d is said to cause reduced hatchability and increased embryonic mortality in chickens (Fasenko *et al.*, 2001; Petek & Dikmen, 2006; Khan *et al.*, 2014) and in Japanese quail (Mani *et al.*, 2008; Romao *et al.*, 2008). The difference observed may be due to genotype and/or breeder age (Tona *et al.*, 2003). The onslaught of heat stress in the humid tropical region requires a genotype of chickens that is thermotolerant and possess a high production profile concurrently. This is difficult because of the existence of an inverse relationship between productive potential and thermotolerance in chickens. However, Transylvanian naked neck (TN) chicken is a well-known thermotolerant strain (Pârva *et al.*, 2007), that has just been recently introduced into the humid tropical region of south-western Nigeria (Abioja *et al.*, 2020b) and possesses a capacity for good growth rate, impressive feed

conversion ratio and good hen-day production, as well. The TN chicken is indigenous to Hungary in Central Europe and found in the hill regions of Transylvania in Romania. The physical features of Transylvanian naked neck chickens were described in the literature (Roberts, 2008). This genotype needs to be studied, conserved and explored in the tropics to improve the local chicken population. Reports on comparative studies of adaptation, egg production, quality and hatchability between Nigerian indigenous and FUNAAB-alpha chickens with Transylvanian naked neck chickens had been given in literature. The strain (TN chicken) was found to possess the capacity to adapt and survive under the prevailing hot conditions of the tropics (Abioja *et al.*, 2020b; Omotara *et al.*, 2020). But information on the hatching traits of TN chickens is not readily available. Therefore, the present study aimed at determining the effect of egg storage duration on egg quality, embryonic mortality and hatchability in Transylvanian naked neck chickens.

Materials and methods

Experimental location

The experiment was carried out at the Poultry Unit of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. The location (latitude 7° 13'N; longitude 3° 26'E; and altitude 76 m above sea level; Google Earth, 2021), found within the rain forest region of south-western Nigeria, has an annual temperature ranging between 27 and 28°C while the annual rainfall is 1217.27mm (Adepani *et al.*, 2017).

Experimental materials and procedures

Fertile eggs used for this study (n=350; weighing 53.0±0.23 g) were obtained from a flock of Transylvanian naked neck hens, aged 30 weeks, artificially inseminated twice weekly. The eggs were carefully selected to be without cracks or visible dirt and be of a similar weight range. Egg collection was done in batches based on the length of storage required. Eggs laid between 18.00h of the previous night and 06.00h the following morning were

collected from pens and moved immediately into the cold room of PEARLS-FUNAAB Hatchery (which is in close proximity to the pens), in crates for storage. Egg movement and fumigation with formaldehyde gas were done within the next one hour.

Egg storage: Before storage, eggs were weighed with Mettler-Teledo® PB3002 electronic balance to the nearest 0.01g and labelled. Fifty eggs were stored in an egg tray with broad end up under 16±1.5°C and 75±1.5% relative humidity condition for each of 0, 3, 6, 9, 12, 15 and 18d. Eggs for 0d storage were collected at 06.00h of the incubation day, weighed, labelled and moved into the hatchery immediately for fumigation and setting without storage. All the (stored and non-stored) eggs were set within 2 to 14 hours post-oviposition.

Incubation, candling and hatching: Three hundred and eight eggs (44 eggs per group with 4 replicates of 11 eggs per replicate) were set at the same time (08.00h) in a 2-stage incubator (N.V. Petersime® EV1/EN2 Incubator, Belgium) kept under uniform conditions of 29.5 and 37.5°C wet-bulb and dry-bulb temperature. The candling was done on d18 of incubation before transfer.

Data collection

Determination of egg quality traits: On incubation day 0, the eggs were weighed again to determine egg weight loss (EWL). Six eggs from each storage batch were opened for the determination of both external and internal egg quality.

Egg weight was measured with the sensitive weighing balance. Egg length and width were taken with a digital caliper (0.001mm sensitivity). Egg surface area and shape index were determined as described in previous studies (Carter, 1975; Anderson *et al.*, 2004). Yolk weight, percentage, diameter, height, index, albumen weight, length, diameter, percentage, height, index and yolk-albumen ratio were calculated (Alsaffar *et al.*, 2013). Haugh unit and internal quality unit were calculated as described previously (Haugh, 1934; Kondaiah *et al.*, 1983). Blastoderm diameter was measured on the yolk surface with

the aid of a caliper (Addo *et al.*, 2018). Shell weight and percentage were taken with the electronic balance mentioned above. Shell thickness was measured with a digital micrometer gauge (Model: 395-741-10, Mitutoyo, Kawasaki, Japan).

Determination of fertility, embryonic mortality and hatchability: Fertility was calculated as the ratio of fertile to set eggs in percentage. Blank eggs were opened to determine the percentage dead-in-germ. Embryonic mortality and hatchability were determined as described previously in the literature (Ishaq *et al.*, 2014). Hatchability of set and fertile eggs were calculated as the ratio of the hatched to set eggs (HS) and fertile eggs (HF) respectively in each storage batch.

Statistical analyses

The obtained data were analysed using a complete randomised design with Minitab Statistical software, model: $Y_{ij} = \mu + D_i + \xi_{ij}$, where Y_{ij} is a trait of interest; μ is the population mean; D_i is the i^{th} effect due to duration of egg storage ($i = 0, 3, 6, 9, 12, 15, 18$); and ξ_{ij} is the residual error. Means were separated with Tukey's HSD test. Means were considered significantly different at $p \leq 0.05$. Regression analysis was carried out on egg storage length with percentage egg weight loss and surface area shrinkage.

Results

Figures 1 and 2 present the influence of storage duration on weight loss (EWL) and surface area shrinkage (ESAS) in Transylvanian naked neck (TN) chicken eggs respectively. Length of storage had a significant ($P < 0.001$) effect on EWL and ESAS. The two parameters increased as the length of storage extended from 0 to 18d in a linear pattern. Though, the shrinkage was similar in eggs stored for 15 and 18d. Regression analysis of egg storage duration yielded R^2 of 0.9756 ($Y = 0.97X - 1.2713$) and 0.9469 ($Y = 0.6336X - 0.6914$) for EWL and ESAS respectively. Table 1 shows the effect of storage duration on external quality characteristics in TN chicken eggs. Fresh eggs had significantly ($P < 0.001$) broader surface area than 6, 12, 15 and 18d stored eggs. Egg length, width and shape index were not ($P > 0.05$) affected by storage length. The effect of storage duration on shell characteristics in TN chicken eggs is shown in Table 2. Length of storage significantly influenced wet ($P < 0.001$) and dry shell weight ($P < 0.01$) but not ($P > 0.05$) shell percentage and thickness. The shell of eggs stored for the 0d was heavier than eggs stored for 18d when dried, and those of 15 and 18d when wet.

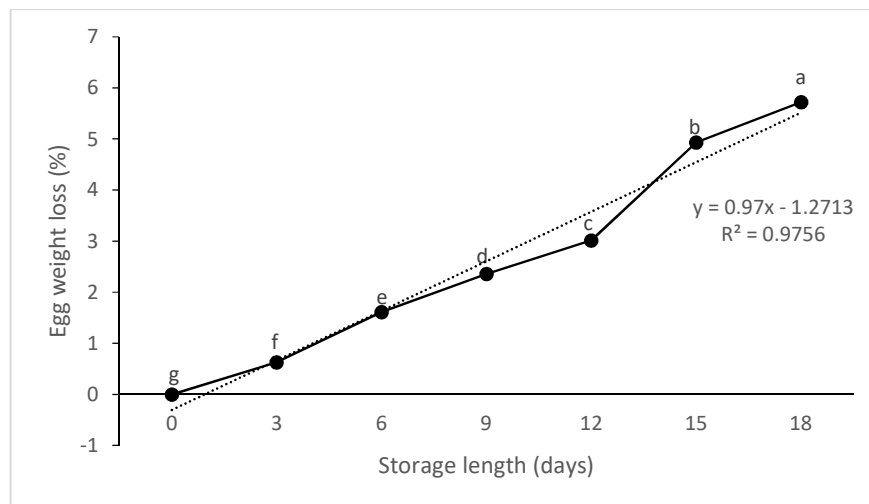


Figure 1: Effect of storage length on weight loss in Transylvanian naked neck chicken eggs
a,b,c,d,e,f,g Means with different letters differ significantly ($p = 0.000$)

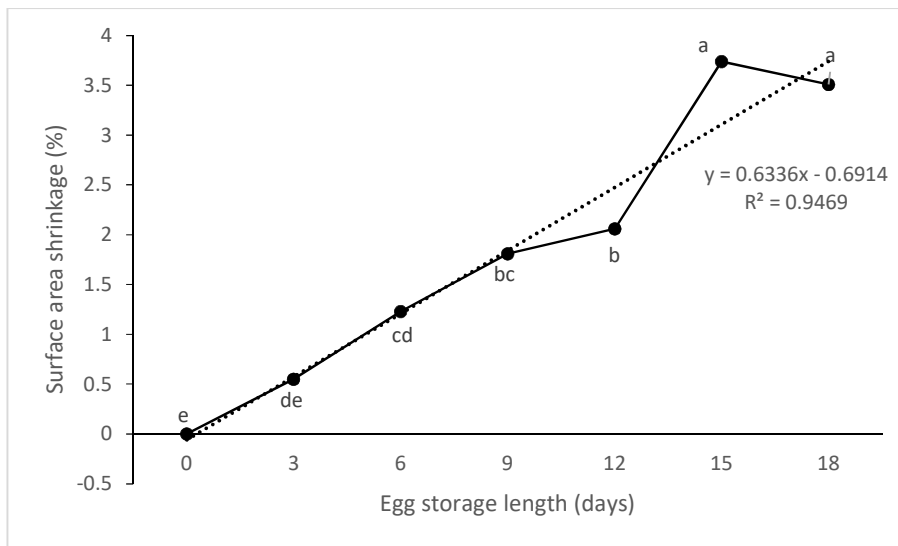


Figure 2: Effect of storage length on surface area shrinkage in Transylvanian naked neck chicken eggs
a,b,c,d,e Means with different letters differ significantly ($p = 0.000$)

Effect of storage duration on internal quality characteristics in TN chicken eggs is presented in Table 3. Yolk weight was not ($P > 0.05$) affected by storage duration. Yolk percentage ($P < 0.001$), diameter ($P < 0.01$), height ($P < 0.001$) and index ($P < 0.001$) were affected by storage length. Eggs on 0 and 3d storage groups had a lower yolk percentage than eggs on 15 and 18d storage. Yolk diameter was higher in 9 to 18d of storage than in fresh eggs. Higher yolk height was observed in eggs stored for either 0, 3 or 6d than in eggs stored for 15 and 18d. The yolk index gradually decreased as the length of storage increased. Length of storage had a significant effect on albumen weight ($P < 0.001$), length ($P < 0.01$), percentage ($P < 0.01$), diameter

($P < 0.01$), height ($P < 0.001$) and index ($P < 0.001$). Albumen weight was reduced in stored eggs, though not following a regular pattern. Fresh eggs had a shorter albumen length than eggs stored for 15 and 18d. Egg stored for 15d recorded a smaller albumen percentage compared to 0 and 3d storage. Albumen diameter was higher in fresh eggs compared to eggs in 12, 15 and 18d storage duration. Albumen height and index were higher in fresh eggs than in stored eggs. Yolk to albumen ratio was higher ($P < 0.01$) in 15d stored eggs than in 0 and 3d stored eggs. Haugh unit and internal quality unit were significantly ($P < 0.001$) higher in fresh eggs than the stored eggs, except for 3d storage.

Table 1: Effect of storage duration on external quality characteristics in TN chicken eggs

Parameter	Egg storage duration (days)							SEM	P
	0	3	6	9	12	15	18		
Number of observations	6	6	6	6	6	6	6		
Egg surface area (cm ²)	66.5 ^a	65.7 ^{ab}	64.3 ^{bc}	65.0 ^{ab}	63.2 ^{cd}	62.0 ^{de}	61.7 ^e	0.34	0.000
Egg length (mm)	54.9	54.9	53.9	56.4	55.0	57.2	53.8	1.40	0.569
Egg width (mm)	41.6	41.4	41.1	41.5	41.4	41.1	41.0	0.22	0.530
Egg shape index (%)	75.8	75.5	76.4	74.1	75.4	72.5	76.3	1.87	0.760

a,b,c,d,e Means along the row with different superscripts differ significantly

Table 2: Effect of storage duration on shell characteristics in TN chicken eggs

Parameter	Egg storage duration (days)							SEM	P
	0	3	6	9	12	15	18		
Number of observations	6	6	6	6	6	6	6		
Shell weight (wet; g)	6.76 ^a	6.57 ^{ab}	6.33 ^{ab}	6.42 ^{ab}	5.97 ^{abc}	5.75 ^{bc}	5.20 ^c	0.221	0.000
Shell weight (dry; g)	5.68 ^a	5.30 ^a	5.50 ^a	5.50 ^a	5.12 ^{ab}	4.91 ^{ab}	4.38 ^b	0.205	0.001
Shell percentage (%)	12.5	12.4	12.3	12.3	11.9	11.8	10.7	0.42	0.061
Shell thickness (mm)	0.68	0.64	0.67	0.69	0.65	0.64	0.60	0.021	0.095

^{a,b,c} Means along the row with different superscripts differ significantly

Table 3: Effect of storage duration on internal quality characteristics in TN chicken eggs

Parameter	Egg storage duration (days)							SEM	P
	0	3	6	9	12	15	18		
Number of observations	6	6	6	6	6	6	6		
Yolk weight (g)	13.8	13.8	14.6	14.5	14.1	15.6	14.8	0.52	0.202
Yolk percentage (%)	25.5 ^c	25.9 ^c	28.4 ^{abc}	27.7 ^{bc}	28.0 ^{abc}	31.9 ^a	30.4 ^{ab}	0.95	0.000
Yolk diameter (mm)	38.4 ^b	39.6 ^{ab}	41.4 ^{ab}	41.8 ^a	41.9 ^a	42.0 ^a	42.5 ^a	0.72	0.002
Yolk height (mm)	15.1 ^a	14.3 ^{ab}	13.7 ^{ab}	13.1 ^{bc}	12.9 ^{bc}	12.1 ^c	11.7 ^c	0.32	0.000
Yolk index (%)	39.4 ^a	36.1 ^{ab}	31.7 ^{bcd}	32.9 ^{bc}	30.9 ^{cd}	28.8 ^{cd}	27.8 ^d	1.04	0.000
Albumen weight (g)	31.7 ^a	30.3 ^{ab}	27.8 ^{cd}	28.5 ^{bc}	28.4 ^{bc}	25.4 ^d	26.6 ^{cd}	0.55	0.000
Albumen length (mm)	88.8 ^b	102.4 ^{ab}	108.1 ^{ab}	108.5 ^{ab}	105.1 ^{ab}	115.1 ^a	116.4 ^a	4.49	0.003
Albumen percentage (%)	58.6 ^a	56.9 ^a	53.9 ^{ab}	54.5 ^{ab}	56.3 ^{ab}	51.8 ^b	54.6 ^{ab}	1.13	0.005
Albumen diameter (mm)	69.3 ^b	79.5 ^{ab}	81.4 ^{ab}	84.1 ^{ab}	90.5 ^a	91.7 ^a	93.1 ^a	4.52	0.008
Albumen height (mm)	7.33 ^a	4.71 ^b	4.12 ^b	3.89 ^b	3.94 ^b	3.24 ^b	3.12 ^b	0.494	0.000
Albumen index (%)	9.47 ^a	5.21 ^b	4.37 ^b	4.07 ^b	4.06 ^b	3.41 ^b	3.22 ^b	0.714	0.000
Yolk:Albumen	0.44 ^b	0.46 ^b	0.53 ^{ab}	0.51 ^{ab}	0.50 ^{ab}	0.62 ^a	0.56 ^{ab}	0.028	0.001
Haugh unit	86.8 ^a	68.7 ^{ab}	63.6 ^b	62.2 ^b	60.1 ^b	52.5 ^b	51.8 ^b	4.65	0.000
Internal quality unit	180.4 ^a	158.3 ^{ab}	150.8 ^b	148.0 ^b	146.2 ^b	131.5 ^b	129.9 ^b	6.60	0.000

^{a,b,c,d} Means along the row with different superscripts differ significantly

Table 4 presents the effect of egg storage duration on egg fertility, embryonic mortality and hatching traits in TN chicken eggs. Extension in storage length significantly ($P < 0.001$) decreased egg fertility. The decline started when the length reached 12d, and drastically fell further in 15 and 18d stored eggs. Dead in germ (DIG) was affected ($P < 0.001$) by storage duration. The least DIG (3.33%) was recorded in fresh eggs and the

highest (38.71%) in 18d stored eggs. Percentage dead in shell increased significantly ($P < 0.001$) from 0 to 6d before it started decreasing from 9 to 18d. Duration of storage significantly ($P < 0.001$) influenced the hatchability of fertile (HF) and set eggs (HS). There was no hatching in 15 and 18d eggs. Hatchability followed the pattern: $0 > 3 > 6 = 9 > 12 > 15 = 18$.

Table 4: Effect of egg storage duration on embryonic mortality and hatching traits in TN chickens

Parameter	Egg storage duration (days)							SEM	P
	0	3	6	9	12	15	18		
Number of observations	4	4	4	4	4	4	4		
Egg fertility (%)	91.7 ^a	92.6 ^a	91.3 ^a	93.0 ^a	46.2 ^b	21.9 ^d	38.7 ^c	0.42	0.000
Dead in germ (%)	3.33 ^f	9.26 ^e	13.04 ^d	16.28 ^c	17.96 ^{bc}	18.75 ^b	38.71 ^a	0.417	0.000
Dead in shell (%)	10.0 ^e	25.9 ^c	41.3 ^a	34.9 ^b	18.0 ^d	3.1 ^f	0.0 ^g	0.36	0.000
Hatchability of set eggs (%)	75.0 ^a	55.6 ^b	34.8 ^c	32.6 ^c	10.3 ^d	0.0 ^e	0.0 ^e	0.64	0.000
Hatchability of fertile eggs (%)	84.9 ^a	66.7 ^b	44.4 ^c	42.4 ^c	36.4 ^d	0.0 ^e	0.0 ^e	0.48	0.000

^{a,b,c,d} Means along the row with different superscripts differ significantly

Discussion

It is well known that deterioration of quality comes up when chicken eggs are stored for a long time (Reijrink *et al.*, 2008; Khan *et al.*, 2014; Mwesigwa *et al.*, 2015). Egg weight loss (EWL) is unavoidable in stored chicken eggs. A direct linear relationship with the length of storage was obtained in FUNAAB-alpha chickens (Akter *et al.*, 2014; Abioja *et al.*, 2021). There are continuous losses of moisture and gases from eggs in storage. Moisture is lost from egg content by evaporation. Carbon dioxide is lost through eggshell pores, leaving the eggs alkaline (Garcia *et al.*, 2010). Storage temperature, humidity, air-flow and the density of pores on the shell affect the rate of EWL. Eggs lose weight irrespective of the storage temperature. However, the rate of EWL in low-temperature condition is lower than in high storage temperature (Akter *et al.*, 2014). In the present trial, EWL increased with storage length. This is in agreement with the previous reports (Alsobayel & Albadry, 2011; Jin *et al.*, 2011; Alsobayel *et al.*, 2013; Khan *et al.*, 2014; Goliomytis *et al.*, 2015; Abioja *et al.*, 2021). There was a significant difference between weight loss in eggs stored for 5 and 15d (Petek & Dikmen, 2006). The present finding agrees with the fact that prolonged storage length increases weight loss in chicken eggs. The EWL was duration-dependent.

In the present trial, there were no significant differences in length and width of stored eggs compared with the freshly laid eggs as earlier reported (Raji *et al.*, 2009). This corroborates the findings in FUNAAB-alpha eggs stored up to 20d (Abioja *et al.*, 2021). In the same vein, there was no difference in egg shape index in this study. Previous studies show that egg shape index was not affected by storage length (Tilki & Saatci, 2004; Raji *et al.*, 2009; Akter *et al.*, 2014; Abioja *et al.*, 2021).

Shell percentage and thickness were not affected by the length of storage in this study. However, the shell weight gradually decreased with the increased length of storage. Previous studies show that dry shell weight and thickness were similar in eggs stored for different

durations (Akter *et al.*, 2014; Addo *et al.*, 2018; Abioja *et al.*, 2021). Yolk percentage and diameter increased with an increase in storage duration. This may be due to loss in egg weight and yolk membrane integrity as eggs aged. Besides, as the storage duration increases, moisture is lost from the yolk and gained by the albumen (Ansah *et al.*, 2014; Addo *et al.*, 2018). The same reasons apply to the decreased yolk height in eggs stored for 12 to 18d in the present study. A decreased yolk index is an indication of deterioration in the yolk quality of long-stored eggs. All the traits examined in the albumen of stored eggs revealed a conspicuous decline. This aligns with the previous studies (Tebesi *et al.*, 2012; Akter *et al.*, 2014; Addo *et al.*, 2018). Similar to previous works on the effect of storage time on egg quality, Haugh and internal quality unit of eggs declined significantly as eggs aged (Akter *et al.*, 2014; Abioja *et al.*, 2021). Some authors reported that the decline in Haugh unit (HU) in chicken eggs started when the storage length was extended beyond 5d (Khan *et al.*, 2014). Irrespective of the age of breeder hens, storage length was found to lower Haugh unit in a study (Tona *et al.*, 2004).

Further storage of TN breeder hens from 12d and beyond drastically affected egg fertility in the present finding. This agrees with other authors who stated that apparent fertility is affected by extended egg storage (Petek and Dikmen, 2006; Addo *et al.*, 2018). Though in another study, no difference in egg fertility of eggs stored between 0 and 9 days was observed (Khan *et al.*, 2014). The present finding that the percentage dead in germ increased with the increase in storage length is in line with the reports in the literature (Petek and Dikmen, 2006; Khan *et al.*, 2014). These authors stated that embryonic mortality in incubated chicken eggs increased with an increase in storage length. Many authors had confirmed that hatchability is lowered by extended storage of chicken eggs (Petek & Dikmen, 2006; Khan *et al.*, 2014; Abioja *et al.*, 2021), especially in older breeder hens (Tona *et al.*, 2004). A similar report was obtained in studies with quail eggs

(Mani *et al.*, 2008; Romao *et al.*, 2008; Çopur Akpınar & Güneç, 2019). The report in a study had stated that hatchable eggs stored for more than 10-14d would have a decline in egg hatchability (King'ori, 2011). There was a significant difference in hatchability of non-transported Japanese quail eggs stored for 7 or 14d (Çopur Akpınar & Güneç, 2019). There was also a negative relationship between hatchability and egg storage duration ($R^2 = 0.53$). Hatchability decreased as storage duration increased (Figure 2). However, the very large scatter between hatchability for single storage duration indicates that other factors were interacting with storage duration (Tona *et al.*, 2007). Hatchability in poultry eggs increases from the point of oviposition until a peak is reached between 3 and 5d. Thereafter, further extension in storage lowers drastically the percentage hatch (Nasri *et al.*, 2020; Rahardja *et al.*, 2020). A study even stated that egg storage must not exceed 4d for optimum hatchability and weight of the hatched chicks, without intervention (Ayeeni *et al.*, 2020). Similarly, a study indicated that storage length in chicken eggs should not exceed 3d (Khan *et al.*, 2014). A reduction in the number of blastodermal cells in extensively stored eggs may account for the poor percentage of hatch obtained (Uyanga *et al.*, 2020).

Conclusions

Egg weight loss and surface area shrinkage increased as the length of storage extended from 0 to 18d in a linear pattern. Shell weight decreased in 15 and 18d-stored eggs. However, the yolk percentage increased in longer storage length. Deterioration in albumen traits, HU and internal quality unit (IQU) began in duration longer than 6d, and reached the crest at 15 and 18d. Storing Transylvanian naked neck chicken eggs beyond 6d reduced quality parameters, hampered hatching traits, and could be lethal. Therefore, mitigating intervention is required to ensure hatching success in prolonged storage of TN chicken eggs.

Animal welfare statement:

The experimental procedure has been approved by the Animal Experimental Board of the Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta Nigeria. Also, the Guideline for Animal Research of Nigeria Institute of Animal Science (NIAS) was followed.

Conflict of interest:

There is no conflict of interest of any sort in this work.

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