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# IMPACTS OF SHORT PERIODS OF INCUBATION DURING EGG STORAGE (SPIDES) ON DYNAMIC WEIGHT LOSS, ALBUMEN, YOLK PH VALUES, AND EARLY EMBRYONIC DEVELOPMENT OF EGGS STORED FOR PROLONGED PERIODS

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**ABSTRACT:** This study aimed to explore the impacts of SPIDES every 4 days intervals for 0, 45, 90, and 135 min on internal characteristics, and early embryonic development of eggs stored for various periods (1, 2, 3, 4, and 5 weeks). Total of 7500 eggs, weighing 62 g, were split into five groups at random, each containing 1350 eggs and three duplicates of 450 eggs; 150 eggs were included in each treatment group as a control negative (non-SPIDES). Results indicated the highest dynamic weight loss (DWL) in non-SPIDES eggs at all storage periods. SPIDES decreased DWL on Days 4, 8, 12, 16, and 20 days of storage. The lowest DWL was observed on Day 4 for eggs stored for 2wk and SPIDES for 90 min. Values of pH for pre- and post-SPIDES of albumen and post-SPIDES of yolk were not affected by storage period, SPIDES protocol, and their interaction. Pre-SPIDES, yolk pH values on Day 4 decreased ($P<0.05$) in eggs stored more than 4wk as compared to 1wk storage period, being the lowest in eggs stored for 1 and 4wk with SPIDES for 135 min, eggs stored for 2 and 3 with SPIDES for 45 min, and eggs stored for 5wk with SPIDES for 90 min. Blastoderm diameter was higher ($P<0.05$) in eggs stored for 4wk than other storage periods, and eggs with SPIDES for 135 min than other minuets on all Incubation hours (12, 18, and 24h). In eggs stored for all periods. Blastoderm diameter was the highest in eggs with SPIDES for 135 min and the lowest in non-SPIDES eggs at all Incubation hours.

In conclusion: Short Periods of Incubation During Egg Storage (SPIDES) every 4 days during storage period has been exhibited improvements in dynamic weight loss, maintained pH values of albumin and yolk, and increased diameter of blastoderm of embryos at early embryonic developmental stage for eggs stored for one week or more.

**Keywords**: Egg storage, SPIDES, Internal characteristics, blastoderm.

## INTRODUCTION

Egg storage is a standard procedure for hatchery operations and expected demand in broiler parent and grandparent hatcheries. The physiological zero temperature of the stored eggs is kept between 21°C and 27°C (Edwards, 1902; Funk and Biellier, 1944). It is common practice for hatching eggs to be stored for several days before starting incubation. Eggs can be stored without a significant reduction in hatchability for one week at temperature (18-20°C; 64.5-70 °F) and relative humidity (75%) in storage rooms. Blastodermic cells may be exposed to stop embryonic development (Bakst and Gupta, 1997; Fasenko *et al*., 2001a) or be able to undergo mitotic activity (Arora and Kosin, 1968) under the conditions of egg storage.

Commercially, eggs are kept on the farm for several days before being shipped to a Incubator. Since it is rarely to perform a pre-incubation right after egg collection, it is better to do so during the egg storage period in order to increase the hatchability rate. The embryo undergoes modifications when it is stored for >7 days. When the egg storage period is prolonged beyond one week, the hatching period is delayed (Mather and Laughlin, 1976; Kirk *et al*., 1980; Christensen *et al*., 2002; Reijrink *et al*., 2010), the hatchability rate is decreased (Merritt, 1964; Mather and Laughlin, 1976; Mather and Laughlin, 1977; Mather and Laughlin, 1979; Fasenko *et al*., 2001b; Fasenko, 2007), and the embryo viability is adversely affected, raising the rate of early and latte embryonic mortality (Reijrink *et al*., 2008). Prolonged duration of egg storage may result in cell death of embryo, alterations in egg components such as a decrease in albumen height and yolk membrane strength, and a rise in albumen pH from 7.6 to 9.0 (Fasenko, 2007; Reijrink *et al*., 2008; Dymond *et al*., 2013). Long-term egg storage can also cause embryonic stress, which can show up as increased apoptosis and necrosis, decreased metabolism, and developmental delays. This can cause irreversible harm to the embryo, which would raise its mortality rate and reduce its hatchability (Fasenko, 1996; Bloom *et al*., 1998; Christensen *et al*., 2001; Fasenko, 2007; Hamidu *et al*., 2010).

To overcome this, new approaches for egg storage management are being trialed among hatchery managers. A few strategies have been devised to make up for the low hatchability brought on by extended storage. According to some early authors' reports, hatchability was increased by pre-incubating eggs prior to storage (Kosin, 1956; Kan *et al*., 1962; Coleman and Siegel, 1966). The increase in total cell number and preservation of the pre-gastrulation embryo's developmental plasticity are thought to be at least partially responsible for the slightly developmentally advanced embryo's resistance to storage-induced effects. This allows for a larger reservoir of available cells to offset the increased cell death brought on by extended storage (Bloom *et al*., 1998; Fasenko *et al*., 2001a; Hamidu *et al*., 2011). In this context, Fasenko *et al*. (2001a, b) reported that a completion of hypoblast formation in embryos by a single pre-incubation before egg storage maintained the hatchability of eggs stored over 14 days. Fasenko (2007) mentioned that embryos of chicken reach the more storage resistant hypoblast stage of embryonic development after six hours of pre-storage incubation. Dymond *et al*. (2013) reported that 3-4 SPIDES of 21 day-egg storage period increased hatchability and decreased incubation period as compared to controls.

Meir and Ar (1998) developed a method that involved delivering multiple short PI after an initial storage period, which we built upon in our study. The SPIDES

(short periods of incubation during egg storage) regimen has been adapted to incorporate four 4-hour PIs administered at 4- to 5-day intervals over a 21-day storage period **(**Dymond *et al*., 2012). It was determined that the SPIDES treatment described here offered the best hatchability restoration in a series of trials that looked at the optimal frequency and duration of the PI treatments (French *et al*., 2011; Nicholson *et al*., 2011). Since then, early commercial experiments have successfully employed SPIDES (Nicholson *et al*., 2012). The developmental consequences of the SPIDES treatment and its impact on hatchability are discussed in this manuscript.

Therefore, the current study aimed to explore the impacts SPIDES every 4-d intervals for 0, 45, 90, and 135 min on Dynamic weight loss, Albumin and yolk pH values, and early embryonic development of eggs stored for various periods (1, 2, 3, 4, and 5 wk.).

## MATERIALS AND METHODS

### Experimental design

A total of 7500 eggs (Ross 308, produced by chickens at 38 weeks old, average weight of 62 g) were obtained from Rummana Broiler Breeder Farm. The eggs used in this experiment were carefully inspected for cracks and other abnormality (i.e., round, and rectangular egg, egg with glass cracks, internal cracks……. etc.), and those that were not suitable for hatching were removed. Eggs were stored at 16°C for 4 days in troll's egg. (this storage day is calculated from the total number of storage period" and Virocid® used a spray device to disinfect eggs), Eggs were randomly divided into five storage period groups (n=1500 eggs), each group was further divided into four sub-groups including negative control (n=150 eggs, non-SPIDES) and three sub-groups for three SPIDES protocols (450 eggs in each). The main five groups included storage periods of 1, 2, 3, 4, and 5 weeks. During each storage period (at 16$^{o}$C and 60% RH), the eggs were exposed to a short period of incubation (SPIDES) either for 45, 90, or 135 min, every 4-days interval. Number of eggs in main and sub-groups is shown in Table 1. About 8 hours (the time during which the eggs are acclimated on physiological zero "23-25$^{o}$C" at setter room before storage) is carried out until the next SPIDES each 4 days.

The hatching machine's operating program (SPIDES) involves setting the temperature of the egg to 90°F, which is equivalent to 32.22°C, with 52% relative humidity which is equivalent to 84°F wet bulb temperature. Following each SPIDES protocol, the eggs were kept in the control room for eight hours before being moved back to the storage room, where they were kept at 16°C and 60% relative humidity, depending on stored time.

### Description of the incubator (Pas reform):

Multiple stage conditions are fixed and depend on the different ages in the same unit where there are 6 to 5 different ages to enter a batch of the machine day after day. The temperature was 99.5°F with relative humidity 85$^{o}$F (wet bulb). Eggs were turned automatically through an angle of 45 every hour until the 19$^{th}$ day of the incubation period, ventilation channels were opened automatically and measured as relative value of the air inlet opening area of the ventilation channels as referred in Table 2. After 19$^{th}$ day, during the last two days of the incubation period, the temperature decreased from 98.3 to 96.3$^{o}$F coincided with relative humidity values of 83.4$^{o}$F (wet bulb) to 86.5$^{o}$F (wet bulb), respectively, for the 19$^{th}$; 20$^{th}$ and 21$^{st}$ day.

### Experimental procedures

A weight sample was made for all treatments, where a tray from each storage group was weighed when the

egg was stocked before storage and weighed after each SPIDES protocol. Also, weight of the same tray of eggs after the end of storage and before the egg enter the incubator machine during SPIDES operation, and the weight after 19 days of incubation was recorded to calculate the loss of egg weight relative to the fresh egg weight. The dynamic weight loss (DWL) was calculated by the following equation:

$$DWL\ (\%) = \frac{Full\ tray\ weight\ at\ set - Full\ tray\ weight\ at\ transfer}{Full\ tray\ weight\ at\ set - Empty\ tray\ weight} \times 100$$

The pH value of the albumen and yolk was promptly assessed using a pH meter that had been calibrated (H12212 pH Meter, HANNA Instruments).

In the current study, an exploratory investigation was conducted on several sixty eggs in a commercial hatchery. The study measured the pH values of the albumen and yolk of fresh eggs as well as the extent of change in the pH values of both albumen and yolk due to long-term storage period and hatching egg using the SPIDES system. In this exploratory study, fresh egg albumen had an average pH of 8.37 and fresh egg yolk had a pH of 6.31.

**Early embryonic development**

In the current study, blastoderm diameter was measured by using Digital Vernier Caliper for each group that was stored or exposed to SPIDES at the start of the experiment and during early incubation period (0, 12, 18, and 24h). This allowed for the calculation of the rate of embryonic development. The embryo's diameter was measured at the start of incubation, twelve hours after the primitive streak formed, eighteen hours after the neural plate formed, and twenty-four hours after the neural tube was fully developed (Bertocchini and Stern, 2002, 2008).

**Statistical analysis**

Data were analyzed via a factorial ANOVA using the GLM procedure in SAS (SAS, 2004). The following model was used for statistical analysis: $Y_{ijk}=\mu+ S_i+ T_j+(ST)_{ij}+ E_{ijk}$ where $Y_{ijk}$ is the $k^{th}$ observation; $\mu$ is the overall mean; $S_i$ is effect of the $i^{th}$ storage period; $T_j$ is the effect of the $j^{th}$ SPIDES protocol; $(ST)_{ij}$ is the interaction between i the storage period and j the SPIDES protocol; and $E_{ijk}$ is the experimental error, accordingly zero mean and variance=$\sigma^2$e. Tukey's test were used to identify differences between means at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Egg dynamic weight loss during storage period:**

Dynamic weight loss (DWL%) of eggs as affected by storage period and SPIDES protocol on different storage days is shown in Table 4. The effect of storage period was significant on DWL on Days 4 (P<0001) and 16 (P<0.0026) of storage period.

Results showed that during the first storage period (1wk), in which eggs were exposed to SPIDES protocols on Day 4 and returned to the storage room for an additional 3 days, eggs showed significantly (P<0.05) higher rate of weight loss on Day 4 of storage than in other storage periods. DWL on Day 16 was significantly (P<0.05) higher for 3 and 4wk storage than for 5wk storage. DWL on Days 8, 12, 20, 24, and 28 was not affected significantly by storage period (Table 4).

The effect of SPIDES protocol was significant on DWL on Days 4 (P<0.0002), 24 (P<0.0005), and 28 (P<0.0011) as shown in Table 4. Results revealed that DWL was reduced significantly (P<0.05) by SPIDES for 45 and 90 min on Day 4, SPIDES for 90 and 135 min on Day 24, and SPIDES for 45 min on day 28 of storage (Table 4). These results indicated that SPIDES for 45 min had a positive impact on

decreasing DWL for eggs, regardless storage period.

The effect of interaction between storage period and SPIDES protocol on DWL of eggs was significant only on Day 4 ($P<0.0012$) and Day 16 ($P<0.0260$) of storage period (Table 4). Results of DWL of eggs stored for different weeks (1, 2, 3, 4, and 5 wk) and exposed to different SPIDES protocols (0, 45, 90, 135 min) that illustrated in Fig. 1 revealed the lowest DWL by using SPIDES for 45 min in eggs stored for 1, 3, and 4 wk, and by SPIDES for 90 min in eggs stored for 2 and 5 wk. This finding indicated that SPIDES reduce DWL in eggs stored for prolonged periods up to 5 weeks and even for eggs stored for short period of one week. Therefore, the highest rate of dynamic weight loss was observed in eggs that were kept as a negative control group (non-SPIDES) at different storage periods. Also, the differences in dynamic loss rate on Days 12, 16, and 20 days of storage were lower in eggs stored for 3, 4 and 5 wk than in eggs stored for 1 and 2 wk. The lowest DWL was observed on Day 4 for eggs stored for 2wk and SPIDES for 90 min (Fig. 1).

Fick's laws describe water diffusion as the physical process of water molecules moving down a concentration gradient. Diffusion flow is influenced by temperature and concentration gradients; water diffuses more quickly in these conditions. Due to variations in water pressure between the interior and exterior of the egg, egg lose water through diffusion through the eggshell, based on the relative humidity and temperature on each side. Eggshell porosity, defined by the number, diameter, length, and shape of its pores, determines how much water escapes an egg (Deeming, 2002). Consequently, it is increased in eggs from larger eggs laid by breeders of the same age or incubated at high temperatures and/or low relative humidity levels (Morita *et al.,* 2009, 2010; Sgavioli *et al.,* 2015). Furthermore, even though water loss is not directly impacted by the air velocity on the egg surface (Meijerhof and van Beek, 1993).

By monitoring the weight loss in the egg trays, the current commercial setter models provide control over the weight loss of the eggs during incubation. To ensure that this control is effective, it is necessary to comprehend the amount of water lost from eggs during the pre-incubation period, especially during the storage period, and to ensure that the eggs are evenly distributed in terms of weight and size within the setter trays. The obtained results indicated that DWL was decreased by storage period of 1wk as compared to 2, 3, 4, and 5wk, and by SPIDES protocol as compared to non-SPIDES ones. In comparable with our results, Meijerhof (1994) reported that eggs stored for 14 days experienced a greater weight loss during storage than eggs stored for 7, 21, 28, and 35 days, likely due to the environment storage. It is possible that daily egg weight loss was also impacted by a change in relative humidity during storage. Also, chicken eggs lost 0.50, 1.02, and 1.66% of their weight following storage for 2, 5, and 10 days, respectively (Samli *et al.,* 2005).

Generally, dynamic weight loss has been linked to rates of embryonic metabolism and development (Ar and Rahn, 1980; Tullet and Burton 1983) and utilized to estimate vital gas exchange (Paganelli *et al.,* 1978; Rahn *et al.,* 1979). The shelf life was found to be no more than 3 days for Rohd Island Red eggs (Khan *et al.,* 2013), 10 days for egg and meat type quail (Romao *et al.,* 2008), 7 days for Cobb broiler breeders' eggs (Tona *et al.,* 2003), and 6 days for pullet breeders' eggs (Egbeyale *et al.,* 2013).

**Albumen pH value:**

Albumen pH values recorded immediately pre-SPIDES and 8 h post-SPIDES protocols on Days 4, 8, 12, 16, 20, 24, and 28 of storage period**,** as

affected by storage period and SPIDES protocol are presented in Tables 5 and 6, respectively.

Analysis of variance revealed that albumen pH value (pre- or post-SPIDES) on all sampling days of storage periods was not affected significantly by storage period, SPIDES protocol, and their interaction (Tables 5 and 6).

According to Leeson (2006), the components of a fresh egg are composed of 32% yolk, 58% albumen, and 10% shell. It is well known that four layers is better_combine to form the egg white: the first, making up to 3% of the white, is the chalazae, or chalaziferous layer, which is directly surrounding the yolk. The inner thin layer, which makes up 17% of the white, comes next. It encircles the chalazae. The third layer is thick or firm, acting as a jacket or envelope to contain the yolk and thin white inside. It makes up 57% of the albumen and is attached to the shell membrane at both ends of the egg. Lastly, making up 23% of the egg white, the outer thin layer is located just inside the shell membranes, with the exception of the area where the thick white is affixed to the shell (USDA, 2000).

The internal quality of the egg begins to decline as soon as it is laid; the longer it is stored, the worse it gets. Nonetheless, there is little variation in the chemical makeup of the egg (yolk and albumen). According to the literature results, the pH value of the albumen in a freshly laid egg ranges from 7.6 to 8.5. In accordance with our results, the pH of albumen rises to 9 in just four days of storage (Lapao *et al.,* 1999), possibly as a precaution against microbial contamination. Also, Heath (1977) found that the albumen pH value increased during storage at a rate that is temperature dependent, reaching a maximum of roughly 9.7. The albumen pH value was found to be 9.18 after three days of storage at 3 °C (Sharp and Powell, 1931). Regardless of the storage temperature (3 to 35 °C), the albumen's pH value reaches 9.4 after 21 days of storage (Li-Chan *et al.,* 1995) and 8.3 after seven days of storage at 22 °C, then remained unchanged when $CO_2$ loss was stopped by oiling the shell (Heath, 1977).

Increasing the albumen pH value of eggs stored for different periods or treated with different SPIDES protocols was attributed to the loss of $CO_2$ through the shell pores, which are contingent upon dissolved $CO_2$, bicarbonate ions, carbonate ions, and protein equilibrium. The partial $CO_2$ pressure in the surrounding environment has an impact on the concentration of bicarbonate and carbonate ions. The loss of water and $CO_2$ causes the internal egg quality to decrease compared to fresh eggs. As a result, the egg's pH values is changed, which causes the thick albumen protein structure to be lost and causes watery albumen. The albumen's hazy look is also caused by $CO_2$; when an egg ages, its $CO_2$ content decreases, and the albumen turns transparent in contrast to fresh eggs. Therefore, albumen pH value decreased from 8.3 to 8.1 in just seven days in oiled eggs kept at 7 °C as reported by Li-Chan *et al.* (1995).

Finally, Onagbesan *et al.,* (2007) reviewed the question of whether high $CO_2$ levels in the incubator during the early stages of incubation are beneficial for embryonic development (De Smit, L. *et al.,* 2006; Bruggeman *et al.,* 2007).

**Yolk pH value:**

According to the results in Table 7, overall mean of pre-SPIDES yolk pH value on Day 4 significantly ($P<0.05$) decreased for eggs stored more than 4wk as compared to 1wk storage period, but did not differ significantly from those stored for 2, 3, and 4wk. However, there were no significant difference in yolk pH value among different storage periods on Days 8, 12, 16, 20, and 28. Results also revealed insignificant effect of SPIDES protocol on overall mean of

pre-SPIDES yolk pH value on different storage days (4-28 d).

The effect of interaction between storage period and SPIDES protocol on pre-SPIDES pH value was significant ($P<0.0268$) only on Day 4 (Table 7).

This effect was reflected in lowest pre-SPIDES yolk pH value in eggs stored for 1 and 4wk with SPIDES for 135 min, eggs stored for 2 and 3 with SPIDES for 45 min, and eggs stored for 5wk with SPIDES for 90 min (Fig. 2).

Results presented in Table 8 showed that the effect of storage period, SPIDES protocol, and their interaction were not significant on post-SPIDES yolk pH values on Days 4, 8, 12, 16, 20, 24, and 28 of storage period.

The obtained results indicated that pre- and post-SPIDES yolk pH values were not change in relation with storage period and SPIDES protocol.

Ovalbumen transforms into S-ovalbumen during storage, and the ovomucin-lysozyme complex separates with the ovomucin gel's breakdown and the albumen's subsequent liquefaction both of which are critical for improved incubation outcomes (Melo *et al.,* 2020). According to Seibel *et al.,* (2005) these reactions help water evaporate through eggshell pores. The yolk's pH value rages between 6 and 6.3 at oviposition (Stern, 1991), and gradually increases from 6.5 to 6.8 following oviposition (Shenstone, 1968; Bakst and Holm, 2003). Unlike the albumen, the yolk's buffer system is not dependent on bicarbonate. The yolk index, or the ratio of yolk height to width, varies during storage. The yolk has a propensity to flatten and the vitelline membrane encircling it weakens (Fromm, 1966). Water movement between specific egg morphological elements is primarily responsible for changes in yolk quality parameters. It should be mentioned that the primary cause of this movement is the water's passage from the albumen to the yolk through the vitelline membrane (Eke *et al.,* 2013). The decrease in yolk shape index values and the strength of the vitelline membrane (Jones and Musgrove, 2005 and Marzec *et al.,* 2019) are two obvious effects of water movement that have been noted by numerous authors. If storage times are extended, this could result in the villi's rupturing and combining components of the egg content. The strength of the ovomucin lysozyme complex determines the viscosity of albumen (Freeman *et al.,* 1974). As the length of the egg storage period and PH albumen increase, this complex becomes less stable, which results in a decrease in albumen thickness (Kato *et al.,* 1970, 1981). At the start of the storage days, the albumen's viscosity decreases concurrently with the yolk's PH value rising. According to Heath (1975), the yolk index, or the ratio of the yolk's height to width, rises as the

**Early embryonic development**

Table 9 summarized that the blastoderm diameter at the start of the storage periods and incubation process does not significantly differ from one another. Regarding the diameter of the blastoderm at the start of the incubation process, it is also discovered that there are no appreciable variations between SPIDES protocols. Although the diameter of the embryos was nearly equal at the beginning of the incubation process (0-h) for all storage periods, the effect of storage period on blastoderm diameter was significant after 12, 18, and 24h of incubation.

Overall mean of blastoderm diameter was significantly ($P<0.05$) higher for all storage periods more than one week, being the significantly ($P<0.05$) the highest for storage period of 4wk as compared to those stored for 1, 2, 3, and 5wk after 12h of incubation. After 18h of hatching, diameter of blastoderm was significantly ($P<0.05$) higher for storage periods of 3 and 4wk than 1wk, but did not differ significantly from that in

storage periods of 2 and 5wk. After 24h of incubation, blastoderm diameter significantly ($P<0.05$) increased for storage periods for 4 and 5wk as compared to 1- and 2-wk storage periods, but did not exhibit significant differences with storage period of 3wk (Table 9).

The effect of SPIDES protocol was significant ($P<0.001$) on blastoderm diameter after 12, 18, and 24h of incubation. Overall mean of blastoderm diameter after all incubation hours in embryos of eggs treated with SPIDES for 135 min achieved significantly ($P<0.05$) the highest growth, followed by SPIDES for 90 and 45 min, respectively, while non-SPIDES (control eggs) showed significantly the lowest embryonic growth (Table 9).

The effect of interaction between storage period and SPIDES protocol was not significant on blastoderm diameter at 0 and 18h of incubation. However, the interaction effect was significant on blastoderm diameter after 12 and 24h of incubation. Results of blastoderm diameter after different hours of incubation illustrated in Fig. 3 reveal that blastoderm diameter after 12 or 24h of incubation showed inconsistent trend of differences between SPIDES protocol for 45 and 90 min, while blastoderm diameter was the highest for eggs treated with SPIDES for 135 min, and the lowest for the non-SPIDES control eggs in different storage periods (1-5wk) (Fig. 3).

These results indicated beneficial impacts of SPIDES protocols, regardless storage period, on improving the embryonic development during the incubation, being the most impact by SPIDES for 135 min.

During the first incubation stage, referred to as the developmental phase, the embryo develops from the pellucida. The fluid and membrane compartments mentioned below are produced by the area opaca: Amnion (envelops the embryo by day 4 of incubation), chorion (forms the chorio-allantois, completed around day 11 of incubation), allantois (a sac arising from the embryo's primitive hindgut from day 2 of incubation), and yolk sac membrane (formed by the area vitellina and area vasculosa). The purpose of these compartments and membranes is to shield the embryo during development and support the embryo's respiration, excretion, and nutrition (Nechaeva *et al.,* 2004). It is of interest to note that the diameter of blastoderm showed gradual increase by advancing incubation hour for non-SPIDES eggs stored for 1 and 2 weeks, however, a tendency of increase or decrease was observed in non-SPIDES eggs stored for mor than 2 weeks (Fig. 3).

The mechanisms responsible for the decreased viability of blastodermic cells elevated expression of genes associated with oxidative stress, apoptosis, and fatty acid metabolism are partially explained by some authors (Hamidu *et al.,* 2011; Bakst *et al.,* 2016).

As the number of viable cells or cell proliferation are not likely to be correlated with the increase in blastoderm volume (Bakst *et al.,* 2012), this may be due to that the increase in blastoderm volume might be related to the blastoderm cells dispersing as the vitelline membrane loses its mechanical strength. This hypothesis aligns with the declining yolk index and the shifting physicochemical characteristics of the yolk and white. In agreement with the present results, Benton and Brake (1996) noted that while the length of storage has a negative impact on embryo survival, there is no correlation found between higher embryo survival and incubating freshly laid eggs, which have a neutral pH and high viscosity. Several reports demonstrated that long storage times cause delays in the development of the embryo (Kaufman, 1938; Steinke, 1972).

Although the exact cause of why some eggs are more resistant to prolonged storage than others is unknown, a variety of factors, such as genetics, egg quality (including composition), and embryo specificities, are likely at play (Christensen *et al*., 2001). Pre storage incubation may be most advantageous when embryos are in the pre gastrula stage of development (<EG10) at egg collection (Meir and Ar, 1998). Prolonged egg storage can affect a number of egg quality parameters and even cause the blastoderm to become so damaged that it cannot regenerate during incubation. Numerous investigations have documented a reduction in viable blastodermic cells following an 8-day period of storage (Bakst *et al.,* 2012).

In our study, we used SPIDES techniques to alleviate the adverse effects of extended egg storage period, which increased the viability of embryos until hatching by reactivating embryo metabolism before incubation (Dymond *et al.,* 2013; Damaziak *et al.,* 2018), consequently increasing embryo size, in term of increasing blastoderm diameter. In light of the aforementioned findings of SPIDES protocols, it appears critical to avoid delaying the beginning of embryonic development at the beginning of the hatching period. The obvious differences in embryo size by SPIDES protocols may be attributed to number and duration of each SPIDES protocol. The delay in embryonic development after prolonged storage periods may be avoided if eggs are promptly pre-warmed from storage temperature to the optimal eggshell temperature of 100°F (Lourens *et al*., 2005) at the start of the hatching process. When the rate of pre-warming is low (12 to 24 hours) at the beginning of the incubation process, mitosis occurs at suboptimal temperatures for a predetermined period of time, and abnormal or delayed embryonic development may result (Reijrink *et al.,* 2008). An alternative explanation is that the embryo's microenvironment has a low rate of cell duplication, which means there are fewer viable embryonic cells available to either produce enough carbon dioxide to lower the pH to 8.2 in the first few days of incubation or to form a sufficient barrier between the embryo's inside and outside (Reijrink *et al.,* 2008).

## CONCLUSION

It can be concluded that there is a negative impact on embryo viability and/or cell death during storage. Several factors appear to influence embryo viability, including the overall count of viable cells, the stage of development at which the embryo is, and the pH value of the embryo's medium. The developmental stage of the embryo may not be crucial for maintaining embryo viability during brief storage times, most likely due to the low rate of cell death. In situations where the embryo cannot control its environment, altering it during storage and/or early incubation seems to be crucial for the optimal initiation of embryonic development.

In conclusion: Short Periods of Incubation During Egg Storage (SPIDES) every 4 days during storage period has been exhibited improvements in dynamic weight loss, maintained pH values of albumen and yolk, and increased diameter of blastoderm of embryos at early embryonic developmental stage for eggs stored for one week or more.

**Table (1):** Number of eggs used storage groups and SPIDES sub-groups.

| SPIDES protocol | Number of eggs in storage groups | | | | | Total |
|---|---|---|---|---|---|---|
| | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk | |
| 0 min (neg. control) | 150 | 150 | 150 | 150 | 150 | 750 |
| 45 min | 450 | 450 | 450 | 450 | 450 | 2250 |
| 90 min | 450 | 450 | 450 | 450 | 450 | 2250 |
| 135 min | 450 | 450 | 450 | 450 | 450 | 2250 |
| Total | 1500 | 1500 | 1500 | 1500 | 1500 | 7500 |

**Table (2):** Program of multi-stage (Pas reform) incubator.

| Temperature ° F | Wet bulb ° F | Ventilation% | Turn angle |
|---|---|---|---|
| 99.5 | 84.5 | 35-85 | 90° every hour |

**Table (3):** Program of multi-stage (Pas reform) hatchery.

| Age | | Temperature (°F) | RH ° F (Wet bulb) | Ventilation (%) |
|---|---|---|---|---|
| Day | hour | | | |
| 18 | 00 | 98.0 | 84 | 40-50 |
| 19 | 12 | 98.0 | 85 | 25-25 |
| 20 | 10 | 98.3 | 86 | 25-65 |
| 20 | 16 | 98.0 | 88 | 45-75 |
| 20 | 18 | 97.0 | 89 | 80-100 |

**Table (4):** Dynamic weight loss (%) of eggs as affected by storage period and SPIDES protocol on different storage days

| Item | Egg dynamic weight loss (DWL%) on different days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 4 | Day 8 | Day 12 | Day 16 | Day 20 | Day 24 | Day 28 |
| **Effect of storage period (wk.)** | | | | | | | |
| 1 | 1.139[a] | | | | | | |
| 2 | 0.791[b] | 0.972 | 0.979 | | | | |
| 3 | 0.726[b] | 0.856 | 0.864 | 0.909[a] | | | |
| 4 | 0.724[b] | 0.849 | 0.856 | 0.957[a] | 0.717 | 0.774 | |
| 5 | 0.594[b] | 0.830 | 0.836 | 0.807[b] | 0.655 | 0.732 | 0.790 |
| SEM | 0.0498 | 0.0413 | 0.0411 | 0.0277 | 0.025 | 0.0262 | 0.016 |
| P- value | <.0001 | 0.0826 | 0.0811 | 0.0026 | 0.096 | 0.28 | - |
| **Effect of SPIDES protocol (min.)** | | | | | | | |
| 0.0 | 0.967[a] | 0.862 | 0.871 | 0.921 | 0.664 | 0.658[b] | 0.617[b] |
| 45 | 0.727[b] | 0.875 | 0.882 | 0.865 | 0.709 | 0.648[b] | 0.788[b] |
| 90 | 0.674[b] | 0.918 | 0.924 | 0.930 | 0.643 | 0.819[a] | 0.884[a] |
| 135 | 0.811[ab] | 0.851 | 0.859 | 0.849 | 0.728 | 0.886[a] | 0.870[a] |
| SEM | 0.0446 | 0.0413 | 0.0411 | 0.0320 | 0.0346 | 0.0370 | 0.0311 |
| P- value | 0.0002 | 0.6822 | 0.7072 | 0.2180 | 0.3093 | 0.0005 | 0.0011 |
| **Effect of interaction** | | | | | | | |
| P-value | 0.0012 | 0.9229 | 0.9183 | 0.0260 | 0.3133 | 0.0986 | - |

[a and b]: Means within the same column for each factor with different superscripts differ significantly at $P \leq 0.05$.

**Table(5):** Pre-SPIDES albumen pH value as affected by storage period and SPIDES protocol on different storage days

| Item | Albumen pH value on different days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 4** | **Day 8** | **Day 12** | **Day 16** | **Day 20** | **Day 24** | **Day 28** |
| **Effect of storage period (wk.)** | | | | | | | |
| 1 | 9.013 | | | | | | |
| 2 | 8.965 | 8.912 | | | | | |
| 3 | 9.020 | 8.802 | 8.930 | 9.006 | | | |
| 4 | 9.016 | 8.820 | 8.973 | 8.995 | 9.065 | 9.091 | |
| 5 | 8.952 | 8.930 | 9.045 | 8.957 | 9.030 | 9.012 | 9.0283 |
| SEM | 0.038 | 0.054 | 0.091 | 0.046 | 0.039 | 0.057 | 0.042 |
| P- value | 0.2451 | 0.2659 | 0.410 | 0.7413 | 0.5495 | 0.3454 | - |
| **Effect of SPIDES protocol (min.)** | | | | | | | |
| 0.0 | 9.016 | 8.911 | 8.996 | 8.988 | 8.996 | 9.040 | 9.006 |
| 45 | 9.053 | 8.885 | 8.940 | 8.960 | 9.043 | 9.041 | 9.060 |
| 90 | 8.973 | 8.829 | 9.022 | 9.025 | 9.116 | 9.120 | 8.946 |
| 135 | 8.918 | 8.840 | 8.980 | 8.971 | 9.035 | 9.006 | 9.100 |
| SEM | 0.034 | 0.054 | 0.041 | 0.053 | 0.055 | 0.081 | 0.085 |
| P- value | 0.446 | 0.6840 | 0.5595 | 0.8367 | 0.5093 | 0.7909 | 0.6319 |
| **Effect of interaction** | | | | | | | |
| P-value | 0.0001 | 0.0771 | 0.6098 | 0.0916 | 0.6964 | 0.8814 | - |

**Table (6):** Post-SPIDES albumen pH value as affected by storage period and SPIDES protocol on different storage days

| Item | Albumen pH value on different days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 4** | **Day 8** | **Day 12** | **Day 16** | **Day 20** | **Day 24** | **Day 28** |
| **Effect of storage period (wk)** | | | | | | | |
| 1 | 9.0356 | | | | | | |
| 2 | 8.9556 | 9.1111 | 9.0222 | | | | |
| 3 | 8.9778 | 9.0889 | 9.0556 | 9.0667 | | | |
| 4 | 9.0111 | 9.0889 | 9.0889 | 9.0222 | 9.0889 | 9.1778 | |
| 5 | 9.0889 | 9.1000 | 9.0111 | 9.1000 | 9.0667 | 9.1444 | 9.1222 |
| SEM | 0.0613 | 0.0611 | 0.0645 | 0.0648 | 0.0583 | 0.0533 | 0.0598 |
| P- value | 0.5859 | 0.9927 | 0.8277 | 0.7007 | 0.7920 | 0.6661 | - |
| **Effect of SPIDES protocol (min)** | | | | | | | |
| 45 | 9.0347 | 9.0417 | 9.0333 | 9.0556 | 9.0500 | 9.1333 | 9.1000 |
| 90 | 9.0267 | 9.1167 | 9.0417 | 9.1111 | 9.0667 | 9.2167 | 9.1333 |
| 135 | 8.9800 | 9.1333 | 9.0583 | 9.0222 | 9.1167 | 9.1333 | 9.1333 |
| SEM | 0.0475 | 0.0529 | 0.0559 | 0.0648 | 0.0714 | 0.0653 | 0.1036 |
| P-value | 0.6828 | 0.4394 | 0.9496 | 0.6262 | 0.7931 | 0.5944 | 0.9663 |
| Effect of interaction | | | | | | | |
| P-value | 0.9908 | 0.3885 | 0.9993 | 0.6297 | 0.8817 | 0.1316 | - |

**Table (7):** Pre-SPIDES yolk pH value as affected by storage period and SPIDES protocol on different storage days

| Item | Yolk pH value on different days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 4 | Day 8 | Day 12 | Day 16 | Day 20 | Day 24 | Day 28 |
| **Effect of storage period (wk)** | | | | | | | |
| 1 | 6.067[a] | | | | | | |
| 2 | 5.873[ab] | 6.4442 | 6.051 | | | | |
| 3 | 5.982[ab] | 6.0725 | 6.009 | 6.0167 | | | |
| 4 | 5.915[ab] | 6.0467 | 5.994 | 6.1133 | 6.1175 | 6.2225 | |
| 5 | 5.838[b] | 6.0017 | 6.062 | 6.0817 | 6.1000 | 6.1600 | 6.0917 |
| SEM | 0.0529 | 0.1364 | 0.044 | 0.0461 | 0.0568 | 0.0434 | 0.0505 |
| P- value | 0.0314 | 0.1029 | 0.6578 | 0.3358 | 0.8304 | 0.3232 | - |
| **Effect of SPIDES protocol (min)** | | | | | | | |
| 0.0 | 5.9627 | 6.0325 | 6.0108 | 5.9500 | 6.0700 | 6.0167 | 6.0267 |
| 45 | 5.8967 | 6.0317 | 6.0667 | 6.1089 | 6.1117 | 6.2600 | 6.1333 |
| 90 | 5.9180 | 6.1925 | 5.9875 | 6.1089 | 6.0600 | 6.2667 | 6.1000 |
| 135 | 5.9620 | 6.3083 | 6.0508 | 6.1144 | 6.1933 | 6.2217 | 6.1067 |
| SEM | 0.0474 | 0.1364 | 0.0441 | 0.0532 | 0.0804 | 0.0613 | 0.1010 |
| P- value | 0.6960 | 0.4170 | 0.5721 | 0.1049 | 0.6428 | 0.0337 | 0.8905 |
| **Effect of interaction** | | | | | | | |
| P-value | 0.0268 | 0.9128 | 0.3241 | 0.6743 | 0.3833 | 0.8129 | - |

[a and b]: Means within the same column for each factor with different superscripts differ significantly at $P \leq 0.05$.

**Table (8):** Post-SPIDES yolk pH value as affected by storage period and SPIDES protocol on different storage days.

| Item | Yolk pH value on different days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 4 | Day 8 | Day 12 | Day 16 | Day 20 | Day 24 | Day 28 |
| **Effect of storage period (wk.)** | | | | | | | |
| 1 | 6.1744 | | | | | | |
| 2 | 6.1444 | 6.1222 | 6.1222 | | | | |
| 3 | 6.1333 | 6.1222 | 6.1333 | 6.1333 | | | |
| 4 | 6.2000 | 6.1000 | 6.1222 | 6.0889 | 6.1889 | 6.1333 | |
| 5 | 6.1333 | 6.1778 | 6.1444 | 6.0778 | 6.1778 | 6.0889 | |
| SEM | 0.0474 | 0.0547 | 0.0500 | 0.0509 | 0.0521 | 0.0567 | 6.1444 |
| P- value | 0.7752 | 0.7770 | 0.9869 | 0.7209 | 0.8827 | 0.5893 | 0.0471 |
| **Effect of SPIDES protocol (min.)** | | | | | | | |
| 45 | 6.1667 | 6.1667 | 6.1167 | 6.1000 | 6.1833 | 6.1500 | 6.1333 |
| 90 | 6.1380 | 6.1333 | 6.1333 | 6.0778 | 6.2000 | 6.1000 | 6.0333 |
| 135 | 6.1667 | 6.0917 | 6.1417 | 6.1222 | 6.1667 | 6.0833 | 6.2667 |
| SEM | 0.0340 | 0.0474 | 0.0433 | 0.0509 | 0.0638 | 0.0694 | 0.0816 |
| P- value | 0.7906 | 0.5418 | 0.9175 | 0.8282 | 0.9344 | 0.7828 | 0.2090 |
| **Effect of interaction** | | | | | | | |
| P-value | 0.9848 | 0.9997 | 0.8930 | 0.8193 | 0.1530 | 0.1530 | - |

**Table (9):** Diameter of blastoderm (mm) after different hatching hours as affected by storage period and SPIDES protocol.

| Item | Blastoderm diameter (mm) at hatching hours | | | |
|---|---|---|---|---|
| | 0 | 12 h | 18 h | 24 h |
| **Effect of storage period (wk.)** | | | | |
| 1 | 4.308 | 7.346$^{c}$ | 11.318$^{b}$ | 17.563$^{b}$ |
| 2 | 4.300 | 9.355$^{b}$ | 13.071$^{ab}$ | 18.288$^{b}$ |
| 3 | 4.317 | 8.881$^{b}$ | 14.648$^{a}$ | 18.587$^{ab}$ |
| 4 | 4.308 | 11.634$^{a}$ | 14.302$^{a}$ | 19.986$^{a}$ |
| 5 | 4.333 | 9.131$^{b}$ | 12.555$^{ab}$ | 20.022$^{a}$ |
| SEM | 0.026 | 0.296 | 0.566 | 0.382 |
| P- value | 0.9112 | <.0001 | 0.0010 | <.0001 |
| **Effect of SPIDES protocol (min.)** | | | | |
| 0.0 | 4.347 | 4.565$^{d}$ | 5.550$^{c}$ | 7.309$^{d}$ |
| 45 | 4.300 | 9.268$^{c}$ | 14.249$^{b}$ | 20.517$^{c}$ |
| 90 | 4.327 | 10.759$^{b}$ | 15.199$^{b}$ | 22.755$^{b}$ |
| 135 | 4.280 | 12.485$^{a}$ | 17.717$^{a}$ | 24.975$^{a}$ |
| SEM | 0.023 | 0.265 | 0.506 | 0.342 |
| P- value | 0.1942 | <.0001 | <.0001 | <.0001 |

$^{a, b, and c}$: Means within the same column for each factor with different superscripts differ significantly at $P \leq 0.05$.

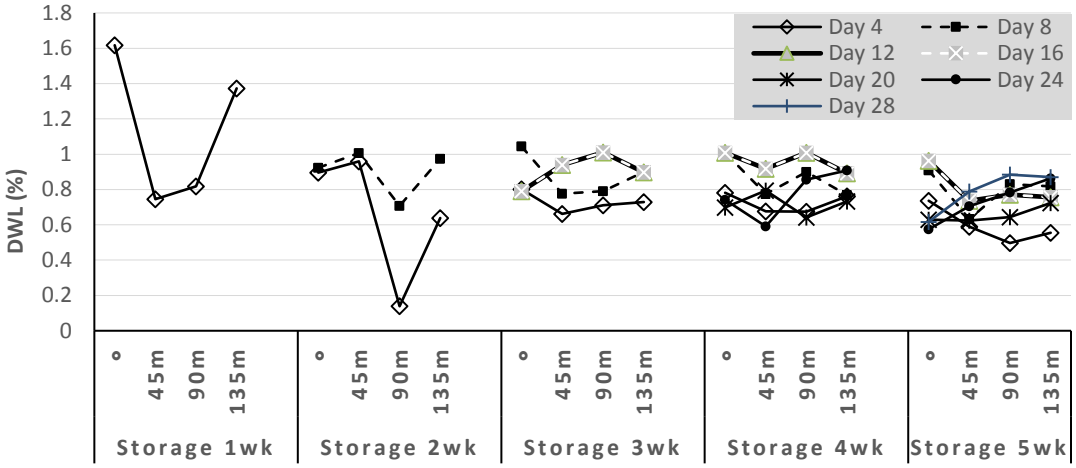



**Fig. (1):** Dynamic weight loss (DWL%) of eggs stored for different weeks and exposed to different SPIDES protocols.

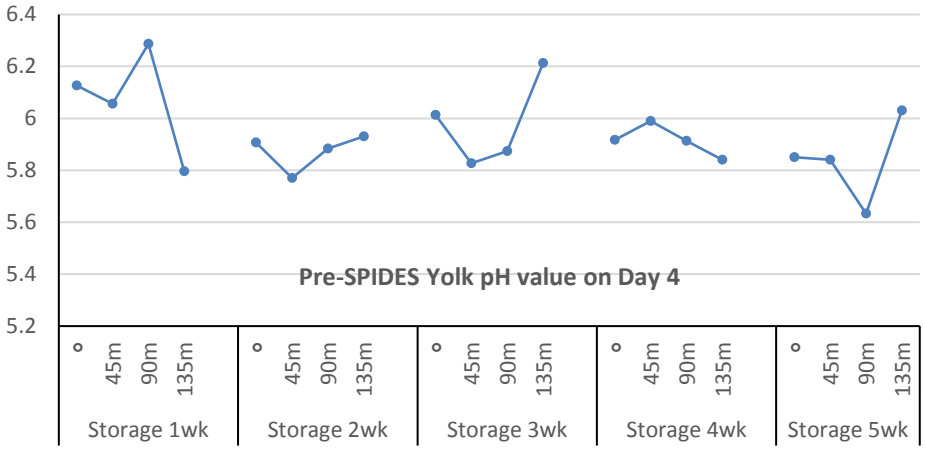
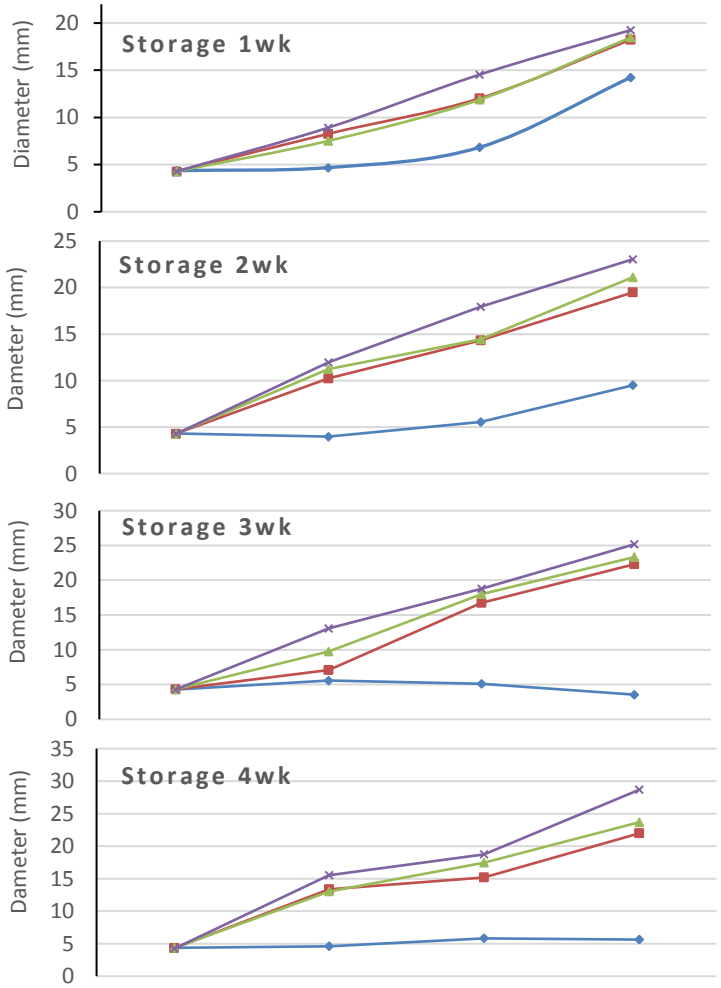



**Fig. (2):** Pre-SPIDES yolk pH value as affected by the interaction effect of storage period x SPIDES protocol on different storage days



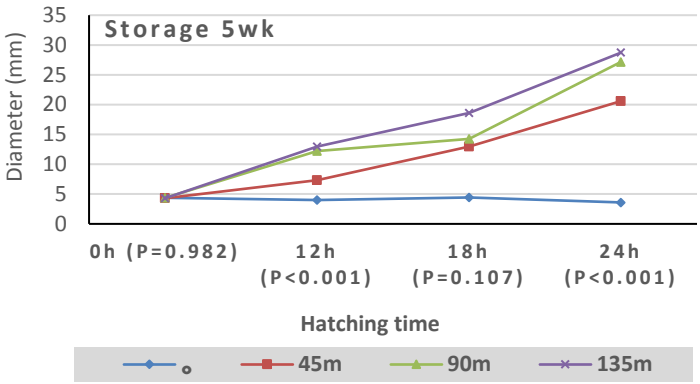



**Fig. (3):**Diameter of blastoderm after incubation for 0, 12, 18, and 24h for eggs stored for different periods and SPIDES protocols.

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## الملخص العربى

## تأثير فترات الحضانة القصيرة أثناء تخزين البيض (SPIDES) على فقدان الوزن الديناميكي وقيم الأس الهيدروجيني للألبومين والصفار والتطور الجنيني المبكر للبيض المخزن لفترات طويلة

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هدفت هذه الدراسة إلى استكشاف تأثيرات SPIDES بفاصل زمني 4 أيام لمدة 0 و 45 و 90 و 135 دقيقة على الخصائص الداخلية والخارجية والتطور الجنيني المبكر للبيض المخزن لفترات مختلفة (1 و 2 و 3 و 4 و 5 أسابيع). تم تقسيم إجمالي 7500 بيضة تزن 62 جرامًا إلى خمس مجموعات عشوائيًا، تحتوي كل منها على 1350 بيضة وثلاث نسخ مكررة من 450 بيضة؛ تم تضمين 150 بيضة في كل مجموعة علاجية كمجموعة تحكم سلبية (غير SPIDES). أشارت النتائج إلى أعلى فقدان للوزن الديناميكي (DWL) في البيض غير SPIDES في جميع فترات التخزين. انخفض DWL بواسطة SPIDES في الأيام 4 و 8 و 12 و 16 و 20 يومًا من التخزين. لوحظ أدنى DWL في اليوم الرابع للبيض المخزن لمدة أسبوعين و SPIDES لمدة 90 دقيقة. لم تتأثر قيم الأس الهيدروجيني لألبومين البيض قبل وبعد استخدام SPIDES وصفار البيض بعد استخدام SPIDES بفترة التخزين وبروتوكول SPIDES وتفاعلهما. انخفضت قيم الأس الهيدروجيني لصفار البيض قبل استخدام SPIDES في اليوم الرابع ($P<0.05$) في البيض المخزن لأكثر من 4 أسابيع مقارنة بفترة تخزين لمدة أسبوع واحد، وكانت الأقل في البيض المخزن لمدة أسبوع و4 أسابيع باستخدام SPIDES لمدة 135 دقيقة، والبيض المخزن لمدة أسبوعين و3 أسابيع باستخدام SPIDES لمدة 45 دقيقة، والبيض المخزن لمدة 5 أسابيع باستخدام SPIDES لمدة 90 دقيقة. كان قطر البلاستوديرم أعلى ($P<0.05$) في البيض المخزن لمدة 4 أسابيع مقارنة بفترات التخزين الأخرى، والبيض المخزن لمدة 135 دقيقة باستخدام SPIDES مقارنة بفترات التخزين الأخرى في جميع ساعات الفقس (12 و18 و24 ساعة). في البيض المخزن لجميع الفترات. كان قطر البلاستوديرم هو الأعلى في البيض المحتوي على SPIDES لمدة 135 دقيقة والأدنى في البيض غير المحتوي على SPIDES في جميع ساعات الفقس.

وفي الختام: أظهرت فترات الحضانة القصيرة أثناء تخزين البيض (SPIDES) كل 4 أيام أثناء فترة التخزين تحسنا في فقدان الوزن الديناميكي، والحفاظ على قيم الرقم الهيدروجيني للألبومين والصفار، وزيادة قطر البلاستودرم في مرحلة النمو الجنيني المبكر للبيض المخزن لمدة أسبوع أو أكثر.