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Breed of Chicken and Frequent Short Periods of Incubation During Different Egg Storage Periods (SPIDES) Differentially Modify the Hatchability %, Chick's Quality and Post-Hatching Bird's Performance

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THE current study aimed to investigate the effect of successive short bursts of warming for 0, 5, and 10h (SPIDES) during egg storage periods (7 and 14 days) on the hatchability %, chick's quality and post-hatching bird's performance among two chicken breeds, Fayoumi and Avian-34. Accordingly, 6300 fertilized eggs (3150 eggs from each breed) were randomly distributed in to 2x2x3 experimental design (2 breeds, 2 storage periods, & 3 warming times) with 5 replicates/ treatment. Eggs' warming was frequently repeated every 3 days for 0, 5, and 10h (SPIDES). The main findings indicated significant reductions of egg weights and increases of egg weight losses with the increase of egg storage and the warming times during storage. The highest egg weight losses were reported for Fayoumi's eggs stored for 7- or 14-days and exposed to frequent SPIDES for 10h as well as Avian-34's eggs stored for 7-days and warmed for 10h. Marked improvements of hatchability % in both Fayoumi, and Avian-34 breeds were recorded following the exposure to frequent SPIDES for 0 and 5h. Additionally, distinct enhancements of post-hatching performance were reported. Chicks hatched from eggs stored for longer periods and exposed to SPIDES possessed less post-hatching weights (W0) in Avian-34 and Fayoumi compared to those directly incubated without warming during storage. However, those chicks succeeded to attain significant heavier body weights at 35 days old. The best post-hatching growth performance was reported for chicks hatched from eggs exposed to 5h of successive warming every three days during storage.

Keywords: SPIDES, Long-term egg storage; hatchability; post hatching performance

Introduction

In the poultry production sector, producing high-quality chicks with a subsequent good post-hatching performance is of an integral concern to producers [1]. This, in turn, requires a fine balance between various management practices in hatcheries including the storage of fertilized eggs [2]. Storing fertilized eggs before artificial incubation is commonly done to synchronize hatchings and fulfil the one-day-old chicks' demand [3]. Normally, fertilized eggs are safely stored for a duration of 7 days at temperatures less than ambient temperature with low negative impacts on hatchability [4]. However, due to variations in the demand for one-day-old chicks and the hatchery capacities, long-term storage may be applied but it impairs egg quality and the viability of hatched chicks [4]. Storage more than 7 days is markedly associated with harming embryonic development because it impairs the blastoderm reactivation [3, 5], depresses the embryonic metabolism, and delays the embryonic development causing irreparable embryonic damage and increases the embryonic mortality, lowers the hatchability [6] and decreases chick performance [7]. Moreover, prolonged egg storage leads to embryonic stress, and increases cell death through increasing embryonic necrosis and apoptosis [8]. Consequently, prolonged storage alters egg quality characteristics such as water loss, yolk, and albumen properties resulting in increasing the hatching time [9] and low chick quality [10]. All of these effects of prolonged storage varies depending on many factors such as breeder's age, breeds, and duration of storage [6].

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In poultry, fertilized eggs require many factors to hatch including temperature, ventilation, turning, and relative humidity [11]. The temperature is the most critical factor as it influences embryo development before and during the incubation [12]. During the storage period, the temperature is reduced (less than ambient temperature) to ensure the survival of the embryo until the start of incubation [13]. Then, at the beginning of incubation, eggs must be slowly warmed by increasing the temperature gradually to the incubation temperature [14]. Therefore, many interventions have been considered to overcome the prolonged storage associated low hatchability and chick quality. During storage, short periods of incubation (SPIDES) are applied to maintain embryonic development and hatchability. SPIDES improve the embryonic development through resisting the storage induced effects via increasing the total cell number and maintaining the developmental plasticity of the pre-gastrulation embryo which provide a larger reservoir of available cells to compensate for the induced cell deaths by prolonged storage [8]. Dymond and his associates in 2012 and 2013 [15] [16] reported that SPIDES treatment (4-h PI delivered at 4- to 5-d intervals) during prolonged egg storage (21 d of storage) increases hatchability and improves chick quality through lowering both early and late embryo mortality, increases the proportion of viable cells and alleviating the negative effects of storage-induced cell death on embryo development. Moreover, the single incubations of 6 or 12 h during the 4 d storage caused similar advances in embryonic development, but the hatchability was worse than in the untreated controls [17, 18]. Thus, small multiple SPIDES during storage have been recommended for greater benefits than a single incubation performed on d 4 of storage. In general, warming during storage is beneficial with the prolonged storage [4].

However, the effect of SPIDES varies based on storage period, breeder age, species, chicken breeds, etc… Thus, in this present study, we hypothesized that applying frequent short bursts of incubation during storage (SPIDES) every three days, instead of a single time, varies depending on the duration of storage, warming time (h), breed of chicken, and their interaction. So, the current study aimed to investigate the effect of SPIDES every three days during storage on hatchability %, embryo quality, and post-hatching performance in different chicken breeds. Additionally, whether the effects of SPIDES vary among different chicken breeds, different warming durations, temperatures, and their interactions.

Material and Methods

This study was conducted at the poultry production department, faculty of agriculture, Kafrelsheikh University, Egypt. The experimental procedures and bird managements were done following the regulations of their animal care and ethics committee.

Egg source and collection

The current study was performed on 6300 fertilized eggs obtained from two different chicken breeds: Egyptian Fayoumi and a commercial broiler strain (Avian-34), with 3150 eggs were used from each chicken breed. Egyptian Fayoumi' eggs were obtained from a 30 to 40 weeks old Fayoumi breeder flock from El-Azab poultry farm, Agriculture Research institute, Ministry of Agriculture, Egypt. While the Avian-34 eggs were got from an Avian-34 broiler breeder flock (almost 30-40 weeks old) from Abd Elsalam Hegazy group, Cairo, Egypt. Eggs from each breed were collected and transferred in a temperature- and relative humidity-controlled vehicle to the artificial hatchery at Faculty of agriculture, Kafrelsheikh University, Egypt. Eggs from each breed were randomly divided according to the experimental design (**Fig.1**) into 5 replicates of 105 eggs/treatment. Then, random 25 eggs/replicate from each breed were examined on arrival to test weight, and the eggshell thickness. After that, eggs then were placed in egg trays and disinfected using Virkon™ S (10 g/L water).

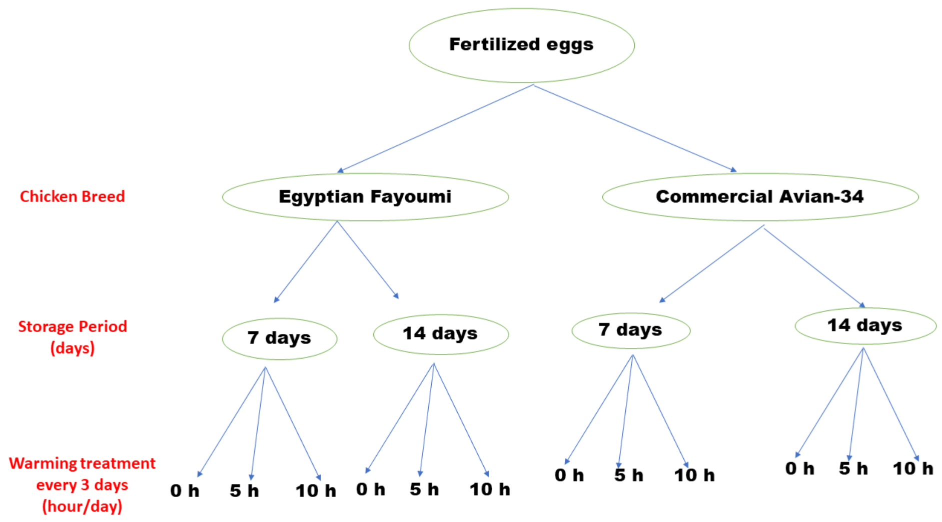

Fig.1.Represents a summary 2x2x3 experimental design used in this study. Fertilized eggs were obtained from two chicken breeds (Egyptian Fayoumi and Avian-34), stored for 7 and 14 days and exposed to 3 SPIDES times during storage for 0, 5, and 10 h.

Experimental Design and SPIDES Treatments

Fig.1 represents a summary of the 2x2x3 factorial experimental design of this study. Briefly, this design depended on two chicken breeds (Egyptian Fayoumi and Avian-34), 2 storage periods (7 and 14 days) and 3 SPIDES times for 0, 5, and 10 h. The experimental design briefly included storing the fertilized eggs in special rooms for 7 and 14 days at 17±1.5 °C, and relative humidity equal 75±2%. Then, frequent incubations every three days were applied. Temperature and relative humidity were checked twice/day to guarantee the stability of temperature, using digital thermometers. A manual thermometer and a hygrometer were also, used two times/ day to check the accuracy of the thermometer's readings according to El-Naggar *et al.* [19]. They were sited nearby the digital thermometers to ensure similar readings. Eggs were subjected to automatic turning twice a day at 90° angle.

Eggs from each breed were subjected to frequent short periods of incubation during egg storage (SPIDES). This was done by warming eggs every three days during storage periods for 0, 5 and 10 h. The warming was occurred by exposing the fertilized stored eggs to room temperature by taking eggs outside the storage room for a period equal to the warming treatment times then returning them back. Different SPIDES treatments were done gradually by getting the eggs from the storage room to ambient temperature then transferred to the incubator where the incubation warming happened to prevent egg sweating. Eggs exposed to 0 h warming were considered as control eggs and were kept in the storage room all time. At the end of storage periods (7 and 14 days), eggs were reweighed then transferred to an artificial incubator after disinfection (Jamesway Chick Master Incubator Inc., Medina, OH 44256, USA). Egg incubation was done at 37.1±0.2 °C and relative humidity 86±2 % for 18 days then eggs were reweighed and moved to hatcher at 36.9±0.2 °C and relative humidity 88±2 % till hatching completed for almost 3 days. During hatching period, hatching eggs were individually counted and checked every 6 h [4] to calculate the hatchability and mortality %.

Egg Weight and Egg Weight Loss

All eggs, from the two breeds, were weighed individually at the egg collection site and on the last day of storage. Egg weight loss (g) that occurred between the start of egg collection and the last day of storage was expressed as a percentage of fresh egg weight.

Post-hatching performance

One-day old chicks from each treatment were individually, wing-banded and weighed to determine one-day-old chick weights (W0). Additionally, chick's length and body temperature were measured. Chick's length was determined by measuring the length (Cm) from the peak to the middle toe. While the body temperature was measured using digital thermometer inserted into cloaca according to El-Kassas, *et al.* [20]. Besides, the navel area was checked for retracted yolk and unhealed navel. Eyes, activity, and appearance scores were also, measure based on Bilalissi, *et al.* [1].

Then, 720 hatched, clean, and dry chicks from each breed were transferred to a rearing farm where they randomly allotted according to the experimental design (Fig.1) with four replicates for each treatment (30 birds/replicate). Chicks had a free access to food and water till the end on the rearing period at 35 days old. Birds fed a commercial starter diet of 22.82

%CP and 3061.99 K.cal/Kg diet as ME then a grower diet with 20.02% CP and 3110 K.cal/Kg diet ME. Weekly individual feed intake was calculated to the nearest gram. Birds were weighed weekly and body weights were determined. Body gain, feed conversion ratio (FCR), housing temperature were frequently reported. Birds had 23:1 light to dark cycle.

Biochemical analysis

At 35 days old, 12 birds/replicate were euthanized, and blood samples were collected to from neck vein with a 27-G needle and 1 mL syringe then sera were isolated by centrifuging blood at 3,000 rpm for 15 min according to ELSaidy, *et al.* [21] and the obtained serum samples were stored at −20°C until analysis. Serum samples were used to analyse the levels of cholesterol, triglyceride (TG), low and high-density lipoprotein (LDL and HDL), total protein, albumin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase as well as serum creatinine level. These blood constituents were measured by a spectrophotometer using commercial kits (Biodiagnostic Co, Egypt) according to the manufacturer's instructions.

Statistical analysis

The obtained results were statistically analyzed by three-way ANOVA using the general liner model procedure of SAS Institute, 2004. Chicken breed (Avain-34 & Egyptian Fayoumi), storage period (7 &14 days), and duration of pre-incubation warming (0, 5, and 10 hours) were the main factors considered in this analysis with their interaction according to the following statistical model; $Y_{ijtk} = \mu + C_i + S_j + H_t + CSH_{ijt} + E_{ijtk}$ where μ = Overall mean, C_i = effect of chicken breed, S_j = Effect of storage period, H_t= Effect of incubation warming during storage (SPIDES), and CSH_{ijt} = Interaction between the three factors and E_{ijtk} = Residual error. Tukey's multiple tests were used at P < 0.05 as statistically significant level to compare means. Results were expressed as means ± SEM.

Results and discussion

Egg weight and egg weight loss

Table 1 illustrates the impact of frequent bursts every three days of SPIDES for 0, 5 and 10 h during different storage periods (7 and 14 days) on egg weights and egg weight losses and how it varied between the two studies chicken populations. The storage period, and the applied frequent SPIDES did not alter the egg weights despite of the significant breed differences (P<0.05). Avain-34's eggs displayed significant higher weights compared to Fayoumi's eggs (P<0.05). However, egg weight losses at 7-days of incubation were significantly altered by the duration of storage period and the time of the applied frequent SPIDES (P<0.05) without breed differences (P>0.05). In this context, the highest egg weight losses were reported for Fayoumi's eggs stored for 7- or 14-days and exposed to frequent SPIDES for 10h as well as Avian-34's eggs stored for 7-days and warmed for 10h (P< 0.05). Whereas the lowest weight losses were usually found in case of Avain-34 and Fayoumi's eggs stored for 7 and 14 days without frequent SPIDES (P<0.05). At the end of incubation (at 18-days), chicken breed, storage period and time of frequent SPIDES distinctly altered egg weight losses at this time (P<0.05). The high losses were measured for both Fayoumi and Avain-34 eggs stored for 14-days and exposed to 5 and 10h of frequent SPIDES (P<0.05). Though, the lowest losses were found in the case of 7-days storage with 5 and 10h frequent SPIDES in Fayoumi, and Avian-34 strains. The statistical analysis of the impacts of the frequent SPIDES, storage times, and chicken breed on the egg weight and egg weight losses revealed no significant interactive effects between these factors on neither the egg weights nor the egg weight loss (P>0.05). Similarly, Melo *et al.* [22] reported that prolonged storage up to 12 days increases weight losses of broiler's eggs. They argued the increases in weight loss of prolonged stored eggs due to increasing water loss [23] and increasing the transformation reactions occur in eggs [22]. For examples; transformation of ovalbumin to S-ovalbumin and the ovomucin-lysozyme complex that assist the evaporation of water via eggshell pores during storage. Because the egg weight loss is not harmful, so, the reported increases in the egg weight loss due to the prolonged storage and the frequent bursts of SPIDES are not harmful to embryos [22]. Additionally, the reported variations between the studied chicken breeds agreed with the previous study of Fathi *et al.* [24] who reported significances for the chicken's genotypes on egg weight losses and the hatchability. Because the avian-34 has heavier eggs than Fayoumi, this may explain the variations in water loss between Fayoumi and the Avian-34. The highest egg weight loss is linked with the smallest eggs because the lighter and smaller the eggs, the higher shell surface-to-mass ratio and increasing this ratio increases the rate of water loss due to increasing evaporation [25].

TABLE 1. Impact of frequent bursts of SPIDES for 0, 5 and 10 h during different storage times (7 and 14 days) on egg weights and egg weight losses between Fayoumi and Avain-34 birds

Breed	storage period (days)	SPIDES time(h)	Egg wt 0 (g)	Egg wt 7d (g)	Egg wt 18d (g)	Egg wt loss 0-7 (g)	Egg wt loss 0-18 (g)
AV-34	**7**	**0**	63.54±0.19^{b}	62.94±0.19^{b}	56.99±0.20^{a}	0.87±0.01^{efg}	9.65±0.11^{d}
		5	63.26±0.12^{b}	62.53±0.11^{b}	56.33±0.13^{b}	1.08±0.03^{cdef}	10.24±0.14^{c}
		10	63.81±0.17^{ab}	62.89±0.15^{b}	56.73±0.22^{ab}	1.35±0.16^{ab}	10.40±0.18^{c}
	Breed* storage period		63.54±0.09	63.54±0.09	62.79±0.09	56.68±0.11	1.10±0.06
	14	**0**	64.15±0.20^{a}	63.56±0.20^{a}	56.55±0.17^{ab}	0.86±0.00^{fg}	11.10±0.09^{b}
		5	63.42±0.12^{b}	62.66±0.12^{b}	55.36±0.17^{c}	1.11±0.01^{cd}	11.89±0.25^{a}
		10	63.40±0.23^{b}	62.68±0.22^{b}	55.23±0.24^{c}	1.05±0.06^{cdef}	12.06±0.18^{a}
	Breed* storage period		63.66±0.11	63.66±0.11	62.97±0.11	55.72±0.13	1.01±0.02
Overall			63.60±0.07	63.60±0.07	62.88±0.07	56.20±0.09	1.05±0.03
Fayoumi	**7**	**0**	44.64±0.18^{d}	44.16±0.18^{d}	39.59±0.16^{de}	0.97±0.01^{defg}	10.31±0.12^{c}
		5	44.01±0.18^{e}	43.47±0.18^{e}	38.87±0.20^{f}	1.12± 0.03^{bcd}	10.64±0.15^{bc}
		10	44.29±0.12^{de}	43.63±0.12^{de}	39.17±0.12^{ef}	1.36±0.14^{a}	10.53±0.19^{c}
	Breed* storage period		44.37±0.09	44.37±0.09	43.81±0.10	39.28±0.09	1.16±0.04
	14	**0**	44.38±0.15^{de}	43.99±0.15^{de}	38.97±0.14^{f}	0.79±0.03^{g}	11.10±0.10^{b}
		5	44.63±0.21^{d}	44.10±0.20^{d}	38.77±0.20^{f}	1.07±0.02^{cdef}	11.96±0.23^{a}
		10	45.89±0.22^{c}	45.27±0.19^{c}	39.88±0.23^{d}	1.22±0.08a^{bc}	11.98±0.198^{a}
	Breed* storage period		44.97±0.13	44.97±0.13	44.46±0.12	39.21±0.12	1.02±0.03
Overall			44.60±0.08	44.05±0.08	39.25±0.07	1.11±0.03	10.92±0.07
MSE			0.971	0.953	0.992	0.389	0.866
R2			0.989	0.990	0.987	0.180	0.440
Source of variation, P value							
Breed			0.001	0.001	0.001	0.423	0.044
Storage period			0.081	0.051	0.0101	0.0134	0.001
SPIDES time			0.071	0.060	0.201	0.001	0.001
Breed*storage period* SPIDES time interaction			0.122	0.311	0.094	0.206	0.287

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

Effect of storage period, frequent SPIDES and chicken breed on hatchability (%) and post-hatching chick quality

Hatchability percentage (**Table 2**) was significantly altered based on the breed of chicken, duration of storage, frequent SPIDES and their interaction (P<0.05). In this regard, the highest percentages of hatchability, both Fayoumi, and Avian-34 breeds, were calculated for eggs stored for 7 days and exposed to frequent SPIDES for 5h (P<0.05). Additionally the eggs stored for 7 days and directly incubated without warming during storage, in both Fayoumi, and Avian-34 breeds, displayed considerably higher hatchability % compared to other SPIEDS conditions (P<0.05). On the other hand, the lowest hatchability percentages were found in the case of eggs stored for 14 days and frequently warmed during storage for 5 or 10 h (P<0.05). For Fayoumi's eggs, the highest hatchability percentages were also, recorded for eggs stored for 7 days and frequently warmed during storage for 5h (P<0.05). Whereas the lowest % was noted for the eggs stored for 14-days and subjected to 5 or 10h frequently warming during storage (P<0.05).

These findings agreed with the previous reports of Okasha *et al*. [26] who reported reduction in hatchability % for eggs stored for long period (15 days). They ascribed the low hatchability % following the long storage to increasing embryonic death with the long-term egg preservation. The embryonic death increases due to the inferior albumen quality, and alteration in its pH and viscosity which in turn harm the viability of the embryo especially during the first developmental stages [27, 28]. Also, Tona et al and Taha *et al.* [29, 30] found that the long-term storage of eggs reduced the albumen's quality and resulting in low hatchability %. Though, the eggs exposed to frequent bursts of SPIDES had higher hatchability % because SPIDES might increase the total cell number and maintain the developmental plasticity of the pre-gastrulation embryo providing a larger reservoir of available cells to compensate for increased cell death induced by prolonged storage [8]. Similar findings were obtained by Dymond *et al.* 2012 and 2013 [15, 16] which stated that SPIDES treatment (4-h PI delivered at 4- to 5-d intervals) during prolonged egg storage (21 d of storage) increases hatchability and improves chick quality through lowering both early and late embryo mortality, increases the proportion of viable cells and alleviating the negative effects of storage-induced cell death on embryo development. The improved hatchability % following the frequent short bursts of SPIDES agreed with the results of Maman and Yildirim [28], Ozlu *et al.* [27], Silva *et al.* [31], and Tag EL-Din *et al.* [32] who noted that short periods of incubation for 6 h before the storing for 14 days improved the hatchability % due to lowering late embryonic mortality. Moreover, our obtained results were in line with the results of Damaziak *et al.* [33] which reported that eggs stored for 7 days had higher hatchability % than freshly laid eggs and prolonged stored ones for 21 to 28 days. This effect may be correlated with in sufficient O_2 levels for early chick embryo's metabolic demands [34] and due to increasing albumen pH with the prolonged storage, respectively.

TABLE 2. Effect of frequent bursts of SPIDES for 0, 5 and 10 h during different storage times (7 and 14 days) on hatchability between Fayoumi and Avain-34 birds

Breed	storage period (days)	SPIDES time(h)	Number of chicks	HATCHABILITY %
AV-34	7	0	145±2.6^{ab}	86.3±1.5^{ab}
		5	151.33±1.2^{a}	90.07±0.7^{a}
		10	112.66±13.9^{c}	67.0±8.2^{c}
	Breed* storage period		136.33±6	81.15±3.6
	14	0	129.6±2.8^{b}	77.18±1.6^{b}
		5	64±2.9^{e}	38.09±1.7^{e}
		10	90.66±1.8^{d}	53.96±1^{d}
	Breed* storage period		94.77±6.6	56.41±3.9
overall			115.55±5.6	68.78±3.37
Fayoumi	7	0	130.33±1.8^{b}	77.5±1.1^{b}
		5	141±0.3^{ab}	83.9±0.2^{ab}
		10	132±3.1^{b}	78.57±1.8^{b}
	Breed* storage period		134.44±1.6	80.02±0.9
	14	0	88.66±7.6	52.77±4.5^{d}
		5	35.3±3.6	21.03±2.1^{f}
		10	55.33±0.2	32.93±0.1^{e}
	Breed* storage period		59.77±5.9	35.58±3.5
Overall			97.11±7.0	57.80±4.1
MSE			16.41	9.76
R2			0.85	0.85
Source of variation, P value				
Breed			0.001	0.001
Storage period			0.001	0.001
SPIDES time			0.001	0.001
Breed*storage period* SPIDES time interaction			0.001	0.001

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

Post-hatching chick quality (**Table 3**) was also, modified according to the applied frequent SPIDES conditions and chicken breeds. In this context, post-hatching chick's body weights were markedly altered by the breed of chicken and duration of frequent SPIDES (P<0.05) without effect of storage period (P>0.05). In Avian-34, highest post-hatching chicks' weights were recorded for chicks hatched from eggs stored for 7 and 14 days and directly incubated without warming during storage. However, the eggs subjected to frequent warming during storage (5 or 10h) resulted in significant low chicks weights (P<0.05). Similarly, in Fayoumi the highest post-hatching weights were also, reported for chicks hatched from eggs stored for 7 and 14 days and incubated without prior warming during storage. Alternatively, the lowest post-hatching weights were reported for the other SPIDES conditions (P<0.05). Besides, all post-hatching weights of Avian-34 were higher than those of Fayoumi breeds (P<0.05). The reported increases in the hatching weights were in line with the results of Nowaczewski *et al.* [25] which recorded higher body weights for chicks emerged from eggs stored for long time despite of increasing egg weight loss. However, our results disagreed with those of Maman and Yildirim [28], Ozlu *et al.* [27], Fasenko [35] and Christensen *et al.* [36] who reported marked decreases in chick's weight following the long-term egg storage due to lowering the metabolic rates, delaying the growth of the internal organs such as heart, liver and lung weight and a consequent poor chick quality [27]. Besides, in the current study, the reported lowering hatching weights following the application of frequent short bursts of SPIDES during egg storage might be correlated with increases of water loss during storage [22]. Besides, the differences in response between the Avian-34 and Fayoumi might be correlated with their variations in body weight hence there is a positive correlation between the body weight, egg weight and chick hatching weight [25]. Chicks' body length was also, varied among the applied frequent SPIEDS conditions and chicken breeds (P<0.05). All Fayoumi chicks had less body length compared with Avain-34 chicks regardless to the applied SPIDES condition (P < 0.05). In addition, for Avian-34 breed, the highest body lengths were reported for chicks hatched from eggs stored for 7 days and exposed to frequent 10h warming during storage (P<0.05). However, the smallest body lengths were reported, in the same breed, for chicks hatched from eggs stored for 14-days and directly incubated without warming during storage (P<0.05). For Fayoumi chicks, the highest body length was measured for chicks hatched from eggs stored for 14 days and subjected to frequent 10h of warming before incubation (P<0.05). Also, eggs that stored for 7days and exposed to 5 or 10h frequent incubation during storage resulted in chicks with nearly the same body length as those hatched from 14-days storage and 10h warming eggs (P>0.05). Whereas chicks hatched from eggs stored for 14days and incubated without warming during storage had the smallest body length (P<0.05). Activities of the hatched chicks, although did not vary between the two studied chicken breeds (P>0.05), were modified by the storage period and duration of frequent incubation during storage (P<0.05). The highly active chicks, from Avain-34 chicken, were hatched from eggs stored for 14 days and frequently warmed during storage for 10 h (P<0.05). Chicks hatched from eggs were subjected to 7 days storage and exposed to frequent 10h warming or 14 days storage and frequently warmed for 0h or 5h in the case of Avain-34 and stored for 14 days and warmed for 5 or 10 h during storage in the case of Fayoumi displayed similar activities (P>0.05). These findings were in agreement with the results of Ebeid *et al.* [37] which stated a decline in the chick quality following the long term egg preservation (for up to 14 days). On the other hand, an improved chick quality was observed for chicks emerged from egg subjected to frequent periods of SPIDES during storage [26, 28]. Though, Reijrink *et al.,* 2010 [38] did not prove improving the chick quality following pre-storage incubation. Therefore, increasing egg storage harm post hatching chick quality but applying frequent short periods of SPIDES during storage relieves this effect.

Post-hatching performance following different SPIDES conditions

The post-hatching performance (**Table 4**) was obviously modulated by the different applied SPIDES conditions and the studied chicken breeds (P<0.05). The Avain-34 chicken had obvious heavy hatching weights (BW0) compared to Fayoumi ones (P<0.05). Within Avian-34, chicks hatched from the eggs stored for 14 days and directly incubated without frequent incubation during storage had the heaviest BW0 followed by those stored for 7 days without frequent incubation during storage. However, the lowest BW0s were recorded for those hatched from eggs stored for 14 days and subjected to frequent incubation for 10 hours during storage. Whereas, among Fayoumi chickens, the largest BW0s were reported for chicks hatched from eggs stored for either 7 or 14 days without frequent incubation during storage. While the lowest BW0s were recorded for chicks hatched from eggs stored for 14 and 7 days and exposed to 10 hours of frequent incubation during storage, respectively. The high body weights following the long term egg preservation agreed with the findings of Nowaczewski et al [25] which recorded increases of chicks' body weights following the long term egg storage. Again, decreasing the chicks hatching weights following the application of SPIDES may be linked with increasing water loss during long term storage [26]. Also, the heavy weights in the case of Avian-34 compared to Fayoumi were perhaps correlated to the differences in egg weight between the two breeds hence there is a positive correlation between egg weight and chick's weight.

Chick's body temperature also, varied among different applied SPIDES and chicken's breeds (P<0.05). The highest body temperature was reported in the case of Fayoumi chicks hatched from eggs stored for 14 days and directly incubated without any incubation during storage periods. Whereas Fayoumi chicks hatched from eggs stored for 7 days and exposed to either 5 or 10h frequent incubation during storage before incubation exhibited the lowest body temperatures (38.72 and 38.62 ^{o}C, respectively). The Avian-34 chicks hatched from eggs stored for 7 or 14 days and subjected to 10 hours frequent incubation during storage had similar lower body temperatures (38.8 and 38.76 ^{o}C, respectively). The reduction of body temperatures following the application of SPIDES may be explained by lowering the body weights due to lowering the metabolic rate [27, 28, 35, 36]. However, the increases body temperature might be argued to the higher heat production found in birds emerged from eggs stored for 14 days that may be elucidated by the higher energy used for development. This explanation might support the increases of body weights for chicks hatched from long-term stored eggs.

TABLE 3. Post-hatching quality of Fayoumi and Avain-34 chicks in response to SPIDES for 0, 5 and 10 h during different storage times (7 and 14 days).

Breed	Storage period (days)	SPIDES time(h)	BW0 (g)	Length_0	ACTIVITY	EYES
AV-34	7	0	45.41±0.4^{a}	20.01±0.0^{c}	9.49±0.0^{e}	10.0±0.0^{a}
		5	43.91±0.3^{b}	20.3±0.0^{b}	9.79±0.0^{bcd}	10.0±0.0^{a}
		10	43.66±0.41^{b}	20.58±0.0^{a}	9.89±0.0^{abc}	10.0±0.0^{a}
	Breed* storage period		44.33±0.24	20.3±0.0	9.72±0.0	10.0±0.0
	14	0	46.11± 0.4^{a}	19.72±0.0^{d}	9.85±0.0 ^{abc}	9.97±0.0^{ab}
		5	44. 0±0.4^{b}	20.08±0.0^{c}	9.81±0.0^{abcd}	9.99±0.0^{a}
		10	41.18±0.4^{c}	20.36±0.0^{b}	9.93±0.0^{a}	10.0±0.0^{a}
	Breed* storage period		43.76±0.2	20.05±0.0	9.86±0.0	10.0±0.0
Overall			44.05±0.1	20.18±0.02	9.79±0.0	9.99±.00
Fayoumi	7	0	30.06±0.3^{d}	17.54±0.0^{gh}	9.7±0.0^{d}	10.0±0.0^{a}
		5	29.53±0.3^{de}	17.8±0.0^{ef}	9.9±0.0^{ab}	10.0±0.0^{a}
		10	28.61±0.3^{e}	17.55±0.0^{ef}	9.77±0.0^{cd}	9.95±0.0^{bc}
	Breed* storage period		29.4±0.2	17.63±0.0^{gh}	9.79±0.0	9.98±0.0
	14	0	30.35±0.3^{d}	17.38±0.0^{h}	9.77±0.0^{cd}	9.93±0.0^{c}
		5	29.65±0.3^{de}	17.69±0.0^{gf}	9.84±0.0^{abc}	9.98±0.0^{ab}
		10	28.68±0.3^{e}	17.9±0.0^{e}	9.87±0.0^{abc}	10.0±0.0^{a}
	Breed* storage period		29.56±0.2	17.67±0.0	9.83±0.0	9.97±0.0
Overall			29.48±0.1	17.65±0.0	9.81± 0.0	9.97±.00
MSE			2.99	0.49	0.31	0.10
R2			0.86	0.87	0.11	0.04
Source of variation, P value						
Breed			0.001	0.001	0.437	0.030
Storage period			0.357	0.006	0.0002	0.149
SPIDES time			0.001	0.001	0.001	0.203
Breed* storage period * SPIDES time interaction			0.001	0.001	0.001	0.003

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

The body weights at 35 days old also, displayed variations because of the chicken breeds under study and the time of frequent incubation during storage (P<0.05) without noticeable effect for the duration of storage period (P>0.05). The Avian-34 birds showed significant higher body weights in all SPIDES conditions compared to the Fayoumi ones. Among the Avian-34, the birds hatched from eggs stored for 14 days and exposed to 5 hours frequent incubation during storage displayed the highest body weights followed by those hatched from eggs stored for 7 and 14 days and exposed to (5h) or (0h) frequent incubation during storage, respectively. Whereas among Fayoumi, birds hatched from different SPIDES conditions showed similar weights. These variations in body weights were linked with significant differences in total body gains between the two chicken breeds, Avain-34 and Fayoumi without any effect for the duration of the storage period and the different SPIDES. In this context, all Avain-34 birds exhibited significant higher body gains compared to the Fayoumi ones (P<0.05). While within the Avain-34 and Fayoumi birds did not display any differences among different SPIDES conditions (P>0.05). Improving post hatching body weights and gain in response to applied SPIDES during egg storage might be correlated with the variations in the thyroid hormone (T3 and T4) in the new hatched chicks that influencing its post-hatching growth and metabolic rates [37]. In addition, it might be linked with increasing the hatching weights of chicks hatched from eggs stored for long time (14 days) without SPIDES exposure due to the quality of day-old chick has a linear relationship and considered as relatively good indicator of broiler performance

The differences in body weights and gains were associated with changes in feed intake and FCR between different applied SPIDES and chicken breeds. In general, Avian-34 birds consumed more food compared with the Fayoumi ones (P<0.05). Among Avain-34, birds hatched from eggs stored for 7 days and incubated without pre-incubation warming consumed more food compared with their contemporaries. However, increasing the egg's storage period to 14 days significantly altered the birds' subsequent feed intake which changed with the application of pre-incubation warming. In this regard, the direct incubation without warming after

14 days storage decreased birds' feed intake while increased with the pre-incubation warming for 5 hours. Nevertheless, increasing the pre-incubation warming to 10 hours significantly lowered the bird's subsequent feed intake. For Fayoumi birds, storing eggs for 14 days with 5 hours pre-incubation warming resulted in higher feed intake of the hatched birds compared with the other Fayoumi birds. Whereas the lowest feed intake was reported for those hatched from eggs stored for 14 days and warmed for 10 hours before incubation. The reported variations in feed intake and body gains obviously correlated with changes in the FCR. Again, the Avain-34 displayed better FCR compared with Fayoumi birds (P<0.05). These variations in feed intake and FCR also, might be attributed to the duration of egg preservation and the frequent bursts of incubation during storage. The improved weight gain and FCR as a result of SPIDES agreed with the findings of Yousaf et at [39] who recommended that SPIDES is a beneficial regimen enhances the post-hatch performance of broilers. Again the different responses between the Avian-34 and Fayoumi chicks might be due to the genetic variations between the two breeds [24].

TABLE 4. Post-hatching performance of Fayoumi and Avain-34 following different SPIDES conditions

Breed	Storage period (days)	SPIDES time(h)	BW0 (g)	Chick's body temp. (oC)	BW35 (g)	Total body gain (g)	Feed intake (g)	FCR
AV-34	7	0	45.41±0.0^{b}	39.28±0.1^{b}	1983.26±29.7^{b}	1898.19±34.39^{a}	3039.55±0.0^{a}	1.53±0.0^{h}
		5	43.91±0.0^{d}	38.98±0.0^{cd}	1863.33±26.0^{c}	1819.42±31.39^{a}	2882.00±0.0^{e}	1.54±0.0^{g}
		10	43.66±0.0^{e}	38.8±0.1^{de}	1954.63±33.3^{b}	1889.73±32.49^{a}	2978.79±0.0^{c}	1.52±0.0^{i}
	Breed* storage period		44.33±0.34	39.02±0.0	1928.63±17.5	1866.87±26.03	2966.78±29.01	1.53±0.00
	14	0	46.11±0.0^{a}	39.3± 0.0^{b}	1971.9±27.4^{b}	1927.90±31.93^{a}	2920.50±0.0^{d}	1.48±0.0^{j}
		5	44.00±0.0^{c}	39.4±0.1^{b}	2057.88±32.4^{a}	1953.44±33.72^{a}	3016.39±0.0^{b}	1.46±0.0^{k}
		10	41.18±0.0^{f}	38.76±0.0^{de}	1826.59±35.0^{c}	1782.92±36.66^{a}	2598.54±0.0^{f}	1.42±0.0l
	Breed* storage period		43.76±0.9	39.15±0.0	1959.42±19.4	1895.10±27.44	2845.14±79.92	1.45±0.01
overall			44.05±0.4	39.08±0.0	1943.73±13.0	1880.45±18.86	2905.96±44.49	1.49±0.01
F	7	0	30.06±0.0^{g}	38.94±0.1^{cd}	433.62± 6.4^{d}	401.44±12.90^{b}	990.17±0.0h	2.28±0.0^{b}
		5	29.53±0.0^{j}	38.72±0.1^{de}	425.17±9.7^{d}	395.64±13.87^{b}	944.53±0.0^{j}	2.22±0.0^{c}
		10	28.61±0.0^{l}	38.62±0.0^{e}	430.5±6.6^{d}	401.89±9.54^{b}	907.00±0.0^{k}	2.10±0.0^{e}
	Breed* storage period		29.40±0.26	38.76±0.0	430.67±4.3	399.66±6.10	947.23±15.20	2.20±0.03
	14	0	30.35±0.0^{g}	39.78±0.1^{a}	449.33±10.5^{d}	418.98±15.06^{b}	955.33±0.0^{i}	2.12±0.0^{d}
		5	29.65±0.0^{i}	39.12±0.0^{bc}	401.2±8.8^{d}	371.55±12.67^{b}	995.46±0.0^{g}	2.44±0.0
		10	28.68±0.0^{k}	38.96±0.1^{cd}	396.9±9.7^{d}	368.22±14.03^{b}	766.43±0.0^{l}	1.93±0.0^{a}
	Breed* storage period			39.28±0.0	419.01±6.0	389.35±8.58	905.74±44.65	2.16±0.09 f
Overall			29.48±0.19	39.02± 0.0	426.11±3.5	394.94±5.46	926.49±23.34	2.18± 0.04
MSE			0	0.31	156.18	42.31	0	0
R2			1.00	0.53	0.95	0.951	1.0	1.0
Source of variation, P value								
Breed			0.001	0.2744	0.001	<0.001	0.001	0.001
storage period			0.001	0.001	0.8555	0.885	0.001	0.001
SPIDES time			0.001	0.001	0.0007	0.099	0.001	0.001
Breed* storage period * SPIDES time interaction			0.001	0.002	0.001	0.031	0.001	0.001

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

The effect of different applied SPIDES conditions extended to the levels of biochemical where different responses were recorded in the two studied breeds and following the different SPIDES (**Table 5&6**). In this regard, directly after hatching at (0day old), the significant highest glucose level was measured for Avian-34 chicks hatched from eggs stored for 7 days and directly incubated without pre-incubation warming (P<0.05). While Avian-34 chicks hatched from eggs stored for 7 days and exposed to 5h pre-incubation warming or stored for 14 days and directly incubated as well as the Fayoumi chicks hatched from eggs directly incubated without pre-incubation warming either stored for 7 or 14 days displayed less levels (P<0.05). The lowest glucose levels were noticed in the case of Avain-34' and Fayoumi' chicks hatched from eggs stored for 14days and warmed for 5h or 10h, respectively before incubation (P<0.05). These variations in the glucose levels following different storage period and the applied SPIDES conditions are possibly correlated with the differences in the carbohydrates contents (glycogen) in different embryonic organs and the rate of gluconeogenesis [36]. In this regard, the prolonged egg storage results in prolonged incubation period which increases the oxygen consumption and increases in the glucose demand by the embryos to survive [40]. For other assessed biochemical parameters such as GOT, GPT, ALB, total protein, and cholesterol, no significant effects were reported for the studied chicken breed (P>0.05). Whereas marked differences were noted for the different SPIDES conditions (storage and pre-incubation warming) at the levels of GOT, ALB, total protein, and cholesterol (P<0.05). The highest GOT levels were reported for both Avain-34 and Fayoumi chicks hatched from eggs stored for 7 days and warmed for 0, 5, and 10h before incubation. While storing the eggs for 14 days decreased its levels. However, increasing the storage time to 14days with 0, 5, and 10 pre-incubation warring significantly lowered the GOT levels (P<0.05). For ALB, only the duration of the pre-incubation warming significantly altered its levels (P<0.05). The highest levels were recorded for both Avain-34 and Fayoumi chicks hatched from eggs stored for either 7 or 14 days and warmed for 10 hours before incubation (P<0.05). Whereas incubating eggs without pre-incubation warming significantly decreased its levels (P<0.05). For total protein levels, the highest levels were measured in Avain-34 and Fayoumi chicks hatched from eggs warmed for 10h either stored for 7days or 14days, respectively. The lowest levels were associated with the absence of the pre-incubation warming (0h) or warming for 5h only in Avain-34 and Fayoumi. The cholesterol levels did show any significant changes between the studied chicken, storage time and the duration of the pre-incubation warming (P>0.05). The variations in these biochemical parameters might be linked with the differences in the growth rate of the embryos among different storage period and the applied SPIDES conditions.

TABLE 5. Post-hatching biochemical profile of Fayoumi and Avain-34 following different SPIDES conditions

Breed	Storage period (days)	SPIDES time(h)	Glucose (g/dL)	GPT (U/L)	GOT (U/L)	ALB (U/L)	Total protein (g/dL)	Creatinine (mg/dl)
AV-34	7	0	197.66±2.08^{a}	4.50±0.48^{bcd}	150.00±12.00^{a}	0.96±0.04^{e}	1.04±0.08^{abcd}	0.39±0.010^{ab}
		5	178.00±2.02^{bc}	5.16±0.13^{abc}	162.33±6.93^{a}	1.25±0.02^{bc}	1.22±0.01^{ab}	0.39±0.00^{ab}
		10	153.33±6.58^{de}	5.43±0.16^{ab}	165.33±7.25^{a}	1.41±0.09^{ab}	1.30±0.08^{a}	0.44±0.01^{ab}
	Breed* storage period		176.33±4.23	5.03±0.18	159.22±5.17	1.20±0.05	1.19±0.04	0.40±.00
	14	0	177.33±3.24^{bc}	5.00±0.30^{abc}	10.30±0.80^{c}	0.95±0.03^{e}	0.63±0.05^{e}	0.43±0.01^{ab}
		5	132.66±3.52^{f}	3.10±0.23^{d}	10.10±1.17^{c}	0.03±0.69^{cd}	0.69±0.04^{de}	0.40±0.02^{ab}
		10	161.33±7.37^{cde}	4.00±0.87^{bcd}	18.10±3.50^{c}	1.52±0.06^{a}	1.23±0.06^{ab}	0.40±0.01^{ab}
	Breed* storage period		157.11±4.58	4.03±0.34	12.83±1.39	1.21±0.05	0.85±0.06	0.41±0.011
Overall			166.72±3.36	4.53±0.20	86.02±10.39	1.21±0.03	1.02±0.04	0.41±0.00
Fayoumi	7	0	180.66± 4.20^{b}	3.80±0.52^{cd}	127.66±10.04^{b}	1.13±0.05^{cde}	0.88±0.08^{bcde}	0.46±0.01^{a}
		5	168.66±4.51^{cde}	4.00±0.24^{bcd}	161.66±8.57^{a}	1.44±0.03^{ab}	1.27±0.09^{ab}	0.45±0.02^{a}
		10	151.66±0.92^{e}	4.46±0.03^{bcd}	170.00±10.25^{a}	1.32±0.04^{bc}	1.20±0.03^{ab}	0.37±0.01^{b}
	Breed* storage period		167.00±3.07	4.08±0.19	153.11±6.44	1.29±0.03	1.11±0.05	0.42±0.01
	14	0	170.00±9.46^{bcd}	6.20±0.77^{a}	4.63±0.50^{c}	1.05±0.07^{de}	1.12±0.10^{abc}	0.41±0.00^{ab}
		5	155.00±0.00^{ed}	6.10±0.00^{a}	12.60±0.00^{c}	1.02±0.00^{e}	0.80±0.00^{cde}	0.37±0.00^{b}
		10	121.33±7.33^{f}	5.36±0.21^{ab}	11.76±1.98^{c}	1.43±0.08^{ab}	1.40±0.32^{a}	0.43±0.03^{ab}
	Breed* storage period		147.00±7.10	5.82±0.34	8.82±1.17	1.21±0.06	1.19±0.14	0.41±0.01
Overall			158.25±3.79	4.85±0.22	89.98±11.05	1.25±0.03	1.15±0.07	0.42±0.01
MSE			15.78607	1.319427	20.83076	0.176968	0.356517	0.058765
R2			0.664100	0.331536	0.933306	0.572015	0.345899	0.202734
Source of variation, P value								
Breed			0.0085	0.1002	0.2869	0.6089	0.2111	0.5473
Storage period			0.001	0.1482	0.001	0.1129	0.0216	0.0913
SPIDES time			0.001	0.7045	0.0012	0..001	0.001	0.0698
Breed* storage period * SPIDES time interaction			0.125	0.221	0.214	0.321	0.415	0.091

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

TABLE 6. Lipid profile of Fayoumi and Avain-34 following different SPIDES conditions

Breed	Storage period (days)	SPIDES time(h)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total lipid (mg/dl)	CHO (mg/dl)
AV-34	7	0	82.66±8.23^{bc}	33.100±5.78^{a}	266.00±62.25^{b}	739.67±125.30^{c}	316.00±58.57^{cd}
		5	59.66±7.35^{ed}	21.26±2.16^{b}	289.00±25.75^{b}	729.33±65.27^{c}	322.33±28.96^{cd}
		10	46.00±3.17^{e}	17.20±2.95^{bc}	429.33±20.19^{a}	983.00±40.83^{ab}	456.00±21.46^{a}
	Breed* storage period		62.77±4.72	23.85±2.56	328.11±26.60	817.33±52.40	364.77±25.40
	14	0	81.66±4.20^{bc}	3.03±0.34^{de}	325.66±13.42^{ab}	797.33±23.59^{bc}	345.33±13.14^{bcd}
		5	81.33±1.76^{bc}	17.43±3.33^{bc}	287.00±13.05^{b}	748.33±22.70^{c}	321.00±10.61^{cd}
		10	78.66±1.92^{bcd}	19.10±6.44^{bc}	351.66±43.67^{ab}	877.67±73.24^{abc}	386.66±37.17^{abcd}
	Breed* storage period		80.55±1.60	13.18±2.72^{bc}	321.44±16.08	807.77±27.74	351.00±14.11
Overall			71.66±2.75	18.52±1.99	324.77±15.40	812.55±29.37	357.88±14.42
Fayoumi	7	0	87.33±0.44^{bc}	0.65±0.19^{e}	268.66±11.91^{b}	686.33±23.58^{c}	287.00±11.75^{d}
		5	73.66±4.91^{cd}	3.44±0.74^{de}	325.66±13.62^{ab}	786.00±34.00^{bc}	343.66±14.58^{bcd}
		10	47.66±2.68^{e}	12.56±4.00^{bcd}	337.66±24.15^{ab}	792.00±48.24^{bc}	359.66±23.06^{abcd}
	Breed* storage period		69.55±3.69	5.55±1.64	310.66±11.32	754.77± 22.46	330.11±11.31
	14	0	164.00±13.51^{a}	3.73±0.58^{de}	379.00±44.61^{ab}	1020.33±81.57^{a}	415.66±43.63^{abc}
		5	95.00±0.00^{b}	7.60±0.00^{de}	422.00±0.00^{a}	1018.00±0.00^{a}	449.00±0.00^{ab}
		10	86.66±5.36^{bc}	12.80±3.37^{bcd}	331.00±34.25^{ab}	833.67±74.47^{abc}	361.00±34.59^{abcd}
	Breed* storage period		121.00±10.29	8.17±1.70	364.57±24.38	940.00±50.15	397.00±24.21
Overall			92.06±6.15	6.70±1.19	334.25±12.87	835.81±28.37	359.37±13.12
MSE			17.87550	10.12032	94.99088	188.2682	91.13141
R2			0.755613	0.490398	0.230557	0.258045	0.240732
Source of variation, P-value							
Breed			0.001	0.001	0.3329	0.2693	0.5473
Storage period			0.001	0.0550	0.1322	0.0156	0.0913
SPIDES time			0.001	0.0907	0.0671	0.3560	0.0698
Breed* storage period * SPIDES time interaction			0.324	0.142	0.741	0.275	0.542

TG= triglycerides, HDL=High density lipoprotein, LDL=Low density lipoprotein CHO= cholesterol. Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

After 35 days old (**Table 7&8**), the chicks hatched form eggs stored for 7 days (in Avian-34 and Fayoumi) or 14 (in Fayoumi) and directly incubated exhibited the highest glucose levels (P<0.05). Whereas the lowest glucose levels continued to be associated with increasing the storage period (14 days) with 5 and 10 h pre-incubation warming. The lowering the glucose levels with increasing egg storage may be correlated with depression in the messenger RNA (mRNA) level of the transporter Na-dependent glucose (responsible for glucose absorption) as a result of the long-term egg storage [41]. The GOT and GPT highest levels were measured for Avain-34' chicks hatched from eggs stored for 7 days and warmed for 5 and 10 h before incubation. Whereas increasing storage period with 0, 5, or 10 h pre-incubation warming lowered their levels. In general, Fayoumi birds had less levels of GOT and GPT compared to Avain-34 birds (P<0.05). For ALB and total protein levels, the Fayoumi birds showed higher levels than the Avain-34' bird. Among Fayoumi birds, those hatched from eggs stored for 7 days and exposed to 5 and 10 h pre-incubation warming displayed the highest levels (P<0.05). Whereas increasing eggs' storage even with or without warming lowered the ALB and total protein levels in the two studied chicken breed. Creatinine levels were also, similarly altered in response to the different storage periods and short time of incubation during storage in both Fayoumi and Avain-34 chicken. Avain-34 birds emerged from eggs stored for 7 days and directly incubated without warming during storage had the highest creatinine levels (P<0.05) compared with the other birds. Increasing egg storage period to 14 days with applying short times of SPIDES during storage for 5 and 10 h significantly lowered the creatinine levels in Fayoumi and Avain-34 birds.

For cholesterol, storing eggs for 14 days with and without warming before incubation was linked with significant reduction in the cholesterol levels in both Avain-34 and Fayoumi birds. Again, these different biochemical parameters might be linked with the differences in the growth rate of the embryos among different storage period and the applied SPIDES conditions.

TABLE 7. biochemical profile of Fayoumi and Avain-34 following different SPIDES conditions at 35-days old.

Breed	Storage period (days)	SPIDES time(h)	Glucose (g/dL)	GPT (U/L)	GOT (U/L)	ALB (U/L)	Total protein (g/dL)	Creatinine (mg/dl)
AV-34	7	0	122.33±1.20^{ab}	14.00±0.28^{cdef}	294.33±12.22^{cd}	0.90±0.05^{ef}	1.00±0.05^{ef}	0.50±0.02^{a}
		5	122.66±3.75^{ab}	16.33±0.60^{a}	516.66±52.88^{a}	1.23±0.03^{cd}	1.19±0.01^{d}	0.28±0.01^{cd}
		10	99.33±3.38^{cd}	16.00±0.28^{ab}	578.00±29.59^{a}	1.23±0.07^{cd}	1.23±0.02^{cd}	0.35±0.01^{bc}
	Breed* storage period		114.77±2.71	15.44±0.30	463±31.03	1.12±0.04	1.14±0.02	0.37±0.02
	14	0	70.33±4.00^{g}	14.33±0.44^{cde}	421.66±44.75^{b}	0.73±0.01^{g}	1.00±0.08^{ef}	0.40±0.03^{b}
		5	80.33±4.60^{efg}	13.66±0.16^{cdef}	329.00±40.46^{c}	0.76±0.04^{fg}	0.95±0.06^{f}	0.25±0.01^{d}
		10	108.33±5.18^{bc}	15.00±0.28^{bc}	279.00±40.32^{cde}	1.23±0.06^{cd}	1.18±0.07^{d}	0.30±0.03^{cd}
	Breed* storage period		86.33±4.06	14.33±0.20	343.22±25.97	0.91±0.05	1.04±0.04	0.31±0.02
Overall			100.55±3.11	14.88±0.19	403.11±21.66	1.01±0.03	1.09± 0.02	0.34±0.01
Fayoumi	7	0	134.00±7.97^{a}	14.66±1.09^{cd}	193.33± 6.41^{e}	1.26±0.01^{cd}	1.71±0.01^{a}	0.34±0.01^{bc}
		5	101.00±7.02^{cd}	14.33±0.16^{cde}	231.00±5.48^{de}	1.56±0.03^{a}	1.74±0.04^{a}	0.30±0.02^{cd}
		10	96.00±2.92^{cde}	13.33±0.16^{def}	226.00±6.17^{de}	1.46±0.01^{ab}	1.62±0.02^{a}	0.31±0.02^{cd}
	Breed* storage period		110.33±4.83	14.11±0.37	216.77±4.68	1.43±0.02	1.69±0.02	0.32±0.01
	14	0	118.66±5.44^{ab}	12.66±0.44^{f}	210.66±22.53^{de}	1.13±0.08^{d}	0.32±0.02^{bcd}	0.32±0.01
		5	77.66± 7.37^{fg}	14.00±0.28^{cdef}	217.33±7.88^{de}	0.93±0.04^{e}	0.33±0.03^{bcd}	0.32±0.02^{bcd}
		10	87.66±8.19^{def}	13.00±0.28^{ef}	193.66±5.62^{e}	1.33±0.04^{bc}	0.31±0.02^{cd}	0.33±0.03^{bcd}
	Breed* storage period		94.66±5.21	13.22±0.22	207.22±8.09	1.13±0.04	1.31±0.03	0.31±0.02^{cd}
Overall			102.50±3.68	13.66±0.22	212.00±4.67	1.28±0.03	1.50±0.03	0.32±0.00
MSE			16.51704	1.354006	85.72862	0.142887	13.80444	0.701444
R2			0.606951	0.408735	0.705054	0.780022	0.701444	13.80444
Source of variation, P-value								
Breed			0.5422	0.001	0.001	0.001	0.001	0.6615
Storage period			0.001	0.0002	0.0002	0.001	0.001	0.001
SPIDES time			0.0001	0.1134	0.0650	0.001	0.001	0.0018
Breed* storage period * SPIDES time interaction			0.258	0.217	0.324	0.841	0.742	0.541

Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

TABLE 8.Lipid profile of Fayoumi and Avain-34 following different SPIDES conditions at 35-days old

Breed	Storage period (days)	SPIDES time(h)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total lipid (mg/dl)	CHO (mg/dl)
AV-34	7	0	132.66±7.74^{b}	411.66±19.48^{a}	41.66±1.16^{cde}	31.33±1.85^{bc}	99.00±3.61^{c}
		5	113.33±4.34^{c}	415.33±17.94^{a}	59.33±1.87^{a}	39.33±2.20^{ab}	121.00±2.56^{ab}
		10	132.33±6.72^{b}	378.33±7.09^{a}	40.66±1.64^{de}	29.66±0.88^{bc}	96.33±2.16^{c}
	Breed* storage period		126.11±3.98	401.77±9.36	47.22±1.89	33.44±1.27	105.44±2.68
	14	0	99.33±6.80^{cd}	277.66±13.54^{c}	36.33 ±1.64^{ef}	21.00 ± 1.80^{c}	76.66±4.04^{d}
		5	89.00±0.28^{d}	255.33±11.84^{c}	50.66±1.69^{b}	2.66±7.65e	70.66±5.94^{d}
		10	110.00±3.78^{c}	323.00±22.14^{b}	48.33±2.04^{bc}	24.00±12.04^{bc}	94.00±10.25^{c}
	Breed* storage period		99.44±3.01	285.33±10.67	45.11±1.58	15.88±4.96	80.44±4.45
Overall			112.77±3.07	343.55±10.65	46.16±1.23	24.66± 2.80	92.94±3.09
Fayoumi	7	0	165.33±8.57^{a}	391.00±18.33^{a}	49.00±0.57^{bc}	18.66±3.84^{cde}	100.33±5.16^{c}
		5	148.00±7.11^{ab}	374.33±15.92^{a}	51.33±5.73^{b}	49.33±3.89^{a}	130.00±4.19^{a}
		10	166.33±12.17^{a}	416.00±13.20^{a}	44.00±1.60^{bcd}	39.00±1.73^{ab}	116.00±0.57^{b}
	Breed* storage period		159.88±5.53	393.77±9.46	48.11±2.00	35.66±3.10	115.44±3.19
	14	0	97.33±2.02^{cd}	265.66±8.74^{c}	48.33±1.36^{bc}	4.33±4.34^{de}	71.66±3.56^{d}
		5	110.33±3.94^{c}	280.66±6.00^{c}	30.66±0.44^{f}	20.33±1.92^{cd}	72.66±1.58^{d}
		10	100.00±3.75^{cd}	273.00±9.83^{c}	43.00±3.05^{cde}	4.00±8.67^{de}	74.00±3.61^{d}
	Breed* storage period		102.5±2.16	273.11±4.78	40.66±1.80	9.55± 3.50	72.77±1.71
Overall			131.22±4.91	333.44±9.81	44.38±1.43	22.61±2.93	94.11±3.43
MSE			19.18604	43.64177	6.955214	16.12581	13.80444
R2			0.664884	0.696793	0.549898	0.471940	0.701444
Source of variation, P-value							
Breed			0.001	0.2316	0.1873	0.5093	0.6615
Storage period			0.001	0.001	0.0006	0.001	0.001
SPIDES time			0.0277	0.2795	0.0188	0.0607	0.0018
Breed* storage period * SPIDES time interaction			0.324	0.741	0.412	0.654	0.341

TG= triglycerides, HDL=High density lipoprotein, LDL=Low density lipoprotein, CHO= cholesterol. Data are presented as means ± SEM. Different letters represent statistical differences (P < 0.05).

TABLE 9. Blood picture of Fayoumi and Avain-34 following different SPIDES conditions at 35-days old

Breed	Storage period (days)	SPIDES time(h)	HB (g/dL)	RBCS(X10-6)	WBCS(X10-3)	Heterophils	Lymphocytes	Monocytes
AV-34	7	0	94.66±2.16d	2.24±0.06cd	106.46±1.01ab	18.33±3.80a	57.22±3.32bc	28.78±0.99a
		5	88.33±0.72e	2.13±0.01d	98.60±0.37bc	13.18±2.71b	63.34±4.55b	20.09± 2.13b
		10	96.00±0.00d	2.33±0.03c	71.60±3.24f	6.93±1.05cd	47.92±1.42cde	15.74±1.74a
	Breed* storage period		93.00±0.98	2.23±0.02	92.22±3.12	12.81± 1.78	56.16±2.24	21.54±1.41
	14	0	93.33±2.72de	2.32±0.09c	66.00±5.32f	8.77±1.51bcd	41.34±6.02de	14.56±0.56bc
		5	93.00±1.04de	2.23±0.03cd	57.03±0.93g	4.99±0.68d	38.92±1.51e	12.14±1.55c
		10	104.33±1.83c	2.66±0.04a	82.06±4.00de	6.62± 1.64cd	58.47±2.48bc	15.42±1.75bc
	Breed* storage period		96.88±1.51	2.40±0.05	68.36±2.96	6.79±0.80	46.24±2.73	14.04±0.82
Overall			94.94±0.93	2.32±0.03	80.29±2.68	9.80±1.05	51.20± 1.87	17.79±0.96
Fayoumi	7	0	105.66±0.88c	2.49±0.02b	113.96±0.60a	11.80±1.05bc	81.48±4.07a	18.39±3.92bc
		5	111.33±3.17b	2.75±0.07a	108.23±1.68a	10.14±0.50bcd	77.66±3.66a	18.26±4.05bc
		10	112.00±2.29ab	2.68±0.01a	95.70±3.40c	8.54± 1.33bcd	57.27±1.95bc	27.96±0.78a
	Breed* storage period		109.66±1.40	2.64±0.03	105.96±1.93	10.16±0.62	72.14±2.79	21.53±2.03
	14	0	117.33±0.33a	2.76±0.04a	83.86±5.69d	5.78±0.80d	61.80±4.50b	15.23±2.41bc
		5	113.33± 2.16ab	2.73±0.04a	75.60±3.82def	7.41±0.76cd	54.46±4.13bc	12.50±1.55c
		10	113.66±1.42ab	2.73±0.04a	73.06±1.09ef	9.10±1.53bcd	49.98±1.74cb	13.01±1.05c
	Breed* storage period		114.77±0.90	2.74±0.02	77.51±2.40	7.43±0.66	55.41±2.24	13.58±1.00
Overall			112.22±0.89	2.69±0.02	91.73±2.48	8.80±0.48	63.77±2.11	17.56±1.2
MSE			5.482928	0.152596	9.488754	5.134362	10.70787	6.500099
R2			0.775588	0.717592	0.793485	0.350469	0.595274	0.429583
Source of variation, P value								
Breed			0.001	0.001	0.001	0.3115	0.001	0.8538
Storage period			0.006	0.001	0.001	0.0211	0.0180	0.0738
SPIDES time			0.001	0.001	0.001	0.001	0.001	0.001
Breed* storage period * SPIDES time interaction			0.547	0.674	0.178	0.278	0.317	0.498

HB= haemoglobin. Data are presented as means ± SEM. Different letters represent statistical differences ($P < 0.05$).

At 35-days old post hatching, blood picture (Table 9) was also, modulated in response to the different applied SPIDES conditions and studied chicken strains. For example: Fayoumi birds exhibited higher HB% in all applied SPIDES conditions compared to Avain-34 ($P<0.05$). Among Fayoumi birds, the highest HB% was in the case of birds hatched from eggs stored for 14 days and directly incubated without pre-incubation warming. Whereas the lowest HB% was in the case of birds hatched from eggs stored for 7 days and also, directly incubated without warming. Among Avain-34 birds, the highest HB% was measured for birds hatched from eggs stored for 14 days and warmed for 10h before incubation. While the lowest HB% was found in the case of birds hatched from eggs stored for 7 days and exposed to 5 h warming before incubation. For RBCs count, Fayoumi birds also, had higher RBCs counts in all the applied SPIDES conditions compared with the Avain-34 birds ($P<0.05$). Among Fayoumi birds hatched from eggs stored for 7 or 14 days and subjected to pre-incubation warming for 5 or 10 h displayed higher RBCs counts compared to those hatched from eggs stored for 7 days and directly incubated without pre-warming. Whereas among Avian-34, birds hatched form eggs stored for 14 days and exposed to 10h warming before incubation had the highest RBCs count similar to Fayoumi ($P>0.05$). Whereas other SPIDES conditions resulted in lower RBCs counts with the lowest counts found in the case of birds hatched from eggs stored for 7 days and either directly incubated or exposed to 5h pre-incubation warming. For WBCs counts, the highest counts were found in the case of both Fayoumi and Avain-34 birds hatched from eggs stored for 7 days and directly incubated or exposed to 5h warming before incubation. While increasing both storage time (to 14 days) and pre-incubation heating to 5 or 10 h were associated with less WBCs counts in both Fayoumi and Avain-34 birds. For heterophils count both Fayoumi and Avian-34 birds showed similar responses ($P>0.05$). The highest counts were found for Avian-34 birds hatched from eggs without SPIDES conditions application. However, all the applied SPIDES conditions, in both Fayoumi and Avian-34, were linked with lowering of heterophils counts. Where the more the increase of storage times and pre-incubation warming duration, the lower the heterophils counts. On the other side, the lymphocytes count in Fayoumi was higher than

Avain-34 in most of applied SPIDES conditions. The highest counts were reported in the case of Fayoumi birds hatched from eggs stored for 7 days and exposed to 0 or 5 h warming before incubation. In poultry, haematological indices are good indicator on body condition and the physiological and pathological status of birds [42]. Accordingly, the alterations in the Avain-34 and Fayoumi blood parameters in response to increasing egg storage period and the applying SPIDES might be ascertain the significance of the duration of egg storage and SPIDES on post-hatching birds' health. In this context, lowering the heterophils count and increasing the lymphocytic count in responding to the applied SPIDES perhaps establish the significant effects of short periods of warming during egg storage on birds immunity and stress response. As described by Ebeid *et al.* [37] who concluded significant enhancements of birds' antioxidant status and humoral immunity as a result of frequent short bursts of incubation during storage.

Conclusion

In summary, applying subsequent repeated short periods of warming (SPIDES) every three days during storage period of eggs resulted in decreasing the weights of stored eggs due to increasing the egg weight losses. However, distinct improvements of hatchability % in both Fayoumi, and Avian-34 breeds were documented following the exposure to repeated SPIDES for 0 and 5h every three days. Additionally, distinct enhancements of post-hatching performance were reported despite of the SPIDES-associated decreases of post-hatching weights (W0). Chicks hatched from eggs subjected to repeated bursts of incubation during storage (SPIDES) interestingly succeeded to attain significant heavier body weights at 35 days old. The best post-hatching growth performance was reported for chicks hatched from eggs exposed to 5h of successive warming every three days during storage. Therefore, frequent repeated warming for 5h during egg storage is recommended for improving hatchability and post-hatching performance.

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سلالة الدجاج وفترات الحضانة القصيرة المتكررة خلال فترات تخزين البيض المختلفة (SPIDES) تغيير نسبة الفقس، جودة الكتكوت وأداء الطيور بعد الفقس

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هدفت الدراسة الحالية إلى دراسة تأثير تعرض بيض التفريخ الى مدد قصيرة متتالية من التسخين لمدة 0 و 5 و 10 ساعات (SPIDES) خلال فترات تخزين البيض (7 و 14 يوما) على نسبة الفقس وجودة الكتكوت وأداء الطيور بعد الفقس بين سلالتين من الدجاج ، الفيومي و Avian-43. وفقا لذلك ، تم توزيع 6300 بيضة مخصبة (3150 بيضة من كل سلالة) بشكل عشوائي على تصميم تجريبي x2x32 (سلالتان ، فترتان تخزين ، و 3 مدد تسخين) مع 5 مكررات / معاملة. تم تسخين البيض بشكل متكرر كل 3 أيام لمدة 0 و 5 و 10 ساعات (SPIDES). أشارت النتائج الرئيسية إلى انخفاض كبير في أوزان البيض وزيادة فقدان وزن البيض مع زيادة تخزينه ومدد التسخين أثناء التخزين. لوحظ أعلى فقد في وزن البيض لبيض الفيومي المخزن لمدة 7 أو 14 يوما والذي تعرض ل SPIDES المتكرر لمدة 10 ساعات وكذلك بيض Avian-43 المخزن لمدة 7 أيام وتسخينه لمدة 10 ساعات. تم تسجيل تحسن ملحوظ في نسبة الفقس في كل من سلالات الفيومي و Avian-43 بعد التعرض المتكرر ل SPIDES لمدة 0 و 5 ساعات. بالإضافة إلى ذلك ، تم رصد تحسن واضح في أداء ما بعد الفقس. تمتلك الكتاكيت التي تفقس من البيض المخزن لفترات أطول والمعرضة ل SPIDES أوزانا أقل بعد الفقس (W0) في Avian-43 و Fayoumi مقارنة بتلك التي تم تحضينها مباشرة دون تسخين أثناء التخزين. ومع ذلك، نجحت تلك الكتاكيت في تحقيق أوزان جسم أثقل بكثير في عمر 35 يوما. و أفضل أداء نمو بعد الفقس للكتاكيت التي تفقس من البيض المعرض ل 5 ساعات من التسخين المتتالي كل ثلاثة أيام أثناء التخزين.