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EFFECT OF PRE-STORAGE HEATING AND PERIOD OF STORAGE ON HATCHABILITY TRAITS OF DOKKI-4 EGGS

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ABSTRACT

This study investigated the effects of pre-storage heating and storage period of hatching eggs on hatchability traits and chick quality of Dokki-4 (Egyptian local strain of chickens) laying hens. A total of 3600 eggs were collected from 46-week-old laying hens. Eggs were distributed in a 3x4 factorial arrangement, with three storage times (4, 8 and 12 days at 18°C and 75% RH) and four heat treatments prior to storage (0, 3, 6 and 9 hours at 37.5°C and 56% RH). Eggs were distributed to twelve treatments of 20 replicates. After storage, eggs were incubated under the normal conditions of incubation at the same time. The results showed that the long storage period increased egg weight loss. Hatchability and chick quality results from 8-12 days stored eggs were lower than eggs stored for 4 days. The 6-hour pre-storage heating system substantially improved egg hatchability and chick quality relative to non-heated or 9-hour heating. Important interactions were observed during pre-storage heating × egg storage time for loss in egg weight, hatchability of total and fertile eggs, embryonic mortality and chick quality. When eggs were stored for more than four days, pre-storage heating of hatching eggs for six hours improved hatchability and chick quality compared to unheated eggs or heated for 9 hours. Conclusively, pre-storage heat treatment beneficially affects hatchability traits and chick quality, especially when hatching eggs are stored for long periods.

Contribution/Originality: This study contributes to existing literature by investigating the effects of pre-storage heating and storage period of hatching eggs on hatchability traits and chick quality of Dokki-4 (Egyptian local strain of chickens) laying hens.

1. INTRODUCTION

Cooling of hatching eggs before incubation is common practice in poultry industry. It is well known that incubation length increases when the storage period is more than seven days [1, 2] and this may had adverse effects on hatchability [3, 4] and chick quality [1, 4]. Furthermore, previous reports have established the adverse effects of long storage period on embryos development and viability, hatching rate [1, 5]. Many publications have shown that storage of hatching eggs for long period decreased egg hatchability in aged breeder hens more than in young breeder hens [4, 5]. It was suggested that the adverse effects of long storage period of eggs could be attributed to the deterioration of egg quality, primarily albumen quality [6]. In this regard, Lapao, et al. [5] found that albumen pH was raised with increasing storage period. Albumen height was also decreased with increasing egg storage

period [5]. Also, Jones and Musgrove [7] yolk sac membrane elasticity decreased as storage time increased. Nahm [8] found that mortality of chicken embryos during storage was strongly related to length of storage. Warming of hatching eggs prior to storage was suggested as a managerial strategy for reducing the negative effects of long storage periods via improving the embryonic development. Fasenko, et al. [3] and Gucbilmez, et al. [9] suggested a correlation between pre-storage heat treatment and improved hatchability of chicken eggs. The same relationship was observed in turkeys [10] and quails [11]. Hamidu, et al. [12]; Hamidu, et al. [13] and Dymond, et al. [14] found that prolonged storage of hatching eggs can cause embryonic stress, leading to increased embryonic mortality, depressed embryonic metabolism, and impaired development. Therefore, it could be assumed that pre-storage incubation might accelerate the embryonic development and enhance the viability of the developing embryos. The present study therefore aimed to investigate the effects of pre-storage heating and storage period of hatching eggs on hatchability and chick quality of Dokki-4 laying hens.

2. MATERIALS AND METHODS

This study was performed at Sakha Research Station, Animal Production Research Institute, Ministry of Agriculture, Egypt.

2.1. Experimental Design and Treatments

In this trial, 3600 hatching eggs were collected from Dokki-4 chicken hens (Egyptian local strain of chickens) at 42 weeks old which maintained under similar environment and management conditions (male: female ratio was 1:10). Light schedule included 16 h of light and 8 h of darkness). The eggs were distributed in a 3 x 4 factorial design, three storage periods (4, 8 and 12 days at 17ºC and 75 % relative humidity) and four incubation times prior to storage (0, 3, 6 and 9 hours at 37.5ºC and 56% relative humidity), summing up 12 treatments with 20 replicates of 15 eggs each. In order to simultaneously incubate all eggs, the eggs were collected in three groups, one group per day, representing the three storage periods (4, 8 and 12 days). The first category therefore corresponded to eggs stored for twelve days; the second to eggs stored for eight days, and the third to eggs stored for 12 days.

2.2. General Management

Immediately after collection, the eggs was fumigated by paraformaldehyde gas [15] through the interaction of 35 ml formalin, 17.5 grams of potassium permanganate and 50 ml of warm water per cubic meter of the volume of evaporation room for 30 minutes then it was ventilated chamber by fresh air through the operation the air fan and opening the windows for about an hour to get rid of the residues of paraformaldehyde gas. All eggs in each group were individually weighed, numbered and randomly distributed into the treatments. The heating timing started when the temperature reached 37.5°C and the relative humidity 56% inside hatchery. After heating, eggs were kept for one hour at room temperature, then eggs stored at 17 ° C in a storage room and 75 percent relative humidity for the periods corresponding to the treatments. After storage, the eggs were kept at 25 ° C and 75% relative humidity for six hours before incubation, then incubated at a temperature of 37.5 °C and relative humidity of 56.5% for 18 days in a Chick Master incubator. After that, eggs were transferred to a Chick Master ® hatcher at 36.6°C temperature and 61.2% (relative humidity).

2.3. Measurements

2.3.1. Egg Weight Loss

Egg weight loss was calculated individually twice at the end of storage period and at day 18 of incubation. Egg weight loss during storage was determined as the difference in egg weight between initial egg weight (at the day of egg collection) and the last day of storage as a percentage of egg weight at collection. Egg weight loss during incubation was determined as the difference in egg weight between the last day of storage and day 18 of incubation

as a percentage of egg weight at the last day of storage. Total egg weight loss was the sum of egg weight loss during storage and incubation.

2.3.2. Embryonic Mortality

At 7 and 14 days of incubation all eggs were candled, and all clear eggs were removed from the trays. At the end of 18 day of incubation, all eggs were candled again and those with evidence of living embryos were transferred from the setter trays to the hatcher trays. [16] categorized the embryonic mortality into three categories: early from days 1 to 7, middle from days 8 to 14 and late from days 15 to 21.

2.3.3. Piped Eggs

The formula referenced by Lourens [17] was applied in determining the rate of Piped eggs:

Rate of Piped eggs % = Number of piped eggs / Number of fertilized eggs*100

2.3.4. Fertility and Hatchability

Fertility was calculated as total fertile eggs as a percentage of total eggs set into the incubator. Hatchability was calculated as a percentage of fertile eggs and total eggs set.

2.3.5. Chick Quality

All the chicks were classified to first grade 'A' and second grade "B.'. A chick was grouped in grade A when it was completely healthy, clean, dry, free of deformities, with bright eyes [4]. The other chicks were classified in grade B. The chicks in both grades were expressed as a percentage of total hatched chicks.

2.3.6. Chick Length

Twenty chicks randomly selected from each treatment for individual measurement of chick length (cm) directly after hatch. The length of the chick was described as the length from the beak tip to the nail implantation on the middle toe [18].

2.4. Statistical Analysis

Program of SAS [19] was used to statistical analysis of the data according to a factorial experiment (3 × 4) applied in a complete randomized design (CRD) to study the effect of three storage periods of eggs and four incubation time prior to storage eggs and their interference in different qualities. The differences between the averages were compared [20].

$Y_{ijk} = \mu + S_i + H_j + S_{ij} + E_{ijk}$

μ = Overall mean.

S_i = Effect of storage period (I=1, 2,3).

H_j= Effect of incubation time prior to storage of eggs (j=1, 2, 3, 4).

S_{ij}= Interaction between storage period and incubation time before storage.

E_{ijk}= Residual error.

3. RESULTS

3.1. Egg Weight Loss (LEW)

In Table 1, mean percentage of LEW during storage, first eighteen days of incubation and total LEW were significantly higher for longer storage period (12 days) compared to the shorter periods (4 and 8 days). In the interaction between storage duration and pre-incubation periods, as pre-incubation periods increased, incremental increase with substantial impact in the percentage of egg weight loss was observed across all the periods studied.

Group of eggs that incubated for 9 hours before 12 days of storage had the highest and most important percentage of egg weight loss during storage, first eighteen days of incubation and total percentage of egg weight loss compared to the other groups and the lowest one with significant value was reported for egg group that was not incubated before storage of 4 days.

Table-1. The effects of storage period, pre-storage incubation and their interaction on fresh egg weight and egg weight loss percentage of Dokki4 eggs.

Treatments	Traits			
	Fresh egg weight (g)	Egg weight loss during storage, %	Egg weight loss after 18 days of incubation, %	Total egg loss, %
Egg storage period (days)				
4	50.42	0.84^{C}	10.57^{C}	11.41^{C}
8	50.45	1.17^{B}	11.62^{B}	12.80^{B}
12	50.50	1.52^{A}	12.51^{A}	14.03^{A}
SEM	0.0562	0.0639	0.2020	0.2593
p-value	0.865	0.0001	0.0001	0.0001
Pre-storage incubation (hours at 37.5°c)				
0	50.50	0.83^{C}	10.73^{C}	11.56^{C}
3	50.43	1.20^{B}	11.84^{AB}	13.04^{B}
6	50.53	1.17^{B}	10.98^{BC}	12.15^{BC}
9	50.36	1.50^{A}	12.73^{A}	14.24^{A}
SEM	0.0562	0.0639	0.2020	0.2593
p-value	0.747	0.001	0.0001	0.0001
Interaction between storage period and pre-storage incubation				
4 days * 0 hours	50.50	0.63^{e}	10.06^{h}	10.69^{g}
4 days * 3 hours	50.30	0.88^{d}	10.34^{gh}	11.22^{fg}
4 days * 6 hours	50.50	0.76^{de}	10.10^{h}	10.86^{fg}
4 days * 9 hours	50.40	1.10^{c}	11.80^{de}	12.90^{cd}
8 days * 0 hours	50.40	0.82^{d}	10.80^{fgh}	11.62^{ef}
8 days * 3 hours	50.50	1.15^{c}	12.06^{cd}	13.21^{c}
8 days * 6 hours	50.50	1.20^{c}	11.05^{efg}	12.25^{de}
8 days * 9 hours	50.40	1.52^{b}	12.60^{bc}	14.12^{b}
12 days * 0 hours	50.60	1.05^{c}	11.33^{def}	12.38^{d}
12 days * 3 hours	50.50	1.58^{b}	13.12^{ab}	14.70^{b}
12 days * 6 hours	50.60	1.56^{b}	11.80^{de}	13.36^{c}
12 days * 9 hours	50.30	1.90^{a}	13.80^{a}	15.70^{a}
SEM	0.0562	0.0639	0.2020	0.2593
p-value	0.996	0.0001	0.0001	0.0001

Note: *Means, within columns, for the main treatment effects or the interaction effects, with no common superscript, differ significantly (P≤0.05). ** SEM = Stander error of the mean.

3.2. Fertility and Hatchability

Table 2 demonstrates the effects of the egg fertility, hatchability of fertile and total eggs. There were no significant effects on the apparent percentage of fertility from the egg storage period, the pre-storage heating duration or the interaction between them. The hatchability of fertile and total eggs was significantly influenced by both the experimental factors and their interaction. Longer egg storage time resulted in a significant linear decrease in the hatchability of fertile and total eggs, irrespective of the length of the preheating. Overall, eggs heated for six hours had substantially higher hatchability of fertile and total eggs relative to non-heated or nine-hour heated eggs. Pre-storage heating of eggs in eggs stored for more than 4 days for six hours substantially improved hatchability (Table 2). During the storage time of four days, eggs heated for nine hours had significantly lower hatchability compared to the non-heated ones. Pre-storage eggs heated for six hours had higher hatchability when stored for four or eight days as compared to eggs stored for 12 days Table 2. The long storage or pre-storage heating eggs didn't affect apparent fertility in the present study.

Table-2. The effects of storage period and pre-storage incubation and their interaction on fertility, hatchability and embryonic mortality of Dokki4 eggs.

Treatments	Traits								
	Fertility, %	Hatchability of total eggs %	Hatchability of fertile eggs %	Embryonic mortality %					
				Early (1-7 days)	Mid (8-14 days)	Late (15-21days)	Piped egg	Rotten egg	Total
Egg storage period (days)									
4	90.37	83.97^{A}	92.77^{A}	2.79^{C}	0.633^{B}	2.10^{C}	0.650^{C}	0.450^{C}	6.62^{C}
8	90.00	80.00^{B}	89.57^{B}	3.97^{B}	0.650^{B}	3.20^{B}	1.050^{B}	0.550^{B}	9.42^{B}
12	90.10	73.10^{C}	81.97^{C}	8.17^{A}	0.733^{A}	4.95^{A}	2.025^{A}	1.375^{A}	17.25^{A}
SEM	0.174	0.858	0.863	0.435	0.014	0.240	0.118	0.101	0.852
p-value	0.675	0.0001	0.0001	0.0001	0.008	0.0001	0.0001	0.0001	0.0001
Pre-storage incubation (hours at 37.5°c)									
0	90.33	78.33^{B}	86.96^{B}	5.86^{A}	0.666	3.80^{A}	1.70^{A}	0.666^{B}	12.70^{A}
3	90.06	78.40^{B}	87.43^{B}	5.32^{A}	0.666	3.56^{A}	1.20^{B}	0.533^{B}	11.28^{B}
6	90.33	82.10^{A}	91.20^{A}	3.56^{B}	0.655	2.40^{B}	0.800^{C}	0.566^{B}	7.98^{C}
9	89.90	77.26^{B}	86.83^{B}	5.16^{A}	0.700	3.90^{A}	1.26^{B}	1.400^{A}	12.43^{A}
SEM	0.1745	0.8583	0.8636	0.4356	0.0147	0.2405	0.1188	0.1019	0.8526
p-value	0.325	0.028	0.033	0.035	0.526	0.022	0.018	0.005	0.0001
Interaction between storage period and pre-storage incubation									
4 days * 0 hours	91.00	84.50^{a}	93.20^{a}	2.50^{h}	0.633^{d}	2.10^{gh}	1.30^{cd}	0.100^{h}	6.63^{f}
4 days * 3 hours	90.00	84.60^{a}	93.60^{a}	2.66^{h}	0.634^{d}	2.00^{g}	0.600^{fg}	0.200^{gh}	6.10^{f}
4 days * 6 hours	90.70	84.20^{a}	94.00^{a}	2.70^{h}	0.633^{d}	1.70^{g}	0.200^{h}	0.500^{ef}	5.73^{f}
4 days * 9 hours	89.80	82.60^{b}	90.30^{b}	3.30^{g}	0.635^{d}	2.60^{ef}	0.500^{gh}	1.000^{c}	8.03^{e}
8 days * 0 hours	90.00	79.50^{c}	88.00^{c}	5.10^{e}	0.633^{d}	3.30^{d}	1.500^{c}	0.400^{fg}	10.93^{c}
8 days * 3 hours	90.00	79.30^{cd}	88.70^{bc}	4.30^{f}	0.634^{d}	3.00^{de}	1.000^{de}	0.400^{fg}	9.33^{d}
8 days * 6 hours	90.30	83.20^{ab}	92.80^{a}	2.30^{h}	0.633^{d}	2.20^{gh}	0.800^{efg}	0.500^{ef}	6.43^{f}
8 days * 9 hours	89.70	78.00^{d}	88.80^{bc}	4.20^{f}	0.700^{c}	4.30^{c}	0.900^{ef}	0.900^{cd}	11.00^{c}
12 days * 0 hours	90.00	71.00^{e}	79.70^{d}	10.00^{a}	0.733^{b}	6.00^{a}	2.3^{ab}	1.500^{b}	20.53^{a}
12 days * 3 hours	90.20	71.30^{e}	80.00^{d}	9.00^{b}	0.735^{b}	5.70^{a}	2.00^{b}	1.000^{c}	18.43^{b}
12 days * 6 hours	90.00	78.90^{cd}	86.80^{c}	5.70^{d}	0.700^{c}	3.30^{d}	1.400^{c}	0.700^{de}	11.80^{c}
12 days * 9 hours	90.20	71.20^{e}	81.40^{d}	8.00^{c}	0.7667^{a}	4.80^{b}	2.400^{a}	2.300^{a}	18.26^{b}
SEM	0.1745	0.8583	0.8636	0.4356	0.0147	0.2405	0.1188	0.1019	0.8526
p-value	0.982	0.0001	0.0001	0.0001	0.044	0.0001	0.0001	0.0001	0.0001

Note: *Means, within columns, for the main treatment effects or the interaction effects, with no common superscript, differ significantly ($P \leq 0.05$). ** SEM = Stander error of the mean.

3.3. Embryonic Mortality

Early and late embryo mortality Table 2 above showed that eggs sorted for 12 days had significantly higher early and late mortality, piped eggs, rotted eggs and overall percentages of embryonic mortality relative to the other sorted period (4 days).

Table-3. The effect of storage period and pre-storage incubation and their combination on chick quality.

Treatments	Traits				
	Chick quality			Day old chick quality measur□ments	
	Grade A[1]	Grade B[2]	Grade C[3]	Body weight□g)	Chick length (cm)
Egg storage period (days)					
4	94.46^{A*}	3.27^{B}	2.25^{B}	35.97^{A}	16.27
8	93.30^{A}	3.58^{B}	3.11^{B}	35.20^{B}	16.22
12	88.26^{B}	7.84^{A}	3.89^{A}	33.97^{C}	16.15
SEM	0.532	0.413	0.200	0.146	0.025
p-value	0.0001	0.0001	0.002	0.0001	0.141
Pre-storage incubation (hours at 37.5°c)					
0	90.71^{B}	6.01^{A}	3.27^{A}	35.00	16.21
3	92.41^{AB}	3.78^{C}	3.80^{A}	35.06	16.21
6	94.11^{A}	4.10^{C}	1.78^{B}	35.13	16.25
9	90.81^{B}	5.70^{B}	3.48^{A}	35.01	16.18
SEM**	0.5328	0.413	0.2002	0.146	0.025
p-value	0.012	0.037	0.0001	0.989	0.845
Interaction between storage period and pre-storage incubation					
4 days * 0 hours	93.30^{cd}	4.13d^{e}	2.56^{de}	36.00^{a}	16.30
4 days * 3 hours	94.36^{b}	2.66^{g}	2.96^{cde}	36.00^{a}	16.30
4 days * 6 hours	98.20^{a}	1.30^{h}	0.500^{f}	36.00^{a}	16.30
4 days * 9 hours	92.00^{e}	5.00^{cd}	3.00^{cde}	35.90^{a}	16.20
8 days * 0 hours	92.33^{e}	4.30^{de}	3.36^{bcd}	35.20^{b}	16.20
8 days * 3 hours	93.70^{bc}	3.10^{fg}	3.20^{cd}	35.20^{b}	16.20
8 days * 6 hours	94.33^{b}	3.00^{fg}	2.66^{de}	35.30^{b}	16.30
8 days * 9 hours	92.83^{de}	3.93^{ef}	3.23^{cd}	35.13^{b}	16.20
12 days * 0 hours	86.50^{h}	9.60^{a}	3.90^{bc}	33.80^{c}	16.13
12 days * 3 hours	89.16^{f}	5.60^{c}	5.23^{a}	34.20^{c}	16.13
12 days * 6 hours	89.80^{f}	8.00^{b}	2.20^{e}	34.80^{c}	16.16
12 days * 9 hours	87.60^{g}	8.16^{b}	4.23^{b}	34.20^{c}	16.16
SEM	0.5328	0.4137	0.2002	0.1466	0.0259
p-value	0.0001	0.0001	0.0001	0.0001	0.920

Note: *Means, within columns, for the main treatment effects or the interaction effects, with no common superscript, differ significantly (P≤0.05). ** SEM = Stander error of the mean.
1- Grade A = Good commercial Chicks. 2- Grade B = Bad Chicks. 3- Grade C = Very bad Chicks.

3.4. Chick Quality

Different metrics were used for assessing the chick quality. These indicators included a grade of commercial chick quality, chick body weight and 1day old chick length. All chick quality characteristics studied were significantly affected by both storage period and duration of pre-storage heating Table 3. Long storage time of eggs was associated with a decrease in the percentage of chicks in grade A, body weight and length of chicks, at one day. In comparison, the levels of Grade B and C chicks for eggs stored for more than four days were significantly higher than those stored for only four days. Pre-storage heating of eggs for six hours resulted in significant improvements in all characteristics of chick quality compared to unheated eggs Table 3. There were significant interactions for the grade of chicks between the storage period and the duration of incubation before storage. The data obtained indicated that the chicks produced from heated eggs for six hours and stored for 4–12 days had significantly higher percentages of A-grade chicks than non-heated eggs Table 3. Only when eggs were stored for four, eight or twelve days Table 3 was observed the significant improvement in grade A chick's percentage in the six-hour heating group as compared to three- or nine-hours heating group. When eggs were stored for 4, 8 or 12 days, chicks with a significantly heavier weight were produced by heating eggs for three, six or nine hours compared to the same storage period with unheated ones. The chicks hatched from six hours of heated eggs and kept for 4 to 12 days were considerably longer than those hatched from unheated or nine hours of heated eggs. No interaction between storage

time and pre-incubation heating profile was observed chick length Table 3. Chicks of the 4-d storage time were longer than chicks of the 8 and 12-d storage time. Chick length reduced when storage time increased. The pre-incubation heating profile did not affect chick length.

4. DISCUSSION

4.1. Egg Weight Loss

Increasing duration of pre-incubation periods means increasing eggs exposure time for the incubation temperature leading to increased loss of egg weight because the pressure of water vapor increases as the duration of pre-incubation rises. In addition, prolonged storage time of the eggs has resulted in a decrease in cuticle consistency, which may contribute to increased water vapor pressure, raising the loss of egg weight [21]. Such findings are consistent with those reported by Reijrink, et al. [6] who observed that storage of commercial Cobb broiler breeder eggs for 3, 5, 8 or 12 days prior to hatching increased the percentage of LEW during storage by 0.24, 0.53, 0.74 and 1.28 percent, respectively. Also, Reijrink, et al. [2] reported that the percentage of LEW during incubation and the percentage of total LEW for 4 hours pre-incubation was lower than for the 24 hours pre-incubation period.

4.2. Fertility and Hatchability

The two main treatments should not have affected fertility because fertilization would have occurred or would not have occurred before the eggs were exposed to the treatments. Fasenko, et al. [3]reported similar suggestions in chicken eggs, and Petek and Dikmen [11] in quail eggs. The latter authors found that the differences for the apparent fertility among the main groups of pre-storage heating and storage duration were not significant. Hatchability in Table 2, was substantially lower hatchability percentages of both fertile and total eggs were observed as storage periods increased because prolonged storage eggs leading to changes in egg components such as increased albumen pH and reduced albumen height and Haugh units [5] which decreased embryonic viability [22] because albumen structure is said to be a dominant factor in successful development of the germ from anaerobic to aerobic metabolism, thereby hatchability percentage decreased [23]. In the long storage period (12 days), hatchability percentage was increased after exposure of the eggs for pre-storage incubation compared to non-pre-storage incubation (control) eggs but was still significantly lower than the same pre-storage incubation level in the short stored period (4 days) may be because pre storage incubation provides more incubation time for the egg to hatch [3], but the changes in the internal egg quality (albumen) due to prolonged eggs stored (14 days) are not prevented by pre storage incubation [6]. Thus, in each pre-storage incubation procedure for the long storage period (12 days), the hatchability percentage is still significantly lower compared with the same amount of pre-storage incubation over the short-stored period (4 days). Dokki4 eggs exposed to warming for six h during the long storage time (12 days) had higher and significant hatchability percentages of fertile and total eggs compared with the control and other pre-heating eggs for 3 and 9h prior to eggs storage, may be because pre-incubation of eggs for 6 h prior to eggs storage for a long time (12 days) allowing the embryos to reach a developmental stage more suitable to survive the long storage period (12 days), which characterized by stopping embryonic development as measured by microscopic staging methods during eggs storage [24, 25] compared to the stages of embryos development that reach to it when pre-warming eggs for 0, 10 and 15h prior to eggs storage for 14 days. Our findings are in accordance with those reported by Fasenko, et al. [3], who demonstrated that pre-incubation periods before storage 6h treatment for broiler breeder eggs took the majority of eggs embryos to a development stage [26] which hypoblast formation is complete and cell migration and differentiation are minimal [27], at this developmental stage, embryos are at a relatively quiescent state which promotes embryos survival to prolonged storage and embryonic death and are better able to withstand developmental arrest during prolonged storage of 14 days [3]. These embryos that displayed an enhanced development due to pre-storage heat treatment may be were

better able to form an effective pH barrier between the inside of the embryo (pH ranges from 7.9 to 8.4; [28]) and its surroundings (albumen pH around 9.5, because after oviposition carbon dioxide is released from the embryo, resulting in an increase in albumen pH from about 7.6 to 9.5 within a short period of time, whereas the yolk remains slightly acidic, at a pH around 6.5 during early incubation than the less developed embryos (control) [2] due to that control treatment did not expose to any worming treatments and thereby its embryonic development which characterized by, area pellucida formation is complete [26], and the more advanced once because worming eggs for12 h or 18 h allowing the majority of eggs embryos to reach an embryonic development Stage 3 or 4 which characterized by, primitive streak formation is approximately half complete or complete respectively [29] with extremely active periods of cellular division, migration and differentiation and do not respond favorably to developmental arrest during 14 d storage resulted in similar hatchability percentages of fertile and set eggs compared to the control group and both of them lower than group wormed for 6 h [3].

4.3. Embryonic Mortality

Extended egg-storage periods leads to; 1) allow albumen to degrade excessively. This deterioration allows the blastoderm to pass close to the shell of the egg resulting in early embryonic mortality resulting from dehydration during the early incubation phases [30] Increases sensitivity to suboptimal conditions of incubation which means that embryos are more sensitive to changes in temperature from storage to incubation time, leading to their higher early mortality [31] the number of viable embryonic cells is small as a function of long-term storage that may result in different steps in the development of the embryo at the beginning of the incubation period due to the lack of adequate cells in the embryo, unable to use the available O_2 efficiently to break down the required nutrients in the yolk in order to release the energy needed for embryonic growth, leading to abnormal development or early embryonic death [13]. On the other hand, prolonged storage of eggs leads to reduced embryonic growth in some muscles such as the breast muscle (pectoralis major) and the hatching (complex) muscle, which are essential for metabolically mobilizing stored glycogen to help the embryo penetrate the shell of the egg and its membranes during the hatching process thereby increasing late embryonic mortality [32]. In the same storage period Table 2, there was a steady rise of negligible mid-mortality values and significant values in early, late embryonic mortality, piped eggs, rotten eggs, and total percentages of embryonic mortality as the pre-incubation time before storage increased. This increase could be explained by Silva, et al. [31] who stated that increasing eggs exposure period for the incubation temperature before storage then storage temperature then incubation temperature being embryos were more susceptible to temperature changes leading to their higher embryo mortality at all studied periods.

4.4. Chick Quality

Pre-storage egg heating for 6 hours improved chick quality in terms of grade A percentage of chicks, chick weight and length, regardless of storage time compared to unheated eggs. These results are consistent with those reported by Yalçin and Siegel [33] and Reijrink, et al. [6]. Reijrink, et al. [2] who demonstrated that heating the hatching eggs before storage improved chick length. They said, this difference in chick length between the control and the pre-storage heated groups could be caused by a difference in hatch time. Pre-storage heating improved embryonic development, and thus pre-storage heated eggs hatched earlier than the control group chicks [6, 33]. This might explain why chick length and weight of the pre-storage heated eggs was higher at the moment of measurement than those of the non-heated eggs [18].

5. CONCLUSIONS

Based on our results, we can conclude that pre-storage heat treatment beneficially affects hatchability traits and chick quality, especially when hatching eggs are stored for long periods (12 days).

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