

EFFECT OF PRE-STORAGE HEATING OF BROILER BREEDER EGGS, STORED FOR LONG PERIODS, ON HATCHABILITY AND CHICK QUALITY

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SUMMARY

This study was undertaken to determine if pre-storage heating of broiler breeder eggs, stored for long periods, would improve the hatchability and chick quality. A total of 2400 broiler breeder eggs were obtained from a 46 weeks old commercial flock. Eggs were distributed in a 3 x 4 factorial design, with three pre-storage heating durations (0, 6, and 9 hours at 37.5° C and 56% RH) and four storage periods (4, 7, 10 and 14 days at 18° C and 75% RH). This adds up to twelve treatments with 20 replicates, of 10 eggs each. After storage, eggs were simultaneously incubated under the usual incubation conditions. Eggs were weighed before heating, after storage and before transfer to the hatcher, to determine the weight loss. Fertility and hatchability of both fertile and total eggs were determined. Embryo mortality was determined in non-hatched eggs. All hatched chicks were weighed and examined to score them for quality. The results indicated that long egg storage period resulted in a significant increase in egg weight loss during storage and after 18 days of incubation, and total egg weight loss. The longer the pre-storage heating duration was significantly increased the egg weight loss percentage during storage. Hatchability and chick quality results of eggs stored from 7 to 14 days were significantly less than eggs stored for 4 days. The 6 hours pre-storage heating treatment, significantly increased the hatchability and chick quality of eggs as compared to the non-heated or 9 hours heated eggs. There were significant pre-storage heating x egg storage length interactions for egg weight loss, hatchability of total and fertile eggs, embryonic mortality and chick quality. When eggs were stored for more than seven days, pre-storage heating eggs for six hours significantly improved the hatchability and chick quality results, as compared to non-heated or heated for 9 hours eggs. In conclusion, pre-storage heating of commercial broiler breeder eggs for six hours at 37.5° C and 56% RH could be used by the poultry industry as a method to improve hatchability and chick quality of eggs stored for longer periods than 7 days.

Keywords: *Pre-storage heating, storage periods, embryonic mortality, hatchability, chick quality*

INTRODUCTION

Storage of hatching eggs is a common practice in commercial breeder farms and hatcheries; the total length of egg storage varies between a few days and several weeks. However, the storage duration depends on the supply of hatching eggs, maximum hatchery capacity, and the variable market demand for 1-day old chicks in the poultry industry. Normally, commercial hatcheries set their eggs after 3 to 5 days of storage, but in some situations, a hatchery needs to increase the storage period beyond 7 days. Egg storage period beyond 7 days is associated with a lag in embryo development (Christensen *et al.*, 2001), altered metabolic rate (Christensen *et al.*, 2001 and Fassenko *et al.*, 2009), a delay in hatch time (Ruiz and Lunam, 2002 and Tona *et al.*, 2003), impairing embryo development and livability (Christensen *et al.*, 2002, Elibol *et al.*, 2002 and Van de Ven, 2004), and decline in hatchability (Tona *et al.*, 2004, Petek and Dikmen, 2006 and Yassin *et al.*, 2008). In addition, Tona *et al.* (2003& 2004) and Willemsen *et al.* (2008) determined the negative effects of prolonged egg storage on chick quality in terms of physical parameters on the day of hatch such as appearance, activity, and quality of the navel area. Besides body weight and other physical

parameters, used by Tona *et al.* (2003), it is also possible to determine the negative effect of long egg storage period on chick quality at hatch with a quantitative method, such as chick length (Wolanski *et al.*, 2004 and Molenaar *et al.*, 2008).

The negative effects of long storage of hatching eggs result from the deterioration of internal egg quality. With long storage time, albumen pH increases whereas, albumen height decreases (Lapao *et al.*, 1999) and yolk sac membrane elasticity decreases (Jones and Musgrove, 2005). Embryonic mortality during storage is highly related to egg weight loss, and increases with storage time (Nahm, 2001 and Reijrink *et al.*, 2010). Reijrink *et al.* (2008) stated that the negative effects of prolonged egg storage may be caused by changes in the embryo or in the egg characteristics, or by both.

During storage, as eggs remain at cold temperature, below physiological zero (20 - 21°C), embryos stop developing at a stage characterized by the complete formation of zona pellucid. However, later, as temperature increase during incubation, embryo development is resumed (Fassenko *et al.*, 2001a). Higher embryo livability and hatchability, and shorter incubation period were observed when the hypoblast stage is achieved before long storage periods (Fassenko *et al.*, 2001a and Reijrink *et al.*,

2009). Before storage, heating chicken eggs for six hours (Fasenko *et al.*, 2001a), turkey eggs for 12 hours (Fasenko *et al.*, 2001b) and quail eggs for 8 hours (Petek and Dikmen, 2004) allows the complete formation of hypoblast. Therefore, pre-storage heating could improve the incubation results of eggs stored for long periods (Fasenko *et al.*, 2001a & b, Renema *et al.*, 2006 and Silva *et al.*, 2008).

When working with long storage periods, the adoption of management practices such as storing the egg with the thin tip down, egg turning during storage and pre-storage heating should be considered to reduce the negative effects on the incubation yield (Rocha *et al.*, 2013). It was reported that pre-heating of poultry eggs, before storage, resulted in more live chicks and a lower level of embryonic mortality compared to eggs that were not heated (Lourens, 2002, Petek and Dikmen, 2004 & 2006 and Marandure *et al.*, 2012). On the other hand, Reijrink *et al.* (2009) stated that pre-storage heating can have a negative as well as a positive effect on hatchability and chick quality. They suggested that pre-storage heating affects embryo viability and therefore survival rate of the embryo during early incubation. Moreover, Reijrink *et al.* (2010) reported that pre-storage heating of eggs (for seven hours) was probably not necessary to make the embryos more resistant to prolonged egg storage (15 days) and did, therefore, not improve hatchability or chick quality.

According to Fasenko *et al.* (2001b), the effect of pre-storage heating on hatchability results, when storage time is prolonged, depends on the developmental stage of the embryo after pre-storage heating. Various pre-incubation storage practices, of hatching eggs, have different considerable influences on the hatching results. However, the effect of pre-storage heating of hatching eggs on chick quality is not studied extensively. Therefore, the specific goal of this study was to determine if pre-storage heating would improve the hatchability and chick quality of broiler breeder eggs stored for long periods.

MATERIAL AND METHODS

Experimental design

A total of 2520, clean, not cracked or miss-shaped, hatching eggs produced by a 46-week-old Hubbard Classic broiler breeder flock were collected from a commercial farm and were transported to the experimental hatchery in four groups (630 eggs each). Out of the total number of eggs, 2400 eggs were distributed in a 3 x 4 factorial design, with three pre-storage heating durations (0, 6, and 9 hours at 37.5° C and 56% RH) and four storage periods (4, 7, 10 and 14 days at 18° C and 75 % RH), summing up twelve treatments with 20 replicates of 10 eggs each. Eggs were hand collected directly from the nests during the second daily collection in order to ensure lay time uniformity. Eggs were collected between five and seven hours after light was switched on,

when the outside temperature was around 20°C. In order to incubate all eggs at the same time, eggs were collected in four groups, one group every four days, representing the four storage periods (4, 7, 10 and 14 days). Therefore, the first group corresponded to eggs stored for 14 days; the second to eggs stored for ten days; the third to eggs stored for seven days and the fourth to eggs stored for four days. Out of the 630 eggs obtained in each collection, 30 were chosen at random (Table 1) to evaluate internal and external egg quality, using egg weight, shell thickness, and percentages of albumen, yolk and shell weights.

General Management

Immediately after collection, the eggs were disinfected by simple fumigation with 7.7g paraformaldehyde/m³ area for 15 minutes (Khan *et al.*, 2012), and then transported to the experimental hatchery. The eggs were then disinfected by formaldehyde fumigation for 20 min, identified, weighed in pools of ten eggs, and randomly distributed into the treatments. Average time between egg collection and beginning of the experiment was four hours. Pre-storage heating for six or nine hours (depending on treatment) was carried out in a Chick Master ® incubator at an average temperature of 37.5°C and relative humidity of 56%. The heating timing started when the temperature reached 37.5°C. After heating, eggs were kept at room temperature for one hour. Eggs were then stored in a storage room at 18°C and 75% relative humidity for the periods corresponding to the treatments. After storage, eggs were maintained at 25°C and 75 % relative humidity for six hours before incubation. Eggs were incubated in a Chick Master ® incubator for 442 hours (18 days and 10 hours) at 37.5°C, average temperature, and 56.5% relative humidity. Eggs were turned hourly. At 442 hours of incubation, eggs were transferred to a Chick Master ® hatcher at 36.6°C, average temperature, and 61.2% average relative humidity.

Measured parameters

All eggs were weighed, in pools of ten eggs, before; heating, incubation, and transfer to the hatcher to evaluate the egg weight loss. Egg weight loss was determined during the storage periods, after 18 days of incubation and the total of two periods. After 18 days of incubation, all eggs were candled to calculate the fertility percentage, for each replicate (10 eggs each). Eggs with evidence of living embryos were transferred from the incubation trays to the hatcher trays. After the hatch was completed, un-hatched eggs were removed, broken and submitted to embryo diagnosis to determine the percentages of early, intermediate, and late embryo mortality (1 to 7, 8 to 14 and 15 to 21 days of embryo development, resp.). Early dead embryos were differentiated by the absence of an egg tooth; the intermediate dead were differentiated by the presence of an egg tooth, the beginning of feathers

and the yolk sac outward the body cavity. The late dead were differentiated by evidence of the yolk sac entering the body cavity and the beak positioned to pip the air cell. Piped (piped and could not hatch) and rotten (contaminated eggs and non-specific embryonic mortality) chicks were also determined. Total embryonic mortality was determined as the amount of the all dead embryos.

Hatchability of fertile eggs and total hatchability percentages were determined at 21 days of incubation, for each replicate, as follow:

Hatchability of fertile eggs % = (number of hatched chicks/ fertile eggs set)*100

Total hatchability % = (number of hatched chicks/ total eggs set)*100

All hatched chicks were weighed and examined to score them for quality (Tona *et al.*, 2003; 2004). Chicks of good quality (saleable chicks, grade A) were defined as being clean, dry and free from deformities (no skin lesion, well-formed beak, normal conformation of legs), completely sealed navel, and no yolk sac or residual membrane protruding from the navel area (Tona *et al.*, 2004). The un-saleable chicks were examined for classification into grade B or C according to the previous traits (Grade B = Bad Chicks; Grade C = Very bad Chicks.). All day-old chicks were scored with a Tona score as described by Tona *et al.* (2003). Chick length was determined according to Willemsen *et al.* (2008).

Statistical analyses

Data were submitted to two way analysis of variance using the General Linear Model procedure of SAS version 8 (1999). The main factors were storage period and pre storage heating duration. Percentage data were subjected to arc sine transformation prior to analysis. Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) when significant differences existed. Significance was set at $P < 0.05$.

RESULTS

Egg characteristics, on a subsample of eggs ($n = 30$ per group) designated to be stored for 4 - 14 days, were conducted to determine if egg component variations existed between the eggs collected maximum 10 days apart. Fresh egg weight, albumen weight (%), yolk weight (%), dried shell weight (%), and eggshell thickness were not significantly influenced by the egg collection period (Table 1).

Egg weight and Egg weight loss

The main effects of egg storage period and pre-storage heating duration on fresh egg weight and egg weight loss percentage are presented in Table (2). No significant differences were found in fresh egg weights among the different storage period or pre-storage heating duration treatments. However,

significant effect of storage period was observed on egg weight loss percentages during storage period, after 18 days of incubation, and during the two periods together (total egg weight loss %). The longer the storage period or the pre-storage heating duration, the higher the egg weight loss percentage was observed (Table 2). Eggs that were heated for 9 hours, pre-storage, had significantly higher egg weight loss percentage, during the first 18 days of incubation and total egg weight loss, as compared to those non-heated or heated for six hours.

The results indicated that there were significant interactions between storage period and pre-storage heating duration on egg weight loss percentages during storage and after 18 days of incubation, and total egg weight loss percentage (Table 2). Egg weight loss percentage during storage increased as a function of storage period at any preheating duration. However, eggs heated for six or nine hours and stored for ten days had higher weight loss percentage, during storage, as compared to non-heated eggs. During first 18d of incubation, egg weight loss percentage was not different among preheating duration within storage period, except when eggs were stored for 14 days. Where, eggs heated for 6 or 9 hours had significantly higher egg weight loss compared to the non-heated eggs. However, within pre-heating treatments, longer storage period resulted in significantly higher percentage of egg weight loss during incubation. Results of total egg weight loss percentage indicated that eggs stored for 4, 7 or 10 days were not influenced by heating duration. Whereas, within the 14 days storage group, eggs heated for nine hours had higher weight loss percentage as compared to non-heated eggs. On the other hand, within pre-heating treatments, total egg weight loss percentage was proportional to storage period.

Fertility, hatchability and embryonic mortality

The results of the apparent fertility, hatchability of total and fertile eggs, and embryonic mortality are shown in Table (3). There were no significant effects of the egg storage period, pre-storage heating duration or the interaction between them on the apparent fertility percentage.

Hatchability of fertile and total eggs was significantly affected by both the experimental factors and the interaction between them. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs, independent of the pre-heating duration. In general, eggs heated for six hours, pre-storage, had significantly higher hatchability of fertile and total eggs, as compared to non-heated eggs or those heated for nine hours. Pre-storage heating of eggs for six hours significantly increased hatchability in eggs stored for more than 4 days (Table 3). Within the four days storage period, eggs heated for nine hours had significantly lower hatchability as compared to those non-heated. Eggs heated for six hours, pre-

storage, had higher hatchability when stored for four or seven days as compared to eggs stored for 10 or 14 days (Table 3).

Early and late embryonic mortality (1-7 and 15-21 days of incubation, respectively), piped eggs, and total embryonic mortality percentages were significantly influenced by the experimental treatments (Table 3). Rotten eggs percentage was significantly affected only, by the storage period. On the other hand, intermediate embryonic mortality (8-14 days of incubation) was not influenced by the storage periods or pre-storage heating duration. Higher percentages of early, late and total embryonic mortality; and piped and rotten eggs were associated with longer egg storage period. When eggs were sorted for 14 days, they had significantly the highest early and total embryonic mortality rates, and piped and rotten eggs percentages (Table 3). This was followed by the shorter periods of storage groups.

Significantly higher late embryonic mortality rate was observed in eggs stored for 10 and 14 days, as compared to the other storage period groups. Regardless of storage period, pre-heating eggs for six hours resulted in significantly lower percentages of early, late and total embryonic mortality when compared to the non-heated eggs or heated for nine hours (Table 3).

Significant interactions were also detected between the pre-storage heating duration and storage period on all embryonic mortality rates, and piped and rotten eggs percentages (Table 3). The results indicated that pre-storage heating eggs for 6 hours significantly decreased early embryonic mortality, when eggs were stored for seven and 14 days. Within all storage periods, pre-storage heating eggs for nine hours led to higher intermediate embryonic mortality rate, as compared to those not heated or heated for six hours.

Pre-storage heating eggs for nine hours resulted in significantly higher rates of late embryonic mortalities when eggs were stored for four or ten days, and significantly lower late embryonic mortality in eggs stored for 14 days, as compared to those not heated. Pre-storage heating eggs for six hours and storing them for 14 days resulted in significant decrease in the percentages of piped and rotten eggs, as compared to the non-heated eggs (Table 3). When eggs were stored for more than four

days, total embryonic mortality rates were significantly lower when eggs were pre-storage heated for six hours, as compared to those not heated or heated for nine hours.

Chick Quality

Different indicators for measuring the chick quality were used. These indicators included commercial chick quality grade, Tona score, chick body weight, and chick length at 1 day old. All chick quality traits studied were significantly affected by both storage period and pre-storage heating duration (Table 4). Long egg storage period was associated with the decrease in grade A chicks percentage, Tona score chick quality, body weight, and chick length, at one day old. Whereas, Grade B and C chicks percentages were significantly higher for eggs stored for more than four days, as compared to those stored for only four days.

Pre-storage heating of eggs for six hours resulted in significant improvement in all chick quality traits, as compared to non-heated eggs. This improvement was not significant when compared to pre-storage heated eggs for nine hours (Table 4), except grade C chicks, which were significantly higher in nine hours group.

There were significant interactions between the storage period and pre-storage incubation duration for chicks' grade. The obtained data indicated that the chicks produced from heated eggs for six hours and stored for 4-14 days had significantly higher percentages of grade A chicks, as compared to non-heated eggs (Table 4). The significant improvement in grade A chicks percentage in the six hours heating group, as compared to nine hours heating group was observed, only, when eggs were stored for four or ten days (Table 4).

When storage period was 10 or 14 days, the chicks produced from pre-storage heated eggs for 6 hours had significantly higher Tona scores than those hatched from non-heated eggs or heated for nine hours. When eggs were stored for 10 or 14 days, heating eggs for six or nine hours produced chicks with significantly heavier weight, as compared to non-heated ones. The chicks hatched from six hours heated eggs and stored for 7 to 14 days were significantly longer, as compared to those hatched from non-heated or nine hours heated eggs.

Table 1. Internal and external egg quality profiles of the four groups of eggs before starting the treatments

Egg collection period	Egg weight (g)	Albumen (%)	Yolk (%)	Shell (%)	Eggshell thickness (mm)
First	66.08± 0.95	60.95±0.68	29.94± 1.12	9.11± 0.35	0.43 ± 0.033
Second	66.29 ± 0.85	61.10 ±0.57	29.79 ± 1.05	9.11± 0.31	0.43 ± 0.034
Third	66.38 ± 0.77	61.38 ±0.61	29.60 ± 1.09	9.02 ± 0.28	0.43 ± 0.032
Fourth	66.57 ± 0.89	61.43 ±0.65	29.52 ± 1.11	9.05 ± 0.38	0.43 ± 0.035
<i>P</i>	0.572	0.298	0.630	0.121	0.550

-No significant differences, within trait, among the egg collection groups were observed

Table 2. The effects of egg storage period, pre-storage heating duration and their interaction on fresh egg weight and egg weight loss percentage¹ of broiler breeder eggs

Main treatment effects	Trait			
	Fresh egg weight (g)	Egg weight loss during storage (%)	Egg weight loss after 18 days of incubation (%)	Total egg weight loss (%)
Egg storage period (days)				
4	66.4	0.75 ^{d*}	12.84 ^d	13.59 ^d
7	66.5	0.95 ^c	13.03 ^c	13.99 ^c
10	66.6	1.21 ^b	13.31 ^b	14.52 ^b
14	66.7	1.51 ^a	13.69 ^a	15.20 ^a
SEM ^{**}	0.086	0.020	0.021	0.028
P	0.786	0.026	0.032	0.028
Pre-storage heating duration (hours at 37.5°C)				
0	66.5	1.01 ^b	13.18 ^b	14.19 ^b
6	66.4	1.13 ^a	13.09 ^b	14.22 ^b
9	66.5	1.17 ^a	13.21 ^a	14.38 ^a
SEM	0.093	0.021	0.032	0.035
P	0.802	0.010	0.019	0.023
Storage period x pre-storage heating duration interaction				
4 days * 0 hours	66.5	0.67 ^d	12.90 ^e	13.57 ^e
4 days * 6 hours	66.5	0.77 ^d	12.82 ^e	13.59 ^e
4 days * 9 hours	66.4	0.82 ^d	12.80 ^e	13.62 ^e
7 days * 0 hours	66.4	0.91 ^{cd}	13.08 ^d	13.99 ^d
7 days * 6 hours	66.3	0.96 ^{cd}	13.04 ^d	14.00 ^d
7 days * 9 hours	66.5	0.99 ^{cd}	13.00 ^d	13.99 ^d
10 days * 0 hours	66.6	1.02 ^c	13.35 ^c	14.37 ^c
10 days * 6 hours	66.5	1.31 ^b	13.28 ^c	14.59 ^c
10 days * 9 hours	66.6	1.30 ^b	13.30 ^c	14.60 ^c
14 days * 0 hours	66.4	1.47 ^{ab}	13.63 ^b	15.10 ^b
14 days * 6 hours	66.5	1.49 ^{ab}	13.72 ^a	15.21 ^{ab}
14 days * 9 hours	66.4	1.57 ^a	13.73 ^a	15.30 ^a
SEM	0.071	0.199	0.032	0.039
P	0.651	0.001	0.001	0.002

* 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.

¹ Egg weight loss percentage estimated as a percentage of fresh egg weight.

Table 3. The effects of egg storage period, pre-storage heating duration and their interaction on apparent fertility, hatchability and embryonic mortality of broiler breeder eggs

Main treatment effects	Trait								
	Apparent fertility %	Hatchability of fertile eggs %	Hatchability of total eggs %	Embryonic mortality %					
				Early	Mid ¹	late	Piped egg	Rotten egg	Total
Egg storage period (days)									
4	90.5	92.6 ^{a*}	83.4 ^a	2.9 ^c	0.8	2.1 ^c	0.6 ^b	0.8 ^b	7.2 ^d
7	90.0	89.6 ^b	80.1 ^b	4.4 ^b	0.6	3.6 ^b	1.1 ^b	0.6 ^b	10.3 ^c
10	90.2	87.3 ^c	77.3 ^c	4.4 ^b	0.7	5.8 ^a	1.0 ^b	0.7 ^b	12.6 ^b
14	90.3	82.4 ^d	73.4 ^d	7.6 ^a	1.1	5.0 ^a	2.3 ^a	1.8 ^a	17.8 ^a
SEM ^{**}	0.08	0.31	0.30	0.26	0.25	0.29	0.27	0.21	0.712
P	0.205	0.001	0.001	0.001	0.351	0.002	0.006	0.020	0.001
Pre-storage heating duration (hours at 37.5°C)									
0	90.3	86.8 ^b	77.5 ^b	5.9 ^a	0.6	4.4 ^a	1.6 ^a	0.8	13.3 ^a
6	90.0	91.2 ^a	81.2 ^a	3.4 ^b	0.6	3.2 ^b	1.0 ^b	0.7	8.9 ^b
9	90.2	86.2 ^b	77.1 ^b	5.2 ^a	1.3	4.8 ^a	1.1 ^b	1.4	13.8 ^a
SEM	0.091	0.421	0.410	0.34	0.32	0.29	0.21	0.30	0.61
P	0.264	0.031	0.030	0.023	0.456	0.018	0.022	0.608	0.017
Storage period x pre-storage heating duration interaction									
4days * 0 hours	91.3	93.8 ^a	84.9 ^a	2.6 ^c	0.3 ^d	2.0 ^g	1.2 ^{bc}	0.1 ^d	6.2 ^d
4 days * 6 hours	89.4	94.2 ^a	83.7 ^{ab}	2.7 ^e	0.4 ^d	1.7 ^g	0.2 ^e	0.8 ^c	5.8 ^d
4 days * 9 hours	90.7	90.3 ^b	81.5 ^b	3.4 ^d	1.6 ^a	2.7 ^f	0.5 ^d	1.5 ^{bc}	9.7 ^c
4 days * 14 hours	89.7	87.8 ^{cd}	78.0 ^{cd}	6.2 ^c	0.3 ^d	3.8 ^e	1.5 ^b	0.4 ^{cd}	12.2 ^{bc}
7 days * 0 hours	90.0	93.6 ^a	83.7 ^{ab}	2.3 ^f	0.3 ^d	2.4 ^{fg}	0.9 ^{bc}	0.5 ^{cd}	6.4 ^d
7 days * 6 hours	90.3	87.8 ^{cd}	78.7 ^c	4.8 ^d	1.3 ^a	4.5 ^{de}	0.8 ^c	0.8 ^c	12.2 ^{bc}
7 days * 9 hours	89.7	86.4 ^d	76.5 ^d	5.5 ^{cd}	0.6 ^c	5.5 ^c	1.2 ^b	0.8 ^c	13.6 ^b
7 days * 14 hours	90.6	89.1 ^c	78.9 ^c	3.1 ^{de}	0.6 ^c	5.2 ^c	1.2 ^{bc}	0.8 ^c	10.9 ^c
10 days * 0 hours	90.3	86.4 ^d	76.6 ^d	4.5 ^{de}	0.9 ^b	7.2 ^a	0.5 ^d	0.5 ^{cd}	13.6 ^b
10 days * 6 hours	90.6	79.1 ^e	70.6 ^e	9.3 ^a	1.0 ^b	6.2 ^b	2.6 ^a	1.8 ^b	20.9 ^a
10 days * 9 hours	90.0	87.8 ^{cd}	78.4 ^{cd}	5.6 ^{cd}	0.9 ^b	3.4 ^{de}	1.5 ^b	0.8 ^c	12.2 ^{bc}
10 days * 14 hours	90.4	80.3 ^e	71.2 ^e	8.0 ^b	1.3 ^a	4.8 ^d	2.7 ^a	2.9 ^a	19.7 ^a
14 days * 0 hours	0.72	1.03	0.99	0.46	0.11	0.34	0.23	0.32	1.06
14 days * 6 hours	0.783	0.001	0.001	0.001	0.031	0.001	0.009	0.018	0.009
14 days * 9 hours									
14 days * 14 hours									
SEM									
P									

* 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- Intermediate embryonic mortality percentage.

Table 4. The effects of egg storage period, pre-storage heating duration and their interaction on chick quality of broiler breeder eggs

Main treatment effects	Trait					
	Chick quality (Commercially) ¹			Tona score chick quality ⁵	Day old chick quality measurements	
	Grade A ²	Grade B ³	Grade C ⁴		Body Weight (g)	Chick length (cm)
Egg storage period (days)						
4	96.6 ^{a*}	2.8 ^d	0.6 ^c	95.4 ^a	46.0 ^a	19.3 ^a
7	92.8 ^b	5.0 ^c	2.2 ^b	91.8 ^b	45.2 ^b	19.2 ^b
10	91.4 ^b	6.7 ^b	1.9 ^b	85.4 ^c	43.3 ^c	19.2 ^b
14	87.8 ^c	8.6 ^a	3.6 ^a	82.0 ^d	41.8 ^d	19.2 ^b
SEM**	0.71	0.24	0.29	1.95	0.09	0.01
P	0.009	0.012	0.021	0.001	0.019	0.021
Pre-storage heating duration (hours at 37.5°c)						
0	90.7 ^b	7.0 ^a	2.3 ^a	86.0 ^b	43.8 ^b	19.2 ^b
6	93.6 ^a	4.7 ^b	1.7 ^b	91.4 ^a	44.3 ^a	19.3 ^a
9	92.1 ^{ab}	5.6 ^b	2.3 ^a	88.5 ^{ab}	44.2 ^a	19.2 ^b
SEM	0.63	0.55	0.30	1.36	0.05	0.01
P	0.015	0.032	0.023	0.032	0.019	0.016
Storage period x pre-storage heating duration interaction						
4days * 0 hours	96.0 ^b	3.1 ^g	0.9 ^{de}	94.3 ^{ab}	46.0 ^a	19.3 ^a
4 days * 6 hours	98.0 ^a	1.5 ^h	0.5 ^e	96.5 ^a	46.0 ^a	19.3 ^a
4 days * 9 hours	95.8 ^b	3.7 ^{fg}	0.5 ^e	95.3 ^{ab}	45.9 ^a	19.2 ^b
7 days * 0 hours	91.7 ^e	6.1 ^d	2.2 ^c	91.5 ^c	45.2 ^b	19.2 ^b
7 days * 6 hours	93.8 ^{cd}	4.1 ^f	2.1 ^c	93.1 ^{bc}	45.3 ^b	19.3 ^a
7 days * 9 hours	93.0 ^d	4.7 ^{ef}	2.3 ^{bc}	90.8 ^c	45.2 ^b	19.2 ^b
10 days * 0 hours	89.8 ^f	8.1 ^b	2.1 ^c	83.5 ^e	42.7 ^d	19.2 ^b
10 days * 6 hours	93.6 ^{cd}	5.1 ^e	1.4 ^d	88.3 ^{cd}	43.7 ^c	19.3 ^a
10 days * 9 hours	91.1 ^{ef}	6.8 ^{cd}	2.1 ^c	84.4 ^e	43.6 ^c	19.2 ^b
14 days * 0 hours	85.4 ^g	10.6 ^a	4.0 ^a	74.6 ^f	41.2 ^f	19.2 ^b
14 days * 6 hours	89.1 ^f	8.2 ^b	2.7 ^b	87.8 ^{cd}	42.1 ^e	19.3 ^a
14 days * 9 hours	88.8 ^f	7.1 ^c	4.1 ^a	83.6 ^e	42.0 ^e	19.2 ^b
SEM	0.52	0.24	0.21	0.39	0.11	0.01
P	0.013	0.019	0.025	0.019	0.001	0.014

* 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.

2- Grade A = Good commercial Chicks.

4- Grade C = Very bad Chicks.

1- According to commercial hatchery standards.

3- Grade B = Bad Chicks.

5- Tona Score according to Tona *et al.* (2003).

DISCUSSION

In the present study, egg external and internal quality traits were not significantly influenced by collection day (maximum 10 days apart) indicating that egg collection day did not directly or indirectly affect the evaluated parameters. Similar results were observed by Silva *et al.* (2008). They reported that internal and external egg qualities of eggs were not different among three different collection days; eggs were collected in three five-day intervals throughout 14 days.

The present results indicated that egg weight losses, during storage, were significantly affected by the main effects of the pre-storage heating duration and length of egg storage. Egg weight loss was proportional to the storage period. Egg weight loss percentage in the 14-day storage group was double that of the four-day storage group. Differences in egg weight losses, prior to incubation, were significantly higher with pre-storage heating eggs for nine hours, followed by six hours heating, as compared to the non-heated eggs. These results were expected because an exposure to long-time storage and heat treatment would increase the opportunity for water evaporation from the eggs. This finding is in agreement with that of Silva *et al.* (2008) and Reijrink *et al.* (2010).

In the present study, the long storage period or pre-storage heating eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments. Similar suggestions were reported by Fasenko *et al.* (2001a) in chicken eggs, and Petek and Dikmen (2004) in quail eggs. They found that the differences for the apparent fertility among the main groups of pre-storage heating and storage duration were not significant.

The current results revealed that egg storage for more than four or seven days markedly impaired incubation results due to higher egg weight loss, as shown by the lower hatchability; higher total embryonic mortality percentage, as well as lower chick quality. These results are in agreement with previous reports on broiler breeder's eggs (Fasenko *et al.*, 2001a & b, Fasenko, 2007 and Silva *et al.*, 2008). They observed lower hatchability and higher embryonic mortality percentage of embryos stored for 14 days as compared to 4 days of storage. These results may be due to that some embryos, from eggs stored for a long period, and could not start developing immediately after normal incubation temperatures were provided or they develop at a slower rate (Fasenko *et al.*, 2001a). Haque *et al.* (1996) observed lower embryo metabolic rate, particularly during the last stage of embryo development, as well as changes in the circulatory system during embryogenesis as storage period increased. This could explain the increase in late

embryo mortality and poorer chick quality found in eggs stored for more than seven days, in the present study. Long periods of storage may also increase the probability of bacterial contamination, as cuticle quality deteriorates (De Reu *et al.*, 2006). Our results confirmed this suggestion, with higher rotten eggs percentage and worse chick quality associated with eggs stored for 14 days.

The deleterious effects of long-term egg storage on chick quality could be due to the reduction of embryo weight. This is an indication of decreased embryo quality that could affect hatch quality (Hamidu *et al.*, 2011). Previously, embryos from broiler eggs, stored for 14 days, displayed a reduced growth rate, lower hatchability and poor chick quality compared with eggs stored for 4 days (Fasenko *et al.*, 2001a). In other studies, embryos from eggs stored for long periods had a slower metabolic rate than those from eggs stored for shorter periods (Fasenko and Robinson, 2001), slower growth of heart and liver (Christensen *et al.*, 2001) and a decline in relative lung weight (Yalçın and Siege, 2003) which resulted in poor chick quality.

Strategies are needed, that allows for the storage of eggs for considerably longer periods with minimum deterioration in hatchability and chick quality, thus increasing productivity of commercial hatcheries. In the present experiment, eggs were, pre-storage, heated (0, 6, or 9 hours at 37.5°C and 56% RH), and were subsequently stored at 18°C and 75% RH, thereby ensuring that the embryos developmental hypoblast stage was achieved by heating and was maintained throughout the storage period. Fasenko *et al.* (1992) stated that embryo stage is not changed by storage period when storage temperature is below embryo development physiological zero (20 - 21°C).

Eggs stored for 7 - 14 days and were heated for six hours prior to storage presented significantly lower total embryonic mortality and higher hatchability of fertile eggs, as compared to those not heated or heated for nine hours. Heating eggs for six hours before storing for 10 or 14 days significantly improved the chick quality, as compared to not heated or heated eggs for nine hours. There was a significant depressive effect of heating eggs for nine hours on the hatchability of total and fertile eggs in eggs stored for four days, whereas this effect was not significant when the eggs were stored longer. The results related to pre-storage heating in long period stored eggs were in agreement with previous reports (Fasenko *et al.*, 2001a & b, Petek and Dikmen, 2004 and Silva *et al.*, 2008).

The improvement in the incubation yield and chick quality in pre-storage heating eggs for six hours, as compared to those not heated or heated for nine hours may be related to the embryos stage and total number of viable embryonic cells, prior to storage. Reijrink *et al.* (2010) reported that, pre-storage heating eggs for seven hours increased the

stage of embryonic development, the total number of embryonic cells, and the total number of viable embryonic cells. How far an embryo develops depends on warming duration and temperature. Reijrink *et al.* (2009) showed that the ability of an embryo to survive prolonged egg storage may depend on the cell activity at a particular stage of development but may also depend on the number of viable embryonic cells. When the number of viable embryonic cells is low, at the onset of incubation, due to cell death during storage, particular steps in the embryo development may be impeded. This may result in abnormal development or embryonic death. Therefore, pre-storage heating of eggs for 6 hours may be considered as a good practice to improve incubation results of eggs stored for short, intermediate, and long periods.

The storage immediately after egg collection increased early embryonic mortality and reduced hatchability, probably due to the higher number of embryos in a pre-gastrula stage, which would be more sensitive to cool temperature and storage stress than the embryos at gastrula stage (Fiuza *et al.*, 2006). In a study of Fasenko *et al.* (2001a), after their pre-storage heating treatment of 6 hours, 76.7% of the embryos were at developmental stage EG13 (hypoblast stage). Hypoblast formation is the initial stage of gastrulation, ensuring their survival during prolonged storage. They hypothesized that embryos at developmental stage EG12 or EG13 are less sensitive to prolonged egg storage than embryos that are less or further advanced. At EG13 stage, the embryo has completed hypoblast formation, and cell migration and differentiation is minimal (Bellairs, 1986).

Conversely, pre-storage heating of eggs for nine hours caused worse hatchability in eggs stored for 4 - 14 days, as compared to six hours, probably because it took the embryos to a developmental stage after EG13 (Fasenko *et al.*, 2001a and Malecki *et al.*, 2005). In embryos further advanced, damage caused by prolonged storage times might be irreversible and might cause embryonic mortality. Fasenko *et al.* (2001b) hypothesized that embryos advanced to the developmental stage EG12 or EG13 are more resistant for prolonged egg storage than embryos further advanced. The advanced stage of gastrulation is characterized by active migration and differentiation, and the embryo is highly sensitive to change in temperature over long periods. In addition, eggs heated for nine hours before 14 days of storage had high egg weight loss after storage, which may be related to their low hatchability. These findings indicated that pre-storage heating eggs treatment for nine hours was not a proper for eggs stored for 4 - 14 days.

Pre-storage heating of eggs for 6 hours improved chick quality in terms of grad A chicks percentage, chick weight and length and Tona score, independent of storage time, as compared to the non-heated eggs.

These results are in agreement with those reported by Yalçın and Siegel (2003) and Reijrink *et al.* (2009). Reijrink *et al.* (2010) reported that Pre-storage heat treatment improved chick quality on the day of hatch in terms of chick length. They stated that, it is possible that this difference in chick length between the control and pre-storage heated groups is caused by a difference in hatch time. Pre-storage heating increased embryonic development and therefore chicks of the pre-storage heated group hatched earlier than chicks of the control group (Yalçın and Siegel, 2003 and Reijrink *et al.*, 2009). This might explain why chick length and weight of the pre-storage heated eggs was higher at the moment of measurement than that of the non-heated eggs (Willemsen *et al.*, 2008).

CONCLUSIONS

In conclusion, pre-storage heating of commercial broiler breeder eggs, for six hours, could be used by the industry as a method to improve hatchability of hatching eggs stored for seven days or more. Further research is needed to precisely determine the number of hours of pre-storage heating required to obtain maximum hatchability and chick quality. Meanwhile, it should be kept in mind that the economic cost of pre-storage heating must be evaluated in comparison with its beneficial effects. Data from the present experiment suggested that a six hours pre-storage heating is optimum. In addition, it should be noted that the present study examined specific storage periods of 4 - 14 days; exposure of eggs to other egg storage periods, season and frequency of egg collection could alter the effectiveness of the pre-storage heating treatments.

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تأثير التدفئة قبل التخزين لبيض دجاج امهات انتاج اللحم المخزن لفترات طويلة على نسبة الفقس و جودة الكتاكيت الناتجة

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أجريت هذه الدراسة بغرض تحديد ما اذا كان تدفئة بيض التفريخ قبل تخزينه لفترات طويلة يحسن من نسبة الفقس و جودة الكتاكيت المنتجة من قطعان دجاج امهات انتاج اللحم . تم تجميع عدد 2400 بيضة من قطيع تجارى لدجاج امهات انتاج اللحم. تم استخدام تصميم متعدد العوامل (3 × 4) بثلاث معاملات لتدفئة البيض (لمدة صفر - 6 - 9 ساعات) على درجة حرارة 37,5°م ورطوبة نسبية 56% وأربع فترات لتخزين البيض (4 - 7 - 10 - 14 يوم) على درجة حرارة 18°م ورطوبة نسبية 75%، وبالتالي كان هناك 12 معاملة لكل منها 20 تكرار من 10 بيضات. بعد انتهاء فترات التخزين تم تفريخ كل البيض في دفعة واحدة تحت ظروف التفريخ العادية. تم وزن البيض قبل و بعد التخزين و عند نقله من المحضن للمفقس لتقدير نسبة الفقد في وزن البيضة. تم تقدير نسبة الاخصاب و نسبة الفقس من البيض الكلى و المخصب وكذلك نسب النفوق الجنينى. تم وزن كل الكتاكيت الفاقسة و تقييم درجة جودتها.

أشارت النتائج الى ان زيادة فترة تخزين البيض أو مدة تدفئته أدى الى زيادة معنوية في نسبة الفقد في وزن البيضة. نتائج نسبة الفقس و جودة الكتكوت المنتج من البيض المخزن لفترات ما بين 7 و 14 يوم كانت أقل معنويًا عن البيض المخزن لمدة 4 أيام فقط. تدفئة البيض قبل تخزينه لمدة 6 ساعات حسن معنويًا من نسبة الفقس و جودة الكتكوت مقارنة بعدم التدفئة أو التدفئة لمدة 9 ساعات.

كما اظهرت النتائج تأثير معنوي للتداخل بين فترة التخزين و مدة التدفئة على كل من النقص النسبي في وزن البيضة، نسبة الفقس من البيض الكلى أو المخصب، نسبة النفوق الجنينى و كذلك جودة الكتكوت.

عند تخزين البيض لفترات أطول من 7 أيام فان تدفئته لمدة 6 ساعات قبل تخزينه أدى الى تحسن معنوي في نتائج الفقس و جودة الكتكوت مقارنة بعدم التدفئة أو التدفئة لمدة 9 ساعات.

الخلاصة ان تدفئة بيض التفريخ المنتج من القطعان التجارية لدجاج امهات انتاج اللحم لمدة 6 ساعات على درجة حرارة 37,5°م ورطوبة نسبية 56% قبل تخزينه هو اجراء يمكن تطبيقه في صناعة الدواجن كطريقة لتحسين نسب الفقس و جودة الكتكوت المنتج من بيض مخزن لفترات أطول من 7 أيام.