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Effect of Pre-Storage Heating of Late Dekalb Breeder's Eggs, Breeder Age and Storage Time on Egg Weight Loss, Hatchability and Chick Quality

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ABSTRACT

This study aimed to evaluate the effect of pre-storage heating, hen age and storage period on the hatchability, embryonic mortality, egg weight loss and chick quality of DeKalb breeder's eggs. A total of 810 fertile eggs were randomly selected and allocated into three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial arrangement in a complete randomized design with three pre-storage heating durations (0, 3 and 6 h), age (75, 80, and 85 weeks) and three storage periods (4, 9 and 14 days). Each treatment was replicated three times with 10 eggs each. Eggs were weighed before heating, incubation and transfer to the hatcher to estimate the egg weight loss. Fertility and hatchability and embryonic mortality were recorded. All hatched chicks were weighed and graded. Results indicated that pre-storage heating eggs for six hours significantly improved the hatchability and chick quality. Pre-storage heating of eggs for three hours, 75-weeks-old breeders, and eggs stored for 4 days significantly improved the chick yield. The lowest embryonic mortality values were observed with75 weeks old breeders and eggs stored for 4 days. The best hatchability and chick quality percentages were recorded with75 weeks of age breeders. In conclusion, pre-storage heating of breeder's eggs for six hours at 37.5°C and 53% RH and storage period of 4 to 9 days could be used to improve hatchability, decrease the embryonic mortality and increase the number of saleable first-grade chicks.

Keywords: Egg storage, Embryos, Hatchability, Quality of chicks

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INTRODUCTION

Storage of hatching eggs is a common practice in commercial breeder farms and hatcheries. The length of the egg storage period varies between a few days and several weeks. Many factors affecting storage duration such as hatching eggs supply, the hatchery capacity and changeable in one-day-old chicks market demand in poultry industry. In general, commercial hatcheries set their eggs after a short period of storage. In contrast, sometimes hatcheries need to extend the storage period exceeding 7 days. Egg storage period beyond 7 days could make a lag in embryonic development (Christensen et al., 2001), change in metabolism (Christensen et al., 2001; Fasenko et al., 2009), postponed the hatching period (Ruiz and Lunam, 2002), affecting negatively the hatchability and the livability (Christensen et al., 2002; Elibol et al., 2002; Van de Ven, 2004), and decline in hatchability (Tona et al., 2004; Petek and Dikmen, 2006; Yassin et al., 2008). Data concerning the appropriate storage conditions for optimal hatchability are contradicting. Storage conditions increase the moisture loss during incubation and consequently impair the chick weight and hatchability (Ruiz and Lunam, 2002). Embryonic mortality during storage increased as storage time increased (Nahm, 2001; Reijrink et al., 2010). During storage, as eggs remain at cold temperature, below physiological zero (20 to 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 (Fasenko et al., 2001a). Reijrink et al. (2008) reported that the negative effects resulted from long period of egg storage may be attributed to changes in the embryo and/or in the egg characteristics. Higher embryo livability and hatchability, and shorter incubation period were observed when the hypoblast stage is achieved before long storage periods (Fasenko et al., 2001a; Reijrink et al., 2009). Before storage, heating chicken eggs for six hours (Fasenko et al., 2001a), turkey eggs for 12 h (Fasenko et al., 2001b) and quail eggs for 8 h (Petek and Dikmen, 2004) allowed the complete formation of hypoblast. Pre-storage heating could alleviate the negative impact of long storage period (Fasenko et al., 2001a; Silva et al., 2008). Improvement has been achieved in hatching chick quality parameters when the fertile eggs were heated before storage (Petek and Dikmen, 2004; Petek and Dikmen, 2006; Marandure et al., 2012). On the other hand, Reijrink et al. (2009) revealed that pre-storage heating can have a negative as well as a positive effect on hatchability and 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. The current study aims to evaluate the effect of pre-storage heating times (zero, 3 and 6 h), breeder age (75, 80, and 85 weeks) and storage periods (4, 9 and 14 days) on egg weight loss, hatchability and chick quality of late DeKalb breeder's eggs.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at Hatchery Unit of Coral Company for Feed and Chicks Production. A total of 810 clean, normal and fertile eggs from DeKalb White layer breeder flock at different ages were randomly selected and transported to the hatchery in three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial arrangement in a complete randomized design with three pre-storage heating durations (0, 3 and 6 h at 37.5°C and 53% RH), age (75, 80, and 85 weeks) and three storage periods (4, 9 and 14 days at 18°C and 75%) summing up to twenty-seven treatments with three replicates ten eggs each placed in setting trays.

General Management

The parent stock was reared in an environmentally controlled house using a deep litter system with automatic feeding and drinking. Eggs were collected three times a day and transported to the hatchery and immediately disinfected by simple fumigation with 3.2g Elphagen (paraformaldehyde) per m^3 for 20 min heated in an electric pan to 105°C in the fumigation room at 25°C and relative humidity 70%. The fumigation room was left for one and half hours for fresh air ventilation. Hatching eggs in the control (0 hours) were kept in the cooler at 18°C and relative humidity 75% during the entire storage period. The other two groups were placed in a setter (Pas Reform, Zeddam, the Netherlands) with a capacity of 38400 eggs operating at 37.5°C and 53% RH, removed after 3 and 6 heating hours, respectively and transferred to the egg storage room. After 4, 9 and 14 days of storage period the eggs were set in a setter (Pas Reform, Zeddam, the Netherlands) with a capacity of 19200 eggs at 37.5°C average temperature and 53% RH, eggs were hourly turned for 18 days using single-stage incubation program of layer eggs. On day 18 of incubation eggs were placed in the hatcher cabinets (Pas Reform, Zeddam, the Netherlands) at 36.6°C average temperature and 75% RH.

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. After 18 days of incubation, all eggs were candled. The eggs containing clear yolk were removed, opened for microscopic differentiation between infertile and stage of embryonic mortality to calculate the true fertility for each replicate (10 eggs each). The eggs with evidence of living embryos were transferred from incubation trays to the hatchery baskets. At the end of the hatching process, un-hatched eggs were removed, broken and submitted to embryo diagnosis to determine the percentage of early, mid and late mortalities as (1 to 7, 8 to 14 and 15 to 21 days of embryo development, respectively). Early dead embryos were identified by the absence of an egg tooth, the mid dead were identified by the existence of an egg tooth, the beginning of feathers and the yolk sac outward the body cavity and the late dead were identified by evidence of the yolk sac inside the body cavity and the beak located to pip the air cell. All hatched chicks were weighed and examined to score them for quality. The chick quality was assessed using the methodology of Tona et al. (2003). The saleable chicks of good quality (first grade) were defined as being clean, dry and free from deformities (no skin lesion, well-formed beak and normal conformation of legs), completely sealed navel, and no yolk sac or residual membrane protruding from the navel. The un-saleable chicks were examined to be classified as second-grade chick’s area according to the scoring method described by (Tona et al., 2004). Hatchability of fertile eggs and total hatchability percentages were determined at 21 days of incubation, for each replicate, according to (Erensayin, 2000) as follow:

$$\text{Hatchability of fertile eggs \%} = \frac{\text{No. of hatched chicks}}{\text{fertile eggs set}} \times 100$$

Table 1. Effect of pre-storage heating period, breeder's age and storage period on egg weight loss and chick yield.

Main Factors	Fresh Egg Weight (g)	Egg wt. Loss During Storage (%)	Egg wt. Loss During Incubation (%)	Total wt. Loss (%)	Chick Yield (%)
Overall mean	65.00	1.78	11.44	13.21	64.34
±SEM	0.104	0.01	0.019	0.023	0.125
Heating (hours)					
0	64.98	1.67^{c}	11.59^{a}	13.26^{a}	60.51^{c}
3	65.07	1.91^{a}	11.13^{b}	13.04^{b}	66.82^{a}
6	65.21	1.75^{b}	11.59^{a}	13.33^{a}	65.68^{b}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**
Age (weeks)					
75	64.78	1.37^{b}	9.68^{b}	11.05^{b}	68.12^{a}
80	64.88	1.99^{a}	12.33^{a}	14.31^{a}	64.43^{b}
85	65.02	1.97^{a}	12.30^{a}	14.27^{a}	60.46^{c}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**
Storage (days)					
4	65.10	1.10^{c}	11.74^{a}	12.84^{b}	67.59^{a}
9	64.93	1.33^{b}	10.79^{b}	12.12^{c}	66.78^{b}
14	65.12	2.89^{a}	11.78^{a}	14.67^{a}	58.64^{c}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences. NS=No significant differences, **=significant difference at P≤0.01, *=significant difference at P≤0.05.

$$\text{Total hatchability\%} = \frac{\text{No. of hatched chicks}}{\text{Total eggs set}} \times 100$$

Statistical Analysis

Data were subjected to ANOVA using the General Linear Model procedure of SPSS (2008). Duncan's multiple range test used to assess the significant differences among treatment means according to the method described by (Steel et al., 1996).

RESULTS AND DISCUSSION

Egg weight, weight loss and chick yield

Table 1 shows that eggs' weight loss and chick yield parameters were significantly (P ≤ 0.01) affected by heating duration, breeder's age and storage period. No significant differences were found in fresh egg weights among the different pre-storage heating duration treatments, breeder's age and storage period. Eggs heated for three hours pre-storage heating duration had the highest weight loss percentage during storage followed by six hours and non-heated eggs. While eggs heated for three hours pre-storage heating times showed a significantly (P ≤ 0.01) lower weight loss percentage during incubation and total weight losses compared to the six hours and non-heated eggs. Pre-storage heating of eggs for three hours significantly (P<0.01) improved chick yield percentage followed by six hours and non-heated eggs. On the other hand, the weight loss percentage during storage, weight loss percentage during incubation, total weight losses and chick yield percentage parameters were significantly (P ≤ 0.01) better for 75-weeks-old breeders compared to 80- and 85-weeks old breeders. However, storage period (days) was significantly (P ≤ 0.01) affected the egg weight loss during storage, incubation, total weight loss and chick yield. Four days storage significantly (P ≤ 0.01) had the lowest egg weight loss percentages during the storage period and chick yield followed by nine and fourteen days, while nine days storage period significantly (P ≤ 0.01) improved egg weight loss percentages during incubation and total weight loss followed by four and fourteen days storage period, respectively.

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 three or six hours and stored for fourteen days had higher weight loss percentage, during storage, as compared to non-heated eggs at 80 or 85-weeks-old breeders. Results of total egg weight loss percentage indicated that eggs stored for 14 days were influenced by heating duration, eggs heated for six or three hours had higher weight loss percentage as compared to non-heated eggs at 75, 80 and 85-weeks-old breeders. These results were observed because exposure to long-time storage and heat treatment would increase the opportunity for water evaporation from the eggs. These findings are in agreement with that of Silva et al. (2008) and Reijrink et al. (2010). Moreover, Fasenko and O'Dea (2009) reported that pre-heating eggs for long periods

Table 2. Interaction effect of pre-storage heating period, breeder's age and storage period on egg weight losses and chick yield.

	Breeder's Age (Week)														
	Fresh Egg Weight (g)			Egg Weight Loss During Storage (%)			Egg Weight Loss During Incubation (%)			Total Weight Loss (%)			Chick Yield (%)		
	75	**80**	**85**	**75**	**80**	**85**	**75**	**80**	**85**	**75**	**80**	**85**	**75**	**80**	**85**
0 h × 4 days	64.9	65.0	65.3	0.9^{e}	0.8^{f}	0.8^{d}	11.2^{b}	12.5^{bc}	13.1^{bc}	12.1^{b}	13.3^{d}	13.8^{c}	68.1^{abc}	64.7^{b}	70.6^{a}
3 h × 4 days	65.3	64.9	65.0	1.8^{a}	1.2^{e}	0.8^{d}	8.5^{f}	12.5^{bc}	13.8^{a}	10.3^{e}	13.7^{b}	14.6^{b}	68.1^{abc}	68.5^{a}	67.2^{b}
6 h × 4 days	65.1	65.3	64.9	1.1^{de}	1.2^{e}	1.4^{b}	7.9^{g}	12.8^{b}	13.4^{b}	9.0^{f}	14.1^{b}	14.8^{b}	68.7^{abc}	64.8^{b}	70.8^{a}
0 h × 9 days	65.1	65.2	64.9	1.2^{cd}	1.6^{c}	1.4^{b}	9.8^{e}	13.9^{a}	10.4^{d}	11.0^{d}	15.5^{a}	11.8^{d}	69.3^{ab}	67.7^{a}	68.1^{b}
3 h× 9 days	65.3	64.8	65.0	1.5^{b}	1.4^{d}	1.3^{c}	7.6^{h}	11.9^{d}	10.4^{d}	9.1^{f}	13.3^{d}	11.7^{d}	70.0^{a}	67.4^{a}	69.2^{ab}
6 h× 9 days	64.8	65.0	65.2	1.1^{de}	1.2^{e}	1.2^{c}	10.1^{d}	12.4^{c}	10.6^{d}	11.2^{cd}	13.7^{c}	11.8^{d}	68.6^{abc}	64.1^{b}	66.5^{c}
0 h × 14 days	64.9	64.8	64.8	1.4^{bc}	3.3^{b}	3.4^{a}	10.0^{e}	10.1^{e}	13.1^{bc}	11.3^{c}	13.6^{cd}	16.5^{a}	65.6^{d}	61.5^{c}	0.0^{d}
3 h× 14 days	65.0	65.1	65.1	1.9^{a}	3.5^{a}	3.9^{a}	10.5^{c}	12.3^{c}	12.7^{c}	12.4^{b}	15.8^{a}	16.5^{a}	66.9^{cd}	62.0^{c}	68.0^{b}
6 h× 14 days	65.4	65.3	65.3	1.5^{b}	3.6^{a}	3.6^{a}	11.6^{a}	12.5^{bc}	13.4^{b}	13.1^{a}	15.8^{a}	16.7^{a}	67.7^{bc}	59.2^{d}	67.7^{b}
SEM	0.247	0.268	0.227	0.063	0.210	0.236	0.259	0.188	0.264	0.261	0.201	0.399	0.297	0.863	4.219
Significant	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean, Different superscript letters under the same factor in the same column means significant differences, NS=No significant differences, **=significant difference at $P \le 0.01$, *=significant difference at $P \le 0.05$.

increased weight loss. They later attributed this weight loss to the evaporation of moisture from eggs. The moisture loss is progressively enhanced by continued exposure of eggs to high temperatures. Similar results were observed by Petek and Dikmen (2004), who reported that egg weight losses during the storage were significantly increased by main effects of pre-storage incubation treatment and the length of egg storage. This result could be justified, since exposure to PRESI and along time of storage would increase water evaporation from the eggs.

Embryonic Mortality

Early, mid and late death of embryos and unhatched egg percentages were significantly influenced by the experimental treatments (Table 3). Pre-heating eggs for six hours resulted in significantly lower percentages of early, late and total unhatched eggs when compared to the non-heated eggs or heated for three hours. Early, mid and late death on shell and unhatched eggs were increased as breeder's age increased. Late death was not influenced by the storage periods. Higher percentages of early death and unhatched eggs were associated with longer egg storage period. When eggs were stored for 14 days, they had significantly ($P \le 0.01$) increased early, mid death and total embryonic mortality when compared to the other storage period groups (9 and 4 days). Table 4 shows that regardless of the storage period, pre-heating eggs for six hours resulted in significantly ($P \le 0.01$) lower percentages of early, late and total unhatched eggs at all ages when compared to the non-heated eggs or heated for three hours. The results indicated that pre-storage heating eggs for 6 h significantly decreased early embryonic mortality when eggs were stored for four, nine and fourteen days at 75, 80 and 85 weeks of age breeder's eggs. When eggs were stored for more than four days, total embryonic mortality rates were significantly ($P \le 0.01$) lower when eggs were pre-storage heated for six hours, as compared to those not heated or heated for nine hours at 75 as compared to 80 or 85-weeks-old breeder's eggs. Eggs stored for 4, 9 and 14 days and were heated for six hours prior to storage presented significantly lower total embryonic mortality and as compared to those not heated. The improvement in the incubation yield in pre-storage heating eggs for six hours, as compared to those not heated may be related to the embryos stage and the total number of viable embryonic cells, prior to storage.

The results related to pre-storage heating for long period stored eggs were in agreement with previous reports. 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. The stage of embryonic

Table 3. Effect of pre-storage heating period, breeder's age and storage period on embryonic mortality.

Main Factors	Early Death (%)	Mid Death (%)	Late Death (%)	Unhatched (%)
Overall mean	19.01	3.33	6.34	28.68
SEM	0.319	0.170	0.226	0.334
Heating (hours)				
0	26.05^{a}	4.07^{a}	8.40^{a}	38.52^{a}
3	18.27^{b}	2.84^{b}	6.05^{b}	27.16^{b}
6	12.72^{c}	3.08^{b}	4.57^{c}	20.37^{c}
SEM	0.552	0.294	0.391	0.579
Significant	**	*	**	**
Age (weeks)				
75	15.80^{c}	2.71^{c}	4.81^{c}	23.33^{c}
80	18.27^{b}	3.08^{b}	5.19^{b}	26.54^{b}
85	22.96^{a}	4.20^{a}	9.01^{a}	36.17^{a}
SEM	0.552	0.294	0.391	0.579
Significant	**	**	**	**
Storage (days)				
4	11.48^{c}	2.84^{b}	6.17	20.49^{c}
9	18.03^{b}	3.21^{ab}	6.05	27.28^{b}
14	27.53^{a}	3.95^{a}	6.79	38.27^{a}
SEM	0.552	0.294	0.391	0.579
Significant	**	*	NS	**

N=27/treatment, SEM=standard error of mean, Different superscript letters under the same factor in the same column means significant differences. NS=No significant differences, **=significant difference at P≤0.01, *=significant difference at P≤0.05.

Table 4. Interaction effects of pre-storage heating period, breeder's age and storage period on embryonic mortality percentage.

	Embryonic Mortality (%) at Different Breeder's Age (Week)											
	Early Death %			Mid Death %			Late Death (%)			Un-hatched (%)		
	75	80	85	75	80	85	75	80	85	75	80	85
0 h × 4 days	13.3^{d}	16.7^{cd}	15.6^{e}	3.3	3.3	3.3^{bc}	6.7	5.6^{abc}	10.0^{ab}	23.3^{cd}	25.6^{c}	28.9^{d}
3 h × 4 days	10.0^{de}	12.2^{de}	12.2^{ef}	2.2	2.2	3.3^{bc}	6.7	3.3^{c}	10.0^{ab}	18.9^{de}	17.8^{de}	25.6^{d}
6 h × 4 days	5.6^{e}	8.9^{e}	8.9^{f}	2.2	2.2	3.3^{bc}	3.3	2.2^{c}	7.8^{bc}	11.1^{f}	13.3^{e}	20.0^{e}
0 h × 9 days	26.7^{b}	20.0^{bc}	31.1^{bc}	3.3	3.3	5.6^{ab}	5.6	8.9^{a}	12.2^{a}	35.6^{b}	32.2^{b}	48.9^{b}
3 h× 9 days	13.3^{d}	16.7^{cd}	22.2^{d}	2.2	3.3	2.2^{c}	4.4	5.6^{abc}	5.6^{c}	20.0^{cde}	25.6^{c}	30.0^{d}
6 h× 9 days	7.8^{de}	11.1^{e}	13.3^{e}	3.3	3.3	2.2^{c}	2.22	4.4^{bc}	5.6^{c}	13.3^{ef}	18.9^{d}	21.1^{e}
0 h × 14 days	33.3^{a}	36.7^{a}	41.1^{a}	3.3	4.4	6.7^{a}	6.7	7.8^{ab}	12.2^{a}	43.3^{a}	48.9^{a}	60.0^{a}
3 h× 14 days	20.0^{c}	23.3^{b}	34.4^{b}	2.2	2.2	5.6^{ab}	4.4	5.6^{abc}	8.9^{b}	26.7^{c}	31.1^{b}	48.9^{b}
6 h× 14 days	12.2^{d}	18.9^{bc}	27.8^{c}	2.2	3.3	5.6^{ab}	3.3	3.3^{c}	8.9^{b}	17.8^{def}	25.6^{c}	42.2^{c}
SEM	1.775	1.595	2.115	0.309	0.247	0.382	0.482	0.514	0.528	2.220	1.969	2.661
Significant	**	**	**	NS	NS	**	NS	*	**	**	**	**

N=27/treatment, SEM=standard error of mean, Different superscript letters under the same factor in the same column means significant differences. NS=No significant differences, **=significant difference at P≤0.01, *=significant difference at P≤0.05.

development 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 h may be considered as a good practice to improve incubation results. 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 by Fasenko et al. (2001a), after their pre-storage heating treatment of 6 h, 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

Table 5. Effect of pre-storage heating period, breeder's age and storage period on fertility and hatchability percentage.

Main Factors	Fertility and Hatchability Percentage		
	True Fertility	Hatchability from Total Eggs	Hatchability from Fertile Eggs
Overall mean	67.46	38.77	56.54
±SEM	0.436	0.393	0.426
Heating (hour)			
0	67.07	28.15^{c}	41.05^{c}
3	67.78	40.99^{b}	59.39^{b}
6	67.53	47.16^{a}	69.17^{a}
±SEM	0.755	0.680	0.738
Significant	NS	**	**
Age (weeks)			
75	73.83^{a}	50.87^{a}	68.87^{a}
80	67.16^{b}	40.25^{b}	59.67^{b}
85	61.40^{c}	25.18^{c}	41.07^{c}
±SEM	0.755	0.680	0.738
Significant	**	**	**
Storage period (day)			
4	67.16	47.04^{a}	69.43^{a}
9	67.90	40.25^{b}	58.72^{b}
14	67.32	29.01^{c}	41.46^{c}
±SEM	0.755	0.680	0.738
Significant	NS	**	**

N=27/treatment, SEM=standard error of mean, Different superscript letters under the same factor in the same column means significant differences **=significant difference at P≤0.01, NS=No significant differences.

completed hypoblast formation, and cell migration and differentiation are minimal (Bellairs, 1986). Petek and Dikmen (2004) observed that pre-storage warming of poultry eggs resulted in more live chicks and lower level of embryonic mortality. In quail eggs, 7 h of pre-storage warming for two days stored eggs as a short-term storage period, improved hatchability percentage as it decreased embryonic mortality rate (Abdel-Azeem,2009). These findings were consistent with the findings of Petek and Dikmen (2006) who indicated that total embryonic mortality rate during incubation was significantly affected by pre-storage incubation warming and egg storage periods. They found that embryonic mortality of eggs of 5 h pre-storage incubation warming was lower compared to the control group (0 h). Atif et al. (2015) showed that warming hatching eggs of White Hisex breeders at 37.5°C for four hours before storage improved hatchability reduced embryonic mortality and increased the percentage of first-grade chicks.

Fertility and Hatchability

The results of the true fertility, hatchability of total and fertile eggs are shown in Table 5. There were no significant effects of the pre-storage heating duration (0, 3 and 6 h), storage period (4, 9 and 14 days) on the true fertility percentage. On the other hand, true fertility percentage was significantly affected due to the breeder's age. Fertility and hatchability decreased as the age of the breeder stock advanced (P ≤ 0.01). Hatchability was improved when the period of the pre-storage heating period increased (P ≤ 0.01). Deterioration in hatchability has been reported when the period of storage increased (P ≤ 0.01). Table 6 shows that the true fertility percentage was not affected by the interaction of pre-storage heating duration, breeder's age and storage period. 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) who showed that fertility of broiler breeder eggs was not affected by the interaction as fertilization. The lower percentage fertility of the eggs stored for 14 days and pre-storage incubation for 18 h occurred as a result of an underestimation of fertility, germinal discs that were fertile, but had died very early during development were likely misclassified as infertile.

This overestimation of infertility occurred because of the difficulty in distinguishing between fertile germ and embryos that died at very early stages of development. Petek and Dikmen (2004) found that the pre-storage incubation treatments or the interaction with the duration of the storage period did not significantly affect apparent fertility. In all ages of the breeder stock, the best hatchability was observed with 6 h pre-storage heating and 4 days of storage period.

The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 weeks old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. The current results revealed that egg storage

Table 6. Interaction effect of pre-storage heating period, breeder's age and storage period on fertility and hatchability percentage.

	Fertility and Hatchability Percentage at Different Breeders Age (Week)								
	True Fertility			**Hatchability from Total Eggs (%)**			**Hatchability from Fertile Eggs (%)**		
	75	**80**	**85**	**75**	**80**	**85**	**75**	**80**	**85**
0 h × 4 days	73.3	67.8	60.0	50.0^{b}	42.2^{bcd}	31.1^{b}	68.1^{cd}	62.3^{c}	51.9^{c}
3 h × 4 days	74.4	66.7	60.0	58.9^{a}	48.9^{ab}	34.4^{b}	79.5^{ab}	73.3^{b}	57.4^{b}
6 h × 4 days	73.3	67.8	61.1	62.2^{a}	54.4^{a}	41.1^{a}	84.8^{a}	80.2^{a}	67.4^{a}
0 h × 9 days	71.1	70.0	63.3	35.6^{c}	34.4^{e}	14.4^{d}	50.2^{e}	49.2^{d}	22.8^{e}
3 h× 9 days	75.6	67.8	61.1	55.6^{ab}	42.2^{bcd}	31.1^{b}	73.6^{bcd}	62.3^{c}	51.0^{c}
6 h× 9 days	73.3	66.7	62.2	60.0^{a}	47.8^{abc}	41.1^{a}	81.9^{ab}	71.5^{b}	66.1^{a}
0 h × 14 days	73.3	64.4	60.3	30.0^{c}	15.6^{f}	0.0^{e}	41.0^{f}	24.1^{e}	0.0^{f}
3 h× 14 days	76.7	66.7	61.1	50.0^{b}	35.6^{de}	12.2^{d}	65.1^{d}	52.5^{d}	19.8^{e}
6 h× 14 days	73.3	66.7	63.3	55.6^{ab}	41.1^{cde}	21.1^{c}	75.7^{abc}	61.7^{c}	33.3^{d}
SEM	0.748	0.634	0.663	2.170	2.163	2.634	2.841	3.119	4.324
Significant	NS	NS	NS	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences. NS=No significant differences **=significant difference at P≤0.01, *=significant difference at P≤0.05.

for more than four or nine days markedly impaired incubation results due to higher egg weight loss, as shown by the lower hatchability; higher total embryonic mortality percentage. These results are in agreement with previous reports on broiler breeder's eggs (Fasenko, 2007; 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 the storage period increased. Heating the eggs for six hours before storage may be considered as a good practice to improve incubation results of eggs stored for short, intermediate and long periods. Also, pre-heating the eggs for six hours resulted in the highest average hatchability when stored for four or nine days as compared to eggs stored for 14 days at 75 as compared to 80 or 85-weeks-old breeder's eggs. These results agree with Lotfi et al. (2011) who found that warming quail eggs for short-term before storage increased total hatchability and decreased incubation length without any negative effect on chick quality. These reports indicated that hatchability was improved by pre-storage warming of hatching eggs. Lourens et al. (2006) confirmed a positive effect of pre-storage warming time on the hatchability of broiler breeder eggs. In quail eggs, seven hours of pre-storage warming for two days stored hatching eggs as a short-term storage period, improved hatchability percentage as it decreased embryonic mortality rate (Abdel-Azeem, 2009).

Chick Quality

Commercial chick quality grades were used for measuring chick quality. Chick quality grades were significantly affected by pre-storage heating duration, breeder's age and storage period (Table 7). Pre-storage heating of eggs for six hours resulted in significant (P ≤ 0.01) improvement in both chick quality grades followed by pre-storage heating of eggs for three hours, as compared to non-heated eggs. Egg produced from 75-weeks-old breeders resulted in significant (P ≤ 0.01) improvement in the chick quality grades compared to those produced from 80 and 85-weeks-old breeders. First-grade chick's percentage was significantly (P ≤ 0.01) decreased by the increased storage period, whereas second-grade chick's percentage was significantly increased. 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 showed a reduction in growth rate, 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 showed a reduction in the rate of metabolism than those from eggs stored for a shorter period (Fasenko and Robinson, 2001) and a decline in relative lung weight (Yalçin and Siege, 2003) which resulted in poor chick quality. Significantly (P ≤ 0.01) higher percentage of first-grade chicks obtain from eggs for four days followed by those stored for nine days while the lower percentage obtained from eggs stored for 14 days, respectively. There were significant interactions between the storage period and pre-storage incubation duration for chicks' grade (Table 8). The obtained data indicated that the chicks produced from heated eggs for six hours and stored for 4to 14 days at 75, 80 and 85 weeks of age breeder's eggs, respectively had higher percentages of grade A chicks. The significant improvement in grade A chicks' percentage in the six hours heating group, as compared to three hours heating group was observed, when eggs were stored for four, nine or fourteen days at

Table 7. Effect of pre-storage heating period, breeder's age and storage period on chick quality.

	Chick Quality Percentage	
Main Factors	**1st Grade**	**2nd Grade**
Overall mean	84.72	15.28
±SEM	0.817	0.817
Heating (hour)		
0	67.45^{c}	32.55^{a}
3	90.84^{b}	9.16^{b}
6	95.88^{a}	4.12^{c}
±SEM	1.414	1.414
Significant	**	**
Age (weeks)		
75	91.14^{a}	8.86^{c}
80	86.52^{b}	13.48^{b}
85	76.51^{c}	23.49^{a}
±SEM	1.414	1.414
Significant	**	*
Storage period (day)		
4	90.52^{a}	9.48^{b}
9	90.21^{a}	9.79^{b}
14	73.45^{b}	26.55^{a}
±SEM	1.414	1.414
Significant	**	**

N=27/treatments=standard error of mean. Different superscript letters under the same factor in the same column means significant differences. **=significant difference at P≤0.01, *=significant difference at P≤0.05.

Table 8. Interaction effect of pre-storage heating period, breeder's age and storage period on chick quality.

	Chick Quality Percentage at Different Breeders' Age (Week)					
	1st Grade			2nd Grade		
	75	80	85	75	80	85
0 h × 4 days	87.0^{bcd}	86.9^{b}	71.1^{b}	13.0^{abc}	12.5^{bc}	13.1^{bc}
3 h × 4 days	96.3^{ab}	95.5^{ab}	87.0^{ab}	3.7^{cd}	12.5^{bc}	13.8^{a}
6 h × 4 days	98.0^{a}	98.1^{a}	94.7^{a}	2.0^{d}	12.8^{b}	13.4^{b}
0 h × 9 days	84.2^{cd}	73.9^{c}	83.3a^{b}	15.8^{ab}	13.9^{a}	10.4^{d}
3 h× 9 days	94.0^{abc}	94.8^{ab}	92.6^{a}	6.0^{bcd}	11.9^{d}	10.4^{d}
6 h× 9 days	96.4^{ab}	97.9^{a}	94.7^{a}	3.6^{cd}	12.4^{c}	10.6^{d}
0 h × 14 days	77.2^{d}	43.3^{d}	0.0^{c}	22.8^{a}	10.1^{e}	13.4^{b}
3 h× 14 days	90.9^{abc}	90.9^{ab}	75.6^{ab}	9.1^{bcd}	12.3^{c}	12.7^{c}
6 h× 14 days	96.2^{ab}	97.2^{a}	89.7^{ab}	3.8^{cd}	12.5^{bc}	13.1^{bc}
SEM	1.564	3.427	5.755	1.564	0.188	0.264
Significant	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences. **=significant difference at P≤0.01.

75, 80- and 85-weeks old breeder's eggs, respectively. These results are in accordance with Reijrink et al. (2009) who suggested that pre-storage warming can affect the chick quality positively or negatively depending on the duration of pre-storage incubation. Marandure et al. (2012) found that pre-incubation of broiler breeder hatching eggs significantly improved hatchability and post-hatch chick uniformity. Atif et al. (2015) showed that warming hatching eggs of White Hisex breeders at 37.5°C for four hours before storage improved hatchability reduced embryonic mortality and increased the percentage of first-grade chicks. In conclusion, pre-storage heating of late DeKalb breeder's (75 weeks old) eggs for six hours at 37.5°C and 53% RH and storage period 4 to 9 days improved hatchability, decreased embryonic mortality percentage and increased the number of saleable first-grade chicks which by far increases profits.

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