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Influence of Egg Pre-storage Heating Period and Storage Length on Incubation Results

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ABSTRACT

This experiment aimed at evaluating the influence of different heating times of settable eggs of Cobb 500® broiler breeders before submitting them to different storage periods on egg weight loss, embryo mortality, and hatchability. A total number of 1,980 eggs were distributed in a completely randomized experimental design with a 3 x 3 factorial arrangement, comprising nine treatments with 22 replicates of 10 eggs each. The following factors were analyzed: pre-storage heating periods (0, 6, 12 hours at 36.92°C) and storage periods (4, 9, 14 days at 12.06°C). After storage, eggs were incubated under usual conditions, and were transferred to the hatcher at 442 hours of incubation. Eggs were weighed before heating, incubation, and transference to determine weight loss. Partial hatchability was determined at 480 hours, and total hatchability at 498 hours of incubation. Embryo mortality was determined in non-hatched eggs. It was concluded that heating eggs for six hour before storage improves incubation results as it decreases incubation length and late embryo mortality, therefore its use can be indicated in commercial operations. Storing eggs for 14 days and pre-heating for 14 days and pre-heating for 12 hours severely impair incubation results, and therefore are not recommended.

INTRODUCTION

A common practice in commercial breeder farms and hatcheries is to store eggs from one to four days to obtain the amount of eggs required for the normal flow of the incubation process. However, sometimes eggs need to be stored for longer than a week. Optimal egg storage conditions depend on the proper control of environmental temperature and air relative humidity, air renewal, turning frequency, breeder flock age, breeder genetic line, time of egg collection, and storage time. Some of these factors present interactions, and further studies are required to determine the optimal egg storage conditions (Fasenko *et al.*, 1992; Meijerhof *et al.*, 1994; Ruiz & Lunam, 2002; Tona *et al.,* 2003).

Longer storage periods lead to longer incubation, impairing embryo development and livability, hatchability, and chick quality and weight (Christensen *et al.*, 2002; Ruiz & Lunam, 2002; Elibol *et al.*, 2002; Tona *et al.*, 2003). These conditions result from the deterioration of internal egg quality. Albumen pH increases with storage time, and this effect is more pronounced in young breeder eggs (Lapão *et al.*, 1999). Albumen height decreases with storage time and breeder age (Lapão *et al.*, 1999) and yolk sac membrane elasticity decreases with storage time (Jones & Musgrove, 2005). Embryo mortality during storage is highly related with egg weight loss, and increases with storage time (Fasenko *et al.*, 1992; Nahm, 2001).

Before lay, the embryo blastoderm starts to differentiate in two germ layers, starting gastrulation (Gonzales & Cesario, 2003). As eggs remain at room temperature after lay, embryos stop developing at a stage characterize by the complete formation of zona pellucida, but later, as temperature and relative humidity increase during incubation, embryo development is resumed (Fasenko *et al.*, 2001a). However, there is higher embryo livability and hatchability, and shorter incubation when hypoblast stage is achieved before long storage periods. Before storage, heating chicken eggs for six hours (Fasenko *et al.*, 2001a) or turkey eggs for 12 hours (Fasenko *et al.*, 2001b) allows the complete formation of hypoblast. Therefore, pre-storage heating could improve the incubation results of eggs stored for long periods of time (Kraszewska-Domanska & Pawluczuk, 1977; Fasenko *et al.*, 2001a,b; Renema *et al.*, 2006).

An experiment was carried out to evaluate the effects of 0, 6, or 12 hours of heating fertile eggs of broiler breeders before 4, 9, or 14 days of storage on egg weight loss, embryo mortality, incubation length, and hatchability.

MATERIAL AND METHODS

A total number of 2,130 fertile eggs produced by a 44-week-old Cobb 500® breeder flock was collected in a commercial farm located in the region of São Carlos, SP, Brazil, and transported to the experimental hatchery. Out of the total number of collected eggs, 1,980 eggs were distributed in a completely randomized experimental design in a 3 x 3 factorial arrangement, with three pre-storage heating periods (0, 6, and 12 hours at 36.92°C) and three storage periods (4, 9, and 14 days at 12.06°C), summing up nine treatments with 22 replicates of 10 eggs each. Eggs were collected directly from the nest during the second daily collection in order to ensure lay time uniformity. In order to incubate all eggs at the same time, eggs were collected in three five-day intervals, representing the three storage periods (4, 9, and 14 days). Therefore, the first collection corresponded to eggs stored for 14 days; the second to eggs stored for nine days; and the third to eggs stored for four days. Immediately after collection, eggs were disinfected by simple fumigation with 7.7g paraformaldehyde/m^3 for 15 minutes, and transported to the experimental hatchery (Poultry Sector, University of São Paulo, Piracicaba, SP), where they were submitted to simple fumigation with 20mL liquid formaldehyde for 20 minutes, 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. Out of the 710 eggs obtained in each collection, 50 were selected at random to evaluate internal and external egg quality, using weight, apparent density, eggshell thickness, and albumen, yolk, and eggshell percentages.

Pre-storage heating for six and 12 hours (depending on treatment) was carried out in an Avimatic® with 14.400-egg capacity at an average temperature of 36.92°C and relative air humidity of 61.33%. After heating, eggs were kept at room temperature for one hour. Eggs were then stored in a chamber at 12.06°C and 76.5% relative air humidity for the periods corresponding to the treatments. After storage, eggs were maintained at 21.33°C and 75.6% air relative humidity for five hours before incubation. Eggs were incubated in an Avimatic® incubator for 442 hours (18 days and 10 hours) at 37.61°C average temperature and 55.9% relative air humidity. Eggs were turn hourly. Eggs were fumigated with paraformaldehyde (15g for 15 minutes) at 12 hours and seven and 14 days of incubation. At 442 hours of incubation, eggs were transferred to an Avimatic® hatcher at 36.37°C average temperature and 60.7% average air relative humidity. When eggs were transferred from the incubator to the hatcher, the number of eggs with cracked shells was recorded to determine the percentage of pipped eggs.

All eggs were weighed before heating, incubation, and transference to the hatcher to evaluate the effects of treatments on egg weight loss.

In order to determine incubation length, newly-hatched chicks were removed from the hatcher in two periods, as chicks hatched. In some treatments, more than 50% of the chicks hatched at 20 days of incubation, and therefore these were removed in the first period, and hatching percentage at 480 hours was recorded for all treatments. Total hatching percentage was determined at 498 hours of incubation. Non-hatched eggs were submitted to embryo diagnosis to determine the percentages of early (1 to 7 days of embryo development), intermediate (8 to 14 days of embryo development), and late (15 to 21 days of embryo development) embryo mortality.

Data were verified as to the presence of outliers, variance homogeneity, and error normality. After these assumptions of the model were verified, data were submitted to analysis of variance using the General Linear Model procedure of SAS® version 8 (1999). When significantly different, means were compared by the test of Tukey at 5% probability. Pipped eggs and early, intermediate, and late embryo mortality did not comply with the error normality assumption, and were transformed [$Y=\sqrt{(Y+0.5)}$] to be submitted to analysis. Tables present the transformed data and the raw data are shown between parentheses.

RESULTS

Internal and external egg quality of the eggs evaluated in the present experiment was not different among the three different collection times (Table 1).

Pre-storage heating and storage duration interaction significantly ($p<0.0001$) affected egg weight loss during storage, incubation period, and total experimental period (storage + incubation) (Table 2). Egg weight loss increased as a function of storage period at any pre-heating period. Eggs stored for nine or 14 days also lost weight as a function of heating period. However, eggs stored for four days presented higher weight loss when heated for six and 12 hours as compared to non-heated eggs (Table 2). During incubation, egg weight loss was not different among heating periods when stored for nine and 14 days, but when stored for four days, weight loss was higher in eggs heated for 12 hours as compared to the non-heated eggs. In these eggs, weight loss increased as storage period increased. On the other hand, weight loss in eggs heated for 12 hours decreased as storage period increased, whereas eggs heated for six hours did not present weight loss differences as a function of storage period (Table 2). Egg weight loss measured during the entire experimental period (storage plus incubation) showed that eggs stored for nine and 14 days were not influenced by heating period. Eggs stored for four days and heated for six or twelve hours presented higher weight loss as compared to non-heated eggs. Weight loss of non-heated eggs was proportional to storage period, whereas eggs heated for six hours lost more weight as compared to those stored for 14 days, and those heated for 12 hours were not affected by storage period (Table 2).

Hatchability results are shown in Table 3. There was a significant effect of the interaction between experimental factors on hatchability at 480 hours ($p<0.0001$). In addition, this parameter decreased as storage period increased, independent of heating period. When eggs were stored for four days, heating for six and 12 hours promoted higher hatchability as compared to non-heated eggs. Eggs stored for nine and 12 days presented higher hatchability when heated for six hours as compared to non-heated eggs or those heated for 12 hours (Table 3).

Table 2 - Interaction between factors on egg weight loss percentage during storage, incubation, and total period.

Heating (hours)	Storage (days)		
	4	9	14
	During storage		
0	0.25 Bc	0.59 Cb	0.93 Ca
6	0.46 Ac	0.75 Bb	1.03 Ba
12	0.49 Ac	0.90 Ab	1.14 Aa
CV (%)		8.62	
	During incubation		
0	9.77 Bb	10.07 Aab	10.21 Aa
6	10.14 ABa	9.93 Aa	10.10 Aa
12	10.30 Aa	9.93 Aab	9.86 Ab
CV (%)		6.38	
	Total period: storage+incubation		
0	10.00 Bc	10.60 Ab	11.04 Aa
6	10.55 Ab	10.61 Ab	11.03 Aa
12	10.75 Aa	10.74 Aa	10.89 Aa
CV (%)		6.24	

Means followed by the same letters – capital letters in the same column, and small letters in the same row – are not different by the test of Tukey ($p>0.05$).

Table 3 - Effect of the interaction among factors on the percentage of partial hatching at 480 hours e and total hatching at 498 hours of incubation.

Heating (hours)	Storage (days)		
	4	9	14
	at 480 hours		
0	60.00 Ba	41.82 Cb	25.91 Bc
6	83.63 Aa	69.54 Ab	46.04 Ac
12	85.00 Aa	51.82 Bb	18.18 Bc
CV (%)		29.84	
	at 498 hours		
0	85.91 Aa	84.09 Aab	78.64 Ab
6	90.00 Aa	86.82 Aa	76.67 Ab
12	86.82 Aa	62.73 Bb	38.64 Bc
CV (%)		15.56	

Means followed by the same letters – capital letters in the same column, and small letters in the same row – are not different by the test of Tukey ($p>0.05$).

Table 1 - Internal and external egg quality profile of the eggs of the present experiment.

Egg collection	Egg weight (g)	%			Eggshell thickness (mm)	Apparent density (g/mL H_20)
		Albumen	Yolk	Shell		
1	66.29	61.10	29.79	9.11	0.43	1.0820
2	66.98	61.38	29.60	9.02	0.43	1.0821
3	66.57	61.63	29.52	8.85	0.43	1.0805
P>F	0.73	0.36	0.73	0.19	0.65	0.19
CV (%)	6.57	2.98	5.97	7.89	7.33	0.45

No significant difference was observed among means ($p>0.05$).

There was a significant effect (p<0.0001) of the interaction between heating and storage periods on total hatchability at 498 hours of incubation. Eggs stored for nine and 14 days presented lower hatchability when heated for 12 hours, whereas hatchability was not affected by heating period when eggs were stored for four days. Non-heated eggs had lower hatchability when stored for 14 days as compared to those stored for four days. Eggs heated for six hours presented higher hatchability when stored for four and nine days as compared to 14 days. The hatchability of eggs heated for 12 hours decreased as storage period increased (Table 3).

Intermediate embryo mortality (8 to 14 days) was not influenced by the experimental treatments (p>0.05) (Table 4). On the other hand, individual factors affected late embryo mortality (15 to 21 days) (p<0.005), with heating for six hours resulting in the lowest late embryo mortality (0.15%) and not heating, the highest (3.94%). Storage for 14 days led to higher late embryo mortality as compared to four-day storage (0.75%). Late embryo mortality of eggs stored for nine days (1.82%) was not significantly different from eggs stored for four or 12 days.

Table 4 - Embryo mortality of eggs submitted to different heating and storage periods.

Factor	Embryo mortality (%)		
	Early[1]	Intermediate	Late
Hours of heating (H)			
0	1.04 (6.82)[2]	0.72 (0.30)	0.90 A (3.94)
6	1.11 (8.67)	0.73 (0.45)	0.72 C (0.15)
12	1.70 (29.09)	0.74 (0.61)	0.81 B (2.12)
Days of storage (S)			
4	1.04 (6.82)	0.72 (0.30)	0.75 B (0.91)
9	1.32 (14.85)	0.71 (0.15)	0.79 AB (1.82)
14	1.51 (22.91)	0.75 (0.91)	0.87 A (3.48)
Probability			
H	<0.0001	0.7236	<0.0001
S	<0.0001	0.0878	0.0047
H x S	<0.0001	0.6023	0.0677
CV (%)	28.76	14.78	26.83

Means followed by the same letters in the same column and for the same factor are not different by the test of Tukey (p>0.05). 1 - The observed interaction between factors is detailed in Table 5. 2 - Data were transformed for analysis [Y= √ (Y+0.5)]. Non-transformed data are presented between parentheses.

A significant interaction (p<0.0001) was detected between the experimental factors on early embryo mortality (1 to 7 days) (Table 5), showing that heating for 12 hours increased early embryo mortality as storage period increased. Non-heated eggs and those heated for six hours did not present differences in early embryo mortality, independent of storage period. There was no difference in the early embryo mortality of eggs stored for four days, independent of heating period, whereas those stored for nine and 14 days presented higher early embryo mortality when heated for 12 hours (Table 5).

Table 5 - Effect of the interaction between factors on early embryo mortality (1-7 days of embryogenesis).

Hours of heating	Days of storage		
	4	9	14
0	1.10 Aa (8.18)[1]	1.05 Ba (7.27)	0.96 Ba (5.00)
6	0.98 Aa (5.45)	1.11 Ba (9.09)	1.21 Ba (11.46)
12	1.03 Ac (6.82)	1.78 Ab (28.18)	2.35 Aa (52.27)
CV (%)		73.15	

Means followed by the same letters – capital letters in the same column, and small letters in the same row – are not different by the test of Tukey (p>0.05). 1 - Data were transformed for analysis [Y= √(Y+0.5)]. Non-transformed data are presented between parentheses.

The percentage of pipped eggs at the transference from the incubator to the hatcher was influenced by the interaction between the experimental factors (Table 5). Pre-storage heating increased that percentage, except for eggs stored for 14 days. Also, storage period had no effect on pipped egg percentage when eggs were not heated. Heating for six hours resulted in the lowest pipped egg percentage of eggs stored for nine and 14 days, whereas when eggs were heated for 12 hours pipped egg percentage decreased as storage period increased (Table 6).

Table 6 - Percentage of pipped eggs at transference.

Hours of heating	Days of storage		
	4	9	14
0	0.82 Ba (2.27)[1]	0.80 Ba (1.82)	0.82 Aa (2.27)
6	1.32 Aa (15.00)	0.95 ABb (5.45)	0.75 Ab (0.91)
12	1.48 Aa (19.54)	1.11 Ab (9.54)	0.80 Ac (1.82)
CV (%)		137.17	

Means of transformed data followed by the same letters – capital letters in the same column, and small letters in the same row – are not different by the test of Tukey (p>0.05). 1 - Data were transformed for analysis [Y= √(Y+0.5)]. Non-transformed data are presented between parentheses.

DISCUSSION

The different collection times could influence internal and external egg quality, as egg weight and yolk percentage increase and albumen and eggshell percentages decrease as a function of broiler breeder age (Vieira & Moran, 1998), and these changes affect embryo development (Burke *et al.*, 1997; Peebles *et al.*, 2000). In the present study, egg external and internal quality was not influenced by collection time (Table 1), which evidences sampling consistence and that collection day did not directly or indirectly affect the evaluated parameter.

Embryo stage is not changed by storage period when storage temperature is below embryo development physiological zero (20 - 21°C). However, due to long storage, blastoderm degenerates, presenting vacuoli in the zona pellucida and stains in the yolk (Fasenko *et al.,* 1992). In the present experiment, eggs were heated before storage (0, 6, or 12 hours at 36.92°C), and were subsequently stored at 12.06°C, thereby ensuring that the embryo development stage achieved by heating was maintained throughout storage.

The experiment showed that storage extended for 14 days markedly impaired incubation results due to higher egg weight loss (Table 2), to longer incubation period, as shown by the percentage of pipped eggs at transference (Table 6) and the percentage of late embryo mortality (Table 4), as well as to lower hatchability (Table 3). Haque *et al.* (1996) observed lower embryo metabolic rate (oxygen consumption) 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 incubation period and in late embryo mortality found. In addition, when eggs were pre-heated for 12 hours, early embryo mortality increased (Table 5). The former results are consistent with the findings of Fasenko *et al*. (2001a), who observed lower hatchability and higher late embryo mortality of embryos stored for 14 days as compared to four days of storage. Early and late embryo mortalities usually occur during incubation. Early embryo mortality is due to high embryo susceptibility to metabolic or genetic errors, or to stressing factors during the first stages of development (Boleli, 2003), whereas late embryo mortality is caused by adverse conditions during incubation, transference, or hatching (López de Alda, 2003).

Late mortality in the present study increased with the period of egg storage before hatching. Eggs stored for 14 days and pre-heated for six hours presented lower late embryo mortality (from 3.54% in non-heated eggs to 0.15%) and lower incubation length, with 46% of hatching in eggs heated for six hours, and only 25% of hatching in non-heated eggs. This effect was also observed with eggs were stored for four and nine days. Therefore, 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. In addition, pre-heating the eggs for six hours resulted in the highest average hatchability (90%) at 498 hours.

On the other hand, in the study of Fasenko *et al.* (2001a), incubation results of eggs pre-heated for 12 hours before storage for 14 days were similar to those of non-heated eggs stored for four days. These authors observed that six hours is the heating duration required to the complete formation of hypoblast in the embryo, and suggest a pre-storage heating period of chicken settable eggs between 0 and 2 hours. Conversely, in the present experiment, heating for 12 hours caused worse hatchability in eggs stored for nine or 14 days, probably because it took the embryos to a development stage after XIII EGK, which is the stage that promotes the highest embryo survival when eggs are submitted to long storage periods (Fasenko *et al*., 2001a; Malecki *et al*., 2005). In addition, eggs heated for 12 hours before nine and 14 days of storage presented high egg weight loss after storage, which may be related to their low hatchability. Long periods of storage may also increase the probability of bacterial contamination, as cuticle quality deteriorates (De Reu *et al*., 2006). Also, these embryos were more susceptible to temperature changes from storage to incubation, contributing to their higher early embryo mortality. These findings indicated that heating the eggs for 12 hours before storage was not a proper treatment for eggs stored for nine or 14 days.

Finally, it is important to highlight that, independent of heating period (0, 6, or 12 hours), storing the eggs for 14 days markedly impaired incubation results. Despite the positive effect of heating the egg for six hours before storage on late embryo mortality and incubation length, the results of eggs stored for 14 days for all studied parameters were always worse as compared to the other storage periods, and therefore this practice is not recommended.

CONCLUSIONS

Heating eggs for six hour before storage improves incubation results as it decreases incubation length and late embryo mortality, therefore its use may be indicated in commercial operations. Storing eggs for 14 days and pre-heating for 12 hours severely impair incubation results, and therefore, are not recommended.

REFERENCES

Boleli IC. Estresse, mortalidade e malformações embrionárias. In:

Macari M, Gonzales E, editores. Manejo da incubação. Campinas-SP: FACTA; 2003. p. 394-434.

Burke WH, Henry MH, Elezaj I. Comparison of embryo and chicks that developed as single individuals in double yolk eggs with those that developed in single yolk eggs. Poultry Science 1997; 76:901-907.

Christensen VL, Wineland MJ, Fasenko GM, Donaldson WE. Egg storage alters weight of supply and demand organs of broiler chicken embryos. Poultry Science 2002; 81:1738-1743.

De Reu K, Grijspeerdt K, Messens W, Heyndrickx M, Uyttendaele M, Debevere J, Herman L. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. International Journal of Food Microbiology 2006; 112:253-260.

Elibol O, Peak SD, Brake J. Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. Poultry Science 2002; 81:945-950.

Fasenko GM, Robinson FE, Hardin RT. Variability in preincubation embryonic development in domestic fowl. 2. Effects of duration of egg storage period. Poultry Science 1992; 71:2129-2132.

Fasenko GM, Robinson FE, Whelan AI, Kremeniuk KM, Walter JA. Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. Poultry Science 2001 a; 80:1406-1411.

Fasenko GM, Christensen VL, Wineland MJ, Petitte JN. Examining the effects of prestorage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. Poultry Science 2001b; 80:132-138.

Gonzales E, Cesario MD. Desenvolvimento embrionário. In: Macari M, Gonzales E, editores. Manejo da incubação. Campinas-SP: FACTA; 2003. p. 51-64.

Haque MA, Pearson JT, Hou P-CL, Tazawa H. Effects of pre-incubation egg storage on embryonic functions and growth. Respiration Physiology 1996; 103:89-98.

Jones DR, Musgrove MT. Effects of extended storage on egg quality factors. Poultry Science 2005; 84:1774-1777.

Kraszewska-Domanska B, Olszanska B, Pawluczuk B. Incorporation of uridine and leucine by the germinal discs of quail eggs kept cool or warmed periodically during storage. British Poultry Science 1977; 18(5):535-7.

Lapão C, Gama LT, Chaveiro Soares M. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poultry Science 1999; 78:640-645.

López de Alda TRB. Embrio-diagnóstico. In: Macari M, Gonzales E, editores. Manejo da incubação. Campinas-SP: FACTA; 2003. p. 51-64.

Malecki IA, Horbanczuk JO, Reed CE, Martin GB. The ostrich (*Struthio camelus*) blastoderm and embryo development following storage of eggs at various temperatures. British Poultry Science 2005; 46(6): 652-660.

Meijerhof R, Noordhuizen JPTM, Leenstra FR. Influence of pre-incubation treatment on hatching results of broiler breeder eggs produced at 37 and 59 weeks of age. British Poultry Science 1994; 35:249-257.

Nahm KH. Effects of storage length and weight loss during incubation on the hatchability of ostrich eggs (*Struthio camuelus*). Poultry Science 2001; 80:1667-1670.

Peebles ED, Gardner CW, Brake J, Benton CE, Bruzual JJ, Gerard PD. Albumen height and yolk and embryo compositions in broiler hatching eggs during incubation. Poultry Science 2000; 79:1373–1377.

Renema RA, Feddes JJR, Schmid KL, Ford MA, Kolk AR. Internal egg temperature in response to preincubation warming in broiler breeder and turkey eggs. Journal of Applied Poultry Research 2006; 15:1-8.

Ruiz J, Lunam CA. Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. British Poultry Science 2002; 43:374-383.

SAS Institute. SAS user's guide: statistics, version 8. Cary; 1999.

Tona K, Bamelis F, Ketelaere de B, Bruggeman V, Moraes VMB, Buyse J, Onagbesan O, Decuypere E. Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poultry Science 2003; 82:736-741.

Vieira SL, Moran Jr. Eggs and chicks from broiler breeders of extremely different age. Journal of Applied Poultry Research 1998; 7:339-346.