

# Effect of Flock Age, Length of Egg Storage, and Frequency of Turning During Storage on Hatchability of Broiler Hatching Eggs

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**ABSTRACT** Broiler hatching eggs from two young (30 and 31 wk) or two old (52 and 53 wk) breeder flocks were stored for 3, 7, or 14 d at 18 C and 75% RH while being turned 0, 4, or 24 times per day in two experiments. Apparent fertility and hatchability of total eggs differed with flock age in both experiments, but the effect was greater in Experiment 1. Hatchability of total and fertile eggs were decreased with flock age in Experiment 1 (31 and 52 wk flocks), but only hatchability of total eggs was decreased in Experiment 2. Hatchability of fertile eggs declined with length of storage period in both experiments with the most obvious effect observed in eggs

stored 14 d. There was an increase in percentages of early and late embryonic mortality with length of storage period in both experiments. Hatchability of fertile eggs was increased by turning 4 or 24 times per day during storage in Experiment 1 or by four times per day, with 24 times per day intermediate, in Experiment 2. There were no storage time × turning in storage interactions. A significant interaction of flock age × turning in storage for hatchability of fertile eggs in Experiment 1 suggested that eggs from an older broiler breeder flock that exhibit reduced fertility benefited more from turning during storage than did eggs from a young broiler breeder flock.

(Key words: turning, egg storage, hatchability, embryonic mortality, broiler hatching egg)

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## INTRODUCTION

Early reports by Waite (1919), Jackson (1921), and Funk (1934) did not recommend turning hatching eggs during storage prior to incubation. Subsequently, Funk and Forward (1951) found no advantage for turning chicken hatching eggs held 10 d or less. However, if eggs held 11 to 14 d were turned daily during the entire storage period, hatchability was 8% greater (Funk and Forward, 1951). Proudfoot (1966) also reported turning during storage to be beneficial when eggs were packed in the usual small end down position and stored for more than 2 wk. However, turning was not beneficial when eggs were packed small end up and stored for up to 4 wk. Becker et al. (1969) examined hatching egg storage periods of 1, 2, 3, 4, and 5 wk. The treatments were the absence of turning or turning cases 90° or 180° twice per day. The 90° turning treatment exhibited consistently higher hatchability of fertile and total eggs compared to the other two treatments for every storage period examined. The overall hatchability of fertile eggs was 53.7, 58.8, and 50.7 % for the no turning, 90°

turned, and 180° turned treatments, respectively. These data suggested that excessive turning during storage could be harmful and reflects the findings of Robertson (1961a,b) who found excessive turning during incubation per se could also be detrimental to hatchability. Bowman (1969) used eggs from a white egg hybrid and brown egg hybrid and found that turning during storage significantly reduced mortality at all stages of embryonic development and the advantage due to turning increased with longer storage. Increases in early and late embryonic mortalities associated with improper turning during incubation per se have been previously reported (Insko and Martin, 1933; Deeming et al., 1987). The literature has also clearly shown that good quality hatching eggs were less sensitive to decreased turning during incubation than poor quality eggs (Lundy, 1969; Wilson, 1991).

These previous reports suggest that some variability in response to hatching egg storage may be expected. However, hatching eggs from a current strain of broiler breeder should respond positively to turning during storage, and the response should be better when an older broiler breeder flock produces the eggs. Further, there should be evidence of an optimum turning frequency during storage. The objective of the present study was to examine the interaction of flock age, length of storage period, and turning frequency during storage on hatchability of fertile eggs and embryonic mortality of broiler hatching eggs.

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## MATERIALS AND METHODS

Hatching eggs were produced from flocks of Ross 308 feather-sexable strain females mated to Ross males. Males and females were grown sex-separate in light controlled facilities on an 8-hr photoperiod. The feeding and BW programs were as described by Ross Breeders (1998).

In the first experiment, hatching eggs were obtained from two flocks aged 31 and 52 wk. Eggs were stored for 3, 7, or 14 d at 18 C and 75% RH. Three turning treatments were used during storage. These were no turning, turning four times per day, or turning 24 times per day. The eggs were held in setter buggies and the turning angle was 90° as in the setter. The unturned eggs were held with the trays in a position horizontal to the floor with the large end of the eggs being up.

The second experiment was conducted as the first experiment, except that two different broiler breeder flocks aged 30 and 53 wk were used and the experiments were separated in time by 5 mo. In both experiments, an incubation tray of 150 eggs constituted a replicate. There were 64 trays in each trial. Treatment groups had four replicate trays, except for the 14-d storage treatments and the four turns daily for 7 d of storage treatment that had only three trays.

The machines used were a Petersime model 576 setter and a model 192 hatcher.<sup>3</sup> The setter was operated at 37.4 ± 0.2 C dry bulb temperature and 28.9 ± 0.2 C wet bulb temperature. The hatcher was operated at 37.2 ± 0.2 C dry bulb temperature and 30 ± 0.2 C wet bulb temperature. Machines were monitored by computer four times daily for proper operation. Trays representing all flock age-storage days-turning treatment combinations were distributed throughout all positions in the setter and hatcher to account for possible small machine position effects that could be due to differences in air flow. In other words, random trays representing all treatment combinations were equally placed in all areas of the machines.

At the time of removing the chicks from the hatchers, all unhatched eggs were opened and examined macroscopically by a single experienced individual to determine percentage fertility, percentage embryonic mortality [early (0 to 6 d), middle (7 to 17 d), late (18 to 21 d plus pipped)], and percentage hatchability of fertile and total eggs. Hatchability of fertile eggs was calculated as the number of chicks hatched per fertile egg set. The percentage middle dead embryos was low and showed no treatment effects, therefore these data were omitted for brevity. It has been reported that macroscopic evaluation to differentiate fertility and early embryonic mortality becomes more difficult with extended periods of storage (Landauer, 1967). Therefore, the fertility results were reported as "apparent fertility" and presented in concert with hatchability of fertile and total eggs and early dead mortality to provide an unbiased view of the data. The results for the incubation variables were analyzed by ANOVA with the general linear model procedure (SAS Institute Inc., 1990). Between-tray variation

(residual) was the source of the error term. Flock age, storage period, and turning treatments were the main effects.

## RESULTS AND DISCUSSION

The apparent fertility, hatchability, and embryonic mortality results of Experiment 1 are shown in Table 1. Apparent fertility was significantly decreased due to flock age but was not affected significantly by storage period or turning in storage. Hatchability of total and fertile eggs was also significantly decreased due to flock age (Table 1). The decrease in hatchability of fertile eggs was due primarily to an increase in percentage late dead embryos. Hatchability of total and fertile eggs also decreased in a time-dependent manner with increasing length of storage. This was due to increases in both percentage early and late dead embryos. Hatchability of fertile eggs was improved by turning 4 or 24 times daily during storage, but there was no obvious relationship to percentage early or late dead embryos. An increase in hatchability of total eggs was found as frequency of turning during storage increased. There were significant interactions of flock age × storage period for apparent fertility and hatchability of total eggs as well as interactions for flock age × turning in storage for apparent fertility, hatchability of total and fertile eggs, and percentage early dead embryos. The interaction means regarding apparent fertility and hatchability of total and fertile eggs are presented in Tables 3 and 4.

The apparent fertility, hatchability, and embryonic mortality results of Experiment 2 are shown in Table 2. Apparent fertility and hatchability of total eggs were decreased significantly by flock age in Experiment 2, as in Experiment 1, but to a lesser degree. However, hatchability of fertile eggs was not affected significantly by flock age in Experiment 2. As in Experiment 1, apparent fertility was not affected significantly by storage period or turning in storage in Experiment 2. Hatchability of total and fertile eggs was decreased by 14 d of storage due to increases in percentage early and late embryonic mortality (Table 2). Hatchability of total and fertile eggs were also improved by turning four times daily during storage compared to not turning (0), whereas turning 24 times per day produced intermediate results for both variables. Similar to the results of Experiment 1, there was no obvious relationship between the change in hatchability of total and fertile eggs due to turning during storage and percentage early or late dead embryos. The effects of turning during storage on each category of embryonic mortality were small and distributed evenly as was suggested previously by the work of Bowman (1969). There were significant interactions of flock age × storage period for apparent fertility, hatchability of fertile eggs, and percentage late dead. The interaction means for apparent fertility and hatchability of total and fertile eggs are presented in Table 3.

Interactions that affect apparent fertility can confound hatchability data and must be carefully considered. In the case of the interaction of flock age × storage period, inspection of the data shown in Table 3 reveals the interaction

<sup>3</sup>[www.petersime.com/](http://www.petersime.com/)

TABLE 1. The effect of flock age, length of storage period, and turning during storage on hatchability of fertile eggs and embryonic mortality of broiler hatching eggs in Experiment 1

Flock <sup>1</sup> age (wk)	Storage <sup>1</sup> period (d)	Turning <sup>1</sup> in storage (times/d)	Apparent fertility (%)	Hatchability (%)		Early dead (%)	Late dead (%)		
				Of total eggs	Of fertile eggs				
31	3	0	96.0	88.3	92.0	5.2	2.1		
		4	96.3	87.8	91.2	5.0	3.0		
	7	0	95.7	85.5	89.3	5.3	4.9		
		4	94.7	84.9	89.6	5.2	4.2		
	14	0	94.3	83.9	89.0	5.8	4.2		
		4	92.2	80.9	87.7	6.0	5.1		
	52	3	0	91.8	80.3	87.5	7.7	4.3	
			4	91.1	77.1	84.6	9.5	4.7	
		7	0	86.1	72.2	83.9	6.7	8.2	
			4	80.0	65.0	81.3	8.2	8.0	
		14	0	80.3 <sup>b</sup>	67.7 <sup>b</sup>	84.3 <sup>b</sup>	6.9	6.6 <sup>a</sup>	
			4	80.0	65.0	81.3	8.2	8.0	
		SE <sup>2</sup>	3	0	0.6	0.6	0.5	0.4	0.3
				4	86.7 <sup>a</sup>	77.9 <sup>a</sup>	89.8 <sup>a</sup>	5.3 <sup>b</sup>	3.5 <sup>b</sup>
7			0	88.2 <sup>a</sup>	76.9 <sup>a</sup>	87.1 <sup>b</sup>	5.7 <sup>b</sup>	5.5 <sup>ab</sup>	
			4	86.8 <sup>a</sup>	72.4 <sup>b</sup>	83.4 <sup>c</sup>	8.5 <sup>a</sup>	6.6 <sup>a</sup>	
14	0		0.7	0.8	0.6	0.5	0.4		
	4		86.8 <sup>a</sup>	74.1 <sup>b</sup>	85.4 <sup>b</sup>	7.2	5.8		
SE <sup>2</sup>	3	0	86.7 <sup>a</sup>	75.8 <sup>ab</sup>	87.4 <sup>a</sup>	6.3	4.7		
		4	88.2 <sup>a</sup>	77.2 <sup>a</sup>	87.6 <sup>a</sup>	6.0	5.2		
	14	0	0.7	0.8	0.6	0.5	0.4		
		4	86.8 <sup>a</sup>	74.1 <sup>b</sup>	85.4 <sup>b</sup>	7.2	5.8		

<sup>a-c</sup>Means with different superscripts differ significantly ( $P \leq 0.05$ ).

<sup>1</sup>Significant interactions: apparent fertility: flock age  $\times$  storage period, flock age  $\times$  turning in storage. Hatchability of total eggs: flock age  $\times$  storage period, flock age  $\times$  turning in storage. Hatchability of fertile eggs: flock age  $\times$  turning in storage. Early dead: flock age  $\times$  turning in storage.

<sup>2</sup>Pooled SE for main effects with 64 total trays of 150 eggs per trial. There were four replicate trays for all interaction means except three trays for the 14 d storage treatments and the four turns per day within 7 d of storage treatment.

to be due to a decline in apparent fertility at 14 d in the 31 wk flock combined with a low apparent fertility in the 3 d group at 52 wk in Experiment 1. The decline at 14 d in the younger flock is to be expected (Landauer, 1967) as the incidence of difficult to discern very early deaths should increase with prolonged storage. The low apparent fertility at 3 d in the 52 wk flock was probably due to an actual lower fertility in this group of eggs, as this effect was not observed elsewhere in the data and this flock did exhibit the lowest fertility observed in these experiments.

In the case of Experiment 2, the interaction of flock age and storage period appeared to be due to a slight decrease in apparent fertility in the 30 wk flock at 14 d of storage that was not observed in the 50 wk flock (Table 3). In any case, apparent fertility appeared to be quite similar among the treatment groups within each flock. With respect to the interaction of flock age  $\times$  turning in storage found in Experiment 1, inspection of Table 4 shows that the interaction was due to apparent fertility increasing with turning in storage in the 52 wk flock but not in the 31 wk flock. This result was consistent with the probable, but difficult to discern (Landauer, 1967), decrease in very early embryonic mortality due to turning in storage that was reflected in improved hatchability of total and fertile eggs (Table 4).

This interaction was not repeated in Experiment 2 in which overall fertility was better (Table 4). These data are consistent in many ways with reports that suggest a positive relationship between apparent fertility and hatchability of fertile eggs (Cooper and Rowell, 1958; McDaniel et al., 1981; Eslick and McDaniel, 1992). The existing literature demonstrates that embryonic mortality, early and late, is increased in the presence of lower fertility.

For the data from Experiment 1 (Table 4), turning 4 or 24 times daily in storage obviously decreased embryonic mortality in the older flock that had poorer fertility. Further, the pattern of treatment effects for hatchability of total and fertile eggs showed equal or greater differences than those due to any differences in apparent fertility. This result provided confidence that there were no spurious results due to disproportionate fertility among the various treatment groups and that the results of both experiments could be interpreted on the basis of hatchability of total and fertile eggs in concert with the embryonic mortality data.

Hatchability of total and fertile eggs decreased with increasing egg storage period, as percentage early and late embryonic mortality increased, as expected, in both experiments (Tables 1 and 2). This result was expected and consistent with previous reports (Mather and Laughlin, 1977;



TABLE 4. Interaction of flock age and turning in storage on apparent fertility and hatchability in Experiments 1 and 2

Experiment	Flock age (wk)	Turning in storage (d)	Apparent fertility <sup>1</sup> (%)	Hatchability (%)	
				Of total eggs <sup>1</sup>	Of fertile eggs <sup>1</sup>
1	31	0	94.6	84.9	89.7
		4	94.3	84.3	89.5
		24	93.5	82.8	88.7
	52	0	78.9	64.1	81.2
		4	79.2	67.5	85.3
		24	82.9	71.7	86.5
2	30	0	97.0	85.1	87.7
		4	97.9	89.1	90.9
		24	97.5	86.9	89.2
	53	0	93.3	81.9	87.8
		4	94.6	84.2	89.0
		24	93.6	82.0	87.6

<sup>1</sup>Significant interactions in Experiment 1 only. Data regarding Experiment 2 are presented for comparison only.

al., 1987; Deeming, 1989), but this appears to be most critical from 3 to 7 d of incubation.

The early work of Card (1926) suggested that turning very early in incubation was critical in that he reported that turning eggs for the first 6 d of incubation to be almost as effective as turning for 18 d of incubation. The work of Bowman (1969) and the present work demonstrate a reduction in all stages of embryonic mortality with turning during storage and extend the findings of Card (1926) earlier into the pre-incubation storage period. This conclusion is not unreasonable if one considers egg storage to be a form of low temperature incubation. Insight into the mechanism of this effect was given by Proudfoot (1966) who found improvements in hatchability due to turning in storage for eggs held large end up but not small end up. The small end up position would apply greater hydrostatic pressure directly on the shell membranes. This suggests a shell membrane-based mechanism that is somehow influenced by the changes in albumen during storage (Brake et al., 1997). Water loss and albumen degradation occur during storage and have been shown to have significant effects on embryonic mortality (Walsh et al., 1995; Brake et al., 1997). The shell membranes "dry" during the period following oviposition (Kutchai and Steen, 1971). Further, shell membrane thickness has been reported to increase with large end up storage (Benton, 1998), which suggests conformational changes in the proteins comprising the shell membranes. These changes could affect the manner in which the chorioallantoic membrane interacts with the inner shell membrane and develops. It is suggested that storage in the small end up position or adequate turning ameliorates the detrimental effects on the shell membranes of storage and facilitates normal development of the chorioallantoic membrane after normal incubation temperatures are achieved.

Hatchability of fertile eggs increased with increasing frequency of turning during storage, without clear relationship to any single stage of embryonic development, in both experiments (Tables 1 and 2). However, the general effect was for turning to reduce percentage early and late embryonic mortality relative to no turning. These data were con-

sistent with the prior findings of Bowman (1969) who found that turning during storage decreased all stages of embryonic mortality. The improvement in hatchability of fertile eggs was evident for 4 and 24 times per day turning in Experiment 1 (Table 1), in which fertility and hatchability of fertile eggs were also reduced with flock age, compared to a significant effect only with turning four times daily in Experiment 2. Data shown in Table 4 also reflect numerically poorer results from turning 24 times daily in the younger flock of Experiment 1. These data appear to somewhat reflect the results of Becker et al. (1969) who found improvement in hatchability with eggs turned 90° but not 180° for various lengths of storage. It is important to note that Robertson (1961a,b) also reported a decreased hatchability with excessive turning during incubation per se. Apparently, hatching eggs can be subjected to excessive turning during storage and incubation.

Lundy (1969) stated that good quality hatching eggs were less sensitive to decreased turning during incubation than poor quality hatching eggs as might be expected for eggs from young versus old flocks. Eggs with poor quality albumen or increased shell porosity (e.g., eggs from older flocks) would be expected to lose water vapor more rapidly and cause the albumen to change more rapidly as well (Brake et al., 1997). Wilson (1991), in his review, also stated that the reduced hatchability and increased malpositioned embryos reported as a result of failure to turn eggs during incubation was more detrimental to poor quality eggs. There was an interaction of flock age (hatching egg quality and fertility) × turning in storage with respect to apparent fertility and hatchability of total and fertile eggs due to turning being clearly more beneficial for eggs from the older flock with reduced fertility than with eggs from the younger flock in Experiment 1 (Table 4). Inspection of the data relating the non-significant interaction of flock age × turning in storage in Experiment 2 (Table 4) revealed a numerical increase in hatchability of fertile eggs for the older flock with turning four times daily during storage of a similar direction, if not magnitude, as that demonstrated in Experiment 1 (Table 1). With the exception of the older flock in Experiment 1, the best results in both

experiments appeared to be produced by turning four times daily during storage. These data also appear to show that turning hatching eggs four times daily during storage produced results superior to no turning or turning 24 times daily unless the overall fertility of the flock was low. The absence of a significant storage time  $\times$  turning in storage interaction in either experiment argues that there was no relationship between length of storage, which would degrade albumen quality, and turning frequency. This finding appears to differ somewhat from the finding that eggs of low quality are more sensitive to decreased turning during incubation per se (Lundy, 1969; Wilson, 1991).

It was concluded that turning broiler hatching eggs four times daily during pre-incubation storage was adequate to improve hatchability of fertile eggs in most cases, irrespective of flock age. The effect appeared to be greater when fertility was low and embryonic mortality was higher than normal.

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