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Influence of pre-incubation storage period on fertility, hatchability and embryonic mortality pattern of two pedigreed flocks of White Leghorn

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ABSTRACT

A study was conducted to assess the effect of length of pre-incubation storage on fertility, hatchability and mortality pattern of embryos in 2 White Leghorn breeding flocks aged from 67 to 80 weeks. Eggs from IWN strain (8478 eggs) and from IWP strain (8801 eggs) were utilized in 5 hatches in this experiment. All the traits except hatchability on total egg set and DIS differed with the strain. Fertility was significantly higher in IWN strain; while hatchability (on fertile eggs set) was significantly better in IWP strain. Compared to IWP, IWN strain showed significantly higher early embryonic death (EED), total embryonic mortality (TEM), weaklings (WL) and rotten eggs (RE); however, incidence of dead germs (DG) was significantly higher in IWP than IWN strain. Hatchability of total and fertile eggs had significant negative correlation with egg storage period in both the strains. DG and TEM had significant positive correlation with storage length in both the populations; while, EED had significant positive correlation with storage length in IWN strain alone. EED, DG and TEM had significant negative correlations with hatchability on both total and fertile eggs in both the strains. TEM had significant positive correlation with EED and DG in both the strains. Regression analysis revealed significant reduction of hatchability of total and fertile eggs over storage period (d) in both the strains. On the other hand DG and TEM significantly increased with increasing storage length in both the flocks.

Key words: Chicken, Embryonic mortality, Hatchability, Pre-incubation storage

Hatchability declines gradually with the storage of hatching eggs over an extended period even under optimum conditions (Schneider 2007). In experimental or nucleus flocks, hatching eggs are sometimes saved over a week period to reduce the number of hatches in pedigree breeding (Fairfull and Gowe 1987). Mostly studies examined the influence of pre-incubation storage periods on hatchability traits on intervals of more than 1 day (Elibol *et al*. 2002). Moreover, these parameters are poorly studied in White Leghorn populations undergoing selection for egg production, in which, pedigree hatching is carried out at very old age. Therefore, this study was designed to examine the influence of pre-incubation storage at daily intervals on fertility, hatchability and mortality pattern of embryos at different stages of development in 2 strains of White Leghorn during pedigree hatching.

MATERIALS AND METHODS

The hatching eggs of base populations of 2 strains of White Leghorn namely IWN and IWP were received from the ICAR in 1978. These strains were selectively bred for egg production based on part-record egg production to 40 weeks until 16th generation, to 60 weeks in 17th generation and to 64 weeks of age from 18th through 22nd generation at All India Co-ordinated Research Project on Poultry Improvement, Mannuthy, Kerala, India. Intra-population combined selection is being practiced using an index (Osborne 1957) based on information from individual and half-sib and full-sib families. Pressure for egg weight was included in the selection programme from third generation as an independent culling level.

Hatching eggs

The hatching eggs produced by the selected birds in both IWN and IWP strains of S22 generation were utilized in this study. A total of 8478 and 8801 hatching eggs for IWN and IWP strains, respectively, incubated in 5 hatches of 10 days interval, from 300 selected parents (aged between 67 and 80 weeks) for each population provided the data. All the birds were artificially inseminated every fifth day. The eggs were collected 4 times daily and were marked on the shell. Immediately after collection the eggs were dry cleaned with sterile cotton cloth, fumigated with 3× concentration of formaldehyde gas and stored in a cooler room at 18ºC with

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relative humidity of 60–70% until they were incubated together on day 10, thus forming 10 treatment groups with the length of storage period varying from 1-day to 10 d. During storage the eggs were placed with broad end up and were not turned. The setter was operated at 37.4±0.2ºC dry bulb and 28.9±0.2ºC wet bulb temperatures. The respective temperatures in the hatcher were 37.0±0.2ºC and 31±0.2ºC. The eggs in the setter were turned once in 1h. Eggs representing all the experimental groups were distributed in all positions in the setter and hatcher.

At the time of removing the chicks from the hatchers, all unhatched eggs were opened and examined macroscopically to determine the number of infertile eggs. Macroscopic evaluation to differentiate fertility and embryonic mortality becomes more difficult with extended periods of storage (Landauer 1967). Therefore, the fertility results were reported as 'apparent fertility'. Mortalities were calculated in 3 periods based on the stages of embryonic development (Morton *et al*. 1965), as a per cent to the total fertile egg set, as follows: *Early embryonic deaths* (EED): deaths on days 1 to 5; *dead germs* (DG): deaths on days 6 to 19; and *dead-in-shell* (DIS): deaths on days 20, 21 and those which failed to hatch. In addition, the *total embryonic mortality* (TEM) ascribable to different days of storage length was calculated as a per cent of total mortality including EED, DG and DIS to the fertile eggs incubated. The chicks which were live, piped but not hatched and those hatched but weak, undersized, poorly feathered, parrot beaked, micromelia, blind, lame, unhealed naveled were classified as '*weaklings*' (WL). The rotten egg (RE), if encountered on the break open study was also counted against different storage periods. Hatchability on fertile egg set (FES) was calculated as the ratio of number of healthy chicks hatched to the total number of fertile eggs incubated. Hatchability on total egg set (TES) was determined as the ratio of number of good chicks hatched to the total number of eggs incubated.

The data from all the 5 hatches were pooled separately for different length of storage period ranging from 1 to 10 days. All the data were analyzed for strain difference by ANOVA using a completely randomized design (CRD). Nature of relationship among fertility and different hatchability traits was examined by correlation. The time trend in fertility and different hatchability traits against storage period was examined by regressing these traits on storage duration in days (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The overall apparent fertility was significantly ($P<0.001$) higher in IWN (98.48%) than IWP (96.86%) strain (Table 1). Correlation of apparent fertility with length of

Table 1. Mean values for day-wise storage length and overall mean±SE values of fertility and different hatchability traits in IWN and IWP strains of White Leghorn

	Length of storage (d)	n	Fertility (%)	Hatchability (TES) (%)	Hatchability (FES) (%)	EED	DG	DIS	TEM	WL (%)	RE (%)
	1	827	98.55	84.04	85.28	6.63	2.33	4.17	13.13	1.23	0.37
	2	844	98.10	81.04	82.61	6.64	3.26	3.62	13.53	0.97	0.36
	3	843	97.75	82.80	84.71	7.16	3.16	2.79	13.11	1.21	0.49
	4	847	98.94	82.41	83.29	8.23	3.10	3.46	14.80	1.43	0.48
IWN	5	842	99.17	81.35	82.04	8.50	3.35	3.47	15.33	0.48	0.36
	6	861	97.68	79.79	81.69	9.39	3.92	3.09	16.41	1.90	0.59
	7	887	97.97	80.50	82.16	8.40	3.91	2.99	15.30	2.65	0.46
	8	842	98.69	82.19	83.27	9.87	3.25	3.25	16.37	3.01	0.72
	9	851	99.18	80.02	80.69	8.89	3.79	3.08	15.76	2.61	0.59
	10	834	98.80	79.98	80.95	8.25	4.37	4.73	17.35	2.06	0.24
	Overall	8478	98.48	81.40	82.66	8.20	3.45	3.46	15.12	1.76	0.47
			±0.13^{A}	±0.42	±0.41^{B}	±0.30^{A}	±0.20^{b}	±0.20	±0.39^{x}	±0.14^{A}	±0.07^{x}
IWP	1	877	97.49	84.72	86.90	6.20	3.04	2.69	11.93	1.05	0.12
	2	891	97.31	82.38	84.66	6.69	4.15	3.81	14.65	0.46	0.23
	3	861	97.10	83.16	85.65	6.94	2.87	3.83	13.64	0.48	0.24
	4	897	96.66	82.72	85.58	5.77	4.04	2.77	12.57	1.50	0.35
	5	875	97.49	84.11	86.28	5.63	3.52	3.28	12.43	1.17	0.12
	6	883	95.92	82.67	86.19	6.14	3.31	3.42	12.87	0.24	0.71
	7	850	97.29	82.94	85.25	6.05	3.87	3.63	13.54	0.97	0.24
	8	900	96.33	80.33	83.39	7.50	4.73	3.34	15.57	0.92	0.12
	9	884	96.95	80.32	82.85	7.23	5.13	4.08	16.45	0.47	0.23
	10	883	96.15	76.67	79.74	9.07	6.01	3.42	18.49	1.18	0.59
	Overall	8801	96.86	81.99	84.65	6.72	4.07	3.43	14.22	0.84	0.29
			±0.18^{B}	±0.41	±0.39^{A}	±0.27^{B}	±0.22^{a}	±0.20	±0.38^{y}	±0.10^{B}	±0.06^{y}

$^{x, y}$ Means bearing different superscripts within each column differ significantly ($P<0.05$); $^{a, b}$ means bearing different superscripts within each column differ significantly ($P<0.01$); $^{A, B}$ means bearing different superscripts within each column differ significantly ($P<0.001$)

Table 2. Estimates of correlation co-efficient (r) among fertility and different hatchability traits in IWN (above diagonal) and IWP (below diagonal) strains of White Leghorn

Traits	1	2	3	4	5	6	7	8	9	10
1		0.189	–0.787**	–0.814**	0.734*	0.820**	0.054	0.896**	–0.131	–0.068
2	–0.580		0.031	–0.288	0.274	–0.032	0.276	0.297	0.004	–0.110
3	–0.799**	0.634*		0.948**	–0.861**	–0.683*	0.435	–0.750*	–0.362	–0.019
4	–0.767**	0.481	0.983**		–0.911**	–0.644*	0.330	–0.812**	–0.349	0.016
5	0.600	–0.482	–0.914**	–0.922**		0.561	–0.345	0.839**	0.172	–0.208
6	0.785**	–0.441	–0.928**	–0.947**	0.774**		0.004	0.819**	–0.269	–0.255
7	0.360	0.021	–0.310	–0.353	0.330	0.251		0.123	–0.738	–0.486
8	0.746*	–0.443	–0.959**	–0.981**	0.933**	0.911**	0.497		–0.259	–0.442
9	0.041	0.120	–0.049	–0.088	–0.061	0.203	–0.703*	–0.086		0.598
10	0.353	–0.776**	–0.432	–0.305	0.287	0.233	0.035	0.259	–0.215	

Traits: 1, length of storage in days; 2, fertility (%); 3, hatchability on total eggs set (%); 4, hatchability on fertile eggs set (%); 5, early embryonic death (%); 6, dead germ (%); 7, dead-in-shell (%); 8, total embryonic mortality (%); 9, weaklings (%); 10, rotten eggs (%). *Significant (P<0.05); ** significant (P<0.01)

storage period in IWN and IWP strains were not significant (Table 2) in magnitude with varied direction indicating that the fertility is determined before lay and therefore storage length had no influence on it. Regression analysis also revealed insignificant change on storage in fertility status of both the strains. Our result was in conformity with the previously published results (Elibol *et al*. 2002). However, Mani *et al*. (2008) have shown decline in apparent fertility due to prolonged storage, which is attributable to the inability to discern very early deaths by visual examination, incidence of which could increase as the storage length is increased. Fertility had significant relationship of positive direction with TES and of negative direction with RE, but at large, for all other traits, it had nonsignificant association of inconsistent sign for strain. Though, Islam *et al*. (2002) observed positive significant correlation between fertility and hatchability on both total and fertile eggs in White Leghorn, the present study revealed such relationship only between fertility and hatchability on total egg set in IWP strain.

The overall hatchability on total egg set showed closer values (81.4 and 81.99% in IWN and IWP strains respectively) with no significant difference between both strains. However, the overall hatchability on fertile egg set was significantly (P<0.001) higher in IWP (84.65%) compared to that of IWN strain (82.66%). Similar to the present findings, significant effect due to genetic strain in Australorp was earlier reported by Yoo and Wientjes (1991). Hatchability of total and fertile eggs had significant negative association of very high magnitude with the length of preincubation egg storage. The significant negative association of hatchability of total and fertile eggs with EED, DG and TEM observed in the present study was in expected line and consistent with the results of Islam *et al*. (2002). Linear regression analysis revealed that the hatchability on total eggs set was significantly (P<0.01) affected by storage period in both the flocks. The hatchability in terms of fertile eggs set also reduced significantly (P<0.01) at the rate of 0.403% per day in IWN strain, while, IWP strain also showed significant (P<0.05) reduction of 0.542% for every increase of one day storage period. Similar to the observations made in the present study, reduction in hatchability on both total and fertile egg set due to preincubation storage in chicken has been reported earlier (Elibol *et al*. 2002, Mani *et al*. 2008, Elibol and Brake 2008). The effect of pre-incubation storage period may be partly due to their inability to initiate growth after the eggs were placed in the incubator and/or to their inability to sustain growth.

The overall early embryonic death (EED) percentage out of total fertile eggs in IWN (8.20) and IWP (6.72) strains was significantly (P<0.001) different. The incidence of EED in these strains had positive correlation with length of storage in these strains; however, the magnitude was significant (P<0.05) only in IWN strain. Regression analysis conducted to study the nature of relationship with storage length revealed that the EED was 6.71 and 5.597% for the eggs stored for a single day in IWN and IWP strains respectively with the daily increment of 0.271 and 0.205% respectively; the increase was statistically significant (P<0.01) in both the populations. Increased EED with prolonged storage was expected and in consistent with previous reports (Elibol *et al*. 2002). However, Mani *et al*. (2008) found no significant relationship of EED with the length of storage.

The incidence of dead germs (DG) was significantly (P<0.01) higher in IWP (4.07%) compared to that of IWN strain (3.45%). In the present study, DG had significantly (P<0.01) high positive correlation with the length of storage in both IWN and IWP strains. The regression of DG on the storage length (days) revealed that the increase in DG percentage was significant (P<0.01) and more sharp in IWP (0.256%) compared to IWN (0.161%) strain over the initial value of 2.661 and 2.562% respectively. Similar results of increased DG with increasing length of egg storage period have been reported previously (Elibol *et al*. 2002). However, Mani *et al*. (2008) found no significant relationship of DG with the length of storage. The possible explanation for increased percentages of EED and DG could be that the egg storage should be taken as low temperature incubation and prolonged storage might weaken the embryo, making it less viable and more susceptible to death once incubation is resumed.

The overall dead-in-shell (DIS) percentages in both the strains (3.46 and 3.43% for IWN and IWP strains respectively) were similar with no significant difference. The correlation between the length of storage (d) and DIS was not significant and varied in sign in IWN and IWP strains indicating no clear direction of association between these traits. Regression analysis also failed to reveal any obvious trend in the DIS due to length of storage period, similar to the findings of Mani *et al*. (2008). The results clearly demonstrated that the embryos weakened due to long storage, perish early in the development and all those survived incubation to the final stages of development hatched out equally well irrespective of the length of storage. However, Schneider (2007) observed increasing tendency of dead-in-shells with the increasing length of pre-incubation holding period.

The total embryonic mortality (TEM), which is inclusive of EED, DG and DIS was significantly ($P<0.05$) higher in IWN (15.12%) compared to IWP strain (14.22%). The correlation between TEM and storage length (d) was significantly positive in sign and significantly high in magnitude in IWN and IWP strains. TEM had significant negative association with hatchability on total and fertile eggs and significant positive associations with EE and DG in both the strains, the results were plausible and bound to occur. Linear regression analysis revealed that the TEM increased @ 0.442 and 0.53% for one day increase in storage period in IWN and IWP strains from the initial value of 12.68 and 11.393% respectively. Increased TEM with increasing storage period was expected and consistent with previous reports (Elibol *et al*. 2002).

The incidence of weaklings (WL) was significantly ($P<0.001$) high in IWN (1.76%) than in IWP strain (0.84%). The percentage of weaklings in IWN and IWP strains had correlation of low magnitude with inconsistent direction with the length of storage (d). There was a decrease of 0.036% weaklings for every single day increase in length of storage in IWN strain. On the other hand, in IWP strain, the incidence of weaklings increased @ 0.006% per day prolongation in storage period. This trait is poorly studied earlier to corroborate the results of the present study.

The per cent rotten eggs (RE) encountered in the break-open study revealed that the incidence was significantly ($P<0.05$) high in IWN (0.47%) compared to IWP strain (0.29%). The correlation coefficients were varied in direction and not significant in both the populations. However, the change in RE due to storage as analyzed by regression analysis was 0.003 and 0.02% per day from the initial value of 0.484 and 0.165, respectively, for IWN and IWP strains. Such information is not available in literature to compare the results of the present study.

From this study, it can be concluded that the fertility and most of the hatchability traits and influenced by genetic strain in White Leghorn. The present study also revealed that the hatchability, EED, DG and TEM in aged White Leghorn flocks can be influenced by length of storage period much similar to those reported earlier for other breeds and younger White Leghorn stocks. However, further research is needed to determine the nature of association of DIS, WL and RE with storage period in chicken to confirm the results of present study.

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