



## Effect of Storage Period and Egg Weight on Embryo Development and Incubation Results

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### ABSTRACT

A total number of 300 females and 30 males of two experimental broiler lines, TT (male) and PP (female), with 39 and 36 weeks of age, respectively. Experiment 1 (TT) evaluated the effect of storage time (ST) on hatchability and embryo mortality (EM). Eggs were stored for 2 (ST1), 4 (ST2), 6 (ST3), 8 (ST4), 10 (ST5), 12 (ST6), and 24 (ST7) days and EM was assessed in the periods of 0 to 6 (EM1), 7 a 13 (EM2), 14 to 17 (EM3), 18 to 21 (EM4), and 0 to 21 (TEM) days. Experiment 2 (PP) evaluated the effect of egg weight (EW) on embryo development. Eggs were individually collected and weighed. Embryos were collected and weighed on days 9 (EW9), 11 (EW11), 13 (EW13), EW15, 17 (EW17) days of incubation and at hatching (EW21). ST linearly influenced hatchability and EM, with an estimated 1.17% reduction and a 1.15% increase, respectively for each 1.0 day of storage. Hatchability was reduced in 21% between ST2 and ST7, resulting from a 462% increase in EM. The correlation between egg weight and embryo weight (EW) was significant after EW3 (0.25), reaching 0.72 for EW21, which corresponded to 70.92% of egg weight. The estimated increase in embryo weight for each 1.0g increase in egg weight was 0.71g for EW21.

### INTRODUCTION

Egg hatchability and chick quality have been used as hatchery performance indicators. Hatchery results are primarily influenced by the broiler breeder farm, which must supply good quality eggs. After that, the hatchery must account for factors affecting performance indicators such as egg management, storage time and conditions, incubation conditions, etc.

Physical egg quality is influenced by bird genotype, general management, health, climate conditions, and breeder age. These factors can interact and affect optimal incubation conditions. Egg water loss and gas exchange are affected by egg size and shape, as well as eggshell porosity, influencing incubation temperature and humidity, particularly during the last week of incubation.

Egg weight increases with breeder age, and eggs of different sizes have different physical and chemical qualities that affect hatchability and chick quality. Chick weight at hatching is strongly related to egg weight. Heavier chicks may present higher body development and smaller yolk sacs due to higher development during incubation, or less developed bodies and larger yolk sacs, allowing them to survive longer before exogenous feed is provided (Skewe *et al.*, 1988).

The correlation between egg weight and embryo weight changes according to embryo development stage. The correlation is not significant during the first half of the incubation period and reaches its



peak (0.50-0.95) at hatching (Shanaway, 1987). Chick weight is primarily determined by egg weight (62 - 78%), and secondarily by weight loss during incubation, eggshell and egg residue weight, genetic line, incubation time and conditions, breeder age, and sex (Shanaway, 1987; Yannakopoulos & Tserveni-Gousi, 1987; Wilson & Harms, 1988). In a comprehensive literature review on egg weight to chick weight ratios in six poultry species, Shanaway (1987) observed that chick weight increases 0.59g for each 1.0g of increase in weight of eggs heavier than 1.91g.

Egg storage is a common practice and often required in commercial incubation, aiming at avoiding the mixture of eggs from different flocks and breeder ages, or of eggs from flocks with undetermined health status. It also allows incubating a larger number of eggs to supply a scheduled demand. Storage management includes several factors that may affect hatchability and hatchling quality.

Short storage periods (2-4 days) do not require particular management procedures, but when eggs are stored for long periods, special techniques are required. The use of an egg-turning system and plastic bags, and the introduction of nitrogen gas to eliminate oxygen when eggs are stored for more than 14 days has promoted improvements in hatchability (Gustin, 1994).

Several studies show that storing eggs for 5 and 10 days reduce hatchability in 0.8 and 2.8%, respectively. In average, increasing storage day for one day can reduce hatchability in 1.0% and add 1.0 hour in incubation time (Decuypere & Micheles, 1992).

The present study aimed at evaluating storage time and egg weight on embryo development and the hatchability of settable eggs.

## MATERIAL AND METHODS

The present study was carried out at Embrapa Suínos e Aves, using a male line (TT) and a female line (PP) of broiler breeders of 39 and 36 weeks of age. Two experiments were carried out with 300 females and 30 males of each line housed in individual cages. Females were fertilized by two artificial inseminations weekly at a ratio of 1 male for every 10 females.

Experiment 1 was carried out with the TT line, and evaluated the effect of storage time on hatchability and embryo mortality.

Eggs were daily collected and stored at 15°C and 90% relative humidity for 3 periods of 14 days. Eight hours before incubation eggs were transferred to the

pre-heating room and grouped according to storage time (ST), with ST1, ST2, ST3, ST4, ST5, ST6, and ST7 corresponding to 2, 4, 6, 8, 10, 12, and 14 days of storage, respectively. Eggs were then incubated at 38°C and 64% relative humidity. After hatching, incubation residues were examined to determine embryo mortality, which was classified as EM1 (0 to 6 days), EM2 (6 to 13 days), EM3 (14 to 17 days), EM4 (18 to 21 days), and total embryo mortality (TEM) from 0 to 21 days of incubation.

Experiment 2, carried out with the PP line, evaluated the effect of egg weight on embryo development. Eggs were collected for 4 periods of 5 consecutive days, with two-week intervals, and 4 incubations were conducted. Egg storage and incubation were managed as described in Experiment 1. In each period, a total number of 960 eggs were individually weighed, identified, separated in 4 groups of 240 eggs (replicates) as a function of weight, and stored in the cold chamber for subsequent incubations. At 9 (EW9), 11 (EW11), 13 (EW13), 15 (EW15), and 17 (EW17) days of incubation, eggs were collected. The live embryos were removed from the eggs, dried in absorbent paper, and individually weighed. On day 18, the remaining eggs were transferred to the hatcher, and separated in trays placed in individual cages, where they remained until day 21, when the hatched chicks were weighed (EW21).

The effects of storage time on hatchability and embryo mortality, and of egg weight on embryo development were analyzed using the statistical procedures of SAS software package (SAS, 1996).

## RESULTS AND DISCUSSION

Table 1 presents the analysis of variance, analysis of regression and coefficient of variation used to determine the effect of storage time on hatchability and embryo mortality (Experiment 1), and Table 2 shows the means of these traits in Table 2.

The results show a significant effect of storage time on hatchability and embryo mortality. Hatchability started to be reduced on day 4 of storage (ST2), with losses of 1.38% per day up to ST4. The analysis of regression shows a linear effect of storage time on hatchability (Figure 1), with an estimated reduction of 1.17% per 1.0 day of egg storage. Similar results were obtained by Decuypere & Micheles (1992), who found that for each 1.0 day in storage time, hatchability was reduced in 1.0% and added 1.0 hour in incubation time.

Hatchability is a direct result of embryo livability,



**Table 1** - Analysis of variance and coefficient of variation (CV) of hatchability (HATCH) and embryo mortality (EM) as a function of storage time (ST).

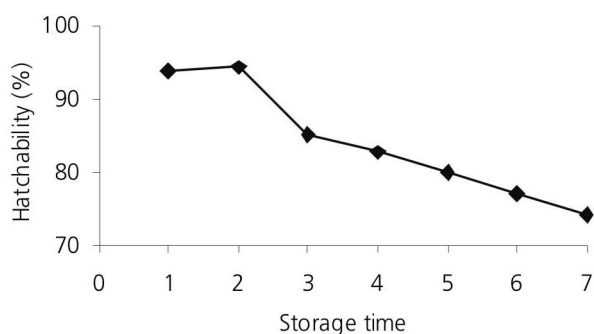
Parameters	Mean square		CV	Regression equation
	ST	Residue		
HATCH	41.105*	22.211	5.57	$Y = 8.82 - 1.17 ST$
EM1	2.428*	1.083	6.25	$Y = 0.50 + 0.41 ST$
EM2	0.291*	0.197	7.42	$Y = 0.10 + 0.12 ST$
EM3	0.226*	0.169	5.82	$Y = 0.12 + 0.14 ST$
EM4	2.148*	1.075	4.98	$Y = 0.44 + 0.48 ST$
TEM	15.925*	6.46	4.54	$Y = 1.17 + 1.15 ST$

( $p < 0.05$ ).

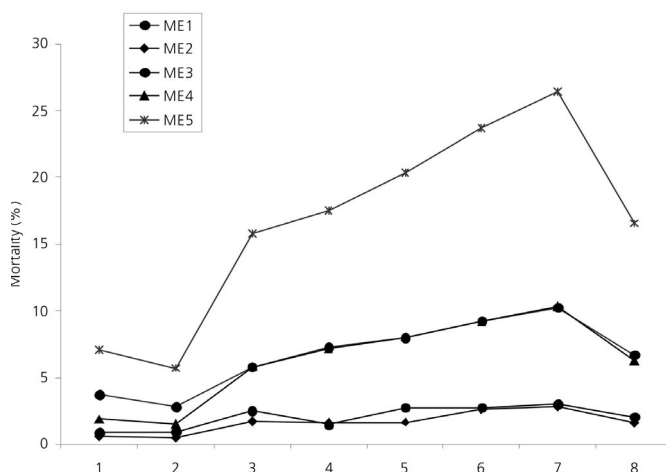
**Table 2** - Effect of egg storage time hatchability (HATCH) and embryo mortality (EM).

Storage time	HATCH	EM1	EM2	EM3	EM4	TEM
ST1	93.83a	3.72d	0.60c	0.87c	1.86e	7.05f
ST2	94.30a	2.79d	0.50c	0.93c	1.48e	5.70e
ST3	85.05b	5.80c	1.70b	2.50a	5.80d	15.80d
ST4	82.75c	7.26b	1.64b	1.46b	7.18c	17.54d
ST5	79.99d	7.95b	1.65b	2.75a	8.02ab	20.37c
ST6	77.04e	9.24a	2.57a	2.76a	9.16a	23.73b
ST7	74.13f	10.25a	2.78a	3.01a	10.35a	26.39a
Mean	83.87	6.72	1.63	2.04	6.26	16.56

Means followed by the same letter within the same parameter are not statistically different.



**Figure 1** - Effect of storage time on hatchability.



**Figure 2** - Effect of storage time on embryo mortality

which is influenced by genetic and environmental factors. The significant increase in embryo mortality was the main responsible factor of hatchability reduction after ST2. There was a linear effect of storage time on embryo mortality, with increases estimated by the analysis of regression of 0.41 (EM1), 0.12 (EM2), 0.14 (EM3), 0.48 (EM4), and 1.15% for total mortality for each 1.0 day of increase in storage time (Figure 2). The highest impact occurred between 4 (ST2) and 6 (ST3) storage days, with a 277% increase in EM, particularly due to early (0 a 6 days) and late (18 a 21 days) embryo mortality. Considering the optimal storage time as 4 days, increasing it to 14 days causes 21% losses in hatchability, which results from an increase in 462% of embryo mortality.

Estimated mean embryo mortalities, considering 14 storage days, were 10.25%, 2.78%, 3.01%, 10.35%,

and 26.39% for EM1, EM2, EM3, EM4, and TEM, respectively. Early (EM1) and late (EM4) embryo mortalities contributed the most to total embryo mortality, independent of storage time. The contributions for total mortality were 40.32% (EM1), 9.81% (EM2), 12.25% (EM3), and 37.61% (EM4), considering 14 days of egg storage.

Considering ST2 as the optimal egg storage time, extending it for 2 days (ST3) caused increases of 108% (EM1), 240% (EM2), 169% (EM3), and 292% (EM4) in embryo mortality. The largest effects of storage time were between ST2 and ST7 on EM4 (599%), EM2 (456%), EM1 (267%), and EM3 (223%).



**Table 3** - Estimates of average egg weight, embryo weight, and percentage of egg weight relative to egg weight (%WW).

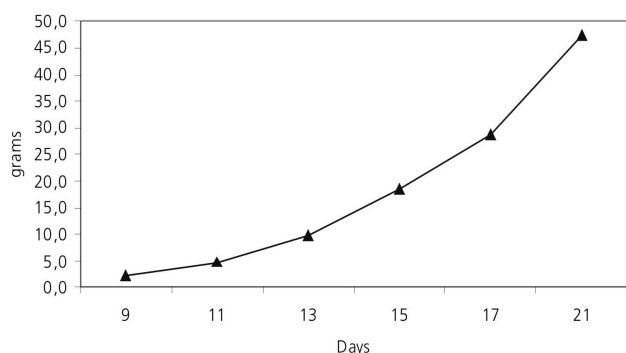
Embryo age	Egg weight	Embryo weight	%WW	r	b
EW9	66.3	2.3	3.4	0.1	0.03
EW11	65.8	4.8	7.2	0.16	0.07
EW13	66.7	9.8	14.7	0.25*	0.15
EW15	66.1	18.5	28	0.28*	0.28*
EW17	65.9	28.7	43.5	0.34*	0.43*
EW21	66.9	47.4	70.9	0.72*	0.71*

\* ( $p < 0.05$ ).

Estimates of mean egg and embryo weights, embryo weight relative to egg weight (%WW), correlation between egg weight and embryo weight, and the effect of increasing egg weight in 1.0g on embryo weight as a function of incubation time is presented in Table 3.

Average egg weight was similar for all evaluated embryo ages.

Embryo development as a function of incubation time is represented by the regression equation presented in Figure 3. The cubic model explained 95% of the variation in embryo development. Other models have been proposed in literature, including non-linear models (Freitas *et al.*, 1983). Embryo weight up to day 9 of incubation (EW9) represented only 4.85% of the final weight, and the largest development occurs in the last third of incubation, corresponding to 61% of the final weight.



**Figure 3** - Embryo development curve as a function of incubation time.

The correlation between egg weight and embryo weight ( $r$ ) varied as a function of embryo development stage. The correlation was significant after EW13 (0.25), and reached 0.72 for weight at hatching. These results are consistent with the values of 0.30 to 0.95 published in literature (Shanaway, 1987). Chick weight corresponded to 70.9% of the egg weight, in agreement with the results of 62-78% described in published studies (Shanaway, 1987; Yannakopoulos & Tserveni-Gousi, 1987; Wilson & Harms, 1988).

After day 13 of incubation (EW13) the estimated increase in embryo weight for each 1.0g of increase in egg weight was 0.15g (EW13), 0.28g (EW15), and 0.43g (EW17). As for weight at hatching (EW21), the obtained increase of 0.71g was higher than that obtained by Shanaway (1987) probably due to the genetic improvement in body weight gain of the modern broiler lines. Schmidt & Figueiredo (2005) verified genetic gains of 0.54% per generation in chick weight at hatch when genetically selected lines were compared to a control line.

The impact on egg weight, and consequently of chick weight, on broiler performance is very variable, but positive effects were found on market weight (Whiting & Pesti, 1983), mortality (Hearn, 1986), feed conversion ratio and absence of effects on mortality (Proudfoot *et al.*, 1982), and feed conversion ratio (Hearn, 1986).

## CONCLUSIONS

Egg storage for more than four days should only be used under special circumstances, as long storage times reduce hatchability due to increased embryo mortality.

Embryo development is correlated to egg weight, and chick weight at hatching corresponds to 70.9% of initial egg weight.

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