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Effect of Egg Weight Categories, Storage Time and Storage Temperature on Incubation Length in Duck Eggs (*Cairina moschata L.* and *Anas platyrhynchos domestica L.*)

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The effect of storage time (3, 7 and 14 days), storage temperature (18±1℃ and 15±1℃) and egg size (average weight −2*std.dev., −1*std.dev., +1*std.dev., and +2*std.dev.) on the incubation length of Muscovy and Pekin duck eggs was studied. Incubation was carried out in a commercial multi-stage incubator and the eggs were daily sprayed from the 10th to the day of egg transfer.

Pekin duck results showed that storage from 3, through 7, to 14 days, significantly prolonged the incubation time either at 15℃ or 18℃ (15℃ : 27.92, 28.11 and 28.31 days ; 18℃ : 27.89, 28.08 and 28.25 days ; Hatch-time=27.82+0.0343 storage). Similarly, egg size influenced the incubation time (27.89, 28.02, 28.11, 28.18 days, for 72.2 g < >77.4 g, 77.5 g< >82.8 g, 82.9 g< >88.1 g and 88.2 g< >93.5 g, respectively).

Muscovy duck results showed that storage prolonged the incubation time : at 18℃, 33.74, 34.18 and 34.60 days, for 3, 7 and 14 days of storage, respectively ; at 15℃, 35.20 and 33.91–34.00 for 14 days and 3–7 days, respectively, ($P<0.05$) Hatch-time=33.48+0.097 storage. Egg size influenced the incubation time but significant differences ($p<0.05$) were observed only between the lighter eggs (70.4–76.2 g) compared to all the other categories : 4h and 35' less than in the reference category (76.3 g–82.1 g).

Since optimum hatchability and duckling quality can only be achieved when chicks hatch contemporaneously, in commercial duck hatcheries the correction for the storage length and for lighter eggs in Muscovies and lighter and heavier eggs in Pekins is strongly advised.

Key words : duck, egg-storage, incubation-length

Introduction

Three factors influence the total incubation time of eggs. The first factor which affects incubation time is the age of eggs. It is well documented that storing eggs reduced embryonic viability (hatchability of fertile eggs) and extended incubation (Proudfoot, 1969 ; Sauveur and De Reviers, 1988 ; Decuypere and Michelis, 1992 ; Meijerhof, 1992 ; Fasenko *et al.,* 2002). Stored eggs took longer to incubate so that a "setting time correction" was commonly done in poultry (in broilers, for each day's

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storage beyond 2 days, one hour was added to the incubation time ; Cobb, 1991). Basal research indicated that during storage there was no discernible embryonic development and the embryo remains in a state of embryonic diapaused when the eggs were held at temperatures below their physiological zero (19°–21℃). However, embryo development still occured during storage, although at a minimum rate. The physiological zero, in fact, is defined the temperature to whom the cell number of the embryo remain constant, since the embryo development still occurs but the new formed cells succeed only in replacing the dead cells. The stage of embryo development and the number of cells contained by the blastoderm determine viability and setting time (Lundy, 1969 ; Mayes *et al.,* 1984 ; Meijerhof, 1992 ; Narushin and Romanof, 2002). The number of cells contained by the blastoderm before incubation was affected by the length and temperature of the eggs in the storage period, since the environment in the storage room affected the rate of survival of the original cells and the rate of replacing with the new cells (Petitte, 1991). Therefore these facts contributed to the decline in viability as the storage period increased, the embryos needed more time to restart their development and the embryo growth rate slowed (Christensen, 2001). Finally longer periods of storage increased the spread of time over which hatching took place and this may affect the total hatchabilty as well as the overall quality of chicks (Decuypere *et al.,* 2001).

The second factor which affects the setting time is the size of the eggs. Larger eggs take longer to incubate. So that also for the size of the eggs a "setting time correction" was commonly done (in broilers, for each 2.5 g above 50 g, 30 minutes were commonly added to incubation time ; Cobb, 1991). The setting time can also be influenced by time of the year, the number and type of other eggs in the setter and indeed, the type of setter used - single-stage, multi-stage rack or multi-stage trolley. Each of these may modify the "effective" incubation temperature, which is the third, and most important factor influencing the total incubation time of all the set eggs. The developing embryo hatched earlier if it was incubated at high temperatures (up to a maximum of 39℃) and later if it was incubated at low temperature (up to a minimum of 36.5℃), however a continuous 37.65℃ gave the best hatch (Freeman and Vince, 1974 ; Sauveur and De Reviers, 1988 ; Rose, 1997).

The synchronization of the embryos is of fundamental importance to obtain good hatches. In fact before pipping the humidity must be reduced, after pipping the humidity must be increased (to avoid the dry of the shell membranes) and after hatch the humidity must again be reduced (to dry the wet chicks). Of course, if the embryos are not synchronized, the humidity conditions in the setter cannot be optimal for every egg and an increase of embryo mortality is sure.

For these reasons we aimed to study the incubation length in duck eggs (Muscovies and Pekins), so that a specific setting time correction could be applied. In addition to time and egg size we aimed to test also the storage temperature, since different temperatures were commonly used for different storage times of duck eggs.

Materials and Methods

Stocks and Housing

Eggs were collected from Italian strains of Muscovy and Pekin ducks (white feathered). The birds were housed in pens with outside runs, at a room temperature of 20℃ and a ratio of one drake to five ducks. Pekin ducks were 36 wk of age and Muscovy ducks were 55 wk of age during egg collection, photoperiod was 14 h light : 10 h dark with lights on at 07 : 00 h. Each deep litter rearing pen had about 400 ducks and the eggs were laid on colony nests or on the litter. A commercial duck layer diet containing 4.37% Ca, 0.69% P, 17% CP, and 11.24 MJ/kg was *ad libitum* fed to both species.

Experimental Design and Methodology of Storage and Incubation

The experiment involved collection of 4,602 eggs over a period of 2 days (3,396 Muscovy duck eggs and 1,206 Pekin duck eggs). On each day the laid eggs were gathered, placed on metallic egg flats with their small ends down, washed and fumigated. On the second day all eggs were transported to the hatchery for the storage and divided randomly into six experimental groups. The experimental groups were subjected to one of the three duration of storage : 3, 7, or 14 days. Half eggs were stored at a constant temperature of 18±1℃, the other half were stored at 15±1℃. All eggs were daily turned and relative humidity was maintained at 70±5%. The temperatures were chosen as it has been recommended that duck eggs be stored at 16–18℃ for storage less than 7 days, and 11–15℃ for storage longer than 7 days (De Carville *et al.,* 1985, Sauveur and De Reviers, 1988 ; De Carville *et al.,* 1990 ; Bagliacca *et al.,* 1991, Bagliacca *et al.,* 1995 a, Bagliacca *et al.,* 1995 b). All eggs were weighed before storage. Incubation was carried out in an automatic multi-stage incubator with hourly turning of 60, without change of temperature during the embryonic period. Muscovy and Pekin duck eggs were daily sprayed starting from the 10th and the 8th day of incubation respectively, till the transfer in the hatcher, according to the technology used for ducks eggs (De Carville and De Croutte, 1985 ; Bagliacca *et al.,* 1989 ; Pingel *et al.,* 1989 ; Sauveur and De Carville, 1990 ; Bagliacca *et al.,* 1991). Candling was done at the 10th day of incubation and at the transfer in the hatcher (the 24th day for Pekin eggs and the 30th day for Muscovy eggs). At the transfer each egg was put inside a gauze-bag and the hatcher was checked every 4–6 hours starting from the 806th hour (33 days and 14 hours) for the Muscovy duck eggs and starting from the 660th hour (27 days and 12 hours) for the Pekin duck eggs. The moment of hatch was estimated by the observation of the conditions of the hatched duckling compared with the former observation on the hatching egg.

Egg weight categories were decided after the incubation by calculating the distribution of the weight in the sample of fertile eggs used for the experiment (avg. and standard deviations). Four categories were determined : average weight minus 2*std. dev., minus 1*std.dev., plus 1*std.dev., and plus 2*std.dev. (see Fig. 1). For this reason 4.56% of the eggs were not used in the analysis since results reported for investigations into incubating eggs, whose weights are not within the average values, are contradictory

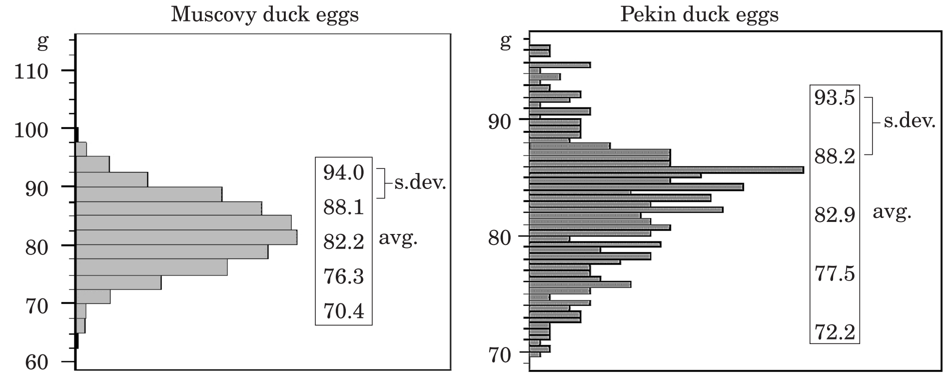


Fig. 1. Egg weight distribution in Muscovy and Pekin duck with the selected categories.

(Narushin and Romanov, 2002).

Statistical Analysis

The data were analysed using least squares analysis (SAS, 2002). For each specie, sources of variation were days of storage (3, 7 or 14 days), storage temperatures (15℃ or 18℃) and first level interaction storage-time*storage-temperature and four egg weight categories. Storage time was also analysed as continuous variable by linear and quadratic regressions (least square distances).

Results and Discussion

The interaction temperature*storage-length was always significant, for the incubation length, either in Muscovy or Pekin eggs. The two factors, temperature and storage length, in fact, differently acted on the embryo development. This interactive effect could be summarized by the final results of the embryo development (the hatching rates) in poultry : lower temperatures were always better for longer storage and higher temperatures were better for shorter storage (Lundy, 1969 ; Mayes and Takeballi, 1984 ; De Carville and De Croutte, 1985). The main factors, which could be useful for a general explanation of the phenomenon were however reported in the tables, in addition to the effect of each cell (sub-factor), which were required for the accurate evaluation of the factors influencing the incubation time.

Effect of Storage Day

Storage significantly prolonged the incubation time in Muscovy duck (see Table 1) but the increase in storage period was different at different temperatures. When the eggs were stored at 18℃ (around the physiological zero) the incubation time greatly differed between the different storage times (33.74, 34.18 and 34.60 days, for 3, 7 and 14 days of storage, respectively ; $P<0.05$). When the eggs were stored at 15℃, the average total incubation time differed only between 14 days and 3–7 days of storage (35.20 days *vs.* 33.91 and 34.00 days ; $p<0.05$). Also in Pekin duck storage significantly prolonged the incubation time (see Table 2) either at 18℃ (around the physiological zero) or 15℃ (at 18℃ of storage, 27.89, 28.08, and 28.25 days, for 3, 7 and 14 days, respectively ; at

Table 1. Muscovy duck incubation length, response in relationship to temperature* storage-length, least squares means table

Interaction : Temperature*		Storage Length			Main Effect Temperature
		3 days	7 days	14 days	
15℃	Number	405	395	250	1050
	Least Sq. Mean days	33.91 d	34.00 d	35.20 a	34.37 a
	Std Error	0.050	0.051	0.064	0.032
18℃	Number	442	362	267	1071
	Least Sq. Mean days	33.74 c	34.18 e	34.60 b	34.18 b
	Std Error	0.048	0.053	0.062	0.031
Main Effect Storage	Number	847	757	517	2121
	Least Sq. Mean days	33.83 c	34.09 b	34.90 a	34.18
	Std Error	0.035	0.037	0.045	0.022

Note : means with different letters differ per P<0.05.

Table 2. Pekin duck incubation length, response in relationship to temperature* storage-length, least squares means table

Interaction : Temperature*		Storage Length			Main Effect Temperature
		3 days	7 days	14 days	
15℃	Number	172	136	92	400
	Least Sq. Mean days	27.92 d	28.11 c	28.31 a	28.11 a
	Std Error	0.009	0.010	0.013	0.006
18℃	Number	176	134	94	404
	Least Sq. Mean days	27.89 d	28.08 c	28.25 b	28.08 b
	Std Error	0.009	0.011	0.013	0.006
Main Effect Storage	Number	348	270	186	804
	Least Sq. Mean days	27.91 c	28.09 b	28.28 a	28.06
	Std Error	0.007	0.007	0.009	0.004

Note : means with different letters differ per P<0.05.

15℃ of storage, 27.92, 28.11, and 28.31 days, for 3, 7 and 14 days, respectively ; P< 0.05).

Effect of Storage Temperature

Interesting to note the overall effect of the temperature. Either in Muscovy duck eggs or in Pekin duck eggs, the lower storage temperature induced a longer incubation time (Bagliacca *et al.,* 2003 a, b) (34.37 *vs.* 34.18 in Muscovies ; 28.11 *vs.* 28.08 in Pekins ; p<0.05) (Table 2). In Pekin duck the longer incubation time was observed in the eggs stored at 15℃ than that observed in the eggs stored at 18℃, and it could be explained by the longer time the former need to reach the temperature of the incubator

(99.7°F or 37.61℃). In Muscovies the increased embryonic time could not be explained only by the longer time the former needed to reach the incubator temperature. The dormiency of the embryo (the development of the embryo was arrested during cool storage) seemed to became "deeper" with lower temperatures so that a delay in the initiation of development following storage or a slower growth rate, probably related to a shrinkage of the blastoderm, might explain the observed prolonged incubation time in these species.

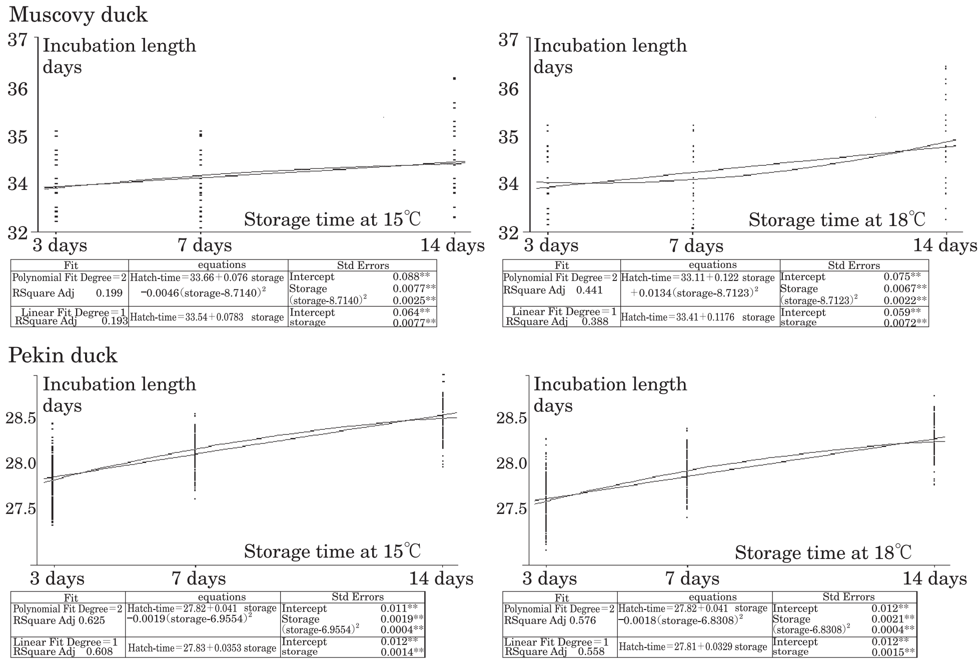
Regarding the general effect of the storage time (independently to the particular temperature), significant differences could be observed for each category of storage either in Muscovy duck eggs or in Pekin duck eggs (Muscovies : 33.83, 34.09 and 34.90 days, for 3, 7 and 14 days of storage, respectively ; Pekins : 27.91, 28.09 and 28.28 days, for 3, 7 and 14 days of storage, respectively ; $P<0.05$).

The biological phenomenon was however continuous and could be explained either by a linear or polinomial regression (see Fig. 2) so that the incubation times for every storage length, between 3 and 14 days, and the two storage temperatures could be easily estimated.

Effect of Egg Size

The size of the eggs influenced the incubation time (see Table 3).

In Muscovy duck eggs, the size of the eggs influenced the incubation time but significant differences were observed only between the lighter eggs compared to all the others ($p<0.05$). Within the central categories (egg weight ±1*std. dev. = 68.37% of



Muscovy duck, storage at 15℃:

Fit	equations	Std Errors
Polynomial Fit Degree = 2 RSquare Adj 0.199	Hatch-time = 33.66 + 0.076 storage −0.0046 (storage-8.7140)^2	Intercept 0.088** Storage 0.0077** (storage-8.7140)^2 0.0025**
Linear Fit Degree = 1 RSquare Adj 0.193	Hatch-time = 33.54 + 0.0783 storage	Intercept 0.064** storage 0.0077**

Muscovy duck, storage at 18℃:

Fit	equations	Std Errors
Polynomial Fit Degree = 2 RSquare Adj 0.441	Hatch-time = 33.11 + 0.122 storage + 0.0134 (storage-8.7123)^2	Intercept 0.075** Storage 0.0067** (storage-8.7123)^2 0.0022**
Linear Fit Degree = 1 RSquare Adj 0.388	Hatch-time = 33.41 + 0.1176 storage	Intercept 0.059** storage 0.0072**

Pekin duck, storage at 15℃:

Fit	equations	Std Errors
Polynomial Fit Degree = 2 RSquare Adj 0.625	Hatch-time = 27.82 + 0.041 storage −0.0019 (storage-6.9554)^2	Intercept 0.011** Storage 0.0019** (storage-6.9554)^2 0.0004**
Linear Fit Degree = 1 RSquare Adj 0.608	Hatch-time = 27.83 + 0.0353 storage	Intercept 0.012** storage 0.0014**

Pekin duck, storage at 18℃:

Fit	equations	Std Errors
Polynomial Fit Degree = 2 RSquare Adj 0.576	Hatch-time = 27.82 + 0.041 storage −0.0018 (storage-6.8308)^2	Intercept 0.012** Storage 0.0021** (storage-6.8308)^2 0.0004**
Linear Fit Degree = 1 RSquare Adj 0.558	Hatch-time = 27.81 + 0.0329 storage	Intercept 0.012** storage 0.0015**

Fig. 2. Relationship between incubation length and storage time (overall effect on storage temperatures).

Table 3. Duck egg incubation length : response in relationship with egg weight categories, least squares means table

Pekin duck - Egg weight categories (g) :	72.2–77.4	77.5–82.8	82.9–88.1	88.2–95.4
Egg number	134	240	326	104
Least Sq. Mean	27.89 c	28.02 b	28.11 ab	28.18 a
Std Error	0.015	0.011	0.009	0.017
Muscovy duck - Egg weight categories (g) :	**70.4–76.2**	**76.3–82.1**	**82.2–88.0**	**88.1–94.0**
Egg number	315	797	697	312
Least Sq. Mean	34.02 b	34.21 a	34.16 ab	34.28 a
Std Error	0.070	0.044	0.047	0.070

Note : means with different letters differ per $P < 0.05$.

the eggs), the eggs of the category 76.3 g-82.1 g did not differ from the eggs of the category 82.2 g-88.0 g in the total incubation time and the least square means of the former was greater than the latter (the general phenomenon where larger eggs took longer to incubate was not observed within these categories). Also the eggs of the heaviest tested category required to hatch only 0.07 days (1 h and 28′) more of the reference category (76.3–82.1 g), with no statistically significant difference.

In Pekin duck eggs significant differences were observed between the two central categories and the lighter eggs ; the heavier eggs took longer to hatch, but the incubation length statistically differed only from the lighter and the medium-light category (77.5–82.8), confirming the general phenomenon where larger eggs took longer to incubate.

The corrections which must be done at incubation-start to obtain the contemporaneous hatch of duck eggs are summarized in Tables 4 and 5. Since Muscovy duck eggs were commonly incubated twice a week while Peckin's were commonly incubated once a week (De Carville and De Croutte, 1985 ; Bagliacca *et al.,* 2003) the days of standard from Muscovy and Peckin are 3 and 7, respectively. For storage temperature of 15℃ or 18℃, the specific values found in the experiment could be adopted, while, for different storage temperature, the overall effect of storage temperature was the best estimation of the correction for each specie. Regarding the egg weight in Muscovy duck, the only significant correction which must be done was the correction for the lighter eggs (contrast of the category 70.4–76.2 *vs.* all the others statistically significant for $p < 0.05$) while in Pekin duck either the correction for the lightest or the heaviest should be done (contrasts with the reference category, 77.5–82.8, statistically significant for $p < 0.05$).

Table 4. Corrections which must be done at incubation-start to obtain the contemporaneous hatch of Muscovy duck eggs (3 days represents the reference category, anticipated times are represented by "minus" and delayed times are represented by "plus")

Storage temperature	15℃	18℃
Effect Temperature	$+4^{h}43'$*	0

Storage length		3 days day or standard	7 days	14 days
Effect storage time at 15℃		0	$+2^{h}08'$ ns	$+30^{h}48'$*
Effect storage time at 18℃		0	$+10^{h}42'$*	$+20^{h}45'$*
Overall effect of storage temperature		0	$+6^{h}24'$*	$+25^{h}46'$*
Linear continuous effect of storage length		$+2^{h}49'$ for each day of storage		

Egg weight categories	70.4–76.2	76.3–82.1	82.2–88.0	88.1–94.0
Effect egg weight	$-4^{h}35'$*	0	$-1^{h}12'$ ns	$1^{h}28'$ ns

* correction statistically significant ; ns correction not statistically significant.

Table 5. Corrections which must be done at incubation-start to obtain the contemporaneous hatch of Pekin duck eggs (Zero represents the reference category, anticipated times are represented by "minus" and delayed times are represented by "plus")

Storage temperature	15℃	18℃
Effect Temperature	$+0^{h}52'$*	0

Storage length		3 days	7 days day or standard	14 days
Effect storage time at 15℃		$-4^{h}41'$*	0	$+4^{h}41'$*
Effect storage time at 18℃		$-4^{h}25'$*	0	$+4^{h}14'$*
Overall effect of storage temperature		$-4^{h}33'$*	0	$+4^{h}28'$*
Linear continuous effect of storage length		$+0^{h}50'$ for each day of storage		

Egg weight categories (g) :	72.2–77.4	77.5–82.8	82.9–88.1	88.2–95.4
Effect egg weight	$-3^{h}11'$*	0	$+2^{h}11'$ ns	$+3^{h}42'$*

* correction statistically significant ; ns correction not statistically significant.

Conclusions

In Muscovy duck eggs the effect of the different thesis has a very big impact on the length of the embryonic period. The Italian strains of Muscovy duck used in the experiment were not well defined from a genetic point of view. The duck are mainly white but coloured offspring are not a so rare exception. This could explain the greater variability and the greater spread of the hatch observed in this specie than in the Pekin duck.

Since optimum hatchability and duckling quality could only be achieved when chicks hatch contemporaneously, also in commercial duck hatcheries, the "setting time correction" has been required to reduce the hatch spread either in Muscovies or in Pekins.

If the eggs are incubated once a week, the most widespread storage time, the correction for the storage length and at least for the lighter and heavier eggs should be calculated so that fresh and stored eggs, and lighter and heavier eggs have to be set at different times.

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