Egyptian J. Anim. Prod. 35, Issue, Dec. (1998):653-664.

THE INFLUENCE OF PARENT AGE AND PREINCUBATION STORAGE CONDITIONS ON RELATIVE ORGANS WEIGHT AND MATERNAL ANTIBODY OF BROILER CHICKS

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SUMMARY

The purpose of the present study was to investigate the effects of temperature of short or long-term preincubation storage of eggs from broiler breeder flocks with two ages on the relative yolk sac weight of 18 - day chick embryo, the relative weight of liver, yolk sac and maternal antibody titer against Newcastle disease virus (NDV) of newly hatched chicks as well as the relative weight of bursa, thymus, and spleen of 8-weeks old chicks. Eggs from two different Arbor Acres breeders flocks aging 45 and 56 weeks were stored for zero (fresh) , 5, 10 or 15 days under room temperature (24-30 °C) or in egg storage room (18-20 °C).

In general, the obtained results revealed that relative liver weight and maternal antibody titer against NDV decreased whereas the relative weight of residual yolk sac elevated with the increase of preincubation storage time. Also, the relative weight of liver significantly increased when eggs were stored at 24-30 °C than those stored at 18-20°C. On the other hand, neither broiler breeder age nor temperature and length of storage period affected the relative weight of thymus and spleen at 8 weeks of age. The relative weight of bursa surpassed significantly in chicks hatched from younger hens over that from older ones.

In conclusion, the present study reveals that parent age and preincubation storage condition influenced the physiological processes that occur in the chicks. Also, the preincubation storage period should be less than ten days.

Keywords: Egg storage , broiler breeder , maternal antibody , relative organs weight.

INTRODUCTION

Storing hatching eggs over an extended period is one of the major problems confronting Egyptian poultry industry, especially in small farms. This problem becomes more aggravated in summer months or when chicks

marketing is exposed to slack. The deleterious effect of bad storage depends on length of storage period , temperature, humidity, gaseous environment, and the orientation and positional changes of the eggs .

Long-term storage depresses hatchability, prolongs incubation time , and reduces chick quality (Merritt,1964; Proudfoot, 1969; Meyes and Takeballi, 1984; Butler, 1991 and Meijerhof,1992). Whereas, conflicting results have been reported concerning the short-term storage, e.g. Walsh (1993) reported that, fresh eggs just laid have a maximum hatching potential, but Brake (1995) stated that , eggs set fresh, without a period of storage , hatch poorly and later and than average, whereas, Meijerhof (1992) and Reis *et al.* (1997) concluded that fertile eggs can be stored for several days without a major loss in hatchability when appropriate conditions are maintained.

In general, optimal storage period and conditions are not fixed . They vary with the different strains as well as the age of the flock . Brake *et al.* (1997) explained that eggs from younger flocks should be stored at slightly higher temperature and lower humidity than those from an older flocks if they are to be stored for the same period . This difference could be attributed to albumin quality.

Investigations on the influences of prolonged storage period of hatching eggs on some physiological parameters of the hatched chicks are limited .The effects of broiler breeder age on chicks quality showed that, chicks from old hens exhibited lower mortality (Hays and Spear, 1952 and McNaugton *et al.*, 1978) heavier body weight, higher growth rate (Sincilair *et al.*,1990), higher relative residual yolk as well as higher antibody titer against sheep red blood cells antigen and lower bursa weight than chicks from young hens (Atta *et al.*, 1996) .

The present experiment was conducted to study the effects of the preincubation storage period of hatching eggs as well as broiler breeder age on some physiological parameters of broiler chicks .

MATERIAL AND METHODS

The present experiment was carried out during fall 1996 ; using hatching eggs collected four times daily from two Arbor Acres broiler breeder flocks that were 45 and 56 weeks of age. Cracked, dirty, misshapen or of extreme size were culled and removed . 770 eggs (61 - 63 grams) were collected from each flock, fumigated and divided into seven groups of 110 eggs each. The first group was set in the incubator in the same day (no storage) whereas the other six groups were stored for either five, ten or fifteen days. Three groups were stored in cooled storage room at 18 - 20°C while the other three groups were stored under room temperature at 24 - 30°C. After storage, eggs were set in a forced air incubator that provided 37.5°C and 60% R.H. in setter and 36.9°C and 80% R.H. in hatcher .

On the 18*th* day of incubation all eggs were candled to discard infertile (clear) eggs and dead embryos. Ten eggs containing viable embryos from each group were broken, the residual yolk sac was carefully removed from the embryo, weighed and expressed as relative weight(grams yolk/100 grams of embryo weight). The remaining fertile eggs were transferred to hatching basket and returned to hatcher. All chicks were removed from the hatcher at 21 days, wing-banded, weighed to nearest 1 gram. Ten newly hatched chicks from each group were randomly chosen, and blood samples were collected by cardiac puncture to detect maternal antibody titer against Newcastle disease virus (NDV) using hemagglutination inhibition (HI) test described by Beard (1980). Then chicks were sacrificed and both residual yolk and liver were removed weighed and expressed as relative weight (grams of organ/100 grams of live body weight) .

The rest of the chicks were placed intermingled in floor pens with wood shaving litter, fed *ad libtum* on a commercial starter ration (22% protein, 3000 Kcal ME/Kg) and exposed to continuous illumination throughout the experiment. Feed and water were available all the time. At eight weeks of age ten chicks from each group were randomaly chosen weighed and sacrificed. Bursa, spleen and thymus were removed and expressed as relative weight (milligram of organ / 100 grams of body weight).

Statistical analysis of data

The data were statistically analyzed according to the General Linear Model Procedure (SAS Institute, 1988) with three-way ANOVA model using age of parent, preincubation storage period and temperature of storage as main effects. Appropriate, means were compared using Duncan's multiple range test (1955).

RESULTS AND DISCUSSION

All tables in this study display the main effects (parent age , preincubation storage period and temperature) that only had a significant influence on traits. Yolk sac is the source of the earliest haemopoietic, including the lymphocyte precursors (Playfair, 1987). Comparison the relative yolk sac weight (RYSW) of 18- day embryos between groups of each parent age exhibited that: within the group of 45 weeks-old hens; 18-day embryos displayed the lowest RYSW when eggs were stored for 15 days at room temperature and the difference was significant ($P<0.05$) when compared with those counterpart from eggs stored for 10 days. While within groups of 56 weeks-old hens no significant differences were observed in RYSW (Table 1).

Regardless, of the parent age and temperature of preincubation storage, the RYSW of 18-day embryos increased considerably with the length of the preincubation storage period and reached significant higher weight when the

storage period prolonged to ten days than the corresponding counterparts from fresh or 15 - days stored eggs .

Table 1. Influence of parent age and preincubation storage condition (length and temperature) on relative yolk sac weight of day 18-embryo

Parent age (week)	Storage temperature °C	Length of storage (day)			
		Fresh	5	10	15
45	Fresh	20.9±1.4^{b}			
	24 - 30		21.6 ±1.4^{ab}	26.0 ± 1.4^{a}	19.7 ± 1.8^{b}
	18 - 20		21.4 ±1.4^{ab}	23.3 ± 1.4^{ab}	22.2 ± 1.4^{ab}
56	Fresh	22.3 ±1.3^{ab}			
	24 - 30		24.5 ±1.4^{ab}	24.8 ± 1.4^{ab}	22.7 ± 1.6^{ab}
	18 - 20		25.4 ±1.6^{a}	23.4 ± 1.8^{ab}	21.8 ± 2.2^{ab}
Pooled		21.67±0.9^{y}	23.09±0.72^{xy}	24.5 ± 0.72^{x}	21.7 ± 0.84^{y}

a b c Means between groups with different letters are significantly differ (P< 0.05) .
x y Pooled means with different letters are significantly differ (P< 0.05) .
* Since no significant differences were found between pooled means of parent age or temperature, they are excluded .

The RYSW of newly hatched chicks had a similar pattern , but reached the highest significant figures when eggs were stored for ten or fifteen days (Table 2). On the other hand, neither the temperature of storage nor the age of parent had significant effect on RYSW of both 18-day embryos or newly hatched chicks. The previous investigations that had been studied the effect of parent age on RYSW had contradicting results. For example, Latour *et al.* (1996) reported that RYSW of 18-day embryos from 35-week old hens were higher than those from younger or older hens, whereas, RYSW of newly hatched chicks from young hens were higher than those from old-hens. But Atta *et al.*(1996) demonstrated that the relative RYSW of newly hatched chicks was positively increased with the age of parent. This contradiction may be due to the variation in parent age or weight of eggs in each study .

In general, 18-day embryos exhibited the greatest RYSW at both ages (weeks) of hens when compared to newly hatched chicks. The same result was stated by Latour *et al.* (1996).The antibodies transferred from the hen to chick named maternal antibody (passive immunity). In birds, immunoglobulin G (IgG) pass from the hens to chicks through the yolk. In this species , IgG is actively concentrated in the yolk through a receptor- mediated pathway (Rose *et al.*, 1974; Griffin *et al.*, 1984) resulting in yolk IgG concentration proportionate to those found in the hen's serum (Brown et al. 1989) . Maternal antibodies to bacteria and viruses can be transferred to the chicks through the yolk resulting antibody titers in newly hatched chicks represent half of those present in the hens (Eidson *et al.*, 1980). The half - life of the maternal antibody, is approximately three days (Malkinson ,1965 ; Davelaar and

Kouwenhoven, 1977). Until maternally derived antibody titers decline, they provide an important protective benefit to the immunologically immature chicks (Parry and Porter,1981). Immunoglobulin M and A are not incorporated into the yolk , but are found in low concentration in the egg white (Albumin) and are believed to be deriven from oviduct secretions (Rose *et al.* , 1974) .

Table 2. Influence of parent age and preincubation storage condition (length and temperature) on relative yolk sac weight of newly hatched chicks

Parent age (week)	Storage temperature °C	Length of storage (day)			
		Fresh	5	10	15
45	Fresh	13.4±1.2^{ab}			
	24 - 30		10.4 ± 1.5^{bc}	15.2 ± 1.3^{a}	15.9 x 1.7^{a}
	18 - 20		12.3 ± 1.3^{ab}	14.0 ± 1.3^{ab}	15.7 ± 1.3^{a}
56	Fresh	10.4±1.3^{bc}			
	24 - 30		7.9 ± 1.3^{c}	15.7 ± 1.5^{a}	16.3 ± 1.3^{a}
	18 - 20		13.1 ± 1.3^{ab}	15.8 ± 1.3^{a}	16.2 ± 1.3^{a}
Pooled		12.1±1.2^{y}	11.0 ± 1.4^{y}	15.1 ± 1.5^{x}	16 ± 1.5^{x}

a b c Means between groups with different letters are significantly differ ($P<0.05$) .

x y Pooled means with different letters are significantly differ ($P< 0.05$) .

* Since no significant differences were found between pooled means of parent age or temperature, they are excluded .

The results of maternal antibody titer presented in table (3) indicate that newly chicks hatched from eggs stored for five days at room temperature had the highest maternal antibody titer against NDV at both ages of hen age but storing eggs more than five days markedly decreased maternal antibody titer (especially in chicks from young hens), and reached a significantly lower level when the storage prolonged to fifteen days. This phenomenon may be due to the degradation of immunoglobulin G when preincubation storage time increased. On the other hand, neither age of parent nor temperature of storage influenced the level of maternal antibody titer .

During embryonic life, liver is the major haemopoietic and lymphopoietic organ (Playfair,1984). The relative liver weight (RLW) of newly hatched chicks was affected by the length of preincubation storage and temperature (Table 4). Chicks from eggs stored for 15 days exhibited a significant lowest RLW when compared with those from another storage time, while RLW of chicks hatched from 5-day stored eggs occupied first .Regardless the length of storage, the RLW of chicks hatched from eggs stored at room temperature was superior and was significantly higher than those counterpart hatched from eggs stored at storage room temperature (18 -20°C).

Table 3. Influence of parent age and preincubation storage condition (length and temperature) on maternal antibody titer

Parent age (week)	Storage temperature °C	Length of storage (day)			
		Fresh	5	10	15
45	Fresh	4.8 ± 0.4^{ab}			
	24 - 30		5.8 ± 0.4^{a}	4.8 ± 0.4^{ab}	3.0 ± 0.5^{c}
	18 - 20		4.6 ± 0.4^{ab}	4.2 ± 0.4^{bc}	3.0 ± 0.4^{c}
56	Fresh	4.2 ± 0.4^{ab}			
	24 - 30		4.4 ± 0.4^{abc}	4.0 ± 0.4^{bc}	3.6 ± 0.4^{bc}
	18 - 20		4.0 ± 0.4^{bc}	4.0 ± 0.4^{bc}	4.2 ± 0.4^{bc}
Pooled		4.5 ± 0.4^{x}	4.7 ± 0.4^{x}	4.2 ± 0.4^{x}	3.5 ± 0.4^{y}

a b c Means between groups with different letters are significantly differ ($P<0.05$).
x y Pooled means with different letters are significantly differ ($p< 0.05$).
* Since no significant differences were found between pooled means of parent age or temperature, they are excluded.

Table 4. Influence of parent age and preincubation storage condition (length and temperature) on relative liver weight of newly hatched chicks

Parent age (week)	Storage temperature °C	Length of storage (day)				Pooled
		Fresh	5	10	15	
45	Fresh	2.3 ± 0.13^{abc}				2.4 ± 0.14^{xy}
56		2.5 ± 0.14^{ab}				
45	24 - 30		2.7 ± 0.16^{a}	2.5 ± 0.14^{ab}	$\pm0.18^{abc}$	2.5 ± 0.16^{x}
56			2.6 ± 0.14^{ab}	2.7 ± 0.16^{a}	2.2 ± 0.14^{bc}	
45	18 - 20		2.6 ± 0.14^{ab}	2.2 ± 0.14^{bc}	1.9 ± 0.14^{c}	2.3 ± 0.14^{y}
56			2.5 ± 0.14^{ab}	$\pm0.14^{abc}$	1.9 ± 0.11^{c}	
Pooled		2.45 ± 0.14^{x}	2.6 ± 0.13^{x}	2.4 ± 0.13^{x}	2.1 ± 0.13^{y}	

a b c Means between groups with different letters are significantly differ ($P<0.05$).
x y Pooled means with different letters are significantly differ ($P< 0.05$).
* Since no significant differences were found between pooled means of parent age or temperature, they are excluded.

Among groups of 45 weeks-old hens, the RLW of chicks from eggs stored at room temperature for five days occupied first and was significantly higher than the counterparts from 15-days stored eggs at storage room. While within groups of 56 weeks-old hens, the chicks hatched from 10-days stored eggs at room temperature displayed significantly higher RLW when compared to chicks from eggs stored for 15 days at storage room temperature (18-20°C).

The reduction in RLW when preincubation storage time was prolonged was accompained by a significant increase in RYSW (Table 2 and 4). The negative relationship between RYSW and RLW has been observed by Pinchasov and Noy (1993) and Noble and Cocchi (1990) who showed that yolk triglycerides are mobilized and accumulated in liver. According to this interpretation, increasing the storage time may increase the degradation of fat which in turn obstruct its mobilization and accumulation in liver.

Both bursa of Fabricious and thymus gland are the primary immune organs in which B and T lymphocytes, respectively differentiate and mature to become antigenically committed, immunocompetent cell, whereas spleen is the secondary lymphoid organ that ensures the maximum contact of antigen-presenting cells that have newly encountered antigen, with B and T lymphocytes to start the immune response (Kuby, 1992).

Since the hatchability of eggs obtained from 45 weeks - old hens, stored for 15 days at room temperature declined to 2.06% (Atallah *et al.*,1998), this group did not have available chicks for sacrificing to detect the relative bursa, thymus and spleen weight. As shown in Tables (5, 6 and 7) no differences were observed in relative bursa, thymus and spleen weight (mg/100 g body weight) between all groups at eight weeks of age, also neither length nor temperature of the preincubation storage influenced the relative weight of the immune organs, whilst, the age of the parent had only significant influence on the relative bursa weight, but did not influence the relative thymus or spleen weight, e.g. the relative bursa weight of chicks hatched from 45 weeks - old hens was significantly higher than that from 56 weeks - old hens. This is in accordance with the previous finding of Atta *et al.* (1996) who have shown that relative bursa weight of chicks hatched from 45 weeks - old hens ranked first with significant difference at 6 weeks of age than that from 37 or 67 weeks - old hens, also they reported that no influence of parent age on relative spleen weight. Because a functioning bursa is essential for normal immunocompetence, an enlarged bursa may suggest an alteration in antibody production (Yumamoto and Glick,1982). This suggestion was tested in the experiment of Dafwang *et al.* (1985) which indicate that higher antibody response to sheep red blood cells was obtained in birds that had heavier bursa weight at three weeks of age, whereas the results of Atta *et al.*,(1996) revealed that relative bursa weight at early stage of age (less than four weeks, not more) may be an indicator to humoral immune response activity, e.g. chicks hatched from 67 weeks - old hens produce higher antibody titer against sheep red blood cells which have heavier relative bursa weight at two weeks of age but inversely, lighter weight at sex weeks of age when compared with the counterpart hatched from 45 weeks - old hens .

In conclusion, the results reported here would imply that many of the physiological processes that occur in the chicks may be directly related to parent age and preincubation storage condition and might directly influence

livability during the growth period. Also, the present study suggests that preincubation storage period should be less than ten days.

Table 5. Influence of parent age and preincubation storage condition (length and temperature) on relative bursa weight of 8-weeks-old chicks

Parent age (week)	Storage temperature °C	Length of storage (day)				Pooled
		Fresh	5	10	15	
45	Fresh	38.0±2.3				35.7±3.5[x]
	24 - 30		36.4±2.2	25.0±4.9		
	18 - 20		34.5±2.4	37.4±2.5	31.2±4.9	
56	Fresh	34.7±3.3				31.8±3.2[y]
	24 - 30		33.0±3.1	24.3±5.2	31.4±5.2	
	18 - 20		30.5±3.3	31.3±3.6	35.0±6.9	

* No significant differences (P< 0.05) were found between mean of groups .
x y Pooled means with different letters are significantly differ (P< 0.05) .
* Since no significant differences were found between pooled means of parent age or temperature, they are excluded .

Table 6. Influence of parent age and preincubation storage condition (length and temperature) on relative thymus weight of 8-weeks-old chicks

Parent age (week)	Storage temperature °C	Length of storage (day)			
		Fresh	5	10	15
45	Fresh	208.2±33.6			
	24 - 30		197.9±31.9	205.0±70.5	-----
	18 - 20		210.0±34.7	202.8±77.4	247.5±70.5
56	Fresh	210.0±48.4			
	24 - 30		203.0±44.6	202.8±75.4	221.4±75.4
	18 - 20		158.8±48.4	207.3±51.5	206.0±84.0

* No significant differences (P<0.05) were found between any of the values .

Table 7. Influence of parent age and preincubation storage condition (length and temperature) on relative spleen weight of 8-weeks-old chicks

Parent age (week)	Storage temperature °C	Length of storage (day)			
		Fresh	5	10	15
45	Fresh	100.0±8.3			
	24 - 30		108.7±7.8	87.5±17.3	
	18 - 20		113.0±8.5	94.8±8.3	106.2±17.3
56	Fresh	92.9±11.9			
	24 - 30		98.0±10.9	95.7±18.5	117.1±18.5
	18 - 20		106.4±11.8	121.3±12.6	87.5±24.5

* No significant differences (P< 0.05) were found between any of the values

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تأثير عمر الأمهات وظروف تخزين بيض التفريخ على الوزن النسبى للأعضاء الداخلية والمناعة الأمية لبدارى التسمين .

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استهدف هذا البحث دراسة تأثير درجة حرارة ومدة التخزين القصيرة أو الطويلة لبيض التفريخ المأخوذ من أمهات بدارى التسمين عند عمرين مختلفين على الوزن النسبى لكيس الصفار بالجنين عند عمر ١٨ يوم ، الوزن النسبى للكبد ولكيس الصفار والمناعة الأمية للكتاكيت عمر يوم والوزن النسبى لغدد البرسا والتيموسية وكذلك الطحال لبدارى التسمين عند عمر ٨ أسابيع . تم تخزين بيض التفريخ المأخوذ من قطيعين لأمهات تسمين أربوراكرز ، الأول فى عمر ٤٥ أسبوع والثانى فى عمر ٥٦ أسبوع لمدد : صفر (البيض الطازج) ، ٥ ، ١٠ ، ١٥ يوم على درجة حرارة الغرفة (٢٤-٣٠°م) أو فى غرفة تخزين البيض (١٨-٢٠°م) .

وبصفة عامة لوحظ انخفاض الوزن النسبى للكبد والمناعة الأمية بينما إزداد الوزن النسبى لكيس الصفار مع زيادة مدة التخزين . ولقد تأثر الوزن النسبى للكبد بدرجة حرارة التخزين ، حيث إزداد معنوياً عندما تم تخزين البيض على درجة حرارة ٢٤-٣٠°م عنه عندما تم التخزين على درجة حرارة ١٨-٢٠°م .

ومن ناحية أخرى لم يؤثر عمر الأمهات أو درجة حرارة التخزين أو طول مدة التخزين على الوزن النسبى للغدة التيموسية والطحال عند عمر ٨ أسابيع .كذلك إزداد الوزن النسبى لغدة البرسا معنوياً فى الكتاكيت التى تم فقسها من الأمهات الأصغر سناً بالمقارنة بمثيله من الأمهات الأكبر سناً.