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EFFECTS OF FLOCK AGE, PRESTORAGE HEATING OF EGGS, EGG POSITION DURING STORAGE AND STORAGE DURATION ON HATCHABILITY PARAMETERS IN LAYER PARENT STOCK

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ABSTRACT

This study was carried out to determine the effect of different preincubation storage periods, different prestorage egg treatments and different egg positions during storage on egg weight loss, embryo mortality, hatchability, hatchling weight and chick sex ratio at hatch in layer parent stock. The following factors were analyzed: flock age (24 weeks, 65 weeks), prestorage heating periods (0, 5 hours at 37.8 °C), storage periods (0, 3, 6, 9, 12, 15 days at 15.0 °C) and egg positions (pointed end up, pointed end down). Parent stock age had a significant ($p < 0.05$) effect on chick weight. This increase in chick size due to advancing age of layer parent stock is attributable to the fact that egg weight significantly ($p < 0.05$) improved as the age of parent stock increased. Significant differences were found in absolute egg weight loss during the storage period, as a function of flock's age ($p < 0.05$). Eggs stored with the small end down lost less weight during storage compared with the eggs stored with the small end up. Hatchability of fertile eggs did significantly decline after storage for 9, 12, and 15 days. Flock age, prestorage heating and egg position during storage had no positive or detrimental effect on hatchability of set and fertile eggs and to male:female ratio under the conditions of this study. A flock age x egg position interaction indicated that females, hatched from eggs derived from old flock exhibited a tendency to be heavier when eggs are stored pointed end down whereas the egg position did not affect the female weight when eggs came from young flock.

Key words: poultry, layer parent stock, hatchability, prestorage heating, egg position, flock age

1 INTRODUCTION

In commercial poultry production, storage of hatching eggs is an indispensable part of hatchery operation and long egg storage times are sometimes unavoidable due to flock logistic issues and market availability (Lima *et al.*, 2012). One of the most frequently used methods to reduce the negative effects of long-term storage has been to heat eggs for short periods before storage. Pre-storage heating is based on the fact that embryos at the pre-gastrula stage at oviposition are less able to withstand the stress of storage compared with embryos at the later, gastrula stage (Schulte-Drüggelte, 2011). In the recent decades, several studies reported that pre-heating of poultry eggs before storage resulted in more live chicks and a lower level of embryonic mortality compared to the eggs that remained un-heated (Petek and Dikmen, 2004; Reijrink *et al.*, 2009). The traditional way to store hatching eggs is in the large end up position. It is assumed that in the case of large end up position the embryo has a higher chance to dehydrate or to stick to the internal eggshell membrane than when the egg is stored in the pointed end up position (Reijrink, 2016). Beside length of storage, embryo developmental stage at the start of storage, the position of eggs during storage there are several other aspects of egg storage that can influence subsequent successful incubation: flock age, genetics, environmental conditions and egg chemical and physical characteristics (Schmidt *et al.*, 2002). Some of these factors present interactions, and the work reported here was carried out with the aim to obtain further information on this subject through the use of layer parent stock's eggs subjected

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to different treatments prior to incubation. Most studies associated with the effect of egg storage period on hatchability characteristics are focused on Japanese quail and broiler parent stock. However, these parameters, especially sex ratio at hatch have not been fully examined in layer parent stock.

2 MATERIAL AND METHODS

In total, 4,349 freshly laid eggs (unwashed, feces-free) were obtained from two flocks of Slovenian layer parent stock Prelux-G. The parent stocks were housed in floor housing system. One flock consisted of young parent stock (24 weeks of age at the start of egg collection), and the other of old parent stock (65 weeks of age at the start of egg collection). In order that all the eggs from all groups could be set in the incubator at the same time, eggs were collected in time intervals, representing the six storage periods (0, 3, 6, 9, 12 and 15 days). Therefore, the first collection corresponded to eggs stored for 15 days and the sixth to fresh eggs (0 days). At the day of collection all the eggs belonging to the particular flock were randomly distributed into the following four treatments: (1) prestorage heating and further storage in egg cooling room with the pointed end up; (2) prestorage heating and further storage in egg cooling room with the pointed end down; (3) holding eggs in egg cooling room with pointed end up; and (4) holding eggs in egg cooling room with pointed end down. A total of 48 interactive groups constituted this study: 2 flocks (young-old) x 6 storage periods x 2 systems of storage treatments (prestorage heating: yes-no) x 2 positions of the hatching eggs during storage period (pointed end up – pointed end down). Prestorage heating was practiced in a single stage setter at a standard incubation temperature of 37.8 °C and 60 % RH on day 1 of storage and lasted 5 hours. Eggs were preheated and incubated in a Petersime model S168 setter and a Petersime model H168 hatcher (Petersime, Zulte, Belgium). After the prestorage heating treatments were completed, all the eggs including eggs from control and prestorage heating groups were stored in egg cooling room at a temperature of 15 °C and relative humidity of 75 % for 3, 6, 9, 12 or 15 days. After storage, eggs were transported from the cooling room to a commercial hatchery where they were in order to prevent condensation of moisture from the air on egg shell surface maintained at 22.0 °C and 60 % air relative humidity for three hours before incubation. Thereafter they were marked, weighed, set on the setter trays with pointed end down and disinfected by formaldehyde fumigation for 20 min in the incubator before the incubation was initiated. For the first 18 days of incubation, the setter temperature was 37.8 °C and humidity 60 %. For the rest period of 3 days the temperature in the hatcher was 37.2 °C and the humidity was 75 %. To determine weight loss eggs were weighed before incubation and at the moment of transference from setter to hatcher. Each setter tray contained eggs of 1 replication (out of 3) and therefore, the setter tray was used as the smallest experimental unit. Hatching was completed on the 21st day. The chicks were carefully removed from the duly identified hatching boxes according to treatment group, feather sexed and their weights were determined by using an electronic scale. Eggs which did not hatch were broken onto a tray and identified for infertility or embryonic mortality. Fertility was calculated as a percentage of set eggs. Hatchability was calculated as a percentage of set eggs and as a percentage of fertile eggs. Embryonic mortality was calculated as a percentage of set eggs. Data were submitted to analysis of variance using the the PROC GLM procedure of SAS software (Sas Inst., 2011). To obtain a normal distribution all the data in percentage form were transformed using arc-sine transformations prior to analysis. The Tukey test was applied to detect statistically significant ($p < 0.05$) differences between treatment groups.

3 RESULTS AND DISCUSSION

As it is indicated in Table 1 egg weight at set was significantly ($p < 0.05$) affected by layer parent stock's age. The eggs from old flock were ($p < 0.05$) larger than those from young flock. It is well recognized that there is a strong relationship between parent stock age and egg weight such that older hens produce larger eggs (Lourens *et al.*, 2006). Significant differences were found in absolute egg weight loss during the storage period, as a function of flock's age ($p < 0.05$).

Data showed that egg weight losses expressed in grams increased with advancing the age of parent stock hens (Table 1). Large eggs have a greater proportion of albumen (and thus greater moisture content) than small eggs. On the other hand eggs from older hens generally have a higher number of pores per egg and a very poor degree of cuticle coverage (Navarro *et al.*, 2013). The cuticle somewhat seals the pores and is useful in reducing moisture losses. Egg weight loss expressed in percentages did not differ between two flocks. Age of the hens had a significant ($p < 0.05$) effect on chick weight (Table 1). Data revealed that male and female chick weights were amplified ($p < 0.05$) with increasing the flock age and consequently egg size. Since chick weight increased with advancing age of layer parent stock it is advisable to set eggs from older flocks in order to obtain havier chicks. Lower hatchability and higher percentage of dead em-

***Table 1:** The effect of parent flock age and egg storage treatment prior incubation on hatchability parameters*

Hatchability parameters	Parent flock age		Egg storage treatment	
	Young flock (LSM ± SE)	Old flock (LSM ± SE)	Prestorage heating (LSM ± SE)	Without prestorage heating (LSM ± SE)
Egg weight at set (g)	56.77^{a} ± 0.07	66.63^{b} ± 0.13	61.46^{a} ± 0.09	61.94^{b} ± 0.09
Egg weight loss (g)	6.32^{a} ± 0.03	7.49^{b} ± 0.05	6.97^{a} ± 0.04	6.85^{b} ± 0.04
Egg weight loss (%)	11.14^{a} ± 0.05	11.26^{a} ± 0.09	11.34^{a} ± 0.07	11.05^{b} ± 0.07
Female weight (g)	46.82^{a} ± 0.27	53.28^{b} ± 0.48	49.65^{a} ± 0.36	50.45^{a} ± 0.36
Male weight (g)	47.43^{a} ± 0.26	53.33^{b} ± 0.45	50.09^{a} ± 0.34	50.68^{a} ± 0.34
Dead embryos (%)	11.36^{a} ± 0.010	13.52^{a} ± 0.030	12.61^{a} ± 0.018	12.24^{a} ± 0.018
Infertile eggs (%)	9.37^{a} ± 0.007	9.05^{a} ± 0.022	9.01^{a} ± 0.013	9.42^{a} ± 0.013
Hatchability of eggs set (%)	78.46^{a} ± 0.007	76.10^{a} ± 0.021	77.40^{a} ± 0.012	77.19^{a} ± 0.012
Hatchability of fertile eggs (%)	87.27^{a} ± 0.010	85.52^{a} ± 0.031	86.22^{a} ± 0.018	86.60^{a} ± 0.018
Male:female ratio	1.09^{a} ± 0.041	1.16^{a} ± 0.072	1.09^{a} ± 0.058	1.16^{a} ± 0.058

LSM – least square mean; SE – standard error; $^{a, b}$ – means within the same row with different superscripts are significantly ($p < 0.05$) different

bryos were recorded in older hens at the age of 65 weeks, however, non-significant difference ($p > 0.05$) was observed between these parameters at the age of 24- and 65-weeks-old hens (Table 1). In general, the decline in hatchability of fertile eggs with increasing age of the parent stock is caused by embryonic mortality. However many other contributing factors exist including larger egg size, poorer shell quality due to bigger surface area, albumen quality deterioration and increased the yolk cholesterol content (Iqbal *et al.*, 2016). Egg weight at set was significantly ($p < 0.05$) reduced when eggs were preheated for 5 hours before storage (Table 1). These results were expected because an exposure to heat treatment would increase the opportunity for water vapour to escape from the egg. Although prestorage heating treatment increased egg weight loss compared with the not preheated group, the differences in egg weight loss during storage did not affect the female or male weight, embryonic mortality, infertility and hatchability (Table 1). Some researchers claimed that warming of eggs did not affect hatchability when the eggs were stored for 4 to 7 days (Reijrink *et al.*, 2009) or had even detrimental effect on the hatchability when eggs were stored for 15 days (Petek and Dikmen, 2006). These differences might be due to variations in the stage of embryonic development at egg collection (before prestorage heating), genotype, flock age, egg yield, hatching performance, storage period and conditions, time of warming, as stated by other researchers (Elibol, 1997). The results presented in Table 2 gave a strong indication that egg position during storage has an influence ($p < 0.05$) on egg weight loss; positioning hatching eggs with pointed end up caused an increase in egg weight loss. It was suggested that storing eggs small end up may result in the central placement of the yolk in the albumen, giving the embryo greater protection from dehydration and adhesions (Mayers and Takeballi, 1984). These results do not agree with the results of our study since storing the eggs pointed end up just insignificantly improved hatch of eggs set by 2.53 % and hatch of fertile eggs by 2.23 % which was probably due to less embryonic mortality (Table 2).

No differences were found in the eggs' fertility among the different storage groups ($p > 0.05$) (Table 2). Hatchability of fertile eggs decreased significantly ($p < 0.05$) with increasing egg storage period after the 6th day of storage. The former results are consistent with the findings of (Elibol *et al.*, 2002), who reported that hatchability after 7 days of storage was higher than that of eggs stored for 14 days in broiler hatching eggs. Storage for 6 days elevates the hatchability of fertile eggs over the fresh egg (Table 2). As an egg ages, it loses CO_2 and moisture through shell pores, the albumen and yolk pH increase, the albumen height and vitelline strength decrease. The egg must lose some of the CO_2 to improve its hatchability but the loss must come at the right time. Excess carbon dioxide loss leading to a high albumen pH may have a negative effect upon initiation of embryonic growth and viability (Onagbesan *et al.*, 2007). This situation may explain why the eggs incubated the same day as they were laid did not hatch as well as those stored for period of 6 days (Table 2). Overall embryo mortality went from 8.44 % in embryos of 6-days stored eggs to 16.86 % in embryos from 9-days stored eggs and this difference was found to be significant due to the main effect of egg storage duration ($p < 0.05$) (Table 2). When eggs age, the vitelline membrane which is an important

Table 2: *Effects of storage time and of egg position during storage on hatchability parameters*

	Egg storage duration						Egg position during storage	
Hatchability parameters	0 d (LSM ± SE)	3 d (LSM ± SE)	6 d (LSM ± SE)	9 d (LSM ± SE)	12 d (LSM ± SE)	15 d (LSM ± SE)	Pointed end down (LSM ± SE)	Pointed end up (LSM ± SE)
Egg weight at set (g)	62.37^{a} ± 0.18	61.79^{ab} ± 0.18	61.81^{ab} ± 0.18	62.22^{a} ± 0.18	61.22^{bc} ± 0.18	60.77^{c} ± 0.18	61.79^{a} ± 0.10	61.61^{a} ± 0.10
Egg weight loss (g)	6.86^{ab} ± 0.08	6.73^{b} ± 0.08	7.04^{ab} ± 0.08	7.17^{a} ± 0.08	6.81^{b} ± 0.08	6.84^{ab} ± 0.08	6.81^{a} ± 0.04	7.01^{b} ± 0.04
Egg weight loss (%)	11.02^{ab} ± 0.13	10.92^{a} ± 0.13	11.39^{ab} ± 0.13	11.51^{b} ± 0.13	11.12^{ab} ± 0.13	11.23^{ab} ± 0.13	11.03^{a} ± 0.07	11.37^{b} ± 0.07
Female weight (g)	49.13^{ab} ± 0.68	49.01^{ab} ± 0.68	48.53^{a} ± 0.68	49.96^{abc} ± 0.68	51.57^{bc} ± 0.68	52.10^{c} ± 0.68	50.57^{a} ± 0.39	49.53^{a} ± 0.39
Male weight (g)	50.69^{ab} ± 0.63	48.44^{a} ± 0.63	48.82^{a} ± 0.63	52.36^{b} ± 0.63	50.26^{ab} ± 0.63	51.72^{b} ± 0.63	50.53^{a} ± 0.36	50.23^{a} ± 0.36
Dead embryos (%)	10.40^{abc} ± 0.060	9.70^{ab} ± 0.060	8.44^{a} ± 0.060	16.86^{c} ± 0.060	14.16^{abc} ± 0.060	16.08^{bc} ± 0.060	13.57^{a} ± 0.020	11.32^{a} ± 0.020
Infertile eggs (%)	8.36^{a} ± 0.044	7.95^{a} ± 0.044	9.13^{a} ± 0.044	7.73^{a} ± 0.044	10.39^{a} ± 0.044	12.05^{a} ± 0.044	9.48^{a} ± 0.015	8.95^{a} ± 0.015
Hatchability of eggs set (%)	80.57^{ab} ± 0.043	81.62^{a} ± 0.043	81.32^{ab} ± 0.043	74.27^{bc} ± 0.043	74.35^{bc} ± 0.043	70.85^{c} ± 0.043	76.02^{a} ± 0.014	78.55^{a} ± 0.014
Hatchability of fertile eggs (%)	88.44^{abc} ± 0.063	89.41^{ab} ± 0.063	91.74^{a} ± 0.063	81.57^{c} ± 0.063	84.13^{bc} ± 0.063	81.72^{c} ± 0,063	85.28^{a} ± 0.021	87.51^{a} ± 0.021
Male : female ratio	1.04^{a} ± 0.101	1.21^{a} ± 0.101	1.06^{a} ± 0.101	0.96^{a} ± 0.101	1.23^{a} ± 0.101	1.23^{a} ± 0.101	1.14^{a} ± 0.058	1.10^{a} ± 0.058

LSM – least square mean; SE – standard error; $^{a, b}$ – means within the same row with different superscripts are significantly ($p < 0.05$) different

barrier to prevent bacteria transfer between the albumen and yolk weakens (Gast *et al.*, 2005; Kirunda and McKee, 2000). This weakening of the vitelline membrane, exposes the embryo to levels of high alkaline pH, which it is suggested, is responsible for early embryonic mortality (Reijrink *et al.*, 2008). Egg weights at set remained fairly constant till the 9th day of storage (Table 2). After that day egg weight significantly ($p < 0.05$) decreased. Statistical differences were detected for egg weight loss when eggs were stored for 15 days. A number of environmental factors have been suggested to influence the sex of the avian offspring produced (Pike and Petrie, 2003). Due to financial implications and animal welfare concerns skewing the sex ratio at hatch simply by prestorage treatment would be beneficial to the commercial poultry industry. Because only the hen is productive for the egg industry, considerable value would be added if the sex ratio of egg laying breeds was altered towards production of females. Our study did not find any skew in chicken sex ratios at hatch due to preincubation treatments. Males were more common than females but differences were insignificant ($p > 0.05$). These results are not in agreement with the findings of Boerjan (2016) who reported that the introduction of 3–6 hours' prestorage heating in Lohmann layer hen hatcheries produced 3–7 % more females from eggs stored for more than 11 days. Interestingly, there was a significant interaction of flock age with egg position for female weight. The interaction was due to female weight decreased (2.4 g) with setting hatching egg with pointed end up in the old flock but not in the young flock. Also consistent with this result, there was a significant flock age x egg position interaction observed for egg weight loss due to higher egg loss for eggs set pointed end up from old flock (0.45 g; 0.73 %) but not from young flock.

4 CONCLUSIONS AND RECOMMENDATIONS

a. Prestorage heating of hatching eggs for 5 hours at 37,8 °C was not an effective way to reduce the loss of hatchability in eggs stored for long periods.

b. Storing eggs pointed end up significantly increased egg weight loss and brought some insignificant improvements in hatching results. The insignificant positive effect of storing eggs with pointed end up on hatchability varied between 2,2 % and 2,5 %. Thus, loses of moisture as water vapour through the pores of the shell could be reduced by turning the eggs with the small end down.

c. Research evidence agree that hatchability and viability of embryos deteriorate with egg storage in excess of 6 days.

d. Neither storage time nor prestorage heating, flock age or egg position during storage affected sex ratio at hatch.

e. Age of flock had a non-significant effect on percentage fertility and hatchability of layer parent stocks. However, heavier eggs from old flock produced chicks with higher hatch-weight than lighter eggs from young flock.

5 REFERENCES

Boerjan M. (2016) Pre-storage incubation and SPIDES: New procedures in hatching egg storage. Pas Reform. Retrieved from http://www.pasreform.com/academy/frequently-asked-questions/incubation/932-pre-storage-incubation-and-spides-new-procedures-in-hatching-egg-storage.html.

Elibol O. (1997). Investigation of some factors affecting hatchability results and troubleshooting. Ankara University, Ph.D. Thesis. Retrieved from http://bornova.ege.edu.tr/%7Efenbenst/.

Elibol O., Peak S.D., Brake J. (2002). Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. *Poultry Science, 81*, 945–950.

Gast R.K., Holt R.P., Murase T. (2005). Penetration of Salmonella Enteritidis and Salmonella Heidelberg through the vitelline membrane in an *in vitro* egg contamination model. *Poultry Science, 84,* 621–625.

Iqbal J., Khan S.H., Mukhtar N., Ahmed T., Pasha R.A. (2016). Effects of egg size (weight) and age on hatching performance and chick quality of broiler breeder. *Journal of Applied Animal Research, 44*, 54–64.

Kirunda D.F.K., McKee S.R. (2000). Relation quality characteristics of aged eggs and fresh eggs to vitelline membrane strength as determined by a texture analyzer. *Poultry Science, 79,* 1189–1193.

Lourens A., Molenaar R., Van Den Brand H., Heetkamp M.J.W., Meijerhof R., Kemp B. (2006). Effect of egg size on heat production and the transition of energy from egg to hatchling. *Poultry Science, 85*, 770–776.

Lima J.C.S., Silva P.L., Coelho L.R., Borges M.S., Freitas A.G., Fonseca B.B. (2012). Effects of inverting the position of layers eggs during storage on hatchery performance parameters. *Brazilian Journal of Poultry Science, 14*, 245–248.

Mayes F.J., Takeballi M.A. (1984). Storage of the eggs of the fowl (Gallus domesticus) before incubation: a review. *World's Poultry Science Journal, 40*, 131–140.

Navarro A.B.R., Dominguez-Gasca N., Munoz A., Ortega-Huertas M. (2013). Change in the chicken eggshell cuticle with hen age and egg freshness. *Poultry Science, 92*, 3026–3035.

Onagbesan O., Bruggeman V., De smit L., Debonne M., Witters A., Tona K., Everaert N., Decuypere E. (2007). Gas exchange during storage and incubation of avian eggs: effects on embryogenesis, hatchability, chick quality and post-hatch growth. *World's Poultry Science Journal, 63*, 557–573.

Petek M., Dikmen S. (2004). The effects of prestorage incubation of quail breeder eggs on hatchability and subsequent growth performance of progeny. *Animal Research, 53,* 527–534.

Petek M., Dikmen S. (2006). The effects of prestorage incubation and length of storage of broiler breeder eggs on hatchability and subsequent growth performance of progeny. *Czech Journal of Animal Science, 51*, 73–77.

Pike T.W., Petrie M. (2003). Potential mechanisms of avian sex manipulation. *Biological Reviews of the Cambridge Philosophical Society, 78,* 553–574.

Reijrink I., Meijerhof R., Kemp B., Van den Brand H. (2008). The chicken embryo and its microenvironment during egg storage and early incubation. *World's Poultry Science Journal, 64,* 581–598.

Reijrink I.A.M., Meijerhof R., Kemp B., Graat E.A.M., Van den Brand H. (2009). Influence of prestorage incubation on embryonic development, hatchability, and chick quality. *Poultry Science, 88*, 2649–2660.

Reijrink I.V.R. (2016). Alternative egg storage methods: small end up or turning. Technical information. Hatchtech. Retrieved from http://hatchtechgroup.com/media/document-en/alternativeeggstoragemethodssmallenduporturning(1).pdf.

SAS Inst. Inc. (2011). The SAS System for Windows, Release 9.3. Cary, NC.

Schmidt G.S., Figueiredo E.A.P., Avila V.S. (2002). Incubação: estocagem de ovos férteis. *Embrapa – Comunicado Técnico*, n. 303, 5 p.

Schulte-Drüggelte R. (2011) Recommendations for hatching egg handling and storage. *Lohmann information, 46*(1), 55–58.