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Original Article

Comparison of Several Turning Frequencies During the Storage Period of Red-Legged Partridge (*Alectoris rufa*) Hatching Eggs

Author(s)

González-Redondo P[I]
https://orcid.org/0000-0002-3324-2861
Martínez-Domínguez AR[I]
https://orcid.org/0000-0003-3155-2812

[I] Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, Universidad de Sevilla, 41013 Sevilla, Spain.

Mail Address

Corresponding author e-mail address
Pedro González-Redondo
Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, Universidad de Sevilla, 41013 Sevilla, Spain.
Phone: +34 954486454
Email: pedro@us.es

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ABSTRACT

The effects of three egg-turning regimes during the pre-incubation storage period on egg weight loss, hatchability, embryonic mortality, chick weight at hatching and incubation length of red-legged partridge (*Alectoris rufa*) compared with unturned eggs were investigated. Two hundred eggs were allocated to four 50-egg batches stored at 15ºC and 80% RH that being turned one, four and 24 times a day or remaining unturned, respectively. Eggs were incubated at 37.8ºC and 55% RH during the first 21 days and at 37.5ºC and 75% RH until hatching. Fertility was 70.5% and a good hatchability performance was obtained, characterised by 81.6% hatchability of fertile eggs, and weight losses of 0.78% during storage and of 10.04% during the first 21 days of incubation, 13.6 ±0.1 g hatchling weight, and incubation length of 23.45 ± 0.07 days (mean ± SEM). Hatchability, embryonic mortality developmental stage, egg weight loss during storage and incubation, hatchling weight and length of the incubation period were not affected by the turning frequency or the absence of turning during storage. Higher hatching synchrony was observed for eggs turned four times a day compared with unturned eggs and eggs turned once a day. In conclusion, turning red-legged partridge eggs during medium-term storage periods does not improve egg viability compared with unturned eggs.

INTRODUCTION

Red-legged partridges (*Alectoris rufa*) are raised in Mediterranean European countries (Portugal, Spain, Italy, and France) to provide birds in order both to ensure hunting stocks and to restock game reserve. This has given rise to a well-developed subsector of game farms (González-Redondo *et al.*, 2010). There are two keys to enhance *A. rufa* game farming productivity: egg handling before incubation and their artificial incubation. These aspects have been recently researched in order to review, assess, and improve the farmers' empirical know-how (e.g., González-Redondo & De la Rosa Sánchez, 2009; González-Redondo, 2010; Gómez-de-Travecedo *et al.*, 2014a, 2014b, 2014c; González-Redondo & Díaz-Merino, 2016). However, scientific assessment of several aspects of red-legged partridge egg handling during storage before incubation is still lacking. One of these aspects is egg turning during storage before incubation, which is a management practice applied in other poultry species to promote embryonic development and performance during subsequent incubation. Egg turning improves the interaction of the chorioallantoic membrane with the inner shell membrane, allowing adequate chorioallantoic membrane development when the typical incubation temperature is reached (Elibol *et al.*, 2002; Damaziak *et al.*, 2018). However, there is no consensus in the poultry literature regarding the mechanisms involved in the effects of egg turning during storage, nor on the optimal turning regime (Elibol *et al*., 2002; Damaziak *et al*., 2018). The most positive effects of egg turning during storage have been reported in eggs from older hens (Damaziak *et al*., 2018), or in low-quality eggs (Elibol *et al*., 2002). In general terms, more egg turning benefits are observed when eggs are stored for longer (over 14 d) than shorter periods (reviewed by Elibol *et al*., 2002).

In this regard, literature recommendations for egg turning during storage in red-legged partridges are controversial. *Alectoris rufa* eggs are usually stored pointed-end down, and Setién (1991) recommends turning them every 30-60 min, Llauradó (1987) and Peña & Caballero (1997) state turning twice a day suffices, while Cancho (1991) considers turning the eggs before incubation unnecessary. However, none of these authors base their recommendations on experimental data. In this context, scientific research has not yet elucidated the need for turning *A. rufa* eggs and, if turning were indeed necessary, the optimal turning frequency during the storage period. Moreover, red-legged partridge eggs store well for long periods (González-Redondo, 2010; Gómez-de-Travecedo *et al*., 2014a) and it is possible that the potential effects of egg turning would be more clearly manifested if the eggs are stored for periods longer than 7-10 d recommended in the farming practices of this species (Cancho, 1991). Therefore, this research was conducted to evaluate the effects of several egg-turning frequencies during a 15-day pre-incubation storage period on hatchability, embryonic mortality, egg weight loss, hatchling weight, and incubation length of artificially incubated red-legged partridge (*A. rufa*) eggs.

MATERIAL AND METHODS

Breeder flock and husbandry

The hatching eggs used in this study were collected on a red-legged partridge game farm in El Ronquillo (province of Seville, southern Spain). Breeders were 2 and 3 yrs old, kept in pairs (1 male and 1 female) in outdoor cages (50 × 65 cm), and fed a commercial feed containing 20% crude protein, 3.3% calcium, and 11.7 MJ metabolizable energy/kg (Avipacsa A-78®, Sanders, Dos Hermanas, Spain) *ad libitum.* Water was supplied *ad libitum*. During the reproductive resting period, the breeding flock was maintained under natural photoperiod. From December, birds were supplemented with artificial light, which was increased by 15 min/d until a total 16-h photoperiod (natural+artificial light) was achieved by January.

Experimental procedure

On March 5th, 200 recently-laid hatching eggs were divided into four treatments, consisting of 50 randomly-selected eggs weighing 19.64±0.10 g (mean ± SEM). After disinfection for 10 min in a UV chamber (UV-BOX 25®, Light progress, Anghiari, Italy), eggs were placed pointed end down for 15 d in a storage chamber (Vinotek®, Liebherr, Biberach an der Riss, Germany) set at 80% RH and15ºC. During this storage period the eggs were subjected to four experimental treatments, characterised by different turning frequencies at regular intervals: no turning (control treatment) and turning once, four times, or 24 times a day, respectively. The turning angle was 45º from vertical plane. After the storage period, and 18 h before being loaded in the incubator, all eggs were pre-warmed by maintaining them at room conditions (65% RH and 23ºC). The four experimental egg groups were submitted to incubation in the same automatic incubator (Masalles HS25®, Masalles, Ripollet, Spain) set at 55% RH and 37.8ºC and were automatically turned every hour. On day 21 of incubation, eggs from all treatments were transferred to the same independent hatcher (1-2 SA® hatcher, Maino Enrico-Adriano S.n.c., Oltrona di San Mamette, Italy) set at 75% RH and 37.5ºC, and egg turning ceased. In order to isolate and identify each hatchling, eggs were individually marked with a number and were subsequently placed in the hatcher trays inside individual, isolated boxes (5 × 5 cm) made of wire net.

Data recorded

All eggs were individually weighed before storage, at setting in the incubator after the storage period, and on day 21 of incubation. Individual egg weight loss percentages were calculated during storage (relative to egg weight at the beginning of storage period), during the first 21 d of incubation (relative to egg weight at setting), and total weight loss (between the beginning of the storage period and on day 21 of incubation). After hatching, the number of hatchlings and unhatched eggs were recorded. Unhatched eggs were broken to determine true fertility (for the sole purpose of determining the real number of fertile eggs stored and incubated per treatment) and, if fertile, they were classified into the following categories (Ernst *et al*., 2004; Gómez-de-Travecedo *et al*., 2014a): fertile eggs without development (FND; the fertilized blastodisc, that is regular and has a distinct circular edge, dies and never develops), eggs showing positive development (PD; there is cell growth over the surface of the yolk, but embryo dies early, before blood formation), early embryonic mortality (EEM, before day 8 of incubation, when only the development of blood vessels can be distinguished), late embryonic mortality (LEM, from day 9 of incubation onwards, when body structures can be distinguished), or pipped embryos dead in the shell (P). The length of the incubation period (measured in d) was obtained as the difference between setting and hatching dates of each single egg, determined through hatching controls carried out every 12 h (following the method of González-Redondo *et al*., 2012; Gómez-de-Travecedo *et al*., 2014c; González-Redondo & Díaz-Merino, 2016). All chicks were individually weighed at hatch.

Statistical analyses

Fertility, hatchability of total eggs set, hatchability of fertile eggs, and embryonic mortality at each development stage as a function of the turning frequency during storage before incubation were analysed using contingency tables on which Pearson's chi-square tests were calculated. Homoscedasticity was determined by Levene's test. One-way analysis of variance was used to analyse statistical differences among treatments of homoscedastic variables, including egg weights (before storage, after the storage period and at 21 d of incubation), egg weight loss during storage, during incubation and total weight loss (storage + incubation) of the fertile eggs, and hatchling weight. The test of Kruskal-Wallis was applied for heteroscedastic variables, such as incubation length. Tukey's multiple range tests at the 0.05 level of significance were used to separate means. Pearson's correlation was calculated to study the relationship between pre- and post-storage weight of fertile eggs. Range (minimum and maximum), as well as coefficient of variation (%) statistics were obtained to describe and analyse incubation length. Incubation length was tested for heteroscedasticity by Levene's test and differences in the variance of this variable among turning frequencies were also analysed by F tests. Mean ± SEM was used to indicate values of the quantitative variables. The statistical program SPSS v. 15.0 (SPSS Inc., 2006) was used to perform the statistical analyses.

RESULTS

Average fertility was 70.5%. Hatchability of total eggs set was 57.5% and hatchability of the fertile eggs reached 81.6% (Table 1). Turning frequency did not affect the fertility of incubated eggs (p=0.573),the hatchability of total eggs set (p=0.444) or the hatchability of fertile eggs (p=0.439). In addition, turning frequency during the storage period did not influence the development stage at embryonic mortality in fertile eggs (p>0.05; Table 2).

Table 1 – Fertility and hatchability of red-legged partridge eggs according to the turning frequency during the storage period.

Turning frequency during the storage period (times a day)	Number of eggs			Fertility[1] (%)	Hatchability of eggs set[2] (%)	Hatchability of fertile eggs[3] (%)
	Incubated	Fertile	Hatched			
0	50	33	29	66.0	58.0	87.9
1	50	33	24	66.0	48.0	72.7
4	50	38	31	76.0	62.0	81.6
24	50	37	31	74.0	62.0	83.8
Total	200	141	115	70.5	57.5	81.6
p-value				0.573	0.444	0.439

[1]Percentage of incubated eggs that were fertile. [2]Percentage of incubated eggs that hatched. [3]Percentage of fertile eggs that hatched.

Table 2 – Developmental stage at embryonic mortality of red-legged partridge fertile eggs according to the turning frequency during the pre-incubation storage.

Turning frequency during the storage period (times a day)	Number of fertile eggs	Development stage at embryonic mortality[1] (% of fertile eggs)					
		FND	PD	EEM	LEM	P	Total
0	33	6.1	3.0	0.0	0.0	3.0	12.1
1	33	12.1	6.1	0.0	3.0	6.1	27.3
4	38	7.9	5.3	2.6	2.6	2.6	21.1
24	37	10.8	5.4	0.0	0.0	0.0	16.2
Total	141	9.2	5.0	0.7	1.4	2.8	19.1
p value		0.821	0.947	0.435	0.568	0.506	0.431

[1]FND: fertile, no development; PD: positive development; EEM: early embryonic mortality; LEM: late embryonic mortality; P: Pipped, dead in the shell.

Average weight of fertile eggs before storage was 19.64±0.10 g, and mean egg weight at setting was 19.48±0.10 g. At these two stages, eggs turned four times a day were lighter than those that had not been turned (*p*=0.034 and *p*=0.031, respectively; Table 3). Moreover, egg weight at setting was positively correlated with initial egg weight (r=0.999, *p*<0.001). Considered jointly, egg weight losses during storage (0.78±0.03%), incubation (10.04±0.12%) and during the storage and incubation periods (10.75±0.13%) did not differ among turning frequency treatments (*p*>0.05; Table 3). After 21 d of incubation, the average weight of the fertile eggs was 17.53±0.09 g, with no difference among experimental groups (*p*=0.077; Table 3).

Mean hatchling weight was 13.62±0.09 g, with no difference among the experimental treatments (*p*=0.082; Table 4).

Table 3 – Egg weight losses during incubation in red-legged partridge fertile eggs according to the turning frequency during the storage period (mean±SEM).

Turning frequency during the storage period (times a day)	Number of fertile eggs	Egg weight before storage (g)	Egg weight before incubation (g)	Egg weight loss during the storage period[1] (%)	Egg weight at 21 d of incubation (g)	Egg weight loss during the first 21 d of incubation[2] (%)	Total egg weight loss[3] (%)
0	33	20.09±0.20[a]	19.93±0.20[a]	0.79±0.06	17.88±0.18	10.28±0.33	10.98±0.34
1	33	19.67±0.20[ab]	19.53±0.20[ab]	0.71±0.05	17.59±0.20	9.97±0.23	10.62±0.24
4	38	19.28±0.20[b]	19.11±0.20[b]	0.88±0.05	17.21±0.19	10.00±0.21	10.78±0.22
24	37	19.57±0.16[ab]	19.42±0.16[ab]	0.74±0.05	17.49±0.15	9.96±0.21	10.63±0.22
Total	141	19.64±0.10	19.48±0.10	0.78±0.03	17.53±0.09	10.04±0.12	10.75±0.13
p value		0.034	0.031	0.133	0.077	0.768	0.730

[1]Values expressed as a percentage of egg weight at the beginning of storage period. [2]Values expressed as a percentage of egg weight at the beginning of incubation. [3]Values are expressed as a percentage of egg weight loss between the beginning of the storage period and day 21 of incubation. [a,b]Means in the same column with different superscripts are significantly different (*p*<0.05).

Table 4 – Hatchling weight and incubation length in red-legged partridge eggs according to the turning frequency during the pre-incubation storage.

Turning frequency during the storage period (times a day)	Number of chicks hatched	Hatchling weight (g; Mean ± SEM)	Incubation length (d)				
			Mean ± SEM	Variance	CV[1] (%)	Min	Max
0	29	13.98±0.17	23.29±0.16	0.76[x]	3.74	22.50	26.00
1	24	13.51±0.22	23.63±0.20	0.92[x]	4.06	22.50	25.50
4	31	13.37±0.16	23.40±0.09	0.27[y]	2.24	22.50	24.50
24	31	14.61±0.14	23.50±0.13	0.52[xy]	3.06	22.50	25.50
Total	115	13.62±0.09	23.45±0.07	0.59	3.29	22.50	26.00
p value		0.082	0.497	0.002			

[1]CV: Coefficient of variation. [x-y]Variances in the same column with different superscripts are significantly different (*p*<0.05).

The mean incubation period lasted 23.45±0.07 d and was independent of egg-turning frequency during the storage period (*p*=0.497; Table 4). A lower variance of the incubation length was found for eggs turned four times a day compared with unturned eggs and eggs turned once a day, while eggs turned 24 hours a day showed an intermediate variance of this variable.

DISCUSSION

The fertility, hatchability of the incubated eggs and hatchability of the fertile eggs found in this trial were within the ranges described for farmed red-legged partridge by several authors (fertility: 50.2-89.7%, hatchability: 30.6-86.4%, and hatchability of the fertile eggs: 57.5-91.6%; Bagliacca *et al.*, 1988; Paci *et al.*, 1992; González-Redondo, 2006, 2010; Mourão *et al.*, 2010; Gómez-de-Travecedo *et al.*, 2014a, 2014b, 2014c; González-Redondo & Díaz-Merino, 2016). As hatchability did not vary among turning frequency treatments, it can be considered that all turning frequencies tested during storage, including the absence of turning, provided suitable hatchability. Our results confirm previous knowledge in alternative poultry species. They agree, therefore, with Woodard & Morzenti (1975), who found that turning chukar partridge (*Alectoris chukar*), pheasant (*Phasianus colchicus*) and Japanese quail (*Coturnix coturnix japonica*) eggs once a day before incubation had no appreciable effect on the hatchability of eggs stored up to 28 d. The results are also consistent with those of Miller & Wilson (1976), who reported that turning bobwhite quail (*Colinus virginianus*) eggs during the pre-incubation storage period does not affect hatchability. Our results do not support, however, several published recommendations concerning red-legged partridge game farming; Setién (1991), for example, advises turning eggs every 30-60 min, while Llauradó (1987) and Peña & Caballero (1997) state that turning them twice a day suffices. However, the present study agreed with Cancho (1991), who considers it unnecessary to turn *A. rufa* eggs during storage.

The negative effect on hatchability due to the absence of egg turning described in the literature for the chicken (*Gallus gallus*) is mainly caused by abnormal adhesion of the embryo to the inner shell membrane, leading to higher incidence of embryo malposition, as well as impairment of albumen utilisation, yolk sac development and fluids distribution in the egg (Decuypere & Bruggeman, 2007). However, the effect of turning of chicken eggs during storage before incubation on hatchability remains controversial. The studies of Mahmud & Pasha (2008) on broiler eggs turned 24 times a day during a 5-d storage period; Khan *et al*. (2012) on broiler eggs turned 3 to 12 times a day during a 6-d storage period and Tiwary & Maeda (2005) in layer eggs turned twice a day during 3- to 4-wk storage periods did not find any improvement in hatchability compared with unturned eggs. Conversely, improved hatchability was reported by Elibol *et al*. (2002) in broiler eggs turned 4 or 24 times a day during 3- to 14-d storage periods; by Mousa-Balabel & Saleem (2004) in broiler eggs turned 3 to 12 times a day during a 6-d storage period, and by Machado *et al*. (2010) in broiler grandparents' eggs turned 24 times a day during 16 d of storage, compared with unturned eggs. As reviewed by Elibol *et al*. (2002), there seems to be no advantage in turning when chicken hatching eggs are stored pointed end down for short periods (mostly less than 10 d), while it seems to be beneficial in longer storage periods. This could explain the absence of positive effects of egg turning during storage in red-legged partridges in the present study because the eggs were stored for a medium-term period of 15 d, while it is known that *A. rufa* eggs can be stored for at least 4 wks without significant loss of viability (González-Redondo, 2010; Gómez-de-Travecedo *et al*., 2014a, 2014b), as also occurs in other *Alectoris* species (Woodard & Morzenti, 1975). In this context, further research should elucidate whether egg turning during storage periods longer than a month could improve the hatchability of red-legged partridge eggs.

Embryonic mortality rate and the development stage at which embryonic mortalities recorded in the present trial are consistent with those of similar studies related to artificial incubation of red-legged partridge eggs (Gómez-de-Travecedo *et al*., 2014a, 2014b, 2014c). As embryonic mortality and the development stage at which embryonic mortalities occurred did not vary among turning frequency treatments, it may be considered that all turning frequencies tested during storage, including the absence of turning, provided suitable embryo performance. Furthermore, Elibol *et al*. (2002) state that there is no obvious relationship between turning 4 and 24 times a day during storage and the percentage of early or late dead embryos in broiler eggs stored between 3 and 14 d. However, Machado *et al*. (2010), in broiler grandparents' eggs stored for 16 d and turned 24 times a day, and Bowman (1969), in layer eggs stored and turned between 7 and 28 d, found a reduction in embryonic mortality at all stages of the incubation compared with unturned eggs. Khan *et al*. (2012) also report lower early embryonic mortality during incubation in broiler eggs turned 3 to 12 times a day during a 6-d storage period, compared with unturned eggs. As for hatchability, the advantage for embryo survival due to turning increases with longer storage periods (Bowman, 1969). This could explain the absence of effects in the present trial because a medium-term storage period was chosen.

It is relevant, however, to note that while the absence of egg-turning during the storage period does not have any detrimental effects on hatchability and embryonic performance, turning them during incubation is essential in red-legged partridges due to the fact that ceasing to turn eggs early during incubation increases embryonic mortality (Llauradó, 1987; Cancho, 1991; Setién, 1991; Peña & Caballero, 1997) and reduces hatchability (González-Redondo & De la Rosa Sánchez, 2009).

Mean weight of recently-laid fertile eggs and egg weight loss during storage and during the first 21 d of incubation results agree with those described for red-legged partridges reared in game farms (González-Redondo, 2010; Mourão *et al*., 2010; Gómez-de-Travecedo *et al*., 2014a, 2014b, 2014c; González-Redondo & Díaz-Merino, 2016). As expected, egg weight losses were not affected by the turning frequency during egg storage. In fact, none of the studies reviewed in the literature assess the potential effect of turning eggs on weight loss during storage and incubation.

Hatchling weights recorded in this trial was similar to the mean values described for red-legged partridges in captivity (13.8-14.3 g, according to Gómez-de-Travecedo *et al*., 2014a, 2014b, 2014c; González-Redondo & Díaz-Merino, 2016) and was not modified by the turning frequency during storage. A visual inspection showed that hatchling quality and performance was normal. Our results do not support those of Mousa-Balabel & Saleem (2004) and Khan *et al*. (2012), who describe an increase in broiler hatchling weight when the eggs were turned 3 to 12 times a day compared with unturned eggs stored for 6 d.

The mean length of the incubation period obtained in this research fitted the 23.4-d period reported for artificially-incubated red-legged partridge eggs (González-Redondo *et al*., 2012). While the modification of other parameters, e.g., storage period length (Gómez-de-Travecedo *et al*., 2014a, 2014b) or incubation temperature (Gómez-de-Travecedo *et al*., 2014c) alters incubation length, egg-turning frequency during storage did not affect its average value. The lower variance of the incubation period found in this trial for eggs turned four times a day compared with unturned eggs and eggs turned once a day partially agrees with Mousa-Balabel & Saleem (2004), who describe a reduction in the hatching time in broiler eggs turned 3 to 12 times a day compared with unturned eggs stored for 6 d. However, it must be taken into account that our data are not strictly comparable with those of Mousa-Balabel & Saleem (2004) because these authors measured hatching time as the time elapsed from loading the eggs in the incubator to the hatching of the last egg in the batch. We, however, calculated the mean incubation length for each experimental batch as the difference between incubator loading and hatching date of each single egg. In the present study, the maximum incubation length of the last egg hatched in the unturned batch was 26.0 d, while the last eggs hatched at 25.50 and 24.50 d in the batches submitted to turning (Table 4), which partially agrees with Mousa-Balabel & Saleem (2004). Consequently, further research is necessary to elucidate whether egg turning during storage modifies hatching synchrony, as suggested by the results of Mousa-Balabel & Saleem (2004) results and by our data. However, it is not clear why the variance of incubation length of the eggs turned 24 times a day had an intermediate value compared with those turned four times a day and eggs turned once a day or unturned. In this sense, it will be interesting to investigate the effects of egg turning during long storage periods on egg quality, embryo viability, and embryo developmental stage at the end of storage period.

CONCLUSION

Turning red-legged partridge eggs one, four or 24 times a day during a storage period of 15 d before incubation did not influence hatchability, hatchling weight, or incubation length compared with unturned eggs. Hatching synchrony seemed to be affected by the egg-turning regime. Therefore, further research is necessary to confirm the recommendation from several informative publications advising *A. rufa* eggs be turned during medium-term storage periods before incubation.

CONFLICT OF INTEREST

No potential conflict of interest was found by the authors.

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