PRODUCTION, MODELING, AND EDUCATION

Effects of storage temperature and length of the storage period on hatchability and performance of red-legged partridge (*Alectoris rufa*) eggs

P. Gómez-de-Travecedo, F. P. Caravaca, and P. González-Redondo¹

Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, Universidad de Sevilla, 41013 Sevilla, Spain

ABSTRACT The aim of the present study was to investigate, in red-legged partridge (Alectoris rufa) eggs, the effects of 7- and 42-d storage periods with different storage temperatures (15, 12, and 9°C) on egg weight loss, hatchability, chick weight at hatch, incubation length, and development stage at embryonic mortality. A total of 420 red-legged partridge eggs were arranged in a 2 \times 3 factorial design with 2 levels of storage length and 3 levels of storage temperature, resulting in 6 treatments consisting of 10 replications of 7 eggs each. We found that the storage length significantly reduced hatchability of the fertile eggs (P = 0.001), increasing late embryonic mortality (P = 0.001). Storage temperature did not influence on the embryonic mortality at any stage (P > 0.05). Egg weight loss during storage increased with the storage length (P < 0.001), storage temperature (P < 0.001), and their interaction (P < 0.001). Incubation length increased with the storage length (P < 0.001); however, it was not influenced by the storage temperature (P > 0.05). Nevertheless, incubation period decreased with the storage temperature for 7-d storage, and increased with the storage temperature for 42-d storage (P = 0.005). It can be concluded that in this study red-legged partridge eggs stored well with little deterioration up to 42 d at 9 and 12°C and 80% RH, in contrast to the lesser durability of eggs described in the literature for other poultry species. In case of 7-d storage periods, hatchability of A. rufa fertile eggs is higher when they are stored at 15°C. These findings are useful to address specific demands of game farms that require fertile eggs for hatching whose shelf-life should be long enough to maintain hatchability until further incubation. And, due to the marked reproductive seasonality of red-legged partridge, long-term storage of hatching eggs could permit the distribution of batches of chicks throughout the year.

Key words: red-legged partridge, Alectoris rufa, egg storage length, storage temperature, hatchability

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INTRODUCTION

To ensure availability of birds for hunting and provide birds for reestablishment purposes, the red-legged partridge (Alectoris rufa) is raised in southwestern European countries, where it is endemic, and in other countries and areas where it has been successfully introduced such as the United Kingdom, Azores, Canary Islands, and Madeira (Coles, 1971; Aebischer and Lucio, 1997, González-Redondo, 2004). Complete-cycle farms coexist with others specialized only in rearing and preparing partridges for their release (González-Redondo et al., 2010). The latter specialized farms, as well as small complete-cycle farms that do not produce sufficient eggs to address specific demands of partridges, require fertile eggs for hatching whose shelf-life should be long enough to maintain hatchability until further incubation, being necessary to store these eggs

©2014 Poultry Science Association Inc. Received May 19, 2013. Accepted November 10, 2013. for a period of time. Another reason to store eggs for long periods derives from the fact that the red-legged partridge has a marked reproductive seasonality (Beer and Jenkinson, 1981). Thus, depending on the area's climate and latitude, egg-laving starts in late February and ends in July or early August (Torres and Garcés, 1995; Molleda, 1998; García Martín and Dalmau, 2003), lasting more than 14 wk (Gaudioso et al., 2002). At the beginning and at the end of the reproductive season, laying frequency is low (Pérez y Pérez, 1981; Bagliacca et al., 1988; González-Redondo et al., 2003; González-Redondo, 2006), whereas laying rate and hatchability peaks between 5 and 9 wk (Gaudioso et al., 2002). Under these conditions, long storage periods might be useful to permit subsequent incubation of the surplus eggs and to distribute the batches of chicks throughout the year. The long-term storage of the eggs may also be useful at the beginning and at the end of the laying season, when the time required to gather the eggs to fill the incubator and to rear a large enough batch of chicks is longer, especially in small farms, due to the low egg-laying rate.

¹Corresponding author: pedro@us.es

Woodard and Morzenti (1975) state that long-term storage of chukar partridge (Alectoris chukar) eggs at 16°C and 70% RH up to 28 d before incubation has little effect on subsequent hatchability. Similarly, González-Redondo (2010) found no significant effect of storage time on hatchability of red-legged partridge (A. rufa) eggs stored at 15°C and 80% HR up to 28 d, although eggs stored in the same conditions for 35 d showed a significant decrease in hatchability. Furthermore, other studies with avian eggs show that the storage temperature affects the success of incubation, lower temperatures being more suitable for longer storage periods and vice versa (Brake et al., 1997). Specifically, optimum hatchability of hen (Gallus gallus) eggs after a storage period longer than 14 d was achieved when storage temperature was about 12°C (Olsen and Haynes, 1948; Funk and Forward, 1960), whereas 15°C was better for eggs stored for 8 d, and 18°C better for those stored for 2 d (Kirk et al., 1980). Nevertheless, there are no scientific studies in this regard with red-legged partridge eggs, although informative publications recommend maintaining A. rufa eggs at 20°C for eggs to be stored up to 3 d, at 13 to 16°C for eggs to be stored for 7 d, and at 11 to 12°C for eggs to be stored for periods longer than 7 d (Cancho, 1991). However, this information is not supported by experimental data. Therefore, the aim of the present study is to investigate the effect of 7- and 42-d storage periods with different storage temperatures (15, 12, and 9°C) on weight losses during the storage and incubation periods, length of the incubation period, hatchability, and embryonic mortality of red-legged partridge (A. rufa) eggs.

MATERIALS AND METHODS

Birds and Husbandry

This experiment was carried out using 420 hatching eggs from a red-legged partridge farm located in the province of Seville, Southern Spain. The breeding partridges were housed in pairs in outdoor cages measuring 50×65 cm. They were fed with commercial feed (20% CP and 3.3% Ca) and subjected to artificial lighting from December onward. Starting from December, the photoperiod was increased by a quarter of an hour every day until a complete photoperiod of 16 h of light (natural light + artificial light) was reached by January. Egg laying started in mid-January. All eggs were collected from partridges aged between 2 and 3 yr.

Experimental Design

The 420 eggs were arranged in a 2×3 factorial design with 2 levels of storage length (7 and 42 d) before incubation and 3 levels of storage temperature (9, 12, and 15°C), resulting in 6 treatments consisting of 10 replications of 7 eggs each. The 3 batches of eggs to be stored for 42 d (210 eggs) were collected on April 15, and the other 3 batches of eggs to be stored for 7

d (210 eggs) were collected on May 20. All eggs were laid within 3 d before the collection day and kept in the farm at standard conditions (15°C and 80% RH) until then, as this was the time required to gather 210 eggs in the cited farm, and 3-d gathering interval for each treatment does not influence the viability of Alectoris eggs (Cağlayan et al., 2009; González-Redondo, 2010). In all cases eggs were kept with the small end down at 80% RH in a storage chamber (Vinotek, Liebherr, Biberach an der Riss, Germany), being turned 45° once a day at regular intervals.

Egg Incubation

Following storage, the eggs were prewarmed before incubation by maintaining them in the room where the incubator was located during 12 h at 24°C and 43% RH. All the eggs were loaded into the incubator on the same date (May 28th). The incubator (Masalles HS25, Masalles, Ripollet, Spain) was set at 37.8°C and 55% RH, and the eggs were turned 45° every hour. On d 21 from the beginning of incubation, the eggs were transferred to a hatcher (Maino Incubators 2–630 XHM, Maino Enrico-Adriano S.n.c., Oltrona di San Mamette, Italy), which was set at 37.5°C and 80% RH, without turning of eggs.

Data Recorded

To determine egg weight losses, all eggs were weighed before storage, after storage, and after 21 d of incubation. For each individual egg, weight loss during storage was calculated as a percentage of egg weight at the beginning of storage period. Egg weight loss during the first 21 d of incubation was calculated as a percentage of egg weight at the beginning of incubation, and total egg weight loss was calculated as a percentage of egg weight loss between the beginning of the storage period and 21 d of incubation. To determine hatchability, the number of hatched chicks and unhatched eggs were recorded after the incubation period. Incubation length was measured, through hatching controls carried out every 12 h, as the difference between the hatching date (when the chick becomes out of the shell) and the date when the incubator was loaded. All chicks were weighed at hatch. Unhatched eggs were broken and examined macroscopically to determine fertility and, if fertile, they were assigned to the following categories: fertile without development, positive development, early embryonic mortality, late embryonic mortality, or pipped but not out of shell (Juárez-Caratachea and Ortiz, 2001; Ernst et al., 2004).

Statistical Analysis

Fertility, hatchability of incubated eggs, hatchability of fertile eggs, weight losses of fertile eggs, chick weight at hatch, incubation length, and embryonic mortality of fertile eggs, as dependent variables, were analyzed

Table 1. Fertility and hatchability of red-legged partridge eggs according to the length of the egg storage period and storage temperature¹

Item	n^2	Fertility ³ (%)	Hatchability ⁴ (%)	Hatchability of the fertile eggs ⁵ (%)	
Storage time (d)					
7	30	57.14	50.48	89.39^{a}	
42	30	59.52	44.29	$74.50^{\rm b}$	
Storage temperature (°C)					
9	20	60.00	49.29	83.58	
12	20	57.14	47.86	83.00	
15	20	57.86	45.00	79.25	
Storage time (d) \times storage temperature (°C)					
7×9	10	61.43	52.86	86.83	
7×12	10	55.71	50.00	90.00	
7×15	10	54.29	48.57	91.33	
42×9	10	58.57	45.71	80.33	
42×12	10	58.57	45.71	76.00	
42×15	10	61.43	41.43	67.17	
SEM		1.90	1.97	2.34	
P-value					
Storage time		0.545	0.126	0.001	
Storage temperature		0.825	0.673	0.673	
Interaction		0.580	0.945	0.252	

a,bValues in the same column with different superscripts are significantly different (P < 0.05).

using the univariate GLM procedure, with storage length and storage temperature before incubation as fixed effects. Interactions between the factors were also analyzed. When significant differences were found by the GLM, means were separated using Tukey's multiple range tests. All data are expressed as mean and SEM. For all comparisons, statistical significance was accepted when P < 0.05. The analyses were performed using SPSS v. 15.0 software (SPSS Inc., 2006).

RESULTS AND DISCUSSION

Fertility and Hatchability

The average fertility of the eggs recorded in this study (58.33%; Table 1) was lower than in previous reports on A. rufa under farming conditions (73.5 to 85.6%; Bagliacca et al., 1988; Paci et al., 1992; González-Redondo, 2006, 2010; Mourão et al., 2010). Differences in eggs fertility between this study and normal values recorded in the literature might be because, in this experiment, eggs were collected after the peak of the breeding season, when fertility of red-legged partridge decreases (Gaudioso et al., 2002; González-Redondo, 2006). Other conditions such as differences in the fertility selection in the breeding flocks, housing type, or kind of feed used (Hernando, 1990) could also influence of the low average fertility observed. However, no differences were found in fertility according to the storage length, storage temperature, or their interaction (P >0.05), which indicates that eggs were randomly distributed among batches.

The average hatchability of the incubated eggs (47.39%; Table 1) was also below the range of values found in the literature (53.4 to 84.1%; Mori et al., 1985; Paci et al., 1992; González-Redondo et al., 2003; González-Redondo, 2006, 2010; Mourão et al., 2010). No differences were found in the hatchability of all incubated eggs according to the storage length, storage temperature, or their interaction (P>0.05).

Hatchability of the fertile eggs (81.95%) was within the mean values found in literature for this species (72.6 to 91.6%; Bagliacca et al., 1988; Paci et al., 1992; González-Redondo, 2006, 2010). This means that storage and incubation conditions were adequate to the requirements of this species. No significant differences were found in hatchability of fertile eggs according to the storage temperature (P > 0.05). However, hatchability of fertile eggs was significantly higher for eggs stored for 7 d than for eggs stored for 42 d (89.39 and 74.50%, respectively). There was no interaction storage length × storage temperature on hatchability of fertile eggs (P > 0.05). These results agree with previous studies showing that, when storage period is lengthened, the internal quality of the fertile egg (yolk ratio, yolk index, albumen ratio, albumen index, and Haugh unit) progressively deteriorates (Tilki and Saatci, 2004; Cağlayan et al., 2009). With storage time, albumen pH increases, albumen height decreases (Lapão et al., 1999), and yolk sac membrane elasticity decreases (Jones and Musgrove, 2005), impairing embryo development and hatchability. Thus, prolonged storage of hatching partridge eggs interferes with their normal progress and hatchability decreases rapidly after 28 d of

¹Mean

²Number of replications of 7 eggs each.

³Percentage of incubated eggs that were fertile.

⁴Percentage of incubated eggs that hatched.

⁵Percentage of fertile eggs that hatched.

storage (Woodard and Morzenti, 1975; Woodard, 1982; González-Redondo, 2010). Nevertheless, mean hatchability value found in our study for the eggs stored for 42 d was within values reported for this species (72.6 to 91.6%; Bagliacca et al., 1988; Paci et al., 1992; González-Redondo, 2006, 2010). This confirms previous studies demonstrating that Alectoris eggs can be hold without decline in hatchability for longer periods than other poultry species eggs (Woodard and Morzenti, 1975; Wilson et al., 1997; Fasenko, 2007; Romao et al., 2008; González-Redondo, 2010). In particular, Woodard and Morzenti (1975) demonstrated that chukar partridge eggs can be stored for 28 d at 16°C and 70\% RH without decline in hatchability, and González-Redondo (2010) observed the same results in red-legged partridge eggs stored for 28 d at 15°C and 80% RH. Nevertheless, González-Redondo (2010) proved a significant decline in hatchability of A. rufa eggs stored for 35 d at 15°C and 80% RH. In fact, we observed in our study that, in case of 42-d storage at 15°C the hatching rate of the fertile eggs (67.17%) was below the average reported for this species under farming conditions. However, hatchability of fertile eggs stored for 42 d at 12°C (76.00%) and at 9°C (80.33%) was within the mean values found in the literature for this species. This matches previous studies in poultry species showing that lower temperatures slow down the internal deterioration of the embryo, which makes them more suitable for longer storage periods (Olsen and Haynes, 1948; Funk and Forward, 1960; Brake et al., 1997). This also agrees with informative publications which recommend to maintain A. rufa eggs at 20°C for eggs to be stored up to 3 d, at 13 to 16°C for eggs to be stored for a week, and at 11 to 12°C for eggs to be stored for periods longer than 7 d (Cancho, 1991; García Martín and Dalmau, 2003).

Egg Weight and Egg Weight Losses

In this study, mean values of recently laid egg weight (19.63 g; Table 2) matched well with those described for A. rufa (Beer and Jenkinson, 1981; Pérez y Pérez, 1981; Mourão et al., 2010) and other species of the Alectoris genus (Kırıkcı et al., 2004; Tilki and Saatci, 2004; Cağlayan et al., 2009) under farming conditions. The effect of storage treatments on the percentage of fertile egg weight loss during storage was highly significant (P < 0.001). In fact, eggs stored for 42 d lost 3.04\% of their initial weight, whereas those stored for 7 d lost only 0.63%. These variations of egg weight loss during storage, depending on the storage length, followed the pattern found by Tilki and Saatci (2004) for rock partridge (Alectoris graeca) eggs stored at 15 to 18°C and 70% RH during 7, 14, 21, 28, and 35 d; and by González-Redondo (2010) for red-legged partridge (A. rufa) eggs stored at 15°C and 80% RH during 7, 14, 21, 28, and 35 d. When storage period is lengthened, moisture losses from the egg increase, which contributes to the deterioration of the internal quality of the fertile egg (Tilki and Saatci, 2004; Cağlayan et al., 2009). The fertile egg weight loss during storage was also influenced by previous storage temperature (P < 0.001). Eggs stored at 9 and 12°C lost 1.18 and 1.49%, respectively, of their initial weight, whereas losses in eggs stored at 15°C were significantly higher (2.82%). A highly significant interaction was also found between storage length and storage temperature on the fertile egg weight loss during storage. Eggs stored for 42 d at 15°C lost 4.60% of their initial weight, whereas eggs stored for 7 d at 9°C lost only 0.43% of their initial weight. Thus, as occurs in other poultry species, during storage the fertile eggs weight loss increased progressively both with temperature and length of storage (Walsh et al., 1995).

Average weight loss of the fertile eggs during the first 21 d of incubation amounted to 11.11% of the egg weight before incubation (Table 2). This value was slightly higher than the values found by González-Redondo (2010) for A. rufa eggs stored for periods varying between 0 and 35 d at 15°C, although it represented a lower weight loss than that found for A. graeca eggs by Kırıkcı et al. (2004). During the first 21 d of incubation, no significant differences among treatments were found in the fertile eggs weight loss (P > 0.05). However, total egg weight loss of the fertile eggs increased progressively for batches of eggs stored during longer periods (P < 0.001; 13.70% in 42-d and 11.75% in 7-d storage)treatments). Total egg weight loss of the fertile eggs increased also progressively with storage temperature (P< 0.001; 12.06% at 9°C, 12.48% at 12°C, and 13.65% at 15°C). No interaction was found between storage length and storage temperature for total egg weight loss (P =0.205). Total weight loss, highly potentiated in batches of eggs stored for long periods, was already observed for rock partridge by Kırıkcı et al. (2004) and for redlegged partridge by González-Redondo (2010). In these cases the egg weight loss, related to progressive deterioration of the internal quality of the egg, is responsible for the decreased in hatchability observed (González-Redondo, 2010).

Incubation Length

Mean value of incubation length in this study (23.22 d) was within those found in the literature for A. rufa under farming conditions (Flores, 1979; Torres and Garcés, 1995; García Martín and Dalmau, 2003; González-Redondo et al., 2012). Incubation length was highly influenced (P < 0.001) by the length of the storage period before incubation (Table 3). Eggs stored for 7 d had significantly shorter incubation periods (23.04 d) than eggs stored for 42 d (23.39 d). These results follow the pattern of previous studies in broiler and duck species (Tona et al., 2003; Bagliacca et al., 2005; Fasenko, 2007), in which longer storage periods prolonged incubation. Long-term egg storage influences embryo development and metabolism so that embryos grow at a slower rate than short-term stored eggs, and subse-

Table 2. Egg weight losses during storage and incubation periods in red-legged partridge fertile eggs according to the egg storage temperature and the length of the storage period¹

Item	n^2	Egg weight before storage (g)	Egg weight loss during storage ³ (%)	Egg weight loss during incubation ⁴ (%)	Total egg weight loss ⁵ (%)
Storage time (d)					
7	30	$19.43^{\rm b}$	$0.63^{\rm b}$	11.20	$11.75^{\rm b}$
42	30	$19.82^{\rm a}$	$3.04^{\rm a}$	11.01	13.70^{a}
Storage temperature (°C)					
9	20	19.49	$1.18^{\rm b}$	11.01	$12.06^{\rm b}$
12	20	19.79	$1.49^{\rm b}$	11.15	$12.48^{\rm b}$
15	20	19.60	2.82^{a}	11.16	13.65^{a}
Storage time (d) × storage temperature (°C)					
7×9	10	19.34	0.43^{e}	10.88	11.26
7×12	10	19.54	$0.41^{\rm e}$	11.34	11.69
7×15	10	19.42	$1.05^{ m d}$	11.39	12.30
42×9	10	19.65	1.93^{c}	11.14	12.85
42×12	10	20.03	$2.58^{\rm b}$	10.97	13.26
42×15	10	19.79	4.60^{a}	10.92	14.99
SEM		0.06	0.19	0.13	0.21
P-value					
Storage time		0.001	< 0.001	0.473	< 0.001
Storage temperature		0.112	< 0.001	0.871	< 0.001
Interaction		0.824	< 0.001	0.486	0.205

 $^{^{\}mathrm{a-e}}$ Values in the same column with different superscripts are significantly different (P < 0.05).

quently produces longer incubation periods (Fasenko, 2007).

In our study, storage temperature did not influence on the incubation length (P>0.05). However, a significant interaction was observed between storage length and storage temperature on incubation length (P=0.005). Thus, eggs stored during 42 d at 15°C had lon-

ger incubation periods (23.49 d) than eggs stored for 42 d at 9°C (23.26 d), and eggs stored during 7 d at 15°C hatched earlier (22.93 d) than eggs stored for 7 d at 9°C (23.23 d). This interactive effect matches with the results of previous researches on embryo development in poultry and duck species in which, for shorter incubations, lower temperatures were always better for longer

Table 3. Chick weight at hatch and length of the incubation period in red-legged partridge according to the egg storage temperature and the length of the storage period¹

Item	$\rm n^2$	Chick weight at hatch (g)	Incubation length (d)	
Storage time (d)		<u> </u>		
7	30	13.99	$23.04^{\rm b}$	
42	30	14.03	23.39^{a}	
Storage temperature (°C)				
9	20	13.92	23.25	
12	20	14.08	23.19	
15	20	13.03	23.21	
Storage time (d) × storage temperature (°C)				
7×9	10	13.79	$23.23^{ m ab}$	
7×12	10	14.13	$22.96^{\rm b}$	
7×15	10	14.04	$22.93^{\rm b}$	
42×9	10	14.05	$23.26^{ m ab}$	
42×12	10	14.02	23.41^{a}	
42×15	10	14.02	23.49^{a}	
SEM		0.07	0.04	
P-value				
Storage time		0.773	< 0.001	
Storage temperature		0.692	0.784	
Interaction		0.580	0.005	

 $^{^{\}mathrm{a,b}}\mathrm{Values}$ in the same column with different superscripts are significantly different (P < 0.05).

¹Mean.

 $^{^2}$ Number of replications of 7 eggs each.

 $^{^3}$ Values are expressed as a percentage of egg weight at the beginning of storage period.

 $^{^4\}mathrm{Values}$ are expressed as a percentage of egg weight at the beginning of incubation.

⁵Values are expressed as a percentage of egg weight loss between the beginning of the storage period and 21 d of incubation.

¹Mean.

²Number of replications of 7 eggs each.

Table 4. Effect of storage length and storage temperature on embryonic mortality of red-legged partridge eggs

Item		Development stage at embryonic mortality ² (% of fertile eggs)					
	n^1	FND	PD	EEM	LEM	Pip	Total
Storage time (d)							
7	30	2.22^{a}	1.78	3.44	$3.17^{\rm b}$	0.00	$10.61^{\rm b}$
42	30	$0.00^{\rm b}$	4.94	5.78	$13.94^{\rm a}$	0.83	25.50^{a}
Storage temperature (°C)							
9	20	2.08	4.25	2.25	7.83	0.00	16.41
12	20	0.00	2.08	7.08	7.83	0.00	17.00
15	20	1.25	3.75	4.50	10.00	1.25	20.75
Storage time (d) × storage temperature (°C)							
7×9	10	4.17	2.00	0.00	7.00	0.00	13.17
7×12	10	0.00	1.67	8.33	0.00	0.00	10.00
7×15	10	2.50	1.67	2.00	2.50	0.00	8.67
42×9	10	0.00	6.50	4.50	8.67	0.00	19.67
42×12	10	0.00	2.50	5.83	15.67	0.00	24.00
42×15	10	0.00	5.83	7.00	17.50	2.50	32.83
SEM		0.64	1.07	1.29	1.68	0.41	2.34
P-value							
Storage time		0.084	0.151	0.370	0.001	0.322	0.001
Storage temperature		0.405	0.697	0.318	0.801	0.375	0.673
Interaction		0.405	0.749	0.421	0.118	0.375	0.252

a, b Values in the same column with different superscripts are significantly different (P < 0.05).

storage periods, and higher temperatures were better for shorter storage (Mayes and Takeballi, 1984; De Carville and De Croutte, 1985; Bagliacca et al., 2005).

Chick Weight at Hatch

In this study, the average chick weight at hatch was 14.01 g (Table 3). This value matched well with that described for A. rufa (Pérez y Pérez, 1981) and A. graeca (Kırıkcı et al., 2004) in captivity. Neither the storage length nor the storage temperature influenced chick weight at hatch (P > 0.05). No interaction between the 2 factors was observed on chick weight at hatch (P > 0.05). However, in previous researches on broiler eggs, chick weight at hatch tends to decline with long storage periods (Christensen et al., 2002; Elibol et al., 2002; Tona et al., 2003). Furthermore, Ruiz and Lunam (2002) found that for short storage (1 to 3 d) the storage temperature (20, 16.5, or 10°C) does not affect chick weight at hatch; however, for long storage period (9 to 11 d), storage at 16.5°C compared with 10°C reduced chick weight at hatch.

Embryonic Mortality

Storage length influenced significantly the rate of late embryonic mortality and, in consequence, on the total embryonic mortality (P=0.001; Table 4). In the 7-d storage treatment, the percentage of late embryonic mortality was 3.17%, whereas it increased to 13.94% in the 42-d storage treatment. Mortality at other embryonic developmental stages was not affected by the stor-

age length (P > 0.05). Our results concur with previous studies (Ozbey and Esen, 2007) that found, in rock partridge fertile eggs stored for 14 d, more embryonic deaths in late periods than in early or medium periods versus eggs stored for 1 d, even though those differences were not significant.

However, neither the storage temperature nor the interaction of storage length and storage temperature influenced the total embryonic mortality or the embryonic mortality at each embryonic development stage studied (P > 0.05).

In conclusion, red-legged partridge (A. rufa) eggs store well with little deterioration up to 42 d at 9 and 12°C and 80% RH, in contrast to the lesser durability of eggs from other poultry species. However, hatchability is liable to decline rapidly and incubation period tends to increase if stored at 15°C during the same period and RH. On the other hand, for 7-d storage periods, increasing storage temperature increases hatchability and shortens incubation length of A. rufa eggs. Informative publications do not recommend maintaining A. rufa eggs more than 7 to 10 d (Pérez y Pérez, 1981; Cancho, 1991; Torres and Garcés, 1995; Molleda, 1998) to prevent unnecessary loss of hatchability. However, if longer storage periods were necessary for market or management reasons, it is feasible to store A. rufa eggs for longer periods obtaining an acceptable hatchability performance: up to 28 d at 15°C (González-Redondo, 2010) or up to 42-d storage periods at 9 or 12°C, according to the results of this study. These findings are highly relevant for the handling of A. rufa fertile eggs in game farms and for the marketing of long shelf life hatching eggs because they show that it is possible to

¹Number of replications of 7 eggs each.

²FND: fertile, no development; PD: positive development; EEM: early embryonic mortality; LEM: late embryonic mortality; Pip: pipped but not out of shell.

store A. rufa eggs for a long period while maintaining optimum hatchability rates.

Nevertheless, further research could be done to investigate whether, in addition to storage temperature, other factors like breeding flock degree of selection or pair age affect storage performance of red-legged partridge eggs.

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REFERENCES

- Aebischer, N. J., and A. Lucio. 1997. Red-legged partridge (Alectoris rufa). Pages 208–209 in The EBBC Atlas of European Breeding Birds: Their Distribution and Abundance. E. J. M. Hagemeijer and M. J. Blair, ed. Poyser, London, UK.
- Bagliacca, M., B. Mori, and L. Gualterio. 1988. Egg laying under artificial photo-regulation in the red partridge. Pages 657–659 in Proc. 18th World's Poultry Congress, Nagoya, Japan. Japan Poultry Science Association, Nagoya, Japan.
- Bagliacca, M., G. Paci, and M. Marzoni. 2005. Effect of egg weight categories, storage time and storage temperature on incubation length in duck eggs (*Cairina moschata L.* and *Anas platyrhynchos domestica L.*). J. Poult. Sci. 42:205–214.
- Beer, J., and G. Jenkinson. 1981. The storage of red-legged partridge eggs. A suggestion for trials. Game Conserv. Annu. Rev. 12:80–82.
- Brake, J., T. J. Walsh, C. E. Benton Jr., J. N. Petitte, R. Meijerhof, and G. Peñalva. 1997. Egg handling and storage. Poult. Sci. 76:144–151.
- Cağlayan, T., S. Alaşahan, K. Kırıkcı, and A. Günlü. 2009. Effect of different egg storage periods on some egg quality characteristics and hatchability of partridges (*Alectoris graeca*). Poult. Sci. 88:1330–1333.
- Cancho, M. 1991. Incubación. Equipo y técnicas de manejo. Control. Pages 21–27 in La Perdiz Roja. Fundación La Caixa-AEDOS, Barcelona, Spain.
- Christensen, V. L., M. J. Wineland, G. M. Fasenko, and W. E. Donaldson. 2002. Egg storage alters weight of supply and demand organs of broiler chicken embryos. Poult. Sci. 81:1738–1743.
- Coles, C. 1971. The Complete Book of Game Conservation. Barrie & Jenkins Ltd., London, UK.
- De Carville, H., and A. De Croutte. 1985. Allevamento dell'anatra. Edagricole, Bologna, Italia.
- Elibol, O., S. D. Peak, and J. Brake. 2002. Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. Poult. Sci. 81:945–950.
- Ernst, R. A., F. A. Bradley, U. K. Abbott, and R. M. Craig. 2004. Egg candling and breakout analysis. ANR Publication 8134. Accessed Mar. 2007. http://anrcatalog.ucdavis.edu/pdf/8134.pdf.
- Fasenko, G. M. 2007. Egg storage and the embryo. Poult. Sci. 86:1020-1024.
- Flores, A. J. 1979. Contribución al estudio de algunos caracteres étnicos de la perdiz roja española (*Alectoris rufa*) en cautividad. Nuestra Cabaña 76:48–53.
- Funk, E. M., and J. E. Forward. 1960. Effect of holding temperature on hatchability of chicken eggs. University of Missouri. Agricultural Experiment Station. Research Bulletin 732, Columbia, MO.
- García Martín, E., and A. Dalmau. 2003. Reproducción de la perdiz roja y la codorniz. Pages 457–495 in Reproducción e incubación en avicultura. Real Escuela de Avicultura, Arenys de Mar, Spain.

- Gaudioso, V. R., M. E. Alonso, R. Robles, J. A. Garrido, and J. A. Olmedo. 2002. Effects of housing type and breeding system on the reproductive capacity of the red-legged partridge (*Alectoris rufa*). Poult. Sci. 81:169–172.
- González-Redondo, P. 2004. Un caso de cambio en el manejo de los recursos cinegéticos: La historia de la cría en cautividad de la perdiz roja en España. Rev. Esp. Estud. Agrosoc. Pesq. 204:179–203.
- González-Redondo, P. 2006. Influence of the laying date on the fertility and hatchability of red-legged partridge (*Alectoris rufa*) eggs. J. Appl. Poult. Res. 15:579–583.
- González-Redondo, P. 2010. Effect of long-term storage on the hatchability of red-legged partridge (*Alectoris rufa*) eggs. Poult. Sci. 89:379–383.
- González-Redondo, P., M. Delgado, and M. Reina. 2003. Caracterización de la puesta y su viabilidad en una granja cinegética de perdiz roja (*Alectoris rufa*). Pages 182–183 in Proc. 2nd Jornadas Ibéricas de Razas Autóctonas y sus Productos Tradicionales: Ganadería Ecológica. Consejería de Agricultura y Pesca. Junta de Andalucía, Sevilla, Spain.
- González-Redondo, P., M. Delgado-Pertiñez, S. Toribio, F. A. Ruiz, Y. Mena, F. P. Caravaca, and J. M. Castel. 2010. Characterisation and typification of the red-legged partridge (*Alectoris rufa*) game farms in Spain. Span. J. Agric. Res. 8:624–633.
- González-Redondo, P., R. Gutiérrez-Escobar, R. Díaz-Merino, P. Panea-Tejera, and A. R. Martínez-Domínguez. 2012. Duración de la incubación artificial en perdiz roja (*Alectoris rufa*). ITEA-Inf. Tec. Econ. Ag. 108:289–297.
- Hernando, A. A. 1990. Factores que influyen sobre el huevo incubable. Selecciones Avícolas 32:295–301.
- Jones, D. R., and M. T. Musgrove. 2005. Effects of extended storage on egg quality factors. Poult. Sci. 84:1774–1777.
- Juárez-Caratachea, A., and M. A. Ortiz. 2001. Estudio de la incubabilidad y crianza de aves criollas de traspatio. Vet. Méx. 32:27–32.
- Kirikçi, K., D. C. Deeming, and A. Günlü. 2004. Effects of egg mass and percentage mass loss during incubation on hatchability of eggs of the rock partridge. Br. Poult. Sci. 45:380–384.
- Kirk, S., G. C. Emmans, R. McDonald, and D. Arnot. 1980. Factors affecting the hatchability of eggs from broiler breeders. Br. Poult. Sci. 21:37–53.
- Lapão, C., L. T. Gama, and M. Chaveiro Soares. 1999. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poult. Sci. 78:640–645.
- Mayes, F. J., and M. A. Takeballi. 1984. Storage of the eggs of the fowl (*Gallus domesticus*) before incubation: A review. World's Poult. Sci. J. 40:131–140.
- Molleda, V. 1998. Cría y manejo de la perdiz en cautividad. Pages 107–115 in La perdiz roja. I curso. Fedenca, Madrid, Spain.
- Mori, B., M. Bagliacca, M. Chiarcossi, and I. Romboli. 1985. Performances riproduttive della pernice rossa allevata in Liguria. Riv. Avic. 54:27–32.
- Mourão, J. L., Á. C. Barbosa, D. Outor-Monteiro, and V. M. Pinheiro. 2010. Age affects the laying performance and egg hatchability of red-legged partridges (*Alectoris rufa*) in captivity. Poult. Sci. 89:2494–2498.
- Olsen, M. W., and S. K. Haynes. 1948. The effect of different holding temperatures on the hatchability of hen's eggs. Poult. Sci. 27:420–426.
- Ozbey, O., and F. Esen. 2007. The effect of storage period on hatchability characteristics of rock partridges (*Alectoris graeca*). J. Anim. Vet. Adv. 6:466–469.
- Paci, G., M. Marzoni, N. Benvenuti, and M. Bagliacca. 1992. Breeding technology of red-partridges: Colonies or couples. Pages 351–352 in Proc. 19th World's Poult. Congr., Amsterdam, the Netherlands. World's Poult. Sci. Assoc., Netherlands Branch, Wageningen, the Netherlands.
- Pérez y Pérez, F. 1981. La perdiz roja española. Científico-Médica, Barcelona, Spain.
- Romao, J. M., T. G. V. Moraes, R. S. C. Teixeira, W. M. Cardoso, and C. C. Buxade. 2008. Effect of egg storage length on hatchability and weight loss in incubation of egg and meat type Japanese quails. Braz. J. Poult. Sci. 10:143–147.

- Ruiz, J., and C. A. Lunam. 2002. Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. Br. Poult. Sci. 43:374–383.
- SPSS Inc. 2006. Manual del Usuario de SPSS Base 15.0. SPSS Inc., Chicago, IL.
- Tilki, M., and M. Saatci. 2004. Effects of storage time on external and internal characteristics in partridge (*Alectoris graeca*) eggs. Rev. Med. Vet. 155:561–564.
- Tona, K., F. Bamelis, B. De Ketelaere, V. Bruggeman, V. M. B. Moraes, J. Buyse, O. Onagbesan, and E. Decuypere. 2003. Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poult. Sci. 82:736–741.
- Torres, A., and C. Garcés. 1995. La explotación de la perdiz. Pages 345–363 in Zootecnia. Bases de Producción Animal. Vol. 5: Avicultura clásica y complementaria. Mundi-Prensa, Madrid, Spain.

- Walsh, T. J., R. E. Rizk, and J. Brake. 1995. Effects of temperature and carbon dioxide on albumen characteristics, weight loss, and early embryonic mortality of long stored hatching eggs. Poult. Sci. 74:1403–1410.
- Wilson, H. R., A. R. Eldred, and C. J. Wilcox. 1997. Storage time and ostrich egg hatchability. J. Appl. Poult. Res. 6:216–220.
- Woodard, A. E. 1982. Raising chukar partridges. University of California. Publication 21321e. Accessed Feb. 2013. http://anrcatalog.ucdavis.edu/pdf/21321e.pdf.
- Woodard, A. E., and A. Morzenti. 1975. Effect of turning and age of egg on hatchability in the pheasant, chukar, and Japanese quail. Poult. Sci. 54:1708–1711.