

SHORT COMMUNICATION

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## Effects of the time to change from incubation to hatching temperature on the artificial incubation of red-legged partridge (*Alectoris rufa*) eggs

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### Abstract

This study investigates, in red-legged partridge (*Alectoris rufa*), the effects of the time to change from incubation to hatching temperature on egg weight loss, hatchability, chick weight at hatch, incubation length, and development stage at embryonic mortality. Five batches of 80 eggs each were incubated at 37.8°C during the first 18, 19, 20, 21 or 22 d of incubation, and subsequently at 37.5°C until hatching. Hatchability, development stage at embryonic mortality and chick weight at hatch were not affected by the time of temperature change ( $p > 0.05$ ). However, incubation length and egg weight loss after 21 d of incubation as representative of that of developed embryos were influenced by the incubation treatment ( $p < 0.001$  and  $p < 0.05$ , respectively). Thus, eggs maintained at the incubation temperature of 37.8°C for 22 d not only hatched earlier (23.04 d) but also with lower dispersion than eggs from the other treatments. As hatching may start around day 22 of incubation, to improve hatching synchrony we could recommend to move *A. rufa* eggs from the incubator, set at 37.8°C, to the hatcher on the 21<sup>st</sup> d of incubation keeping the temperature unchanged, and reduce it to 37.5°C on the 22<sup>nd</sup> d. Nevertheless, further research should be carried out to study the effect of this temperature scheduling on chick growth and performance.

**Additional key words:** egg weight loss; game farming; hatchability; incubation length.

The red-legged partridge (*Alectoris rufa*) is a game species endemic to south-western Europe (Iberian Peninsula, continental France, north-western Italy and some Mediterranean islands). It has also been successfully introduced in the United Kingdom, the Azores, the Canary Islands, and Madeira (Aebischer & Lucio, 1997). Nevertheless, wild populations of *A. rufa* have decreased due to several factors like predation, problems related to the release of farm-reared partridges (sanitary and hybridisation risks), deterioration of their natural habitat due to changes in land use, and increase of hunting pressure (Buenestado *et al.*, 2009; Casas *et al.*, 2012; Delibes-Mateos *et al.*, 2012). Although it is known that releases of red-legged partridges raised under farming conditions do not contribute to an effective conservation of their wild

populations and it may even be a threat to it (Negro *et al.*, 2001; Pérez *et al.*, 2004; Barbanera *et al.*, 2010), *A. rufa* is raised in these countries for hunting purpose in order to ensure hunting bags and provide birds for re-establishment purposes (Sokos *et al.*, 2008; Díaz-Fernández *et al.*, 2012). In fact, its captive breeding has been successfully developed from the mid-1960's onwards, leading to a well-developed sub-sector (González-Redondo *et al.*, 2010). However, various aspects of the artificial incubation, which is one of the keys to the productivity of these game farms, have not yet been rigorously investigated. Specifically, incubation temperature is one of the main factors to achieve good hatchability and viable chicks. Several authors recommend incubating *A. rufa* eggs at 37.8°C at least during the first 20-21 d of incubation, and then transferring

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Abbreviations used: EA (early abortion); FWD (fertile without development); LA (late abortion); P (pipped but not out of shell); PD (positive development); RH (relative humidity).

them to the hatcher, where temperature is lowered to 37.5°C, until hatching on day 23-24 (García Martín & Dalmau, 2003; González-Redondo *et al.*, 2012). The reason for reducing the temperature at the end of the incubation is that some studies on hen eggs report that the temperature of the embryo at the end of its development is almost 2°C higher than at its beginning, due to the extra heat caused by the movement and metabolic activity of the embryos. As a consequence, heating needs are different at this late stage (Romijn & Lokhorst, 1956; Decuypere & Michels, 1992). However, hatching temperature scheduling has not yet been contrasted in red-legged partridge by scientific studies. Therefore, the present study aimed to investigate the effects of the time when the temperature is lowered from the incubation to the hatcher temperature, on hatchability, weight loss, incubation length and embryonic mortality of red-legged partridge eggs.

A total of 400 hatching eggs were collected from a game farm located in the province of Seville (Spain). The breeding partridges, aged between 2 and 3 years, were fed with commercial feed (2,775 kcal kg<sup>-1</sup> metabolizable energy, 200 g kg<sup>-1</sup> crude protein, 42.5 g kg<sup>-1</sup> crude fat, 40 g kg<sup>-1</sup> crude fibre and 30 g kg<sup>-1</sup> Ca; Avipacsa A-78®, Sanders, Dos Hermanas, Spain) and were housed in pairs in outdoor cages measuring 50 × 65 cm. The partridges were initially subjected to natural lighting but, from December, artificial lighting was added, increasing the photoperiod by a quarter of an hour every day until 16 h of light (natural + artificial) were reached per day by January. Egg laying started in mid January.

Eggs used in this trial were laid and collected between 23<sup>rd</sup> and 25<sup>th</sup> March. On March 25<sup>th</sup>, the eggs were randomly distributed in five batches of 80 eggs each. Twelve hours before being loaded in the incubator, all batches were pre-warmed at 23°C and 43% relative humidity (RH) by maintaining them in the room where the incubator itself was located. Then, all batches were loaded into the incubator (Masalles HS25®, Masalles, Ripollet, Spain) on the same date (March 26<sup>th</sup>). The incubator was set at 37.8°C and 55% RH, and eggs were turned 45° every hour. Egg batches were transferred to the hatcher (Maino Incubators 2-630 XHM®, Maino Enrico-Adriano S.n.c., Oltrona di San Mamette, Italy) on days 18, 19, 20, 21 or 22 from the beginning of the incubation, respectively. The hatcher temperature was 37.5°C. To maintain identical the other incubation parameters across batches, the hatcher was at 55% RH from the beginning of the

incubation until day 21, and eggs went on being turned 45° every hour. On day 21 of incubation, RH was increased to 80% and the turning of eggs was stopped in all the experimental batches.

All eggs were weighed before incubation and on day 21 of incubation as the time at which the embryos can be considered developed, since in this species hatching can start on day 21.5 under farming conditions (González-Redondo *et al.*, 2012). Weight losses after 21 d of incubation were calculated for each egg, as a percentage of the initial weight. After the incubation period, the number of hatched chicks and unhatched eggs were recorded. Unhatched eggs were opened to determine macroscopically infertility or the following stages of embryonic mortality: fertile without development (FWD) when the blastodisc still had the characteristic shape and size of a fertile one but its outline was deteriorated, positive development (PD) when the blastoderm had further developed but there was still absence of blood formation, early abortion (EA) when blood rings or dead embryos at early stages were observed, late abortion (LA) in case of chicks fully formed but dead without pipping, or pipped but not out of shell (P) (Juárez-Caratachea & Ortiz, 2001; Ernst *et al.*, 2004). The length of the incubation period was measured as the difference between the incubator loading and hatching dates, which were determined through hatching controls carried out every 12 hours. All the chicks were weighed at hatching.

Statistical differences in the fertility, hatchability of the incubated eggs, as well as hatchability and embryonic mortality of the fertile eggs, as a function of the time of change (days 18, 19, 20, 21 or 22 of incubation) of the incubation temperature (37.8°C) to the hatching temperature (37.5°C), were analysed using contingency tables on which Pearson's  $\chi^2$  tests were performed. Statistical differences in the initial and final weights, weight losses of the fertile eggs during the first 21 d of the incubation period, chick weight at hatch and incubation length, as a function of the time of change in temperature during incubation, were analysed by one-way analysis of variance. When differences among time of change of temperature treatments were significant, means were separated using Duncan's multiple range tests at the 0.05 level of significance. All results of the quantitative variables are expressed as mean  $\pm$  standard error of the mean. The descriptive statistics maximum, minimum, coefficient of variation, and skewness and kurtosis coefficients were calculated for the variable incubation length. Differences in the variance

**Table 1.** Fertility, hatchability, embryonic mortality and chick weight at hatch of red-legged partridge eggs according to the time of change from incubation temperature (37.8°C) to hatching temperature (37.5°C)

Time of temperature change <sup>1</sup> (d)	Eggs incubated (No.)	Fertility <sup>2</sup> (%)	Hatchability <sup>3</sup> (%)	Hatchability of the fertile eggs <sup>4</sup> (%)	Embryonic mortality <sup>5</sup> (% of the fertile eggs)					Chick weight at hatch (g) (mean ± SEM)
					FWD	PD	EA	LA	P	
18	80	48.8	38.8	79.5	0.0	5.1	10.3	5.1	0.0	14.36 ± 0.17
19	80	47.5	43.8	92.1	0.0	0.0	5.3	2.6	0.0	14.36 ± 0.16
20	80	58.8	55.0	93.6	0.0	0.0	2.1	4.3	0.0	14.21 ± 0.16
21	80	42.5	36.3	85.3	0.0	2.9	2.9	5.9	2.9	14.36 ± 0.21
22	80	56.3	48.8	86.7	0.0	4.4	6.7	2.2	0.0	14.13 ± 0.20
$\chi^2$		5.661	7.450	4.896	—	4.067	3.320	1.019	4.995	
F										0.393
df		4	4	4	—	4	4	4	4	4
p-value		0.226	0.114	0.301	1.000	0.400	0.508	0.910	0.290	0.813

<sup>1</sup> Time elapsed from the start of incubation. <sup>2</sup> Percentage of incubated eggs that were fertile. <sup>3</sup> Percentage of incubated eggs that hatched. <sup>4</sup> Percentage of fertile eggs that hatched. <sup>5</sup>FWD: fertile without development; PD: positive development; EA: early abortion; LA: late abortion; P: Pipped but not out of shell.

of the incubation length among treatments were also analysed. The analyses were conducted using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Average fertility (50.8%; Table 1) and hatchability of the total of the incubated eggs (44.5%; Table 1) were lower than in previous reports on *A. rufa* under farming conditions (73.5 to 89.7% and 53.4 to 86.4%, respectively; Paci *et al.*, 1992; González-Redondo, 2010; Mourão *et al.*, 2010) or in the wild (93% in hatchability; Tavares *et al.*, 2001) and did not differ among treatments ( $\chi^2_{4,400} = 5.661$ ,  $p = 0.226$  and  $\chi^2_{4,400} = 7.450$ ,  $p = 0.114$ , respectively). The breeding partridges had a similar age, characterised by the maximum fertility (Mourão *et al.*, 2010), and eggs were collected in the middle of the reproductive season, when laying rate and fertility of red-legged partridge peaks (Fernandes Barbosa, 2009). Thus, differences between the fertility obtained in this research and the mean values found in the literature could be due to other farming conditions such as differences of the fertility selection in the breeding flocks, housing type or kind of feed used (King'ori, 2011). The low hatchability of the eggs set was subsequently caused by the low fertility.

Hatchability of the fertile eggs (87.7%; Table 1) matched with values described for captive-bred red-legged partridges (72.6 to 91.6%; Bagliacca *et al.*, 1988; Paci *et al.*, 1992) and did not differ among experimental batches ( $\chi^2_{4,203} = 4.896$ ,  $p = 0.301$ ). Furthermore, no differences were found among treatments in the mortality at each embryonic development phase,

whose mean values were 0% in FWD, 2.5% in PD, 5.4% in EA, 3.9% in LA, and 0.5% in P phase (Table 1,  $\chi^2_{16,203} = 13,656$ ,  $p = 0.624$ ).

Chick weight at hatch was 14.27 ± 0.08 g, in agreement with values described for *A. rufa* under similar conditions (Beer & Jenkinson, 1981), and it was not influenced by the time of change from the incubation to the hatching temperature (Table 1,  $F_{4,173} = 0.393$ ,  $p = 0.813$ ).

The average weight of the fertile eggs before incubation was 19.84 ± 0.09 g, regardless of treatment (Table 2;  $F_{4,395} = 1.015$ ,  $p = 0.911$ ) and, after 21 d of incubation, the average weight loss of the fertile eggs was 10.22 ± 0.12% of their initial weight. Both values matched with those previously described for *A. rufa* under similar conditions (Beer & Jenkinson, 1981; Mourão *et al.*, 2010). Differences were found among treatments in the egg weight loss during incubation ( $F_{4,395} = 1.239$ ,  $p = 0.036$ ). Thus, although there was not a linear progression, eggs whose incubation temperature was reduced from 37.8 to 37.5°C on the 20 or 22<sup>nd</sup> d of incubation lost more weight than eggs whose temperature was reduced on the 19<sup>th</sup> d (Table 2).

The average incubation period lasted 23.39 ± 0.03 d, within the range of values described for this species (González-Redondo *et al.*, 2012). Incubation length was highly influenced by the incubation treatment ( $F_{4,173} = 10.175$ ,  $p < 0.001$ ), being shortened as the change of the incubation to hatcher temperature delayed (Table 2). Thus, eggs whose temperature was

**Table 2.** Egg weight, egg weight loss after 21 d of incubation and length of the incubation period in red-legged partridge fertile eggs according to the time of change from incubation temperature (37.8°C) to hatching temperature (37.5°C)

Time of temperature change <sup>1</sup> (d)	Fertile eggs (No.)	Egg weight of the fertile eggs before incubation (g)	Egg weight loss of the fertile eggs after 21 d of incubation <sup>2</sup> (%)	Incubation length (d)						
				Mean ± SEM	Variance	CV <sup>3</sup> (%)	Skewness	Kurtosis	Min	Max
18	39	19.78 ± 0.22	10.03 ± 0.31 <sup>ab</sup>	23.63 ± 0.07 <sup>a</sup>	0.17 <sup>x</sup>	1.72	1.06	3.07	23.00	25.00
19	38	19.88 ± 0.17	9.72 ± 0.24 <sup>b</sup>	23.40 ± 0.08 <sup>b</sup>	0.25 <sup>x</sup>	2.12	1.76	4.88	22.50	25.00
20	47	19.89 ± 0.19	10.56 ± 0.20 <sup>a</sup>	23.51 ± 0.07 <sup>ab</sup>	0.19 <sup>x</sup>	1.86	1.48	3.02	23.00	25.00
21	34	19.96 ± 0.22	9.91 ± 0.22 <sup>ab</sup>	23.41 ± 0.09 <sup>b</sup>	0.22 <sup>x</sup>	1.98	1.80	4.43	23.00	25.00
22	45	19.70 ± 0.23	10.68 ± 0.30 <sup>a</sup>	23.04 ± 0.05 <sup>c</sup>	0.08 <sup>y</sup>	1.26	1.71	5.71	22.50	24.00
F		1.015	1.239	10.175						
df		4	4	4						
p-value		0.911	0.036	<0.001						

<sup>1</sup> Time elapsed from the start of incubation. <sup>2</sup> Values are expressed as a percentage of egg weight at the beginning of incubation.

<sup>3</sup> CV: Coefficient of variation. <sup>a-b</sup> Means in the same column with different superscripts are significantly different ( $p < 0.05$ ).

<sup>x-y</sup> Variances in the same column with different superscripts are significantly different ( $p < 0.05$ ).

reduced from 37.8°C to 37.5°C between days 18<sup>th</sup> and 21<sup>st</sup> of incubation had incubation lengths according to that described in literature for *A. rufa*. This also agrees with the usual handling of *A. rufa* fertile eggs in game farms, which is transferring eggs from the incubator to the hatcher on day 20 or 21 of incubation (García Martín & Dalmau, 2003). However, eggs kept at the incubation temperature of 37.8°C for 22 d hatched significantly earlier ( $23.04 \pm 0.05$  d) than the eggs from the other treatments and in agreement with the modal value of 23 d for the incubation length found by González-Redondo *et al.* (2012).

Furthermore, eggs kept at the incubation temperature for 22 d hatched within a brief interval of 36 h and with lower dispersion than eggs from the other treatments, showing the lowest coefficient of variation (1.26) and the highest kurtosis (5.71) for the incubation length. No difference was found in the variance of the incubation length among the treatments submitted to change of the incubation temperature on days 18 to 21, which showed a mean variance of 0.21. However we found a difference ( $p < 0.05$ ) in the variance of the incubation length between these treatments and the 22-d treatment, which showed a variance of 0.08. Previous studies on artificial incubation of *A. rufa* eggs state that hatching can start on day 21.5 and finish on day 26 from the beginning of the incubation, within an interval that might vary up to 4 d according to the incubation conditions (González-Redondo *et al.*, 2012). Thus the lower hatching dispersion achieved by

the eggs kept at the incubation temperature of 37.8°C for 22 d is a relevant finding because it could enable game farms to improve hatching synchrony, useful to extract all the chicks from the hatcher at the same time, thus minimizing extraction queues. This optimises chick batches management, and chicks that hatch earlier would not have to wait long for extractions and their dehydration risk would be minimised.

In conclusion, changing the temperature from 37.8 to 37.5°C between day 18 and 22 of incubation did not affect hatchability, embryonic mortality and chick weight at hatch in red-legged partridge. Changing the temperature on day 22 shortened incubation length and reduced hatching dispersion, with respect to the standard length of incubation. Considering that *A. rufa* eggs must be turned at least up to day 20 of incubation (González-Redondo & De la Rosa Sánchez, 2009) and that hatching may start around day 22 of incubation (Table 2 and González-Redondo *et al.*, 2012), to improve hatching synchrony it could be recommended to move eggs from the incubator (set at 37.8°C, 55% RH, with regular turning of eggs) to the hatcher (set at 37.8°C, 80% RH, without turning of eggs) on the 21<sup>st</sup> d of incubation, and reduce hatcher temperature to 37.5°C on the 22<sup>nd</sup> d. Nevertheless, since the final purpose of red-legged partridge farms is to rear healthy and vigorous partridges for release in nature, further research should investigate whether maintaining high the temperature until day 22 of incubation could have any negative effect on chick growth and survival rate after hatch.

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