



Effect of sex ratio, storage time and temperature on hatching rate, fertility and embryonic mortality in Chukar partridge (*Alectoris chukar*)



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ABSTRACT

Chukar partridges (*Alectoris chukar*) are frequently reared in captivity with the aim of producing fertile eggs and chicks. The aims of the present study were to evaluate the effect of male to female sex ratio on hatching rate and fertility and to determine the optimal temperature and time for egg storage and its effect on hatching rate and embryonic mortality in Chukar partridges. Maximum hatching rate and fertility rate were affected by male:female ratios during breeding ($P < 0.05$). The greatest embryonic mortality rate was with eggs that were stored at 25 °C for 15–20 d, however, the greatest hatching rate was when eggs were stored at 10 and 15 °C for 5 days. Storage time of Chukar partridge eggs can be extended for as long as 5 d, but longer storage periods can negatively affect hatching rate. In conclusion, a reduction of storage temperature and duration of time of storage in combination with reducing the sex ratio of the egg producing females and males during the breeding period might improve fertility, hatching rate and fertility of Chukar partridge eggs.

1. Introduction

The Chukar partridge (*Alectoris chukar*) is a Eurasian gamebird in the family Phasianidae of the order Galliformes. The native range of this partridge includes Eurasia, from China and Mongolia in the east, to southeastern Europe in the west. It is also a valuable gamebird for people living within the habitat range for these chukars, as well as in other countries where it has been introduced for hunting (Barbanera et al., 2007). Chukar partridges are primarily produced for hunting, meat and egg production as a protein source, and for hunting tourism (Pourghanbari et al., 2016).

Considering the reproductive seasonality of the Chukar partridge (Mohammad Rezazadeh et al., 2017, 2018), frequency of egg laying is less at the beginning and end of the breeding season for birds of this species. This feature could potentially be manipulated for eggs to be stored for longer periods, exceeding the recommended time for storage of eggs with retention of embryo viability (i.e., > 7–15 d) (González-Redondo, 2010). Comparison of hatching rate and embryo losses after extended durations of egg storage in several avian species indicated there was a sustainability in hatching rate but zygote viability decreasing rapidly with advancing age of the birds (Mani et al., 2008). In particular, Chukar partridge eggs can be stored for 28 d at 16 °C and 70% relative humidity (RH) without a decrease occurring in hatching rate. There have been similar results in studies with the closely related red-legged partridge

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(*Alectoris rufa*) where eggs were stored for 28 d at 15 °C and 80% RH (Gomez-de-Travecedo et al., 2014). Storage for longer periods, however, has led to decreases in hatching rate and increases in proportion of non-viable embryos (Mani et al., 2008). Fertility, hatching rate and embryonic mortality can be affected by duration of storage period, age of breeding birds, composition of hen diets and sex ratio in poultry (Uğurlu et al., 2017).

Sex ratios are a key aspect of a species' natural history and are particularly important, as this influences behavior, social structure, and breeding system dynamics (Riordan et al., 2015). Chukar partridges normally mate in pairs when in their natural habitat. In captivity, however, one male can be mated with three or four females (Alkon, 2015). Male to female ratio in a poultry flock is a major factor in determining behavior (Haghighi et al., 2016). Overall fertility, overall hatching rate and hatching rate of fertile eggs of closely related rock partridges (*Alectoris graeca*) were 75%–97%, 59%–88% and 7%–97%, respectively (Yilmaz and Tepeli, 2009). The objectives of this study were to evaluate differing sex ratios during breeding of the Chukar partridge and its effect on hatching rate and fertility and to determine the optimal temperature and duration in time for Chukar partridge egg storage and effects on hatching rate and embryonic mortality.

2. Materials and methods

There were two components to these studies: the first part to determine the effect of male (M) to female (F) sex ratio during breeding on fertility and hatching rate, and the second to determine the effect of different durations in egg storage time and temperature on hatching rate and embryonic mortality.

2.1. Sex ratio experiment

There was an imposing of a one to three male to female ratio (75 M:225 F) during the breeding period of 38 week-old Chukar partridges with the birds being housed in pens of identical size (2 × 2 m). All of the birds were reared in the same conditions and were consuming the same diet during the 7 months prior to initiation of the experiment. The birds were randomly divided into five experimental groups with five replicates each, and were assigned to one of the following ratios: 1 M:1 F; 1 M:2 F; 1 M:3 F; 1 M:4 F; and 1 M:5 F.

The diet (Table 1) was offered to the birds daily at the 06:00 h, and water was provided *ad libitum* to all the birds. Strict sanitation

Table 1
Composition of basal diet of Chukar partridge.

Ingredient	g/kg
Corn	518.00
Soybean meal	355.00
Soybean oil	31.40
Dicalcium phosphate	7.00
Limestone	75.00
Sodium chloride	2.80
Sodium bicarbonate	1.00
L-Lys-HCl	1.30
DL-Met	3.40
Vitamin and mineral premix ¹	5.00
Phytase 10,000	0.10
Total	1000.00
Analysis	
Metabolizable energy (Kcal/kg)	2800.00
Crude protein (%)	19.84
Calcium (%)	3.10
Available phosphorous (%)	0.32
Sodium (%)	0.15
Chloride (%)	0.23
Lysine (%)	1.08
Methionine (%)	0.48
Methionine + Cysteine (%)	0.88
Threonine (%)	0.65
Tryptophan (%)	0.22
Arginine (%)	1.26
Isoleucine (%)	0.77
Valine (%)	0.83

The following was provided per kg of diet: vitamin A, 10,000 IU; vitamin D3, 4500 IU; vitamin E, 65 IU; vitamin K3, 3 mg; vitamin B1, 2.5 mg; vitamin B2, 6.5 mg; vitamin B3, 60 mg; vitamin B5, 18 mg; vitamin B6, 3.2 mg; vitamin biotin, 0.22 mg; folic acid, 1.9 mg; vitamin B12, 0.017 mg; choline chloride, 1400 mg, Mn, 120 mg; Zn, 110 mg; Fe, 20 mg; Cu, 16 mg; I, 1.25 mg; Se, 0.3 mg.

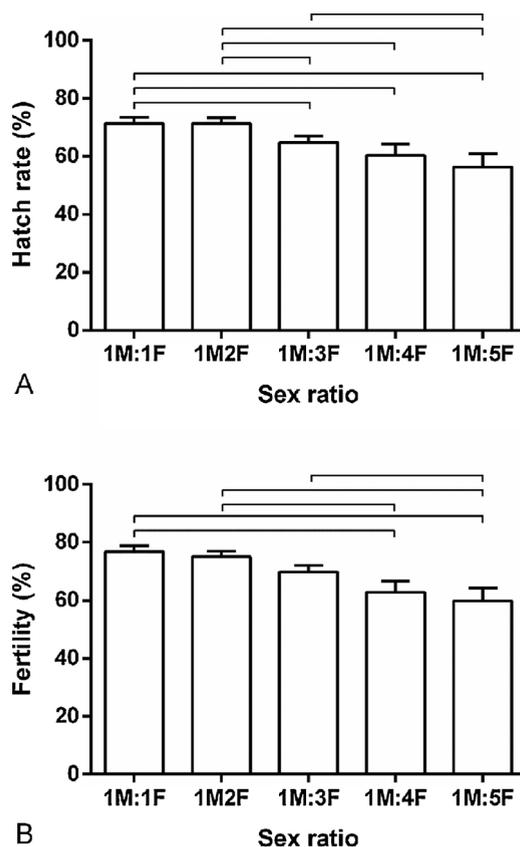


Fig. 1. Effect of sex ratio of males (M) and females (F) during breeding on mean and standard errors for hatching rate and fertility of Chukar partridge eggs; Lines above the columns indicate differences between each group ($n = 4$, $P < 0.05$).

practices were maintained in the facility throughout the experiment. The cages were cleaned daily to reduce the probability of any disease outbreak. Vaccinations and medications were imposed when deemed important to do so during the experimental period (Karousa et al., 2015). Eggs were stored (maximum 5 d) in a room at 20 °C with 78% mean RH, until setting of eggs in an incubator. All the experimental batches were subsequently loaded into an incubator on the same date. The setter part of incubator was calibrated at 37.8 °C and 55% RH, and eggs were automatically turned every hour. On day 20 of incubation, all the experimental eggs were transferred to hatchery set at 37.0 °C temperatures and RH was increased to 75% and there was a cessation in turning of eggs in all batches (Gomez-de-Travededo et al., 2014). After the end of the incubation period (24 d), unhatched eggs were collected and counted, then opened and the contents were macroscopically examined to determine fertility and embryonic death (Haghighi et al., 2016). Fertility was calculated by using the formula: $\text{Fertility (\%)} = (\# \text{ fertile eggs} / \# \text{ total eggs}) \times 100$. Hatching rate of the eggs was calculated by using the formula: $\text{Hatching rate} = (\# \text{ hatched chicks} / \# \text{ total eggs}) \times 100$.

2.2. Egg storage time and temperature experiments

Clean eggs with normal size and shape, and shell quality were collected for incubation. All eggs which were too small (i.e., < 10 g) or too large (i.e., > 20 g) in size, or that were soft shelled, cracked, broken, dirty, misshapen and otherwise having a poor shell structure were discarded. A total of 900 eggs were arranged with five durations of storage time (0, 5, 10, 15 and 20 d) and at four storage temperatures (10, 15, 20 and 25 °C), resulting in 20 treatments consisting of seven replications of seven eggs/replication (Karousa et al., 2015).

All the experimental batches were subsequently loaded into an incubator on the same date. The setter part of incubator was set at 37.8 °C and 55% RH, and eggs were automatically turned every hour. On day 20 of incubation, all the experimental eggs were transferred to hatchery set at 37.0 °C temperatures and RH was increased to 75% and there was a cessation of turning of eggs in all batches (Gomez-de-Travededo et al., 2014). At the end of the incubation period, unhatched eggs were collected and counted, then opened and the contents were macroscopically examined to determine cause of embryonic death.

2.3. Data analysis

The data were analyzed statistically using an ANOVA, and the means were compared using the Least Significant Difference Test

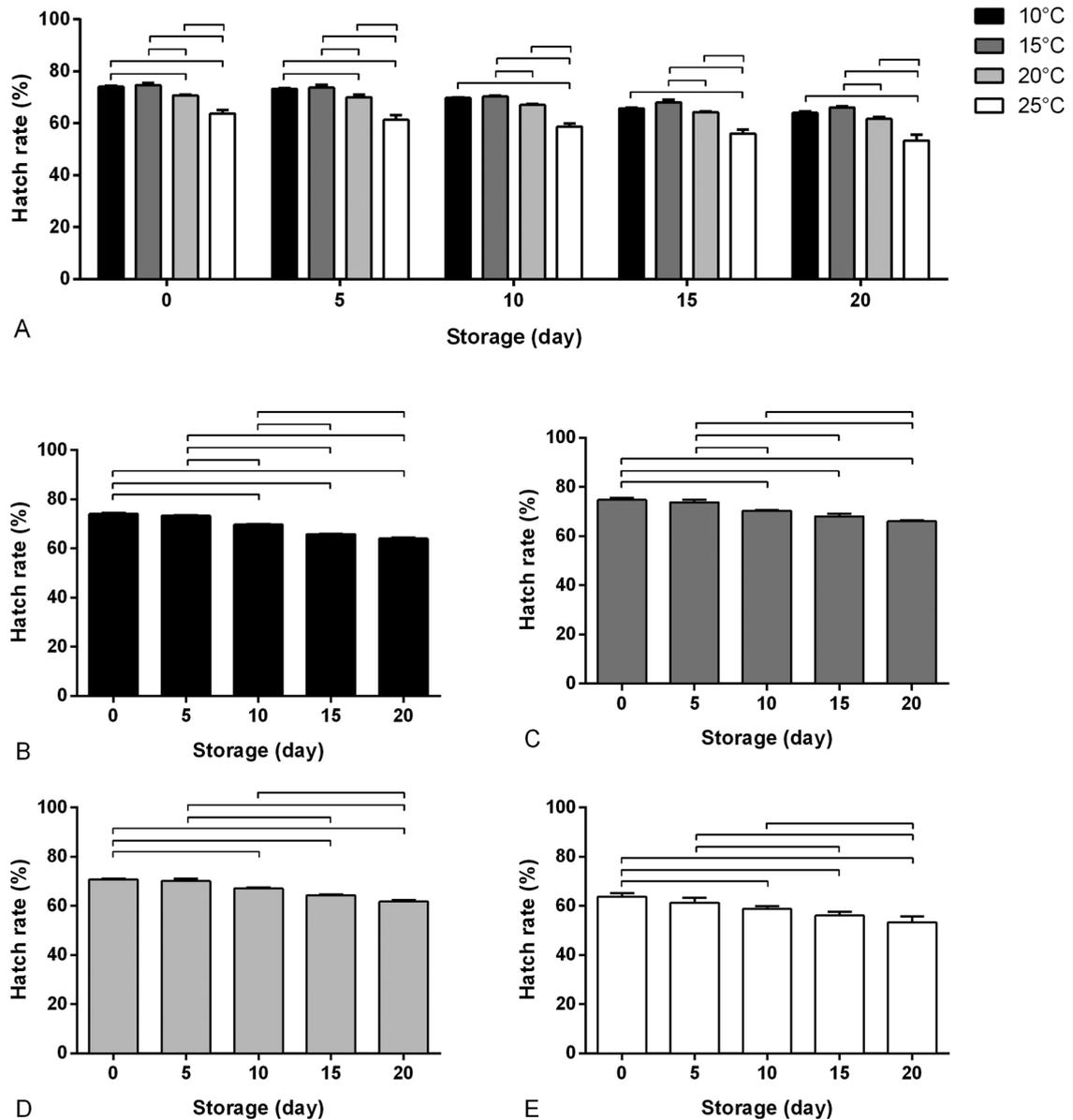


Fig. 2. Effect of storage temperature (°C) and duration of storage time (day) on hatching rate of Chukar partridge eggs; A, comparisons of different temperatures in the same day. B, comparisons of different durations of storage time at a 10 °C storage temperature; C, comparisons of different durations in storage times at a 15 °C storage temperature; D, comparisons of different durations in storage time at a 20 °C storage temperature; E, comparisons of different durations in storage time at a 25 °C storage temperature; Lines above the columns indicate differences between each group ($n = 3, P < 0.05$).

with the General Linear Model with use of the Minitab Micro Computer Software (SPSS 16.00 for windows). Differences were considered significant at $P < 0.05$ (Duncan, 1955).

3. Results

The male to female sex ratios affected egg hatching rate and fertility (Fig. 1). The male to female sex ratio was 1:1 or 1:2 that resulted in the greatest fertility and embryonic development compared to when the sex ratio was greater. Fertility percentage was greater with sex ratios of one male: one or two females than with one male to three to five females ($P < 0.05$). The greater average percent fertility with the one male to one or two female sex ratio may be attributed to there being copulations with female(s) as compared with when there were three or more females/male.

Hatching rate decreased with storage period with less than 5 days of egg storage appearing to be optimal for maximal hatching rates (Fig. 2). There were effects of duration of storage period and temperature on hatching rate and embryonic mortalities

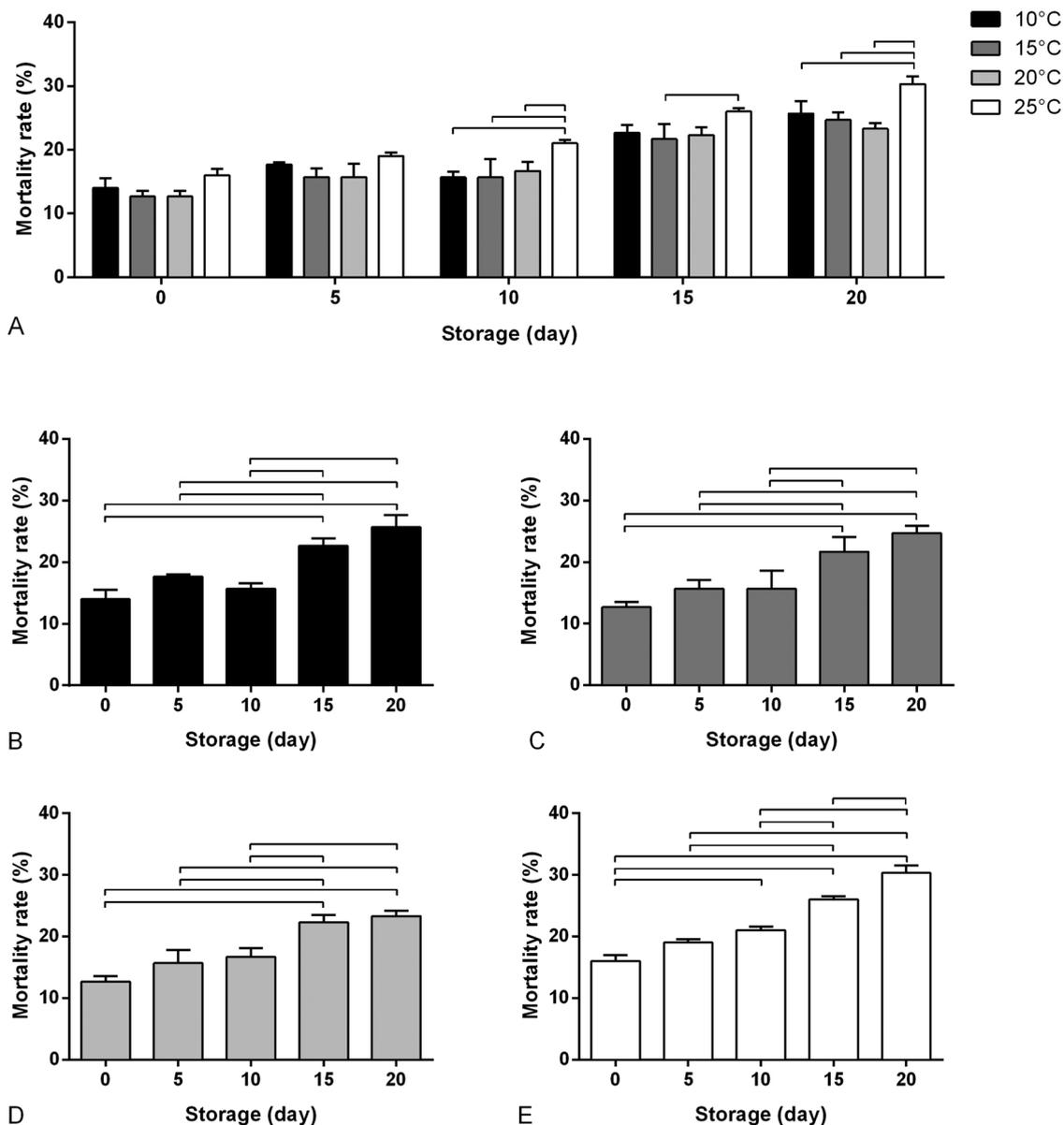


Fig. 3. Effect of storage temperature ($^{\circ}\text{C}$) and duration of time of storage (day) on embryonic mortality of Chukar partridge eggs; A, comparisons of different temperatures when there was the same duration of storage (day); B, comparisons of different durations of storage time at a 10°C storage temperature; C, comparisons of different durations of storage time when there was storage at a 15°C storage temperature; D, comparisons of different durations in storage time when there was storage at a 20°C storage temperature; E, comparisons of different durations in storage time when there was storage at a 25°C storage temperature; Lines above the columns indicate differences between each group ($n = 3$, $P < 0.05$).

($P < 0.05$). Egg hatching rate percentage loss was greater with increased durations of storage and at greater temperatures ($P < 0.05$).

The greatest rate of embryonic mortality occurred when there was storage at 25°C with a duration of storage of 15–20 d, and the greatest hatching rate was when storage temperatures were at 10 and 15°C for a period of 5 d (Figs. 2 and 3). The least hatching rate when there was storage periods of less than 5 days was when there was storage at 25°C (61.3%).

4. Discussion

Fertility depends on various factors such as storage period, breeding season, pre-incubation conditions, lighting, nutritional state of birds, mating and time of mating (Miazi et al., 2012). Although Chukar partridges normally mate in pairs when in their natural habitat, in captivity the ratios are often one male to three or four females (Alkon, 2015). As a result, understanding what factors affect these skewed sex ratios (i.e., a sex ratio different from 1:1) can be important in predicting a species' future population growth,

viability and vulnerability to extinction (Riordan et al., 2015).

Juvenile sex ratios are generally one male to one female. Adult sex ratios in birds, however, are generally biased towards males, but in mammals are frequently skewed towards females (Nadal et al., 2016). Different genetic, behavioral and ecological theories have been proposed to explain biased sex ratios during different life stages, and these theories have been based on characteristics ranging from genetic sex determination to parental condition, behavior, environment and weather as primary factors modulating juvenile sex ratios (West and Sheldon, 2002; Schindler et al., 2015).

Hatching rate for fertile eggs may also be affected by several factors such as storage duration, care of hatching eggs, age of breeding birds, quality of eggs, season and nutrition (Khan et al., 2013). The results in the present study are inconsistent with those of González-Redondo (2010) where it was concluded that there was a unique feature of Chukar partridge eggs that allowed for eggs to be stored for as long as 28 d without appreciably affecting hatching rate. Duration of egg storage period affects developmental processes and constituent contents of eggs in chickens. There is a marked decrease in hatching rate after 6 or 7 d however, when there is use of properly stored eggs that have been in storage for 10–14 d reasonable hatching rates can occur (Brake et al., 1997). For example, Kirk et al. (1980) reported that eggs stored for 2 d had greater hatching rates when stored at 18 °C as compared with 15 °C, whereas there were different results for eggs stored 8 d; although outcomes after long-term storage appears to be optimal when there is storage at near 12 °C.

Temperature during storage affects fertility of eggs (Miazi et al., 2012). The mean value for egg hatching rate (74.6%) at 15 °C in the present study were similar to the values previously reported for this species (ranging 72%–92%,) (González Redondo et al., 2014). Storage of Japanese quail (*Coturnix japonica*) eggs for a period of 1–7 d resulted in the greatest fertility and hatching rates when eggs were stored for 1–3 d (Khan et al., 2013). Mani et al. (2008) reported that hatching rates were greater in quail when egg storage was for less than 7 d as compared with 10 d of storage at 15 °C and 70%–80% RH.

5. Conclusions

After a 5 d of storage period of fertilized chukar partridge eggs, there was a lesser hatching rate and greater embryonic mortality. Change in storage temperature and time of storage duration might indirectly affect chukar partridge egg fertility and hatching rate. Storage time of Chukar partridge eggs can be extended for as long as 5 d, but periods of storage of longer than 5 d can negatively affect egg hatching rate. The greatest hatching rate occurred when there was egg storage at 10–15 °C with durations of storage time of 0–5 d. The optimal male to female ratio during breeding for the greatest fertility was one male to one female for Chukar partridges.

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