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Reproductive performance of red-winged tinamous (*Rhynchotus rufescens*) using different mating strategies in captivity – case report

[*Desempenho reprodutivo de perdizes (Rhynchotus rufescens) utilizando diferentes estratégias de acasalamento em cativeiro – relato de caso*]

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ABSTRACT

The aim of this study was to evaluate the reproductive performance of red-winged tinamous in captivity using different mating systems during three breeding seasons. The data were collected from August to March during three breeding seasons of the species. Ten couples and four groups of red-winged tinamous were randomly selected for formation of the monogamous and polygynous mating system, respectively, with egg collection from both systems. The traits evaluated were number of eggs per female, egg breaking and egg fertilization. Data were statistically analyzed by the least square method and logistic regression. Animals in couples mating strategy were superior ($P<0.05$) in terms of number of eggs per female compared to the group mating strategy. High egg breaking rate ($P<0.05$) in the red-winged tinamous groups was observed. Eggs of animals in couples mating strategy were 5.24 and 11.5 more likely to be fertilized than those of group strategy in two breeding seasons. In general, the reproductive efficiency in terms of low egg breaking, high egg production and fertility was observed for red-winged tinamous from the monogamous mating system.

Keywords: egg-laying, fertility, monogamy, Tinamidae

RESUMO

O objetivo deste estudo foi avaliar o desempenho reprodutivo dos perdizes de asas vermelhas em cativeiro utilizando diferentes sistemas de acasalamento durante três épocas de reprodução. Os dados foram coletados de agosto a março, durante três épocas de reprodução da espécie. Dez casais e quatro grupos de perdizes de asas vermelhas foram selecionados aleatoriamente para a formação do sistema de acasalamento monogâmico e poliginoso, respectivamente, com coleta de ovos de ambos os sistemas. Os traços avaliados foram o número de ovos por fêmea, a quebra dos ovos e a fertilização dos ovos. Os dados foram analisados estatisticamente pelo método dos mínimos quadrados e pela regressão logística. Os animais na estratégia de acasalamento de casais foram superiores (P<0,05) em termos de número de óvulos por fêmea, em comparação com a estratégia de acasalamento do grupo. Foi observada uma alta taxa de quebra de ovos (P<0,05) nos grupos perdizes de asas vermelhas. Ovos de animais em estratégia de acasalamento de casais foram 5,24 e 11,5 mais propensos a serem fertilizados do que os de estratégia de grupo em duas épocas de reprodução. Em geral, a eficiência reprodutiva em termos de baixa quebra de ovos, alta produção de ovos e fertilidade foi observada para os tinâmicos de asas vermelhas do sistema de acasalamento monogâmico.

Palavras-chave: fertilidade, monogamia, postura de ovos, Tinamidae

INTRODUCTION

The red-winged tinamou *Rhynchotus rufescens* (Temminck, 1815) of Tinamiformes order has a chicken appearance and is considered terrestrial, predominantly South America in semi-open areas. Although classified as “least concern” in terms of extinction risk, the wildlife population of the red-winged tinamou shows a declining trend (The IUCN…, 2016) due to poaching,

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deforestation, and man-made fires. These data highlight the need for special attention to the reproductive management of red-winged tinamous in captivity from a conservational point of view.

The breeding season of red-winged tinamous ranges from August to March in Brazil, a period that corresponds to the long days of the year when luminosity exerts effects on the endocrine system of males and females and controls reproductive traits (Bruneli *et al*., 2005; Paranzini *et al*., 2018). The utilization of fertile eggs of red-winged tinamous reared in captivity can becomes more efficient if adequate management practices are adopted, such as storage process, artificial incubation, and neonatal management, increasing reproductive efficiency.

The monogamous mating system can be considered a reproductive strategy used for raising tinamous in captivity, which has the benefit of individual paternity identification for selection of reproductive traits. However, according to Cromberg *et al*. (2007), the polygynous mating system (one male to two or more females) establishes benefits to genetic breeding program for domestication of red-winged tinamous.

This information is important to improve research on the reproduction of red-winged tinamous, encouraging the increase of the fertility rate of these birds. The aim of this study was to evaluate the reproductive performance of red-winged tinamous in captivity using monogamous and polygynous mating systems during three breeding seasons.

MATERIAL AND METHODS

The experiment was conducted at the red-winged tinamou nursery of the Wild Animal Sector of the Lageado Experimental Farm, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Unesp, Botucatu, São Paulo, Brazil. The nursery is divided into two units for animal management (Units I and II) and one unit for storage and artificial incubation of the eggs (Maternity Unit). The experiment was approved by the Ethics Committee for the Use of Animals of Faculdade de Medicina Veterinária e Zootecnia/Unesp (Protocol number: 0012/2019).

The data were collected from August to March during three breeding seasons of the species (years 2017, 2018, and 2019). For analysis of the distribution of the laying period, four reproductive phases were established per season: phase 1 including August and September, phase 2 including October and November, phase 3 including December and January, and phase 4 including February and March.

Before starting each breeding season, ten couples and four groups of red-winged tinamous with average age of 2.5 years were randomly selected for formation of the monogamous and polygynous mating system, respectively. The couples were housed in 2-m² pens in Unit I, with one couple per pen (Fig. 1), and the groups were housed inside 6-m² pens of Unit II with one group per pen, where each group presented male to female ratio equal to 5:15 (Fig. 2).

The animals had *ad libitum* access to ration and water offered in tubular feeders and pendular drinkers, both cleaned once a day. The layer diet during the breeding seasons consisted of mash corn and soybean meal (Table 1). Starting with the 2019 breeding season, a pelleted diet with the same formulation was offered to the animals.

Eggs were collected four times per day, twice per shift (morning and afternoon). The collected eggs were sent to the maternity unit, sprayed with water containing quaternary ammonium (0.5%) as disinfectant. Broken or cracked eggs were recorded. The eggs were stored in a refrigerator at a temperature of 18ºC and humidity of 75% for 72 hours.

After storage, the eggs were transferred to the incubators (Premium Ecológica IP 120), with automatic turning at an angle of 45º. The temperature and humidity of the incubators were 36 ºC and 60%, respectively, and were controlled with a digital thermohygrometer (Kasvi® K29-5070H). Ovoscopy was performed after 18 days of incubation to identify and quantify fertile eggs by positive visualization of the embryo disc or subsequent stages of embryo development. The fertile eggs were transferred to the hatcher (Premium Ecológica NP-70) where they remained at a temperature of 37 ºC and humidity of 70% inside individual net bags to identify individual animals during the hatching process. Chicks with normal birth and those that died before birth (embryonic death) were recorded.

Figure 1. Couple of red-winged tinamous for monogamous mating system (Source: Personal archive).

Figure 2. Group of red-winged tinamous for polygynous mating system (Source: Personal archive).

Table 1. Formulation of the layer diet offered to red-winged tinamous during the breeding seasons evaluated

Ingredient	Diet composition (%)
Corn	40.40
Soybean meal	37.20
Wheat bran	5.60
Soybean oil	3.50
Salt	0.43
Calcitic limestone	5.56
Dicalcium phosphate	1.61
Premix[1]	5.70
Total	100.0

[1]Composition: vitamin A: 52,800µg; vitamin D3: 12,000µg; vitamin E: 0.5mg; vitamin K3: 0.1mg; vitamin B1: 0.036mg; vitamin B2: 0.2mg; vitamin B6: 0.05mg; vitamin B12: 0.56mg; niacin: 0.7mg; biotin: 0.003mg; pantothenic acid: 0.5mg folic acid: 0.03mg; choline: 0.02mg; iron: 1.1mg; copper: 0.3mg; manganese: 0.18mg; zinc: 0.12mg; iodine: 0.024mg; selenium: 0.003mg; methionine: 0.02mg; calcium: 0.175mg; phosphorus: 68g; sodium: 23g; chlorine: 36g; growth promoter: 2g; coccidiostat: 10g; antifungal: 0.2mg; BHT: 1g; inert: 1,000g.

All data were submitted to quality control before creation of the final archive for data analysis using SAS 9.3 (SAS Institute Inc.). Number of eggs per female (NE), egg breaking (EB) and egg fertilization (EF) were analyzed for each breeding season. The NE trait was analyzed by the least square method using the Glm procedure, with the following statistical model:

$$y_n = \mu + P_j + M_k + P_j x C_k + \varepsilon$$

where
y_n = trait in *y* breeding season,
μ = overall mean of the trait,
P_j = fixed effect of the j^{th} reproductive phase,
M_k = fixed effect of the k^{th} mating strategy,
$P_j x M_k$ = interaction between reproductive phase and mating strategy,
ε = random error.

Logistic regression was used for the analysis of EB and EF traits since they are dependent categorical variables. In this analysis, the regression coefficients (β) were interpreted as the rate of change in a category of the dependent variable (y) per unit of change in the independent variables (x) in relation to a given arbitrary category (Hosmer Júnior *et al*., 2013).
The logistic model establishes a relationship between k variables (categorical or continuous) and the odds of success of a dichotomous dependent variable. It is necessary to establish a linear relationship between the independent variables, as well as transformation of the response variable (Mendes *et al*., 2004). The linear logistic model is given by:

$$\text{logit}(p_i) = \log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_k x_{ki}$$, where i = 1, ..., n,

with logit (p_i) being called the logarithm of the odds of success with

$$p_i = \frac{\exp\,(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_k x_{ki})}{1 + \exp\,(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_k x_{ki})}$$

where
p_i = odds of success of the i^{th} observation,
β_0 = intercept of the model,
$\beta_1, \beta_2, \ldots, \beta_k$ = regression coefficients,
$x_1, x_2, \ldots, x_k$= variables referring to the i^{th} observation.

Reproductive phase and mating strategy were used as the independent variables for the analysis of EB and EF traits. The maximum likelihood estimates of the model parameters were obtained using the Logistic procedure. The reference categories were phase 1 and group mating strategy. The odds ratio was calculated to relate the probabilities of the reference categories to the other categories, as follows:

$$odds\ ratio = \left(\frac{p_i}{1-p_i}\right) \Big/ ref\left(\frac{p_i}{1-p_i}\right)$$

where

$ref\left(\frac{p_i}{1-p_i}\right)$ = probability of the reference category.

The results were considered significant at a level of less than 5% (P<0.05), applying Tukey's multiple comparison test of means if necessary.

RESULTS

The total egg production (fertile and non-fertile) of red-winged tinamous was significantly higher (P<0.01) in the 2019 breeding season (1,140 eggs) when compared to 2017 (303 eggs) and 2018 (472 eggs). The reproductive phase was significant (P<0.01) for egg production, with higher frequencies observed from October to January (46.9% and 31.4% in reproductive phases 2 and 3, respectively) considering all breeding seasons.

Animals in couples mating strategy were superior (P<0.05) in terms of number of eggs per female compared to the group mating strategy, and high egg breaking rate in the groups was observed (P<0.05) in 2018 and 2019 breeding seasons (Table 2). Fertility increased after the 2018 breeding season, with higher rates in reproductive phases 2 and 3, and low fertility in phases 1 and 4 of the 2017 breeding season (Fig. 3).

Table 2. Number of eggs per female, egg breaking rate and fertility in couples and groups mating strategies of red-winged tinamous according to breeding season

Breeding season	Mating strategy	NE[1]	EB[2] (%)	Fertility (%)
2017	Couples	12.4 a	25.3 a	75.9 a
	Groups	5.1 b	28.4 a	41.9 b
2018	Couples	10.6 a	13.2 b	64.0 a
	Groups	6.6 b	23.5 a	64.0 a
2019	Couples	23.8 a	18.4 b	94.6 a
	Groups	14.7 b	25.3 a	60.5 b

[1]Number of eggs per female; [2]Egg breaking. Different letters in the same column indicate significant differences (P<0.05).

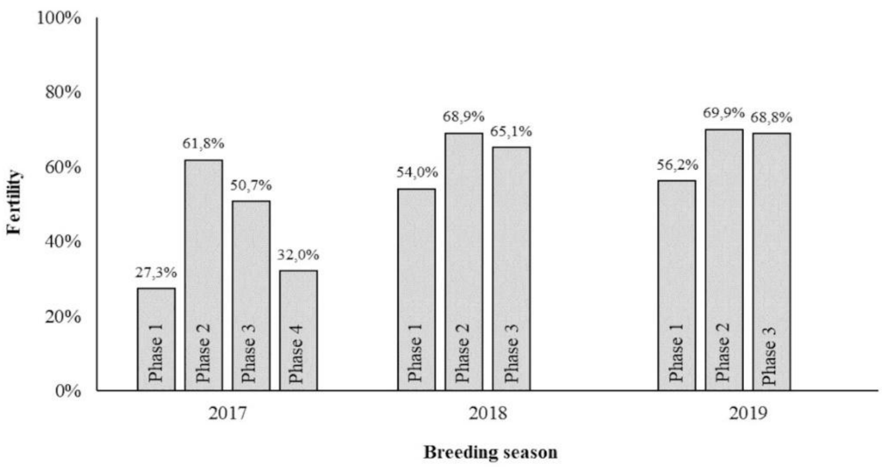

Figure 3. Fertility of red-winged tinamous according to the reproductive phase of each breeding season.

The egg fertilization was significant (P<0.05) for all effects evaluated in the logistic model. According to the odds ratio calculation, fertility was significantly higher (P<0.05) in reproductive phase 2 for each breeding season, which correspond to the months with the highest frequency of mating and egg production, compared to phase 1 (Table 3). Eggs of animals in couples mating strategy were 5.24 and 11.5 more likely to be fertilized than those of group strategy in 2017 and 2019 breeding seasons, respectively, with non-significant difference (P>0.05) in 2018.

Table 3**.** Odds ratio obtained with the logistic regression model for the evaluation of egg fertilization in red-winged tinamous

Effect	*Odds ratio*		
	2017	2018	2019
Reproduction phase			
1	1	1	1
2	6.84*	1.90*	1.82**
3	4.03	1.59	1.43
4	1.55	-	-
Mating strategy			
Groups	1	1	1
Couples	5.24**	0.93	11.5**

*P<0.05; **P<0.01.

DISCUSSION

The low egg laying rate in 2017 can related to stress caused by the formation of breeding batches, because partial transfer of the birds to the pens in newly built barn during the first week of laying. The stress represents the reaction of the animal organism to stimuli that disturb its normal physiological equilibrium or homeostasis and can lead to low egg production (Lara and Rostagno, 2013).

These changes led to an increase of 2018 and 2019 egg production, when the birds were already adapted in the experimental pens. The results demonstrated that changes in breeding batches should occur after the end of the breeding season and not at the beginning. The mean number of eggs per female of all red-winged tinamous in the present study during the 2019 breeding season was considered higher than the average egg laying reported by Kermode (1997) in a study on the Chilean tinamou (*Nothoprocta perdicaria*) reared in captivity, which was 20 eggs per year.

The use of a pelleted diet for red-winged tinamou rearing in 2019 breeding season may have increased feed intake and egg production. Pelleted diet has several benefits in relation to bird performance, one of which is improved feed efficiency and greater egg production (Almirall *et al*., 1997).

Egg production results by reproductive phases corroborate the findings of Bruneli *et al*. (2005) for red-winged tinamous, who reported higher egg production from October to January evaluating two breeding seasons. The low egg production in phases 1 and 4 can be explained by the low natural luminosity annual. According to Scanes (2014), the increase in luminosity stimulates the hypothalamus of domestic birds and induces the secretion of gonadotrophin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), hormones that promote sexual activity and egg laying.

In contrast to the present study, Cromberg *et al*. (2007) found no significant differences (P>0.05) in the egg production of females between monogamous and polygynous mating. The results of the present study show that monogamous tinamous systems in captivity provided better egg production than systems in groups with a high density of animals per pen.

The annual fertility rates of this study were higher than the 42.7% reported by Cavalcante (2006) studying red-winged tinamou and lower than the rate reported by Bruneli *et al*. (2005) who obtained a mean of 84.3%. Eggs collected during phase 4 of the 2018 and 2019 breeding seasons were not sent for artificial incubation because the units of the flock had reached the maximum housing capacity for new animals, which led to the termination of egg incubation.

Fertility results showed better reproductive efficiency of red-winged tinamous in monogamous mating. The stress might have had a determining effect on the low reproductive efficiency of males in group mating systems of the present study. This stress is caused by the presence of more animals in the pen during the reproductive period considering the solitary habits of the species in the wild (Sick, 1997). According to Bruneli (2006), male tinamous (*R. rufescens*) with low fecundity have higher corticosterone concentrations than males with high fecundity. Corticosterone is the main glucocorticoid hormone in birds that is related to stress induction. The serum concentration of this hormone varies among species (Cockrem, 2007; Hau *et al*., 2010).

The fertility rates obtained for couples in the present study were superior to the performance of couples reported by Cromberg *et al*. (2007), who obtained a percentage of 45.69%. The authors found no difference (P>0.05) compared to the group systems. Species such as *Gallus gallus* (chicken) and *Meleagris gallopavo* (turkey) cease egg production when prolactin reaches a certain level in the body, thus inducing the brooding behavior (Boni *et al*., 2007). In the case of red-winged tinamous, the male is responsible for natural incubation of the eggs (brooding), a fact that may negatively influence fertility.

Bruneli (2006) found no variation in the serum concentration of prolactin, the hormone responsible for brooding, in male tinamous during a given breeding season. However, in the present study, brooding was observed in some males of couples and the expression of this behavior ceased after egg collection in the pens. As observed for laying hens (Albino and Bassi, 2005), the selection of individuals without brooding behavior may be considered a criterion for obtaining high fertility rates; however, the number of broken eggs may increase because of the lack of protection of the eggs by males before collection.

CONCLUSIONS

Reproductive efficiency in terms of low egg breaking, high egg production and fertility was observed for red-winged tinamous selected to the monogamous mating system, probably due to the environmental effect inherent to the polygyny. The mating strategy of couples contributes to easier animal handling and greater safety in egg collection from red-winged tinamous in captivity.

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