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Reproductive and Behavioral Aspects of Red-winged Tinamous (*Rhynchotus rufescens*) in Groups with Different Sex Ratios

■ **Author(s)**

Cromberg VU[1]
Stein MS[1]
Boleli IC[2]
Tonhati H[1]
Queiroz SA[1]

[1] Departamento de Zootecnia – Faculdade de Ciências Agrárias e Veterinárias - UNESP.
[2] Departamento de Morfologia e Fisiologia Animal – Faculdade de Ciências Agrárias e Veterinárias - UNESP.

■ **Mail Address**

Sandra Aidar Queiroz
Departamento de Zootecnia
Faculdade de Ciências Agrárias e Veterinárias - UNESP
Via de Acesso Prof. Paulo Castellane
14.884-900. Jaboticabal, SP, Brazil.
Phone 5516 32092678
Fax 5516 32092684

E-mail address: saquei@fcav.unesp.br

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ABSTRACT

The aim of this research study was to evaluate the reproductive performance of tinamous submitted to five different male:female ratios. The study was carried out with 72 birds in a randomized experimental design with 4 replications. Tinamous were housed in cages, using the ratios of one (1:1), two (2:1), three (3:1) and four (4:1) females per male, and also one male was housed with three females individually (3R:1), in a rotational system. Reproductive records of the breeding season from September 2004 to March 2005 were used. The reproductive traits studied were: number of eggs laid, fertility, and percentage of eggs damaged and cracked by pecking. Nonparametric analyses of these traits were performed using Kruskal-Wallis test. Two replications of treatments 1:1 and 4:1, and one of treatment 2:1 were video-taped for three days, 12 hours/day. The videotapes were sampled according to the scan method to fit an ethogram. Birds were also watched for one hour per day to study dominance and agonistic behavior. None of the reproductive traits was affected by mating sex ratio ($p<0.05$). Female dominance could be related to displacement behavior ($r=1.00$), and male sitting in immobility plus sitting in activity behaviors were related to lower number of damaged eggs ($r=-0.90$). Social dominance was indirectly determined by displacement behavior in the study situation. A large number of damaged eggs occurred in all treatments, thereby not allowing a clear conclusion on the best male:female ratio.

INTRODUCTION

Due to its broad geographic dispersion, omnivorous feeding habit, and the taste of its meat, the domestication of the Red-winged Tinamous (*Rhynchotus rufescens*), also called South American partridge, has become attractive for economic purposes. The pressure to domesticate this and other tinamous species also stems from the limited supply of birds in nature (Homma, 1993). Studies carried out using this bird in an environment similar to that used to raise broilers have shown good performance in terms of growth rate (Queiroz *et al.*, 2004; Tholon *et al.*, 2004), excellent carcass and breast yields (Moro *et al.*, 2006), and perfect adaptation to meal (Moro, 1996) and pelleted (Hoshiba *et al.*, 2003) feeds. However, the reproductive performance of this species in captivity is still a problem that needs to be solved. According to Sick (1997), under natural conditions, the breeding season starts in September and ends in March. Females lay around 25 eggs during the reproductive season, and the male is responsible for hatching them. Bump & Bump (1969) studied eggs collected in nature and reported 6 non-fertilized eggs among 114, and a hatchability of 88%, emphasizing that even in nature reproductive problems occur. Similar results have not been demonstrated for tinamous raised in captivity. Carnio *et al.* (1999)

reported fertility, hatchability and birth percentages of 49%, 52%, and 26%, respectively. Cravino (undated) found that an infertility percentage ranging from 30 to 65%, and emphasized the need of studies to determine the causes of decreased reproductive success in captivity of this bird. Bruneli *et al.* (2005), in a study carried out using a production system similar to that used for broilers, found fertility, hatchability and birth percentages of 71%, 54%, and 38%, respectively. Although these results show better efficiency, they are far from those necessary to make the domestication of the Red-winged Tinamou a worthwhile economic activity.

Tinamous are classified as polyandrous (Sick, 1997). In nature, each female mates with 2 to 4 males during the mating season (Magalhães, 1994). The reproductive strategies used for raising tinamous in captivity are mating one male to two or more females in order to improve male sexual potential and to provide genealogical records of the birds to establish a genetic breeding program for domestication. The aim of this research study was to evaluate the reproductive performance of tinamous using different ratios of females per male, and to describe male and females behavioral aspects under these experimental conditions.

MATERIAL AND METHODS

The experiments were carried out during the reproductive season of September 2004 to March 2005. Birds were housed in a commercial poultry house inside cages, measuring 2.0x2.0x1.0 m, in the Wild Animal Sector of the Animal Science Department, at the School of Agricultural and Veterinary Sciences of São Paulo State University (FCAV-Unesp / Jaboticabal, Brazil), geographically located at 21 ºS and 48 ºW. All animals were fed *ad libitum* on a daily basis, with a layer pelleted feed, based on corn and soybean. Water was also supplied *ad libitum*. Sex was determined by cloaca reversion (Moro *et al.*, 1994). All birds were approximately two years old, and were in their second reproductive season. No added light period was used in this trial.

Five different female to male ratios were studied. The seventy-two birds were randomly housed in 24 cages (2 months before the reproductive period), and submitted to the following treatments, with 4 replications each:

- TR: Rotational system => one female per male per week, 3 females per male;
- T2: Couple => 1 male:1 female;
- T3: Trio => 1 male: 2 females;
- T4: Quartet => 1 male:3 females;
- T5: Quintet => 1 male:4 females.

Each TR replication used two cages, one for housing the females while waiting their turn to mate, and the other for housing the mating couple. The male was kept with one female per week.

Eggs were collected four times a day, and their origin and laying order were recorded for each cage and treatment. Damaged and broken eggs due to pecking were quantified and discarded immediately after collection. Eggs were preferentially laid on the grass (*Cynodon dactylon*) hay litter as the artificial nests provided to the birds were hardly ever used.

The following reproductive traits were analyzed: number of eggs laid per female, fertility rate, and percentage of eggs cracked by pecking and discarded eggs. Fertility rate was calculated as the number of fertilized eggs divided by the total number of eggs laid, and expressed as percentage. Non-hatched eggs were opened, and examined for embryonic development and mortality in order to determine fertility. If the eggs were fertile, the period of embryonic death was determined (1 to 7 d = early mortality, 8 to 14 d = mid-mortality, and 15 to 21 d = late mortality). The data were analyzed by the Kruskal-Wallis test (p=0.05).

Two replications of treatments T2 and T5, and one of T3 were videotape recorded for three consecutive days, 12 hours/day, with a total of 36 hours recordings/ treatment. Birds were observed during the day because tinamous show only daytime activity. They received white ink marks on different parts of the body in order to allow the identification of individuals at a distance.

The video tapes were sampled according to the scan method (Lehner, 1992): the tape was stopped every 5 minutes, and the behavior of the birds was observed and recorded according to an ethogram (Table 1). Time budget analysis for the different behavior categories was performed. Birds were also observed for one hour per day (from 10:00 to 10:30 AM, and from 03:00 to 03:30 PM) to study dominance and agonistic behaviors. Threatening, attacking and avoiding behaviors were evaluated for all birds placed in the same cage. The results found in each replication were transferred to a social metric matrix to be computed in a joint analysis. The final matrix was obtained by adding the values in the aggression and threatening matrices, and subtracting these numbers from the avoidance behavior matrix number. Spearman's (rank) correlations

Table 1 - Working ethogram of *Rhynchotus rufescens* observed groups.

Category	Description
Aggression Initiator (AGI)	The bird pecks another one when it goes near it, or when the other bird gets near it.
Aggression Receiver (AGR)	Bird is pecked by other birds.
Threat (TT)	The bird points or hurls its head in direction of another bird, and pursues it.
Preening (PR)	The bird grooms its feathers with the beak.
Dust bathing (DB)	The bird walks and makes oblique movements, lowering its body and pecking the soil. It may straighten its body and continue to walk, or it may turn and sit near the pecking soil area. In this position, it continues to peck the soil in front of its chest, always sprinkling the litter material onto its body.
Drinking (DK)	The bird stops in front of the drinker, lowers its beak and pecks the water trough, and drinks.
Scratching (SC)	The bird uses the beak and/or feet to stir the soil or the litter
Eating (ET)	Bird in front of the feeder, lowers its head, and grabs feed particles with its beak.
Displacement (DI)	The bird, keeping the body erect, moves along or around the cage, putting one leg in front of the other and thrusting its body forward (walking or running).
Bristling (BR)	The bird bristles its plumage, shaking its body laterally with half-circular movements.
Stretching (SO)	The bird stretches out the leg and the wing of the right (left) side of body.
Avoidance (AV)	The bird, motionless or in displacement, changes the direction of its movement as another bird approaches, avoiding it.
Mounting (MO)	The bird approaches another bird and places a foot on its back. The other animal crouches, and allows the first animal to climb its back.
Standing (ST)	The bird stands immobile in erect position at least for 5 seconds.
Sitting in immobility (STI)	The bird remains in sitting position at least for 5 seconds.
Sitting in activity (STA)	The bird, in sitting position, scratches the soil or the litter with its beak, or presens its feathers with beak.

between tinamous behavioral activities and reproductive performance were estimated using the software Statistica 6.0 (P=0.05).

RESULTS

Reproductive Performance

Table 2 shows the average number of eggs laid per female, fertility rate, and the percentage of discarded eggs per treatment. Apparently, treatment means are different, but the Kruskal-Wallis test results did not show any significance (P>0.05). The high standard error of mean values for the analyzed traits indicated a huge variation among replications of the same treatment.

Percentage of total broken eggs, fertility rate, and number of eggs laid per female for each treatment used in the behavioral study are shown in Table 3. The data presented in this table displays the same pattern as the data in Table 2, that is, means differ greatly among treatments and among replications within the same treatment.

Frequency of behaviors

The frequencies of the behaviors listed in Table 1 were analyzed per individual bird. Displacement behavior showed the highest frequency among all behaviors in most birds (Table 4). The exception was the female of Couple 2, which showed higher frequency for standing (ST), sitting in immobility (STI), and sitting in activity (STA).

As to displacement behavior, when treatments were sorted in decreasing order, the following order was found: Quintet 2, Couple 1, Trio, Couple 2, and Quintet 1, with average frequency values of 40, 39, 25, 24.5, and 21.2, respectively. Similarly, we can classify the birds in the treatments according to the categories Sitting in immobility plus Sitting in activity, in ascending order of frequency of occurrence as: Quintet 1, Couple 2, Trio, Couple 1, and Quintet 2 (Table 4).

Considering birds according to sex, including females and males of all treatments, it was observed that the males exhibited more activity than the females, except for the Trio, where the reverse occurred.

Table 2 - Means and standard error of the number of eggs laid per female, fertility rate, and percentage of eggs damaged and cracked by pecking per treatment of tinamous (*Rhynchotus rufescens*) raised in captivity (Kruskal-Wallis Test).

	Couple	Trio	Quartet	Quintet	Rotational	P value
Number of eggs laid /female	20.25±6.18	12.62±6.18	15.75±6.18	16.06±6.18	17.91±6.18	0.9868
Fertility (%)	45.69±14.72	76.23±14.72	61.58±14.72	39.76±14.72	62.35±14.72	0.3350
Eggs damaged and cracked by pecking (%)	6.45± 12.85	35.92±12.85	32.62±12.85	24.62±12.85	32.34±12.85	0.2483

Table 3 - Number of eggs laid per female, fertility percentage, and total percentage of eggs damaged and broken by pecking in the different treatments used in the behavioral study of tinamous (*Rhynchotus rufescens*).

	Couple 1	Couple 2	Trio	Quintet 1	Quintet 2
Number of laid eggs/female	8.00	30.00	3.24	3.90	16.00
Fertility (%)	37.99	92.30	57.78	27.98	42.55
Eggs damaged and cracked by pecking (%)	26.90	13.33	40.47	37.42	26.56

Agonistic Behavior

Table 5 shows the matrices obtained by computing agonistic interactions among birds within of each treatment. The matrices were built by summing the acts of aggression and threat minus avoidance behavior. Information on Table 5 allows us to understand the social interaction present in each treatment, and also to outline a hierarchical arrangement of each group.

Correlations between behavioral and reproductive traits

Table 6 presents the scores assigned to behavioral and reproductive traits of the experimental tinamous. The values of the behavioral traits Displacement and Sitting in immobility (STI) plus Sitting in activity (STA) were assigned as the average frequency of these behaviors per female, the frequency of males in the treatments with more than one female, and the frequency of these behaviors of females and males in the couples. Damaged and broken eggs due to pecking represent the percentage of discarded eggs per replication. The recorded number of eggs laid per female per replication shows the number of eggs laid per female only in the replications of treatments recorded and observed in the behavioral study, and the total number of eggs laid is the total number of eggs laid by all females in each treatment, including the replications that were not recorded and observed. Female dominance assigns the highest values of agonistic behavior directed to the male, and male dominance means the same for males.

Using the values shown in Table 6, we estimated rank correlations among these traits. Table 7 presents only significant rank correlation estimates between behavioral and reproductive traits.

DISCUSSION

Aggression is a ritualized way of communication aimed at establishing a hierarchy in a small group (Loiselet, 2004). Table 5 shows that, although Couple

Table 4 - Percentage of the more frequent behaviors of tinamous *(Rhynchotus rufescens)* raised in captivity.

	Couple 1		Couple 2		Quintet 1					Quintet 2					Trio		
	F	M	F	M	F1	F2	F3	F4	M	F1	F2	F3	F4	M	F1	F2	M
DI	39	39	14	35	21	16	20	16	33	50	32	17	50	51	15	39	21
PR	3	15	11	30	20	14	19	19	7	5	3	14	5	5	29	15	15
ST +STI	17	11	37	10	19	30	18	29	14	8	4	10	10	5	18	18	25
STA	10	0	24	9	2	1	2	19	5	9	13	18	9	13	3	1	5

F = female; M= male; DI = displace; PR = preen; ST = stand; STI = sit in immobility; STA = sit in activity. Values were rouded.

Table 5 - Final agonistic behavior matrices between birds in each of the recorded treatment. The values in columns represent the bird that performs the action, and values in rows the bird that receives the action. M= male; F= female.

Couple 1	F	M	**Couple 2**	F	M	**Trio**	F 1	F 2	M
F	0	12	F	0	-13	F 1	0	-5	2
M	9	0	M	4	0	F 2	5	0	2
						M	2	1	0

Quintet 1	F 1	F 2	F 3	F 4	M	**Quintet 2**	F 1	F 2	F 3	F 4	M
F 1	0	1	0	0	1	F 1	0	7	0	1	8
F 2	1	0	2	2	0	F 2	-3	0	0	1	-1
F 3	1	0	0	0	0	F 3	1	0	0	0	-3
F 4	3	0	0	0	0	F 4	0	4	1	0	1
M	-2	0	0	0	0	M	0	4	1	0	0

Table 6 - Values assigned to behavioral and reproductive traits of South American tinamous (*Rhynchotus rufescens*) raised in captivity.

	Couple 1	Couple 2	Trio	Quintet 1	Quintet 2
Area per animal in each replication (m^2)	1.0	1.0	0.6	0.4	0.4
Male Displacement	39.0	35.0	21.0	33.0	51.0
Female Displacement	39.0	14.0	27.0	18.2	37.2
Male STI+STA	11.0	10.0	19.0	14.0	5.0
Female STI+STA	17.0	37.0	18.0	29.0	8.0
Cracked and discarded eggs per replication (%)	26.9	13.3	40.4	37.4	26.5
Number of eggs laid per female per replication recorded	8.0	30.0	3.2	3.9	16.0
Total number of eggs laid per experimental treatment	20.0	20.0	12.6	16.0	16.0
Female dominance	12.0	-13.0	2.0	1.0	8.0
Male dominance	9.0	4.0	2.0	0.0	4.0

STI+STA = Sitting in immobility plus sitting in activity.

Table 7 - Estimates of rank correlation (Spearman) between behavioral and reproductive traits of South American partridges (*Rhynchotus rufescens*), for the replications recorded and observed.

	Male Displacement	Number of eggs laid per female	Female Displacement	Female dominance	Broken eggs
Broken and discarded eggs		-1.00			
Female Displacement				1.00	
Male STI+STA	- 0.6	0.9	-	-	-0.9
Female STI+STA			-0.9	-0.9	

STI+STA = Sit in immobility plus sit in activity.

1 male received a greater number of aggressive bouts from the female (12), it also showed aggressive behavior and threatened the female. Couple 2 presented a different pattern: the male seemed to dominate the female most of the time. The Trio behavior could be placed in an intermediate position between the behaviors shown by Couples 1 and 2: female 2 dominated both male and female 1, which disputed the dominant position between them. For quintet 2, Table 5 shows female 1 dominance over all the other birds, followed by females 4, 3, and 2. The male could be placed in the same hierarchical level of female 3. The hierarchical positions of animals belonging to Quintet 1 were not clear. Female 1 seemed to have more interaction with the male, and females 2 and 4 dominated female 3. When establishing a possible hierarchical classification in this group, female 2 would be the dominant among all, since it attacked all the others and was threatened only once by female 1. Female 1 was the weakest. It could be placed in this position because all females attacked it, including females 3 and 4, which did not show any dominance sign. However, female 1 was the only one that interacted with the male. In sorting agonistic interaction treatment means in decreasing order, Quintet 2, Couple 1, Couple 2, Trio, and Quintet 1 present values, 36, 21, 17, 17, and 13, respectively (Table 5).

This attempt to establish a hierarchical classification, in addition to displacement analyses, indicated that dominant females were more active, achieving levels comparable to those exhibited by males, and even higher, as shown with the Trio male (Table 4). A perfect match between agonistic encounters and displacement means was found, indicating that as agonistic encounters increase, displacement frequencies also increase.

It was observed that males exhibited more pronounced dominant signs, particularly the males from Couple 2 and Quintet 1. Coincidently, the number of eggs laid per female in these treatments was higher, 30 and 16, respectively (Table 3). Cromberg *et al.* (2003) observed the behavior of two trios (1 male:2 females) of Red-Wing Tinamous in captivity. In that study, one of the females of the second trio wounded its foot, presenting difficult locomotion. This wounded female was then dominated by the male, which bred to the healthy female, which also resulted in a greater number of eggs laid.

The estimated correlations support the previous discussion, and also provide other important information. A perfect correlation was estimated (r=1.00) between female displacement and female dominance behavior (Table 7). Therefore, this result allows us to conclude that social dominance is indirectly determined by displacement behavior in the study situation. Similarly, the measurements of sitting in immobility plus sitting in activity could also be used for the same purpose.

Maximum, but negative, rank correlation (r= -1.00) was determined between damaged and cracked eggs due to pecking and number of eggs laid per female (Table 7). This means that in replications where females laid more eggs there were less cracked and discarded eggs. This result acquired more relevance in that the number cracked eggs was not correlated with available area per bird (Tables 3 and 6). In addition, this variable (available area per bird) was not correlated with dominance.

The high correlation between cracked eggs and male activity frequency, as measured by male sitting in immobility plus sitting in activity (r=-0.90), leads us to believe that the process that culminates in cracked eggs is also related to hierarchical arrangement and dominance. Females are the dominant birds and, in captivity, male displacement can be associated with movements of escaping from females (Cromberg *et al.*, 2003). Therefore, an increase in the frequency of motionless behaviors of males, such as sitting in immobility and sitting in activity, could be considered as a predisposition to confrontation. This seems reasonable, as in this species, the male is responsible for hatching the eggs and taking care of the young, which are biological processes that require at least some degree of fearlessness. Other correlation values in Table 7 also support this interpretation. The negative and high correlation (r=-0.60) found between sitting in immobility plus sitting in activity and male displacement behaviors, and an even higher value (r=-0.90) for the correlation between these traits for females (Table 7), show that these behaviors are connected, and it seemed that females exhibit a more stereotyped process for this behavior.

Another result supporting our concerns as to the importance of social organization in these birds for the success of their reproductive performance in captivity is that replications where males showed less activity resulted in a higher number of eggs laid (sitting in immobility plus sitting in activity x number of laid eggs, r= -0.9, Table 7).

This research study indicates that tinamou male is not always able to exhibit its reproductive potential in order to attract, and eventually to stimulate female oviposition when sharing a limited space with female(s). Therefore, housing these birds in harems, with one male along with several females, does not seem to be recommended for reproductive purposes in this species.

The study of tinamou behavior under the experimental conditions of this research produced important advances in the biological knowledge of these animals, particularly as to the relation between dominance and female displacement, number of cracked and discarded eggs, and male displacement and number of eggs laid and cracked eggs. The characterization of dominant and subordinate birds was improved, and this information may be very useful when selecting sires and dams.

CONCLUSIONS

The social behaviors of this species, including their social-hierarchical structure, should to be taken into account during the domestication process of tinamous. It was possible to indirectly determine social dominance by displacement behavior in the study situation. A large number of damaged eggs occurred in all treatments, not allowing a clear conclusion as to the best male:female ratio.

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