EFFECTS OF GREATER RHEA (*RHEA AMERICANA*) FEMALE ATTRIBUTES ON EGG VIABILITY

Efectos de los atributos de la hembra de ñandú (Rhea americana) sobre la viabilidad del huevo

Labaque[1,2]*, **M.C., Martella**[3,2], **M.B., Bazzano**[2], **G. and Navarro**[3], **J.L.**

Instituto de Investigaciones Biológicas y Tecnológicas (IIByT – CONICET and UNC),
Instituto de Diversidad y Ecología Animal (IDEA-CONICET and UNC)
[2]Catedra de Ecología (Facultad de Ciencias Exactas, Físicas y Naturales), Universidad Nacional de Córdoba

SUMMARY

We conducted a study throughout a breeding season in a captive-bred population of Greater rheas (*Rhea americana*) to evaluate the association between egg viability and the female´s body weight and age. A total of 180 eggs laid by 15 females of three age classes (5 individuals per class) were collected, individually identified, and their morphometric traits, fertility and hatchability were registered. Egg fertility increased with female´s age but was unaffected by female weight. Hatching rate does not correlate with maternal attributes. The current paper contributes towards understanding the role of certain maternal attributes in egg viability. Our findings suggest that breeding Greater rheas in large flocks where free mating occurs, increases the expression of natural sexual behavior and enhances the productivity and welfare of the birds.
Key words. ratites, female age, egg fertility and hatchability, endangered species.

RESUMEN

Realizamos un estudio a lo largo de una temporada reproductiva en una población de Ñandú común (*Rhea americana*) en cautiverio, con el fin de evaluar la asociación entre la viabilidad del huevo y el peso corporal y la edad de la hembra. Se colectaron un total de 180 huevos depositados por 15 hembras de tres clases de edad (5 individuos por clase). Cada huevo se identificó y se registraron sus características morfométricas, su fertilidad y éxito de eclosión. La fertilidad de los huevos se incrementó con la edad de la hembra que lo produjo pero no fue afectado por el peso corporal de la misma. La tasa de eclosión no se correlacionó con los atributos maternos evaluados. El presente trabajo contribuye a comprender el rol de ciertos atributos maternos en la viabilidad del huevo. Nuestros hallazgos sugieren que la cría de ñandúes en grandes grupos, donde el libre apareamiento es posible, aumentaría la expresión de un comportamiento sexual natural mejorando la productividad y el bienestar de las aves.
Palabras clave. ratites, edad de las hembras, fertilidad y eclosión de los huevos, especie amenazada.

Introduction

The Greater rhea (*Rhea americana*) is one of the endangered species of the grassland ecosystems of South America, categorized as 'Near Threatened' by the IUCN (2017) and included in Appendix II of CITES (Navarro and Martella, 2008). Wild populations of Greater rhea are affected by human activities, such as illegal hunting, egg harvesting and the conversion of natural habitats into croplands (Martella and Navarro, 2006; Lèche et al., 2014). As natural populations of Greater rhea continue to decline, captive breeding will become a valuable conservation tool for native ratites (Navarro and Martella, 2008; Labaque et al., 2010; 2013), with farms in Argentina possibly serving as genetic reservoirs and sources of individuals for repopulation (Alonso Roldan et al., 2011). It is therefore crucial to identify the factors accounting for variations in the performance of breeding stocks and to assess different management practices for captive populations, to increase their welfare, and provide a baseline for decision-making on wild or captive populations.

In order to achieve profitable production levels and/or ensure viable populations, it is necessary to obtain the maximum possible number of viable eggs. The relationship between egg and chick traits depends on female egg investment since it is the female who derives the nutrients for the formation of eggs. Recent studies have shown that the body weight of Greater rhea females (at the beginning of the breeding season) determines the date of onset of egg laying, clutch size, and chemical composition of the eggs. At the same time, most of the physical characteristics and unsaturated fatty acid content of eggs are affected by both the age and weight of the female (Labaque et al., 2010). In particular, the female's age and weight are positively correlated with the percentage of some essential fatty acids in the yolk of their eggs. Higher concentrations of these nutrients in the yolk have been associated with greater egg viability in terms of increased hatchability (Navarro et al., 2001; Bonato et al., 2015) and/or the early survival of hatched chicks (Noble et al., 1996; Bonato et al., 2015). However, in Greater rheas it remains unclear how changes in the physical and chemical characteristics of the egg, associated with maternal attributes, can affect the likelihood of hatching. In the present study, we evaluate the relationship between body weight and age of Greater rhea females and the fertility and hatchability of their eggs.

Materials and Methods

During the 2001-2002 breeding season, we studied a captive population of 19 Greater rheas (1 male: 3.5 females) whose founding groups came from two different sites of Central Argentina, and which has most of the genetic variation present in wild populations of that region (Alonso Roldán et al., 2011). Females were assigned to three age classes (5 individuals per class): age class 1 corresponds to those hatched two breeding seasons prior to the study (1999/2000, i.e. the age at which rheas usually start to lay eggs); age class 2 corresponds to those hatched three breeding seasons before the study (1998/1999); and age class 3 to those hatched ≥ 4 seasons previously. Males were hatched 3 and ≥ 4 seasons previously (2 individuals per age class). In mid-August (one month before the average starting date of the laying season), each female was weighed using a 90 × 60 cm platform digital scale (0.1 kg accuracy). The resultant body weight of these females was an average of 22 ± 0.12 kg, ranging between 20 and 26 kg. We individually identified the rheas with neck tags and/or numbered plastic leg bands, and maintained them in a paddock of about 1400 m², fenced with 1.6 m-high rhombic wire mesh, at the Córdoba Zoo (Argentina). Individuals were fed on balanced feed formulated for hens, which is the most widely used feed in commercial Greater rhea farms in Argentina (Labaque et al., 2010; Bazzano et al., 2011). The daily amount of feed provided was estimated based on fulfilling the average consumption of adult rheas (600 g of pelleted feed/individual; Navarro et al., 2000).

During the laying season (September to January), we monitored the Greater rhea females throughout the day to detect egg laying, so that each fresh egg could be assigned to the respective female. Immediately after being laid, all the eggs were collected, individually marked with soft pencil, and their morphometric characteristics registered. We measured fresh egg weight to the nearest 0.1 g and egg length (major axis) and breadth (minor axis) with a calliper (to the nearest 0.1 mm). Their volume was estimated according to Labaque et al. (2007),

$$L \times B^2 \times Kw$$

where L = length (cm), B = breadth (cm), and the constant for Greater rhea Kw = 0.578 (Labaque et al., 2007). Considering that the different females laid eggs at different moments throughout the breeding season (Labaque et al., 2010), we assigned each egg to one of three consecutive periods based on the laying date: early (September-October), mid (November), and late (December-January).

Of the 252 eggs laid throughout the laying season, a total of 180 were randomly assigned to incubation and stored at 13-16 °C for 3 days, in accordance with Labaque et al. (2004). Later, we artificially incubated these eggs following the protocol described by Labaque et al. (2013), and at 34 days of incubation, we transferred them to a hatcher set at the same temperature and humidity as the incubators. The hatcher trays had individual compartments enabling the chicks to be matched with the shells from which they hatched. Hatchability was assessed as the hatching percentage of the total number of incubated eggs (total hatchability).

Statistical Analysis

We analyzed data using the InfoStat statistical software package (InfoStat, Version 2014). Mean ± standard error values are presented throughout the text. Chi-squared contingency tests were used to compare fertility and hatchability of eggs during the early, mid and late laying periods. The average morphometric characteristics of fertile and infertile eggs, and hatched and unhatched eggs were compared using a Mann-Whitney test. For each dependent variable (fertility, and hatchability), we implemented a mixed generalized linear model. The age of females (distributed in three levels), period of the laying season (distributed in three levels), laying order, clutch size, and weight of females were included as predictor variables. To take into account the correlation of response among eggs belonging to the same female, we specified an autoregressive continuous model for eggs laid by the same females, ordered according to their laying date (Pinheiro and Bates, 2004). Categorized variables such as age and laying period were transformed into dummy auxiliary variables for incorporation into the model. The effect of age on reproductive performance was confirmed by comparing females in age classes 2 and 3 with individuals in age class 1, the latter representing sexual maturity in the species. Thus for instance the dummy variable with a value t_2 in the multiple regression model corresponds to age 1 compared with age 2, and t_3 corresponds to age 1 compared with age 3. In order to determine the effect of the laying period, the variables measured in the eggs laid in the mid (November) and late (December-January) periods were compared with those of the early period (September-October). In this case, values t_2 and t_3 represent the comparison of the early period with the mid and late periods, respectively.

Results

We artificially incubated a total of 180 eggs. Their fertility rate was 93% and hatchability was 69.4%. Of all fertile eggs that did not hatch, 46% were discarded in advanced stages of embryonic development (42% of them owing to malpositioned embryo, and 4% to edematous embryo); the cause of failure to hatch could not be ascertained for the remaining eggs. No differences in morphometric characteristics of fertile and infertile eggs were found (p>0.05) (Table 1). Similarly, no differences were detected between hatched and unhatched eggs in terms of practically all size measures (p>0.05), except that hatched eggs were longer than unhatched ones (p<0.05) (Table 2).

We did not find differences in the percentage of fertility, nor in hatchability among eggs collected in the three successive laying periods of the breeding season (p>0.05) (Table 3).

Female body weight did not correlate with egg fertility nor hatchability (t = 0.67, p>0.05, t = 0.027, p>0.05). Likewise, the age of the female did not affect the hatching success of her eggs (t_2 = 1.5, p>0.05, t_3 = 0.14, p>0.05).

However, the fertility of eggs laid by the oldest group of females was higher (99%) than that of eggs laid by the youngest group (86%) (t_3 = 1.98. p<0.05), whereas no difference in fertility was found between the youngest and intermediate-aged females (93%) (t_2 = 1.59, p>0.05).

Discussion

The fertility and hatchability rates of eggs in the studied Greater rhea population are within the published ranges for this species under captive conditions (Navarro and Martella, 2002; 2008; Labaque et al., 2004; 2013). The higher fertility of eggs in older females (age class 3) can be attributed to various factors, one of which is the increasing maturity of the reproductive system with advancing age, impacting positively on other reproductive parameters such as the number, size and quality of the eggs produced (Labaque et al., 2010). This is based on the close correlation between female age and reproductive performance, as also reported in other bird species (i.e. Martin, 1995; Hipfner et al., 1997) including another ratite, the ostrich (Lambrechts, 2004; Bonato et al., 2015). Another possible explanation for the higher fertility rate in eggs of older females relates to the choice of mate, in other words, to the ability of females to recognize the necessary traits in males for mating success (Houston et al., 2005; Olson et al., 2008), favored by the promiscuous mating behavior characteristic of the species (Székely et al., 2000). This explanation is based on the findings of Bonato et al. (2009a), who observed that ostrich (*Struthio camelus*) females are able to differentiate males by their coloration, indicative of their immunological state, and subsequently depositing eggs of various sizes as a consequence of this mating selection (Bonato et al., 2009b). A further possibility is that the older (and possibly more experienced) females select the "best males" for mating purposes, and/or to promote a synergistic effect between their own particular traits and those of the male, ultimately leading to greater reproductive success. The findings of Bonato et al. (2015) in the ostrich also support this hypothesis; the author showed that in this species a group of individuals within the population exhibited certain reproductive advantages directly related to female and male attributes, with 60% of the total chicks in the flock deriving from this limited group of males and females who mated among themselves. In this context, mating choice is favored by large reproductive flocks. This strategy is also seen to be beneficial for the Greater rhea, since this species attains higher levels of productivity when bred in large groups (4 males: 14 females) than in small groups (2 males: 7 females) (Labaque, 2006). The latter author found that the number of laying females, the quantity of eggs laid (in total and per individual), the duration of the laying period and the percentage of hatched eggs and live chicks in the first month were all significantly higher in large flocks. Our findings, in conjunction with those of Labaque (2006) lead us to propose the idea that when breeding in large flocks where free

Table 1. Morphometric characteristics of fertile and infertile eggs laid by captive females of Greater rheas throughout a breeding season. No significant differences were detected (Mann-Whitney test). The "n", refers to the number of eggs
Cuadro 1. Características morfométricas de huevos fértiles e infértiles producidos por hembras de ñandú mantenidas en cautiverio durante la estación reproductiva. No se detectaron diferencias significativas (Mann-Whitney test). La "n", refiere al número de huevos.

Morphometric characteristics	Fertile (n = 168)	Infertile (n = 12)
Weight (g)	546.2 ± 17.6	572.0 ± 5.3
Lenght (cm)	12.7 ± 0.2	12.6 ± 0.1
Breath (cm)	8.9 ± 0.03	8.71 ± 0.01
Volume (cm³)	561.3 ± 14.3	576.9 ± 5.6

Table 2. Morphometric characteristics of hatched and unhatched eggs laid by captive females of Greater rheas throughout a breeding season. The "n", refers to the number of eggs.
Cuadro 2. Características morfométricas de huevos eclosionados y no eclosionados producidos por hembras de ñandú mantenidas en cautiverio durante la estación reproductiva. La "n" refiere al número de huevos.

Morphometric characteristics	Hatched (n = 125)	Unhatched (n = 55)
Weight (g)	577.3 ± 6.1	554.2 ± 8.9
Lenght (cm)	12.7 ± 0.1[a]	12.5 ± 0.1[b]
Breath (cm)	8.9 ± 0.0	8.8 ± 0.1
Volumen (cm³)	583.0 ± 6.4	559.5 ± 9.1

Values within the same row not sharing a common superscript are significantly different at $p<0.05$ (Mann-Whitney test).

Table 3. Percentage of fertility and hatchability of eggs laid by captive Greater rhea females in three successive laying periods in a breeding season. For each parameter, no significant differences were observed among periods (Chi-squared contingency tests. The "n" refers to the number of eggs).
Cuadro 3. Porcentaje de fertilidad y eclosión de huevos producidos por hembras de ñandú mantenidas en cautiverio en tres períodos sucesivos de una temporada reproductiva. Para cada parámetro, no se observaron diferencias significativas entre períodos (test de de Chi cuadrado de contingencia. La "n" se refiere al número de huevos.

Laying Period	Fertility (%)	Hatchability (%)
Early (September-October)	93 (n = 46)	71 (n = 35)
Mid (November)	92 (n = 59)	67 (n = 43)
Late (December-January)	94 (n = 63)	70 (n = 47)

mating occurs, the older and/or more experienced females mate with the best males, maximizing the expression of natural sexual behavior and thus giving rise to higher productivity and better overall welfare of the birds. However, as the conservation status of the species, its large size and housing requirements have imposed us to work with a comparatively limited sample size and no replicates, our results should be taken as a first approach and used cautiously. Thus, to produce more conclusive evidence that might allow extrapolating our findings to different cases it will be desirable to further test this hypothesis in other Greater rhea populations.

Contrary to what one would expect in light of the differences found by Labaque et al. (2010) in terms of the fatty acid composition of the eggs of females with different attributes, the hatching rate was not affected by the age of the mothers. The only difference found was in the length of the eggs that hatched, a parameter unrelated to female attributes (Labaque et al., 2010). One could argue that although the differences found by Labaque et al. (2010) in egg fatty acid composition may be statistically significant, they are biologically irrelevant in terms of detectable effects on hatchability, at least under artificial incubation conditions. One should take into account that artificial incubation ensures that temperature and humidity

conditions, crucial to embryonic development (Labaque et al., 2004), remain constant throughout the whole process, giving rise to a higher hatching rate than in the case of natural incubation (Navarro and Martella, 2008). Natural incubation conditions can vary considerably in accordance with habitat (Bertram, 1992) and the traits of the incubating male (Bellis, 1996), both factors impacting on the success of the clutch (Labaque, 1996; Martella and Navarro, 2006). It is feasible to suppose that certain egg characteristics and/or those of its progenitors that appear insignificant in intensive breeding systems can be adaptive under natural incubation conditions. Future studies could be aimed at evaluating whether naturally incubated eggs of varying size and/or fathered by different females and males have different degrees of hatching success. Studies along these lines are useful not only for the purpose of expanding our knowledge of the biology of the species, but also in terms of providing producers with the means to assess the respective productivity outcomes of the different breeding systems (semi-intensive and intensive). Furthermore, research in this area will improve our understanding of management techniques that foster the viability and welfare of captive-bred Greater rhea.

Our findings show that under intensive breeding conditions with artificial incubation, the age of Greater rhea females has a positive impact on egg fertility but not on hatchability.

Acknowledgements

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