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Effect of maternal environment on yolk immunoreactive corticosterone and its influence on adrenocortical and behavioral activity in chicks of Greater Rhea (*Rhea americana*)

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ABSTRACT

Maternal corticosterone in avian eggs may modify offspring phenotype in order to increase survival in poor environments. In the Greater Rhea (*Rhea americana*), we previously found that yolk immunoreactive corticosterone is influenced by the quality of the maternal environment: eggs laid by females of the intensive rearing system (IRS), living in poor captive conditions, had higher yolk immunoreactive corticosterone than those produced by females of the semi-extensive rearing system (SRS), living in better conditions. Here, we evaluate if these different hormone levels are associated with the production of different phenotypes. We collected eggs from the IRS and SRS for hormonal quantification and artificial incubation. Then, half of the chicks selected from each environment were exposed to a capture and restraint protocol, and the rest remained undisturbed and were used as controls. In the IRS, we found that higher yolk immunoreactive corticosterone was associated with the production of chicks that had reduced hatchability, lower hatchling mass and higher baseline fecal glucocorticoid metabolites (FGM) than those produced by SRS females. Moreover, after capture and restraint, IRS chicks did not modify their FGM nor their behaviors compared to their controls, while SRS chicks increased their FGM and spent more time ambulating and less time pecking, compared to their controls. These results indicate that yolk immunoreactive corticosterone could modify offspring phenotype. Although future studies are needed to elucidate their implications for fitness, our results suggest that yolk corticosterone could be mediating an adaptive maternal effect that allows individuals to better cope with poor conditions.

1. Introduction

In several bird species, it has been shown that corticosterone is transmitted from the mother to the egg (e.g., Hayward and Wingfield, 2004; Hayward et al., 2005; Love et al., 2005; Navara et al., 2006) and could influence embryo development and chick phenotype. For example, it has been reported that egg-corticosterone injections decreased hatchability in chickens (*Gallus gallus domesticus*, Eriksen et al., 2003), increased incubation time in yellow-legged gulls (*Larus michahellis*, Rubolini et al., 2005), and reduced hatchling body size in barn swallows (*Hirundo rustica*, Saino et al., 2005). Moreover, corticosterone injected into the egg also increased baseline corticosterone levels in chickens (*Gallus gallus domesticus*, Ahmed et al., 2014), and decreased stress responsiveness in quails (*Coturnix coturnix japonica*, Hayward

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et al., 2006) and starlings (*Sturnus vulgaris*, Love and Williams, 2008). Although these experimental studies suggest that prenatal exposure to elevated corticosterone has detrimental effects on offspring, it is possible that energetic trade-offs exist that make these effects overall advantageous in certain natural contexts. For species with large brood sizes, elevated egg corticosterone levels could be mediating an adaptive mechanism of brood reduction when environmental conditions are poor, allowing a mother to maximize the quality of the young she does fledge (Love et al., 2005; Sheriff and Love, 2013). Alternatively, being small early in life could be an adaptive response that increases survival in environments where food is scarce or difficult to obtain (Henriksen et al., 2011). Moreover, an attenuated stress response characterized by a quicker return to baseline levels may facilitate adaptive behaviors that allow individuals to better cope with frequent exposure to stressful stimuli (Love and Williams, 2008; Weinstock, 2008).

The Greater Rhea (*Rhea americana*) is a gregarious bird endemic to South America that lives in grasslands and agroecosystems with variable degrees of anthropic disturbance (Giordano et al., 2010). It has been classified as "Near Threatened" by the International Union for Conservation of Nature (IUCN, 2016), because of drastic declines of their free-ranging populations. The species is reared in captivity for production and conservation purposes, as a reservoir of genetic resources and for reintroduction into the wild (Navarro and Martella, 2011). Captive breeding can be performed in intensive rearing systems (IRS) or semi-extensive ones (SRS). In the IRS, 2 males and up to 6 females are kept in small pens with bare ground, and they are daily provided with processed food and chopped alfalfa. Alternatively, in the SRS, several males and females are housed in enclosures larger than 1 ha with natural or implanted vegetation. Birds are generally kept foraging on a rotational basis and receive processed food as a supplement. For the Greater Rhea, environmental conditions of the IRS are considered of lower quality than those of the SRS, because of interference with the development of species-specific natural behaviors (FAWC, 1993). For example, the lack of pastures in the IRS prevents birds from developing their natural foraging behavior (Martella et al., 1996), and the small size of pens does not allow the formation of different reproductive groups. Recently, we found that eggs laid by females of the IRS have on average higher yolk immunoreactive corticosterone than those produced by females of the SRS (Della Costa et al., 2016). However, it is unknown if these elevated corticosterone levels could modify embryo development and chick phenotype. Hypothesizing that this occurs, here we first verified that eggs laid by females of the IRS have elevated yolk immunoreactive corticosterone. Then, we tested the prediction that yolk immunoreactive corticosterone increases incubation time and decreases hatchability and hatchling mass. Finally, using a capture and restraint protocol, we examined if yolk immunoreactive corticosterone could modify adrenocortical and behavioral activity in the chick.

2. Materials and methods

2.1. Study species and maternal environments

Greater Rheas are omnivorous, although fundamentally herbivorous (Martella et al., 1996; Pereira et al., 2003). During the non-breeding season, they form large flocks of 20 to 70 individuals of both sexes and different ages (Bruning, 1974). At the beginning of the breeding season, due to intense male-male competition for females, these flocks disaggregate in different reproductive groups composed of 1 or 2 males and several females (Bruning, 1974). Their mating system combines simultaneous polygyny and simultaneous and serial polyandry (Handford and Mares, 1985), with a high degree of promiscuity (Martella et al., 2014). The male constructs the nest, in which several females lay their eggs. On average, the same female could lay eggs in 1.9 nests and had 3.7 chicks (Martella et al., 2014). The male also incubates the eggs. On average, each male had progeny in 1.75 nests and produced 4.5 chicks (Martella et al., 2014). The chicks are precocial and nidifugous.

In this study, we worked with the IRS population located at the Experimental Farm of the Zoological Garden of Córdoba city, Argentina (31° 25′ S, 64° 10′ W), and with the SRS population, located at the Pampa Cuyen Farm in Balcarce city, Argentina (37° 49′ S, 58° 12′ W). In the IRS, 4 adult males and 10 adult females were housed in 0.02 ha pens with bare ground (2 males and 5 females per pen) and fed ad libitum on processed food and chopped alfalfa (*Medicago sativa*). In the SRS, 20 adult males and 20 adult females were housed in a 1.5 ha enclosure, and feeding involved direct foraging on grasses and dicot herbs and processed food. IRS population was formed in the mid-90′ s, and SRS population in 2003, with several individuals coming from the IRS, so both populations are genetically close related. All procedures developed in this study were conducted with the approval of the ethics committee of the *Consejo Nacional de Investigaciones Científicas y Técnicas* (CONICET) (Resolution N° 1047, Appendix II, 2005).

2.2. Egg collection

We collected 70 eggs from 2 nests of the IRS (one nest per pen) and 72 eggs from 5 nests of the SRS, during the intermediate laying period of the season, when the peak of egg production occurs and 80% of females are laying (Lábaque et al., 2010). Eggs collected remained < 24 h in the nest, to avoid hormone production by the developing embryo (Groothuis and von Engelhardt, 2005). Daily, we checked the nests and collected only half of the freshly laid eggs to maintain an increasing clutch size. We distinguished freshly laid eggs by their intense yellow color, which fades as the days elapse. Egg collection procedure took < 5 min, and males always returned to incubate within 10 min. Eggs were marked with an identifying code and weighed. We used the egg mass as it is the easiest most accurate and measurable direct indicator of egg size and volume (Lábaque et al., 2007). Some eggs collected were frozen at −20 °C until hormone analysis (25 eggs from the IRS and 23 eggs from the SRS) and the rest were artificially incubated.

2.3. Yolk corticosterone assay

We defrosted the eggs until the albumin could be scraped off from the yolk with a spatula. Each yolk was extracted according to Almasi et al. (2012). Briefly, we diluted 0.15 g of yolk in 600 µl of double-distilled water, vortexed for 30 s and froze overnight. On the next day, we added 3 ml of 100% methanol. We shook the sample for 30 min and froze it overnight. After centrifugation (4000 rpm for 10 min), 1 ml of the supernatant was evaporated under a stream of nitrogen and then resuspended in 250 µl of assay buffer and vortexed. The solution was frozen at −20 °C until hormone analysis. We assumed that extraction efficiency was the same for all samples. Yolk corticosterone concentration was determined using a commercially available ^{125}I corticosterone radioimmunoassay kit (MP Biomedicals, Costa Mesa, California, USA). This immunoassay was previously validated for Greater Rhea by Della Costa et al. (2016), who found that cross-reaction of the antibody with progesterone is low (2.5%) compared with the percentage of cross-reaction reported elsewhere using other immunoassays (Quillfeldt et al., 2011; Rettenbacher et al., 2009, 2013). Hormone quantification was performed in a gamma counter (Counter PC-RIA-MAS Stratec Electronic GMBH), sampling the number of counts per minute. The result was provided in nanograms per milliliter (ng/ml), as predetermined by the group diagnostic protocol. The final value of immunoreactive corticosterone was corrected for weight and dilution and expressed in nanograms per gram of yolk (ng/g). Each sample was assayed in duplicate. Yolk samples from both environments (IRS and SRS) were analyzed in the same assay. Mean intra- and interassay coefficients of variation were < 10%. Since Della Costa et al. (2016) showed that two or more hormones or hormone metabolites that bind to the antibody cause the measured signal in the yolk, we will refer to

immunoreactive substances detected by our corticosterone antibody as "immunoreactive corticosterone".

2.4. Artificial incubation and chick rearing

Artificial incubation and chick rearing were carried out at the Experimental Farm of the Zoological Garden, following the protocols described by Navarro et al. (1998, 2005). Eggs collected from both environments (IRS and SRS) were randomly distributed in commercial turning incubators designed for Greater Rhea eggs. We calibrated the incubators at 36.4 °C and humidity that allowed eggs to lose a total of 12 to 15% of their initial mass. Eggs were weighed and candled every 4 to 5 d throughout the incubation period. Eggs with no embryonic development were regarded as infertile, whereas those with arrested embryonic development were classified as embryonic mortality. At 30 days of incubation, we transferred the eggs to a hatcher that maintained the same temperature and relative humidity until hatch. The hatcher was divided into individual compartments for eggs. For each environment (IRS and SRS), we calculated hatchability as the percentage of hatched chicks from the total fertile eggs, and duration of incubation as the number of days elapsed between laying date and hatching date. After hatching, chicks were weighed, identified with numbered leg-bands and placed into a closed nursery room of 3 × 2 m with infrared lamps as a heat source. Once chicks reached 3 days of age, they were placed into an external breeding pen of 9 × 5 m with bare ground. In this pen, chicks had access to a closed shelter with infrared lamps. They were fed on daily rations of processed food, supplied according to the curve of consumption depending on their age (Navarro et al., 2005). All chicks from eggs laid in the IRS and SRS were placed into the same breeding pen, so they were reared under identical environmental conditions.

2.5. Experimental test

We worked with 24 chicks between 18 and 31 days of age (25.7 ± 0.6), from eggs collected in the IRS (n = 12) and in the SRS (n = 12). Half of the chicks from each environment were randomly assigned to a stress group, and the rest remained undisturbed and were used as controls. Since the monitoring of adrenocortical and behavioral activity requires continuous visual attention of at least one person per chick, we worked in 6 successive batches of 4 animals each. Therefore, each batch consisted of representative individuals from each experimental group: one IRS chick and 1 SRS chick of the stress group, and 1 IRS chick and 1 SRS chick of the control group. At 10:00 am, chicks of the stress groups were captured and moved to a pen near the breeding pen, where they were placed in individual cardboard boxes (40 × 14 × 14 cm) for 10 min (standardized capture and restraint protocol). Then, birds were removed from the boxes and returned to their breeding pen. Immediately after, we monitored the adrenocortical and behavioral activity of the stressed and control chicks.

2.6. Monitoring adrenocortical and behavioral activity in chicks

The adrenocortical activity was monitored through the quantification of fecal glucocorticoid metabolites (FGM). This non-invasive method was previously validated for the Greater Rhea by Lèche et al. (2011). During the following 9 h after the stressed chicks returned to the breeding pen, we collected the feces from stressed and control chicks immediately after deposition. It is important to recall that unlike most avian species, Greater Rhea urine is stored and excreted separately from feces (Stewart, 1994) and, therefore, it is not mixed in dropping material. Feces collected were placed individually into labeled plastic bags and frozen at −20 °C until hormone analysis. Steroid extraction was performed following the protocol described by Lèche et al. (2011). Briefly, we added 5 ml of 60% methanol (100% methanol/distilled water) to 0.5 g of homogenized feces and stirred with a shaker for 30 min. After centrifugation (4000 rpm for 10 min), 1 ml of the supernatant was evaporated in a warm water bath (40 °C), and then resuspended in 195 µl of assay buffer + 5 µl of 100% methanol. The solution was vortexed and frozen at −20 °C until hormone analysis. We assumed that extraction efficiency was the same for all samples. FGM concentration was determined using a commercially available ^{125}I corticosterone radioimmunoassay kit (MP Biomedicals, Costa Mesa, California, USA), as described by Lèche et al. (2011). Hormonal quantification was performed in a gamma counter (Counter PC-RIA-MAS Stratec Electronic GMBH), sampling the number of counts per minute. The result was provided in nanograms per milliliter (ng/ml), as predetermined by the group diagnostic protocol. The final value of FGM was corrected for weight and dilution and expressed in nanograms per gram of feces (ng/g). Each sample was assayed in duplicate. Mean intra- and interassay coefficients of variation were < 9%.

The behavioral activity was monitored following a focal-scanning technique. Chicks were video-recorded as described below, using a digital camera (Samsung SMX-F43BM/XB6). For each batch, we performed consecutive series of video recordings of the 4 individualized chicks in sequence. Each video recording had a length of 1 min, with a 5-s interval between successive chicks. The sequential order of video recordings in the series was assigned randomly among the chicks of the batch. We repeated the obtained sequence 5 times in each batch, to obtain 20 videos in total, comprising 5 videos with 4-min intervals for each chick. We analyzed the video recordings second-by-second, to register ambulating, pecking, drinking, preening, and resting (Table 1). These behaviors were selected according to our previous study (Della Costa et al., 2013), in which we reported that adult Greater Rheas modify them in response to acute stressors. We calculated the time spent on each behavior by adding the number of seconds that chick developed the behavior. Behavioral time was expressed in seconds per minute.

2.7. Statistical analysis

We performed general linear mixed models (LMMs) to evaluate the effect of maternal environment (IRS vs. SRS) on yolk immunoreactive corticosterone (LMM1), and on egg and hatchling mass (LMM2 and LMM3, respectively), including the nest as a random factor. Hormonal data were transformed to natural logarithms, and hatchling mass data were transformed to sequential ranks (Shirley, 1987), for normality of residuals. We estimated the effect size (Cohen's d) for the effect of maternal environment for the LMM1, LMM2, and LMM3. Following Cohen (1992), the absolute cut-off values of 0.8, 0.5 and 0.2 were used to identify "large", "medium" or "small" effect sizes, respectively. Hatchability and duration of incubation were compared between environments, using a proportions difference test (Marascuilo and McSweeney, 1977) and a Wilcoxon-Mann-Whitney test (Zar, 1984), respectively. Given that the nest did not have a significant effect on yolk immunoreactive corticosterone (ANOVA, $F_{5,\ 41} = 0.25$, $P = 0.94$), and in order to select the most parsimonious models (following the Bayes and Akaike's information criteria), we did not include the nest as a random factor, in the analysis of adrenocortical and behavioral data. We adjusted an LMM to determine the effect of interaction between maternal environment (IRS vs. SRS) and treatment (stress vs. control group) on FGM levels, including the batch and the identity of the chick

Table 1
Description of behaviors of Greater Rhea (*Rhea americana*) chicks.

Behavior	Description
Ambulating	Moving through steps, walking or running
Pecking	Touching the food or the ground, standing, sitting or lying
Drinking	Tapping the water with the beak
Preening	Touching his feathers with the beak, standing, sitting or lying
Resting	Sitting or lying, with head raised or down

as random factors (LMM4). FGM data were transformed to natural logarithms for normality of residuals. We adjusted an LMM to determine the effect of interaction between maternal environment and treatment on time spent preening, including Identity variance function to the maternal environment, and the batch as a random factor (LMM5). Preening time data were transformed to sequential ranks (Shirley, 1987), for normality of residuals. Finally, we used generalized linear mixed models (GLMMs) with gamma distribution and logarithmic link function, to study the influence of interaction between maternal environment and treatment on time spent ambulating and on time spent pecking, including the batch as a random factor (GLMM6 and GLMM7, respectively). We estimated the effect size (Eta squared) for the effect of the interaction between maternal environment and treatment for the LMM4, GLMM6, and GLMM7. Following Cohen (1988), the cut-off values of 0.14, 0.06 and 0.01 were used to identify "large", "moderate" or "small" effect sizes, respectively. To the best of our knowledge, no method of estimating effect size has been devised for LMM assuming an Identity variance function. Thus, no effect size could be estimated for the effect of the interaction between the maternal environment and treatment on time spent preening (LMM5). The times spent drinking and resting were not statistically analyzed, because only a few chicks showed these behaviors: 4 chicks drinking (1 from the IRS stress group, 2 from the IRS control group, and 1 from the SRS stress group), and 3 chicks resting (1 from the IRS control group, 1 from the IRS stress group, and 1 from the SRS control group). LMMs and GLMMs were adjusted using the Infostat statistical software package (Di Rienzo et al., 2012). Data were tested for normality of residuals using Shapiro-Wilks modified tests. Residues vs. predicted values were plotted to verify homoscedasticity. Means of main effects were compared using Fisher's tests. Differences were considered significant at $P < 0.05$. Non-transformed values were expressed as mean ± standard error (SE). Effect sizes were estimated using "sjstats" (Lüdecke, 2019) and "EMA-tools" (Kleiman, 2017), in R-project software (R Core Team, 2018).

3. Results

Yolk immunoreactive corticosterone was influenced by the maternal environment: eggs produced by females of the IRS showed higher yolk immunoreactive corticosterone than those produced by females of the SRS (Table 2). The effect size (Cohen's d) for maternal environment effect was −2.34. Egg mass, hatchability and hatchling mass were also influenced by the maternal environment: females of the IRS produced eggs with reduced mass and chicks with lower hatchability and hatchling mass than those produced by females of the SRS (Table 2). The effect size (Cohen's d) for maternal environment effect on egg and hatchling mass was 4.47 and 2.33, respectively. Duration of incubation did not differ between the IRS and SRS (Table 2). FGM in chicks were influenced by the interaction between maternal environment and treatment ($F_{1,\ 15} = 9.91$, $P = 0.01$): chicks of the IRS control group showed higher FGM than those of the SRS control group; chicks of the SRS stress group had higher FGM than those of their control group, and there were no differences between chicks of the IRS stress and control group (Fig. 1). The effect size (Eta squared) for the interaction effect was 0.20. The time spent ambulating was also influenced by the interaction between maternal environment and treatment ($F_{1,\ 20} = 5.27$, $P = 0.03$): chicks of the IRS control group ambulated more than those of the SRS control group, chicks of the SRS stress group ambulated more than those of their control group, and there were no differences between chicks of the IRS stress and control group (Fig. 2). The effect size

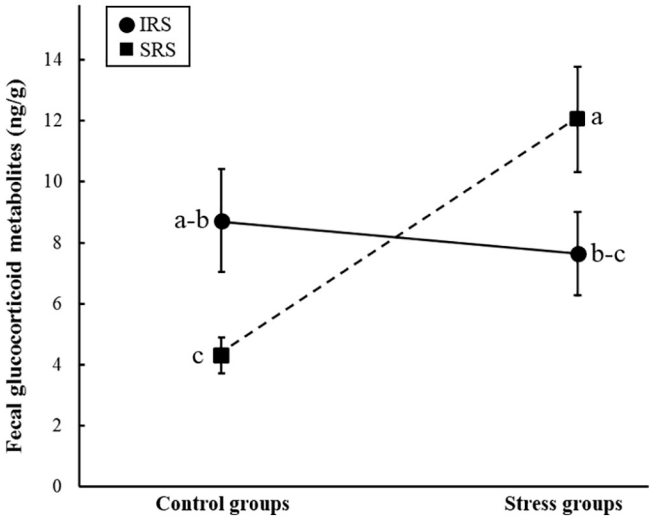

Fig. 1. Fecal glucocorticoid metabolites (mean ± SE) of Greater Rhea chicks from eggs laid by females of the intensive (IRS) and semi-extensive (SRS) rearing systems ($n = 12$ chicks from each environment). Half of the chicks from each environment were randomly assigned to a stress group, and the rest remained undisturbed and were used as controls. Levels not connected by the same letter are significantly different ($P < 0.05$).

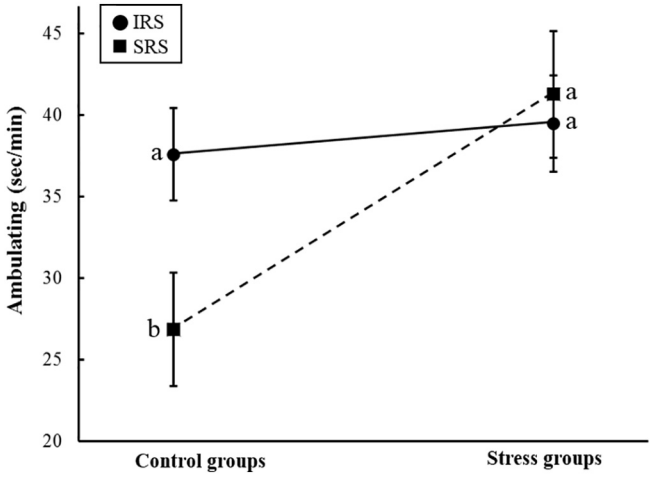

Fig. 2. Ambulating time (mean ± SE) of Greater Rhea chicks from eggs laid by females of the intensive (IRS) and semi-extensive (SRS) rearing systems (n = 12 chicks from each environment). Half of the chicks from each environment were randomly assigned to a stress group, and the rest remained undisturbed and were used as controls. Levels not connected by the same letter are significantly different ($P < 0.05$).

Table 2
Yolk immunoreactive corticosterone and egg and chick traits produced by Greater Rhea females living in the intensive (IRS) and semi-extensive (SRS) rearing systems. Levels not connected by the same letter are significantly different ($P < 0.05$).

Variable	IRS	SRS	Statistic values
Yolk immunoreactive corticosterone	76.53 ± 4.57 ng/g ($n = 25$)[a]	61.52 ± 5.36 ng/g ($n = 23$)[b]	$F_{1,\ 5} = 6.83$, $P < 0.05$
Initial egg mass	573.99 ± 7.22 g ($n = 70$)[a]	630.23 ± 8.6 g ($n = 72$)[b]	$F_{1,\ 5} = 24.96$, $P < 0.01$
Hatchability	77.14%[a]	96.77%[b]	*Dif Prop*: 0.20, $P = 0.03$
Duration of incubation	36.63 ± 0.41 days ($n = 27$)[a]	36.80 ± 0.61 days ($n = 30$)[a]	$W = 780$, $P = 0.96$
Hatchling mass	376.85 ± 7.84 g (n = 27)[a]	411.4 ± 6.5 g (n = 30)[b]	$F_{1,\ 5} = 9.96$, $P < 0.03$

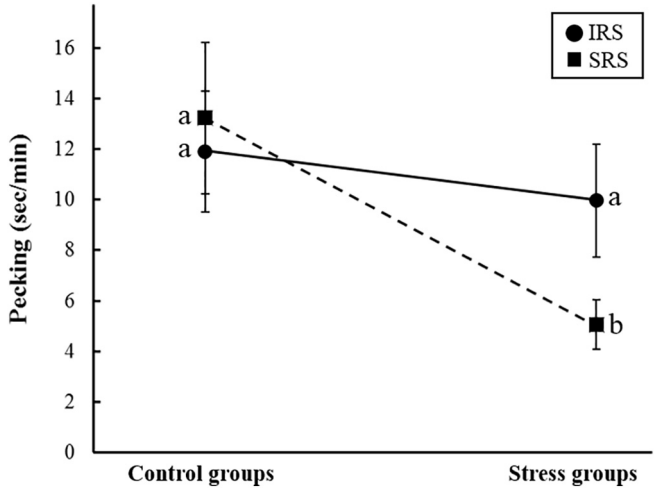

Fig. 3. Pecking time (mean ± SE) of Greater Rhea chicks from eggs laid by females of the intensive (IRS) and semi-extensive (SRS) rearing systems (n = 12 chicks from each environment). Half of the chicks from each environment were randomly assigned to a stress group, and the rest remained undisturbed and were used as controls. Levels not connected by the same letter are significantly different ($P < 0.05$).

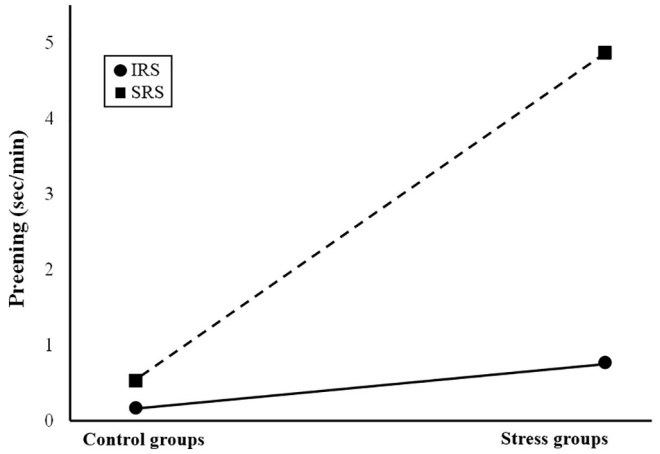

Fig. 4. Preening time (mean ± SE) of Greater Rhea chicks from eggs laid by females of the intensive (IRS) and semi-extensive (SRS) rearing systems (n = 12 chicks from each environment). Half of the chicks from each environment were randomly assigned to a stress group, and the rest remained undisturbed and were used as controls.

(Eta squared) for the interaction effect was 0.13. The time spent pecking was also influenced by the interaction between maternal environment and treatment ($F_{1,\ 20} = 4.78$, $P = 0.04$): chicks of the SRS stress group spent less time pecking than their control group, and there were no differences between chicks of the IRS and the SRS control group, nor between those of the IRS stress and control group (Fig. 3). The effect size (Eta squared) for the interaction effect was 0.12. The time spent preening was not influenced by the interaction between maternal environment and treatment ($F_{1,\ 15} = 0.12$, $P = 0.73$, Fig. 4).

4. Discussion

We found that egg laid by females of the IRS had on average higher yolk immunoreactive corticosterone than those produced by SRS females. These findings are in agreement with our previous study (Della Costa et al., 2016) and indicate that rearing conditions experienced by the females could influence their yolk corticosterone deposition. However, it is not known what environmental factors are determining this influence. The IRS and SRS differ from each other in several factors such as the size of the area, diet composition, breeding density, sex ratio, and probably male-male competition. Also, the state of the females themselves in terms of, for example, their age, physical condition, and past experiences, could be different in both rearing systems. In the Greater Rhea, unlike other bird species, several females lay eggs in the same nest. In this study, given that several nests were used from each rearing system, the individual eggs should have been nested in females that had been in turn nested in nests. However, it was not possible because we did not identify the female that laid each egg, creating a firm possibility for pseudoreplication. Our next best option to account for this potential pseudoreplication was to include the nest as a random effect in those cases where its addition resulted in an improvement in model fit, according to Bayes and Akaike's information criteria.

In the IRS, we found that higher yolk immunoreactive corticosterone was associated with the production of chicks that had reduced hatchability, lower hatchling mass, and higher baseline FGM, in comparison with those produced by females of the SRS. These results are in accordance with our predictions and suggest that prenatal exposure to elevated maternal corticosterone has deleterious effects for the embryo. However, it is also possible that elevated corticosterone in the yolk may be mediating an adaptive maternal effect that allows individuals to better cope with poor environmental conditions (Bowers et al., 2016; Chin et al., 2009; Zimmer et al., 2013). For example, a mechanism of brood reduction could be advantageous for Greater Rheas living in the IRS, where the space is reduced. It could also be that chicks with lower hatchling mass have lower metabolic rate and therefore need less food, which would make them more adapted to the lack of vegetation for foraging. Furthermore, it is possible that the higher FGM and ambulating time observed in chicks produced by IRS females are associated with processes of mobilization of energy that allow chicks to find other food items. Interestingly, since the corticosterone deposited into the yolk comes exclusively from the female's plasma, the higher yolk immunoreactive corticosterone quantified in eggs laid by IRS females, suggests that they also have on average higher plasma corticosterone than those of the SRS.

In agreement with our prediction, we found that different immunoreactive corticosterone levels in the yolks were associated with the production of chicks that exhibited a different adrenocortical and behavioral activity after a stressful event. For example, we observed that chicks produced by females of the IRS did not modify their FGM levels nor their behaviors after a capture and restraint protocol. These findings are similar to those reported on other species, which showed that egg-corticosterone injections decreased stress responsiveness (Hayward et al., 2006; Love and Williams, 2008; Zimmer et al., 2013). In an environment where the small size of pens does not allow the chicks to escape from a threat, a lack of stress response could improve their chances of survival. If an increase of corticosterone levels does not allow the individual to evade a threat, prolonged maintenance of elevated corticosterone could be deleterious for health and well-being. Although we observed that capture and restraint did not trigger a stress response in chicks produced by females the IRS, this does not mean that they could not respond to stressors later in their life. In fact, several studies showed that adult Greater Rheas hatched from eggs laid in the IRS, increase their corticosterone levels and modify their behaviors in response to capture, handling and transportation (Della Costa et al., 2013; Lèche et al., 2013, 2016). However, these stress responses are attenuated compared to those of birds hatched from eggs laid in the SRS (reviewed by Navarro et al., 2018a). For example, it has been recently reported that adult Greater Rheas of the IRS exhibited lower increases in adrenocortical activity when they are exposed to a new environment, in comparison with birds of the SRS (Navarro et al., 2018b). These findings, in agreement with our results, support the idea that prenatal exposure to higher corticosterone decrease stress responsiveness and suggest that these effects may persist into adulthood.

In contrast to the females of the IRS, we found that females of the

SRS produced chicks that increased their FGM levels and ambulating time and decreased their pecking time after capture and restraint. This rapid adrenocortical and behavioral response, characteristic of the emergency life-history state (Wingfield et al., 1998), could be favorable in environments such as the SRS, where birds would be more likely to encounter new stimuli and even possible "predators or threats", and where the availability of more space would allow them to escape. However, in this study, chicks from eggs laid by females of the SRS were reared under the environmental conditions of the IRS. This means that those chicks were exposed after hatching to an environment that differed from the one experienced by their mothers. It would be interesting to evaluate in future studies how this mismatch could affect the fitness of individuals. The environmental mismatching hypothesis proposes that mothers could adjust the offspring phenotype in a maladaptive way if the environment experienced by the offspring does not correlate with the maternal environment (reviewed by Groothuis and Taborsky, 2015). Consequently, we could expect that chicks produced by females of the SRS would experience fitness disadvantages in the IRS.

In conclusion, we verified that eggs laid by Greater Rhea females living under poor environmental conditions have on average higher yolk immunoreactive corticosterone than those produced by females living in better conditions. We also showed, for the first time in the species, that higher yolk immunoreactive corticosterone has the potential to modify adrenocortical and behavioral activity in the chick. Although we do not know if these effects are directly induced by yolk immunoreactive corticosterone or are indirect consequences of effects on other traits, our results suggest that this hormone could be mediating an adaptive maternal effect that allows individuals to better cope with poor environmental conditions. Future studies are needed to confirm this hypothesis.

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