~~Sex-specific~~E ~~e~~ffects of ~~a prelaying~~ Pb exposure on sperm quality and ~~the~~ reproductive output ~~on~~ of ~~the~~ red-legged partridge

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1. Introduction

Lead (Pb) poisoning by the ingestion of shot pellets is a frequent cause of death in wild birds, but also have a wide range of sublethal effects [1]. Among other functions, Pb has been shown to impair the reproductive system of both males [2] and females [3], which can sometimes lead to infertility, alterations during embryo~~s~~ development or in the final reproductive outcome [3].

The aim of this study was to analyze the effects of an experimental Pb shot exposure at sublethal doses in red-legged partridge (*Alectoris rufa*) on sperm quality, egg laying process, egg quality, hatching and reproduction success, levels of dietary antioxidants and carotenoid-based coloration, as well as Pb effects on the body condition of the offspring at birth. We also studied the relationships between carotenoid-based coloration and sperm quality considering Pb toxicity, to better understand carotenoid allocation trade-offs.

2. Materials and methods

A total of 166 partridges were housed in pairs and randomly assigned to one of the three experimental groups: Control (no shot), low dose (1 shot), and high dose (3 shot). Shot were administered by gastric gavage during the non breeding season, and the exposure was repeated before the onset of egg laying in the breeding seeason. All eggs laid were collected daily, weighed, measured and incubated until hatching. Hatched chicks were also weighed and measured. We used a massage technique [4] to collect weekly semen samples to calculate sperm concentration, viability (Figure 1), acrosome integrity and parameters related to the quality and type of movement of spermatozoa. Blood Pb concentration and plasma levels of vitamins (retinol, α-tocopherol) and carotenoids (lutein and zeaxanthin) were analyzed after one month since Pb exposure. Carotenoid-based coloration was studied by beak and eye ring spectrophotometry before and after one month of Pb exposure.

Figure 1: Viability and acrosome integrity of spermatozoa.

Viability of ~~R~~red-legged partridge spermatozoa revealed by ~~stained with~~ nigrosine-eosine staining and acrosome integrity evidenced by aniline-blue staining ~~to reveal viability~~.

3. Results and discussion

Females exposed to 1 Pb shot laid larger (p=0.007) and heavier (p=0.002) eggs, and the same effect was observed in females exposed to 3 Pb shot (p=0.026 and; p=0.046, respectively). The laying of larger eggs has been associated with larger yolks and lipid stores in precocial species, which results in higher hatchling energy [5] and may imply a greater investment of carotenoids into eggs from the mother. In fact, chicks from females exposed to the 1 Pb shot presented greater mass at birth (p=0.017). On the contrary, larger and heavier eggs from females exposed to 3 Pb shot did not result in heavier chicks, and the hatching rate in this group was lower (62%) than in the control group (80.5%) (p=0.042). Furthermore, females mated with Pb exposed males tended to increase clutch size (p=0.053) and increased the ratio between chick mass at birth and egg mass (all p<0.001), reflecting an enhanced reproductive effort.

Males exposed to 3 Pb shot had sperm with lower percentages of motility (p=0.008) and acrosomal integrity (p=0.024) [2] (Figure 2). However, these effects were not associated with a reduced sperm viability or fertilization rate. Males exposedure to 1 Pb shot showed increased vigour of spermatozoa (all p<0.031) but not in progressiveness.

Pb exposed females had a reduced percentage of pigmented eye-ring area (all p<0.016). This reduced allocation of carotenoid to colour the eye rings may be explained by an increased carotenoid allocation to the egg yolk [6], which would be consistent with heavier eggs and chicks produced by these females. Pb exposed males showed greater levels of circulating carotenoids (all p<0.001) and no Pb effects on coloration were detected at the end of the breeding period. Moreover, we found positive relationships between sperm parameters and coloration that were also interrelated with antioxidant levels. Our results indicatesuggest that colourful males may have greater sperm motility (p=0.006) and velocity (all p<0.006) and this was associated with higher levels of tocopherol. In addition, proper antioxidant levels (e.g.: higher levels of retinol) seem wereto be related with higher sperm concentration and acrosomal integrity which might be related to a greater capacity to keep oxidative balance.

Con formato: Justificado

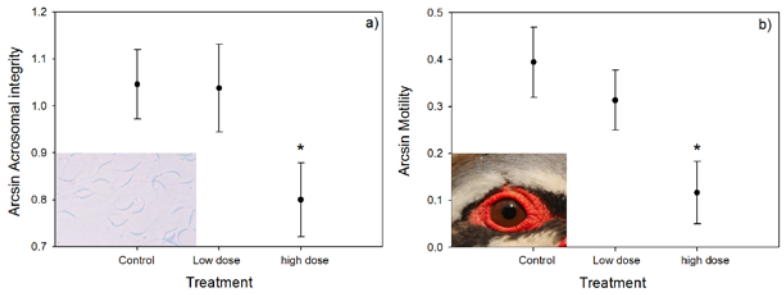

Figure 2: Effects of Pb on sperm quality.

Marginal means (± S.E.) of the percentage of sperm acrosomal integrity (a) and the percentage of sperm motility as a function of treatment group. Asterisks indicates significant differences with the control group. (LSD, p<0.05).

Con formato: Centrado

4. Conclusions

Overall, the sublethal Pb doses used here did not induce spermatozoon death or infertility in males, but rather caused an increase in reproductive investment. Pb exposed females also exhibited increased investment in reproduction, laying larger and heavier eggs and chicks, but had reduced carotenoid-based coloration and hatching rate. Several sperm parameters showed positive relationships with carotenoid-based coloration and levels of antioxidants, both that were influenced by Pb exposure, suggesting that redder males may be more capable to preserve sperm from oxidative stress.

5. References

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