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The effect of maternal immunization on female oxidative status, yolk antioxidants and offspring survival in a songbird

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ABSTRACT

Immune defense involves inflammatory reactions in which immune cells produce reactive oxygen species (ROS) to fight pathogens. ROS may however cause damage to the host if they are not balanced by antioxidant defenses. Therefore, one should expect individuals undergoing an immune reaction to use antioxidants to prevent oxidative stress. Antioxidants are vital compounds that provide important protection against oxidative damage of embryos and newly hatched chicks. Thus, during egg laying a female that contracted an infection may face a trade-off between the allocation of antioxidants into self-maintenance and into her offspring via the eggs. In our study we investigated whether immunized females face this trade-off and consequently modify the antioxidant allocation into the eggs and whether this allocation affects offspring performance. We injected female zebra finches (*Taeniopygia guttata*) with lipopolysaccharide prior to egg laying while some females were left unimmunized. We removed the second egg of each clutch, while we allowed the other eggs to hatch. We assessed oxidative stress in females 24 h after immunization, yolk antioxidant capacity of the second egg of the clutch and survival success of the offspring until adulthood. Compared to controls, immunized females had higher oxidative damage, but similar plasma non-enzymatic antioxidant levels. The treatment did not affect yolk antioxidants, clutch size, laying date and offspring survival. However, we found a positive correlation between yolk antioxidant capacity and offspring survival, irrespective of the treatment. Our study suggests that our immune challenge may not have changed female strategy of antioxidant allocation between self-maintenance and offspring survival.

1. Introduction

Animals are critically reliant on the immune system, which protects them against parasites and pathogens (Bonneaud et al., 2003). Reactive oxygen species (ROS) constitute an important part of an immune defense (Sorci and Faivre, 2009). ROS serve to kill pathogens, but their overproduction may cause oxidative damage to the host if they are not counterbalanced by the antioxidant defenses (Sorci and Faivre, 2009; Costantini and Møller, 2009). Oxidative stress results from a disturbance in the balance between pro-oxidants and antioxidants, leading to oxidative damage to proteins, lipids and nucleic acids (Halliwell and Gutteridge, 2007).

A central idea of the so-called immuno-oxidative ecology is that oxidative stress may provide a currency for quantifying the physiological costs of the immune activation, given the negative impact of oxidative stress on growth, reproduction and senescence (Hasselquist and Nilsson, 2012; Costantini, 2014). These costs may become particularly evident during demanding periods of an individual's life cycle, such as during the breeding season, when individuals have to tradeoff the investment in self-maintenance against that in reproduction. For example, Christe et al. (2012) found that increased parental effort reduces antioxidant protection and increases malaria infection in male great tits (*Parus major*). Egg production is another demanding reproductive activity, which may result in several costs to the laying female. Females transfer into the eggs substances (antibodies, hormones, lysozyme and antioxidants) that are vital for offspring development (Williams, 1994). Given that resources occur in limited supply, their transfer into the eggs would result in lower amount of resources available for the female to sustain her self-maintenance functions (Stearns, 1992). For example, females experimentally forced to produce extra eggs suffered more oxidative stress than females that were not forced to do so (Travers et al., 2010). The oxidative cost of egg production might, however, be dependent on the female's allocation strategy. If females prioritize their own antioxidant protection, an increase in ROS (e.g., induced by increased immune demands) during reproduction might lead to a reduced transfer of antioxidants into the eggs associated with stable or increased antioxidant defenses. However, if females prioritize reproductive investment, they may not alter their antioxidant allocation into the eggs, but show lower antioxidant defenses and suffer higher levels of oxidative damage. Antioxidants are not only important for female's

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antioxidant protection, but also for the offspring development and survival. In fact, it has been shown that carotenoids and other compounds with antioxidant properties (e.g., vitamin E) provide significant protection of embryos and newly hatched chicks against oxidative damage (Surai et al., 1996; Surai and Speake, 1998).

In this study, we tested the hypothesis that a short-term inflammatory process activated by an immunization prior to laying induces oxidative stress in females and, consequently, it influences the deposition of non-enzymatic antioxidants into the eggs. We also hypothesize that a differential antioxidant deposition into the eggs can affect offspring survival. Before egg-laying, female zebra finches (*Taeniopygia guttata*) were injected with lipopolysaccharide (LPS), which mimics a bacterial infection by stimulating the release of cytokines (Akira et al., 2006). LPS induces firstly an inflammatory response by activating the innate immune system (e.g., eosinophils, heterophils and macrophages; Fang et al., 2004; Xie et al., 2000). In birds the acute phase response generally appears within 3 h from the injection of LPS and lasts 24–48 h (Owen-Ashley et al., 2006; Burness et al., 2010; Lopes et al., 2012). Then LPS causes a humoral response with a peak of production of specific antibodies against LPS after 7 days from injection (De Boever et al., 2008; Parmentier et al., 2008). We investigated the effect of the immunization on female's plasma oxidative damage and non-enzymatic antioxidant capacity and the yolk lipophilic non-enzymatic antioxidant capacity. Finally, we assessed the survival of offspring until sexual maturity and whether the egg antioxidant capacity of the second egg of a given clutch predicted the survival of offspring from that clutch. We relied on the non-enzymatic components of the antioxidant defenses because it is the most important barrier against ROS in embryos and hatchlings (Surai and Speake, 1998; Surai, 2002). We predicted that immunization with LPS causes oxidative stress and reduces egg antioxidant capacity because female's antioxidants are depleted due to the immune challenge. We also predicted differences in survival between offspring of immunized mothers and control ones due to the expected differential allocation of antioxidants in the eggs in the experimental groups. Specifically, we predicted that survival probability is lower in the offspring of immunized mothers compared to those of the control group because they should hatch from eggs with lower antioxidant capacity. In statistical terms we expected to find an interaction between egg antioxidant capacity and experimental group.

2. Materials and methods

2.1. Experimental design

Zebra finches used in this study originated from the laboratory colony situated at the Institute of Environmental Sciences of the Jagiellonian University, Cracow, Poland. During the experiments the birds were kept in a climatized room at 20 ± 2 °C, under a 13:11 h incandescent light:dark photoperiod, lights on at 7 am. They were fed ad libitum with a standard mixture of seeds (Megan, Poland), along with a mixture of hard-boiled egg chopped with finely grated carrots. They also received grit and cuttlebone. Vitamins C, A, B1, B6, B12, D3, K (Ornitovit Kanarki, Dolfos, Poland) were added to the food once a week. We randomly paired 64 females with non-related males and we placed the couples into visually separated individual cages (75 × 30 cm and 40 cm high) equipped with external nest-boxes and provided with nest building materials.

Before the experiment the females were allowed to lay one complete clutch in order to gain breeding experience. Once all females had laid the first clutch, we removed their eggs and nests and immunized the females. 34 females were injected intraperitoneally with 100 μl of LPS (Lipopolysaccharide, Sigma Aldrich) suspended in saline (concentration = 1 mg/kg of body weight) and 30 females with 100 μl of saline. A sample of blood was taken from the brachial vein of each female using heparinized capillaries prior to and 24 h after the injection. The capillaries were centrifuged at 2000*g* for 15 min, and then the plasma was separated from the red blood cells and frozen at −70 °C. Because some females did not initiate new clutches after immunization (immunized: 6 out of 34; control: 7 out of 30, $\chi^2 = 0.76$ df = 1 P = 0.38), our final data set consist of 28 immunized and 23 control females.

All eggs were numbered with non-toxic pen just after laying. We removed the second egg from each clutch just after laying and froze it at −70 °C for later analyses of yolk non-enzymatic antioxidant capacity. Given the low variation in the content of single antioxidants among the first eggs in the laying sequence (Griffith et al., 2011; Pariser et al., 2012; Newbrey et al., 2015) or in the yolk antioxidant capacity of the whole clutch (Costantini, 2010; García-Tarrasón et al., 2014), we assumed the antioxidant capacity of the second egg to be representative of that of the whole clutch.

At expected hatching date we inspected the nests hourly during the day, while, during the night (from 8 pm to 8 am), we substituted the eggs with clay models and placed them in an incubator chamber (temperature 36.4 °C, humidity ~70%) to enable determination of which hatchling came from which egg. We returned the unhatched eggs or the chicks hatched during the night in the incubator chamber to the nest in the morning at 8 am.

We marked the nestlings with a non-toxic marker to identify them until they were ringed with an individually numbered aluminum ring at the age of 2 weeks. In order to disentangle pure maternal effects from parental care, half of the nestlings in a given clutch were attempted to be cross-fostered on the day of hatching within a pair of broods (one brood of control and one of immunized female) which started hatching on the same day and had similar clutch size (±1 egg). We matched the nestlings according to the position of the egg in the laying sequence. However, given the small number of nestlings that we were able to cross-fostered, we combined all experimental broods (cross-fostered and untouched) in the statistical analyses (see below). Offspring survival was followed until the third month of life when sexual maturity is achieved and the individual sex can be determined by plumage characteristics. Offspring lived with their parents until adulthood.

2.2. Assessment of female plasma oxidative status

We could not assess oxidative damage compounds and plasma non-enzymatic antioxidant capacity before and after immunization for all females because either the plasma sample was hemolyzed or it was too limited in the amount. Specifically, we could measure the oxidative damage compounds before and after immunization in the plasma of 15 immunized females and 14 control females. We assessed oxidative damage compounds using the d-ROMs test (Diacron International, Italy). This assay quantifies the plasma reactive oxygen metabolites (ROMs), primarily hydroperoxides. In this test the ROMs of the plasma samples, in presence of iron, generate the alkoxyl (R–O•) and alkylperoxyl (R–OO•) radicals that are highly reactive and able to propagate the oxidative cascade. These compounds react with an aromatic amine substituted, contained in the chromogen and oxidized it, changing the color of the mixture to pink. The intensity of the color is directly proportional to the plasma concentration of ROMs (Costantini and Dell'Omo, 2006). We pipetted 4 μl of plasma in a small Eppendorf tube, diluted with 200 μl of a solution containing 0.01 M acetic acid/sodium acetate buffer (pH 4.8) and N,N-diethyl-p-phenylenediamine as chromogen, and incubated for 75 min at 37 °C with mild shaking. Then we centrifuged it at 13,000 rpm for 2 min and pipetted 190 μl of the supernatant into a well of a microplate. We made a calibration curve by 1:1 serial dilution. We read the absorbance at 505 nm with a spectrophotometer (Tecan Infinite M200). Analyses were run in duplicate (repeatability r = 98.87% $F_{72,73} = 88.92$ P < 0.001) and the mean values were used for statistical analyses. The concentration of ROMs was calculated by comparison with a standard curve obtained by measuring the absorbance of a standard solution. The results of the d-ROMs test were expressed as mM of H_2O_2 equivalents.

We measured the plasma non-enzymatic antioxidant capacity before and after immunization in 16 immunized females and 14 control females. We measured the plasma non-enzymatic antioxidant capacity using the Oxy-Adsorbent test (Diacron International, Italy), according to the protocol in Costantini and Dell'Omo (2006). In this test the oxidant solution is put "in excess", compared to the adsorption ability of the sample. The residual HOCl reacts with an alkyl-substituted aromatic amine solubilized in the chromogen. That amine is oxidized and transformed into a pink derivate. The intensity of the colored complex is inversely related to the antioxidant capacity (Costantini and Dell'Omo, 2006). We incubated 2 μl of the diluted plasma with 200 μl of a titrated HOCl solution at 37 °C for 10 min. We used the same relative volumes for the reference standard and blank. We added 2 μl of a chromogen mixture at the end of the incubation and we read the absorbance at 490 nm with a spectrophotometer (Tecan Infinite M200). The samples were run in duplicate (repeatability $r = 85.28\%$ $F_{78,79} = 12.6$ $P < 0.001$) and the mean values were used for statistical analyses. Measurements are express as mM of HOCl neutralized, according to the following formula:

$$\frac{(\text{Blank Absorb.} - \text{Sample Absorb.})}{(\text{Blank Absorb.} - \text{Calibrator Absorb.})} * (\text{Calibrat. Concentration})$$

2.3. Assessment of yolk antioxidant capacity

We quantified the lipophilic antioxidant capacity of the yolk of 24 eggs of the immunized group and 23 eggs of the control group. Firstly, we extracted lipophilic antioxidants from the yolk. We mixed 50 mg of yolk with 0.1 ml 5% NaCl solution. Then we diluted it with 0.5 ml of acetone, vortexed for 2 min, sonicated for 10 min and finally centrifuged at 10,000 rpm for 2 min to extract antioxidants. We collected 100 μl of the acetone phase (where there are the yolk lipophilic antioxidants) and diluted it in 400 μl of acetone (Costantini, 2010). We measured the yolk antioxidant capacity using the Oxy-Adsorbent test (Diacron International, Italy). We used 2 μl of the diluted solution, obtained in the last step of the extraction of the lipophilic antioxidants from the yolk, and we followed the protocol already described for plasma samples. Analyses were run in duplicate (repeatability $r = 68.45\%$ $F_{46,47} = 5.34$ $P < 0.001$) and the mean values were used for statistical analyses. The yolk antioxidant capacity assessed in this way was expressed as mM of HOCl neutralized per mg of yolk. Then we also calculated the total yolk antioxidant capacity by multiplying the yolk antioxidant capacity per mg of yolk for the yolk mass (expressed in mg).

Before applying the above protocol we evaluated the extraction efficiency of the lipophilic antioxidants from the yolk. From each egg we took two aliquots of 50 mg of yolk and put them in separate Eppendorf tubes. We then added 6 μl of OXY standard to one tube and 6 μl of distilled water to the other tube. In a third tube, we added only 6 μl of OXY standard. Then we added 0.1 ml of 5% NaCl solution and later 0.5 ml of acetone to all 3 Eppendorf tubes. We then processed the samples as described above. The extraction efficiency was 92.04% ($N = 5$).

2.4. Statistical analyses

All variables were checked for normality before analyses. In all General Linear Mixed Models (GLMM) the experimental group was included as a fixed factor and female ID as a random effect. We used a General Linear Mixed Model (GLMM) with a repeated measures design to compare the measurements before and 24 h after the injection with LPS in order to detect the effects of the immunization on ROMs and OXY concentrations in female plasma. We included sampling time as fixed factor and the interaction between sampling time and experimental group. We used one-way ANOVA to compare the yolk antioxidant capacity of the second egg, the clutch size and the delay between immune challenge and clutch initiation between the two experimental groups. We also made a General Linear Model (GLM) to test if the treatment affected the covariation between the plasma non-enzymatic antioxidant capacity and yolk antioxidant capacity (either expressed as mg of yolk or total). In the model we included yolk antioxidant capacity as a dependent variable, plasma non-enzymatic antioxidant capacity as covariate, the experimental group as a fixed factor and the interaction between plasma non-enzymatic antioxidant capacity and experimental group.

To test if a differential allocation of yolk antioxidant into the eggs between the experimental groups would have affected the probability of offspring survival (wherever a nestling survived until adulthood = 1 or not = 0) we used GLIMMIX macro in SAS using a logit link function and a binomial error variance (Krackow and Tkadlec, 2001). Offspring sex and experimental group were included as fixed factors, egg antioxidant capacity (either expressed as mg of yolk or total) as covariate and female ID as random effects to avoid pseudoreplications (Hurlbert, 1984). We also tested the interaction between experimental group and egg antioxidant capacity (either expressed as mg of yolk or total). We were able to cross-foster a small number of nestlings (41 out of 153). Hence, in order to avoid loss in statistical power, we combined in the survival analyses both broods in which nestlings were either cross-fostered or were not cross-fostered. This choice was further supported by preliminary analyses which showed that outcomes were unchanged if a fixed factor indicating if the nestling was cross-fostered or was not was included. Moreover, we found no difference in survival between cross-fostered and unfostered nestlings ($\chi^2 = 1.06$ df = 1 $P = 0.3$). All statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC, USA) with a Satterthwaite approximation for the degrees of freedom and type III error.

3. Results

The treatment caused a significant change in the concentration of ROMs in the female plasma, as shown by the significant interaction between time and group (GLMM with repeated measures design, group $F_{1,27} = 1.22$ $P = 0.28$, time $F_{1,27} = 10.7$ $P = 0.003$, time × group $F_{1,27} = 5.71$ $P = 0.024$, Fig. 1). Separated analyses performed within groups showed that the concentration of ROMs increased among immunized females ($F_{1,14} = 14.21$ $P = 0.002$), but did not change among the control ones ($F_{1,13} = 0.46$ $P = 0.51$). The immunization did not affect female plasma non-enzymatic antioxidant capacity as indicated by the lack of interaction between time and group (GLMM with repeated measures design, group $F_{1,28} = 0.15$ $P = 0.71$, time $F_{1,28} = 1.38$ $P = 0.25$, time × group $F_{1,28} = 0.70$ $P = 0.41$, Fig. 1).

The treatment did not affect the clutch size (one-way ANOVA, $F_{1,49} = 0.18$, $P = 0.67$, mean ± S.E. immunized: 5.0 ± 0.2 range: 3–6 eggs, control: 4.8 ± 0.2 range: 3–6 eggs) and the delay between immune challenge and clutch initiation (one-way ANOVA, $F_{1,49} = 0.01$ $P = 0.93$, mean ± S.E. immunized: 6.9 ± 0.6 range: 3–14 days, control: 7.0 ± 0.7 range: 3–16 days). Moreover, the treatment had no effect on the yolk non-enzymatic antioxidant capacity per mg of yolk (one-way ANOVA, $F_{1,45} = 0.64$ $P = 0.43$) and the total yolk non-enzymatic antioxidant capacity (one-way ANOVA, $F_{1,45} < 0.001$ $P = 0.95$).

There was no effect of the treatment on the covariation between the plasma non-enzymatic antioxidant capacity and either the yolk non-enzymatic antioxidant capacity per mg of yolk (GLM, group $F_{1,26} = 2.56$ $P = 0.12$, female plasma non-enzymatic antioxidant capacity $F_{1,26} = 0.93$ $F = 0.34$, group × female plasma non-enzymatic antioxidant capacity $F_{1,26} = 2.53$ $P = 0.12$) or the total yolk non-enzymatic antioxidant capacity (GLM, group $F_{1,26} = 1.82$ $P = 0.18$, female plasma non-enzymatic antioxidant capacity $F_{1,26} = 0.34$ $P = 0.56$, group × female plasma non-enzymatic antioxidant capacity $F_{1,26} = 1.75$ $P = 0.19$). Outcomes of the models did not change when we centered the covariate, female plasma non-enzymatic antioxidant capacity, within groups (all P values ≥ 0.57). Concerning the survival of the offspring, we did not find a significant interaction between either yolk

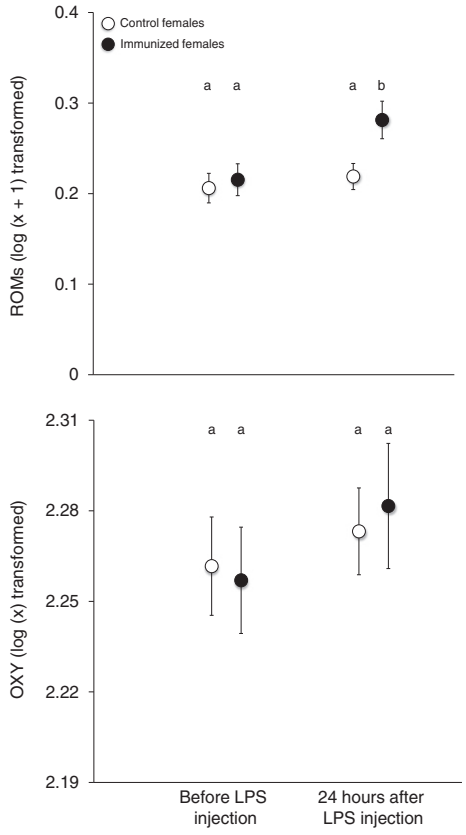

Fig. 1. Changes in plasma oxidative damage compounds (ROMs) and in plasma non-enzymatic antioxidant capacity (OXY) in immunized and control females. Values of ROMs are expressed as mM of H_2O_2 equivalents. Values of OXY are expressed as mM of HOCl neutralized. All values are shown as least square means ± S.E. Means sharing the same letter do not differ significantly.

non-enzymatic antioxidant capacity per mg of yolk or total yolk non-enzymatic antioxidant capacity and the experimental group (GLIMMIX, group × egg antioxidant capacity per mg of yolk, $F_{1, 37.8} = 0.09$ P = 0.76; group × egg total antioxidant capacity, $F_{1,31.1} = 0.07$ P = 0.79). In the same models analyzed without the interactions, we found that offspring survival did not differ between experimental groups (GLIMMIX, $F_{1,36.1} = 0.79$ P = 0.37), nor between males and females (GLIMMIX, $F_{1,138} = 0.33$ P = 0.56). However, we found a positive and significant covariation between the survival of offspring from a given clutch and the egg antioxidant capacity per mg of yolk of the second egg of that clutch (GLIMMIX, $F_{1,37.5} = 4.12$ P = 0.049, Fig. 2). The outcomes of GLIMMIX models did not change when we centered the covariate, egg antioxidant capacity, within groups (egg antioxidant capacity per mg of yolk P < 0.05; all the other P values ≥ 0.11).

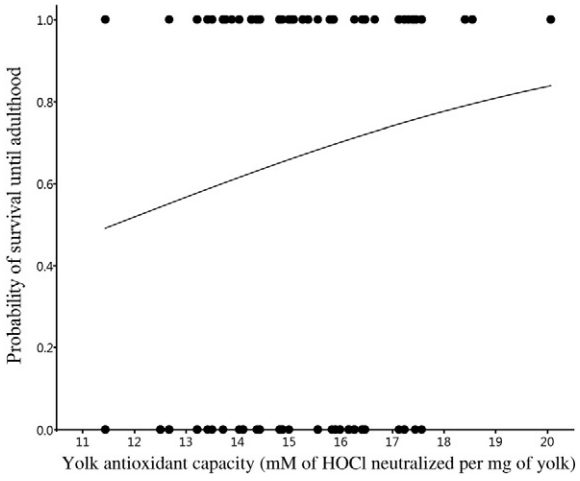

Fig. 2. Survival probability of nestlings until adulthood (wherever a nestling survived until adulthood = 1 and not = 0) in relation to yolk non-enzymatic antioxidant capacity of the second egg laid in a clutch. Values of yolk non-enzymatic antioxidant capacity are expressed as mM of HOCl neutralized per mg of yolk.

4. Discussion

Our study showed that immunization with LPS induced oxidative stress in females, but it did not affect plasma non-enzymatic antioxidant levels. Our treatment did not affect either the level of antioxidants in the eggs and did not significantly influence offspring survival until sexual maturity. We found a positive correlation between yolk antioxidants and offspring survival in both groups combined.

Increased oxidative damage in immunized females corroborates findings of previous studies on avian (e.g., Costantini and Dell'Omo, 2006; Casagrande et al., 2012) and non-avian (e.g., Sirak et al., 1991; Ferretti et al., 2005) species. We did not show any immunization effects on the level of plasma non-enzymatic antioxidants. In fact previous studies on the potential effect of immunization on plasma antioxidant levels are highly inconsistent. Some studies showed either a decrease (e.g. Costantini and Dell'Omo, 2006; Casagrande et al., 2012) or an increase (e.g. Marri and Richner, 2015) in the level of antioxidants, while others did not find any effect of the immunization on the antioxidant level in the plasma (e.g. Cohen et al., 2007; Schneeberger et al., 2013). The apparent stability of plasma non-enzymatic antioxidant defenses after a short-term immune challenge can be explained in different ways. It might be the result of the mobilization of antioxidants from tissues where they are stored, which could mask the depletion in circulating antioxidants. Alternatively, this may be explained by a time lag between an increase of ROS production and the corresponding effect on antioxidant levels: this potential time lag would result in damage to initially increase more rapidly than antioxidants, enabling detection only of an increase of oxidative damage compounds in the plasma (Zhou et al., 1999; Kankaanpaa et al., 2007). It is also possible that in fact our treatment might have negative effect on plasma non-enzymatic antioxidants, but the immunization causes such a short-term increase in ROS production that effects on antioxidants cannot be observed at the time we sampled birds. The level of antioxidants seems to be very flexible and it may quickly return to the level observed prior to the treatment (Teixeira et al., 2013; Xu et al., 2012). The oxidative burst constitutes a first line of immune defense (Sorci and Faivre, 2009), so a 24-hour time lag between immunization and sampling might be too long to observe any effect of immunization on antioxidant capacity. Finally, it might be that females during reproduction were limited in their antioxidant resources, hence they could not increase their antioxidant capacity in response to the immune challenge.

Since we expected a depletion of antioxidants due to immune defense, we also expected that immunized females should produce eggs with a lower concentration of non-enzymatic antioxidants. We did not confirm such a prediction as our treatment did not affect the level of yolk antioxidants. As explained in the previous paragraph it is possible that this may stem from the fact that females might have already restored the balance between pro-oxidants and antioxidants while laying eggs. Eggs were laid on average 7 days after the treatment, i.e., when the inflammatory process is already over and there is the peak of production of specific antibodies against LPS. As females were not facing a depletion of antioxidants, they could allocate to the eggs the same amount of antioxidants as that transferred by control mothers into their eggs.

Conversely to our prediction, the survival probability of the offspring did not differ between groups, possibly because there was no differential allocation of antioxidants into the eggs. We found, however, that the survival probability was significantly higher among offspring

originating from clutches with higher egg antioxidant capacity. This result was consistent in both groups as shown by the non-significant interaction between treatment group and egg antioxidant capacity.

Non-enzymatic antioxidants (e.g., carotenoids and vitamin E) have been shown to play a crucial role during embryo development and at hatching in the protection of the newly hatched chicks against oxidative damage (Surai et al., 1996; Surai and Speake, 1998; Surai, 2002). For example, experimental increase of yolk carotenoids through their supplementation to the mothers or direct injection into eggs showed that they can enhance fledging success (Marri and Richner, 2014; McGraw et al., 2005). In contrast to non-enzymatic antioxidants, protection against oxidative damage afforded by antioxidant enzymes becomes more important with time in the post-hatching growth period, when oxygen concentration in tissues, metabolic activity and production of free radicals increase and concentrations of dietary antioxidants decrease, respectively (Surai et al., 1999; Surai, 2002). Hence, the allocation of non-enzymatic antioxidants of females into their eggs is very relevant for the short-term survival perspectives of offspring. However, the positive effect of egg antioxidants on offspring survival might also be due to carry over effects on the adult antioxidant status. For example, low neonatal availability of dietary antioxidants resulted in a long-term impairment in the capacity to assimilate them, thereby setting up a need to trade off the requirement for antioxidant activity against the need to maintain morphological development and sexual attractiveness (Blount et al., 2003). Hence, *in ovo* exposure to high levels of antioxidants might shape a phenotype better able to assimilate dietary antioxidants in adulthood.

In conclusion, our study shows that a short-term immunostimulation of the female a few days before egg laying increased their oxidative stress but did not affect transfer of non-enzymatic antioxidants into the eggs. Our results also suggest that yolk antioxidants can have a long-lasting effect on offspring survival. It would be interesting in future studies to assess whether a chronic inflammatory process would have a stronger impact on female oxidative status, egg quality and offspring survival.

Competing interests

The authors declare no competing financial interests.

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