Comparative Biochemistry and Physiology, Part A 179 (2015) 192–196

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Immune response, oxidative stress and dietary antioxidants in great tit nestlings

Viviana Marri *, Heinz Richner

Evolutionary Ecology Lab, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

ARTICLE INFO

Article history:
Received 23 June 2014
Received in revised form 22 October 2014
Accepted 22 October 2014
Available online 28 October 2014

Keywords:
Antioxidant capacity
Immune challenge
LPS
Parus major
Reactive species
Vitamin E

ABSTRACT

The activation of immune defences counteracts pathogens, but mounting an immune response is costly and can negatively impact life-history traits. Immune activation releases highly reactive species that kill pathogens but can also cause oxidative damage to host tissues, and these negative effects may therefore constrain further investment in immune responses. To offset these toxic effects, animals rely on a complex system of antioxidants. Here, we tested if vitamin E, a dietary antioxidant, can reduce oxidative damage induced by an immune challenge and thus enhance the immune response. In a 2 × 2 experimental design, we supplemented great tit nestlings with either vitamin E or a placebo, and then injected them with either a bacterial lipopolysaccharide (LPS), or a buffer solution (PBS) as a control. LPS-treated nestlings mounted an inflammatory response and increased antioxidant capacity, without any change in ROM (reactive oxygen metabolites), an index of early oxidative damage. These results suggest that the likely transient increase in reactive species of the LPS injection was counteracted by a rise in endogenous antioxidant defences that was independent of supplementary dietary antioxidants. Indeed, vitamin E supplementation neither affected oxidative status nor enhanced the immune response, suggesting that in our experimental condition great tit nestlings were not limited in vitamin E and in antioxidants in general. Overall, our results show that birds can mount an effective antioxidant response to face an immune challenge, and can therefore avoid stress caused by a transient increase in reactive species generated by immune activation.

1. Introduction

The immune system has evolved to defend individuals against pathogens and parasites. However, mounting an immune response is energetically costly (Lochmiller and Deerenberg, 2000) and can negatively impact growth (Soler et al., 2003; Brommer, 2004; Romano et al., 2011), reproductive success (Bonneaud et al., 2003; Marzal et al., 2007; Chargé et al., 2010) and even survival (Moret and Schmid-Hempel, 2000; Hanssen et al., 2004). A trade-off between immunity and life-history traits seems common in birds, but the mechanism regulating it is less clear (Hasselquist and Nilsson, 2012). Costantini and Møller (2009) in a meta-analysis found that the induction of an immune response may cause oxidative stress in birds. Moreover, Hasselquist and Nilsson (2012) analysed the importance of different potential costs of immune responses, and suggested that oxidative stress could be a key factor mediating both short-term and long-term costs of immune system activation.

Oxidative stress arises when there is an imbalance between reactive species and antioxidants, in favour of the former (Sies, 1991). Reactive species are by-products of the metabolic activity that can cause damage to lipids, proteins and DNA (Finkel and Holbrook, 2000) and are also produced during an immune response. When vertebrates are attacked by pathogens, they first mount an innate non-specific immune response during which fluids, molecules and immune cells such as phagocytes are delivered into the infected tissue (Sorci and Faivre, 2009). Phagocytes release highly reactive species to kill pathogens (Swindle and Metcalfe, 2007). Reactive species are effective antimicrobial agents, but they do not discriminate between pathogens and host cells, and can therefore generate oxidative stress in the host tissues (Sorci and Faivre, 2009). To counteract the toxic effects of reactive species, organisms have a complex antioxidant system that includes enzymes, minerals and vitamins (Surai, 2002). Dietary antioxidants are compounds that cannot be synthetized by animals, and thus have to be acquired with the diet. Among these, vitamin E is a lipophilic antioxidant that scavenges reactive species and protects lipids from peroxidation, which is essential for the protection of cell membranes (Surai, 2002). Vitamin E is considered a powerful antioxidant, such that Traber and Atkinson (2007) in a review interpret all the effects of vitamin E in vivo as a result of its antioxidant properties. Since the activation of the immune system is increasing the production of reactive species, vitamin E could be involved in the immune response through its role as antioxidant. Beneficial effects of vitamin E on the immune system have been shown in experiments with farm and laboratory animals:

* Corresponding author. Tel.: +41 31 631 30 18; fax: +41 31 631 30 08.
E-mail address: viviana.marri@iee.unibe.ch (V. Marri).

http://dx.doi.org/10.1016/j.cbpa.2014.10.013
1095-6433/

vitamin E stimulated the non-specific immune system in gilthead seabream (*Sparus aurata*) (Ortuno et al., 2000), improved both humoral and cell-mediated immune response in quail (*Coturnix coturnix*) (Hooda et al., 2005), and increased secondary antibody titres against SRBC in Japanese quail (*Coturnix coturnix japonica*) (Senobar-Kalati et al., 2012). On the other hand, experiments with wild species are more rare and do not confirm the immuno-stimulatory role of vitamin E: vitamin E did not improve T-cell mediated immune response in barn swallow (*Hirundo rustica*) nestlings in the wild (de Ayala et al., 2006), or in male greenfinches (*Carduelis chloris*) kept in captivity (Hõrak et al., 2007). However, vitamin E increased antioxidant capacity in male great tits (Losdat et al., 2011) and could therefore be involved in the immune response in this species. To our knowledge only one study (Hõrak et al., 2007) investigated if vitamin E boosts the immune system through its role as an antioxidant, and found negative results.

Here, we thus investigate (1) whether the activation of the immune system increases reactive oxygen metabolites (ROM) that are early oxidative damage products (2) whether a pre-treatment with vitamin E can reduce ROM following immune activation, and (3) whether a pre-treatment with vitamin E can enhance immune responses in great tit (*Parus major*) nestlings. Using a 2 × 2 full-factorial design, we pre-treated whole broods of great tit nestlings with vitamin E and then we immune challenged them with LPS (lipopolysaccharide from the cell membrane of *Escherichia coli*). We measured the effects of the experimental treatments on the swelling response to LPS, body temperature, antioxidant capacity, ROM, and body mass. We expected the immune response to generate oxidative stress, and vitamin E to alleviate immune-induced oxidative stress. If antioxidant availability limits the investment into the immune response, we expected vitamin E supplemented birds to show a higher response to LPS treatment.

2. Materials and methods

The experiment was performed in spring 2013 in a natural population of great tits, breeding in nest-boxes in Könizbergwald, a forest near Bern, Switzerland (46°56′ N, 7°24′ E). Nest-boxes were monitored daily from early April onwards to determine laying and hatching dates. The experiment consisted of a 2 × 2 full factorial design; whole broods were randomly assigned to four groups: vitamin supplemented (N = 36), immune challenged (N = 38), vitamin supplemented and immune challenged (N = 35), control (N = 37). Some nests were excluded from the experiment because whole broods died, therefore the nests used for the analyses were respectively: 35, 31, 33, and 34.

2.1. Vitamin E treatment

We aimed to double the daily amount of vitamin E that nestlings naturally acquire from food between days 6 and 10 post-hatch, the period of most intense growth (Gosler, 1993), as previous study showed that doubling the amount of ingested antioxidants had positive effects on different traits in great tit (Helfenstein et al., 2008; Losdat et al., 2011; Marri and Richner, 2014). We therefore calculated the daily food intake (DFI) for great tit nestlings, according to Crocker et al. (2002) and taking into account that altricial developing birds need additional food to allocate to growth (de Ayala et al., 2006). We then multiplied the DFI by the concentration of vitamin E in caterpillar, the main source of food for great tit nestlings (Gosler, 1993), to obtain an estimated daily vitamin E intake (for more details of the method, see Marri and Richner, 2014). We supplemented nestlings with one larva of *Calliphora* spp. either unmanipulated or coated with vitamin E (α-tocopherol acetate, Sigma-Aldrich, Basel, Switzerland) on days 6, 8, and 10 after hatching (Fig. 1). We therefore pre-treated nestlings, as the supplementation with vitamin E occurred before the immune challenge. Since vitamin E is a lipophilic antioxidant and can be stored in the liver and mobilized when needed

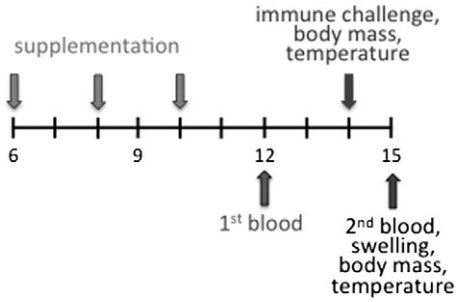

Fig. 1. Timeline of the experiment.

(Elsayed, 2001), the pre-treatment is expected to influence the response to the immune challenge.

2.2. Immune challenge treatment

Immune-challenged nestlings were injected subcutaneously in the right wing with 0.01 mg of LPS (lipopolysaccharide from the cell membrane of *E. coli*) dissolved in 0.02 ml of PBS (phosphate buffered saline) on day 14 post-hatch. Control nestlings were injected with the same quantity of PBS. LPS mimics a bacterial infection by promoting the release of cytokines and by inducing an inflammatory response at the injection site (Dunn and Wang, 1995). LPS can provoke mass loss (Bonneaud et al., 2003) and fever (Maloney and Gray, 1998). To assess the ability to rise an inflammatory response, we measured the wing web thickness before and 24 h after the injection at the inoculation site with a constant-tension dial micrometer (Mitotuyo, Type 2046FB-60, Tokyo, Japan) to the nearest 0.01 mm, a greater swelling reflecting a better inflammatory response (Parmentier et al., 1998). We also measured the skin temperature on the stomach using an auricular thermometer (ThermoScan, Type 6022, Braun, Lausanne, Switzerland) before and after the injection to assess the potential triggering of a fever response that may enhance immunological functions (Ostberg and Repasky, 2006). We decided to measure the skin temperature as it was shown to be highly correlated with the rectal temperature in great tit nestlings (Berthouly et al., 2008). Finally, we measured the difference in body mass before and 24 h after the injection to assess mass loss. Nestlings below 13.5 g were excluded from the immune challenge treatment since a LPS treatment could potentially kill them. Since the number of excluded nestlings is low compared to the injected ones (66 nestlings out of 919) and since the proportion of small nestlings excluded did not differ according to the treatments ($\chi^2 = 0.94$, P = 0.33), it is unlikely that excluding small nestlings could have biased our results.

2.3. Morphological measurement

The body mass of 14 and 15 days post-hatch nestlings was measured with an electronic balance (±0.1 g). Twelve days post-hatch we took a blood sample (30 μl) from the metatarsus vein. A drop of this sample was stored in ethanol 96% until later analyses to determine sex (see Griffiths et al., 1998 for the sexing technique), and the rest was used to analyse oxidative stress.

2.4. Oxidative stress analyses

To measure oxidative stress we took a blood sample from the brachial vein (30 μl) on day 12, two days after the supplementation with vitamin E stopped and 15 post-hatch, one day after the LPS injection, as one day was proven to be enough to register a change in oxidative damage markers (Costantini and Dell'Omo, 2006). We kept blood samples cool in an ice box until centrifugation in the evening, and then stored them at −80 °C. We assessed antioxidant capacity and ROM using the OXY-