Environmental Pollution 229 (2017) 448–458

Contents lists available at ScienceDirect

Environmental Pollution

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

Oxidative status in relation to metal pollution and calcium availability in pied flycatcher nestlings – A calcium manipulation experiment☆

Silvia Espín [a, b, *], Sandra Ruiz [a], Pablo Sánchez-Virosta [a, b], Thomas Lilley [a, c], Tapio Eeva [a]

[a] Department of Biology, University of Turku, 20014 Turku, Finland
[b] Area of Toxicology, Department of Socio-Sanitary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain
[c] Evolution, Ecology and Behaviour, Institute of Integrative Biology, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom

ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form
25 April 2017
Accepted 31 May 2017
Available online 30 June 2017

Keywords:
Antioxidant defense
Oxidative stress
Calcium
Fledging success
Passerine

ABSTRACT

Metal exposure can produce oxidative stress by disrupting the prooxidant/antioxidant balance. It has been suggested that calcium (Ca) may provide protection against metal toxicity in the organism. The objective of this study is to explore the effects of Ca availability and metal pollution on oxidative stress biomarkers in pied flycatcher (*Ficedula hypoleuca*) nestlings. For this purpose, we performed a Ca-supplementation experiment with birds inhabiting a Ca-poor and metal-polluted area in SW Finland. An array of oxidative stress biomarkers (GSH, GSH:GSSG ratio, GPx, GST, CAT, SOD, lipid peroxidation and protein carbonylation) was measured in red blood cells. The effects of antioxidant molecules and oxidative damage on nestling size, growth, fledging success and fledgling number were evaluated. We observed an up-regulation of GST activity and increased protein carbonyl content in the polluted zone, probably related to a combination of higher metal exposure and reduced food quantity and quality in this area. As expected, birds from the unpolluted zone showed higher GSH:GSSG ratio but, unexpectedly, also showed signs of higher lipid peroxidation (not statistically significant, $p = 0.056$), both responses likely being related with the lower Ca availability. Our study suggests that different measures of oxidative damage are affected by different factors: while damage to proteins was the target of metal exposure/food limitation, poor Ca availability may enhance damage to lipids in growing birds. The intercorrelations found between Ca in plasma, metal exposure and the different oxidative stress biomarkers show that the antioxidant defense is finely regulated to cope with increased oxidative challenges. Finally, our results suggest that the antioxidant status during early development, conditioned by environmental pollution and Ca availability, is one factor affecting nestling survival and fledgling number.

1. Introduction

Calcium (Ca) is an essential nutrient involved in diverse roles in birds because it is needed for eggshell formation and bone growth and it participates in many biochemical reactions (Reynolds et al., 2004; Stanford, 2006). Passerine birds need to consume Ca-rich nutrition such as snail shells in addition to their normal diet containing insufficient Ca (Graveland and van Gijzen, 1994). When the availability of Ca-rich material is poor, birds may produce smaller clutches with reduced egg size and eggshell thickness, as well as reduced number of fledglings (reviewed by Reynolds et al., 2004). This may occur especially in acidified or polluted environments because soil acidification and metal pollution may reduce the availability of food rich in Ca (Eeva et al., 2010; Graveland, 1996). Furthermore, dietary Ca deficiency in birds can increase the absorption of metals such as cadmium (Cd) and lead (Pb) (Dauwe et al., 2006; Scheuhammer, 1996), and these metals may alter the internal balance and function of Ca in the organism (Pounds, 1984; Suzuki et al., 2004, 1985).

Redox-active (e.g. copper, Cu and zinc, Zn) and redox-inactive (e.g. Pb, Cd, arsenic, As and mercury, Hg) elements can produce oxidative stress by disrupting the prooxidant/antioxidant balance (Koivula and Eeva, 2010). In general, these metals can generate reactive oxygen species (ROS) and may deplete the major antioxidants of cells (i.e. glutathione, GSH) and alter the levels of

☆ This paper has been recommended for acceptance by editor Dr. J. Rinklebe.

* Corresponding author. Area of Toxicology, Department of Socio-Sanitary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain.
E-mail addresses: silvia.espin@um.es, sieslu@utu.fi (S. Espín).

http://dx.doi.org/10.1016/j.envpol.2017.05.094
0269-7491/

antioxidant enzymes, such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). These changes may lead to oxidative damage in membrane lipids, proteins and DNA (Ercal et al., 2001; Koivula and Eeva, 2010; Sánchez-Virosta et al., 2015). Consequently, cellular damage caused by metal-induced oxidative stress is one of the defining mechanisms of metal toxicity (Ercal et al., 2001; Koivula and Eeva, 2010; Sánchez-Virosta et al., 2015). During the past decade, the number of studies evaluating the effects of metals on the oxidative status in wild birds has increased considerably (Berglund et al., 2007, 2014, Espín et al., 2014a, 2014b, 2016a; Koivula et al., 2011; Martínez-Haro et al., 2011; Rainio et al., 2013, 2015; Stauffer et al., 2017). Experimental evidence suggests that the toxicity of Pb and Cd is influenced by other nutritionally required elements such as Ca, which may reverse or provide protection against metal effects in the organism (Prasanthi et al., 2005; Snoeijs et al., 2005). Previous research suggests that Ca administration in mice and fish may have a protective effect against metal toxicity by decreasing the gastrointestinal absorption of metals but also by modulating metal-induced oxidative stress (Jamakala and Rani, 2012; Prasanthi et al., 2010). However, corresponding experiments are lacking for wild birds.

The objective of this study is to explore the effects of Ca availability and metal pollution on oxidative stress biomarkers in chicks of an insectivorous passerine, the pied flycatcher (*Ficedula hypoleuca*). For this purpose, we performed a Ca-supplementation experiment in 2014 with pied flycatchers inhabiting a Ca-poor and metal-polluted area in SW Finland (Espín et al., 2016b). Because there are different antioxidants involved in protection against ROS and antioxidant activities are linked to each other, we measure a variety of antioxidant concentrations and activities (GSH, GSH:GSSG ratio, GPx, GST, CAT and SOD) and biomarkers of oxidative damage (lipid peroxidation as thiobarbituric acid reactive substances, TBARS and protein carbonylation, PCO) in red blood cells. In addition, we evaluate the effects of antioxidant molecules and oxidative damage on nestling size, growth, survival and number of fledglings in order to better understand the role of the antioxidant status on nestling development. To the best of our knowledge, only two experimental studies have been carried out relating Ca deficiency and metal pollution in wild passerine populations (Eeva, 1996; Espín et al., 2016b), and the effects on growth and biochemistry (Espín et al., 2016b), eggshell characteristics and yolk vitamin and carotenoid levels (Espín et al., 2016c), and vitamins in plasma (Ruiz et al., 2017) have been reported in different publications. However, this is the first experimental study evaluating the potential protective effect of Ca on metal-induced oxidative stress in free-living birds. On the basis of the background information available, we expect (i) increased oxidative stress in pied flycathers living in the metal-polluted zone compared to the control zone, (ii) decreased oxidative damage in calcium supplemented birds in the polluted zone, and (iii) significant effects of oxidative stress biomarkers on growth and survival suggesting that the antioxidant status during early development may compromise breeding parameters.

2. Material and methods

2.1. Experimental set-up

The Ca-supplementation experiment was conducted during the breeding season 2014 in the surroundings of a copper-nickel (Cu-Ni) smelter in Harjavalta (61°20′ N, 22°10′ E), southwestern Finland. Metal concentrations (especially As, Cd, Cu, Ni, Pb and Zn) are elevated in the vicinity of the smelter (hereafter called polluted zone) due to current and long-term deposition. A more detailed description of metal contamination and Ca availability in the study area and the Ca experiment performed is provided by Espín et al. (2016b). The study was carried out on populations of great tit (*Parus major*, scope of a different publication) and pied flycatcher using nest boxes (see Lambrechts et al., 2010) situated along the pollution gradient, divided into a polluted (0–4 km from the smelter) and unpolluted zone (4.1–11 km from the smelter). Old nest material was removed before the start of nest building and nest boxes were checked in mid-April and then periodically to monitor the progress in the nest building and to record the laying date, clutch size, hatching date, brood size, and number of fledglings. When new nests in an advanced building stage were found, they were assigned in turn either to the Ca-supplemented group or to the control group. Consequently, feeders (small cylindrical plastic cups) with 5 g of crushed mussel shells (Versele Laga) were placed inside the experimental nest boxes. Empty feeders were also placed in control nest boxes. The feeders were regularly checked and refilled when needed, so there was always a source of Ca material (*ad libitum* supplementation). The remaining supplement was weighed and replaced at the beginning of the incubation period and just after hatching. At the age of 12 days (hereafter d12) the feeder was removed and the leftover Ca was weighed. Ca consumption during the laying, incubation and chick rearing periods was calculated with these measurements (provided in Espín et al., 2016b). In total, we set 35 Ca-supplemented nests (17 in polluted and 18 in unpolluted zone) and 30 control nests (15 in polluted and 15 in unpolluted zone) of pied flycatcher. The experiment was conducted under licenses from the Animal Experiment Committee of the State Provincial Office of Southern Finland (license number ESAVI/1650/04.10.03/2012) and the Centre for Economic Development, Transport and the Environment, ELY Centre Southwest Finland (license number VARELY/319/07.01/2014).

2.2. Sampling and measurements

One egg from each clutch was collected in order to evaluate the effect of the Ca supplementation in egg parameters and yolk vitamin and carotenoid levels (published in Espín et al., 2016c). On d7 after hatching, birds were ringed and combined feces of several nestlings from the same brood were collected and conserved at −20 °C for metal analysis. On d7 and on d12 post-hatching, pied flycatcher nestlings were weighed, and their maximum wing chord length, minimum tarsus length (Svensson, 1992) and total head length were measured as described in Espín et al. (2016b). Breeding, size and growth parameters are reported in Espín et al. (2016b). Blood samples (approximately 75 μl) were collected on d7 and d12 by venipuncture of the brachial vein with a needle and using sodium-heparinized microhematocrit capillary tubes (80 iu/ml, Marienfeld). Tubes were centrifuged in the field (4400 g, 5 min) and plasma and red blood cells (RBC) were split in different tubes and kept in liquid nitrogen and then conserved at −80 °C in the laboratory. Plasma collected at d7 was pooled per brood (N = 61 broods) for vitamin analysis (Ruiz et al., 2017), while plasma collected on d12 from 92 individuals (normally two randomly selected chicks per brood, N = 47 broods) was used to measure the plasma constituents Ca and uric acid, and the enzyme activities of alkaline phosphatase (ALP; Enzyme Commission number, EC 3.1.3.1) and creatine kinase (CK; EC 2.7.3.2) as described and reported by Espín et al. (2016b). RBC collected at d12 from 91 pied flycatcher nestlings (N = 47 broods) were used to measure oxidative stress biomarkers in the present study.

2.3. Analysis of metals and oxidative stress biomarkers

Brood level fecal samples (N = 65) from the 7-day-old nestlings