Chemosphere 151 (2016) 189–201

Contents lists available at ScienceDirect

Chemosphere

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

Effects of experimental calcium availability and anthropogenic metal pollution on eggshell characteristics and yolk carotenoid and vitamin levels in two passerine birds

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HIGHLIGHTS

- Metal pollution affected maternal lutein and vitamin D3 allocation to yolk.
- Metal pollution did not affect the egg volume, eggshell index and pigmentation.
- Both species were capable to obtain sufficient calcium for eggshell formation.
- Nestling growth and size was influenced by carotenoids and vitamin D3 in yolk.
- The two species differ in their carotenoid and vitamin D3 investment into egg yolks.

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form
14 February 2016
Accepted 16 February 2016
Available online 15 March 2016

Handling Editor: A. Gies

Keywords:
Eggshell pigmentation
Maternal effects
Lutein
Cholecalciferol
Retinol

ABSTRACT

The maternal investment into egg quality depends on the condition of the female, the quality of the mate, and the quality of the environment. In that sense, availability of nutrients and exposure to pollutants are essential parameters to consider. The main aim of this study is to assess the effects of calcium (Ca) availability and anthropogenic metal pollution on early-stage reproduction in two passerine species, great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*), inhabiting a Ca-poor and metal-polluted area in SW Finland. Both species were able to obtain sufficient Ca for eggshell formation, and metal pollution was below the level of having negative effects in the egg size and eggshell characteristics. However, metal polluted environment negatively affected yolk lutein and vitamin D3 levels in both species, probably because of a lower access to carotenoid-rich diet and higher metal interference with vitamin D3 metabolism. The higher levels of vitamin D3 in yolks in the unpolluted zone could also be due to upregulated D3 levels as a response to the lower natural Ca availability. Yolk carotenoids and vitamin D3 were positively associated with nestling growth and size, supporting their importance for the appropriate chick development. The interspecific differences in yolk nutrient concentrations possibly reflect the different growth rate of these species. Pied flycatchers are likely adapted to low Ca availability through an efficient vitamin D3 metabolism, but their Ca intake could be close to a deficient level.

1. Introduction

Early life conditions affect physiology, morphology, health and behavior in birds (Ahmed et al., 2014; Anisman et al., 1998; Desai et al., 1995; Zimmer et al., 2013). Females may change prenatal environment by transferring varying amounts of essential resources into their eggs such as calcium (Ca), vitamins and carotenoids, and thus influence offspring quality (see e.g. Hõrak et al., 2002; Saino et al., 2002). The importance of Ca availability for

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http://dx.doi.org/10.1016/j.chemosphere.2016.02.074
0045-6535/

successful breeding in passerine birds has been widely reported (Graveland et al., 1994; Graveland and van der Wal, 1996; Reynolds, 2001; Reynolds and Perrins, 2010). This macronutrient is required for shell formation during egg laying since up to 98% of the dry mass of eggshell consists of calcium carbonate (Reynolds et al., 2004). The eggshell provides protection and is a source of Ca for the embryo skeletal growth and calcification (Reynolds et al., 2004; Reynolds and Perrins, 2010). Vitamin D3 (cholecalciferol) is required to effectively use Ca (NRC, 1994), and its active metabolite 1,25-dihydroxyvitamin D3 (1,25OHD3) is essential for the transport of Ca from the eggshell to the embryo (Elaroussi et al., 1994; Narbaitz, 1987). Thus, both Ca and vitamin D3 levels in females and their transfer to the egg during oogenesis are of utmost importance for an optimum eggshell quality and embryo development. Furthermore, carotenoids and vitamin A (retinol) are important dietary micronutrients transferred from maternal circulation and deposited in the egg yolk. Carotenoids function as pigments in feathers, precursors of vitamin A, antioxidants, they are involved in cell differentiation and proliferation, and play various roles in the endocrine and immune systems (Eeva et al., 2008; Møller et al., 2000; Surai et al., 2001a,b). Vitamin A is also essential during early stages of embryogenesis for the initiation of organogenesis (Zile, 2004, 2001). In eggs, carotenoids and vitamin A are involved in regulation of embryonic development by way of their antioxidant properties, crucial due to the fast oxidative metabolism in growing embryos associated with oxidative stress (Gaál et al., 1995; Hargitai et al., 2006; Surai, 2002).

The maternal investment into egg quality depends on the condition of the female at breeding and on the quality of the mate, but also on the quality of the environment. In that sense, Ca availability and metal pollution are essential parameters to consider. Small passerine birds cannot store enough Ca in their body for successful reproduction, so they consume Ca-rich foods such as snail shells in addition to their normal food (Graveland, 1996; Graveland and Drent, 1997; Mänd et al., 2000; Tilgar et al., 1999). Ca provisioning experiments have shown that tits consumed more Ca during the egg-laying and nestling period, and less during incubation (Espín et al., 2016; Graveland and Drent, 1997), which supports the special Ca requirement for eggshell formation and skeletal growth (Reynolds et al., 2004; Starck, 1998). Therefore, Ca-poor areas where exchangeable Ca in the soil and snail abundance are depressed, may pose a problem to their reproduction (see Reynolds et al., 2004). Evidence of Ca-limited reproduction in birds has been observed mainly as thin-shelled eggs, and reduced egg and clutch size; thus, egg properties have been preferred as response variables in Ca-provisioning studies (reviewed in Reynolds et al., 2004). Vitamin D deficiency may also cause decreased egg production and egg weight as well as eggshell thinning, and in growing birds it may derive in hypocalcemia, which affects skeletal development (Macwhirter, 1994). Another parameter related to Ca limitation is the eggshell pigmentation. Great tits (*Parus major*), as some other small passerines, lay white eggs speckled with brown protoporphyrin pigment spots (eggshell maculation) (Kennedy and Vevers, 1976; Wegmann et al., 2015). Different authors have evidenced that the eggshell pigmentation increases with decreasing Ca availability and is related to eggshell thickness (Gosler et al., 2005; Higham and Gosler, 2006). Recent findings support the so-called structural-function hypothesis (Hargitai et al., 2013), which argues that protoporphyrin pigments are used to compensate for localized shell-thinning, strengthening it when Ca is scarce (Gosler et al., 2005; Solomon, 1997).

On the other hand, metal-polluted environments represent an additional challenge for passerines, since pollution may affect snail abundance (Eeva et al., 2010b). Along with this, Ca deficiency in the diet is known to increase the absorption and accumulation of harmful metals, such as lead (Pb) and cadmium (Cd), in birds (Dauwe et al., 2006; Scheuhammer, 1996). Furthermore, these metals may alter the homeostasis and function of Ca (Pounds, 1984; Suzuki et al., 1985) and interfere with normal vitamin D3 metabolism by blocking renal synthesis of 1,25OHD3 (Edelstein et al., 1984; Moon, 1994; Smith et al., 1981). Exposure to metals is also known to cause oxidative stress (Koivula and Eeva, 2010; Sánchez-Virosta et al., 2015). Therefore, metals may interfere with levels of antioxidants in birds inhabiting polluted areas, as has been found for vitamin A and carotenoids in birds naturally or experimentally exposed to certain contaminants (Fernie et al., 2005; Ortiz-Santaliestra et al., 2015). Moreover, the secondary environmental effects of metal pollution on food quality are known to affect levels of dietary nutrients such as carotenoids in plasma of birds (Eeva et al., 2009, 2005), although the effects on yolk components have been scarcely evaluated (Hargitai et al., 2016). Since the concentrations of vitamins and carotenoids in the egg yolk depend on the maternal intake and transfer of these components (Bortolotti et al., 2003; Gaál et al., 1995; Griminger, 1966; Hargitai et al., 2006; Karadas et al., 2005; Mattila et al., 2004; Stevens and Blair, 1985), metal exposure and Ca deficiency in laying females could interfere in the yolk concentrations of these compounds, which could compromise hatching success and chick development.

The main aim of this study is to assess the effects of Ca availability and anthropogenic metal exposure on early-stage reproduction in two passerine species inhabiting a Ca-poor and metal-polluted area. For this purpose, we experimentally manipulated the availability of Ca and evaluated different parameters that could be sensitive to Ca deficiency and/or metal effects such as hatching probability, egg size and eggshell characteristics (eggshell index and pigmentation pattern), and vitamin (D3 and A) and carotenoid levels in yolk. Since bird species may differ in their Ca requirement and metal sensitivity (Eeva and Lehikoinen, 2010), differences between great tits and pied flycatchers (*Ficedula hypoleuca*) are also evaluated. Furthermore, we will explore the effects of yolk nutrients (vitamins and carotenoid content) on hatching probability, nestling size and growth, and fledging success.

Since Ca is strongly involved in the first stage of breeding and is closely related to vitamin D, we expect to find a negative effect of metal pollution on eggshell characteristics and yolk vitamin D content, and a positive effect of Ca supplementation on eggshell parameters. Based on previous findings showing higher plasma carotenoid (lutein) levels and plumage carotenoid chroma in great tit nestlings in the unpolluted area than in the polluted area (Eeva et al., 2009), we hypothesize that eggs of the polluted area will contain less carotenoids and vitamin A. Finally, since some studies have shown that yolk micronutrient levels may affect the nestling growth and fledging success (e.g. Marri and Richner, 2014; McGraw et al., 2005), we expect to find an effect of yolk vitamins and carotenoid content on these nestling traits. Although the importance of evaluating Ca availability in conjunction with environmental pollution has been highlighted by different researchers (Graveland, 1995; Poulin and Brigham, 2001; Reynolds, 2001; Scheuhammer, 1991), to the best of our knowledge only two experimental studies have been done relating Ca deficiency and metal pollution in wild passerine populations (Eeva, 1996; Espín et al., 2016).

2. Material and methods

2.1. Ca-provisioning experiment

The feeding experiment with Ca 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