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Levels of antioxidants in breeding female Audouin's gulls and their deposition in eggs across different environments

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ABSTRACT

Diet quality and dietary antioxidants are interrelated factors that influence many animal traits. However, little is known about this relationship in wild birds, especially how it may affect the deposition of antioxidants from the female to the eggs over the laying sequence. In this context, albumen has been far less studied than yolk due to its lack of carotenoids, which are a common focus of dietary antioxidant research. Our study was conducted in the Audouin's Gull (*Larus audouinii*), a seabird that shows a great dependence on trawl discards, although in the Ebro Delta also exploits resources from neighbouring rice fields, especially the American crayfish (*Procambarus clarkii*). We examined the relationship between diet (traced through stable isotope analysis) and the antioxidant capacity (not only carotenoids, but also other non-enzymatic antioxidants, like tocopherols and retinol) of plasma in females from two breeding groups and their clutches. In the eggs we analysed antioxidants in both albumen (hydrophilic antioxidants) and yolk (hydrophilic and lipophilic antioxidants) fractions, taking into account the laying sequence. A decrease in the antioxidant capacity of female plasma was found over the incubation period. We found little difference between groups in the antioxidant deposition pattern in the yolk and whole egg over the laying sequence, but a greater variation was observed in the intra-clutch patterns of albumen antioxidant capacity, probably related to quality differences. When taking into account total antioxidant deposition in the clutch, a declining tendency over the laying sequence was found. Both yolk lipophilic and whole egg antioxidant capacity were negatively related to $\delta^{15}N$, indicating that marine fish diets (with depleted $\delta^{15}N$ values) contain a higher amount of antioxidants than rice field prey.

1. Introduction

Diet quality and dietary antioxidants are interrelated factors that influence many life-history traits of animals (reviewed in Catoni et al., 2008). Indeed, the diet is a source of compounds with antioxidant properties that animals can only acquire from food because they are unable to synthesise them de novo (Cohen et al., 2009). Dietary antioxidants may help protect the organism from oxidative stress arising from oxidative damage to biomolecules (Costantini and Verhulst, 2009; Halliwell and Gutteridge, 2007; Sies, 1991). This is a very important function because accumulation of such damage is thought to contribute to cellular senescence, loss of organ performance and Darwinian fitness (Costantini, 2008; Kirkwood, 2005). However, the antioxidant machinery is particularly complex and, beyond dietary antioxidants, includes many important endogenous molecules, like antioxidant enzymes or repair systems (Pamplona and Costantini, 2011).

Dietary antioxidants can be classified as hydrophilic (e.g. vitamin C and polyphenols) and lipophilic (e.g. vitamin E and carotenoids), according to their chemistry. The mechanisms of action among different classes of antioxidants vary, but their ultimate function is the same, i.e. to neutralise the damaging action of free radicals (Pamplona and Costantini, 2011). In addition, the diet contributes other substances that do not have antioxidant properties but that affect oxidative balance. For example, the polyunsaturated fatty acids in food are also important because they are substrates for lipid peroxidation (Hulbert, 2005).

The antioxidant content of the diet also influences traits other than oxidative stress, including sexual ornamentation, fertility and fecundity, and brood growth (Biswas et al., 2007; Blount et al., 2002; de Ayala et al., 2006; McGraw, 2006). Dietary antioxidants are therefore considered important for reproduction, which represents one of the most demanding periods of animal life (Nager, 2006; Speakman, 2008), as well as for the growth and survival of progeny (Mousseau and Fox, 1998). Since these antioxidants are present in limited amounts, their deposition in the egg may reduce the capacity of the laying female to cope

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with reactive oxygen species, giving rise to trade-offs between self-maintenance and reproductive investment (Costantini, 2010a). Knowing how diet quality relates to the circulating non-enzymatic antioxidant levels in the breeding female and the patterns of allocation over the laying sequence will provide a better insight into how this trade-off influences life-history strategies.

Only a few experimental studies have looked at the link between diet and circulating/deposited antioxidants in wild birds (e.g. Beaulieu et al., 2010; Hipfner et al., 2010a,b). For example, it has been shown that lower-trophic-level feeding is linked to the production of carotenoid-rich yolks (Hipfner et al., 2010a,b). However, recent studies have shown that carotenoids offer limited protection against oxidative stress (Costantini and Møller, 2008; Isaksson and Andersson, 2008; Simons et al., 2012), highlighting the importance of looking at other egg dietary antioxidants as well. In eggs the deposition of antioxidants in albumen has received very little attention compared to their deposition in yolk (Blount et al., 2002, 2004), despite the important role of albumen in hatchling size and offspring development, behaviour and viability (Bonisoli Alquati et al., 2007; Ferrari et al., 2006). Therefore, there is an important need to study the relationships between diet quality and non-enzymatic antioxidant capacity (not only carotenoids) in breeding females and the albumen and yolk fractions of eggs.

To address these issues, we carried out a field study on breeding Audouin's Gulls (*Larus audouinii*), using stable isotope analysis (carbon and nitrogen isotopes) as an integrative tool for tracing the diet of gulls in the wild (Inger and Bearhop, 2008; Kelly, 2000).

The Audouin's Gull is a medium-sized migratory species categorised as "Near Threatened" at a global level by the International Union for Conservation of Nature (IUCN) (BirdLife International, 2012). About two thirds of the world's Audouin's Gull breeding population nest in the Ebro Delta Natural Park, NE Spain (Arcos et al., 2009). Although originally described as a nocturnal predator of pelagic fish, especially clupeids (BWPi, 2004), the species shows a great dependence on human trawling activity in the study area, scavenging around fishing vessels and using discards very efficiently (Oro and Ruiz, 1997; Oro et al., 1996a; Ruiz et al., 1996). However, Audouin's Gulls in the Ebro Delta also exploit resources from neighbouring rice fields, where their main prey is the American crayfish (*Procambarus clarkii*). This invasive species seems to become a more important food resource for gulls during trawling moratorium periods (Oro et al., 1996b, 1997). The isotopic characterisation of the different habitats in the Ebro Delta has been extensively documented, and it is well known that rice field prey present enriched δ^{15}N signatures and depleted δ^{13}C values compared to marine prey (Cotin et al., 2011; García-Tarrasón et al., 2013).

Considering all the above, our main objectives were: (1) to find out the relevance of the main foraging habitats exploited by this species – marine or rice field – (Navarro et al., 2010) on the circulating antioxidant capacity of breeding females (measured in the plasma of incubating females) and their clutch (measured in the albumen and yolk of a three-egg clutch), and (2) to test the laying order effect on the antioxidant deposition, which could indicate a trade-off between antioxidant availability for females and their eggs. In fact, the laying order has been shown to be a very important factor in avian species with a brood reduction strategy such as gulls (Lack, 1954; Ramírez et al., 2011; Rubolini et al., 2011). We hypothesized that (1) the natural feeding resource (marine fish) would be better than the introduced American crayfish, and (2) an important laying order effect would be detected in the egg antioxidant deposition because of a limited availability of antioxidant molecules.

2. Material and methods

2.1. Study area and sampling

The study was carried out during the 2010 breeding season at the Punta de la Banya (40° 40′N, 0° 45′E), a protected sandy peninsula with salt pans in the Ebro Delta Natural Park (NE Spain). Audouin's Gulls nest there in discontinuous groups in natural coastal dunes with vegetation and in salt pans subject to human activity. Two breeding groups (2.5 km apart) of similar age distribution (from plastic ring readings) were selected for comparison: one in the dunes (DG) and another in the salt pans (SG). Besides habitat, they differed markedly in parameters such as the number of pairs, population density and reproductive success (see Supplementary material, Table S1).

During the peak egg-laying period (late April), the nests were tagged when the first egg was laid. Inspections were conducted every two days until clutch completion to establish laying order and the final clutch size. Ten three-egg clutches were selected from each group. Freshly laid eggs were sampled and replaced with dummy eggs. Eggs were marked, placed under refrigeration and transported to the laboratory. Twelve females from these nests (six from each group) were captured with nest traps during the incubation period and measured (weight, tarsus length and skull length — the distance from the supraoccipital to the bill tip). Blood samples (2 mL) were also collected from the brachial vein; 0.5 mL was preserved in a neutral vial for molecular identification of sex and the rest was placed in heparinised vials. Blood samples were kept at +4 °C until centrifugation. Sex of gulls was determined using polymerase chain reaction (PCR) amplification of the CHD genes (Ellegren, 1996; Griffiths et al., 1998).

2.2. Ethical note

This study was authorized by the Autonomous Government of Catalonia (*Departament de Medi Ambient i Habitatge — Generalitat de Catalunya*), as well as by the wildlife personnel of the Ebro Delta Natural Park. Both agreed with the protocol of handling and sampling of Audouin's gulls.

2.3. Laboratory procedures

Eggs were opened and the albumen was separated from the yolk. Each fraction was weighed, homogenised and divided into subsamples prior to storage. Albumen subsamples were frozen at −20 °C and yolk subsamples at −80 °C.

The heparinised vials were centrifuged (2000 rpm for 10 min) within 6 h of collection. The supernatant plasma was pipetted off and stored in 250-μl aliquots at −80 °C.

2.4. Stable isotope analysis

Albumen, yolk and plasma samples were lyophilised to dryness and ground to powder. In the case of yolk, samples were lipid-extracted following the Folch method (Folch et al., 1957) to remove lipid influence on carbon isotope ratios. Subsamples (0.3 mg plasma or 0.35–0.38 mg albumen and yolk) were placed in tin capsules for stable carbon and nitrogen isotope ratio determination. Isotopic analyses were carried out at the Scientific and Technical Services of the Universitat de Barcelona (Spain) by means of a Thermo-Finnigan Flash 1112 elemental analyser (CE Elantech, Lakewood, NJ, USA) coupled to a Delta-C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany), with IAEA standards being applied every 12 samples to calibrate the system. Stable isotope ratios were expressed in the standard δ notation relative to Vienna Pee Dee Belemnite (δ^{13}C) and atmospheric N_2 (δ^{15}N). Replicate assays of standards indicated analytical measurement errors of ±0.1‰ and ±0.2‰ for δ^{13}C and δ^{15}N, respectively.

2.5. Assays of oxidative damage and non-enzymatic antioxidant capacity

Analyses were performed at the Institute of Biodiversity, Animal Health and Comparative Medicine at the University of Glasgow (UK).