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Influences of carotenoid supplementation on the integrated antioxidant system of a free living endangered passerine, the hihi (*Notiomystis cincta*)

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Abstract

The integrated antioxidant system is recognised as an essential component of an organisms self maintenance. Our knowledge of this system, however, is largely restricted to species of economic importance. The health and productivity benefits these dietary based compounds provide make them increasingly relevant for study in wildlife ecology. The aim of this research was to identify numerous components of this integrated system in a free living and endangered passerine bird, the hihi. In addition experimental supplementation with carotenoids was used to investigate the modulatory interactions with other members of the antioxidant system. Our results identified lutein and zeaxanthin as the carotenoids utilised by hihi (82% and 17% of total carotenoids respectively in control samples of egg yolk, 84% and 16% of total carotenoids respectively in control samples of nestling plasma), and that vitamin E was represented by both α- and γ-tocopherol. Retinol was also present, as was selenium in surprisingly high concentrations (599.64, 91.76, 377.72 ng/g fresh weight Se in control samples of yolk, albumin and plasma, respectively). Supplementation of lutein and zeaxanthin not only increased their presence in egg yolk ($F_{1,10}$=14.285, P=0.005 and $F_{1,10}$=9.606, P=0.015, respectively) and nestling plasma ($F_{1,19}$=35.126, P<0.001 and $F_{1,19}$=28.597, P<0001, respectively) but also led to increased selenium concentration in egg yolk ($F_{1,10}$=7.213, P=0.028), increased retinol concentration in nestling plasma ($F_{1,19}$=4.272, P=0.054) and decreased α-tocopherol concentration in nestling plasma ($F_{1,19}$=5.122, P=0.037). These results provide detail of the antioxidant system in novel taxa and importantly highlight interaction between these various compounds. Given their increased application in productivity and health in agriculture and human medicine we highlight the potential application of this knowledge in wildlife ecology and conservation.

Keywords: Antioxidant; Carotenoids; Conservation; Egg yolk; Plasma; Retinol; Selenium; Vitamin E

1. Introduction

All living organisms are under constant attack from free radicals. In particular, superoxide is considered the major free radical stress produced by living cells (Halliwell and Gutteridge, 1999). Its production comes as the unavoidable by-product of metabolism, and as part of the immune systems defence against foreign micro-organisms (Schwarz, 1996; Surai, 2002). To survive individuals need protection from these, and other, radical species. Radical species are known to damage lipids, proteins and DNA and, as a consequence, affect membrane composition and structure, enzymatic activities and cause mutagenesis. As such a delicate balance is required between the sometimes necessary presence of radicals and a protective system to control them. The integrated antioxidant system is the collective term used to describe the varied compounds involved in cell protection. This system includes the fat soluble antioxidants (including vitamins A and E, coenzyme Q and carotenoids), water soluble antioxidants (including ascorbic acid, uric acid), antioxidant enzymes (including glutathione peroxidase (GSH-Px), superoxide dismutase and catalase) and the thiol redox system (including both the glutathione and thioredoxin systems) (Surai, 2002).

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Although each antioxidant has specific roles in this integrated system there is also a level of interaction and modulation between these different compounds. For example, carotenoids are known to be precursors of vitamin A, being converted to retinoids in the intestinal wall (Cheng and Deuel, 1950). However, less than 10% of known carotenoids are converted into vitamin A and the role of more than 500 additional carotenoids awaits investigation (Surai, 2002). Interactions between carotenoids and vitamin E are not clear yet. Numerous studies have shown positive, negative, and no effects of carotenoids on vitamin E levels (discussed in Surai, 2002). Further, synergistic interactions have also been shown. For example, providing lycopene (a red carotenoid) to female rats increased the activities of antioxidant enzymes such as GSH-Px (Breinholt et al., 2000). These interactions depend somewhat on which compounds are utilised within each antioxidant family. Each species has its own combination of these compounds, governed by a mix of dietary availability and phylogeny (e.g., in carotenoids see Tella et al., 2004), making it difficult to determine the optimal antioxidant balance of any given taxa.

Our knowledge is largely restricted to model laboratory species (such as rats and mice), humans and domestic animals. Very little information on antioxidant systems is available in non-domestic and free living wild species. This is somewhat surprising given the growing interest in the fitness advantages antioxidants accrue to individuals (e.g., see Alonso-Alvarez et al., 2004; Blount et al., 2004, 2003; McGraw and Ardia, 2003). Carotenoids in particular are the focus of extensive ecological and evolutionary research due primarily to their dual function in self maintenance and as pigments in animal ornamentation (Moller et al., 2000). The clear health advantages and dietary basis of carotenoids has also resulted in an application to measurements of environmental stress (Eeva et al., 1998; Horak et al., 2000). Unfortunately, most research in this area does not consider the interactions of carotenoids with other antioxidants despite the obvious potential to extend our knowledge of integrated antioxidant systems to varied taxa. In addition, there is obvious potential to apply this knowledge, along with lessons learnt in domestic animal production, to address questions in wildlife health.

The aims of our study were therefore twofold. Firstly, to document the concentrations of various antioxidants in a free living but endangered bird species, and secondly to investigate the effects of supplementing carotenoids on other components of their integrated antioxidant system. We fed breeding hihi (*Notiomystis cincta*) carotenoids known to be utilised by this species. Our first question was to determine whether carotenoids supplemented to breeding females were deposited into egg yolk and whether this, in addition to direct provisioning from parents, resulted in higher carotenoid concentration circulating in nestling plasma. Secondly, we wanted to investigate the effects of this increased carotenoid availability on various additional antioxidants in both egg and plasma samples. Our focus was on the fat soluble antioxidants, vitamins A and E, and Selenium (Se) which is an important component of the antioxidant enzyme glutathione peroxidase (GSH-Px).

2. Materials and methods

The hihi (or stitchbird, *Notiomystis cincta*) is an endangered passerine endemic to New Zealand whose diet consists of nectar, fruit and invertebrates. Although once widespread throughout the North Island of New Zealand the species suffered a rapid decline following European colonisation and by 1890 had become restricted to a single offshore island population (3083 ha. Hauturu). Conservation of this species relies on reintroducing birds to additional island and mainland habitats and has met with limited success (Taylor et al., 2005). One hypothesis for this poor success is pathogen stress on immunocompromised individuals (Alley et al., 1999; Taylor et al., 2005). This study was conducted on Tiritiri Matangi Island, the single reintroduced population (established in 1995) which shows positive population growth.

Previous research has identified lutein and zeaxanthin as the carotenoids circulating in adult hihi plasma, and used as pigments for the males bright plumage (82% and 14%, respectively, Ewen et al. unpublished data). We therefore provisioned these carotenoids in the form of a commercially available product OroGLO® supplied by Kemin Industries. OroGLO® comprises 82.69% *trans*-lutein and 6.07% *trans*-zeaxanthin which provides the approximate ratio of these carotenoids as found in hihi plasma. Hihi on Tiritiri Matangi are provisioned with sugar water (20% by mass) for management and we took advantage of this to conduct our carotenoid supplementation feeding experiment. All breeding pairs were individually provided with supplementary food (placed within 10 m of the nest) from the commencement of nest building until nestlings fledged (at age 30 days). Only nests with simple pairs (i.e., one social male and one female parent) were included in the study. Female age did not vary between treatment groups ($F_{1,46}=2.73$, $P=0.11$) and no obvious health differences were detectable between females in this population. In half these nests carotenoids were added to sugar water to a final concentration of 100 μg/mL following results from immune activation and antioxidant activity to variable dose rates of OroGLO® in the zebra finch (Alonso-Alvarez et al., 2004). Carotenoids were provided in brown plastic bottles to avoid light and all feeders were changed every second day to keep food fresh. A sample of nests was observed for one hour each to determine if supplementary food was taken. There was no significant difference in the use of these feeders between treatment groups (carotenoid $N=11$, mean female visits/hour $4.5=SE=0.45$; sugar $N=7$, mean female visits/hour $3.7=SE=0.57$; $F_{1,16}=1.04$, $P=0.32$). Nests were checked daily following the commencement of egg hatching and all un-hatched eggs were collected three days after their expected hatch date (14 days after incubation starts). Any unfertilised eggs were separated into their yolk and albumen components and stored frozen until antioxidant analyses. Surviving nestlings had blood samples collected at 24 days old. Blood was immediately centrifuged and plasma removed and similarly stored frozen until antioxidant analysis.

Carotenoids and vitamins A and E were determined by high performance liquid chromatography (HPLC) as described