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S16-5 Carotenoid biochemistry, transformation and function

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Abstract Antioxidants (carotenoids, vitamin A and vitamin E) play important roles by eliminating free radicals. These biochemicals are often rare and their availability is limited because of their rate of turnover. Signals such as bright gape color in nestling birds may provide reliable information about the quality of offspring because bright color derives from carotenoids that are used in competition. Hosts defend themselves against parasites by raising immune responses that often depend on rapid cell proliferation, or excessive production of biochemicals, which in turn generate free radicals. Supplementation with carotenoids can increase the strength of immune responses, suggesting that availability of carotenoids can affect host response to parasite attack. Maternal effects are common in birds because of substances deposited in the eggs by females; several studies have shown that quality of coloration in female plumage and integumentary structures directly reflects the quality of carotenoid content in their eggs.

Key words Antioxidants, Immunity, Life history, Maternal effects, Migration, Yolk

1 Introduction

Energy is traditionally considered to be the singlemost important currency in the activities of living organisms. This view in biology has predominated in recent decades, favoring optimality solutions to biological problems as the research approach. Although biological processes, by definition, are fuelled by energy, this does not necessarily mean that energy is the limiting resource. We suggest that many biological phenomena involving biochemical processes may be better understood if the production of free radicals, and the role of carotenoids and other antioxidants in eliminating such free radicals, are considered.

All physical activity gives rise to production of free radicals that can cause damage to molecules (Leffler, 1993). Free radicals are atoms or molecules with unpaired electrons, and they arise as by-products of normal metabolism and rapid cell proliferation during immune function. DNA, proteins and lipids, including molecules used in immune defence, can be damaged by free radicals. If not eliminated, changes in membrane properties (fluidity, flexibility) and functions (intracellular signaling, enzymatic activities) caused by oxidative stress can result in reduced immunocompetence (Chew, 1996). The negative effects of free radicals can be neutralized by antioxidants, such as carotenoids and vitamins A and E (Surai, 1999; Møller et al., 2000).

Production of free radicals and antioxidant defences are usually balanced under normal physiological conditions, but this balance can be disrupted by stress in favor of free radical production, thereby creating oxidative stress (Sies, 1997). Oxidative stress arises from insufficiency of antioxidants in diet, inadequate dietary proteins and synthesis of metal-binding proteins, excess production of free radicals from metabolism of toxic compounds, and excessive activation of radical-producing natural systems such as phagocytosis during inflammation (Halliwell, 1996).

Here we propose that antioxidants may play an important role affecting the evolution of many phenomena in avian biology, even impacting on phenotypic variation within populations. In this overview, we first describe carotenoids and their biochemistry. Then we review examples of the importance of carotenoids in determining the expression of signals, modulating immune responses, mediating maternal effects, and affecting optimal bird migration.

2 Carotenoid biochemistry and function

More than 600 different carotenoids are grouped into xanthophylls and carotenes based on their molecular structure, whether oxygenated or aliphatic chains. Carotenoids can only be synthesized by algae, bacteria, fungi and plants (Fox, 1979; Latscha, 1990; Stradi, 1998), and, therefore, animals must ingest carotenoids for use in physiological processes or display. Animals differ in their rate of carotenoid absorption (Scheidt, 1989; Blount et al., 2002a), in their ability to convert ingested carotenoids (Fox, 1979; Stradi, 1998), and in their rate of carotenoid deposition in signal colors (Brush and Power, 1976). Birds preferentially accumulate xanthophylls, particularly lutein, zeaxanthin and canthaxanthin, and carotenes less readily, e.g., β-carotene (Brush, 1981; Stradi, 1998; Blount et al., 2002a).

Many carotenoids have been found in the feathers of birds, some ingested and others derived from oxidative transformation. β-carotene, for example, may be converted into echinenone, and sometimes canthaxanthin or 3-hydroxy-echinenone, adonirubin and astaxanthin (Stradi, 1998). Furthermore, β-carotene can be converted into vitamin A (Damron et al., 1984; Schaffer et al., 1988), and vitamin A also has antioxidant activity. Xanthophylls are more widely distributed, plentiful and diverse than carotenes (Latscha, 1990). Thus, xanthophylls in circulation and storage seem more likely to be of direct dietary origin than sourced from transformation of carotenes. Occurrence of xanthophyll in a signal color might indicate availability of donor xanthophyll or transformed carotene, and hence reflect allocation to signals.

Carotenoids play important roles in immuno-regulation and immuno-stimulation in vertebrates (Bendich, 1989). β-carotene and other carotenoids enhance T- and B-lymphocyte proliferation, stimulate effector T-cell function, enhance macrophage and cytotoxic T-cell capacities, increase the populations of specific types of lymphocyte, and stimulate the production of cytokines and interleukins (Bendich, 1989). Cockerels fed β-carotene and canthaxanthin produce significantly higher antibody titres against Newcastle disease virus than controls (McWhinney et al., 1989); and experimentally increased dietary intake of β-carotene and vitamin E increase immunity against Escherichia coli (Tengerdy et al., 1990). Laying hens fed supplemental carotenoids transfer these to chicks via egg yolk, and produce chicks with enhanced spleen and bursal lymphocyte function *in vitro* (Haq et al., 1996). Thus, carotenoids affect different immune functions directly.

Carotenoids also have detoxification effects that are related to their effects on immune function. With other antioxidants, they act as free radical scavengers and protectors of biologically important molecules, serving as free radical traps and efficient quenchers of singlet oxygen (Ames, 1983). They effect a decrease in immuno-suppressive peroxides, and maintain membrane receptors that are essential for immune function. Immune cells use free radicals to kill bacteria and other infections by stimulating free radical production by macrophages (Chapple, 1997). Therefore, enhanced immune cell activity can overproduce free radicals and here antioxidant protection is vital (Gille and Sigler, 1995).

3 Specific examples of carotenoid function

3.1 Signaling

Communication is based on signals. Reliability of signals is generally ensured by an absence of conflict of interest between sender and receiver, or, more frequently, by the cost of the signals. More expensive and effective signals can be produced only by signalers of high quality because of costs, ensuring that cheating only occurs rarely. Many visual sexual signals are based on carotenoids, reliably reflecting the amounts of carotenoids ingested (e.g., Hill, 1992). Such signals provide accurate information about the condition of individuals because use of carotenoids for signals competes with use for immune function or free radical scavenging (reviews in von Schantz et al., 1999; Møller et al., 2000).

Begging signals provide reliable information about the condition of offspring, and parents may use such information to allocate limited resources to those most viable (Saino and Møller, 2002). Nestling barn swallows (*Hirundo rustica*) with more brightly colored gapes receive more food than their siblings (Saino et al., 2000). Experimental challenge to their immune system with an antigen reduces gape coloration (Saino et al., 2000). Such a reduction in the level of signaling can be reversed by lutein supplements, causing the nestlings to regain bright gape color (Saino et al., 2000). A subsequent study revealed that nestlings with bright gape colors indeed had superior condition and stronger immune responses than their siblings (Saino et al., 2003). Thus, nestling signals are mediated by carotenoids and reflect offspring condition reliably.

3.2 Host-parasite interactions

Carotenoids have immuno-stimulating and immuno-modulating effects. These effects act through stimulation of immune responses, elimination of free radicals produced during immune responses, and elimination of free radicals after they have been used to combat pathogens susceptible to free radicals. There are, however, relatively few examples of host immune function being affected by carotenoids in free living birds. One example for nestling barn swallows has been described in the previous section. Adult barn swallows have red plumage color on the head, partly dependent on lutein. Males with brightly colored plumage are more healthy than pale males which have lower leukocyte counts and circulating levels of immunoglobulins (Saino et al., 1999). Chicks of moorhens (*Gallinula chloropus*) have brightly colored feathers and skin on the head, and parent moorhens use intensity in chick coloration to allocate parental effort. Moorhen chicks provisioned with extra lutein had a stronger T-cell dependent immune response than controls (Fenoglio et al., 2002). Thus, carotenoids can boost immune responses in free-living birds.

3.3 Maternal effects

Female birds invest in progeny by transferring antioxidants to their eggs. Since the hatching process causes oxidative stress (Surai, 1999), antioxidant protection is vital. Carotenoid and vitamin E concentrations in embryonic tissues reach maximal levels at hatching (Surai et al., 1996), providing maximal antioxidant protection to tissues with high levels of polyunsaturated fatty acids (Speake et al., 1998); such tissues are vulnerable to free radical attack (Surai, 1999). Since carotenoids are not accumulated in the body to any extent (Surai and Speake, 1998), there must be a premium on the capacity of a female to transfer carotenoids to egg yolk. Maternally-derived carotenoids in yolk reduce susceptibility to oxidative stress (Surai and Speake, 1998), and probably preserve passively-acquired maternal antibody from oxidation in embryos and chicks (Blount et al., 2000).

Oxidative stress arising from the production of free radicals is likely to be greatest in rapidly growing embryos because of their high levels of oxidative metabolism (Vleck and Bucher, 1998). Antioxidants transferred to eggs or synthesized by the developing embryo (ascorbic acid, reduced glutathione or antioxidant enzymes) build an effective antioxidant system responsible for maintaining protection against the damaging effects of free radicals (Surai, 1999). Thus carotenoids play a role in the control and destruction of free radicals towards limiting oxidative stress in growing chicks.

A comparative study of eggs from more than 50 different species of birds has shown that concentration of antioxidants varies by more than an order of magnitude among species, and that species-specific differences in yolk carotenoids are statistically highly significant (J.D. Blount et al., unpublished data). Lesser black-backed gulls provided with extra carotenoids before egg formation increased the intensity of yellow coloration of legs, beak and eye ring (Blount et al., 2002b). This increase in female coloration was directly reflected in the quality of eggs in terms of carotenoid concentration and growth performance of young (Blount et al., 2002b; J.D. Blount unpublished results). In great tits (*Parus major*) breeding in two different habitats of differing quality, females were more brightly yellow in the rich habitat (Hõrak et al., 2002). More brightly yellow females also laid eggs with higher concentrations of antioxidants independent of habitat, and nestlings hatched from such eggs grew better than the nestlings of paler females. Thus, female color in these two very different species reflects the amount of carotenoids put into eggs.

Carotenoid deposition in yolk by female barn swallows is affected by the trade-off between self-maintenance and allocation to offspring (Saino et al., 2002). A challenge to the immune system of females before start of laying reduced yolk carotenoids. This result demonstrates that females trade their own use of carotenoids against allocation to eggs. Moreover, female allocation of carotenoids to eggs was found to be related, through experimental manipulation, to the size of a secondary sexual character in their mates. Male barn swallows with longer tails are more attractive to females, and males with such tails have few parasites and strong immune responses (Møller, 1994).

Offspring also resemble parental males with respect to parasite load, suggesting that levels of parasitism have a genetic basis (Møller, 1994). Thus, the young of short-tailed males may have more activated immune systems than those of long-tailed males. Tail length manipulation of males significantly affected the concentration of carotenoids in yolk, since females allocated more carotenoids to eggs when their mates had their tails shortened (Saino et al., 2003). This is in accord with predictions because offspring derived from such eggs are likely to suffer more from parasitism than other offspring; and therefore they have a highly activated immune system that is dependent on egg carotenoids for balance.

4 Future prospects

Carotenoids and other antioxidants have important consequences for birds, as has been illustrated by studies of signaling, host-parasite interactions, and maternal effects. Two particular areas need more research effort. First, there needs to be much more integration of research in energetics, free radicals and antioxidants. Only when we know how levels of activity translate into production of free radicals and use of antioxidants will we begin to understand the magnitude of stores required, from the rate of acquisition of antioxidants compared with their rate of use. Secondly, a broad understanding of carotenoid availability, use and function in relation to ecology of different species is needed. Such comparative analyses could explain the determinants of levels of circulating and stored carotenoids, and potentially their impacts on reproductive strategies and life-history.

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