No Effect of Carotenoid Supplementation on Phytohemagglutinin Response or Body Condition of Nestling House Wrens

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ABSTRACT

Carotenoids are an essential and often limiting resource in animals and play important roles in immune system function. In birds, the period shortly after hatching is an energetically demanding stage characterized by rapid growth in body size and organ systems, including the immune system. Availability of carotenoids for the growing nestlings may be of particular importance and potentially limiting at this stage of development. We tested the hypothesis that the availability of carotenoids for the embryo in the egg and in the diet of nestlings limits the condition and immune responses of nestling house wrens (*Troglodytes aedon* Vieillot 1809), a species with melanin-based plumage pigments. In one experiment, nestlings within females' second broods were randomly assigned to receive either a control or a lutein supplement (2008); in a second experiment, females, before their first broods, were either induced to lay additional eggs or not induced, and nestlings within both kinds of broods were supplemented as in the first experiment (2009). There were no significant effects of lutein supplementation on nestling condition or phytohemagglutinin response. There was a significant effect of lutein supplementation on nestling mass in 2008, but the difference was opposite to that predicted. Moreover, even when breeding females were stressed by inducing them to lay supernumerary eggs, lutein supplementation of nestlings had no effect on the size or condition of nestlings hatching from these eggs. These results suggest that maternally derived lutein in the egg and that provided in the diet of nestlings are not limiting to normal development and to the components of the immune system involved in the phytohemagglutinin response of nestling house wrens.

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Introduction

Carotenoids are a class of naturally occurring, lipid-soluble compounds that play a variety of roles in animals, including scavenging of free radicals, pigmentation of the integument, and stimulation and modulation of the immune system (Møller et al. 2000; Blount et al. 2003; McGraw and Ardia 2003; McGraw et al. 2006). The more than 600 carotenoid compounds are classified into two groups, carotenes (aliphatic) and xanthophylls (oxygenated; Armstrong and Hearst 1996). There is evidence in birds that xanthophylls can enhance immune function by increasing cutaneous immune activity (McGraw and Ardia 2003; Saino et al. 2003) and constitutive innate immunity (McGraw et al. 2006). They also serve as antioxidants (Krinsky 2001; McGraw 2005), scavenging free radicals and helping to protect cells and tissues from oxidative damage. Because birds are unable to produce their own carotenes and xanthophylls and must obtain them from their diet, carotenoids are often considered limiting in both adults and nestlings (Hill 1991; Møller et al. 2000). It is known that female birds store carotenoids and differentially allocate them to their eggs during laying (Koutsos et al. 2003; Helfenstein et al. 2008), suggesting that maternally derived carotenoids are important resources used to enhance egg quality (Blount et al. 2002). Evidence that egg quality is enhanced by increasing carotenoid levels comes from an experiment in which eggs injected with lutein produced nestlings with an increased T cell immune response (Saino et al. 2003). In growing chickens (*Gallus domesticus*), lutein incorporation in immune tissues is modified by maternal carotenoid status (Koutsos et al. 2003). Thus, maternally derived carotenoids may play an important role in the development and expression of nestling immune function.

Despite the well-documented importance of carotenoids in influencing the immunoresponsiveness of adult birds (reviewed in Blount 2004; McGraw et al. 2006; but see Navara and Hill 2003), there is surprisingly little information on how dietary carotenoids influence the condition and immune responsiveness of growing nestling birds. It is at just this time during their ontogeny, however, that individuals are first exposed to an antigen-rich environment, and their rapid structural growth may require them to divert resources from the development and maintenance of their immune system to other components of growth.

Those studies that have investigated the effects of dietary supplements of carotenoids on rapidly growing altricial nestlings have produced conflicting results, with dietary supplements of xanthophylls producing an increased cutaneous immune response in mountain bluebirds (*Sialia currucoides*; O'Brien and Dawson 2008) on the one hand and no response

in great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*; Biard et al. 2006; Fitze et al. 2007) on the other. One possible explanation for such conflicting results may lie in the differences among species in the amount of carotenoid-based pigments incorporated in their integument (O'Brien and Dawson 2008). Species with extensive carotenoid-based pigments in their nestling plumage, such as great tits and blue tits, may preferentially allocate carotenoids to growing feathers at the expense of immune system development, whereas species relying on melanin-based pigments or structural-based colors, such as mountain bluebirds, may be able to allocate carotenoids exclusively to immune function.

In this study, we tested the hypothesis that the availability of the xanthophyll lutein for the embryo in the egg and in the diet of nestlings enhances the condition and immune responses of nestling house wrens (*Troglodytes aedon*). Because pigments in the plumage of house wrens are melanin based, we predicted that if lutein is limiting, nestlings supplemented with dietary lutein would end the nestling period in better body condition and able to mount stronger immune responses than controls. We also predicted that if females were induced to produce additional eggs (i.e., supernumerary eggs) and thus were subject to greater stress (reviewed in Blount 2004), nestlings hatching from these supernumerary eggs would be in poorer condition than those hatching from unmanipulated clutches; in addition, we predicted that provision of dietary supplementation with lutein to these nestlings would help mitigate these effects.

Methods

Study Species and Study Area

The house wren is a small (10–12 g), migratory, sexually monomorphic songbird with a melanin-based integument, except for carotenoid-based coloration of the mouth lining of adults and nestlings and of the gape of nestlings. House wrens are almost exclusively insectivorous, and there are no reports of them consuming carotenoid-containing plant material (Johnson 1998). In the study population, females begin egg laying in late April–early May, laying one egg per day until the clutch of four to eight eggs is completed. After ~12 d of incubation by the female, the first egg of the clutch hatches (brood day 0), after which the nestlings are provisioned by both the male and the female until they leave the nest on brood day 15–17. Females typically produce two or more broods each season, with a modal clutch size of seven eggs in May (early season) and six in early July (late season). The last clutches are laid in early August. Additional details on the biology of the house wren can be found in Johnson (1998).

This study was conducted at the Mackinaw study area in McLean County, Illinois (40°40′N, 88°53′W), which consists of floodplain and upland secondary forest bordering the Mackinaw River. The study area has 700 nest boxes arranged 30 m apart in north/south-oriented rows 60 m apart (5.4 nest boxes/ha). Nest boxes are mounted ~1.5 m above the ground on metal poles, most of which are either greased or have a 48.3-cm diameter aluminum disk mounted below the box to discourage nest predators. Details on nest box dimensions and materials are reported in Lambrechts et al. (2010).

General Methods

From early May to early August, nest boxes were checked twice each week for evidence of nest building and egg laying. Nests used in this study in 2008 were mostly on the floodplain north of the Mackinaw River, and those used in 2009 were on an adjacent upland forest-savanna tract in the northwestern corner of the study area, where nest boxes were first placed in 2004 (see fig. 1 in DeMory et al. 2010). Once egg laying began, nests were checked daily and the attending adults were captured and banded with a U.S. Fish and Wildlife Service numbered aluminum band. Males were also banded with a unique combination of one aluminum and three colored bands (for a total of two bands per leg).

Experiment 1: 2008

To determine the effect of lutein supplementation on nestling condition and immunity, we employed a randomized complete block design in which nestlings within each experimental brood were randomly assigned to receive either a control or a lutein supplement. Because the food supply of house wrens apparently declines as the breeding season progresses (Styrsky et al. 1999; Barnett et al. 2011), the experiment was performed exclusively on late-season (July–August) broods (normally the second brood of the season for females in this population), and none of the breeding adults contributed more than one clutch in the sample, thereby precluding pseudoreplication. To identify control nestlings and lutein-supplemented nestlings, we clipped the right hallux toenail of control nestlings and the left hallux toenail of lutein-supplemented nestlings. Supplemented nestlings received either lutein dissolved in Mazola corn (*Zea mays*) oil (experimentals) or corn oil only (controls). Although corn oil itself contains a small amount of carotenoids (2.3 μg of carotenoids per gram; Moreau et al. 2007), we determined that the amount of carotenoids each nestling would have received from the corn oil alone represented less than 0.5% of the amount of luteins that was added to the corn oil in the supplemented treatment, which is a trivial amount. The liquid was administered using a Gilson Pipetman inserted briefly into the nestlings' esophagus. Nestlings were given 15 μL of a 1 μg/μL lutein solution (15 μg/d) on brood days 4, 6, 8, and 10, for a total of four supplements. This dosage was within the physiological range for daily intake of granivorous passerines of a size similar to that of house wrens (McGraw and Ardia 2004).

All nestlings were weighed to the nearest 0.1 g by means of a digital scale (Acculab Pocket Pro 250-B) on brood days 4 and 11. On brood day 11, we measured nestling right tarsus length to the nearest 0.1 mm by means of dial calipers and collected an ~50-μL blood sample from the left brachial vein in a heparinized capillary tube. Blood samples were stored on ice while in the field. Later the same day, we centrifuged the blood samples at 1,610 *g* (Hematastat II; Separation Technol-

ogies) to separate the plasma from red blood cells and recorded hematocrit (percentage of blood sample comprised of packed red blood cells) as the mean of three readings.

Immediately after the blood sample was collected on brood day 11, nestlings were injected with phytohemagglutinin (PHA; Sigma-Aldrich, L8754) at a concentration of 5 mg/mL dissolved in 50 μL of sterile phosphate-buffered saline. Before injection, the thickness of the web was measured using a digital thickness gauge (Mitutoyo, 547-500). A second measurement of wing-web thickness was made ~24 h later. Three pre- and postinjection measurements (to the nearest 0.01 mm) were averaged to give final mean pre- and postinjection values. Wing swelling (postinjection thickness minus preinjection thickness) over this 24-h period indicates the extent of recruitment of immune cells to the subcutaneous injection site and is a standard method of assessing cutaneous immune response (Martin et al. 2006; Forsman et al. 2008). We manipulated 31 late-season broods with 132 nestlings in this experiment.

Experiment 2: 2009

To increase our probability of detecting an effect of lutein supplementation on nestling condition, we supplemented chicks hatching from supernumerary eggs, which were presumably in poorer condition than those hatching from earlier-laid eggs. We employed a split-plot design in which females were either induced to lay additional eggs or allowed to lay a normal clutch (clutch-size manipulation), and nestlings within both kinds of broods were randomly assigned to receive either a control or a lutein supplement, as in the first experiment. The experiment was performed exclusively on early-season (May–June) broods (the first brood of the season for females in this population) because females breeding later in the season will not produce supernumerary eggs.

The first clutch-size manipulation treatment was determined by a coin toss, and thereafter nests were randomly assigned to a treatment by alternating between treatments as new nests became active. In the treatment in which supernumerary eggs were produced, females were induced to lay larger-than-average clutches by removing their first- through fifth-laid eggs on the day each was laid. To avoid the risk of nest abandonment, the first-laid egg was replaced with an artificial plastic egg of size and coloration similar to that of house wren eggs; these artificial eggs were readily accepted by females. We also replaced the fifth-laid egg with an artificial egg to encourage females to continue to lay (two artificial eggs in nest), then allowed females to lay until incubation began (eggs were warm to the touch). Most females continued to lay until they had produced a total of eight to ten eggs, but the number of eggs in the nests averaged four (not including the two artificial eggs). Thus, females whose clutch size was manipulated incubated a total of six eggs (four real plus two artificial eggs), which was similar to the number of eggs incubated by control females and thereby eliminated any treatment differences in incubation effort (see Dobbs et al. 2006).

Because females induced to lay supernumerary eggs had significantly smaller brood sizes than females from the control broods, nonexperimental nestlings were added and removed as needed to maintain an average brood size of six to avoid differences in parental provisioning between treatments. These nonexperimental nestlings were taken from nests outside the study plot and were matched for mass and age with the nestlings in the foster nest. Foster nestlings are readily accepted by the parents and achieve similar mass and size as their nest mates (Finke et al. 1987). We clipped the middle toenail of transfer nestlings to distinguish them from control or lutein-supplemented nest mates. Transfer nestlings received no supplements.

Nestlings were given 15 μL of a 2 μg/μL lutein solution (30 μg/d) on brood days 4–10 (for a total of seven doses), with condition measures recorded as in experiment 1. We increased the lutein concentration and frequency of supplements in 2009 to ensure that they occurred throughout the early nestling period, when carotenoid supplements have a larger effect on carotenoid-based color expression than later in the nestling period (Fitze et al. 2003). This dosage was also within the physiological range for daily intake of granivorous passerines of a size similar to that of house wrens (McGraw and Ardia 2004). Nestlings in 2009 were also injected with PHA, but these data were discarded when it was discovered that they had been given an incorrect dosage; this error did not affect condition measures, as these were obtained before PHA injection. We manipulated 41 early-season broods with 184 nestlings in this experiment.

Statistical Analysis

We used SAS 9.1 statistical software (SAS Institute 2004) for all analyses. We employed mixed-model ANOVA in PROC MIXED to examine the effect of lutein supplementation on nestling PHA response and condition measures in 2008. Nest was included as a random effect to account for the statistical nonindependence of nestlings within a brood. Treatment was included as a fixed effect, and hatching date (brood day 0, a measure of time of season) and number of nestlings in the nest on brood day 4 were included as covariates. Parameter estimates were obtained using restricted maximum likelihood, and degrees of freedom were estimated using the Satterthwaite approximation (Littell et al. 2006). To obtain minimal adequate models (Crawley 1993), we employed sequential backward elimination to remove nonsignificant terms ($P > 0.15$), beginning with highest-order interactions.

We used a split-plot ANOVA in PROC MIXED with complete randomization of whole plots to examine the effects of treatment on nestling immune function in 2009. Clutch-size manipulation and carotenoid-supplementation treatment were included as fixed effects, with the former applied to the whole brood (whole-plot unit) and the latter applied at the level of the individual nestling (split-plot unit). Nest was included as a random effect to account for the statistical nonindependence of nestlings within a brood.

Results

Experiment 1: 2008

There was no significant effect of lutein supplementation on the PHA response (tables 1, 2). The only factor associated with the PHA response was hatching date, with broods hatching later in the season showing a higher PHA response than those hatching earlier; however, this trend was not quite statistically significant ($P = 0.07$). There was also no effect of lutein supplementation on nestling hematocrit (tables 1, 2). There was, however, a significant effect of hatching date, with broods hatching later in the season having lower hematocrit values than those hatching earlier in the season. There were significant effects of both lutein supplementation and hatching date on nestling mass on brood day 11 (tables 1, 2). Contrary to our expectation, control nestlings were actually slightly heavier than lutein-supplemented nestlings. Nestling mass declined significantly over the course of the breeding season. Analysis of the effect of carotenoid supplementation on nestling tarsus length yielded a significant three-way interaction among lutein treatment, hatching date, and brood size. Because the interaction precluded meaningful interpretation of lower-order effects, we reran the analysis specifying Type II SS using the HTYPE option in SAS. The effect of treatment on tarsus remained unchanged from the original analysis, which suggests that after adjusting for the other effects in the model (except for those terms including the given effect) carotenoid supplementation has a weak effect on structural body size. However, the effect was extremely small and in the opposite direction predicted by the hypothesis.

Experiment 2: 2009

There was no significant effect of lutein supplementation or clutch-size manipulation on nestling hematocrit, mass, or tarsus size, nor were there any significant interactions between the two main effects (tables 3, 4).

Discussion

There were no significant effects of lutein supplementation on nestling PHA response, results that are inconsistent with the hypothesis that the availability of lutein limits nestling immune response in this species with its melanin-based plumage pigments. Our use of lutein for the carotenoid supplement was, of course, based on the assumption that lutein and not another carotenoid plays a role in modulating the components of the immune response that are measured by the PHA assay, an assumption for which, however, there is evidence (Saino et al. 2003; O'Brien and Dawson 2008).

There was no significant effect of lutein supplementation on nestling hematocrit in either experiment, but there was a significant treatment effect of lutein supplementation on nestling mass on brood day 11 in 2008; however, the difference was opposite to that predicted, with nestlings in control broods achieving greater mass than nestlings in lutein-supplemented broods. This result contrasts with that of Biard et al. (2006), who found that carotenoid-supplemented diets of nestling blue tits and great tits led to improved body condition and increased mass compared with that of controls. Perhaps species with carotenoid-based plumage, such as blue tits and great tits, are more likely than species with melanin-based plumage, such as

Table 1: Mixed-model ANOVAs to assess the effect of lutein supplementation on phytohemagglutinin (PHA) response and condition measures of nestling house wrens in late-season broods in 2008

	Estimate ± SE	*F* (df)	*P*
PHA response:			
BD0	.005 ± .003	3.66 (1, 19.1)	.0707
Condition measures:			
Hematocrit:			
BD0	−.38 ± .14	7.62 (1, 25.8)	.0105
Mass:			
Carotenoid treatment[a]	.22 ± .10	4.77 (1, 101)	.0313
BD0	−.036 ± .015	5.42 (1, 27.4)	.0276
Tarsus:			
Carotenoid treatment[a]	62.2 ± 30.4	4.20 (1, 91)	.0433
BD0	.06 ± .18	.23 (1, 24.2)	.6329
NYBD4	2.57 ± 7.21	.30 (1, 24)	.5908
Treatment × BD0[a]	−.29 ± .14	4.31 (1, 91.1)	.0406
Treatment × NYBD4[a]	−12.4 ± 5.4	5.31 (1, 91.1)	.0234
BD0 × NYBD4	−.012 ± .032	.28 (1, 24.1)	.5997
Supplement × BD0 × NYBD4[a]	.057 ± .024	5.46 (1, 91.1)	.0216

Note. BD0 (the day of the year on which the first egg hatched) and NYBD4 (the number of nestlings present in the nest on brood day 4) were included as covariates.

[a]Relative to experimental treatment.

Table 2: Phytohemagglutinin (PHA) response and condition measures (least square mean ± SE) of nestling house wrens in experimental (lutein-supplemented) and control (vehicle-supplemented) broods (2008)

	Control			Experimental		
Variable	*N*	Mean ± SE	Min–Max	*N*	Mean ± SE	Min–Max
PHA swelling (mm)	42	.38 ± .02	.05–.87	44	.36 ± .02	.11–.62
Hematocrit (%)	56	42.7 ± 1.08	28.1–58.47	43	43.5 ± 1.07	31.7–55.8
Mass (g)	64	9.43 ± .1	7.0–10.8	68	9.21 ± .13	6.7–11.0
Tarsus (mm)	59	18.17 ± .15	14.5–19.8	64	18.17 ± .15	14.7–19.8

the house wren, to respond to carotenoid supplements with increased condition and mass because trade-offs between growth and immune function are no longer necessary in the latter. However, in another study of carotenoid-supplemented blue tit nestlings, growth rate did not differ between supplemented and control nestlings (Larcombe et al. 2010).

Although inducing some females to produce extra eggs was designed to subject them to greater stress than control females, it is possible that it did not. However, we have demonstrated that production of supernumerary eggs by female house wrens has a profound effect on their future reproductive success. In a subsequent experiment in which females were subjected to the same protocol of egg removal, incubation, and nestling provisioning as used in this experiment, females producing supernumerary eggs were less likely to produce a second brood, and, if they did so, it took them longer to initiate their clutch. Furthermore, the clutch size of experimental females attempting a second brood was significantly smaller than that of controls, and, if an experimental female returned to breed in the study area the next year, the number of eggs she produced was significantly smaller than that of returning control females (Bowers et al., forthcoming). Despite these dramatic negative effects on females induced to produce supernumerary eggs in this subsequent experiment, lutein supplementation had no effect on the final size or condition of nestlings that hatched from their eggs in the present study. There is evidence from other species that nestlings can pay a cost when different forms of maternal stress occur, including reduced immune response (Rubolini et al. 2005), as well as reduced hatchability and body condition (Saino et al. 2005). When nestlings themselves were stressed by the presence of ectoparasites, carotenoid-supplemented nestling mountain bluebirds, a species with largely structurally based plumage coloration, gained mass more rapidly than unparasitized nestlings (O'Brien and Dawson 2008).

One possible explanation for our results is that female house wrens, stressed or otherwise, allocate sufficient amounts of lutein to their eggs to sustain the normal development and immunity of their offspring from the embryonic stage through the early-nestling stage. Thereafter, the lutein required to promote the health, growth, and immunity of nestlings is presumably obtained through food items brought back to the nest by both parents. In our study population, parental house wrens feed nestlings various insect prey—including butterfly larvae and crickets (Morton 1984), which are rich in carotenoids (Olson 2006)—and thus the food provided to nestlings may have contained sufficient carotenoids to obscure any benefit of lutein supplementation. While it might have been desirable to measure plasma carotenoid concentrations to assess this possibility, other studies have found that carotenoid supplementation does not always result in increased plasma concentrations despite significant treatment effects on nestling phenotype (Biard et al. 2006), perhaps because carotenoids can be sequestered in other tissues (e.g., liver). We propose that deposition of carotenoids in the egg by female house wrens is sufficient to provide for all the needs of the embryo and perhaps even the young hatchlings, because altricial birds hatch with an incompletely developed immune system and receive passive immunity from their mothers (Rose et al. 1974; Grindstaff et al. 2006).

It is also possible that the timing of carotenoid supplementation influenced the outcome of these experiments. Carotenoid supplements administered shortly after hatching resulted in increased feather coloration in great tit nestlings compared with that in controls, whereas those administered later in the nestling period produced no difference in color expression (Fitze et al. 2003). Similarly, carotenoids may also have a larger influence on immunostimulation in the first few days after hatching than

Table 3: Split-plot ANOVAs to assess the effect of lutein supplementation and clutch-size manipulation on condition measures of nestling house wrens in early-season broods in 2009

	F (df)	*P*
Hematocrit:		
Carotenoid treatment	.20 (1, 89.8)	.6595
Clutch-size manipulation	.57 (1, 32.9)	.4553
Carotenoid × clutch size	1.70 (1, 89.8)	.1959
Mass:		
Carotenoid treatment	.31 (1, 127)	.5806
Clutch-size manipulation	.59 (1, 33.5)	.4489
Carotenoid × clutch size	.14 (1, 127)	.7086
Tarsus:		
Carotenoid treatment	.31 (1, 12)	.7020
Clutch-size manipulation	.31 (1, 33.8)	.9495
Carotenoid × clutch size	.31 (1, 124)	.6198

Table 4: Condition measures (least square mean ± SE) of nestling house wrens in relation to carotenoid treatment and clutch-size manipulation (2009)

	Supernumerary eggs laid						Control clutch size					
	Control			Carotenoid supplemented			Control			Carotenoid supplemented		
Variable	*N*	Mean ± SE	Min–Max	*N*	Mean ± SE	Min–Max	*N*	Mean ± SE	Min–Max	*N*	Mean ± SE	Min–Max
Hematocrit (%)	28	41.3 ± 1.7	32.0–68.7	31	42.6 ± 1.7	26.9–72.5	30	41.8 ± 1.87	24.4–53.8	27	39.1 ± 1.9	14.5–54.8
Mass (g)	41	9.86 ± .16	6.2–12.0	42	9.84 ± .16	7.9–11.3	41	10.07 ± .18	8.4–11.9	40	9.96 ± .18	7.4–11.8
Tarsus (mm)	41	18.41 ± .17	16.7–20.6	42	18.42 ± .1	15.6–19.9	39	18.44 ± .20	15.0–19.6	40	18.36 ± .19	11.8–19.8

later, but in this study measures of immune function were made near the end of the nestling period.

Notwithstanding the well-documented beneficial effects of carotenoids on the health, condition, and immunity of adult birds, our results add to a growing number of studies suggesting that maternally derived carotenoids in the egg and those provided later in the diet are often not limiting to the normal development and immunocompetence of nestlings. For example, nestling Eurasian kestrels supplemented with carotenoids showed no decrease in oxidative damage, nor were they heavier or in better condition than control nestlings (Costantini et al. 2007). Larcombe et al. (2010) supplemented nestling blue tits with a carotenoid mixture of 20 : 1 lutein to zeaxanthin daily for a period of 11 d, starting when the chicks were 3 d old. They found no differences between control and carotenoid-supplemented nestlings with respect to oxidative damage, plumage coloration, or growth rate.

In contrast to these negative results, there are studies that have reported significant effects of carotenoid supplementation on altricial nestlings, but most of these have been conducted on species with carotenoid-based plumage coloration (e.g., Biard et al 2006; Berthouly et al. 2008; Eeva et al. 2009). An exception is an experiment on nestling mountain bluebirds that combined carotenoid supplementation and ectoparasite removal (O'Brien and Dawson 2008), which found that the PHA response of nestlings was enhanced by carotenoid supplements but that there was no interaction between the immune response and the presence or absence of ectoparasites. Interestingly, nestlings of females in good condition had stronger cutaneous immune responses than those of females in poorer condition, but this occurred only for the parasites-removed treatment.

In conclusion, there have been few studies of the role played by carotenoids in any aspect of the ontogeny of bird species with melanin-based plumage, such as the house wren. We found no evidence that supplementing the diet of nestling house wrens with lutein unambiguously improved their condition and immune function, even when we stressed females by inducing them to produce supernumerary eggs. Thus, these results are not consistent with the hypothesis that the availability of the carotenoid lutein for the embryo in the egg and in the diet of nestling house wrens limits their development, condition, and immune response. However, studies of a number of different altricial and nonaltricial species have reported context-dependent responses to carotenoid supplements (e.g., Berthouly et al. 2008; Romano et al. 2008; Eeva et al. 2009), which suggests that the negative results that we report here and those in other studies should be interpreted with some caution.

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