

Dietary carotenoids mediate a trade-off between egg quantity and quality in Japanese quail

KEVIN J. MCGRAW¹

Department of Animal Science, University of California-Davis, One Shields Avenue, Davis, California 95616, USA

Carotenoids offer animals many nutritional, health, and reproductive benefits. When in high supply, carotenoids can boost antioxidant protection and immune strength as well as stimulate egg production and enrich the color of sexual ornaments like feathers. Certain reproductive investments, however, often come at the cost of others; for example, the production of many offspring may compromise the quality of those offspring. Under such a scenario, we rarely know the precise intrinsic or extrinsic mechanism that generates such a reproductive trade-off. Here I show that variation in dietary carotenoid intake mediates a trade-off between egg quantity and quality in female Japanese quail (*Coturnix japonica*). Females fed high doses of two common plant carotenoids, lutein and zeaxanthin, during a 1-month diet experiment were more likely to lay eggs, but produced eggs with significantly smaller yolks. Yolk serves as the critical nutritional supply for developing embryos, and several studies show dramatic negative developmental consequences for offspring that are allocated scant yolk reserves. These results demonstrate nutritional control of yolk size and highlight a potential reproductive cost of high carotenoid accumulation in multiparous birds. In future studies, we should consider total yolk-carotenoid reserves rather than simply carotenoid concentration to better understand the cost-benefit balance of these nutrients.

KEY WORDS: *Coturnix japonica*, egg-yolk carotenoids, lutein, maternal effects, maternal investment, reproductive trade-offs, yolk size, zeaxanthin.

INTRODUCTION

Mothers from egg-laying animals shunt nutrients to their offspring, in the form of yolk, prior to hatching. Yolk components nourish embryos during development and facilitate proper growth and survival (WILLIAMS 1994). During these breeding times, however, mothers are faced with the challenge of using nutrients to drive their own energy-demanding reproductive efforts, or to allocate them to offspring. In fact, an inherent trade-off between offspring quality and quantity often emerges

¹ Current address: School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA (Phone: (480) 965-5518; Fax: (480) 965-6899; E-mail address: kevin.mcgraw@asu.edu).

(SMITH & FRETWELL 1974, SINERVO & SVENSSON 1998); mothers may invest nutrients in producing large numbers of eggs, but this comes at the expense of reduced egg quality (e.g. size, nutrient load; NAGER et al. 2000).

One such class of nutrients that plays a valuable role in both maternal reproduction and yolk-mediated embryonic development are the carotenoids (BLOUNT et al. 2002, BLOUNT 2004). Carotenoids are fat-soluble molecules that animals acquire from the diet and use for a variety of physiological and morphological purposes. At the somatic level, carotenoids provide antioxidant defenses to the body's cells and tissues (MØLLER et al. 2000, KRINSKY 2001), modulate responsiveness of the immune system (MCGRAW & ARDIA 2003, CHEW & PARK 2004), and offer photoprotection in sensitive tissues like the eye (THOMSON et al. 2002). Regarding gametic and reproductive investment, carotenoids are incorporated into external tissues like feathers, scales, and skin to become colorful and advertise sexual attractiveness and parental quality (HILL 2002). They have also been shown recently, when in high dietary supply, to stimulate egg-laying in gulls (BLOUNT et al. 2004) and to increase survival of nestling finches (MCGRAW et al. 2005). Interestingly, despite the expectations of the offspring quantity/quality trade-off, few studies have demonstrated negative somatic or reproductive consequences experienced by breeding mothers given high carotenoid supplies. Based on this hypothesis, one would predict that the reproductive benefits of carotenoid accumulation and use are not necessarily universal and that there may be subtle, compromising modifications of egg or offspring quality, especially in species that produce large numbers of clutches or eggs per reproductive attempt or season.

Here, I experimentally investigated the effect of carotenoid intake on egg production and characteristics in Japanese quail (*Coturnix japonica*). I manipulated two naturally occurring dietary carotenoids (the xanthophylls lutein and zeaxanthin) across a broad range of concentrations in captive quail for one month and considered both the eggs, laying capacity of females as well as an important measure of embryonic nutrition in eggs-yolk size. Yolk size is an important determinant of hatchling growth and size in animals from lizards (RADDER et al. 2002, WARNER & ANDREWS 2002) to birds (HILL 1993, DZIALOWSKI & SOTHERLAND 2004).

METHODS

Forty female quail, all of which were 23 weeks of age on the last day of the experiment, were housed in individual pens in the Meyer indoor animal facility on the campus of the University of California-Davis. During the study, ambient temperature was held constant at 22 °C, relative humidity at 55%, and light intensity at 100 lumens. Birds were kept on a constant day:night cycle of 12:12 hr.

I established eight treatment groups of five quail each, varying only in dietary carotenoid concentration (0, 15, 30, 45, 60, 75, 90, and 105 mg carotenoid/kg diet). Though nothing is yet known of natural variation in carotenoid levels in wild quail, I chose this range of doses because it matches that found in the only quantitative study of dietary carotenoids in wild granivorous birds (house finches [*Carpodacus mexicanus*]; HILL et al. 2002). All quail were fed a rice- and soy-based diet that was formulated to meet or exceed all NRC requirements for chickens (Table 1; *sensu* KOUTSOS et al. 2003). To this diet, I added the appropriate amount of xanthophyll carotenoids, in the form of 10% Oro-Glo (Kemin Industries, Inc., Des Moines, IA), for each treatment group during the one-month feeding experiment. Oro-Glo contains two naturally occurring xanthophylls, lutein and zeaxanthin, at a ratio of 93:7%.

For the 2 months before the experiment began, birds were fed the base diet, free of carotenoids, in order to remove any carotenoids that birds had in circulation or tissue storage (e.g. liver). This was done because, prior to the study, birds were fed a commercial diet (Layena 6501, Purina Mills, St Louis, MO) that contained a small amount of carotenoids (ca 10 mg/kg; unpubl. data). After consuming the carotenoid-free diet for 2 months, I visually inspected a subset of yolks to evaluate the success of the 'bleaching' process and all ($n = 6$) were pale.

To examine the effects of carotenoid feeding on egg production and yolk characteristics, I collected eggs at two points during the study: on the 24th and 31st day after carotenoid supplementation was initiated. I evaluated yolk characteristics for the 2nd (larger) set of eggs only; at this time point, 21 eggs were collected ($n = 2, 2, 2, 2, 4, 2, 3,$ and 4 for the respective treatment groups, from 0 up to 105 mg/kg). My measure of egg-laying capacity was determined by averaging the % of females in each treatment group that produced eggs on each collection day. To be sure that the dietary carotenoid treatments significantly impacted yolk-carotenoid contents, I scored the color of yolks using digital photography and computerized image analysis. Fresh eggs were cracked open and the contents gently emptied into a Petri dish. Two photographs were taken of each egg against a neutral-gray photograph board and under standardized indoor lighting conditions. A yellow color chip was included in each image as a reference, to account for any interphotograph differences in color capture and image size. I imported images into Adobe Photoshop (at a resolution of 1760×1168 pixels), highlighted the full area of the yolks with the Lasso marquee, and scored yolk coloration along three traditional axes of color — hue, saturation, and brightness — using the HSB scale under the Color Picker function (sensu MCGRAW et al. 2002). I then measured the area of yolk by determining the number of pixels occupied using the Histogram function (sensu MCGRAW et al. 2002) and comparing it to the yellow color standard of known area.

RESULTS

Treatment effects on yolk coloration, yolk size, and egg-laying capacity

Dietary carotenoid treatment had a significant effect on both yolk hue (ANOVA, $r^2 = 0.77$, $F_{7,20} = 6.12$, $P = 0.003$) and saturation ($r^2 = 0.80$, $F_{7,20} = 7.49$, $P = 0.001$), but not brightness ($r^2 = 0.31$, $F_{7,20} = 0.83$, $P = 0.58$; Fig. 1). Post-hoc pairwise comparisons revealed that yolks from the 0 mg/kg group had significantly higher (less yellow) hue scores than those from the 30, 45, 60, 90, and 105 mg/kg carotenoid group and exhibited significantly less saturated colors than yolks from the 45, 60, 90, and 105 mg/kg carotenoid groups (Tukey-Kramer HSD tests, all $P < 0.05$; Fig. 1).

Experimental variation in dietary carotenoid access also had significant effects on other egg characteristics. Carotenoid dose affected both female egg-laying propensity ($r^2 = 0.84$, $F_{7,15} = 6.1$, $P = 0.01$) and yolk size ($r^2 = 0.73$, $F_{7,20} = 4.96$, $P = 0.006$), but in opposite directions. Females fed high amounts of carotenoids (60 and 105 mg/kg) were more likely to lay eggs on the randomly selected collection days than females fed lower amounts of carotenoids (0 and 75 mg/kg) (Fig. 2a). However, females supplemented with high amounts of carotenoids laid eggs with yolks that were significantly smaller in area; in particular, females fed the two highest carotenoid doses (90 and 105 mg/kg) laid eggs with significantly smaller yolks than females fed 45 and 75 mg/kg (Tukey-Kramer HSD tests, all $P < 0.05$; Fig. 2b).

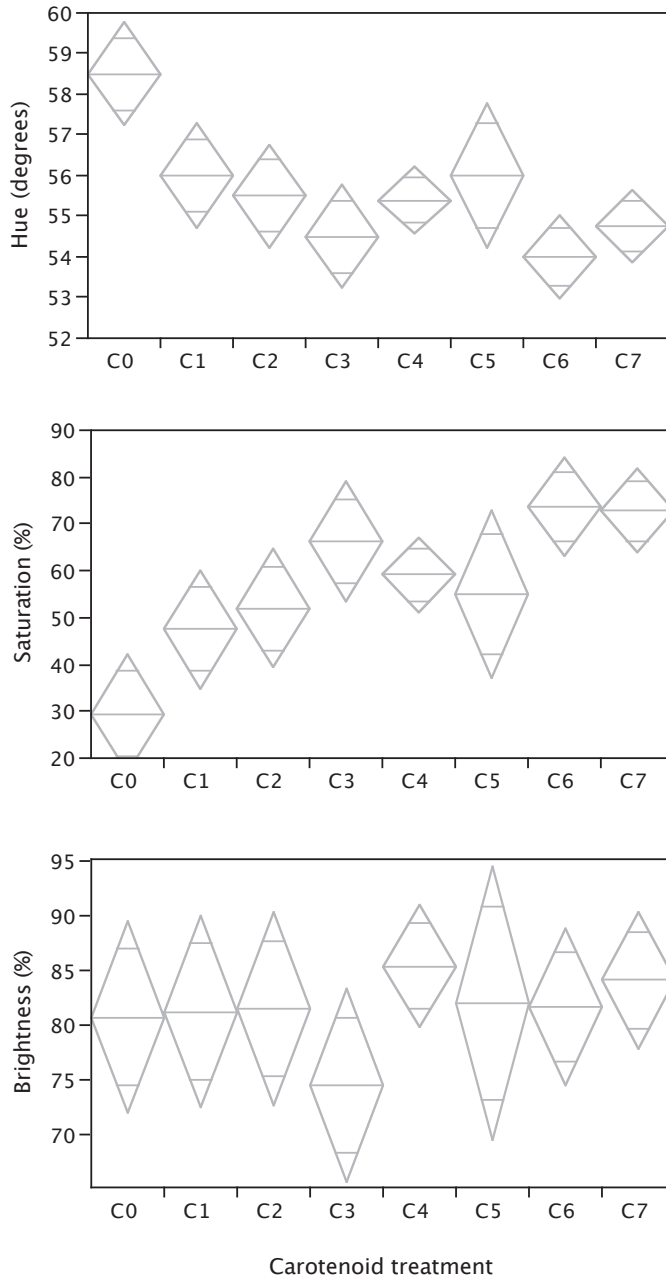


Fig. 1. — Effect of dietary carotenoid supplementation on egg-yolk color in female Japanese quail. C0 through C7 treatments correspond to 0, 15, 30, 45, 60, 75, 90, and 105 mg/kg of carotenoid added to the base diet. Horizontal lines (from top to bottom) in the diamond plot signify the 25th, 50th, and 75th percentiles; outer points (from top to bottom) indicate the 10th and 90th percentiles. Yolk colors were scored in traditional tristimulus color space (hue, saturation, and brightness) using digital photography under standard lighting conditions. Yolks became orange in hue and more saturated with increasing dietary carotenoid content (see text for statistics).

Correlations between yolk color, yolk size, and egg-laying capacity

Because females were randomly assigned to dietary treatments, I expected a notable amount of unaccounted for and inherent (genetically/physiologically based) variation in carotenoid use among groups. Thus, in addition to blocking by treatment group in my statistical analyses, I also took the approach of correlating individual measures of yolk coloration (my mark of their response to the treatment) with both yolk area and egg-laying capacity. I suspected that this might clarify some of the relationships between diet and yolk color/size that were not perfectly dose-dependent

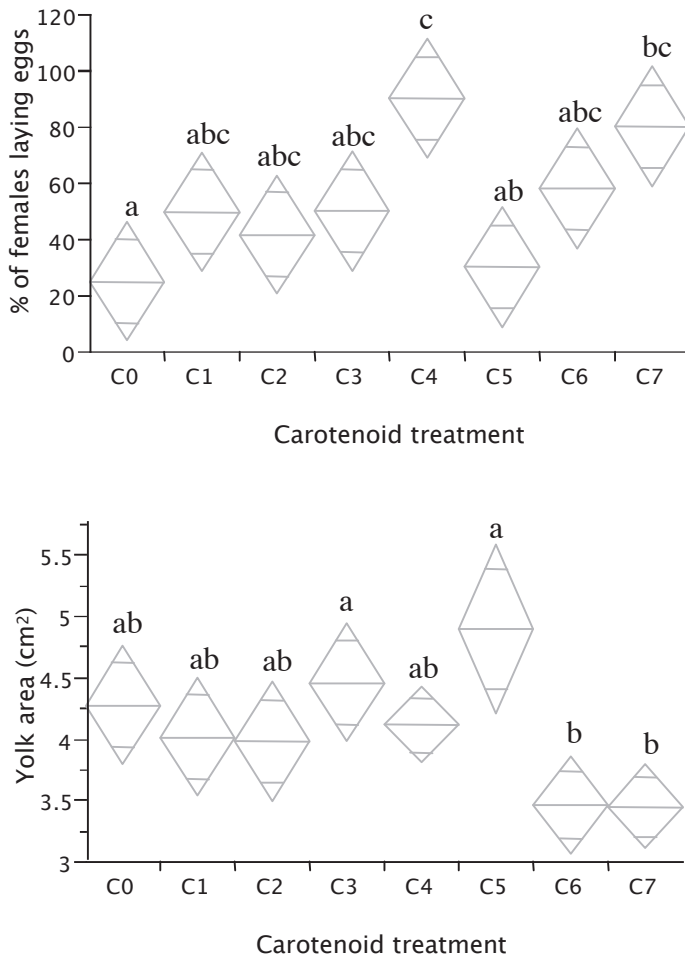


Fig. 2. — Effect of dietary carotenoid supplementation on egg-laying capacity and yolk size in female Japanese quail. See legend of Fig. 1 for more information on carotenoid treatments. Eggs were collected on 2 randomly selected days post-supplementation, and egg-laying capacity represents the average percent of females that produced an egg on each collection day. Yolk area was measured from eggs collected using digital photography, with a size standard included in each photograph to control for (slight) interphoto variation in the distance between camera and subject.

(e.g. the high hue and low saturation values, but large area of the yolks from the 75 mg/kg treatment group). Among all eggs studied, both yolk size (Fig. 3) and the percentage of females laying eggs per treatment group (Fig. 4) were significantly negatively correlated with yolk hue and significantly positively correlated with yolk saturation. Moreover, though not statistically significant, group means of yolk size tended to be negatively correlated with group egg-laying propensity (Fig. 4).

DISCUSSION

I experimentally demonstrate a carotenoid-driven trade-off between offspring quantity and quality in quail. Quail hens fed large supplies of dietary carotenoids were more likely to lay eggs during the study, but their eggs contained a smaller

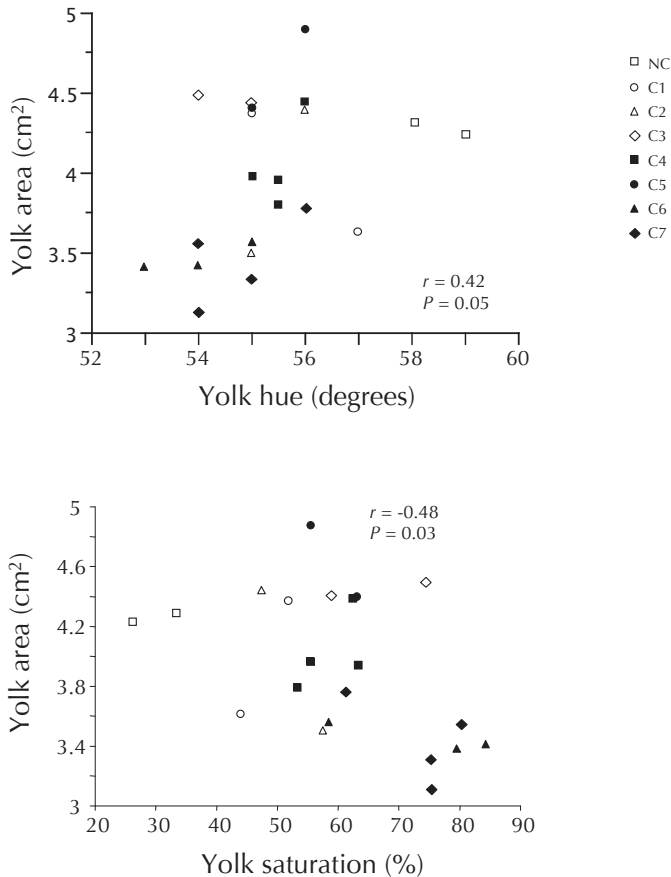


Fig. 3. — Correlations between the yolk-color parameters that significantly varied with diet (hue and saturation) and yolk area for Japanese quail eggs. Points represent individual birds from all treatment groups. Pearson's correlational tests showed that orange and more saturated yolks were smaller in area.

amount of total yolk reserve. The nutritional challenges of producing many, high-quality offspring have been previously examined and confirmed in birds (e.g. lesser black-backed gulls, *Larus fuscus*; NAGER et al. 2000), but to date no individual nutrients have been targeted as potential mediators of this trade-off. Due to their varied physiological effects, carotenoids may be important molecules for limiting and enhancing aspects of reproductive performance in birds and other animals (BLOUNT 2004, BLOUNT et al. 2004).

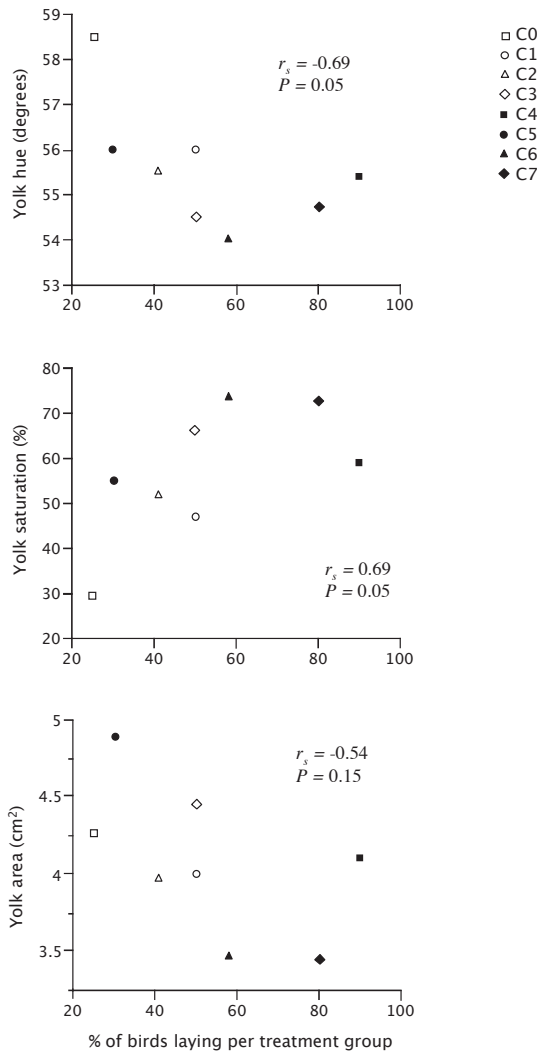


Fig. 4. — Correlations between egg-laying capacity and both yolk color (hue and saturation) and area in Japanese quail. Points represent treatment means. Spearman-rank correlational tests were used, due to small sample sizes, and show that birds who produced more eggs tended to lay eggs with more orange, more saturated, and smaller yolks.

There are several possible molecular mechanisms by which carotenoids dually control these breeding efforts. Carotenoids may directly stimulate egg-production through the upregulation of estrogenic enzymes (e.g. NG et al. 2000) or by offering antioxidant protection to egg precursors and components (e.g. vitellogenin, VLDL; BLOUNT 2004, BLOUNT et al. 2004). Carotenoids could also modify yolk size directly, by accelerating follicular growth rates (CHRISTIANS & WILLIAMS 2001) due to their gene-regulatory or antioxidant capacity. However, this could also occur indirectly, as the act of egg production itself depletes internal yolk supplies (NAGER et al. 2000). Because of different energetic demands, trade-offs that are documented experimentally in the lab are not always borne out in free-living animals (e.g. for testosterone and immunity in birds; PETERS 2000), so it will be important now to examine egg investment in several species of wild birds to determine the prevalence of this nutrient-specific trade-off. For the aforementioned 'indirect' mechanism, one would predict that a quantity/quality trade-off would be common in multiparous species (either with large clutches or many clutches per year). In support of this, gulls lay few (ca 3) eggs per year, and no carotenoid-mediated trade-off in egg production and yolk size has been reported (BLOUNT et al. 2004).

This study provides the second documented negative effect of carotenoid supplementation (at physiological levels) on breeding investment in birds. BLOUNT et al. (2002) showed that high carotenoid levels led to decreased concentrations of maternal antibodies in the yolks of free-living gulls. Taken together, these results suggest that there may be costs to allocating carotenoids to certain reproductive efforts over others, much like LOZANO (1994) proposed for the allocation of carotenoids to health versus sexual coloration. Studies that follow the fitness consequences of these investments will help clarify the relative importance of different means of carotenoid allocation. In my study, for example, it is unclear whether eggs containing carotenoid-deficient but larger yolks would have been more likely to hatch or produce young with different growth rates or survival probabilities than eggs containing carotenoid-rich but small yolks.

The reported findings also support the carotenoid basis of yolk-color variation in birds. Carotenoids and riboflavin (SQUIRES & NABER 1993) give yellow color to yolks, and in animals other than chickens no studies have experimentally manipulated dietary carotenoid content and confirmed that the intensity of yolk color is due to increased carotenoid levels. In fact, yolk coloration is not the lone aspect of carotenoid investment that researchers should be measuring. Total carotenoid investment, in milligrams, is the important biological parameter of interest to breeding mothers and their developing embryos, and to measure this one must quantify both total carotenoid concentration and yolk mass. Future studies should adopt this more comprehensive approach so that we can continue to improve our understanding of the costs and benefits of carotenoids in animal systems.

ACKNOWLEDGMENTS

This research was approved by the Institutional Animal Care and Use Committee at the University of California-Davis. I thank K. Klasing, P. Nolan, and two anonymous referees for constructive comments on the manuscript. Funding was provided by the United States Department of Agriculture (grant to K. Klasing) during data collection and by the School of Life Sciences and College of Liberal Arts and Sciences at Arizona State University during manuscript preparation.

REFERENCES

- BLOUNT J.D. 2004. Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics* 430: 10-15.
- BLOUNT J.D., HOUSTON D.C., SURAI P.F. & MØLLER A.P. 2004. Egg-laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*. *Proceedings of the Royal Society of London (B)* 271: S79-S81.
- BLOUNT J.D., SURAI P.F., NAGER R.G., HOUSTON D.C., MØLLER A.P., TREWBY M.L. & KENNEDY M.W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proceedings of the Royal Society of London (B)* 269: 29-36.
- CHEW B.P. & PARK J.S. 2004. Carotenoid action on the immune response. *Journal of Nutrition* 134: 257-261.
- CHRISTIANS J.K. & WILLIAMS T.D. 2001. Interindividual variation in yolk mass and the rate of growth of ovarian follicles in the zebra finch (*Taeniopygia guttata*). *Journal of Comparative Physiology (B)* 171: 255-261.
- DZIALOWSKI E.M. & SOTHERLAND P.R. 2004. Maternal effects of egg size on emu *Dromaius novaehollandiae* egg composition and hatchling phenotype. *Journal of Experimental Biology* 207: 597-606.
- HILL G.E. 2002. A red bird in a brown bag: the function and evolution of colorful plumage in the house finch. *New York: Oxford University Press*.
- HILL G.E., INOUE C.Y. & MONTGOMERIE R. 2002. Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society of London (B)* 62: 1119-1124.
- HILL W.L. 1993. Importance of prenatal nutrition to the development of a precocial chick. *Developmental Psychobiology* 26: 237-249.
- KOUTSOS E.A., CLIFFORD A.J., CALVERT C.C. & KLASING K.C. 2003. Maternal carotenoid status modified the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). *Journal of Nutrition* 133: 1132-1138.
- KRINSKY N.I. 2001. Carotenoids as antioxidants. *Nutrition* 17: 815-817.
- LOZANO G.A. 1994. Carotenoids, parasites, and sexual selection. *Oikos* 70: 309-311.
- MCGRAW K.J. & ARDIA D.R. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *The American Naturalist* 162: 704-712.
- MCGRAW K.J., MACKILLOP E.A., DALE J. & HAUBER M.E. 2002. Different plumage colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental coloration. *Journal of Experimental Biology* 205: 3747-3755.
- MCGRAW K.J., PARKER R.S. & ADKINS-REGAN E. 2005. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften* 92: 375-380.
- MØLLER A.P., BIARD C., BLOUNT J.D., HOUSTON D.C., NINNI P., SAINO N. & SURAI P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian and Poultry Biology Reviews* 11: 137-159.
- NAGER R.G., MONAGHAN P. & HOUSTON D.C. 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. *Ecology* 81: 1339-1350.
- NG J.H., NESARETNAM K., REIMANN K. & LAJ L.C. 2000. Effect of retinoic acid and palm oil carotenoids on oestrone sulphatase and oestradiol-17 β hydroxysteroid dehydrogenase activities in MCF-7 and MDA-MB-231 breast cancer cell lines. *International Journal of Cancer* 88: 135-138.
- PETERS A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proceedings of the Royal Society of London (B)* 267: 883-889.
- RADDER R.S., SHANBHAG B.A. & SAIDAPUR S.K. 2002. Pattern of yolk internalization by hatchlings is related to breeding timing in the garden lizard, *Calotes versicolor*. *Current Science* 82: 1484-1486.

- SINERVO B. & SVENSSON E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83: 432-442.
- SMITH C.C. & FRETWELL S.D. 1974. The optimal balance between size and number of offspring. *The American Naturalist* 108: 499-506.
- SQUIRES M.W. & NABER E.C. 1993. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: riboflavin study. *Poultry Science* 72: 483-494.
- THOMSON L.R., TOYODA Y., LANGNER A., DELORI F.C., GARNETT K.M., CRAFT N., NICHOLS C.R., CHENG K.M. & DOREY C.K. 2002. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Investigative Ophthalmology and Visual Science* 43: 3538-3549.
- WARNER D.A. & ANDREWS R.M. 2002. Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society* 76: 105-124.
- WILLIAMS T.D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biological Reviews* 68: 35-59.