Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Behavioural Processes 73 (2006) 325–332
BEHAVIOURAL PROCESSES
www.elsevier.com/locate/behavproc

Effects of β-carotene supplementation on chick growth, immune status and behaviour in the grey partridge, *Perdix perdix*

M. Cucco*, B. Guasco, G. Malacarne, R. Ottonelli

University of Piemonte Orientale, DiSAV, via Bellini 25, I-15100 Alessandria, Italy

Received 23 May 2005; received in revised form 26 July 2006; accepted 3 August 2006

Abstract

Carotenoids are important for various functions during chick development. Since these pigments cannot be synthesized, they can be considered limited resources that the mother optimally allocates between herself and her offspring (maternal effect). Some studies have examined the effects of carotenoids on growth and immune function but little is known about their role in behaviour. In this study of the grey partridge, we conducted two supplementation experiments: (1) laying females were fed with β-carotene enriched or impoverished diets; (2) chicks were fed directly with β-carotene enriched or impoverished diets. We then evaluated the effects of this carotenoid on chick growth, immunocompetence and anti-predator behaviour (reactions to a raptor model). In the first experiment, the β-carotene enriched diet given to mothers did not cause any difference in chick physiology. In the second experiment, β-carotene supplementation of chicks had a significant beneficial effect on their growth and immune response, although their behavioural reactions did not differ in relation to the diet. Therefore, β-carotene supplementation had beneficial effects on growth and immunocompetence only when directly supplied to chicks. The beneficial effect reported in other species for begging or pecking behaviours was not confirmed for the anti-predator behaviour of grey partridge chicks.

Keywords: Anti-predator reaction; Carotenoids; Feeding experiment; Growth; Grey partridge; Immune reaction

1. Introduction

The benefits of increased carotenoid intake in animals have usually been analysed by considering their effect on tegumentary colours. For example, in fishes and birds the yellow-red carotenoid-related colours are commonly used in intraspecific visual communication and males typically develop the showiest livery, thus advertising their quality as a mate to females (Hill, 1990; Kodric-Brown, 1989; Lozano, 1994; McGraw and Ardia, 2003; von Schantz et al., 1999). More recently, concurrent effects on immunological condition and antioxidant activity have been examined (Alonso-Alvarez et al., 2004), and a positive effect of carotenoids on these fitness-related traits has been shown (rev. in Møller et al., 2000; Surai, 2002). Since these pigments cannot be synthesized by animals, they can be considered limited resources that individuals allocate optimally among different functions. These considerations favour the hypothesis that, in a reproductive context, colours honestly communicate the individual's foraging and survival qualities (Zahavi, 1975) and, according to the Hamilton and Zuk (1982) hypothesis, the bearer's good health state (free-from-parasites).

Because of their beneficial effects on physiological and immune conditions, carotenoids are probably important both in adulthood and the early phases of development. According to a life-history approach, each mother should optimally allocate these substances between herself and her progeny. The positive effects of β-carotene on cell-mediated and humoral immunity of offspring were demonstrated in growing rainbow trout *Onchorynchus mykiss* (Amar et al., 2000) and in juvenile parrot-fishes *Oplegnatus* sp. (Tachibana et al., 1997). In birds, carotenoid supplementation of chicks increased their immune reaction (Cheng et al., 1999; Fenoglio et al., 2002; Haq et al., 1996; Tanvez, 2004; Tengerdy et al., 1990). An injection of the carotenoid lutein into the egg led to hatchlings with better immune response than controls, and the immune response predicted nestling survival until fledging (Saino et al., 2003).

Contrary to findings on immunocompetence, carotenoids seem to have little influence on growth. Indeed, studies by Haq et al. (1995), Fenoglio et al. (2002) and Royle et al. (1999) did

---

* Corresponding author at: University of Piemonte Orientale, Department of Environmental and Life Sciences, Via Bellini 25, I-15100 Alessandria, Italy. Tel.: +39 0131 360276; fax: +39 0131 360243.

E-mail address: cucco@unipmn.it (M. Cucco).

0376-6357/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.beproc.2006.08.002

not show any effect on growth; the only exception is a recent study of the canary *Serinus canaria* (Tanvez, 2004).

Carotenoids may also have beneficial effects on the developing nervous system and behaviour. In chickens, β-carotene significantly improved visual system development (Neuringer et al., 1988). In the moorhen *Gallinula chloropus* and the canary, carotenoid-fed chicks showed more intense pecking-begging behaviours (Fenoglio et al., 2002; Tanvez, personal communication): this suggests an effect of carotenoids on the visual system, even though the behavioural effect could have been due simply to the improved general condition (as shown by the better immune reaction) (Fenoglio et al., 2002). Such an explanation, linking health state and behaviour, was proposed in barn swallows *Hirundo rustica*: late-hatched chicks with a higher immunoglobulin concentration and more intense T cell-mediated immunity begged more for food (Saino et al., 2001). Furthermore, an experimental increase of carotenoids in the egg improved immunity in barn swallow nestlings (Saino et al., 2003), again suggesting a link between carotenoids, immune status and begging intensity.

Whereas the possible behavioural effects of carotenoids have been poorly studied, there is an extensive literature on the outcomes of early exposure to hormones, both *in ovo* and during the first days of a chick's life. These effects range from changes in chick competitive and begging behaviours (Eising and Groothuis, 2003; Kitaysky et al., 2001) to modifications of locomotor activity (Wada, 1982), attentional processes (Clifton et al., 1988), fear and distress (Jones et al., 1992), and call ontogeny (Fusani et al., 1994, 1997). To date, few studies have explored the possible effects of other regulatory or micronutrient substances (such as vitamins and carotenoids) on chick behaviour (Jones et al., 1999).

The aims of the present study were to evaluate if: (1) carotenoids available to the mother can translate into better condition of the chicks, and (2) if carotenoids supplied directly to the chicks can improve their health state, growth and behaviour. We conducted two carotenoid supplementation experiments in the grey partridge *Perdix perdix*, a socially monogamous precocial bird characterized by large clutches. In the first experiment, 64 pairs of adults were assigned to two groups and fed with a different quantity (low and high) of β-carotene in their diet. Immune, morphological and behavioural parameters were measured in the chicks hatched from these pairs. In the second experiment, two groups of chicks were fed with a β-carotene enriched or impoverished diet and the same parameters were measured.

2. Methods

2.1. Study area and experimental design

The study was conducted on grey partridges reared in 2002 and 2003 at a game breeding farm in S. Giuliano Nuovo, Alessandria, NW, Italy (Cucco et al., 2006). In total, 32 breeding pairs in 2002 and another 32 pairs in 2003 were housed in individual outdoor reproduction cages (4 m long × 1 m wide × 0.5 m high). Throughout the year, the birds were maintained in natural light and temperature conditions.

The influence of dietary carotenoids on chick health and body condition was investigated by: (1) β-carotene supplementation to adults, and (2) β-carotene supplementation directly to chicks. We chose β-carotene because of its known effect on immune condition (Haq et al., 1995; Tengerdy et al., 1990). β-Carotene is efficiently converted to Vitamin A (Moren et al., 2002). However, since we did not use enzyme blockers or radio-labelled and tracked carotenoids to investigate the direct molecular effects, we do not know the active molecular form in the partridge body.

(1) The differential supplementation of β-carotene to adults lasted 5 months, from late January (about 2 months before the egg laying period) to early July (the end of the laying period). In both years the hens were assigned to two groups: the low-carotene group was fed with a standard partridge diet of cereal pellet plus 2.7 mg/kg of β-carotene, while the high β-carotene group received the same standard food plus 27 mg/kg of β-carotene. The high β-carotene level was chosen to match the value usually used in Italian grey partridge breeding farms (near the highest value utilized in poultry, with a high safety margin with respect to the NRC recommendation, Villamide and Fraga, 1999; NRC, 1994). The low β-carotene level was 10 times lower than the high level, near the minimum nutrient requirement reported by NRC (1994). The rearing food was a powdered mixture commonly used by aviculturists to provide proper nutrition during egg laying (nutrition facts: protein 19.5%, fat 3.7%, ash 11.5%). Food and fresh water were always available.
(2) β-Carotene supplementation to chicks. The hens laid a total of 1040 eggs in 2002 and 1037 eggs in 2003. Freshly laid eggs were individually marked with a non-toxic marker to record the position in the laying order and date of laying, and then incubated for 26 days in a commercial incubator at 37.5 °C and 60% humidity. After hatching, chicks were individually tagged with a numbered plastic ring on the leg to allow subsequent identification. We assigned chicks to four groups (fed with low or high β-carotene food, and born from eggs laid by females fed with low or high β-carotene). Chicks in the low β-carotene group received a standard chick partridge diet of cereal pellet plus 2.2 mg/kg of β-carotene, while the high β-carotene group received the same standard food plus 22 mg/kg of β-carotene. The high β-carotene level was chosen to match the value usually used in Italian grey partridge breeding farms, while the low β-carotene level corresponded to the minimum nutrient requirement reported by NRC (1994). Chicks of the different experimental groups were raised for 6 weeks in separate heated pens in a nursery room maintained at 20.5 ± 2.0 °C. After 6 weeks, the chicks were removed from the heated pens and placed in large outdoor aviaries.

2.2. Growth and immunological test

We measured the body mass of all chicks with an electronic balance (±0.01 g accuracy) at hatching and at 10, 21 and 31 days of age.

Cell-mediated immunity was assessed on two occasions, at age 10 and 21 days. We used the PHA test to measure the delayed cutaneous hypersensitivity response to injection of phytohaemoagglutinin (PHA), a foreign antigen that causes T-lymphocyte proliferation and local swelling (Lochmiller et al., 1993). For each chick, a small area on the right wing web (patagium) was marked with non-toxic ink. The thickness of the right wing web was measured with a thickness gauge (Alpha spa, Milan) with an accuracy of 0.01 mm. The right wing web area was then injected intradermally with 0.25 mg of PHA (Sigma L-8754) dissolved in 0.05 ml of phosphate-buffered saline (PBS). After 24 h, the wing web thickness in the marked area was remeasured. The subcutaneous injection with PHA produces local inflammation, and the increased wing web thickness is directly related to the immunological condition (Merino et al., 1999; Smits et al., 1999). The results showed in figures and tables are expressed as variation of thickness of the right wing web between the measure performed before and after the injection. At 21 days of age, we performed a second PHA test.

2.3. Behavioural tests

When the chicks were 90 days old, they were subjected to a behavioural test to measure their anti-predator reaction (Beani and Dessì-Fulgheri, 1998). The young grey partridges were exposed to a predatory stimulus, the “Flying buzzard-like silhouette”, on 7 sunny days in October, in an outdoor aviary 2.5 m wide, 5 m long and 2 m high. The stimulus was a black wooden model of the Buzzard *Buteo buteo* (a natural predator of partridges, Birkan and Jacob, 1988), with a wingspread of 15 cm, pulled by an electric motor at the speed of 1.9 m/s at 3 m above the ground (Gyger et al., 1986). This fast-moving stimulus, with an apparent speed of 12.6 body lengths/s, is more effective than slower stimuli in eliciting anti-predator behaviour (Evans et al., 1993). This apparatus mimicked a raptor with a wingspread of 100 cm, flying at a speed of approximately 45 km/h at 20 m above the ground.

The behavioural responses of birds to the stimuli were grouped into five mutually exclusive categories (vigilance, freezing, crouching, escape, non-anti-predator behaviour) and were recorded during the presentation of the stimulus and 0.5, 1, 2, 3, 4 and 5 min after its disappearance (instantaneous scan sampling method, in which several individuals are scanned at predetermined points in time and their behavioural states are scored: Altmann, 1974).

- Vigilance: upright posture, outstretched neck, and movements of the head, scanning from side to side.
- Freezing: birds adopted a motionless posture.
- Crouching: birds rapidly squatted on the ground after presentation of the raptor.
- Escape: birds ran towards vegetation cover and hid.
- Non-anti-predator behaviour: any behaviour not falling into one of the above categories, usually foraging and exploring.

Chicks were tested in groups of four, in the 2003. Two observers, hidden by a fence, scanned the behaviour of two partridges each. For each individual and behavioural category, a total score was calculated as the sum of all the instantaneous observations recorded during the presentation of the stimulus and 0.5, 1, 2, 3, 4 and 5 min after its disappearance. Preliminary observations lasting 20 min showed that the animals usually returned to their normal activity after the first 5 min. The predatory stimulus test was run twice, the second test occurring 20 min after the first.

2.4. Statistics

To compare the characteristics of chicks in the four experimental groups, we conducted distinct multivariate analyses of covariance (MANCOVAs) with mass, immune response and behaviours as dependent variables, and position in the laying order as covariate, with year, parent, parental diet and chick diet as independent factors (Wilkinson, 1998). In preliminary analyses including interactions between factors, the interaction values were not statistically significant; hence, for the sake of brevity, they are not reported in the text. In the figure showing the relationship between position in the laying order and chick mass, instead of original mass values we plotted the residual chick mass after correcting for differences in year, parental diet and chick diet as we found significant difference related to these variables (see Section 3).

For the comparison of behavioural responses, we randomly selected two chicks from each female, one fed with the high β-carotene diet and the other with the low β-carotene diet.

3. Results

3.1. Effect of β-carotene supplementation on chick growth and immunocompetence

Prolonged supplementation of the laying females with a β-carotene enriched diet did not significantly influence any aspect of chick growth and immunocompetence (Table 1).

In contrast, β-carotene supplementation of the chicks had significant effects on both growth and immune status (Table 1). The difference in mass was observed early in life, at 10 days of age (Table 1). In particular, chicks fed with the high β-carotene diet had greater mass, in both study years (Fig. 1). However, the difference was no longer present in the last stages of growth, at both 21 and 31 days of age (values for the four combinations of hen diet/chick diet are reported in Table 2). Early in life, at age 10 days, the mass values also differed significantly in relation to the parental pair and the study year (lower values in 2002 than in 2003), and there was a slight but significant decrease of mass in relation to position in the laying order (Fig. 2). The slight decrease of chick mass related to laying order represented a decline of only 2–3% of the mean value, hence being one-half the 5–6% difference related to β-carotene treatment. All differences in growth related to diet disappeared in the last stages of growth, at age 21 and 31 days (Tables 1 and 2).

The difference in immune reaction was not present early after hatching (values for the four combinations of hen diet/chick diet are reported in Table 2), but was observed after 21 days of

Table 1
Multivariate ANCOVAs comparison of mass and immune reaction in groups of grey partridges fed with different quantities of β-carotene

Variables	Chick's diet		Parent's diet		Year		Position in the laying order		Parent		N
	F	P	F	P	F	P	F	P	F	P	
Mass age 10	4.0	0.04[*]	0.1	0.78	5.9	0.02[*]	60.2	0.001[***]	4.6	0.001[***]	448
Mass age 21	0.3	0.57	0.2	0.66	1.8	0.17	0.5	0.48	1.9	0.001[***]	301
Mass age 31	0.5	0.49	0.1	0.77	4.4	0.04[*]	3.3	0.07	1.6	0.02[*]	143
Immune response age 10	0.1	0.86	0.1	0.96	29.2	0.001[***]	6.1	0.01[**]	1.2	0.11	402
Immune response age 21	55.5	0.001[***]	0.1	0.97	9.6	0.002[**]	5.3	0.02[*]	1.1	0.34	275

[*] $P<0.05$.
[**] $P<0.01$.
[***] $P<0.001$.

differential nutrition (Table 1), when chicks fed with the high β-carotene diet had higher immunocompetence (Fig. 3). Immune reaction values also significantly differed in relation to the study year (lower values in 2003 than in 2002), and there was a slight but significant decrease of immune reaction in relation to position in the laying order (Fig. 2). The slight decrease of immune reaction related to laying order represented a decline of only 5–6% of the mean value, hence being largely lower than the 20–40% difference related to β-carotene treatment. There were no differences related to the parental pair (Table 1).

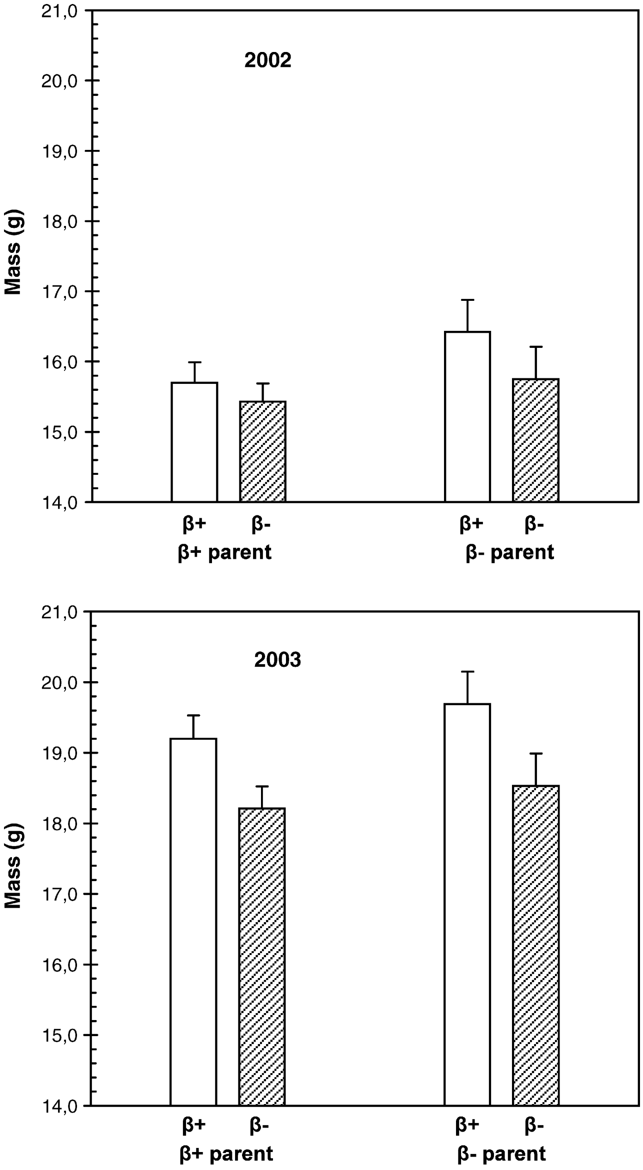

Fig. 1. Comparison of body mass (g; mean + S.E.M.) in 10-day-old chicks fed with a poor (β−) or a rich β-carotene diet (β+), and hatched from eggs laid by females fed with poor or rich β-carotene diets.

Table 2
Comparison of mean values of mass (g) and immune reaction (mm) in groups of grey partridges fed with different quantities of β-carotene

Variable	β+ parent's diet		β− parent's diet	
	Chick β+	Chick β−	Chick β+	Chick β−
Mass age 21				
2002	37.5 ± 1.52	40.3 ± 1.41	38.8 ± 1.61	42.8 ± 5.43
2003	50.5 ± 0.86	47.7 ± 1.09	49.7 ± 1.11	49.6 ± 1.10
Mass age 31				
2002	79.1 ± 4.76	89.4 ± 3.19	99.7 ± 19.2	112.4 ± 8.06
2003	139.1 ± 2.74	130.6 ± 3.53	137.5 ± 3.52	136.1 ± 4.15
Immune response age 10				
2002	0.85 ± 0.06	0.96 ± 0.07	0.58 ± 0.07	0.87 ± 0.09
2003	0.21 ± 0.01	0.11 ± 0.01	0.22 ± 0.02	0.08 ± 0.01

Mean ± S.E.M. for groups differing in diet are reported.

3.2. *Effect of β-carotene supplementation on chick behaviour*

The most frequent anti-predator behaviours were freezing and crouching, while escape and vigilance were rarely observed. Behavioural reactions did not significantly differ in relation to position in the laying order (Table 3). Prolonged β-carotene supplementation of the laying females did not significantly influence any aspect of chick behaviour. Likewise, β-carotene supplementation of the chicks failed to affect the frequency of the anti-predator behaviours (Table 3).

4. Discussion

In this study, supplementation of β-carotene to grey partridge hens had no effect on chick characteristics, while direct β-carotene supplementation of chicks had an evident effect on their immunocompetence and early growth. Our results are in line with recent findings in canaries: the same experimental design showed that the chick immune response and growth rate

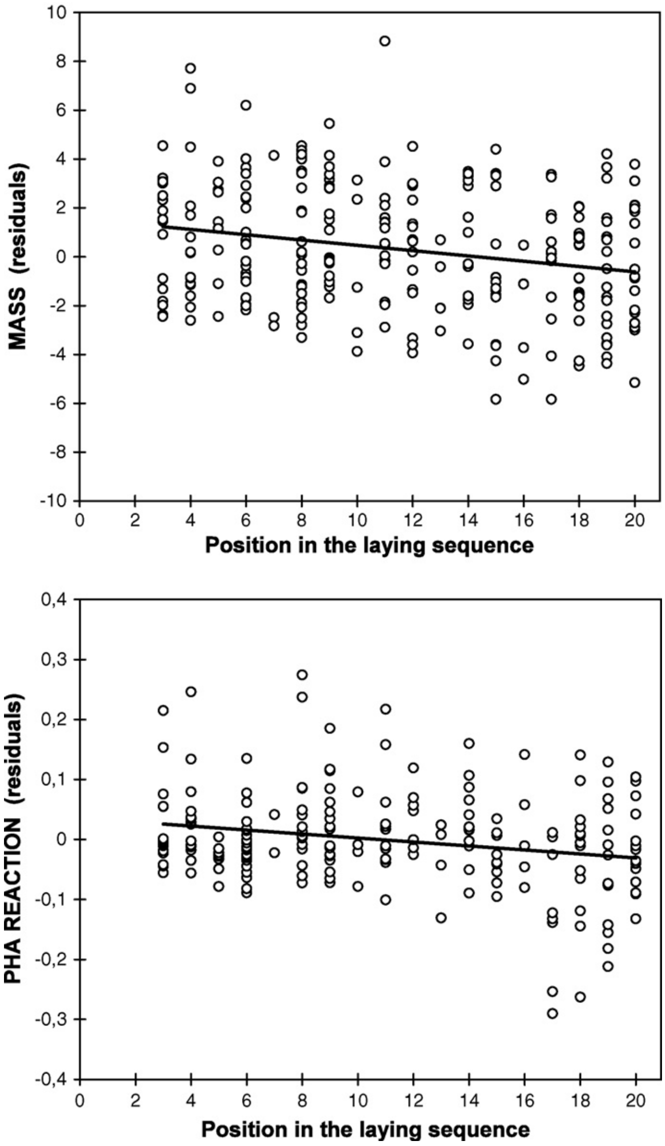

Fig. 2. Mass in 10-day-old chicks, and immune reaction in 21-day-old chicks, in relation to position in the laying order (residuals after correcting for differences in year, chick diet and parental diet).

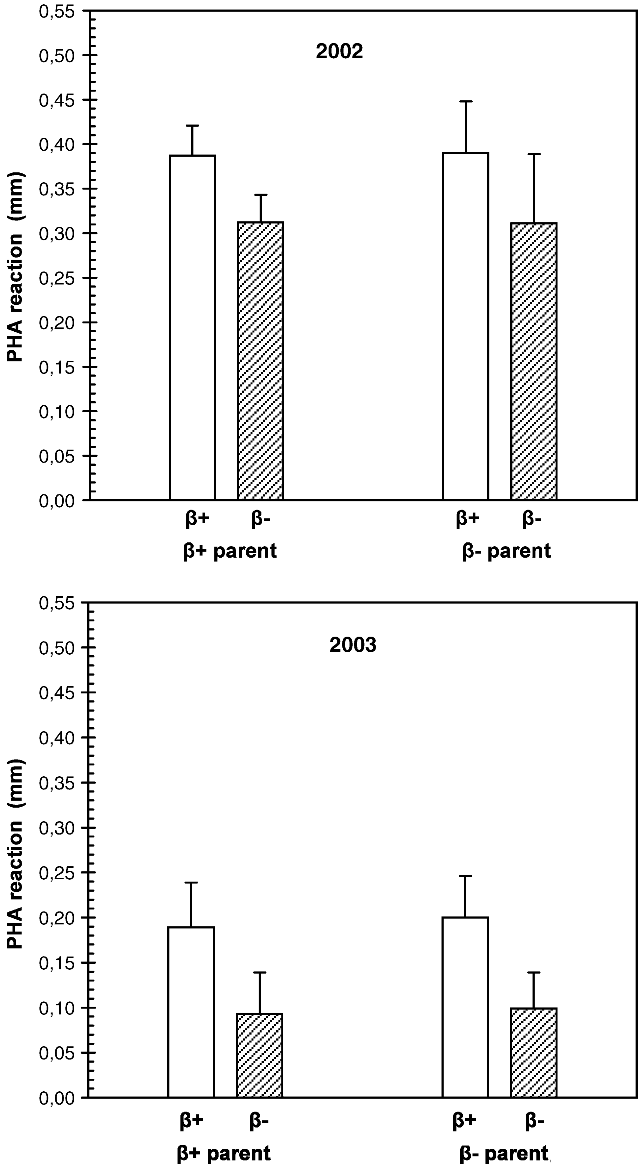

Fig. 3. Comparison of immune reaction (mm, PHA test; mean + S.E.M.) in 21-day-old chicks fed with a poor (β−) or a rich β-carotene diet (β+), and hatched from eggs laid by females fed with poor or rich β-carotene diets.

Table 3
Comparison of percent anti-predatory behaviours in groups of grey partridges fed with different quantities of β-carotene

Behaviour	β+ parent's diet		β− parent's diet		Statistics					
	Chick β+	Chick β−	Chick β+	Chick β−	Effect of parent's diet		Effect of chick's diet		Position in the laying order	
					F	*P*	*F*	*P*	*F*	*P*
Vigilance	5.56 ± 0.27	3.65 ± 0.22	2.28 ± 0.126	6.16 ± 0.37	0.04	0.84	0.29	0.59	0.09	0.76
Freezing	43.83 ± 0.84	35.92 ± 0.66	37.08 ± 0.80	36.58 ± 0.62	0.01	0.97	0.05	0.83	0.01	0.97
Crouching	8.92 ± 0.46	18.75 ± 0.49	16.67 ± 0.41	15.25 ± 0.54	2.41	0.12	0.01	0.91	0.45	0.51
Escape	4.01 ± 0.13	3.13 ± 0.12	5.30 ± 0.16	3.26 ± 0.13	0.32	0.57	1.54	0.22	0.61	0.44
Non-anti-predator	37.67 ± 0.76	38.58 ± 0.68	38.67 ± 0.83	38.75 ± 0.63	0.45	0.50	0.08	0.77	0.05	0.83

Mean ± S.E.M. for groups differing in diet are reported.

were not related to maternal food quality but did benefit from β-carotene enriched food given to the chicks (Tanvez, 2004).

In our first experiment, the β-carotene supplementation to mothers had no effect on any variable measured in the chicks. This could be because the enriched diet did not affect the blood level of circulating carotenoids in the mother or did not increase the β-carotene concentration in the yolk (Ottonelli, 2005). This suggests that grey partridge eggs are supplied with a constant amount of carotene, regardless of the mother's diet. In contrast, it has been shown in other species that carotenoids supplied to mothers can reach the yolk (Blount et al., 2000, 2002a,b; Bortolotti et al., 2003; Royle et al., 2003; Tanvez, 2004). The embryo absorbs large quantities of these antioxidants from the yolk during the last few days of embryonic development, and chicks with a high tissue concentration of maternally derived carotenoids have enhanced antioxidant protection (Surai and Speake, 1998) and immunocompetence (Haq et al., 1996; Koutsos et al., 2003a). It must be stressed that chicks cannot synthesize their own antibodies until several days post-hatching (Blount et al., 2000); thus a high carotenoid content in the egg should be beneficial and more important than carotenoids acquired by young birds in their own diet (Koutsos et al., 2003b). Nevertheless, chicks could be disadvantaged by too many carotenoids early in life. Although chicks with passive immunity can benefit from enhanced defence against disease, they could be disadvantaged in mounting their own humoral immune system too early in life: this could induce an excess of peroxidation produced by the cell-mediated part of the immune reaction chain (Blount et al., 2000). Negative effects of carotenoids have also been suggested for adults, as high concentrations of these substances might be toxic (Mayne, 1996, but see Olson and Owens, 1998). More research is needed to identify potentially detrimental effects of carotenoids (Surai, 2002).

In the second experiment, the direct supply of a high quantity of β-carotene to chicks had a clear effect on chick immunity and growth but not on behaviour. The beneficial effect of β-carotene on immunity and growth was consistently observed in the two study years, even though its magnitude differed. Year-to-year variations in growth and immunity have been reported in birds (Chastel et al., 1995; Korpimäki, 1990; Aquarone et al., 2002) and have frequently been linked to annual variations of ecological (climate, food, etc.) or social (breeder density) factors. However, in our controlled condition, there was no annual variation in food or breeding pair density. Hence, a climatic factor, or other unidentified causes, could have been at work. Interestingly, in the 2002 there was a smaller growth than in 2003, matched with a higher immune reaction in 2002 versus 2003. This observation, consistent with the idea that immune function development involves trade-offs with growth (Soler et al., 2003; Mauck et al., 2005, but see Fair et al., 1999), suggests the relevance and the need of future investigations in our study model.

We also observed a slight but statistically significant decrease of growth and immune reaction in relation to position in the laying order. Yet, the biological significance of this effect is not clear since the magnitude was very low (the percent difference between the first and last born chicks was largely lower than the difference due to the β-carotene supplementation). This is in line with most adaptive explanations related to laying order: such explanations are generally applied to altricial asynchronous species, while laying hierarchies seem to have very little influence on fitness in precocial synchronous birds (e.g. partridges) (Amundsen, 1996). The mass values also differed significantly in relation to parental pair. This fact suggests a genetic basis for mass, and is in line with the significant repeatabilities of chick mass and several egg characteristics (mass, size, total proteins, lysozyme and avidin) previously reported in the same species (Cucco et al., 2006).

The positive effect on growth was present early in life, while the effect on immune response appeared later. This difference could be due to gradual development of the immune system, since chicks progressively acquire their autonomous immunity (Pastoret et al., 1998), and/or it may reflect a trade-off between the immune system and growth (Alonso-Alvarez and Tella, 2001), since response to injections with PHA is energetically costly (Martin et al., 2003; Bonneaud et al., 2003, 2005). The difference in mass between chicks fed with high and low β-carotene content was not present anymore after the first 10 days of growth. This result shows that an effect of carotene supplementation on mass is dependent on the bird age, and points out that studies on the effect of carotenoid supplementation on mass should consider several measurements during growth. However, the physiological mechanisms underlying the better growth of chicks fed a β-carotene-rich diet are not fully understood (Surai, 2002). Uni et al. (2000) suggested that this common effect in birds is due to the beneficial influence of β-carotene (provitamin A) on intestinal absorption, since a deficiency of this substance alters the proliferation and maturation of intestinal mucosa.

The positive effect of β-carotene on the immune system is in line with findings in other bird species (Fenoglio et al., 2002; Koutsos et al., 2003b; Tanvez, 2004; Tengerdy et al., 1990). However, a positive effect of carotenoid supplementation on mass increase has not been reported in other birds (Haq et al., 1995; Fenoglio et al., 2002; Royle et al., 1999; Saino et al., 2003), except for the canary (Tanvez, 2004). Taken together, the positive effects of β-carotene on immunocompetence and growth show that this substance has a real beneficial effect on chicks.

We observed that chicks fed with different β-carotene quantities did not differ in the frequency of vigilant, freezing, crouching and escape anti-predator behaviours when the raptor model "flew" over the arena.

To date, only a few studies have considered the effect of carotenoids on behaviour. Moorhen chicks increased their pecking behaviour when fed with a carotene-rich diet (Fenoglio et al., 2002), while β-carotene fed canaries displayed more intense begging (Tanvez, personal communication). Therefore, we decided to investigate if other chick behaviour might benefit from β-carotene supplementation. Our results show that this substance has no effect on anti-predator behaviour. This finding seems complementary to other results on hormonal control: testosterone-implanted partridges were more vigilant than controls when exposed to the same experimental predator simulation utilized in our study (Fusani et al., 1997).

In conclusion, our study shows that β-carotene has beneficial effects on fitness-related physiological traits of chicks (immunocompetence and mass) but no specific effects on chick anti-predator behaviours.

Acknowledgments

We thank L. Beani and F. Dessì-Fulgheri for lending us the apparatus to mimic raptor flight, L. Borasi, E. Ferrando and V. Ferron for help in the field work, and A. Tanvez for useful comments on the manuscript. This study was supported by 40-60 MURST grants.

References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B., Sorci, G., 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. Am. Nat. 164, 651–659.

Alonso-Alvarez, C., Tella, J.L., 2001. Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. Can. J. Zool. 79, 101–105.

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 49, 227–265.

Amar, E.C., Kiron, V., Satoh, S., Okamoto, N., Watanabe, T., 2000. Effects of dietary β-carotene on the immune response of rainbow trout *Oncorhynchus mykiss*. Fish. Sci. 66, 1068–1075.

Amundsen, T., 1996. Lack's brood reduction hypothesis and avian hatching asynchrony: what's next? Oikos 76, 613–620.

Aquarone, C., Cucco, M., Malacarne, G., 2002. Annual variation of immune condition in the Hooded Crow (*Corvus corone cornix*). J. Ornithol. 143, 351–355.

Beani, L., Dessì-Fulgheri, F., 1998. Anti-predator behaviour of captive grey partridges (*Perdix perdix*). Ethol. Ecol. Evol. 10, 185–196.

Birkan, M.G., Jacob, M., 1988. La perdix grise. Hatier, Paris.

Blount, J.D., Houston, D.C., Moller, A.P., 2000. Why egg yolk is yellow? Tree 15, 47–49.

Blount, J.D., Surai, P.F., Nager, R.G., Houston, D.C., Møller, A.P., Trewby, M.L., Kennedy, M.W., 2002a. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proc. R. Soc. London B 269, 29–36.

Blount, J.D., Surai, P.F., Houston, D.C., Møller, A.P., 2002b. Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. Funct. Ecol. 16, 445–453.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., Sorci, G., 2003. Assessing the cost of mounting an immune response. Am. Nat. 161, 367–379.

Bonneaud, C., Richard, M., Faivre, B., Westerdahl, H., Sorci, G., 2005. An Mhc class I allele associated to the expression of T-dependent immune response in the house sparrow. Immunogenetics 57, 782–789.

Bortolotti, G.R., Negro, J.J., Surai, P.F., Prieto, P., 2003. Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output. Physiol. Biochem. Zool. 76, 367–374.

Chastel, O., Weimerskirch, H., Jouventin, P., 1995. Body condition and seabird reproductive performance: a study of three petrel species. Ecology 76, 2240–2246.

Cheng, Y.H., Shen, T.F., Pang, F., Chen, B.J., 1999. Effect of dietary carotenoids on immune response in mule ducklings. J. Chin. Soc. Anim. Sci. 28, 415–426.

Clifton, P., Andrew, R., Brighton, L., 1988. Gonadal steroids and attentional mechanisms in young domestic chicks. Physiol. Behav. 43, 441–446.

Cucco, M., Malacarne, G., Ottonelli, R., Patrone, M., 2006. Repeatability of cell-mediated and innate immunity, and other fitness-related traits, in the grey partridge. Can. J. Zool. 84, 72–79.

Eising, C.M., Groothuis, T.G.G., 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. Anim. Behav. 66, 1027–1034.

Evans, C.S., Macedonia, J.M., Marler, P., 1993. Effects of apparent size and speed on the response of chickens, *Gallus gallus*, to computer-generated simulations of aerial predators. Anim. Behav. 46, 1–11.

Fair, J.M., Hansen, E.S., Ricklefs, R.E., 1999. Growth, developmental stability and immune response in juvenile Japanese quails (*Coturnix coturnix japonica*). Proc. R. Soc. London B 266, 1735–1742.

Fenoglio, S., Cucco, M., Malacarne, G., 2002. The effect of a carotenoid-rich diet on immunocompetence and behavioural performances in Moorhen chicks. Ethol. Ecol. Evol. 14, 149–156.

Fusani, L., Beani, L., Dessì-Fulgheri, F., 1994. Testosterone affects the acoustic structure of the male call in the grey partridge (*Perdix perdix*). Behaviour 128, 301–310.

Fusani, L., Beani, L., Lupo, C., Dessì-Fulgheri, F., 1997. The sexually-selected vigilance behaviour of the grey partridge (*Perdix perdix*) is affected by androgen plasma levels. Anim. Behav. 54, 1013–1018.

Gyger, M., Karakashian, S.J., Marler, P.M., 1986. Avian alarm calling: is there an audience effect? Anim. Behav. 34, 1570–1572.

Hamilton, W.D., Zuk, M., 1982. Heritable true fitness and bright birds: a role for parasites? Science 218, 384–387.

Haq, A.U., Bailey, C.A., Chinnah, A., 1995. Neonatal immune response and growth performance of chicks hatched from single comb White Leghorn breeders fed diets supplemented with beta-carotene, canthaxanthin, or lutein. Poult. Sci. 74, 844–851.

Haq, A.U., Bailey, C.A., Chinnah, A., 1996. Effect of beta-carotene, canthaxanthin, lutein and Vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. Poult. Sci. 75, 1092–1097.

Hill, G.E., 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim. Behav. 40, 563–572.

Jones, R.B., Satterlee, D.G., Ryder, F.H., 1992. Fear and distress in Japanese quail chicks of two lines genetically selected for low or high adrenocortical response to immobilization stress. Horm. Behav. 26, 385–393.

Jones, R.B., Satterlee, D.G., Cadd, G.G., 1999. Timidity in Japanese quail: effects of Vitamin C and divergent selection for adrenocortical response. Physiol. Behav. 67, 117–120.

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behav. Ecol. 12, 619–625.

Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol. 25, 393–401.

Korpimäki, E., 1990. Body mass of Tengmalm's owls *Aegolius funereus*: seasonal, between-year, and age-related variation. Ornis Scandinavica 21, 169–178.

Koutsos, E.A., Calvert, C.C., Humphrey, B.D., Klasing, K.C., 2003a. Interactions between carotenoids and the inflammatory response in chickens. FASEB J. 17, A1125–A11125.

Koutsos, E.A., Clifford, A.J., Calvert, C.C., Klasing, K.C., 2003b. Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). J. Nutr. 133, 1132–1138.

Lozano, G.A., 1994. Carotenoids, parasites, and sexual selection. Oikos 70, 309–310.

Lochmiller, R.L., Vestey, M.R., Boren, J.C., 1993. Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. Auk 110, 503–510.

Martin, L.B., Scheuerlein, A., Wikleski, M., 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? Proc. R. Soc. London B 270, 153–158.

Mauck, R.A., Matson, K.D., Phillipsborn, J., Ricklefs, R.E., 2005. Increase in the constitutive innate humoral immune system in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. Funct. Ecol. 19, 1001–1007.

Mayne, S.T., 1996. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J. 10, 690–701.

Merino, S., Martínez, J., Møller, A.P., Sanabria, L., De Lope, F., Perez, J., Rodriguez-Caabeiro, F., 1999. Phytohaemoagglutinin injection assay and physiological stress in nestling house martins. Anim. Behav. 58, 219–222.

Moren, M., Næss, T., Hamre, K., 2002. Conversion of carotene, canthaxanthin and astaxanthin to Vitamin A in Atlantic halibut (*Hippoglossus hippoglossus* L.) juveniles. Fish Physiol. Biochem. 27, 71–80.

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 Poult. Biol. Rev. 11, 137–159.

McGraw, K.J., Ardia, D.R., 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am. Nat. 162, 704–712.

NRC (National Research Council), 1994. Nutrient Requirements of Poultry, 9th ed. National Academy of Sciences, Washington, DC.

Neuringer, M., Anderson, G.J., Connor, W.E., 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Ann. Rev. Nutr. 8, 517–541.

Olson, V.A., Owens, I.P.F., 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol. Evol. 13, 511–514.

Ottonelli, R., 2005. Effetti materni nella starna (*Perdix perdix*): il ruolo dei carotenoidi sulla qualità delle uova e sullo stato di salute di adulti e giovani. Ph.D. thesis, University of Piemonte Orientale, Alessandria, Italy.

Pastoret, P., Gabriel, P., Bazin, H., Govaerts, A., 1998. Handbook of Vertebrate Immunology. Academic Press, New York.

Royle, N.J., Surai, P.F., McCartney, R.J., Speake, B.K., 1999. Parental investment and egg yolk lipid composition in gulls. Funct. Ecol. 13, 298–306.

Royle, N.J., Surai, P.F., Hartley, I.R., 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. Funct. Ecol. 17, 472–481.

Saino, N., Incagli, M., Martinelli, R., Ambrosini, R., Møller, A.P., 2001. Immunity, growth and begging behaviour of nestling Barn Swallows *Hirundo rustica* in relation to hatching order. J. Avian Biol. 32, 263–270.

Saino, N., Ferrari, R., Romano, M., Martinelli, R., Møller, A.P., 2003. Experimental manipulation of egg carotenoids affects immunity of barn swallows nestlings. Proc. R. Soc. London B: Biol. Sci. 270, 2485–2489.

Smits, J.E., Bortolotti, G.R., Tella, J.L., 1999. Simplifying the phytohaemoagglutinin skin-testing technique in studies of avian immunocompetence. Funct. Ecol. 13, 567–572.

Soler, J.J., de Neve, L., Perez-Contreras, T., Soler, M., Sorci, G., 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. Proc. R. Soc. London B 270, 241–248.

Surai, P.F., 2002. Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham University Press, Nottingham.

Surai, P.F., Speake, B.K., 1998. Distribution of carotenoids from the yolk to the tissue of the chick embryo. J. Nutr. Biochem. 9, 645–651.

Tachibana, K., Yagi, M., Hara, K., Mishima, T., Tsuchimoto, M., 1997. Effects of feeling β-carotene supplemented rotifers on survival and lymphocyte proliferation reaction of fish larvae of Japanese parrotfish (*Oplegnathus fasciatus*) and spotted parrotfish (*Oplegnathus punctatus*): preliminary trials. Hydrobiologia 358, 313–316.

Tanvez, A., 2004. Effets maternels et qualité de l'oeuf chez le canari domestique commun et le goéland leucophée. Thèse de doctorat. Paris X Nanterre, Paris, France.

Tengerdy, R.P., Lacetera, N.G., Nockels, C.F., 1990. Effect of β-carotene on disease protection and humoral immunity in chickens. Avian Dis. 34, 848–854.

Uni, Z., Zaiger, G., Gal-Garber, O., Pines, M., Rozenboim, I., Reifen, R., 2000. Vitamin A deficiency interferes with proliferation of the cells in the chicken small intestine. Br. Poul. Sci. 41, 410–412.

Villamide, M.J., Fraga, M.J., 1999. Composition of vitamin supplements in Spanish poultry diets. Br. Poul. Sci. 40, 644–652.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D., Wittzell, H., 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proc. R. London B 266, 1–12.

Wada, M., 1982. Effects of sex steroids on calling, locomotor activity and sexual behaviour in castrated male Japanese quail. Horm. Behav. 16, 147–157.

Wilkinson, L., 1998. SYSTAT 8.0. Systat, Evanston, IL.

Zahavi, A., 1975. Mate selection: a selection for a handicap. J. Theor. Biol. 53, 205–214.