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Supplementation of the maternal diet with tomato powder and marigold extract: effects on the antioxidant system of the developing quail

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Abstract 1. The effects of natural dietary carotenoid supplementation of the maternal diet (tomato powder and marigold extract) on transfer to the egg yolk and on the development of the antioxidant system of the young quail liver in early postnatal life were investigated.

2. Sixty Japanese quail (*Coturnix coturnix japonica*) were allocated to four treatment groups, each with three replicates consisting of four females and one male each. The quail were fed on one of four different diets for 23 d, each of them based on a low carotenoid, wheat/barley-based control diet. Tomato powder and marigold extract were added at rates of 20 and 2 g/kg to treatments 2 and 3, respectively. Marigold extract and tomato powder were also used in combination in treatment 4 at 2 g marigold + 20 g tomato powder/kg of diet.

3. At 20 weeks of age, 60 eggs from each treatment were collected and placed in an incubator. After hatching, d-old quail from each group were reared (under standard commercial conditions) up to 14 d of age. They were fed on a low-carotenoid commercial diet. After hatch, at 1, 7 and 14 d, the livers of five young quail from each treatment were assessed for total carotenoid concentration and carotenoid profile.

4. Results indicated that lycopene is transferred from the feed to the egg yolk and further to the liver of the developing embryo. Elevated carotenoid concentration in the egg yolk and correspondingly in the liver of newly hatched quail remains significant during first week posthatch.

5. Lutein and lycopene did not affect vitamin E concentration in the egg yolk or liver of the newly hatched quail. A combination of increased concentrations of lycopene and lutein in the egg yolk results in elevated concentrations of coenzyme Q in the liver of the newly hatched quail.

INTRODUCTION

The success of embryonic and early postnatal development are determined in part by the composition of the hatching egg so chick size, vigour, early feeding behaviour and the immune status of the chick are dependent on the nutrition of the breeder hen (Fisher and Kemp, 2000). The avian embryo develops in a cleidoic egg which contains all the nutrients required for embryogenesis (Rommer, 1957). It has been suggested that embryonic development and especially the hatching process are associated with production of free radicals (Lozano, 1994; Surai, 1999*a*; Møller *et al*., 2000) and that the antioxidant system within the embryo is an important factor in determining the viability of the chick (Surai, 2002). The antioxidant system of the developing embryo and newly hatched chick includes fat-soluble antioxidants such as vitamin E (Surai, 1999*b*), carotenoids (Surai *et al*., 2001*a*,*b*) and water-soluble antioxidants including ascorbic acid and reduced glutathione (GSH), as well as antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Surai, 1999*b*, 2002). During embryonic development carotenoids, vitamin E (Surai *et al*., 1996) and selenium (Surai, 2000) are transferred from egg to the embryo reaching their maximum concentration at the time of hatching. In fact, accumulation of natural antioxidants in the liver of the

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developing chicken at time of hatching is considered (Surai and Speake, 1998; Blount *et al*., 2002*a*,*b*) to be an adaptive mechanism to deal with overproduction of free radicals at this vulnerable period of ontogenesis.

Our previous work has shown that increased levels of dietary vitamin E (Surai *et al*., 1999) or organic selenium (Surai, 2000) decreased lipid peroxidation in the embryonic tissues. Recently, attention has been paid to carotenoids as potential regulators of antioxidant defences in the developing chickens (Surai *et al*., 2001*a*,*b*).

As with vitamin E and selenium it has been shown that carotenoid concentration in the chicken egg yolk could be substantially increased by dietary manipulation (Surai and Speake, 1998). Similarly, the carotenoid concentration has been manipulated in gull eggs (Blount *et al*., 2002*a*,*b*), where the increased carotenoid concentration was shown to have a protective effect against lipid peroxidation in both the egg yolk and developing embryo. It is feasible that these antioxidant effects may be brought about by synergistic interactions between carotenoids and vitamin E, as observed *in vitro* (Edge *et al*., 1997). Furthermore it has been shown that carotenoids in zebra finches have immunomodulating properties (Blount *et al*., 2003*a*) supporting an idea that carotenoids in plumage are indicative of the health status of male birds. It has also been shown that the increased carotenoid status of the newly hatched chicks that resulted from feeding the parent stock a maize-based diet was beneficial for chicken growth and development (Kemp *et al*., 2001). These effects may persist well into the later life of the bird. Thus in poultry production it has long been acknowledged that the first 7 d of growth determines body weight at the end of the growth (d 42–49) period (Portsmouth, 2001). In a recent study (Koutsos *et al*., 2003) it was demonstrated that chicks hatched from eggs depleted of carotenoids had a compromised ability to utilise carotenoids in later life (4 weeks posthatch). Similarly, when laying hens were fed on a diet supplemented with lutein, the concentration of this carotenoid in plasma of newly hatched chicks was elevated and the increased carotenoid level was maintained for up to 5 weeks posthatch (Haq *et al*., 1995). On the other hand work with zebra finches (Blount *et al*., 2003b) showed that, just after hatching, a short period (15 d) of low quality diet significantly decreased plasma carotenoid concentrations at adulthood. Among several naturally occurring carotenoids and xanthophylls lycopene, present mainly in tomato (Mangles *et al*., 1993) has over twice the activity of β-carotene in quenching singlet oxygen and over 10 times that of α-tocopherol, which has the highest antioxidant activities (Di Mascio *et al*., 1989; Stahl and Sies, 1996).

Recently, additional attention has been paid to the health promoting properties of lycopene. It has been reported that a high dietary intake of β-carotene, lycopene, lutein, zeaxanthin or β-cryptoxanthin have been associated with a diminished risk of some cancers (Cohen, 2002; Giovannucci *et al*., 2002). However, data concerning the synergistic effects between lycopene and other carotenoids (especially lutein) and other antioxidants (vitamin E) are not available for avian species.

In previous work, we have shown that alfalfa extract Px Agro™ (Desialis, Chalones-en-Champagne, France) marigold and tomato powder could be used as effective sources of carotenoids for egg yolk coloration (Karadas *et al*., 2005*a*). It has also been reported that retinol oleate and retinol palmitate concentrations in egg yolk and the liver of 1-d-old quail chick significantly increased as a result of supplementation of the maternal diet with these carotenoids (Karadas *et al*., 2005*b*). Similarly, it has been found that maternal feed with a high concentration (3%) of PX alfalfa significantly increased carotenoid concentration in the liver of progeny after about 2 weeks compared to maternal feeding with low carotenoids diets (Karadas *et al*., 2005*c*).

The aim of the present work, therefore, was to study the effect of lycopene and lutein in the maternal diet singly or in combination on carotenoid concentration and composition, as well as vitamin E and coenzyme Q concentrations in the quail egg and tissues of newly hatched quail in early postnatal development.

MATERIALS AND METHODS

Breeders

This study was conducted with 60 quail (*Coturnix coturnix japonica*) breeder birds allocated to four treatment groups, each with three replicates consisting of four females and one male each. The SAC Animal Ethics Committee gave approval for the study to be conducted.

Dietary treatments

All the diets were prepared from a main basal diet with calculated compositions of 200 g/kg crude protein and 12·84 MJ/kg energy, according to the recommendations of Leeson and Summers (1997) for diets suitable for quail layers/breeders (Table 1). The quail in the control group (Treatment 1) were fed on the basal diet, with no added carotenoids, the only available carotenoids being those naturally

Table 1. *The composition of the quail breeder diet (Control)*

Ingredients	g/kg
Wheat	512·00
Barley	108·50
Soyabean meal	192·00
Soyabean oil	54·00
Fishmeal	49·00
Dicalcium phosphate	7·00
Limestone	69·60
Sodium chloride	2·90
Methionine	2·00
Vitamin–mineral mixture*	3·00
Natural pigments	–
Total	1000·00
Calculated composition	
ME (MJ/kg)	12·84
Crude protein (g/kg)	200·00
Crude fat (g/kg)	75·10

*Supplied per kg of diet: 150 µg retinyl acetate, 1250 µg cholecalciferol, 50 mg DL-α-tocopherol acetate, 3 mg vitamin menadione, 2 mg vitamin cyanocoblamin, 50 mg pantothenic acid, 1 mg folic acid, 200 µg D-biotin, 80 mg $FeSO_4 \cdot 7H_2O$, 10 mg $CuSO_4 \cdot H_2O$, 80 mg $ZnSO_4 \cdot H_2O$, 1 mg KI.

present in the components of the feed (Table 2). The three other groups received the standard diet supplemented with a commercially available product, tomato powder (20 g/kg), marigold extract (Kemin, Europe; 2 g/kg) with a combination of tomato powder + marigold extract (20 g + 2 g/kg, respectively). The concentrations of carotenoids in tomato powder and marigold extract were determined by HPLC analysis. Carotenoid sources were kept in a darkened room at −20°C. Experimental diets were prepared weekly and the animal experiment started within 1 week after product analyses. The birds were kept in pens, on litter. Feed and water were provided *ad libitum*.

Sampling of feed, eggs and embryos

Feed

In order to determine the concentration of carotenoids in the tomato powder and marigold extracts, three replicates from each product were analysed. The diets were prepared and samples of the diets were also analysed for carotenoid concentration (Table 2).

Eggs

For evaluation of yolk carotenoids composition and vitamin E, two eggs per replicate (six eggs/treatment) were randomly selected from breeders at 20 weeks of age, just prior to incubation. After selection the eggs were broken and the yolks were separated. The yolks were then frozen at −70°C and stored until they were analysed.

Table 2. *The concentration of individual and total carotenoids in experimental diets (µg/g)*

	Treatments			
Carotenoids	Control	TP	MG	TP + MG
Lutein	0·44	0·78	10·56	11·91
Zeaxanthin	0·06	0·11	1·49	1·56
Lycopene	nd	2·26	nd	2·61
β-Carotene	0·05	0·65	0·70	0·55
Unknown carotenoids	0·01	0·49	0·06	0·05
Total carotenoids	0·56	4·28	12·81	16·68

Values shown are means.
Nd, not detected (no peaks on HPLC chromatogram).

Embryos

Eggs were incubated in a commercial incubator. Fifteen quail in each replicate group (45 quail per treatment) were placed in a controlled environment building and grown on the control diet for 14 d. At d 0, 7 and 14, two chicks from each replicate (six chicks/treatment) were collected, killed by cervical dislocation, and their livers were collected, frozen and stored at −70°C for antioxidant analyses.

Analytical work

Concentration of carotenoids in tomato powder and marigold extract, diets, egg yolk and liver were determined by high-performance liquid chromatography (HPLC) (Surai *et al.*, 1996, 2001*a*). Samples of the tomato powder and marigold extract and then the diets were saponified with ethanolic potassium hydroxide in the presence of pyrogallol (Surai *et al.*, 1996) for 30 min at 70°C, cooled and extracted twice with hexane. After evaporation of hexane under nitrogen, the residue was re-dissolved with dichloromethane–methanol (1:1, v/v) and a 10-µl aliquot was injected into the HPLC for analysis. Samples of egg yolk and liver (200–300 mg) were mixed in 0·7 ml 5% sodium chloride, then 1 ml ethanol was added and samples homogenised. During homogenisation, 2 ml hexane were added. Then samples were centrifuged and the hexane phase, containing the carotenoids, vitamin E and coenzyme Q was collected. Extraction with hexane was performed twice, and the combined phase was evaporated under nitrogen and re-dissolved in mixture of dichloromethane–methanol (1:1, v/v).

Individual carotenoids (lutein, lycopene, *cis*-lutein, anhydrolutein, lycopene, *cis*-lycopene, β-carotene, unknown carotenoids) were determined by HPLC using a Spherisorb type S3ODS2, 5-µm C18, reverse-phase column, 25 cm × 4·6 mm (Phase Separations, Clwyd, UK) with a mobile phase of acetonitrile–methanol (85:15) and acetonitrile–dichloromethane–methanol (70:20:10)

in gradient elution (Granado *et al*., 1998), using detection by absorbance at 445 nm. Peaks were identified by comparison with the retention times of a range of carotenoid standards (variously obtained from Sigma, Poole, UK; Fluka, Gillingham, UK; Apin, Abingdon, UK; and Hoffman-La Roche, Basel, Switzerland) as well as using co-elution of individual carotenoids with known standards.

Vitamin E (α-, γ-, δ-tocopherol and α-, γ-tocotrienols) were determined as previously described (Surai and Speake, 1998) using an HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co Ltd., with a Jasco Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type S30DS2, 3 µm C_{18} reverse-phase HPLC column, 15 cm × 4·6 mm (Phase Separations Limited, UK). Chromatography was performed using a mobile phase of methanol–water (97:3, v/v) at a flow rate of 1·05 ml/min. Fluorescence detection of tocopherols and tocotrienols involved excitation and emission wavelengths of 295 and 330 nm, respectively. Standard solutions of tocopherols and tocotrienols in methanol were used for instrument calibration and tocol was used as an internal standard.

Coenzyme Q was analysed in the same extract by injecting 50 µl into the same HPLC system, but using a Vidac 201TP54 column (5 µm, 25 cm × 4·6 mm) and mobile phase ethanol–methanol–2-propanol (70:15:15, v/v) and flow rate of 1·5 ml/min with a diode array detection at 275 nm (Mattila and Kumpulainen, 2001). Coenzyme Q10 (Sigma) standard was used for calibration.

Statistical analysis

The feeding trial was a completely randomised design. The mean and standard deviation were calculated for all treatment groups and are presented in tables. Data sets were tested for normality by Kolmogorov–Smirnov and Anderson–Darling tests prior to variance analyses. Values of parameters were normally distributed. Each variable was compared using the generalised linear model analysis of variance technique of the SAS software (SAS, 1996) to assess treatment differences. In general, P values $< 0{\cdot}05$ were considered statistically significant. When the general linear model yielded significant differences, Duncan's multiple-range tests were conducted to determine particular differences between treatments (SAS, 1996).

RESULTS

All birds that were on the study survived and were healthy. The antioxidant profile of the quail egg yolk, newly hatched 7- and 14-d-old quail livers are shown in Tables 3–8. Results for total carotenoids of the experimental ingredients (tomato powder and marigold extract) were 233 and 6178 mg/kg, respectively. Therefore, the total carotenoid levels in the diets were 0·6, 4·3, 12·8, 16·7 mg/kg for Treatments 1–4, respectively (Table 2). The major specific carotenoid in the low xanthophyll (control) diet was lutein (79·0% of the total carotenoids content) followed by zeaxanthin (10·5%). The dominating component of the carotenoid profile of diet T2 (tomato) was lycopene (52·7%) followed by lutein and β-carotene (18·2 and 15·2%, respectively). In diets T3 and T4 (marigold; marigold and tomato), lutein was the major component (80·4 and 66·1%, respectively) while diet T4 had lycopene as a second significant component (12·5%). In both T3 and T4, zeaxanthin was also an important feature of the diets (11.6 and 9·3%, respectively).

Table 3. *The concentration (µg/g) of individual carotenoids in the egg yolk of quails before incubation*

	Treatments			
Carotenoids	Control	TP	MG	MG + TP
Lutein	$1{\cdot}04 \pm 0{\cdot}07^{b}$	$1{\cdot}83 \pm 0{\cdot}15^{b}$	$23{\cdot}29 \pm 2{\cdot}19^{a}$	$22{\cdot}24 \pm 1{\cdot}11^{a}$
Zeaxanthin	$0{\cdot}13 \pm 0{\cdot}04^{b}$	$0{\cdot}20 \pm 0{\cdot}02^{b}$	$2{\cdot}53 \pm 0{\cdot}24^{a}$	$2{\cdot}36 \pm 0{\cdot}07^{a}$
cis-Lutein	$0{\cdot}08 \pm 0{\cdot}04^{b}$	$0{\cdot}29 \pm 0{\cdot}16^{b}$	$0{\cdot}21 \pm 0{\cdot}17^{a}$	$2{\cdot}01 \pm 0{\cdot}12^{a}$
Anhydrolutein	$0{\cdot}01 \pm 0{\cdot}00^{c}$	nd	$0{\cdot}16 \pm 0{\cdot}02^{b}$	$0{\cdot}20 \pm 0{\cdot}02^{a}$
Lycopene	nd	$1{\cdot}21 \pm 0{\cdot}10$	nd	$1{\cdot}25 \pm 0{\cdot}25$
cis-Lycopene	nd	$0{\cdot}23 \pm 0{\cdot}04^{a}$	nd	$0{\cdot}04 \pm 0{\cdot}05^{b}$
β-Carotene	nd	$0{\cdot}01 \pm 0{\cdot}00^{b}$	$0{\cdot}51 \pm 0{\cdot}11^{a}$	$0{\cdot}38 \pm 0{\cdot}12^{a}$
Unknown carotenoids	$0{\cdot}07 \pm 0{\cdot}01^{b}$	$0{\cdot}11 \pm 0{\cdot}01^{b}$	$2{\cdot}20 \pm 0{\cdot}26^{a}$	$2{\cdot}00 \pm 0{\cdot}14^{a}$
Total carotenoids	$1{\cdot}33 \pm 0{\cdot}140^{b}$	$3{\cdot}89 \pm 0{\cdot}27^{b}$	$30{\cdot}81 \pm 2{\cdot}86^{a}$	$30{\cdot}80 \pm 1{\cdot}32^{a}$

Values are means ± SE of measurements from six egg yolk samples.
Means of each factor in a row not sharing a common superscript differ significantly ($P < 0{\cdot}05$).
Nd, not detected (no peaks in HPLC chromatogram).

Table 4. *The concentration (μg/g) of vitamin E, and vitamin A in the egg yolk of quails before incubation*

	Treatments			
Antioxidants	Control	TP	MG	MG + TP
Retinol	6·21 ± 0·73	6·88 ± 0·43	6·70 ± 0·31	7·04 ± 0·12
α-Tocopherol	139·14 ± 16·46	149·44 ± 8·66	139·82 ± 15·52	121·10 ± 9·72
γ-Tocopherol	28·48 ± 1·92	30·81 ± 0·91	31·35 ± 2·45	27·50 ± 2·99

Values are means ± SE of measurements from six egg yolk samples.

Table 5. *The concentration (μg/g) of individual carotenoids in the liver of d-old quail chicks*

	Treatments			
Carotenoids	Control	TP	MG	MG + TP
Lutein	0·91 ± 0·10^{b}	1·19 ± 0·35^{b}	16·45 ± 1·89^{a}	16·96 ± 2·11^{a}
Zeaxanthin	0·15 ± 0·01^{b}	0·18 ± 0·07^{b}	1·96 ± 0·27^{a}	1·82 ± 0·25^{a}
cis-Lutein	0·08 ± 0·02^{b}	0·14 ± 0·04^{b}	1·80 ± 0·20^{a}	1·54 ± 0·20^{a}
Anhydrolutein	nd	0·11 ± 0·09^{b}	0·49 ± 0·08^{a}	0·43 ± 0·07^{a}
β-Cryptoxanthin	0·02 ± 0·01^{b}	0·03 ± 0·00^{b}	0·65 ± 0·12^{a}	0·46 ± 0·09^{a}
Lycopene	nd	2·11 ± 0·40	nd	1·77 ± 0·46
β-Carotene	0·09 ± 0·01^{c}	0·01 ± 0·00^{c}	1·13 ± 0·15^{a}	0·73 ± 0·11^{b}
Unknown carotenoids	0·12 ± 0·03^{b}	0·11 ± 0·03^{b}	2·74 ± 0·14^{a}	3·42 ± 0·62^{a}
Total carotenoids	1·36 ± 0·12^{b}	3·88 ± 0·87^{b}	25·19 ± 2·85^{a}	27·12 ± 5·12^{a}

Values are means ± SE of measurements from the liver of six birds.
Means of each factor in a row not sharing a common superscript differ significantly ($P < 0·05$).
Nd, not detectable (no peaks in HPLC chromatogram).

Table 6. *The concentration (μg/g) of vitamin E and coenzyme Q in the liver of d-old quail chicks*

	Treatments			
Antioxidants	Control	TP	MG	MG + TP
α-Tocopherol	462·72 ± 25·01	384·90 ± 37·65	496·12 ± 76·90	522·52 ± 61·78
γ-Tocopherol	47·40 ± 3·39	54·23 ± 4·78	72·53 ± 9·35	55·52 ± 9·96
γ- Tocotrienol	7·18 ± 0·19^{a}	4·85 ± 0·49^{b}	10·46 ± 1·87^{a}	11·63 ± 2·72^{a}
α-Tocotrienol	2·01 ± 0·99^{a}	nd	0·26 ± 0·17^{b}	2·21 ± 0·78^{a}
Coenzyme Q	80·12 ± 4·26^{b}	107·99 ± 8·93^{ba}	110·09 ± 12·62^{ba}	124·01 ± 14·15^{a}

Values are means ± SE of measurements from the liver of six birds.
Means of each factor in a row not sharing a common superscript differ significantly ($P < 0·05$).
Nd, not detectable (no any pick at HPLC chromatogram).

Table 7. *The concentration (μg/g) of vitamin E and coenzyme Q in the liver of 7-d-old quail chicks*

	Treatments			
Antioxidants	Control	TP	MG	MG + TP
α-Tocopherol	19·38 ± 2·79	12·36 ± 1·94	15·06 ± 3·17	14·76 ± 4·06
γ-Tocotrienol	0·70 ± 0·03	0·91 ± 0·17	1·16 ± 0·28	1·07 ± 0·18
Total carotenoids	0·14 ± 0·01^{c}	0·18 ± 0·02^{c}	0·54 ± 0·14^{b}	0·91 ± 0·07^{a}
Coenzyme Q	50·95 ± 3·90	55·40 ± 3·54	44·93 ± 11·02	53·75 ± 6·86

Values are means ± SE of measurements from the liver of six birds.
Means of each factor in a row not sharing a common superscript differ significantly ($P < 0·05$).

The total carotenoid concentrations of egg yolk and the liver of day-old quail were very similar (Tables 3 and 5). The main carotenoid in the egg yolk and liver of d-old quail of the control group was lutein, comprising 78·4 and 66·5% of the total carotenoids, respectively, with zeaxanthin being the second biggest contributor to the carotenoid profile of the egg and liver of the 1-d-old quail. Similarly, with the control group, lutein was also the major carotenoid in most of the treatment groups, comprising 47·1, 75·6 and 72·2% of total carotenoids, respectively, in egg yolk and 30·5, 65·4 and 62·5% in the liver of d-old quail, respectively, in Treatments 2–4. This study demonstrated that lycopene is transferred from the feed to the egg yolk and in T2 it comprised

Table 8. *The concentration (μg/g) of vitamin E and coenzyme Q in the liver of 14-d-old quail chicks*

	Treatments			
Antioxidants	Control	TP	MG	TP + MG
α-Tocopherol	8·97 ± 1·26	9·57 ± 0·76	8·36 ± 0·97	8·37 ± 0·45
γ-Tocopherol	0·99 ± 0·17	1·03 ± 0·12	0·64 ± 0·17	0·83 ± 0·13
γ-Tocotrienol	1·44 ± 0·56	0·61 ± 0·11	0·64 ± 0·14	1·12 ± 0·46
Total carotenoids	0·23 ± 0·05	0·18 ± 0·01	0·21 ± 0·01	0·21 ± 0·00
Coenzyme Q	103·32 ± 6·72	118·26 ± 13·85	98·92 ± 14·12	101·61 ± 5·52

Values are means ± SE of measurements from the liver of six birds.

36·0% of total carotenoids. However, its transfer was much less effective than that for lutein. For example, in the T2 diet, the proportion of lutein was lower than that of lycopene, but in the egg the lutein proportion was much higher than that of lycopene. The lycopene concentration in the egg yolk did not depend on the presence of other carotenoids in the diet, since there was no difference in lycopene concentration between T3 and T4. However, lycopene in the liver of d-old quail was the major carotenoid comprising 54·5 and 30·5% of the total carotenoids in the groups T2 and T4, respectively. Therefore, lycopene was effectively transferred from egg yolk to the developing quail (Table 5). As can be seen from data presented in Tables 3 and 5, carotenoid concentration in egg yolk and liver of d-old quail increased proportionally to their concentration in the diet ($P < 0·05$).

As shown in Table 7, carotenoid concentration in the quail liver markedly decreased for the first week of the postnatal development. However, maternal diet had a significant effect on this variable. Indeed, total carotenoid concentrations in the liver of 7-d-old quail from T3 or T4 were significantly higher than that in the control group (Table 7). Therefore, after hatch all quail were fed on the control, low carotenoid diet and differences seen in the levels of total carotenoids in the liver of the 7-d-old quail were still significant ($P < 0·05$). It is necessary to mention that total carotenoid concentration in the quail liver sharply decreased in postnatal development and at the end of the 14-d period on the control diet there was no difference in carotenoid concentration between control and experimental quail.

Our results indicated that the inclusion of various carotenoid sources in the quail diet did not affect retinol, α-tocopherol and γ-tocopherol concentrations in the egg yolk, and liver of 1-, 7- and 14-d-old quail (Tables 4 and 6–8). Also, our data (Tables 7 and 8) indicate that coenzyme Q concentration in the liver of 1-d-old quail was affected by the maternal diet. Indeed, in the liver of group 4 (marigold + tomato), coenzyme Q concentration was 1.6-fold higher than that in the control group ($P < 0·05$; Table 6). However, in 7- and 14-d-old quail, this difference was no longer significant (Tables 7 and 8). This is the first report to show coenzyme Q concentration in the avian liver.

DISCUSSION

Carotenoids have received a lot of attention recently as a result of their health-promoting properties (Dixon *et al*., 1994; Lepage *et al*., 1996; Riso *et al*., 1999; Zhang and Omaye, 2001). However, while carotenoids are involved in the regulation of many physiological processes in animals and humans, their roles in avian biology need further investigation (Surai, 2002). In this study, we demonstrated that the inclusion in the quail diet of two sources of natural carotenoids was associated with a significant increase in their concentration in the egg yolk and, more importantly, in the liver of newly hatched chicks. These results are in line with our previous observations indicating that carotenoid concentration in the egg yolk and liver of newly hatched chicken reflects their content in the maternal diet (Surai and Speake, 1998; Surai and Sparks, 2001*a*; Surai *et al*., 2003). The obtained results also confirmed our previous observations with chickens in relation to carotenoid profile of chicken eggs and liver of newly hatched chicks. Indeed, lutein is shown to be effectively assimilated from the diet and transferred to the egg yolk and further to the liver of the developing quail.

It has been shown that lutein and zeaxanthin are two major carotenoids in chicken eggs (Surai and Sparks, 2001*a*). However, this contrasts with some avian species in the wild. In particular, gull eggs are shown to contain a wide range of carotenoids including β-carotene, echinenone and canthaxanthin (Royle *et al*., 2001; Blount *et al*., 2002*b*). Similarly, egg yolk from a moorhen and coot were characterised by the presence of a substantial proportion of β-carotene (Surai *et al*., 2001*b*). Our results also showed that extensive transfer of carotenoids from the egg yolk to the embryonic tissues takes place during the last week of incubation in parallel

with increasing lipid unsaturation (Surai *et al.*, 1996). The main site of carotenoid accumulation in the avian embryo is the liver. The concentration of carotenoids in this tissue increased substantially during the last week of incubation reaching its maximum at the time of hatching. This tendency is the same for different avian species including chicken, turkey, duck and goose (Surai *et al.*, 1998). In d-old chickens the concentration of carotenoids in the liver is several times higher than that in other tissues (Surai *et al.*, 1996). The accumulation of carotenoids in the yolk sac membrane takes place in the same manner but begins earlier than in the liver. This is because carotenoids are firstly absorbed from the residual yolk by the yolk sac membrane by phagocytosis (Noble and Cochi, 1990) then transferred from the yolk-sac membrane to the liver (Shand *et al.*, 1994; Surai *et al.*, 1996; Speake *et al.*, 1998). While it is generally accepted that carotenoids possess antioxidant properties, mechanisms of their involvement in antioxidant processes *in vivo* have not been characterised.

It has been shown that carotenoid concentration in the chicken egg yolk could be substantially increased by dietary manipulation (Surai and Speake, 1998; Surai and Sparks 2001*b*). Similarly, carotenoid concentration was manipulated in gull eggs (Blount *et al.*, 2002*a*,*b*). Increased carotenoid concentration was shown to have a protective effect against lipid peroxidation in egg yolk and developing embryo. Similar protective effects of vitamin E and selenium (Surai, 2000; Surai *et al.*, 2000) have been shown.

While the diet is the major determinant of the carotenoid composition of the egg, some results (Blount *et al.*, 2002*a*,*b*) suggest differences in efficiency of individual carotenoid assimilation from the diet and their accumulation in the egg and also indicate metabolic transformation of carotenoids in some avian species. It has been hypothesised that the mechanisms of physiological discrimination among carotenoids for the purposes of yolk production have been shaped by selection because the optimal balance of individual carotenoids for yolk is not matched by their relative availability in the diet (Blount *et al.*, 2002*b*). Also, it has been shown that in the lesser black-backed gull the second and third eggs of a clutch usually contain less carotenoid than the first egg laid (Royle *et al.*, 1999). In this respect, it is interesting that concentrations of all individual carotenoids decreased in the third egg in comparison to the first egg (Royle *et al.*, 2001), and carotenoid dietary supplementation of gulls was not able to overcome this trend (Blount *et al.*, 2002*b*). Data from studies using zebra finches also suggest that females may be able to adjust egg composition to influence offspring quality variation within broods (Royle *et al.*, 2003).

The most important finding of this study is related to the transfer of lycopene from the feed to the egg yolk and embryonic tissues. Lycopene has potent antioxidant properties in biological systems (Di Mascio *et al.*, 1989; Stahl and Sies, 1996), and has well defined health-promoting properties in humans (Cohen, 2002; Giovannucci *et al.*, 2002; Johnson, 2002). Therefore, lycopene transfer to the egg yolk alone or in combination with lutein could potentially improve the antioxidant properties within the developing quail, thus reducing the adverse effects of free radicals generated during maturation of the embryo and growth of the young bird. Previously, it was suggested that carotenoid accumulation in the liver of the developing chicks could be an adaptive mechanism to deal with overproduction of free radicals generated during hatching process (Surai, 1999*a*, 2002).

It is not clear at present if carotenoids express their health-promoting properties directly or as a result of interactions with other antioxidants. For example, they can potentially recycle vitamin E (Surai, 2002) or other antioxidants. However, there was no evidence of interactions with vitamin E in this study, there being no effect of carotenoids on vitamin E concentration in the egg yolk or liver of the developing quail. On the other hand, a combination of increased concentrations of lutein and lycopene was associated with increased levels of another antioxidant, coenzyme Q. Therefore, we cannot rule out carotenoid participation in antioxidant interactions within the egg and embryo. In fact, this is the first study to show coenzyme Q levels in the liver of a developing avian species. In this respect, coenzyme Q is a unique fat-soluble antioxidant that arguably has not received enough attention in relation to its participation in antioxidant defences in the animal body. Coenzyme Q can be obtained from the diet but, more importantly, it is synthesised in the body. Therefore, an increased level of coenzyme Q in the liver of the newly hatched quail as a result of dietary carotenoid supplementation could be considered beneficial.

At time of hatching many quail systems are not mature and continue developing. This includes the immune system, digestive system as well as hormonal regulation. Our previous observations with birds clearly showed immunomodulating properties of carotenoids (Blount *et al.*, 2003*a*). Therefore, increased carotenoid concentration in combination with increased coenzyme Q level in the quail liver could positively affect immunocompetence of the developing quail.

However, this question needs further investigation.

In conclusion, the results of the present experiment could be summarised as follows:

1. Lycopene is transferred from the feed to the egg yolk and further to the liver of the developing embryo.
2. Elevated carotenoid concentration in the egg yolk and correspondingly in the liver of newly hatched quail remains significant during first week posthatch.
3. Lutein and lycopene did not affect vitamin E concentration in the egg yolk or liver of the newly hatched quail.
4. A combination of increased concentrations of lycopene and lutein in the egg yolk is translated in elevated levels of coenzyme Q in the liver of the newly hatched quail.

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