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The Effects of Different Types of Antioxidants (Se, Vitamin E and Carotenoids) in Broiler Diets on the Growth Performance, Skin Pigmentation and Liver and Plasma Antioxidant Concentrations

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■Keywords

Broiler, antioxidants, carotenoids, performance, immune response, pigmentation.

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ABSTRACT

This study investigated the effects of the addition of different antioxidants to broiler diets on their live performance, liver antioxidant composition and concentrations, immune response, and meat and skin color. A total of 945 three-day-old Ross 308 broiler chicks of both genders were randomly allocated to one of nine dietary treatments (n=105), with three replicates 35 chicks per pen, as follows: T1: control (commercially available corn-and soybean-based broiler diet); T2: selenium (control+0.5 mg/kg Sel-Plex™Se yeast); T3: vitamin E (control+200 mg/kg Kavimix-E-50 α-tocopherol acetate); T4: lutein (control+100 mg/kg 5% Lutein Beads XB); T5: lycopene (control+100 mg/kg 5% Lyco Beads XB);T6: canthaxanthin (control+25 mg/kg 10% Carophyll®Red);T7: apo-ester (control+25 mg/kg 10% Carophyll®Yellow); T8: lutein+zeaxanthin (control+25 mg/kg Xamacol®); and T9: β-carotene (control+100 mg/kg 10% Rovimix®). Feed (starter, grower, developer and finisher phases) and water were provided *ad libitum* for 42 days. Body weights, feed intake, feed conversion values and plasma carotene concentrations were recorded weekly, and liver antioxidant concentrations were recorded at the end of the experiment. Newcastle disease (LaSota) vaccination was performed on day 22. HI titers were measured on days 14, 21, 35 and 42 to determine the effects of the antioxidants on the immune system.

The addition of selenium, vitamin E, and carotenoid supplements to the commercial broiler diet significantly increased antioxidant accumulation in the liver and the plasma. All antioxidants assessed significantly improved the immune response. Selenium and vitamin E supplementation also significantly improved total carotenoid concentrations in the plasma. The carotenoids enhanced skin and meat color. None of the supplements tested influenced growth (p>0.05).

INTRODUCTION

Studies of birds in the wild have suggested that antioxidants are a limiting factor for laying females, as supplementation with antioxidants increases their transfer to eggs, which has beneficial consequences for specific offspring traits (Biard *et al.,* 2005). Recent studies have shown that the injection of physiological amounts of antioxidants into eggs increases a major component of the acquired immune system without any adverse consequences on other offspring traits (Saino *et al.,* 2003). Still, antioxidant concentrations are lower in maternal tissue than in yolk, suggesting that mothers actively transfer these compounds to eggs (Sunder & Flachowsky, 2001; Surai, 2002; Rubolini *et al.,* 2006). Therefore, during incubation, the incorporation of these compounds from egg yolk into the developing embryo and chick tissue (e.g., liver and blood) may substantially contribute to the antioxidant defenses of

the offspring (Surai & Sparks, 2001; Surai *et al.,* 2001 a,b; Koutsos *et al.,* 2003a; Karadas *et al.,* 2005a).

Despite some results have suggested that carotenoids exhibit weak antioxidant effects (Costantini & Møller, 2008), it is clear that these molecules also have immune-stimulating functions (Møller *et al.,* 2000; Saino *et al.,* 1997; 2003), which are important to combat the inhibitory effects of oxidative stress on immune responsiveness (Kurien & Scofield, 2008; Costantini & Møller, 2008; Galván *et al.,* 2012). However, the beneficial functions of carotenoids vary depending on their source, and there is no consensus regarding the minimum and maximum dosages in broiler diets. In the wild birds, the egg yolk and tissue concentrations of Se, carotenoids and vitamin E are many times higher than those in captive birds (Karadas *et al.,* 2005b; Surai, 2002; 2006).

Carotenoids have also been demonstrated to affect broiler skin color, which is an important factor for consumer acceptance in many countries (Castañeda *et al.,* 2005; Liu *et al.,* 2008). Xanthophylls, which comprise a particular class of carotenoids, are the most prominent source of pigmentation in poultry feed (Goodwin, 1950); however, the typical corn- and soybean-based commercial poultry diet does not supply the necessary amounts or types of xanthophylls required to produce a deep yellow skin (Castañeda *et al.,* 2005).

Selenium (Se) is an essential trace mineral that is important for growth as a component of poultry nutrition (Surai, 2002; Selle *at al.,* 2013). Se supplementation to animal feeds enhances the immune status of the animal and the ability of the immune system to respond to disease challenges (Tayeb & Qader, 2012).

Vitamin E plays an important role in various biochemical and physiological processes, including antioxidant activity (Litta *et al.,* 2014). Reportedly, the supplementation of 150 and 300 IU/kg vitamin E to feeds was shown to improve the immune response and to reduce the mortality of chickens challenged with *E.coli* (Tengerdy & Nockels, 1975). Similar effects have been obtained with doses ranging from 100-300 mg/kg in turkeys and chickens challenged with colibacillosis, coccidiosis, and listeriosis (Colnago *et al.,* 1984; Julseth, 1974).

In the past decade, considerable information has been published, indicating that vitamin E, Se, and carotenoids are major immune-stimulating agents (Surai, 2002). Despite the enormous amount of research focusing on vitamin E and Se, few of these studies have compared the effects of vitamin E, Se and different sources and levels of carotenoids in broiler diets. Therefore, the present study aimed at evaluating the effects of dietary supplementation with high doses of Se and vitamin E, as well as with low and high doses of carotenoids, on broiler antioxidant systems, skin pigmentation and immune responses.

MATERIALS AND METHODS

Animals and experimental design

In total, 945 one-day-old commercial Ross 308 broiler chicks were purchased from a local supplier. All chicks were wing-banded for individual identification upon arrival at the university's poultry research farm. At 3 days of age, 105 chicks were randomly distributed among nine pens according to antioxidant and pigment treatments, with three replicates that had 35 chicks per pen, as follows:

Treatment 1: control diet (corn- and soybean-based commercial diet)
Treatment 2: control diet+0.5 mg/kg Seyeast (Sel-Plex™ 2000)
Treatment 3: control diet+200 mg/kg vitamin E (Kavimix-E-50)
Treatment 4: control diet+100 mg/kg lutein (Lutein Beads XB 5%)
Treatment 5: control diet+100 mg/kg lycopene (Lyco Beads XB 5%)
Treatment 6: control diet+25 mg canthaxanthin (Carophyll®Red 10%)
Treatment 7: control diet+25 mg/kg apo-ester (Carophyll®Yellow 10%)
Treatment 8: control diet+25 mg/kg lutein (Xamacol®20)
Treatment 9: control diet+100 mg/kg β-carotene (Rovimix® 10%).

The commercial diet was purchased from a commercial supplier (Seher Tavukçuluk, Malatya). The supplements were provided by the manufacturers (Sel-Plex™ 2000 from Alltech-Kentucky; Kavimix-E-50 from Kartal Kimya-Istanbul; 10% Carophyll® Red, 10% Carophyll® Yellow and 10% Rovimix® from DSM Nutrition-Istanbul; Xamacol®20 from Novus-Brussels; and 5% Lutein Beads XB and 5% Lyco Beads XB from Lyco Red Corp., New Jersey, USA) respectively. Detailed information regarding the nutritional content of the basal commercial diet is provided in Table 1.The supplements were added to the commercial feed using an electric mixer with a capacity of 40 kg. The

birds were provided with feed and water *ad libitum* throughout the experimental period. The commercial feed varied according to the following feeding schedule: starter feed, 0-11 days; grower feed, 12-21 days; developer feed, 22-35 days; and finisher feed, 36-42 days (Table 1).

Table 1 – Broiler Feed Composition (g/kg)

Feed ingredients (g/kg)	Phase			
	Starter	Grower	Developer	Finisher
	Days 1-11	Days 12-21	Days 23-35	Days 36-42
Corn	415.82	430.84	460.18	485.75
Full-fat soybean	300.00	250.00	250.00	250.00
Soybean	114.00	120.00	84.23	75.00
Wheat	75.00	75.00	75.00	58.00
Meat-chicken meal	35.00	35.00	40.00	40.00
Meat-bone meal	25.00	25.00	25.00	25.00
Dicalcium phosphate	10.00	13.70	13.40	13.75
Limestone	6.00	5.50	3.63	4.14
DL-Methionine	3.00	3.53	3.44	3.14
Vegetable oil	4.00	28.00	31.14	30.57
Salt	2.77	2.04	1.82	1.61
L-Lysine	2.71	3.65	4.13	4.69
Vitamin mixture[1]	2.00	2.00	2.00	2.00
Mineral mixture[2]	2.00	2.00	2.00	2.00
Antifungal[3]	2.00	2.00	2.00	2.00
Anticoccidial[4]	0.70	0.70	0.70	0.70
Sodium bicarbonate	-	1.04	1.33	1.65
Total	1000.00	1000.00	1000.00	1000.00
M. Energy	3050	3150	3201	3226
Crude protein	23.50	22.50	21.20	20.25
Crude oil Lysine	8.78 1.46	10.38 1.44	10.75 1.40	10.75 1.38
Methionine	0.66	0.66	0.64	0.60
Calcium	1.00	0.98	0.98	1.00
Phosphate (*available*)	0.49	0.49	0.48	0.48

Vitamin premix[1] added at 2 g/kg yielded 12000 IU vitamin A, 2400 IU vitamin D_3, 32 mg vitamin E*, 4 mg K_3, 2.4 mg B_1, 4.8 mg B_2, 4 mg B_6, 0.016 mg B_{12}, 16 mg niacin, 8 mg calcium-D-pantothenate, 1.2 mg folic acid, 0.06 mg biotin, and 360 mg choline chloride. Mineral premix[2] added at 2 g/kg yielded 80 mg manganese, 60 mg iron, 8 mg copper, 0.5 mg cobalt, and 2 mg iodine. Antifungal[3] Toxifix (toxin binder for poultry). [4]Lasalocid sodium 15%.

The birds live weight gain, feed intake, and feed conversion ratio were measured weekly from 1 to 6 weeks of age. A researcher with an animal care research ethics certificate (FK) collected blood and hepatic samples from two chickens from each replicate, for a total of six chickens per treatment. A blood sample was drawn from the jugular vein (Hoysak & Weatherhead 1991) using a heparinized syringe and placed into an ice-cold tube. The plasma samples were stored at –20°C for analyses of total carotenoids each week. At the end of the experiment, the chickens were sacrificed by cervical dislocation immediately after the blood sample was obtained.

Liver analysis

Liver carotenoid, vitamin A (retinol and retinol esters), vitamin E and coenzyme Q_{10} levels were measured using high-performance liquid chromatography (HPLC), as described previously by Surai *et al.,* 2001a. Tissue aliquots were vortexed in 0.7 mL of 5% NaCl. Ethanol (1 mL) was added, and the samples were homogenized for 1 min. Hexane (2 mL) was added during homogenization. Following homogenization, the samples were centrifuged, and the carotenoid-containing hexane phase was collected. The hexane extraction was repeated, and the combined hexane phase was dried using N_2 gas. It was then re-dissolved in dichloromethane/methanol (1:1 v/v).

The concentration of vitamin E (α-, γ-, and δ-tocopherols, as well as α- and γ-tocotrienols) was determined as previously described (Surai *et al.,* 2001a, b) using a Shimadzu Prominence HPLC system (Fluorescence Spectro fluorometer, Japan) fitted with a Hypersil Gold 5µ C18 reverse-phase column (10 cm×4.6 mm; Thermo, US) using a mobile phase of methanol/water (97:3 v/v) at a flow rate of 1.05 mL/min. The 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. Coenzyme Q_{10} was analyzed in the same extract by injecting 50 µl into the same HPLC system but using a Vidac 201TP54 column (5µ m,25 cm×4.6 mm), a mobile phase of ethanol/methanol/2-propanol (70:15:15 by volume) and a flow rate of 1.5 mL/min with diode array detection at 275 nm (Mattila & Kumpulainen, 2001). A coenzyme Q_{10} (Sigma) standard was used for calibration.

Total carotene levels were analyzed by injecting 10 µL sample extraction into the same HPLC system, using a Waters NH2 column (5µm, 25 cm x 4.6 mm), a mobile phase of methanol/water (97:3 by volume) and a flow rate of 1.5 mL/min with diode array detection at 444 nm (Surai & Sparks, 2001).

The levels of retinol and retinol esters were determined by injecting 10 µL sample extraction into the HPLC system using a Waters Supherisorb ODS 2, 5µ C_{18} reverse-phase HPLC column (25 cm x 4.6 mm; Phase Separations Limited, UK), with diode array detection at 325 nm (Karadas *et al.,* 2005a).

Immune response

Half of the control chicks were selected as negative controls to verify effects of the control diet on the immune response to vaccination. Except for the negative controls, all birds were inoculated with a commercial B_1strain LaSota (Lohmann Animal Health International (LAHI), Gainesville, GA) EID_{50} live vaccine tittered to a dose of at least $10^{6.5}$. The vaccines were administered with tap water that was dechlorinated by adding skimmed milk to 20 L of water. Blood samples were collected from the brachial veins of two birds per replicate (total of six birds) and centrifuged (3000×g for 10 min) to obtain the serum for immune response analyses. The antibody response to vaccination was evaluated by NVD hemagglutination inhibition (HI) assays (log_2/8 HA), which were performed as described by Van Eck & Goren (1991) at 13 days and 20 days post-vaccination, when the chickens were 35 and 42 days of age.

Skin color evaluation

Broiler carcasses were refrigerated at 4°C for 24 h. Breast and leg colors were evaluated using a Chromameter (CR-400, Minolta, Japan) and expressed using the CIE-LAB dimensions of redness (a*), yellowness (b*) and lightness (L*); for each bird, three readings were performed, and the averages were calculated and recorded.

Statistical analysis

Statistical analysis was performed using SAS version 13.2 statistical software for Windows. Duncan's multiple range tests were used to assess differences between treatments, and a $p<0.05$ was considered statistically significant (unless otherwise stated).

RESULTS

Diet analyses

Analysis of the feed (n=4 samples) indicated that the concentrations of total carotenoids in the control diet were 7.40±0.69, 9.54±0.76, 13.95±0.12, and 15.18±0.18 µg/g for the starter, grower, developer and finisher phases, respectively. Lutein, zeaxanthin, and β-carotene were the only carotenoids detected in the control diet, and they were found at the following concentrations (% of total carotenoids): 43.51% lutein, 54.46% zeaxanthin, and 2.02% β-carotene for the starter phase; 51.35% lutein, 46.22% zeaxanthin, and 2.43% β-carotene for the grower phase; 49.82% lutein, 48.46% zeaxanthin, and 1.72% β-carotene for the developer phase; and 53.72%, lutein, 44.00% zeaxanthin, and 2.28% β-carotene for the finisher phase.

The total carotenoid concentrations of the experimental groups are listed in Table 2.The carotenoid percentage changed as follows: 95.02% lutein for the 100-ppm-lutein-supplemented treatment (T4); 92.94% lycopene for the lycopene-supplemented treatment (T5); 77.97% canthaxanthin for the canthaxanthin-supplemented treatment (T6); 74.02% apo-ester for the apo-ester-supplemented treatment (T7); 72.08% lutein for the lutein-supplemented treatment (T8); and 88.45% β-carotene for the β-carotene-supplemented treatment (T9).

Bird performance

Body weights, feed intake, feed conversion ratios, and mortality rates are presented in Table 3. In general, the body weights of the birds did not vary significantly among the treatment groups ($p>0.05$).

Overall, feed intakes throughout the course of the experiment (weeks 0-6) were significantly lower in the 200 mg vitamin E and 25 mg/kg apo-ester treatment groups compared with the 100 mg/kg β-carotene treatment group ($p<0.05$).

The feed conversion values did not vary significantly among the treatment groups ($p>0.05$).

The mortality rate was significantly higher in the β-carotene treatment group compared with the

Table 2 – Total carotenoid concentrations of the experimental diets (µg/g)

	Total carotenoid concentration (µg/g) per phase of diet			
Treatments	Starter	Grower	Developer	Finisher
Control (Cont)	7.40±0.69	9.54±0.76	13.95±0.27	15.18±0.19
Cont+0.5 Sel-Plex	6.92±0.39	8.42±0.69	13.91±0.24	16.56±0.27
Cont+200 Vitamin E	7.69±0.58	9.20±0.48	13.18±0.25	16.67±0.16
Cont+100 Lutein Beads	107.11±1.52	110.88±0.69	114.46±5.36	118.09±1.75
Cont+100 Lyco Beads	107.17±5.03	108.87±3.62	114.48±1.22	115.23±0.64
Cont+25 Canthaxanthin	32.73±0.16	33.77±1.30	39.36±0.84	42.93±1.26
Cont+25 Apo-ester	31.76±1.41	32.85±0.30	38.80±0.45	42.21±0.83
Cont+25 Xamacol	29.47±0.47	30.87±0.27	38.77±1.13	39.23±0.55
Cont+100 β-carotene	107.68±1.67	108.98±1.30	112.29±0.92	114.85±0.79

Table 3 – Performance data by supplement treatment group

	Performance data on day 42			
Treatments	Body Weight (BW), g	Feed Intake (FI), g	FCR (FI/BW)	Mortality%
Control (Cont)	2862±22.05	4767±143.25^{ba}	1.66±0.07	5.71^{ba}
Cont+0.5 Sel-Plex	2890±52.92	4409±197.93^{ba}	1.53±0.10	5.71^{ba}
Cont+200 Vitamin E	2823±63.59	4224±48.01^{b}	1.50±0.02	2.86^{b}
Cont+100 Lutein Beads	2833±91.34	4517±34.38^{ba}	1.59±0.07	2.86^{b}
Cont+100 Lyco Beads	2783±112.89	4527±100.21^{ba}	1.63±0.13	7.62^{ba}
Cont+25 Canthaxanthin	2883±74.46	4447±141.26^{ba}	1.54±0.06	2.86^{b}
Cont+25 Apo-ester	2670±55.00	4245±232.26^{b}	1.59±0.07	7.62^{ba}
Cont+25 Xamacol	2760±86.22	4473±235.96^{ba}	1.62±0.09	2.86^{b}
Cont+100 β-carotene	2890±87.83	4979±357.12^{a}	1.72±0.14	9.52^{a}
F	0.93	1.54	0.82	3.03
p	0.51	0.21	0.59	0.03

a-c are used to indicate that within the same column, the values that do not share a common superscript are significantly different at the level of probability indicated.

vitamin E, lutein beads, and canthaxanthin treatment groups ($p<0.05$).

Immune response

The antibody titers for the different treatment groups are presented in Table 4. Before vaccination on day 14, significant differences were found between the other treatment groups and the 25 mg/kg Xamacol® and 100 mg/kg β-carotene groups ($p<0.05$); these differences, however, disappeared on day 21 ($p>0.05$). At 13 days post-vaccination, the antibody titers in all treatment groups improved significantly compared with those in the negative (unvaccinated) control group (Table 4). Significant improvements were also found in the positive (vaccinated) control group ($p<0.05$). No significant differences were found in the stimulated immune responses of the positive (vaccinated) control group and the groups supplemented with 25 mg/kg Xamacol® and 100 mg/kg β-carotene. Interestingly, at 20 days post-vaccination (42 days of age), the HI response of the negative control group was significantly improved compared with those of all of the experimental treatment groups, although it did not improve compared with that of the positive control group ($p<0.05$).

Plasma total carotenoid concentration

The total plasma carotenoid concentrations by week are presented in Table 5. All of the antioxidant treatment groups had significantly higher total plasma carotenoid concentrations compared with those of the control group during week 1 of the experiment. However, in week 2, the total plasma carotenoid concentration of the vitamin E treatment group was significantly higher than those of all of the other experimental groups, and although the total plasma carotenoid concentration of the Se treatment group was lower than that of the vitamin E treatment

Table 4 – Vaccine anticor hemagglutination titers (log_2/8HI) (mean±SE; log_2; n=6)

Treatments	Days			
	D14	D21	D35	D42
Negative control*	*	*	6.17±0.31^{d}	9.50±0.42^{a}
Positive control (Cont)	6.33±0.21^{c}	5.67±0.21	7.83±0.17^{c}	8.17±0.31^{ba}
Cont+0.5 Sel-Plex	5.83±0.17^{c}	5.07±0.22	9.83±0.40^{ba}	8.00±0.36^{b}
Cont+200 Vitamin E	5.83±0.31^{c}	5.66±0.18	10.00±0.26^{ba}	6.67±0.49^{bc}
Cont+100 Lutein Beads	6.00±0.00^{c}	6.00±0.00	10.17±0.40^{a}	7.33±0.56^{bc}
Cont+100 Lyco Beads	6.00±0.26^{c}	5.76±0.12	9.33±0.49^{ba}	6.17±0.31^{c}
Cont+25 Canthaxanthin	6.17±0.17^{c}	6.00±0.02	10.00±0.26^{ba}	7.00±0.63^{bc}
Cont+25 Apo-ester	6.17±0.31^{c}	5.67±0.21	9.83±0.31^{ba}	6.67±0.61^{bc}
Cont+25 Xamacol	7.00±0.26^{b}	6.17±0.40	9.50±0.43^{c}	7.83±0.40^{b}
Cont+100 β-carotene	7.67±0.21^{a}	6.17±0.11	8.83±0.14^{bc}	7.17±0.48^{bc}
P	0.00015	0.97	0.00018	0.00029
F	7.23	0.47	11.97	4.16

*Negative control, chicks from control groups but not vaccinated.

a-d are used to indicate that within the same column, the values that donot share a common superscript are significantly different at the level of probability indicated.

Table 5 – Plasma total carotene concentrations (µg/g) by age

Treatments	Weeks of age, n=6					
	1	2	3	4	5	6
Control (Cont)	5.55±0.36^{f}	6.51±0.72^{cb}	2.49±0.70^{e}	3.36±0.58^{dc}	1.54±0.31^{e}	0.98±0.14^{e}
Cont+0.5 Sel-Plex	8.26±0.25^{e}	8.54±0.60^{b}	4.87±0.63^{dc}	3.88±0.31^{dc}	1.88±0.14^{e}	1.62±0.36^{e}
Cont+200 vitamin E	9.47±0.63^{ed}	11.02±1.08^{a}	7.15±1.22^{bc}	4.92±0.44^{c}	3.32±0.18^{de}	1.51±0.36^{e}
Cont+100 Lutein Beads	11.42±0.93^{cb}	6.16±0.71^{c}	8.69±0.48^{ba}	6.91±0.74^{b}	6.54±0.57^{ba}	7.93±0.91^{b}
Cont+100 Lyco Beads	13.09±0.76^{b}	3.15±0.49^{d}	6.73±0.48^{bc}	3.91±0.57^{dc}	3.72±0.49^{dc}	4.22±0.45^{d}
Cont+25 Canthaxanthin	12.44±0.65^{b}	4.93±0.27^{cd}	10.59±1.15^{a}	9.53±1.27^{a}	5.23±0.64^{bc}	6.29±0.33^{c}
Cont+25 Apo-ester	10.22±0.44^{cd}	6.44±0.83^{c}	7.37±0.49^{b}	2.57±0.41^{de}	8.19±1.04^{a}	7.47±0.70^{cb}
Cont+25 Xamacol	16.57±0.56^{a}	6.81±0.66^{cb}	7.01±0.61^{bc}	3.53±0.41^{dc}	7.93±0.55^{a}	10.38±0.57^{a}
Cont+100 β-carotene	9.37±0.64^{ed}	5.97±0.37^{c}	4.41±0.37^{de}	1.36±0.37^{e}	3.23±0.83^{de}	2.48±0.29^{e}
F	26.72	10.56	10.54	15.44	17.95	44.52
P_{sig}	0.00014	0.00031	0.00014	0.00013	0.00019	0.00029

a-f are used to indicate that within the same column, the values that do not share a common superscript are significantly different at the level of probability indicated.

group, it was significantly higher than those of all of the other pigment-supplemented treatment groups ($p<0.05$). These differences between the vitamin E and Se treatment groups disappeared during weeks 4, 5 and 6. During week 3, all of the experimental groups except for the 100 mg/kg β-carotene-supplemented group had significantly higher total plasma carotenoid concentrations than the control group ($p<0.05$). In week 4 after vaccination, the concentrations in the 25 mg/kg canthaxanthin and the 100 mg/kg Lutein Bead groups were significantly improved compared with the concentrations in all of the other experimental treatment groups. These two groups had been flowed up with the vitamin E supplementation treatment. However, lower total carotenoid concentrations were recorded in the β-carotene-supplemented treatment group ($p<0.05$).

Liver antioxidant concentrations

The liver antioxidant (total carotenoids, α- and γ-tocopherols, total vitamin E and coenzymeQ_{10}) concentrations are presented in Table 6. The total carotenoid concentrations in the liver varied among the treatment groups as follows: canthaxanthin>apo-ester≥Xamacol®>Lutein Beads≥ Lyco Beads>β-carotenoid≥ vitamin E≥ Sel-plex≥ control ($p<0.05$). The levels of α- and γ-tocopherols and coenzyme Q_{10} were not affected by the treatments; however, the liver vitamin E concentrations (total vitamin E and α-tocopherol) were significantly higher in Vitamin E supplemented than those in the other treatment groups (Table 6).

The liver retinol and retinol ester concentrations at 42 days of age are presented in Table 7. As the table shows, β-carotene supplementation significantly improved the total vitamin A concentrations in the liver ($p<0.05$) compared with all of the other experimental treatments. The treatments did not affect the retinol concentrations ($p>0.05$); however, β-carotenoid supplementation had a significant positive effect on the R-linoleate, R-oleate, R-palmitate and R-stearate concentrations ($p<0.05$). In addition, compared with β-carotene supplementation

Table 6 – Antioxidant composition of the liver at 42 days of age (µg/g)

Treatments	Antioxidants in the liver; n=6				
	Total carotene	δ-Tocopherol	α-Tocopherol	Total vitamin E	Coenzyme Q_{10}
Control (Cont)	2.67±0.60^{e}	0.88±0.19	47.94±10.47^{b}	48.82±10.56^{b}	154±9.61
Cont+0.5 Sel-Plex	3.28±0.63^{e}	0.91±0.18	51.41±11.12^{b}	52.32±11.29^{b}	144±12.11
Cont+200 Vitamin E	3.95±0.42^{e}	1.02±0.21	90.32±5.48^{a}	91.35±5.36^{a}	133±15.58
Cont+100 Lutein Beads	9.73±0.96^{cd}	1.45±0.37	47.39±9.31^{b}	48.84±9.11^{b}	139±10.69
Cont+100 Lyco Beads	9.02±1.82^{d}	1.28±0.18	45.63±8.79^{b}	46.91±8.88^{b}	159±21.75
Cont+25 Canthaxanthin	32.54±5.85^{a}	0.95±0.17	47.20±14.96^{b}	48.14±15.12^{b}	141±27.91
Cont+25 Apo-ester	23.11±3.80^{b}	0.93±0.09	33.92±7.40^{b}	34.84±7.43^{b}	130±15.57
Cont+25 Xamacol	16.40±2.34^{cb}	0.87±0.10	42.88±5.37^{b}	43.758±5.36^{b}	152±12.28
Cont+100 β-carotene	6.11±0.52^{d}	1.56±0.40	45.99±1.93^{b}	47.55±1.96^{b}	130±11.14
F	17.45	1.29	3.15	3.11	0.40
P_{sig}	0.0003	0.27	0.007	0.007	0.91

a-b are used to indicate that within the same column, the values that donot share a common superscript are significantly different at the level of probabilityindicated.

Table7 – Liver retinol and retinol ester concentrations at 42 days of age (µg/g)

Treatments	Retinol and retinol esters in the liver; n=6					
	Retinol	R-linoleate	R-oleate	R-palmitate	R-stearate	Total vit. A
Control (Cont)	5.81±0.91	9.22±1.96^{b}	16.49±1.41^{b}	32.48±1.40^{a}	9.12±2.22^{c}	73.10±2.33^{b}
Cont+0.5 Sel-Plex	6.27±1.45	7.99±0.43^{b}	8.52±0.52^{d}	14.34±1.14^{cb}	13.20±1.28^{cb}	50.31±2.72^{fed}
Cont+200 Vitamin E	6.47±1.40	8.64±0.52^{b}	11.53±0.25^{c}	13.00±2.11^{b}	14.90±1.31^{cb}	54.89±3.13^{ced}
Cont+100 Lutein Beads	6.27±0.88	7.49±0.53^{b}	11.59±0.79^{c}	19.36±1.99^{b}	16.26±0.40^{cb}	60.97±2.30^{cbd}
Cont+100 Lyco Beads	4.70±1.11	4.85±0.90^{b}	7.39±0.66^{d}	13.12±1.01^{cb}	17.26±1.24^{cb}	47.32±2.86^{fed}
Cont+25 Canthaxanthin	7.17±2.09	6.97±0.59^{b}	7.38±1.28^{d}	8.46±1.33^{c}	14.03±3.29^{cb}	39.26±3.44^{f}
Cont+25 Apo-ester	8.76±1.85	8.75±1.10^{b}	7.70±1.05^{d}	18.90±2.44^{b}	21.59±1.31^{b}	65.71±4.74^{cb}
Cont+25 Xamacol	9.17±1.21	7.51±0.64^{b}	6.11±0.52^{d}	7.21±0.92^{c}	11.12±1.23^{c}	41.11±2.04^{fe}
Cont+100 β-carotene	5.38±2.54	16.3±2.95^{a}	20.61±1.22^{a}	31.06±4.97^{a}	36.58±7.93^{a}	109.57±10.16^{a}
F	1.03	5.46	26.43	15.97	6.73	23.27
P_{sig}	0.43	0.00011	0.00023	0.00014	0.00019	0.00080

a-f are used to indicate that within the same column, the values that donot share a common superscript are significantly different at the level of probability indicated.

and the control diet, pigment, vitamin E and selenium supplementation had negative effects on retinol ester accumulation ($p<0.05$).

Skin and meat pigmentation

The skin and meat pigmentation findings are presented in Tables 8, 9 and 10. Table 8 shows that significant improvements in breast and leg skin and meat redness (a*) were only found in the 25 mg/kg canthaxanthin treatment group ($p<0.001$). In addition, redness (a*) in the 100 mg/kg β-carotene treatment group was significantly lower compared with the 100 mg/kg Lutein Beads and 25 mg/kg canthaxanthin treatment groups (Table 8).

As shown in Table 9, breast and leg skin and meat yellowness (b*) in the 100 mg/kg Lutein Beads, 25 mg/kg apo-ester and 25 mg/kg Xamacol® groups were significantly higher than those in the control, 100 mg/kg Lyco Beads and 100 mg/kg β-carotene groups ($p>0.05$). No differences were found between the breast and leg skin and meat yellowness (b*) of the control, 100 mg/kg Lyco Beads and 100 mg/kg β-carotene groups ($p>0.05$). Although canthaxanthin is a pigment that is used to enhance redness, it also significantly increased meat and skin yellowness (b*) in the broilers that received it as a supplement compared with the control broilers. By contrast, meat and skin yellowness was negatively affected in the 200 mg/kg vitamin E supplemented group and was similar to that of the control group.

Breast and leg skin and meat lightness (L*) were significantly improved in the canthaxanthin treatment group compared with the other treatment groups (see Table 10).

DISCUSSION

Performance

Compared with the corn-and soybean-based control diet, the supplementation with selenium, vitamin E and carotenoids from various sources did not cause any significant differences in the live weight, feed intake

Table 8 – The effects of different sources of antioxidants on skin and meat pigmentation redness (a*), n=6

Treatments	Skin		Meat	
	breast	Leg	breast	leg
Control (Cont)	1.00±0.45^{b}	0.01±0.42^{cb}	-1.70±0.14^{b}	1.49±0.57^{b}
Cont+0.5 Sel-Plex	1.94±0.54^{b}	-0.05±0.32^{cb}	-1.07±0.76^{b}	1.71±0.94^{b}
Cont+200 Vitamin E	1.60±1.28^{b}	-0.58±0.63^{cb}	-1.06±0.62^{b}	1.16±0.47^{b}
Cont+100 Lutein Beads	1.861±0.41^{b}	1.39±0.63^{b}	-0.01±0.22^{b}	2.69±0.48^{b}
Cont+100 Lyco Beads	1.44±0.97^{b}	0.85±1.05^{cb}	-1.00±0.32^{b}	2.26±1.36^{b}
Cont+25 Canthaxanthin	8.92±0.95^{a}	4.20±0.70^{a}	6.57±0.72^{a}	8.38±0.72^{a}
Cont+25 Apo-ester	3.93±1.48^{b}	0.77±0.45^{cb}	0.36±0.51^{b}	1.55±0.71^{b}
Cont+25 Xamacol	3.65±1.86^{b}	0.52±0.77^{cb}	-0.45±0.54^{b}	2.43±0.71^{b}
Cont+100 β-carotene	0.92±0.80^{b}	-0.83±0.48^{c}	-0.09±1.19^{b}	0.33±0.62^{b}
F	5.86	5.12	15.30	9.08
P	<.0001	<.0002	<.0001	<.0001

a-b are used to indicate that within the same column, the valuesthat donot share a common superscript are significantly different at the level of probability indicated.

Table 9 – The effects of different sources of antioxidants on skin and meat pigmentation yellowness (b*) values, n=6

Groups	Skin color		Meat color	
	breast	leg	breast	leg
Control (Cont)	12.93±0.51^{cd}	11.65±0.64^{c}	9.49±0.55^{d}	8.87±0.42^{ed}
Cont+0.5 Sel-Plex	13.91±0.46^{cd}	11.92±0.61^{c}	11.19±0.88^{dc}	11.11±1.68^{ed}
Cont+200 Vitamin E	12.29±0.52^{d}	9.34±0.39^{d}	10.24±0.76^{dc}	9.98±0.62^{ed}
Cont+100 Lutein Beads	21.95±0.59^{a}	19.96±0.81^{a}	17.88±0.83^{a}	17.65±0.65^{a}
Cont+100 Lyco Beads	15.01±1.32^{cbd}	14.72±0.83^{b}	12.37±0.61^{c}	11.45±0.62^{cd}
Cont+25 Canthaxanthin	22.59±1.73^{a}	15.94±1.07^{b}	16.76±1.03^{ba}	15.01±0.88^{b}
Cont+25 Apo-ester	20.71±0.82^{a}	16.33±0.74^{b}	15.03±0.98^{b}	14.97±1.27^{b}
Cont+25 Xamacol	17.42±0.74^{b}	14.91±0.73^{b}	14.75±1.24^{b}	12.63±1.66^{cb}
Cont+100 β-carotene	15.37±0.78^{cb}	10.06±0.55^{c}	12.48±0.34^{c}	8.70±0.90^{e}
F	18.34	18.17	15.16	14.03
p<	0.00012	0.00019	0.00021	0.00049

a-e are used to indicate that within the same column, the values that donot share a common superscript are significantly different at the level of probability indicated.

or feed conversion ratio (FCR) over the course of the 6-week experimental period (p>0.05).

Our findings for selenium are in line with those of Selle *et al.* (2013); Tayeb & Qader (2012); Skrivan *et al.* (2008), who reported that different doses of selenium and vitamin E had no significant effects on live body weight, body weight gain, feed intake, or FCR. Our findings for Se were not in agreement with those of Hoffman (2007); Ozkan *et al.* (2007) or Zelenka & Fajmonova (2005), who noted that the FCR of broilers fed organic Se supplements was improved compared with that of broilers fed un-supplemented diets.

Our findings for vitamin E supplementation are in agreement with those of Bottje *et al.* (1997); Rama Rao *et al.* (2011) who reported that differences in dietary α-tocopheryl acetate concentrations (0, 17, 46 and 87 mg/kg diet) had no effect on broiler performance. By contrast, Chae *et al.* (2006) and Rebole *et al.* (2006) reported that supplementation of 200 mg/kg α-tocopheryl acetate increased body weight gain and the FCR without affecting feed intake.

Pigment supplementation has previously been reported to have no significant effect on feed intake or body weight (Fletcher *et al.,* 1986; Haq *et al.* 1995, 1996; Royle *et al.* 1999; Jensen *et al.* 1998; Perez-Vendrell *et al.,* 2001; Fenoglio *et al.* 2002; Waldenstedt *et al.* 2003; Castañeda *et al.,* 2005; Li *et al.,* 2012).

In contrast with the above performance parameters, our study found that the mortality rate was significantly affected by antioxidant supplementation. The mortality rate of the group that received supplementary β-carotene was significantly higher than those of the 200 mg/kg vitamin E, 100 mg/kg Lutein Beads, 25 mg/kg canthaxanthin and 25 mg/kg Xamacol® groups. These differences may have been caused by differences in the dosages. Our results are in line with those of Siegel *et al.* (2000), who reported day 41 mortality rates of 8.6% 7.5% and 5.4% for chickens that received dietary vitamin E supplementation of 10, 100, and 300 IU/kg per day, respectively. Similarly, Blum *et al.* (1992) reported an increased mortality rate in cocks and hens that received a low level of

Table 10 – The effects of different sources of antioxidants on skin and meat lightness (L*) values, n=6

Treatments	Skin color		Meat color	
	breast	leg	breast	leg
Control (Cont)	65.30±1.19^{a}	64.12±1.31^{a}	56.13±1.34^{a}	55.84±0.97^{a}
Cont+0.5 Sel-Plex	64.96±1.12^{a}	64.21±0.85^{a}	56.95±1.04^{a}	56.85±1.92^{a}
Cont+200 Vitamin E	64.54±0.72^{a}	63.85±0.88^{a}	56.77±0.59^{a}	56.05±1.04^{a}
Cont+100 Lutein Beads	64.46±0.89^{a}	64.00±0.64^{a}	56.98±0.93^{a}	54.90±1.19^{a}
Cont+100 Lyco Beads	64.24±0.07^{a}	64.72±1.09^{a}	56.61±1.97^{a}	55.70±1.15^{a}
Cont+25 Canthaxanthin	57.72±0.59^{b}	58.51±1.07^{b}	47.84±0.36^{b}	46.62±0.54^{b}
Cont+25 Apo-ester	63.95±0.83^{a}	64.18±0.75^{a}	55.33±1.41^{a}	55.79±1.34^{a}
Cont+25 Xamacol	64.17±1.00^{a}	64.74±0.72^{a}	56.81±0.70^{a}	53.59±1.52^{a}
Cont+100 β-carotene	63.95±1.06^{a}	64.29±0.33^{a}	54.15±1.37^{a}	53.05±1.18^{a}
F	6.17	4.62	6.33	6.80
P	0.0001	0.0004	0.0001	0.0001

a-b are used to indicate that within the same column, the values that donot share a common superscript are significantly different at the level of probability indicated.

vitamin E supplementation (20 mg/kg diet) (3.2% and 2.9%, respectively) compared with that of hens that received a higher (160 mg vitamin E/kg diet) level, for which the mortality rates decreased (1.7% and 1.5%, respectively). In other previous animal studies, the survival rates of parrot fish larvae were found to increase with β-carotene supplementation (Tachibana *et al.* 1997), and lycopene was shown to enhance mouse resistance to bacterial infection with *Klebsiella pneumoniae* (Lingen *et al.* 1959). However, in our study, β-carotene and lycopene supplementation did not improve survival rates.

Immune response

The immune responses of the birds were significantly stimulated by all experimental treatments compared with those in the unvaccinated controls. Interestingly, immunity was significantly higher in the vaccinated controls compared with the unvaccinated controls ($p<0.05$), and there were no differences in immune stimulation between the vaccinated control group and the birds fed the Xamacol®- and β-carotene-supplemented diets. It is possible that HI was stimulated by both treatments on day 14 prior to vaccination, which could account for these results. Newcastle disease challenges are often used to assess the immune-stimulating properties of antioxidants. Surai (2006) reported that Se and vitamin E significantly improved the immune responses of chicks vaccinated with a live Newcastle vaccine. Shekaro *et al*. (2012) have reported that the addition of 0.5 ppm organic Se to broiler diets enhanced the immune response against infectious bursal disease, and a number of other studies (El- Sheik *et al.,* 2010; Erf *et al.,* 1998*;* Arshad *et al.*2005; Hegazy & Adachi, 2000; Denghua *et al.,* 2001; Ez-Vendrell *et al.,* 2001) have found that humoral antibody titers increase when feed is supplemented with Se or vitamin E. Our findings that organic selenium supplements (0.5 ppm) significantly increased the 10-day post-vaccination HI antibody titers against NDV (compared with both the unvaccinated and vaccinated controls, as well as the birds given 25 ppm Xamacol® supplements ($p<0.05$), are in agreement with the findings of the studies mentioned above.

In our study, dietary supplementation with 200 mg/kg α-tocopherol significantly increased the antibody response to the NDV vaccine compared with the response in both the unvaccinated and vaccinated controls, as well as that of the broilers given 25 ppm Xamacol® ($p<0.05$). These results are in line with those of a number of studies (Boa-Amponsem *et al.,* 2006; Leshchinky & Klasing, 2001) that have reported significant increases in the humoral immune responses of chickens provided with higher concentrations of dietary α-tocopherols (200-300 mg/kg). By contrast, Rama Rao *et al.* (2011) reported that α-tocopherol (<100 mg/kg) had no significant effect on the humoral immune response to NDV, and Marsh et al. (1981) and Qureshi *et al.* (1993) reported a similar lack of an effect of dietary supplementation with higher levels of α-tocopherol (100-250 mg/kg).

Links between immune function and carotenoids have been experimentally demonstrated (Penissi 2003; Blount *et al.,* 2003; Moller *et al.,* 2000; Camplani *et al.,* 1999; McGraw&Ardia2003; McGraw *et al.* 2006; Aguilera & Amat, 2007; Fitze *et al.,* 2007; Alonso-Alvarez *et al.,* 2004; Schiedt *et al.,* 1985; Sepp *et al.,* 2011). Our results are in agreement with those of several studies (Tengerdy *et al.,* 1990; Cheng *et al.,* 2001;Cucco *et al.,* 2006) that have demonstrated that β-carotene does not effectively protect chickens against *E.coli* or enhance antibody protection; however, our findings conflict with those of Bendich & Shapiro (1986) and Jyonouchi *et al.* (1994), who have reported that β-carotene enhances the immune response in rats. Our findings of the positive effects of other carotenes on the immune system are in line with previous findings indicating that carotenoids have immune-suppressive effects in bird species (Fenoglio *et al.,* 2002; Koutsos *et al.,* 2003a,b; Tengerdy *et al.,* 1990; Blount *et al.,* 2003; Pennisi, 2003; Moller *et al,* 2000; Peters *et al.,* 2004; Klasing, 2007).

Plasma carotene concentrations

With the optimum level of carotenoid supplementation of broiler diets, the degree of carotene accumulation varied according to the supplement provided as follows: Xamacol®>canthaxanthin>apo-ester>vitamin E>selenium>control ($p<0.05$) (Table5).

The significant increases in the plasma total carotenoid concentrations that were observed when the basal diet was supplemented with pigments, as well as with vitamin E and Se, demonstrated the synergistic effects of substances with antioxidant properties. However, unlike the optimum level of carotenoid supplementation (25 mg/kg) in the control diet, Table 5 shows that the total carotenoid accumulation in the plasma of the chickens was only increased by dietary supplementation with 100 µg/kg Lutein Beads and not by high-dose (100 µg/kg diet) carotenoid supplementation (except for the first week over the course of the experiment) compared with that in the control group.

As shown in (Table 5). Lyco Beads and β-carotene had no significant effects on the total plasma carotene concentrations ($p>0.05$). Further research is necessary to explore the differences related to the chemical compositions of lycopene, lutein and β-carotene.

Table 5 shows that the best results for total plasma carotenoid accumulation were obtained with supplementation of 25 mg/kg Xamacol®, followed by 25 mg/kg canthaxanthin, 25 mg/kg apo-ester, 100 mg/kg Lutein Beads and 100 mg/kg β-carotene. These results, which suggest that quality is more important than quantity, are not in agreement with those of Sepp *et al.* (2011), who have shown that differences in carotenoid supplement dosages do not significantly affect plasma carotenoid levels. The low level of plasma carotenoid accumulation found with high doses of supplementary β-carotene in our study might indicate pro-oxidant activity, which would supports the findings of Ruiz *et al.* (1999), who observed antioxidant activity in both the fresh and cooked meat of broilers that were fed diets containing 15 ppm β-carotene and found pro-oxidant activity when the amount was increased to 50 ppm. In a study of rats, Alam & Alam (1983) found that plasma and liver peroxide levels increased as the dose of β-carotene increased, but no beneficial effects were observed. Similar data were reported by Lomnitski *et al.* (1991), who described increases in testes TBARS values as a result of β-carotene dietary supplementation.

Lutein is a carotenoid that constitutes major parts of the avian diet, egg yolk and tissue. It has also been shown to perform better than β-carotene in quenching lipid peroxyl radicals and to be a more efficient antioxidant than α-tocopherol *in vitro* (Chopra *et al.,* 1993). Woodall *et al.* (1996) reported that 37-day-old chickens provided with dietary supplementation of 100 mg/kg of either β-carotene, zeaxanthin, or canthaxanthin had total plasma carotene levels of 3.3, 15.5, and 7.1 µg/mL, respectively, compared with the level of only 2.6 µg/mL that was found in birds fed a commercial diet with no supplements. Their results for β-carotene are very similar to ours, but their findings for canthaxanthin are different than ours (8.18±1.26 µg/mL with 25 mg/kg dietary supplementation). Our findings also showed that Lutein Beads were more efficient than Lyco Beads in transferring dietary carotenoids to the plasma. These results are consistent with previous observations that have indicated that lycopene is much less efficient than other carotenoids in terms of egg yolk deposition (Karadas *et al.,* 2006). Sikder *et al.* (1998) has attributed these differences to the different characteristics of the various types of carotenoids. A previously published lycopene dose-response study has demonstrated the saturation of lycopene absorption at increasing doses (Diwadkar-Navsariwala *et al.,* 2004). Increasing serving sizes of a tomato beverage were administered to adult men; these servings delivered lycopene doses ranging from 10 to 120 mg. Compartmental modeling showed that the lycopene absorption rates for 10, 30, and 60 mg were 34, 14, and 7%, respectively (Novotny *et al.,* 2010). In our study, we found that over the dose range studied, absorption efficiencies decreased with increasing dose.

Studies have revealed much higher carotenoid concentrations in the plasma of wild birds (Surai,2002). For example, Tella *et al.* (1998) investigated plasma carotenoid concentrations in 356 birds from 26 wild species and found concentrations ranging from 0.4 to 74.2 µg/mL, with an average concentration of 9.4 µg/mL. The plasma carotenoid concentrations in white storks were shown to increase from 2.2 µg/mL to 11.1 µg/mL after they were fed a diet of crayfish (Negro *et al.,* 2000). Plasma carotenoid concentrations in wild American Kestrels raised in captivity were found to vary from approximately 4 to 87 µg/mL, depending on the season and bird sex and age (Negro *et al.,* 1998). Plasma carotenoid concentrations in wild shrikes were found to vary from approximately 10 to 20 µg/mL (Bortolotti *et al.,* 1996). Unlike those in the wild birds, the plasma total carotenoid accumulation for the broilers in our study did not exceed 16 µg/mL, regardless of the dose of supplementary carotenoid. Further research should be conducted to better elucidate the effects of dosage.

Liver antioxidant (total carotenoid, vitamin E, and coenzyme Q_{10}) concentrations

The liver is considered the main storage site for fat-soluble antioxidants (carotenoids, vitamins A, E and coenzyme Q_{10}) (Tyczkowski & Hamilton, 1986; Schiedt, 1998, Galvan *et al.,* 2012). The analysis of bird livers performed in the present study showed that the total carotenoid levels in the livers of the birds that received carotenoid dietary supplements were significantly higher than those in the controls ($p<0.05$) (Table 6). The total liver carotenoid levels in the canthaxanthin treatment group were also significantly higher than those in the Lutein Bead and Lyco Bead treatment groups ($p<0.05$). The finding that canthaxanthin accumulates at higher rates than other synthetic red pigments is consistent with reports in the literature (Klasing, 1998; Waldenstedt *et al.,* 2003).

Vitamin E supplementation was found to significantly increase the liver vitamin E concentrations ($p<0.05$); however, the different antioxidant treatments did not significantly affect the liver coenzyme Q_{10} levels (Table 6).

The dietary supplements did not significantly affect the liver retinol levels, but they did have significant effects on the liver retinol ester concentrations ($p<0.05$) (Table 7). The supplementation with β-carotenoid significantly increased the liver R-linoleate and R-oleate concentrations, whereas all of the other treatments significantly decreased the R-oleate concentrations compared with that in the control group. With the exception of β-carotenoid, all of the other dietary supplements decreased liver R-palmitate concentrations compared with that in the control group. Compared with the concentrations in the controls, both apo-ester and β-carotenoid supplementation significantly increased the R-stearate concentrations, which, however, were not affected by the other experimental treatments. Moreover, the R-stearate concentrations in the β-carotenoid treatment group were higher than those in the apo-ester treatment group. The total vitamin A concentration was significantly higher in the β-carotenoid treatment group, whereas the concentrations were significantly lower in all of the other treatment groups compared with the control group, with the exception of the apo-ester treatment group.

These results are in accordance with reports in the literature and indicate that rather than influencing skin pigmentation, β-carotene, which is a precursor of vitamin A, tends to be converted into retinoids (Barua & Olson 2000; Poor *et al.,* 1987), which mainly accumulate in tissues as oxy-carotenoids (Surai, 2002). A study conducted by Yeum & Russell (2002) has demonstrated that orally administered physiological doses of β-carotene are completely converted into vitamin A in the small intestine enterocytes of rats, producing retinoid forms that are incorporated into chylomicrons, secreted into lymph, injected into the blood stream and then delivered to the liver for storage. The direct measurement of liver vitamin A reserves has been described as the gold standard for assessing vitamin A status (Barua & Olson, 2000;Tanumihardjo, 2004; Howe & Tanumihardjo, 2006). However, it has been suggested that the dose may influence the conversion of β-carotene to vitamin A (Brubacher & Weiser 1985).

Skin and meat pigmentation

Whether consumers consider a product attractive will influence their purchasing decisions. The color of raw poultry meat is important in terms of consumer expectations regarding freshness; raw breast meat is expected to be pale pink in color, and raw thigh and leg meat is expected to be dark red (Northcutt, 2009).

Redness (a* values)

Our study found that supplementation of broiler diets with canthaxanthin significantly improved breast and leg skin and meat redness (a*) (Table 8). The leg skin pigmentation a* values in the canthaxanthin treatment group were higher than those in the other treatment groups. However, the a* values in the β-carotene group were lower than those in both the canthaxanthin and Lutein Bead groups. It is possible that rather than contributing to pigmentation, the majority of β-carotenoid accumulated in the liver as retinol and retinol esters, which would explain these findings.

Canthaxanthin is commonly used to provide a yellowish-reddish color to broiler carcasses (Pérez-Vendrell *et al.,* 2001), which is an important factor for consumer acceptance in various countries (Castaneda *et al.,* 2005; Liu *et al.,* 2008). The commercial poultry industry considers an a* value of at least 4 to be desirable (Castaneda *et al.,* 2005). Castaneda *et al.* (2005), who investigated the effects of different pigment types and amounts in broiler diets, reported that a synthetic pigment provided results closest to the commercially prescribed a* value. The canthaxanthin-supplemented group in our study not only met this goal but also had close to twice the minimum recommended commercial a* value.

Tyczkowski & Hamilton (1986) studied the absorption, transport and deposition of canthaxanthinin broilers that were provided with dietary supplements ranging from 5-80 mg/kg feed and found that the canthaxanthin concentrations in the jejunum, large intestine, serum, liver and toe web were directly proportional to those provided in the diet. Our study used the level of canthaxanthin recommended for broiler diets (25 mg/kg) and obtained significant improvements in skin and meat redness values ($p<0.05$).

Canthaxanthin has been reported to accumulate in the tissue and eggs of wild birds such as mallards and gulls (McGraw & Toomey 2010; Surai *et al.,* 2001b; Prager *et al.,* 2009; Surai, 2012), and commercial broiler producers should take this into consideration when contemplating the canthaxanthin supplementation of broiler diets.

Yellowness (b* values)

The present study found b* values ranging from 1 to 9 (Table 9). Bianchi *et al.* (2005) reported yellowness

(b*) values of 6.2 for normal breast meat and 7.6 for pale breast meat. Castroman et al. (2013) reported breast meat and leg meat yellowness values of 16.9 and 16.7, respectively, for organic broilers and 19.7 and 19.1, respectively, for conventional broilers. Although our values are higher than those reported by Bianchi *et al.* (2005), they are very close to those reported by Castroman *et al.* (2013) and other studies in the literature (Waldenstedt *et al.,* 2003; Inborr & Lignell, 1997). Yellowness in breast skin is a good indicator of the xanthophyll content in feed (Pérez-Vendrell *et al.,* 2001), with more intense yellow tones of broiler carcasses suggesting a higher intake of yellow pigments from through the diet (Mourao *et al.,* 2008).

Lightness (L* values)

At 24 hours post-mortem, the L* values in the group that received supplementary canthaxanthin (L*=46-47) were significantly lower than those in all of the other experimental groups in our study (Table 10). The findings for these groups (L*=54-56) are in line with the values (L*=53-57) reported by Castromán *et al.* (2013). Although Van Laack *et al.* (2000) used an L* value of 60 as the limit for pale poultry breast meat, other studies (Petracci *et al.,* 2004; Bianchi *et al.,* 2005; Castromán *et al.,* 2013) used an L* value of 53 as a cut-off to distinguish between pale and normal poultry meat. Accordingly, the values found in the present study would be considered pale, with the exception of the canthaxanthin group, for which the values would be considered normal.

CONCLUSIONS

The present study showed that supplementation of commercial broiler diets with antioxidants significantly improved the immune response and liver antioxidant concentrations. Carotenoids were also found to improve skin and meat pigmentation. Total plasma carotenoid levels were also improved by Se and vitamin E supplementation, indicating the synergic effects of antioxidants. However, the fact that high doses of β-carotene also increased mortality rates must be considered.

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