



Research paper

Dietary astaxanthin enhances immune response in dogs

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ABSTRACT

No information is available on the possible role of astaxanthin on immune response in domestic canine. Female Beagle dogs (9–10 mo old; 8.2 ± 0.2 kg body weight) were fed 0, 10, 20 or 40 mg astaxanthin daily and blood sampled on wk 0, 6, 12, and 16 for assessing the following: lymphoproliferation, leukocyte subpopulations, natural killer (NK) cell cytotoxicity, and concentrations of blood astaxanthin, IgG, IgM and acute phase proteins. Delayed-type hypersensitivity (DTH) response was assessed on wk 0, 12 and 16. Plasma astaxanthin increased dose-dependently and reached maximum concentrations on wk 6. Dietary astaxanthin enhanced DTH response to vaccine, concanavalin A-induced lymphocyte proliferation (with the 20 mg dose at wk 12) and NK cell cytotoxic activity. In addition, dietary astaxanthin increased concentrations of IgG and IgM, and B cell population. Plasma concentrations of C reactive protein were lower in astaxanthin-fed dogs. Therefore, dietary astaxanthin heightened cell-mediated and humoral immune response and reduced DNA damage and inflammation in dogs.

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1. Introduction

Astaxanthin, a keto oxycarotenoid found in high amounts in the carapace of crustaceans, flesh of salmon and trout, and other marine organisms, possesses important biological actions. For instance, astaxanthin is a potent antioxidant (Martin et al., 1999); its antioxidant activity was reported to be higher than that of β -carotene, α -carotene, and lutein (Naguib, 2000), and is higher than α -tocopherol against certain reactive oxygen species (Miki, 1991). Rainbow trout fed astaxanthin-rich red yeast had greater ability to reduce oil-induced oxidative stress (Nakano et al., 1999), lower serum lipid peroxides and

transaminase activity (Nakano et al., 1995). Whether directly or indirectly related to its antioxidant activity, astaxanthin enhanced both humoral (Jyonouchi et al., 1995) and cell-mediated (Chew et al., 1999a) immune responses, and inhibited mammary (Chew et al., 1999b) and bladder (Tanaka et al., 1994) tumor growth in rodents. In *Helicobacter pylori*-infected mice fed astaxanthin-rich algae extract, the bacterial load and gastric inflammation were reduced, apparently due to a shift in T lymphocyte response from Th1 dominated by IFN γ to a mixed Th1/Th2 response with IFN γ and IL-4 (Bennedsen et al., 1999). Park et al. (2010b) reported that domestic dogs and cats absorb astaxanthin into the blood; the astaxanthin is then taken up by all subcellular organelles of blood leukocytes. While previous studies have shown an immuno-enhancing action of dietary lutein (Kim et al., 2000a) and β -carotene (Chew et al., 2000), similar reports on astaxanthin are unavailable. In this study, we used dogs as the animal model to study the possible immuno-modulatory action of dietary astaxanthin.

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2. Materials and methods

2.1. Animals and experimental design

Neutered female Beagle dogs (9–10 mo old; 8.2 ± 0.2 kg body weight) were randomly assigned ($n = 14/\text{treatment}$) to be fed 0, 10, 20, or 40 mg astaxanthin (109 g astaxanthin/kg oleoresin concentrate from *Haematococcus pluvialis*, astraZanthin™, La Haye Laboratories, Redmond, WA) daily for 16 wk. Astaxanthin was incorporated into a commercial basal diet (The Iams Co., Lewisburg, OH) and fed two times daily (200 g food/d). The diet met or exceeded the requirements for all essential nutrients, and contained (g/kg): 85.3 moisture, 275.8 protein, 60.7 ash, 115.0 fat, 9.9 Ca, 9.3 P, 21.3 crude fiber, and 18,914 kJ/kg gross energy; the n-6:n-3 fatty acid ratio was 7.9. Dogs were housed in 2 m × 3 m pens (2 dogs/pen) in a temperature- (20–22 °C) and light- (14 h light) controlled facility. All animals had access to numerous enrichment items and were socialized using human interaction daily. Body weight was recorded on wk 0, 4, 8, and 16. Blood was collected by jugular venipuncture into evacuated tubes containing heparin or EDTA on wk 0, 6 and 12 for assessing immune function. All dogs were vaccinated (Vanguard 5™, Smithkline Beecham, West Chester, PA) on wk 12 and 14 and blood collected on wk 16 to assess post-vaccination immune responses. Dogs were previously vaccinated (distemper, adenovirus, parainfluenza, parvovirus, leptospirosis) approximately 12 mo prior to this study. The research protocol was approved by the Washington State University Institutional Animal Care and Use Committee.

2.2. HPLC

Astaxanthin concentration in plasma was analyzed by HPLC (Alliance 2690 Waters HPLC system fitted with a photodiode array detector, Waters, Milford, MA) as previously described (Park et al., 2010a). Trans-β-apo-8'-carotenal (Sigma Chem. Co., St. Louis, MO) was used as the internal standard.

2.3. Delayed-type hypersensitivity

On wk 12 (before vaccination) and 16 (after vaccination), all dogs were injected intradermally with 100 μL of physiologic saline (negative control), an attenuated polyvalent vaccine containing canine distemper virus, adenovirus type-2, parainfluenza virus and parvovirus (Vanguard 5™, Smithkline Beecham, West Chester, PA), and phytohemagglutinin (PHA, 0.5 g/L saline), as previously described (Chew et al., 2000) to assess cutaneous delayed-type hypersensitivity (DTH). Skin induration was measured at 0, 24, 48 and 72 h post-injection.

2.4. Hematology

Complete blood count (white blood cell, RBC and platelet counts, lymphocyte, monocyte and granulocyte differential counts, hematocrit, hemoglobin, and mean corpuscular volume, hemoglobin and hemoglobin concentration, and platelet volume) was performed on

a hematology analyzer (Vet ABC-Hematology Analyzer, Heska, Fort Collins, CO) using EDTA-treated blood.

2.5. Lymphoproliferation

The proliferation response of peripheral blood mononuclear cell (PBMC) to phytohemagglutinin (PHA, 2 and 10 mg/L final concentration), concanavalin A (Con A; 1 and 5 mg/L), and pokeweed mitogen (PWM; 0.25 and 1.25 mg/L) was assessed on wk 0, 6, 12 and 16 using whole blood cultures (Chew et al., 2000). Whole blood was cultured in order to mimic *in vivo* conditions.

2.6. Leukocyte subset

Monoclonal antibodies (mAbs) used to quantitate canine lymphocyte subsets were purchased from Dr. Peter Moore (UC Davis, CA). The mAb used were specific for the following lymphocyte subsets: CD3+ (CA17.2A12, total T cells), CD4+ (CA13.1E4, helper T cells, Th), CD8+ (CA9.JD3, cytotoxic T cells, Tc), MHC II+ (CA2.1C12) and CD21+ (CA2.ID6, mature B cells). Isolated cells were stained as previously described (Kim et al., 2000a,b) and single-color flow cytometry was used to determine the changes in lymphocyte subsets on wk 0, 6, 12 and 16.

2.7. IgG and IgM

Concentrations of IgG and IgM in plasma were analyzed by ELISA (Bethyl Lab., Inc., Montgomery, TX).

2.8. Natural killer cell cytotoxic activity

Canine thyroid adenocarcinoma cells (target cells) were resuspended to 2×10^5 cells/mL in DMEM (Sigma, St. Louis, MO) containing 100 mL/L fetal bovine serum, 100,000 U/L penicillin and 100 mg/L streptomycin sulfate. PBMC (effector cells) were resuspended to 1×10^6 and 2×10^6 cells/mL and 100 μL added to the target cells in 96-well flat-bottom plates to provide effector:target ratios of 5:1 and 10:1. After incubating for 8 h, 20 μL (5 g/L) of MTT were added, incubated 4 h, supernatant removed and the formazan resuspended in 100 μL isopropanol. Optical density was measured at 550 nm and the percent of specific cytotoxicity calculated.

2.9. C-reactive protein

Changes in acute phase proteins were assessed in plasma by measuring C-reactive protein (CRP) using in a solid-phase sandwich immunoassay (Tri Delta Diagnostics, Morris Plains, NJ).

2.10. DNA oxidative damage

Oxidative damage was assessed by measuring 8-hydroxy-2'-deoxyguanosine (8-OHdG) in plasma with a commercial ELISA (BIOXYTECH™ 8-OHdG-EIA Kit, Oxis-Research, Portland, OR).

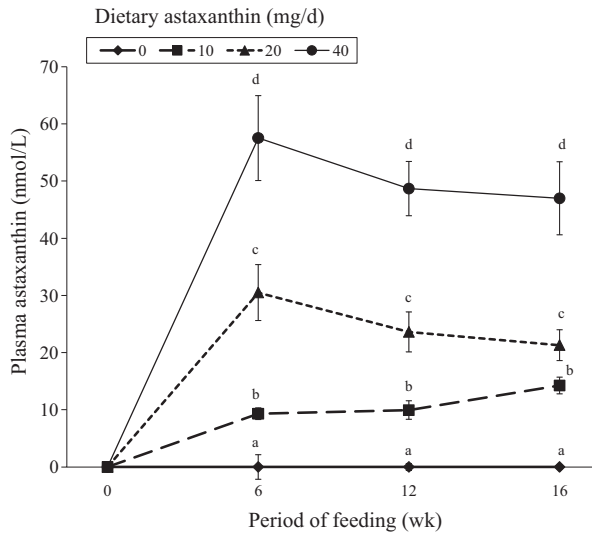


Fig. 1. Plasma astaxanthin concentrations in dogs fed diets containing 0, 10, 20 or 40 mg astaxanthin for 16 wk. Values are means \pm SEM ($n = 14$). Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

2.11. Statistics

Data were analyzed by repeated measures ANOVA using the General Linear Model of SAS (1991). Differences among treatment means were compared by a protected LSD test and considered different at $P < 0.05$.

3. Results

3.1. Animals and diet

Diet did not significantly influence body weight throughout the study; average body weight for all dogs on wk 0 and 16 was 8.18 ± 0.16 and 8.57 ± 0.11 kg, respectively. Astaxanthin was not detectable in the plasma of all dogs prior to supplementation (Fig. 1). However, astaxanthin increased ($P < 0.05$) in a dose-dependent manner, with maximal astaxanthin concentrations in blood observed by wk 6 in all supplemented dogs.

3.2. Delayed-type hypersensitivity

Diet did not significantly influence skin induration response to saline, PHA or vaccine on wk 0 and 8 (not shown). However, on wk 12, all dogs fed astaxanthin had higher ($P < 0.05$) DTH response at 48 and 72 h after an intradermal challenge with the vaccine (Fig. 2). After the dogs were vaccinated on wk 12 and 14, a significant earlier DTH response was observed at 24 and 48 h. No similar heightened post-vaccination response was observed with PHA.

3.3. Hematology

Blood hematology generally showed no dietary effects throughout the study (Table 1).

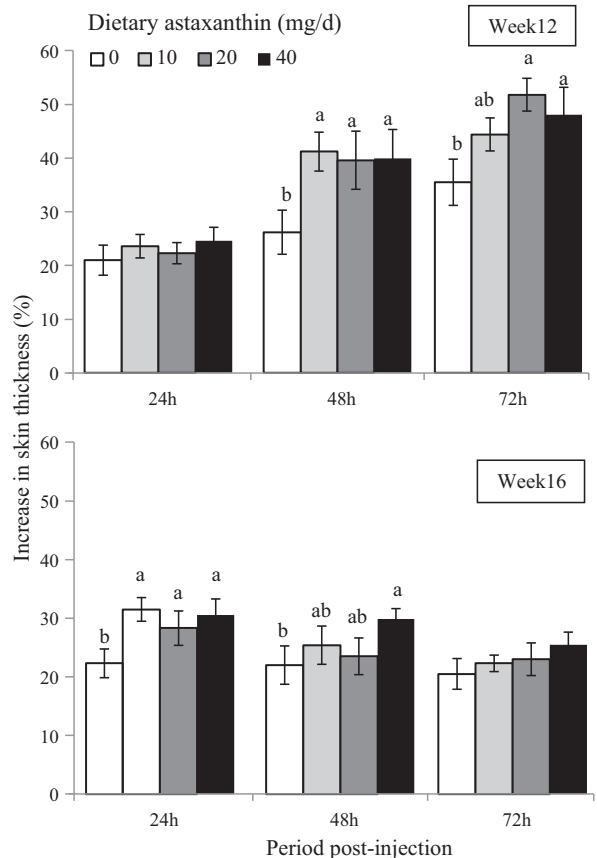


Fig. 2. Delayed-type hypersensitivity response (expressed as a percentage of skin thickness measured at 0 h) to an intradermal injection with a polyvalent vaccine in dogs fed 0, 10, 20 or 40 mg astaxanthin daily for 12 (top panel) or 17 (bottom panel) wk. Skin induration was measured at 0, 24, 48 and 72 h post-injection. Values are means \pm SEM ($n = 14$). Data were analyzed by repeated measures ANOVA. ^{a,b,ab}Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

3.4. Lymphoproliferation

Dogs fed 20 mg astaxanthin had higher ($P < 0.05$) Con A-induced PBMC proliferation compared to unsupplemented dogs on wk 12. However, astaxanthin did not influence changes in PHA- or PWM-stimulated PBMC proliferation during the study (Table 2).

3.5. Phenotyping

Dietary astaxanthin generally increased ($P < 0.05$) the population of B cells at wk 12 in a dose-dependent manner (Table 3). On wk 16 (post-vaccination), dogs fed 20 mg astaxanthin had the highest B cell population. Astaxanthin did not influence changes in the populations of CD4⁺, CD8⁺, and MHC class II⁺ cells throughout the study (Table 3).

3.6. Immunoglobulin production

Concentration of plasma IgG increased ($P < 0.05$) on wk 12 in dogs fed 20 mg astaxanthin and again post-vaccination on wk 16 (Fig. 3). Higher (40 mg) dietary

Table 1
Blood hematology results for dogs fed 0, 10, 20 or 40 mg astaxanthin for 16 wk.

Variable	Dietary astaxanthin (mg/d)			
	0	10	20	40
WBC	9.26 ± 0.5	9.80 ± 0.4	9.39 ± 0.6	9.79 ± 0.5
RBC	6.78 ± 0.1	6.81 ± 0.2	6.81 ± 0.2	6.73 ± 0.2
HGB	15.9 ± 0.3	15.9 ± 0.5	15.9 ± 0.4	15.7 ± 0.4
HCT	46.4 ± 1.1	46.5 ± 1.2	46.5 ± 1.2	45.7 ± 1.4
MCV	68.6 ± 0.4	68.4 ± 0.4	68.1 ± 0.4	67.9 ± 0.6
MCH	23.5 ± 0.2	23.6 ± 0.2	23.4 ± 0.1	23.4 ± 0.2
MCHC	34.3 ± 0.2	34.5 ± 0.2	34.3 ± 0.1	34.4 ± 0.2
RDW	13.9 ± 0.2	13.9 ± 0.2	13.9 ± 0.1	13.8 ± 0.2
PLT	257 ± 19	350 ± 13	366 ± 16	366 ± 33
MPV	8.27 ± 0.2	8.22 ± 0.2	7.99 ± 0.2	8.09 ± 0.2
LYM%	12.2 ± 0.9	12.1 ± 1.1	12.6 ± 1.0	12.5 ± 0.6
LYM10 ³ /mm ³	1.09 ± 0.1	1.13 ± 0.1	1.36 ± 0.1	1.24 ± 0.1
MON%	3.85 ± 0.3	3.78 ± 0.2	3.98 ± 0.3	3.80 ± 0.2
MON10 ³ /mm ³	0.30 ± 0.0	0.31 ± 0.0	0.31 ± 0.0	0.31 ± 0.0
GRA%	84.0 ± 1.1	84.1 ± 1.2	81.4 ± 5.7	83.7 ± 0.8
GRA10 ³ /mm ³	7.93 ± 0.4	8.44 ± 0.3	8.07 ± 0.6	8.17 ± 0.4

Values are means ± SEM (*n* = 14).

astaxanthin did not produce the same stimulation of IgG production. Overall, vaccination on wk 12 and 14 generally increased plasma IgM concentrations approximately 300% in all dogs. Even though plasma IgM concentration was not influenced by dietary astaxanthin pre-vaccination (wk 0–12), concentrations were higher (*P* < 0.05) in dogs fed 20 mg astaxanthin post-vaccination (wk 16, Fig. 3). At the commencement of the study, concentrations of IgG and IgM were similar among treatment groups.

3.7. Natural killer cell cytotoxic activity

Dietary astaxanthin produced a dose-dependent increase (*P* < 0.05) in NK cell activity on wk 6; dogs fed 40 mg astaxanthin had a significantly higher response (*P* < 0.05) than dogs in control (Fig. 4). On wk 12, only dogs fed 20 mg astaxanthin had higher (*P* < 0.05) NK cell cytotoxic activity than control. This same trend continued through wk 16. No treatment difference was observed on wk 0.

Table 2
Mitogen-induced proliferation response in dogs fed 0, 10, 20 or 40 mg astaxanthin for 16 wk.

Mitogen	Wk	Dietary astaxanthin (mg/d)			
		0	10	20	40
PHA	0	9234 ± 1557	7202 ± 955	6282 ± 873	7656 ± 1204
	6	11667 ± 1518	8071 ± 676	14416 ± 2466	10451 ± 3160
	12	20646 ± 2062	15796 ± 2115	23052 ± 3971	15845 ± 2337
	16	11610 ± 1348	7590 ± 807	12491 ± 1560	13190 ± 3550
Con A	0	10633 ± 1574	6375 ± 816	7548 ± 1115	9073 ± 1130
	6	14802 ± 1569	11145 ± 1786	18336 ± 2239	11457 ± 2242
	12	16531 ± 2043	11104 ± 1738	22723 ± 3208*	12347 ± 1594
	16	12876 ± 1877	9508 ± 1525	17051 ± 2683	15685 ± 3114
PWM	0	18742 ± 2339	15942 ± 1555	17889 ± 1426	16005 ± 1760
	6	23039 ± 3081	15410 ± 1332	23856 ± 2953	13749 ± 1713
	12	20008 ± 2799	16338 ± 1928	22715 ± 2393	14789 ± 1659
	16	17490 ± 2816	12733 ± 1015	16358 ± 2240	14129 ± 2098

Values are means ± SEM (*n* = 14).

* Statistically different (*P* < 0.05) from wk 0 as analyzed by ANOVA.

3.8. C-reactive protein

Concentrations of plasma CRP were similar through wk 12 of the study with an average of 4.48 mg/L (Fig. 5). However, plasma C-reactive protein concentrations were lower (*P* < 0.05) on wk 16 in all dogs fed astaxanthin.

3.9. DNA oxidative damage

There was no dietary influence on the concentration of 8-OHdG on wk 12 (Fig. 6). As with CRP, dietary astaxanthin inhibited (*P* < 0.05) plasma 8-OHdG production of astaxanthin-supplemented dogs. There was no further decrease in plasma 8-OHdG concentrations in dogs fed 40 mg astaxanthin.

4. Discussion

Astaxanthin enhanced both cell-mediated and humoral immune responses in dogs. Daily doses 10–40 mg astaxan-

Table 3
Phenotyping of lymphocyte subpopulations response in dogs fed 0, 10, 20 or 40 mg/d astaxanthin for 16 wk.

Phenotype	Wk	Dietary astaxanthin (mg/d)			
		0	10	20	40
Total T (%)	0	82.2 ± 1.7	78.9 ± 1.9	78.4 ± 2.4	77.5 ± 1.9
	6	82.8 ± 2.4	77.7 ± 2.0	78.6 ± 2.7	76.0 ± 2.0
	12	79.8 ± 3.1	77.2 ± 1.4	77.6 ± 3.0	77.2 ± 2.2
	16	77.5 ± 3.9	75.9 ± 2.2	71.8 ± 5.4	70.6 ± 5.9
Total Th cells (%)	0	56.2 ± 2.5	53.7 ± 1.7	52.5 ± 2.1	52.4 ± 2.0
	6	55.4 ± 2.4	52.2 ± 1.9	53.4 ± 2.4	51.0 ± 1.9
	12	54.6 ± 2.8	51.2 ± 1.9	50.7 ± 2.8	49.5 ± 1.8
	16	51.7 ± 3.8	50.1 ± 1.8	52.3 ± 2.1	52.7 ± 1.7
Total Tc cells (%)	0	18.0 ± 4.0	25.0 ± 7.0	22.0 ± 4.0	28.0 ± 8.0
	6	14.0 ± 0.2	12.0 ± 2.0	10.0 ± 2.0	10.0 ± 2.0
	12	11.0 ± 2.0	7.0 ± 1.0	10.0 ± 2.0	8.0 ± 2.0
	16	27.0 ± 4.0	30.0 ± 5.0	26.0 ± 4.0	53.0 ± 32.0
Total B cells (%)	0	12.9 ± 1.6	14.7 ± 1.4	15.3 ± 2.2	16.2 ± 1.2
	6	17.3 ± 2.4	21.5 ± 1.9	22.2 ± 2.6	22.9 ± 1.7
	12	16.8 ± 2.6	18.0 ± 1.5	18.5 ± 2.5	20.1 ± 1.7*
	16	19.3 ± 2.9	21.3 ± 1.9	23.1 ± 2.1	22.3 ± 1.5
MHCII+ cells (%)	0	99.3 ± 0.1	99.3 ± 0.2	99.4 ± 0.2	99.6 ± 0.1
	6	97.8 ± 0.6	93.7 ± 5.2	98.0 ± 0.5	90.9 ± 6.3
	12	99.1 ± 0.2	99.1 ± 0.1	98.8 ± 0.2	99.1 ± 0.1
	16	99.1 ± 0.4	98.3 ± 0.8	99.1 ± 0.2	99.2 ± 0.2

Values are means ± SEM (n = 14).

* Significantly different from control, $P < 0.05$.

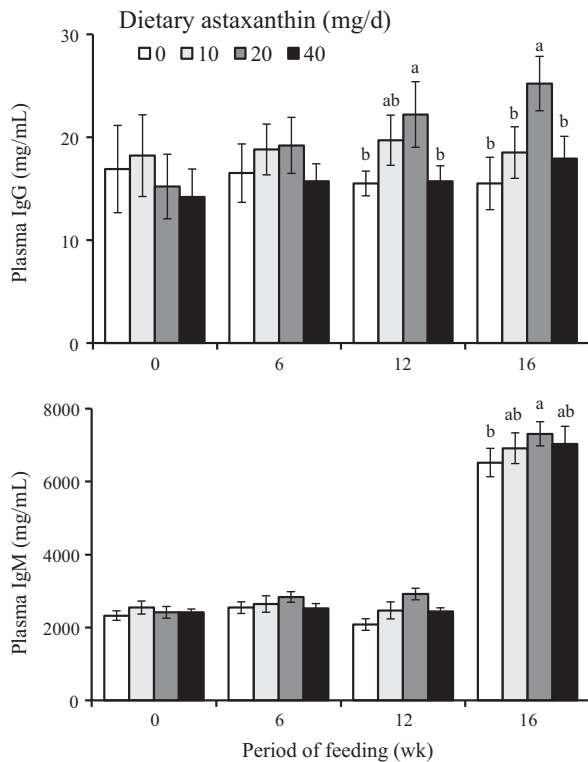


Fig. 3. Concentrations of plasma IgG (top panel) and IgM (bottom panel) in dogs fed 0, 10, 20 or 40 mg astaxanthin daily for 16 wk. All dogs were vaccinated with a polyclonal vaccine on wk 13 and 15. Values are means ± SEM (n = 14). Data were analyzed by repeated measures ANOVA. ^{a,b,ab}Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

thin increased DTH response to specific antigens (vaccine) both before and after the dogs were vaccinated. However, the overall skin induration response was more rapid post-vaccination than before vaccination. We have reported similarly heightened DTH responses in dogs fed β -carotene (Chew et al., 2000) and lutein (Kim et al., 2000a), in cats fed astaxanthin (Chew et al., 2004), β -carotene (Park et al., 2004), and lutein (Kim et al., 2000b), and in humans administered astaxanthin (Park et al., 2010a). The cutaneous response is a reliable clinical test to assess in vivo T-cell function in dogs (Miyamoto et al., 1995). Therefore, astaxanthin (a ketocarotenoid) joins β -carotene (a nonpolar carotenoid) and lutein (a hydroxycarotenoid) in enhanc-



Fig. 4. Natural killer cell cytotoxic activity in dogs fed 0, 10, 20 or 40 mg astaxanthin daily for 16 wk. Values are means ± SEM (n = 14). Data were analyzed by repeated measures ANOVA. ^{a,b,ab}Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

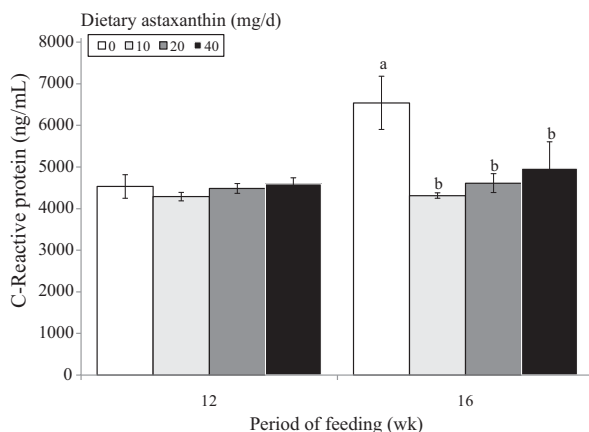


Fig. 5. Concentrations of plasma C-reactive proteins in dogs fed 0, 10, 20, or 40 mg astaxanthin daily for 16 wk. All dogs were vaccinated with a polyclonal vaccine on wk 12 and 14. Values are means \pm SEM ($n = 14$). Data were analyzed by repeated measures ANOVA. ^{a,b}Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

ing T cell immune response in dogs, as assessed by DTH test.

The heightened DTH response is consistent with a higher lymphocyte proliferative response to Con A, a T-cell mitogen. The highest blastogenic response was observed with 20 mg astaxanthin. Higher mitogen-induced lymphocyte blastogenic responses have also been observed in dogs fed lutein (Kim et al., 2000a), and in cats fed β -carotene (Park et al., 2004) and lutein (Kim et al., 2000b). In humans, dietary astaxanthin increases T and B cell mitogen-induced PBMC proliferation (Park et al., 2010a). Similarly, higher PHA-induced splenocyte proliferation ex vivo (Chew et al., 1999a) and splenocyte and thymocyte proliferation in vitro

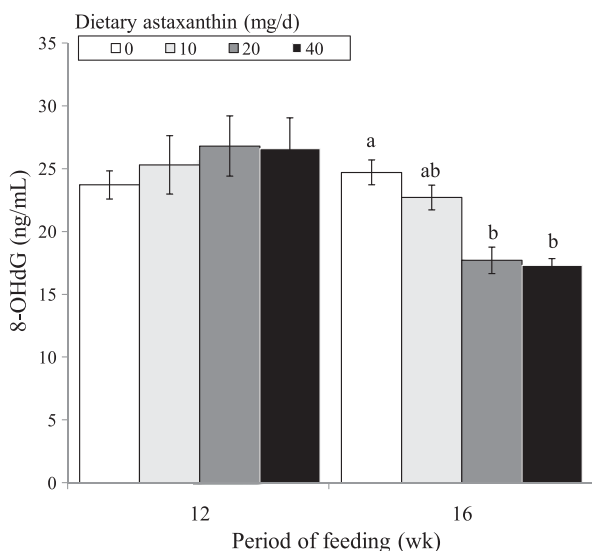


Fig. 6. Concentrations of plasma 8-hydroxy-2'-deoxyguanosine (8-OHdG) in dogs fed 0, 10, 20, or 40 mg astaxanthin daily for 16 wk. All dogs were vaccinated with a polyclonal vaccine on wk 12 and 14. Values are means \pm SEM ($n = 14$). Data were analyzed by repeated measures ANOVA. ^{a,b,ab}Means within a sampling period with different superscripts differ significantly, $P < 0.05$.

(Okai and Higashi-Okai, 1996) were reported in mice. In this study, dietary astaxanthin also increased NK cell cytotoxic activity. Others have shown that astaxanthin increased NK cell cytotoxic activity in humans (Park et al., 2010a), increased murine cytotoxic T lymphocyte activity (Jyonouchi et al., 2000), and inhibited stress-induced suppression of murine NK cell activity (Kurihara et al., 2002). The adaptive branch of the immune system also is regulated by NK cells that serve as an immuno-surveillance system against tumors and virus-infected cells; therefore, astaxanthin may play a role in cancer etiology. Indeed, tumor immunity against Meth-A tumor antigen has been reported in mice fed astaxanthin (Jyonouchi et al., 1994). In addition to cell-mediated immune response, dietary astaxanthin also stimulated humoral immunity. Astaxanthin increased IgG production both pre- (wk 12) and post-vaccination (wk 16) but increased IgM production after vaccination only. The greatest response was observed in dogs fed 20 mg astaxanthin. Cats fed astaxanthin also showed higher ex vivo antibody response (Chew et al., 2004). Astaxanthin partially restored humoral immune response in old mice (Bejma and Ji, 1999), enhanced Ig production in response to T-dependent stimuli in human blood cells (Jyonouchi et al., 1995) and induced production of polyclonal antibody G and M in murine spleen cells (Okai and Higashi-Okai, 1996). Interestingly, we previously reported higher antibody production in dogs fed 20 mg β -carotene (Chew et al., 2000) or 20 mg lutein (Kim et al., 2000a).

The enhanced immune status of dogs fed astaxanthin may explain the lower concentrations of circulating CRP in these animals after the vaccination challenge. Blood CRP production generally increases in response to infection, inflammation and other disease states involving tissue necrosis (Griselli et al., 1999; Lopez-Garcia et al., 2005; Smolen et al., 2006), all of which may be indicative of high oxidative stress. In fact, this study and others (Park et al., 2010a,b) showed that dietary astaxanthin reduced DNA damage as measured using the oxidative biomarker 8-OHdG. Because of the high content of polyunsaturated fatty acids in the membranes, immune cells are particularly sensitive to oxidative damage by free radicals. Reactive oxygen species (ROS) are produced via the mitochondria electron transport system during ATP production, as well as xanthine oxidase and phagocytes (Bejma and Ji, 1999; Ji, 1999). Cumulative oxidative damage to the mitochondria is considered the main culprit of cell senescence which in turn is responsible for aging and the development of age-related chronic diseases (Gershon, 1999). On the other hand, astaxanthin is a potent antioxidant (Martin et al., 1999; Naguib, 2000). Astaxanthin is approximately 100 fold more protective than lutein and β -carotene against UVA-induced oxidative stress in vitro (O'Connor and O'Brien, 1998). It can attenuate exercise-induced neutrophil infiltration and subsequent delayed-onset damage to the skeletal and cardiac muscle in mice (Aoi et al., 2003). Astaxanthin also decreased gastric inflammation in mice infected with *Helicobacter pylori* by shifting the T-lymphocyte response from a Th1 response dominated by IFN- γ to a Th1/Th2 response dominated by IFN- γ and IL-4 (Bennedson et al., 1999). The action of ROS is likely mediated through its ability to induce NFkB and AP-1 which are redox-sensitive transcrip-

tion factors that regulate the production of chemokines, inflammatory cytokines, and adhesion molecules (Aoi et al., 2003). Therefore, astaxanthin can decrease inflammation by inhibiting ROS-induced production of NF κ B and AP-1.

Reactive nitrogen species also play an important role in inflammation. Astaxanthin decreased nitric oxide (NO) production and iNOS activity in mouse macrophages, resulting in the inhibition of COX, which down-regulates the production of PGE₂ and TNF- α (Ohgami et al., 2003). TNF- α is a pleiotropic cytokine produced by activated macrophages and monocytes. The TNF- α and IL-1 cascade activates p38 MAPK, thus promoting proinflammatory gene expression and cytokine production. Therefore, in this study, astaxanthin may be exerting its anti-inflammatory action by inhibiting reactive oxygen and nitrogen species.

Maximum concentration of astaxanthin in the plasma of dogs fed 40 mg astaxanthin reached was 0.06 μ mol/L, and was observed by wk 6. Recently we (Park et al., 2010b) reported that plasma astaxanthin concentration in dogs fed 40 mg astaxanthin daily for 16 d was 0.2 μ mol/L. This discrepancy in uptake efficiency is likely due to differences in the source of the astaxanthin used in the two studies. The astaxanthin complex used in this study is from *Haematococcus pluvialis* and exists primarily in an esterified 3S,3'S stereoisomer while synthetic astaxanthin (Park et al., 2010b) contains primarily the 3R,3'S form. Human subjects fed 2–8 mg astaxanthin daily for 4 wk had plasma astaxanthin concentrations of 0.09–0.13 μ mol/L while subjects administered 100 mg astaxanthin once per orally had plasma concentrations of 2.3 μ mol/L (Osterlie et al., 2000). Furthermore, astaxanthin in the blood was present primarily in the HDL in both dogs and cats (Park et al., 2010a,b) while in humans, most of the astaxanthin is found in the VLDL chylomicra, with lesser amounts in the LDL and HDL (Osterlie et al., 2000). High density lipoprotein is the major lipoprotein in the blood of dogs and cats.

Indeed, Chew et al. (Park et al., 2010b) reported that lymphocyte mitochondria accounted for 40–50% of total astaxanthin taken up by blood leukocytes in dogs, while the microsomes and nuclei also take up significant amounts of astaxanthin, suggesting that astaxanthin is strategically located to exert its antioxidant function in the cell. For instance, the mitochondria produce large quantities of reactive oxygen species, and changes in mitochondrial and plasma membrane potential can influence cell-mediated immune function (Shigenaga et al., 1994).

In summary, dietary astaxanthin enhanced cell-mediated and humoral immune response, and exerted its antioxidant action by decreasing DNA damage and acute phase protein production. Dietary amount of 20 mg or less seems to provide adequate immune protection in the event of stress.

Conflict of interest

The authors declare that they have no conflicts of interest.

Contributions

J.S.P. and B.P.C. designed research, analyzed data, and wrote the paper; J.S.P., B.P.C., B.D.M. conducted research; M.G.H., S.M., G.A.R. provided essential materials; B.P.C. had primary responsibility for final content. All authors read and approved the final manuscript.

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