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Dietary lutein stimulates immune response in the canine

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Abstract

The possible immuno-modulatory action of dietary lutein in dogs is not known. Female Beagle dogs (17–18-month old; 11.4±0.4 kg body weight) were supplemented daily with 0, 5, 10 or 20 mg lutein for 12 weeks. Delayed-type hypersensitivity (DTH) response to saline, phytohemagglutinin (PHA) and a polyvalent vaccine was assessed on Weeks 0, 6 and 12. Blood was sampled on Weeks 0, 2, 4, 8 and 12 to assess (1) lymphocyte proliferative response to PHA, concanavalin A (Con A), and pokeweed mitogen (PWM), (2) changes in peripheral blood mononuclear cell (PBMC) populations, (3) interleukin-2 (IL-2) production and (4) IgG and IgM production. After the completion of 12-week study, we continued to collect the blood weekly up to 17 weeks to evaluate the changes in immunoglobulin production upon first and second antigenic challenges on Weeks 13 and 15. Plasma lutein+zeaxanthin was undetectable in unsupplemented dogs but concentrations increased ($P<0.05$) rapidly on Week 2 in lutein-supplemented dogs. Thereafter, concentrations generally continued to increase in dose-dependent manner, albeit at a much slower rate. Dogs fed lutein had heightened DTH response to PHA and vaccine by Week 6. Dietary lutein increased ($P<0.05$) lymphocyte proliferative response to all three mitogens and increased the percentages of cells expressing CD5, CD4, CD8 and major histocompatibility complex class II (MHC II) molecules. The production of IgG increased ($P<0.05$) in lutein-fed dogs after the second antigenic challenge. Lutein did not influence the expression of CD21 lymphocyte marker, plasma IgM or IL-2 production. Therefore, dietary lutein stimulated both cell-mediated and humoral immune responses in the domestic canine.

Keywords: Canine; Lutein; Immunity; Lymphocyte; Vaccine; DTH

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1. Introduction

The importance of carotenoids in promoting health is well documented in both humans (Burri, 1997) and animals (Chew, 1995a). Evidence has accumulated showing that carotenoids can modulate immune response (Chew, 1993; Mayne, 1996). Most studies have concentrated on the biological action of β-carotene (Bendich and Shapiro, 1986; Chew, 1987; Prabhala et al., 1991). However, more recent studies have begun to address the importance of other carotenoids, including lutein.

Lutein is abundant in nature and is a major carotenoid found in the plasma of human and certain animals. Recently, we reported that dogs can absorb lutein from the diet and circulating lutein is subsequently taken up by blood lymphocytes (Cerveny et al., 1998). Lutein has been shown to inhibit mammary tumor growth in mice (Chew et al., 1996; Park et al., 1998) and prevent lipid peroxidation (Stahl et al., 1998). Lutein is also known to possess immune regulatory activity. Mice fed lutein had increased phytohemagglutinin (PHA)-stimulated lymphocyte proliferation response (Chew et al., 1996) and enhanced antibody production in response to a T cell dependent antigen (Jyonouchi et al., 1994).

However, no study is available on the immunomodulatory action of dietary lutein in dogs. Therefore, our objective is to study the possible immuno-modulatory action of dietary lutein in the domestic canine.

2. Materials and methods

2.1. Animals and diet

Fifty-six female Beagle dogs (17–18-month old, mean body weight 11.4±0.4 kg; Summit Ridge Farms, Susquehanna, PA) were randomly assigned to be supplemented daily with 0, 5, 10 or 20 mg lutein (FloraGlo™ crystalline lutein, Kemin Industries Inc., Des Moines, IA) for 12 weeks. After completion of 12-week study, the experimental period was extended up to 17 weeks to evaluate the changes in immunoglobulin (Ig) production upon antigenic challenge. The lutein supplement contained 76.66% lutein and 5.23% zeaxanthin (Kemin Foods, Des Moines, IA) and was resuspended in soybean oil to the appropriate concentration and administered perorally at 0800 h daily. Food (200 g/dog/day) was offered immediately after the lutein supplementation. The basal diet (Table 1) met

Table 1
Composition of the basal diet

Item	g/kg
Moisture	66
Protein	262
Ash	74
Fat	160
Calcium	14.8
Phosphorus	10.3
Gross energy (kcal/kg)	4783

or exceeded the requirements for all essential nutrients (NRC, 1985). All dogs were housed in 2 m×2 m pens (2 dogs/pen) in a temperature (20–22°C) and light (14 h light)-controlled facility. Body weight was recorded at Weeks 0, 6, and 12. The research facility is accredited by the American Association for the Accreditation of Laboratory Animal Care and the research protocol was approved by the Washington State University, Institutional Animal Care and Use Committee. Blood was collected by jugular venipuncture into heparinized evacuated tubes on Weeks 0, 2, 4, 8 and 12 and aliquots used for HPLC analysis and for assessing immune responses (described later).

2.2. Extraction and HPLC analysis

Plasma was extracted for analysis of lutein, zeaxanthin, retinol and α-tocopherol (Alliance 2690, Waters, Milford, MA) as previously described (Park et al., 1998). Plasma was extracted using a 1:1 mixture of petroleum ether:anhydrous diethyl ether. The mobile phase was a 47:47:6 (v/v/v) mixture of HPLC-grade acetonitrile:methanol:chloroform (Fisher Scientific, Fair Lawn, NJ). The identity of the eluted compounds was confirmed by comparing their absorption spectrum with that of pure standards on a photodiode array detector (Waters, Milford, MA). Because baseline separation of lutein and zeaxanthin was not accomplished, plasma concentrations are reported as lutein+zeaxanthin.

2.3. Delayed-type hypersensitivity (DTH) response

Skin induration response was assessed in all dogs on Weeks 0, 6, and 12. Dogs were injected intradermally in the flank area with saline (8.5 mg/ml; control), an attenuated polyvalent vaccine containing canine distemper virus, adenovirus type-2, parainfluenza virus and parvovirus (Vanguard 5^{TM}, Smithkline Beacham, West Chester, PA; specific antigen), and PHA (0.5 mg/ml; non-specific antigen). All animals were vaccinated with the same vaccine prior to the commencement of the study. This technique has previously been used to assess cell-mediated immunity in dogs (Miyamoto et al., 1995) and cats (Otto et al., 1993). The doses of vaccine and PHA used were previously determined to provide optimal skin response in Beagle dogs of similar age. The injection site was clipped and wiped with 70% ethyl alcohol. The injection volume was 100 μl. Skin induration was measured at 0, 24, 48 and 72 h post-injection with the aid of a pressure-sensitive digital micrometer (Mitsutoyo, Tokyo, Japan) and response was expressed as a percentage of skin thickness measured at 0 h.

2.4. Mitogen-induced peripheral blood mononuclear cell (PBMC) proliferation

Blood collected on Weeks 0, 2, 4, 8 and 12 were used to assess mitogen-induced lymphocyte proliferation by PBMC. Whole blood culture was used to mimic in vivo conditions. Mitogens used were PHA, concanavalin A (Con A) and pokeweed mitogen (PWM). Whole blood was thoroughly mixed and then diluted 1:12 with RPMI-1640 containing 25 mM Hepes, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Sigma, St. Louis, MO). Preliminary studies using undiluted blood and blood diluted 1:2, 1:4, 1:8,

1:12 and 1:16 showed optimal PBMC proliferative response with a 1:12 dilution. Triplicate 150 μl volumes were pipetted into 96-well round bottom plates and 50 μl of the appropriate mitogens added. The final concentrations of the mitogens in culture were 10 and 2 μg/ml for PHA, 5 and 1 μg/ml for Con A and 2.5 and 0.5 μg/ml for PWM, representing higher and lower mitogen concentrations, respectively. The mixture was incubated for 72 h at 37°C in a humidified incubator under 5% CO_2 atmosphere. A time of 4 h prior to the termination of the incubation, 20 μl of [^{3}H]-thymidine (1 μCi/well) was added. Cells were harvested and radioactivity was counted by liquid scintillation. The proliferative response of PBMC was expressed as stimulation indices (SI), which were calculated as follows:

$$\mathrm{SI} = \text{Mean counts per minute (cpm) of stimulated cultures} / \text{mean cpm of unstimulated cultures}$$

2.5. Monoclonal antibodies and flow cytometric analysis

Mouse monoclonal antibodies (mAbs) used to quantitate the canine lymphocyte subsets were purchased from VMRD (Pullman, WA). The mAb used were specific for the following lymphocyte subsets: anti-CD5 (DH3B, total T cells), anti-CD4 (DH29A, helper T cells), anti-CD8 (CADO46A, cytotoxic T cells), anti-MHC class II (CAT82A) and anti-CD21 (F46A, mature B cells). Single color flow cytometry was used to determine the changes in lymphocyte subsets. Briefly, PBMC were separated using Histopaque-1119 (Sigma, St. Louis, MO) and resuspended to 1×10^7 cells/ml in phosphate buffered sline (PBS) supplemented with 2% gamma globulin-free horse serum, 5% goat serum and 0.2 mg/ml sodium azide. For immunofluorescence analysis, a total of 5×10^5 cells were incubated for 30 min on ice with mouse anti-canine lymphocyte mAbs. Cells were then washed three times and labeled with fluorescein isothiocyanate (FITC)-conjugated goat $F(ab')_2$ anti-mouse IgG+IgM (H+L) (Caltag, Burlingame, CA) to visualize the bound mAb. Stained cells were fixed in 4% paraformaldehyde in preparation for acquisition and determined by flow cytometer (FACScan, Becton Dickinson, San Jose, CA) after gating the lymphocyte population by forward and side scatter analysis. For each sample, 5000 total events were acquired for analysis. Appropriate negative controls were included to correct for background fluorescence. Data were expressed as the percentage of positive-staining cells corrected for cells stained non-specifically with the secondary antibody.

2.6. Interleukin-2 (IL-2) production

Whole blood was diluted 1:2 with RPMI-1640 and cultured for 48 h in the presence of 5 mg/ml PHA. The IL-2 concentration of the culture supernatant was determined in triplicate by ELISA kit (Cytokine Total™, Intergen, Purchase, NY). The polyclonal anti-human IL-2 cross-reacted with canine IL-2 and recombinant human IL-2 was used as the standard. The detection limit of the assay was <2 ng/ml and the coefficient of variation was 4.4%.

2.7. Plasma IgG and IgM

Plasma collected on Weeks 0, 2, 4, 8,12 were analyzed for IgG and IgM concentrations by single radial immunodiffusion (SRID). In addition, all dogs were challenged with the same polyvalent vaccine on Week 13 and again on Week 15 to determine the changes in plasma Ig concentrations. Blood was collected weekly from Weeks 13 through 17 for Ig analysis. Goat antiserum to canine IgG or IgM (ICN, Aurora, OH) was used and ring diameters were measured using a SRID reader (Transidyne General Corp., Ann Arbor, MI).

2.8. Statistical analysis

Data were analyzed by split-plot ANOVA using the General Linear Model of SAS (1991). Differences among treatment means within a sampling period were compared using the Student's *t*-test.

3. Results

3.1. Body weight

Body weight of dogs did not significantly differ among treatment groups during the experimental period. Mean body weight of dogs across all diets and periods was 11.4±0.4 kg.

3.2. Plasma carotenoids, Vitamin A and E

Plasma concentrations of lutein+zeaxanthin in lutein-supplemented dogs increased rapidly during the first 2 weeks of feeding (Fig. 1). In contrast, plasma lutein+zeaxanthin was not detectable in unsupplemented dogs. Concentrations of plasma lutein+zeaxanthin on Weeks 2 through 12 was significantly higher ($P<0.05$) in dogs fed lutein than in unsupplemented dogs. Lutein supplementation did not influence concentrations of plasma retinol and α-tocopherol. Concentrations of these vitamins across all treatments and all periods averaged 3.63±0.14 and 37±2 μmol/l, respectively.

3.3. DTH response

During all periods studied, DTH response to saline as a control did not differ significantly among treatment groups (data not shown). Prior to lutein feeding (Week 0), DTH responses to PHA and vaccine were similar across all dietary groups. Irrespective of treatment period, maximal DTH response to PHA was observed around 24 h post-injection, whereas maximal response to vaccine occurred between 48 and 72 h. Also, skin thickness response to PHA was approximately two times higher than to vaccine.

On Week 6, there was a dose-related DTH response to PHA at 24 h post-injection (Fig. 2). The DTH response decreased by 48 and 72 h and no significant treatment

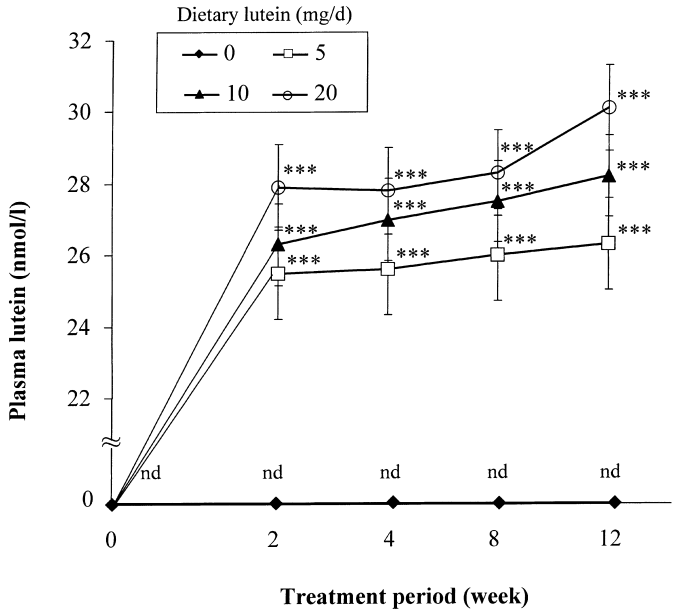

Fig. 1. Concentrations of plasma lutein+zeaxanthin in dogs fed diets containing 0, 5, 10 or 20 mg lutein for 12 weeks. Values are means±S.E.M. (n=14). Statistical significance is as follows: ***P<0.001 compared with control group; nd: undetected.

difference was observed during these times. The DTH response to vaccine was dose-related at 48 and 72 h post-injection and was not significant at 24 h.

On Week 12, the DTH response to PHA was significantly higher for dogs fed 20 mg lutein compared to unsupplemented dogs at 48 and 72 h (Fig. 2). In contrast to Week 6, the DTH response to vaccine was not significantly influenced by lutein supplementation and averaged 36.7±3.9% across all treatments and all periods.

3.4. Mitogen-induced PBMC proliferation

Dietary lutein did not significantly influence both concentrations of PHA-, Con A- and PWM-stimulated proliferative response of PBMC on Weeks 0 and 4 (data not shown). However, proliferative responses in whole blood cultures to both concentrations of mitogens were enhanced by lutein supplementation on Weeks 8 and 12. For the higher concentrations of mitogens, proliferative response was increased on Weeks 8 (P<0.05) and 12 (P<0.01) in dogs fed 20 mg lutein compared to unsupplemented dogs. Dogs fed 5 and 10 mg lutein also had higher PBMC proliferation on Week 8 in response to PHA (Fig. 3).

The effects of dietary lutein on Con A-stimulated PBMC proliferative response (Fig. 3) were generally similar to those observed with PHA-induced proliferation. On Weeks 8 and 12, dogs fed 20 mg lutein had higher PBMC proliferation in response to both concentrations of Con A. Dogs fed 10 mg lutein also showed higher PBMC proliferation

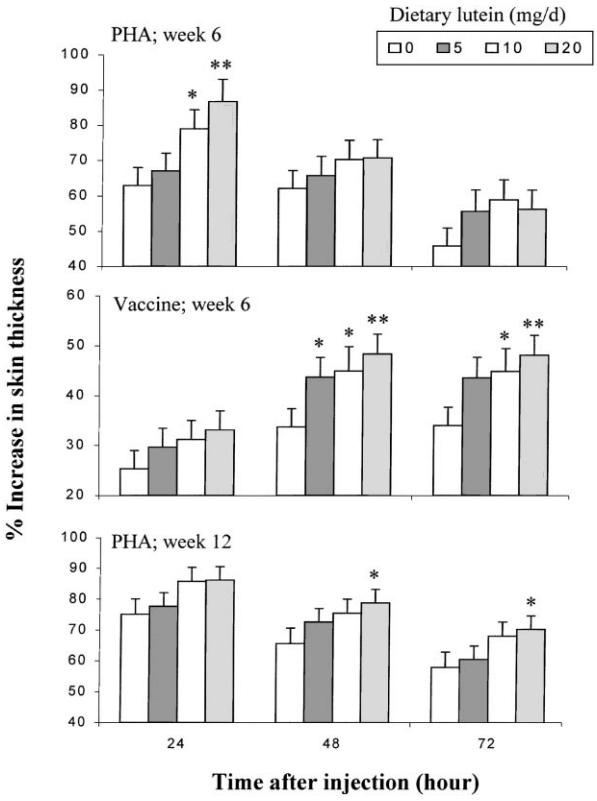

Fig. 2. DTH response (expressed as a percentage of skin thickness measured at 0 h) in dogs fed 0, 5, 10, or 20 mg lutein for 12 weeks. Dogs were challenged with PHA and polyvalent vaccine at Weeks 6 and 12. Skin induration was measured at 0, 24, 48 and 72 h post-injection. Values are means±S.E.M. (n=14). Statistical significance is as follows: *P<0.05, **P<0.01 compared with control group.

on Weeks 8 and 12 in response to 5 μg/ml Con A. Generally, PBMC proliferation was higher with 5 μg/ml than with 1 μg/ml Con A.

Proliferation of PBMC in response to PWM (Fig. 3) was generally similar to those observed with PHA and Con A. Dogs fed 20 mg lutein had significantly higher PBMC proliferation on Weeks 8 and 12 in response to both concentrations of PWM as compared to unsupplemented controls. Those fed 10 mg lutein also had higher proliferative response on Week 8.

3.5. Lymphocyte subsets

The effects of dietary lutein on the percentages of CD5+ total T cells, CD4+ Th cells, CD8+ Tc cells, CD21+ B cells and MHC class II+ lymphocytes are illustrated on Table 2. Dietary lutein supplementation did not significantly influence the percentages of any of the lymphocyte markers on Weeks 0 and 4 (data not shown). On Weeks 8 and 12, dogs fed lutein generally had higher percentages of total T cells than unsupplemented

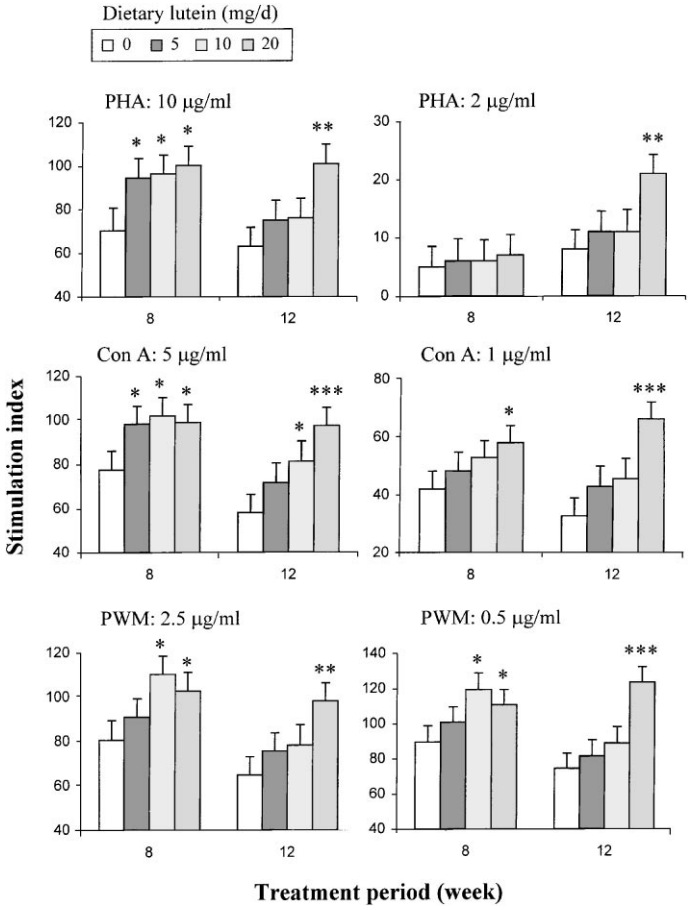

Fig. 3. Proliferative responses of PBMC to higher (left column) and lower (right column) concentrations of PHA, Con A and PWM measured on Weeks 8 and 12. Values are means±S.E.M. (n=14). Statistical significance is as follows: *P<0.05, **P<0.01, ***P<0.001 compared with control group.

control and was statistically significant (P<0.05) with dogs fed 5 and 20 mg lutein. Dogs fed 20 mg lutein also had a higher (P<0.05) percentage of MHC class II+ cell populations on Weeks 8 and 12 compared to unsupplemented dogs.

The percentage of Th cells was higher (P<0.05) on Week 12 in dogs fed 5 mg lutein and that of the Tc cell population was higher (P<0.05) on Week 8 in dogs fed 20 mg lutein compared to unsupplemented dogs. In contrast to other lymphocyte subpopulations, dietary lutein did not significantly influence the percentage of CD21+ B cell population.

3.6. IL-2 production

The production of IL-2 by PHA-stimulated PBMC in whole blood cultures did not differ significantly among dietary treatments throughout the experimental period (data not

Table 2
Population changes in lymphocyte subsets in dogs fed 0, 5, 10 or 20 mg lutein daily for 12 weeks[a,b]

Lymphocyte subsets (%)	Dietary lutein (mg/day)			
	0	5	10	20
Week 8				
CD5	79.7±1.4	83.9±1.4*	81.5±1.4	85.1±1.4**
CD4	40.9±2.4	42.9±2.6	44.5±2.4	46.4±2.4
CD8	21.6±1.8	25.3±1.9	23.1±1.7	27.9±1.7*
MHC class II	53.6±4.6	56.0±4.8	54.6±5.5	65.4±4.8*
CD21	11.3±1.2	12.8±1.2	10.6±0.7	10.3±0.8
Week 12				
CD5	81.4±1.4	86.1±1.4*	84.5±1.4	83.8±1.5
CD4	41.1±2.4	47.8±2.4*	47.2±2.4	43.5±2.5
CD8	21.6±1.7	23.4±1.7	20.4±1.7	21.8±1.8
MHC class II	46.3±6.3	55.3±5.0	59.1±5.9	68.0±6.8**
CD21	8.4±0.7	7.4±0.7	7.2±0.6	8.3±0.6

[a] Values are means±S.E.M. (n=14) and expressed as a percentage of total lymphocyte population.
[b] Statistical significance is as follows: *P<0.05, **P<0.01 compared with control group.

shown). Concentrations of IL-2 in culture medium averaged 15.7±0.4 ng/ml across all treatments and all periods.

3.7. Ig production

The concentrations of plasma IgG and IgM did not differ among dietary treatment groups during the first 12 week of dietary supplementation and averaged 12.3±0.75 and 1.57±0.11 mg/ml, respectively. However, after the second antigenic challenge on Week 15, the concentration of plasma IgG was higher (P<0.05) on Week 16 in dogs fed 5 mg

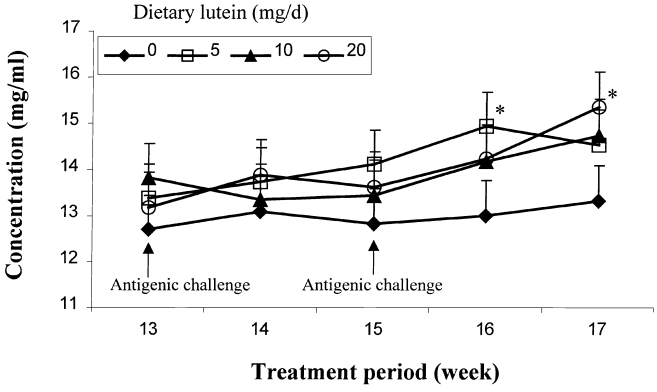

Fig. 4. Changes in plasma polyclonal IgG concentrations in dogs fed 0, 5, 10, or 20 mg lutein from Week 13 through 17. All dogs were challenged with a polyvalent vaccine on Weeks 13 and 15. Values are means±S.E.M. (n=14). Statistical significance is as follows: *P<0.05 compared with control group.

lutein ($P<0.05$) and on Week 17 in dogs fed 20 mg lutein (Fig. 4) whereas plasma IgM was not changed (data not shown).

4. Discussion

In this study, we showed for the first time that dietary lutein significantly enhanced cell-mediated and humoral immune response in dogs. Lutein supplementation stimulated the proliferative response of PBMC to PHA, Con A and PWM. There was a marked increase in PBMC proliferation in response to PHA and Con A at Week 12 in dogs fed 20 mg lutein. This is in agreement with Chew et al. (1996) who reported that dietary lutein enhanced PHA-induced splenocyte proliferation in mice. Similarly, enhanced PBMC proliferation was observed in humans and animals given carotenoids. Young healthy males given β-carotene also had higher PBMC proliferation in response to PHA and Con A (Moriguchi et al., 1996). Weanling pigs injected with β-carotene had increased lymphocyte proliferative response to both Con A and PHA (Hoskinson et al., 1990). However, in vitro studies with β-carotene have not yielded similar stimulatory responses (Tjoelker et al., 1988; Jyonouchi et al., 1991; Lessard and Dupuis, 1994). The discrepancy between ex vivo and in vitro studies may be explained by the presence of cleavage products in vivo (Akporiaye et al., 1993). In this study, dogs supplemented with lutein had higher percentages of total T and Th cells. Thus, enhanced mitogenesis by dietary lutein is likely attributed to an increase in these lymphocyte subpopulations. Alexander et al. (1985) reported increased numbers of total T cells and Th cells in humans supplemented with β-carotene for 2 weeks. Alternatively, the enhanced mitogenic response may be mediated by alterations in the expression of surface markers such as IL-2 receptors and MHC II molecules, which are responsible for PBMC clonal expansion and antigen presentation, respectively. Habu and Raff (1977) showed that a marked decrease in proliferative response of mouse T lymphocytes to Con A and PHA in the absence of monocytes. They also suggested that T cell proliferation induced by mitogens might involve the recognition of stimulatory ligand in association with major histocompatibility complex molecules. Our whole blood cultures contain adherent and non-adherent lymphoid cells. Therefore, increased expression of MHC II molecules in this study may partly support the enhanced mitogenic response. The lack of a significant mitogenic response due to lutein feeding on Week 4 suggests a lag time for lutein to exert its cellular actions. Prabhala et al. (1991) demonstrated an enhancing effect of β-carotene on the percentage of cells bearing IL-2 receptors and NK cell markers with peak effects after 8 weeks of supplementation in humans. In this study, lutein did not influence PHA-induced IL-2 production despite observed increases in PHA-, Con A- and PWM-induced PBMC proliferation. These results are consistent with Chew et al. (1996) who reported increased PHA-stimulated mitogenesis and no significant influence on IL-2 production in mice fed 0.1 or 0.4% lutein. In contrast, β-carotene supplementation has been shown to increase IL-2 receptors in human (Prabhala et al., 1991; Watson et al., 1991). Therefore, different carotenoids seem to exert different effects on IL-2 response.

The DTH response is a good in vivo indicator of cell-mediated immunity. At Week 6, an intradermal injection with PHA (non-specific immunity) produced a dose-dependent

increase in DTH skin induration. Maximal response was observed at 24 h after challenge. This is in agreement with others who reported similar DTH responses to PHA in rats (Mendenhall et al., 1989) and puppies (Taura et al., 1995). Dietary lutein also significantly increased the DTH response to vaccine, indicative of a specific immune response. This heightened DTH response to the vaccine may be mediated through the action of Th cells that play a central role as effector cells at the reaction site or through increased expression of MHC II molecules, resulting in more effective antigen presentation. Unlike PHA in which maximal DTH response was observed at 24 h after injection, DTH response to the vaccine was the highest between 48 and 72 h after challenge. The latter is typical of a specific DTH response where there is a delayed response time for cytokines to induce a localized influx of macrophages and for cell activation. Cytokines, such as IL-1, IL-2, TNF-α and IFN-γ are also important participants in DTH response. These cytokines may play important roles in the observed DTH response. However, IL-2 production by PBMC was not influenced by dietary lutein in this study.

Another functional arm of the immune system responding to pathogens and other foreign intrusions is the humoral immune response. Lutein did not significantly influence the concentrations of plasma IgM or IgG during the first 12-week of the study when no antigenic challenge was administered. After the initial 12-week feeding period, all dogs were inoculated with a polyvalent vaccine on Weeks 13 and 15. Interestingly, upon re-exposure to the antigen at Week 15, plasma IgG concentrations increased significantly in lutein-fed dogs compared to unsupplemented dogs whereas plasma IgM was not influenced. This finding is of clinical importance because dietary lutein may enhance the antibody response of dogs given routine vaccinations. The observed increase in the populations of Th cell and MHC II-positive cells could enhance the secondary IgG response by B cells; the latter is likely mediated through the action of cytokines and through antigen presentation. However, corresponding flow cytometry data on changes in leukocyte subpopulations are not available to permit direct comparisons to the elevated IgG on Weeks 13–17.

High intakes of dietary carotenoids are correlated with lower risk of developing certain types of cancers (Ziegler, 1989; Singh and Gaby, 1991; Block et al., 1992) and improved protection of the host through enhanced immune response (Bendich, 1991; Chew, 1993). Lutein, an oxycarotenoid (β,ε-carotene-3,3′-diol) found in plants and microorganisms, is absorbed into the plasma of humans (Parker, 1989; Olmedilla et al., 1997) and animals including calves (Bierer et al., 1995), chickens (Tyczkowski and Hamilton, 1986; Allen, 1992) and mice (Chew et al., 1996; Park et al., 1998). Little is known about the absorption and possible biological action of lutein in dogs. The present study showed that dogs, like humans and certain animal species, can absorb lutein. The concentration of plasma lutein increased rapidly after 2 weeks of supplementation. Dietary lutein did not influence concentrations of plasma retinol and α-tocopherol in this study. Similarly, other studies have failed to show any influence of dietary carotenoids on concentrations of plasma retinol and α-tocopherol in cats (Kim et al., 2000).

In summary, dietary lutein enhanced canine Th and Tc cell populations and the expression of MHC class II molecules, possibly resulting in increased mitogen-induced PBMC proliferation and DTH response. Also, lutein increased the antibody response to a

vaccine. Herein, we provide evidence for the role of dietary lutein in enhancing both the cell-mediated and humoral immune responses of the domestic canine.

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