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Effects of a *Saccharomyces cerevisiae* fermentation product-supplemented diet on circulating immune cells and oxidative stress markers of dogs

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Abstract

Feeding *Saccharomyces cerevisiae* fermentation product (SCFP) has previously altered fecal microbiota, fecal metabolites, and immune function of adult dogs. The objective of this study was to investigate measures of skin and coat health, changes in circulating immune cell numbers and activity, antioxidant status, and oxidative stress marker concentrations of healthy adult dogs fed a SCFP-supplemented extruded diet. Sixteen adult English Pointer dogs (8 M, 8 F; mean age = 6.7 ± 2.1 yr; mean BW = 25.9 ± 4.5 kg) were used in a randomized crossover design study. All dogs were fed a control diet for 4 wk, then randomly assigned to either the control or SCFP-supplemented diet (0.13% of active SCFP) and fed to maintain BW for 10 wk. A 6-wk washout preceded the second 10-wk experimental period with dogs receiving opposite treatments. After baseline/washout and treatment phases, skin and coat were scored, and pre and postprandial blood samples were collected. Transepidermal water loss (TEWL), hydration status, and sebum concentrations were measured (back, inguinal, ear) using external probes. Oxidative stress and immune cell function were measured by ELISA, circulating immune cell percentages were analyzed by flow cytometry, and mRNA expression of oxidative stress genes was analyzed by RT-PCR. Change from baseline data was analyzed using the Mixed Models procedure of SAS 9.4. Sebum concentration changes tended to be higher ($P < 0.10$; inguinal, ear) in SCFP-fed dogs than in controls. TEWL change was lower ($P < 0.05$) on the back of controls, but lower ($P = 0.054$) on the ear of SCFP-fed dogs. Delayed-type hypersensitivity response was affected by diet and time post-inoculation. Other skin and coat measures and scores were not affected by diet. Changes in unstimulated lymphocytes and stimulated IFN-γ secreting T cells were lower ($P < 0.05$) in SCFP-fed dogs, while changes in stimulated T cells were lower ($P < 0.05$) in control-fed dogs. Upon stimulation, the percentage of cytotoxic T cells delta trended lower ($P < 0.10$) in SCFP-fed dogs. Change in serum superoxide dismutase concentrations was higher ($P < 0.05$) and change in catalase mRNA expression was lower ($P < 0.05$) in SCFP-fed dogs. All other measurements of immune cell populations, oxidative stress markers, and gene expression were unaffected by treatment. In conclusion, our data suggest that SCFP positively impacts indicators of skin and coat health of dogs, modulates immune responses, and enhances some antioxidant defense markers.

Lay summary

Saccharomyces cerevisiae fermentation product (SCFP) is a yeast product containing bioactive fermentation metabolites, residual yeast cells, and yeast cell wall fragments. In this study, SCFP was investigated for its impacts on immune health, oxidative stress, and skin and hair coat health in dogs. Using a randomized crossover study design, 16 adult pointer dogs were used to compare changes in immune cell numbers and activity, antioxidant status and oxidative stress marker concentrations, and skin and coat health markers when fed a SCFP-supplemented diet or control diet. Skin sebum concentrations increased in dogs fed SCFP, but transepidermal water loss changes depended on body location (ear, inguinal, or back). Delayed-type hypersensitivity response was affected by diet and time. Changes in unstimulated lymphocytes and stimulated IFN-γ secreting T cells were lower in SCFP-fed dogs, while changes in stimulated T cells were lower in control dogs. Changes in stimulated cytotoxic T cells tended to be lower in SCFP-fed dogs. Change in serum superoxide dismutase concentrations were higher, while change in catalase mRNA expression was lower in SCFP-fed dogs. In conclusion, our data suggest that SCFP positively impacts indicators of skin and coat health of dogs, modulates immune responses, and enhances some key antioxidant defense markers.

Key words: canine nutrition, immunity, skin and coat health, yeast product

Abbreviations: AAFCO, Association of American Feed Control Officials; AAPH, 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride; APC, antigen-presenting cell; BW, body weight; CAP-e, cell-based antioxidant protection in erythrocytes; CBC, complete blood count; ConA, concanavalin A; COX-2, cyclooxygenase-2; DM,

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dry matter; DTH, delayed-type hypersensitivity; GI, gastrointestinal; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; MDA, malondialdehyde; MHC-II, major histocompatibility complex class II; MOS, mannanoligosaccharides; MPO, myeloperoxidase; NFE, nitrogen-free extract; NK, natural killer; OM, organic matter; PBMC, peripheral blood mononuclear cell; PHA, phytohaemagglutinin; RBC, red blood cell; SCFP, *Saccharomyces cerevisiae* fermentation product; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TEAC, Trolox equivalent antioxidant capacity; TEWL, transepidermal water loss; TLR, toll-like receptor

Introduction

Functional ingredients in pet foods and treats fall into the category of "wellness-related products," as they provide health benefits beyond the provision of essential nutrients (i.e., water, vitamins, minerals, proteins, and fats). Several studies have investigated the health benefits of functional ingredients in humans, which has led pet owners to believe functional ingredients will exert the same positive effects on their pets (Swanson et al., 2002; Flickinger et al., 2003; Cortese et al., 2015; Di Cerbo et al., 2016; Sechi et al., 2017). However, the mechanisms of action, optimal inclusion levels, and possible harmful effects of most functional ingredients are not completely understood in dogs (Swanson et al., 2003).

Saccharomyces cerevisiae fermentation product (SCFP) used in this study is a dry, proprietary product produced via *S. cerevisiae* fermentation and includes residual yeast cells, yeast cell wall fragments, fermentation metabolites, and media used during fermentation. Because SCFP has been shown to positively impact performance, health, and immunity in humans and several animal species, including swine, poultry, and cattle, it may aid as a functional ingredient in pet foods (Moyad et al., 2009; Shen et al., 2011; Kidd et al., 2013; Zaworski et al., 2014). Components of the yeast cell wall [i.e., mannanoligosaccharides (MOS) and β-glucans] and a wide variety of yet to be described metabolites and bioactive components from yeast fermentation have been implicated in yeast's gastrointestinal (GI)- and immune-modulatory properties. In adult dogs, supplementation of SCFP has been shown to positively impact GI health and immune function. Lin et al. (2019) demonstrated that SCFP (125, 250, and 500 mg/d) modulated fecal microbiota and fermentative end-products in adult beagles by increasing *Bifidobacterium* and decreasing phenol and indole concentrations. Dietary SCFP was also shown to elevate immune capacity by enhancing T helper-1 cell responses and decreasing toll-like receptor (TLR) responses following ex vivo ligand stimulation (Lin et al., 2019).

In another study, supplementing adult Labrador Retrievers with SCFP (500 mg/d) led to decreased thiobarbituric acid reactive substances (TBARS) and improved total antioxidant capacity (TAC) in serum, suggesting a reduction in lipid peroxidation and increased antioxidant capacity when subjected to exercise and transport stress (Varney et al., 2021). SCFP-fed Labradors also exhibited increased serum concentrations of TNF-α and decreased serum concentrations of immunoglobulin E and immunoglobulin G, suggesting immunomodulatory effects (Varney et al., 2021). Additionally, SCFP has been shown to alter fecal microbiota, fecal metabolites, antioxidant status, and immune cell function of adult dogs; however, SCFP supplementation has previously been administered via gelatin capsule or top dressing. Further investigation of its ability to affect oxidative stress, antioxidant status, and immune responses in dogs fed with an SCFP-supplemented extruded kibble diet is warranted and impacts on skin and coat health have not been determined.

The objective of this study was to determine the effects of an SCFP-supplemented extruded diet on the circulating immune cell percentages and effector function, antioxidant status, and oxidative stress marker concentrations, and measures of skin and coat health of adult dogs. We hypothesized that dogs fed the SCFP-supplemented diet would have enhanced immune cell numbers and functionality, reduced concentrations of markers associated with oxidative stress and inflammation, and improved skin and coat health compared with dogs fed a control diet.

Materials and Methods

All experimental procedures were approved by the Kennelwood Inc. IACUC prior to experimentation and were performed in accordance with the U. S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Animals and housing

Sixteen adult pointer dogs [8 intact males, 8 intact females; mean age = 6.7 ± 2.1 yr; mean body weight (BW) = 25.9 ± 4.5 kg] were used in a crossover design. All dogs were housed individually (inside run = 1.17 m × 1.42 m; outside run = 1.08 m × 3.05 m) at Kennelwood, Inc. (Champaign, IL). Dogs had free access to fresh water and were fed once daily to maintain BW throughout the study. The amount of food offered was based on previous feeding records and the estimated caloric content of the diets.

Experimental timeline and diets

Prior to the study, dogs were vaccinated (Duramune Max 5 Dog Vaccine; Elanco Animal Health, Greenfield, IN), and blood samples were collected for serum chemistry measures and complete blood count (CBC) to confirm health. Dogs were vaccinated with Duramune Max 5 Dog Vaccine prior to the start of the adaption phase to serve as a specific antigen for the DTH test (described later). A crossover study began with a 4-wk adaptation phase (weeks −4 to −1) followed by two 10-wk experimental periods. There was a 6-wk washout phase (weeks −6 to −1) between experimental periods. Dogs were fed the control diet during the adaptation and washout phases, then randomly allotted to an SCFP-supplemented or control diet after baseline measurements (week 0) were taken. Both dietary treatments tested were extruded kibble diets formulated to meet all Association of American Feed Control Officials (AAFCO, 2022) nutrient recommendations for adult dogs at maintenance and were formulated with similar ingredients and nutrient targets (Blue Buffalo Co. Ltd., Wilton, CT; Table 1). The SCFP-supplemented diet was formulated to include approximately 0.13% of the active SCFP ingredient (0.4942% of TruMune; Diamond V Mills, Inc., Cedar Rapids, IA). Based on the food intake measured in the study, this inclusion level resulted in an average intake of 30.1 ± 3.6 mg SCFP/kg BW per day (ranged from 21.7 to 38.1 mg SCFP/kg BW). At baseline (week 0), after week 5, and after week 10, blood samples were collected, and skin measurements were conducted. Dogs were weighed and body condition scores were assessed using a 9-point scale (Laflamme, 1997) each week prior to the morning feeding.

Table 1. Ingredient and chemical composition of experimental diets tested

Ingredient	Control	SCFP
	--- %, as-basis ---	
Chicken, deboned	18.23	18.23
Chicken meal	18.05	18.05
Barley	18.05	18.00
Oats	18.05	18.00
Brown rice	16.58	16.54
Chicken fat	4.79	4.79
Liquid digest	1.71	1.71
Powdered cellulose	1.61	1.61
Powder digest	0.86	0.86
Potassium chloride	0.52	0.52
SCFP[1]	–	0.49
Calcium carbonate	0.36	–
Salt	0.42	0.42
Trace mineral mix	0.23	0.23
Vitamin mix	0.23	0.23
Choline chloride	0.19	0.19
Mixed tocopherols	0.07	0.07
DL-methionine	0.07	0.07
Analyzed composition		
Dry matter (DM), %	90.97	90.59
	--- %, DM basis ---	
Acid-hydrolyzed fat	12.81	12.62
Crude protein	25.61	25.32
Total dietary fiber	14.03	12.94
Insoluble fiber	9.50	7.91
Soluble fiber	4.53	5.03
Ash	8.76	8.34
NFE[2]	38.79	40.61
Gross energy, kcal/kg	5,076	5,125
Calculated metabolizable energy[3], kcal/kg	3,342	3,386

[1]TruMune; Diamond V Mills, Inc., Cedar Rapids, IA.
[2]Nitrogen-free extract % = 100 % – (% Acid Hydrolyzed Fat + % Crude Protein + % Moisture + % Ash + % Total Dietary Fiber).
[3]Metabolizable energy estimated with modified Atwater factors: 10 × [(3.5 × % Crude Protein) + (8.5 × % Crude Fat) + (3.5 × % NFE)].

Blood sample collection

Fasted (12 h overnight) blood samples for serum chemistry and CBC were collected at baseline, week 5, and week 10 of each experimental period. Blood samples for immune cell numbers and effector function, antioxidant status, and oxidative stress measures were collected at baseline and week 10 of each experimental period. Blood samples for the cell-based antioxidant protection in erythrocytes (CAP-e) assay were collected at baseline (before and 2 h after meal), and week 10 (before and 2 h after meal) of each experimental period. Blood samples were collected via jugular or cephalic puncture using 19- to 22-gauge needles. Samples were immediately transferred to appropriate vacutainer tubes, with 0.5 mL going into BD Vacutainer Plus plastic whole blood tubes (#363706; Lavender with K_2EDTA additive; Becton Dickinson, Franklin Lakes, NJ), 17.5 mL going into BD Vacutainer SST tubes (#367988 and #367983; Becton Dickinson) for serum separation, 20 mL going into BD Vacutainer Heparin tubes (#366480; Becton Dickinson), and 7.5 mL going into PAXgene Blood Tubes (#762165; Qiagen, Valencia, CA).

Serum chemistry and CBC

Serum was isolated by centrifuging SST tubes at 2,000 × *g* at 4 °C for 15 min (Beckman CS-6R centrifuge; Beckman Coulter Inc., Brea, CA). Once serum was harvested, it was transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for serum chemistry analysis. The K_2EDTA tubes, containing uncoagulated blood, were cooled (but not frozen) and transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for CBC analyses.

Immune cell populations

Ficoll (Sigma, St. Louis, MO) was added to blood in a 1:1 volume ratio and centrifuged at 300 × *g* at 4 °C for 30 min to separate peripheral blood mononuclear cells (PBMC) from blood samples (acceleration = 9, deceleration = 0). The percentage of T cells, natural killer (NK) cells, and antigen-presenting cells (APC) was evaluated by flow cytometry. For T cell populations, PBMC were distributed into two tubes (1×10^6 cells/tube). One tube was incubated with cell stimulation cocktail [phorbol 12-myristate 13-acetate, ionomycin, brefeldin A, and monensin (eBioscience, San Diego, CA)]. Both tubes of cells were incubated at 37 °C in 5% CO_2 for 4 h followed by surface marker labeling on ice with fluorophore conjugated antibodies to CD3 (FITC; Clone CA17.2A12; BioRad, Cat. No. MCA1774F), CD4 (APC; Clone YKIX302.9; BioRad, Cat. No. MCA1038APC), and CD8 (Pacific Blue; Clone YCATE55.9; BioRad, Cat. No. MCA1039PB) to identify T cells. After labeling, samples were fixed and permeabilized with fixation buffer and permeabilization buffer (eBioscience, San Diego, CA) and then stained with IFN-γ (PE; Clone; BioRad, Cat. No. MCA1783PE). NK cells were identified by staining 1×10^6 cells with fluorophore conjugated antibodies to CD3 (FITC; Clone CA17.2A12; BioRad, Cat. No. MCA1774F), and CD5 (APC; Clone YKIX322.3; BioRad, Cat. No. MCA1037APC). Antigen presenting cells of interest included B cells and monocytes presenting major histocompatibility complex class II (MHC-II) on the cell surface. The percentages of MHC-II expressing APC were identified using by staining 1×10^6 cells with fluorophore conjugated antibodies to CD14 (Pacific Blue; Clone TUK4; BioRad, Cat. No. MCA1568PB), CD21 (PE; Clone CA2.1D6; BioRad, Cat. No. MCA1781R), and MHC-II (FITC; Clone YKIX334.2 BioRad, Cat. No. MCA1044F). Populations of T cells, NK cells, and APC were then acquired on a LSRII Flow Cytometer (Becton Dickinson). Gates were determined using unstained and single-stained samples obtained from the same PBMC of origin. Results were analyzed using FlowJo version 10.6.2 flow cytometry software (Becton Dickinson). The gating strategy used to determine immune cell populations is shown in Supplementary Figures S1–S3. For NK cells, the population was determined according to Huang et al. (2008).

Responsiveness of lymphocytes to toll-like receptor (TLR) agonists

A total of 1×10^5 PBMC/ well were seeded into 96-well plates. Cells were stimulated with media or agonists of TLR2 (100 μg/mL zymosan; Invivogen, San Diego, CA), TLR3 [50 μg/mL polyinosinic–polycytidylic acid sodium salt, poly(I:C); Sigma], TLR4 (100 ng/mL lipopolysaccharides, LPS; Sigma), or TLR7/8 (5 μg/mL resiquimod,

Invivogen). After 24 h of incubation, supernatants were collected for measurement of TNF-α concentration using a commercial ELISA kit (#MBS761131; MyBioSource, San Diego, CA).

Serum oxidative stress and immune markers

Circulating oxidative stress and immune markers [8-isoprostane, Trolox equivalent antioxidant capacity (TEAC); malondialdehyde (MDA); superoxide dismutase (SOD)], LPS-binding protein (LBP), and lysozyme concentrations were measured using commercial ELISA kits (8-isoprostane: #MBS2611970, TEAC: # MBS169313, MDA: #MBS2605193, SOD: #MBS2104718, LBP: #MBS093112, Lysozyme: #MBS2604408, MyBioSource, San Diego, CA) according to the manufacturer's instructions. The remaining serum samples were shipped overnight on dry ice to Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) for serum α-tocopherol analysis and to NIS labs (Klamath Falls, OR) for the CAP-e assay.

Cellular antioxidant protection and bioavailability

To test for antioxidants in serum that were likely to be bioavailable at the cellular level in vivo, the canine serum samples were tested ex vivo using the CAP-e assay (Jensen et al., 2008; Phillips et al., 2019). Human erythrocytes were purified and washed four times in phosphate-buffered saline, and stored at 4 °C until use, and used for testing within 4 d. For the CAP-e cellular antioxidant protection assay, each canine serum sample was tested in quadruplicate, using human erythrocytes to detect antioxidant compounds present in the canine serum. The canine serum samples were kept at −80 °C until testing. All long-term samples from each dog were tested in the same run, and all acute samples from each dog were tested in a parallel run using the same batch of human erythrocytes. Serum samples were thawed, briefly vortexed, and kept at 4 °C until testing was initiated within the hour. To avoid antibody-mediated lysing of the human erythrocytes by Ig present in the canine serum samples, a 10× solution of EDTA buffer was added to each serum sample immediately prior to testing. The erythrocytes were treated with the canine serum samples in quadruplicate for 20 min. During this incubation time, antioxidant compounds were able to cross the cell membrane and enter the erythrocyte cell. Following the incubation of erythrocytes with serum, the erythrocytes were washed twice with PBS to remove any compounds from the test products that were not absorbed by the cells. Cell cultures were then treated with the indicator dye 2′,7′-dichlorofluorescin diacetate, which becomes fluorescent when oxidized. The peroxyl-free radical generator 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH) was added to trigger oxidation. Control cultures were performed in hexaplicate and included untreated erythrocytes as a negative control (not exposed to serum or AAPH) and erythrocytes treated with AAPH in the absence of serum (positive control). After exposure to AAPH for 1 h, the fluorescence intensity was measured at 488 nm using a Tecan Spectrafluor plate reader (Tecan, Männedorf, Switzerland). When a reduction of fluorescence intensity was observed in erythrocytes exposed to a serum sample prior to exposure to AAPH, this was indicative that the serum contained antioxidants that were able to penetrate the erythrocyte cells and protect them from AAPH-mediated oxidative damage.

Whole blood gene expression

Total RNA from blood cells was isolated using a PAXgene Blood RNA Kit (#762331; Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA concentrations were determined using a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA). Gene expression was measured by real-time two-step RT-qPCR using an Applied Biosystems 7900HT real-time PCR system (Applied Biosystems, Waltham, MA) and was carried out with SYBR Green chemistry (Bio-Rad Laboratories, Hercules, CA) in a QuantStudio 7 instrument (Thermo Fisher Scientific, Waltham, MA) using validated forward and reverse primers (Bio-Rad Laboratories). Genes of interest included the following: glutathione peroxidase (UniqueAssayID: qCfaCED0030791), glutathione reductase (UniqueAssayID: qCfaCED0031064), catalase (UniqueAssayID: qCfaCED0028561), SOD (UniqueAssayID: qCfaCED0038911), myeloperoxidase (MPO, UniqueAssayID: qCfaCID0034597), and cyclooxygenase-2 (COX-2, UniqueAssayID: qCfaCED0024663; PrimePCR SYBR Green Assay, Bio-Rad Laboratories). All gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method, represented as gene expression relative to the housekeeping gene (*RPS5*, UniqueAssayID: qCfaCED0028510).

Skin and hair coat condition

At baseline and week 10, skin and coat were scored by three blinded researchers according to Rees et al. (2001). Hair was scored using the following scale: 1: dull, coarse, dry; 2: poorly reflective, nonsoft; 3: medium reflective, medium soft; 4: highly reflective, very soft; and 5: greasy. Skin condition was scored using the following scale: 1: dry; 2: slightly dry; 3: normal; 4: slightly greasy; and 5: greasy. After skin and coat scoring was done, dogs were sedated by an intramuscular injection of a combination of butorphanol (Torbugesic; 0.2 mg/kg BW) and dexmedetomidine (0.02 mg/kg BW) so that transepidermal water loss [TEWL; Tewameter TM 300 MDD (Courage + Khazaka Electronic GmbH, Cologne, Germany)], hydration status [Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany)], and sebum concentrations [external Sebumeter SM 815 (Courage + Khazaka Electronic GmbH, Cologne, Germany)], and delayed-type hypersensitivity (DTH) response could be measured.

Delayed-type hypersensitivity

DTH response was tested at week 10 as described by Kim et al. (2000). Briefly, dogs were injected intradermally in the flank area with 100 μL of saline (0.85%; functioned as control), an attenuated vaccine (Duramune Max 5 Dog Vaccine; Elanco Animal Health; functioned as specific antigen), and phytohaemagglutinin (PHA; 0.5 mg/mL) and concanavalin A (ConA; 0.5 mg/mL), which both functioned as nonspecific mitogens. Injections were performed in triplicate along the flank area after the injection site was clipped and wiped with 70% ethyl alcohol. After skin measurements and DTH injections were completed, an injection of the reversal agent for dexmedetomidine, atipamezole (0.2 mg/kg BW), was administered intramuscularly. Skin induration was measured at baseline, 15, 30, 45, and 60 min after injection, and 24, 48, and 72 h after injection using a digital caliper. The DTH

responses were reported as average wheal diameter (mm) as described by van der Valk et al. (2016).

Diet chemical analyses

Both diets were ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen and then analyzed for dry matter (DM) and ash according to AOAC (2006; methods 934.01 and 942.05), with organic matter (OM) being calculated. Crude protein was calculated from Leco (FP2000 and TruMac) total nitrogen values according to AOAC (2006; method 992.15). Total lipid content (acid-hydrolyzed fat) was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Total dietary fiber was determined according to Prosky et al. (1988). Gross energy was measured using an oxygen bomb calorimeter (model 6200, Parr Instruments, Moline, IL). Recovery of SCFP and validation of dietary concentration were measured using inert, identifiable iron particles (Microtracers F and a Microtracer Rotary Detector magnetic separator; Micro-Tracers, San Francisco, CA).

Statistical analyses

Data were analyzed using the Mixed Models procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC). The fixed effect of treatment was tested, and the dog was considered a random effect. Change from baseline differences between treatments was determined using a Fisher-protected least significant difference with a Tukey adjustment to control for experiment-wise error. A probability of $P < 0.05$ was accepted as statistically significant and $P < 0.10$ was the trends when considering the effects of diet, time, and their interactions. Reported pooled standard errors of the mean was determined according to the Mixed Models procedure of SAS 9.4.

Results

One dog was removed from the study immediately following the washout phase for medical reasons (pain and enlarged mammary tissue). Therefore, one less dog was allotted to the control group before the second treatment period began. The remaining 15 dogs completed the study. Most dogs maintained their BW, food intake, and health throughout the study. Two dogs were administered oral antibiotics during the study period: cephalexin (525 mg, twice per day) for 10 d during the adaptation phase to treat an infected wound in one dog, and enrofloxacin (136 mg, twice per day) for 15 d during the first treatment period to treat a urinary tract infection in the other dog. Baseline measures are presented in Supplementary Tables S1–S3. Due to differences observed at baseline, change from baseline values was used to evaluate outcomes in the present study.

Change from baseline CBC and serum chemistry profiles were slightly affected by dietary treatment and time (Supplementary Tables S4 and S5). Change from baseline platelet concentrations and basophil % were affected ($P < 0.05$) by a time–diet interaction (Supplementary Table S4). Platelet concentrations were variable over time in dogs fed with the control diet but decreased over time in dogs fed with the SCFP diet. Basophil % increased slightly over time in dogs fed the control diet but was more variable in dogs fed the SCFP diet. Red blood cell (RBC) concentrations, hemoglobin concentrations, and hematocrit % changed ($P < 0.01$) over time but were not affected by diet. Change from baseline serum total protein, globulin, gamma glutamyltransferase, and triglyceride concentrations were lower ($P < 0.05$), while the albumin:globulin ratio tended to be higher ($P < 0.10$) in dogs fed the SCFP diet in than those fed the control diet (Supplementary Table S5). Change from baseline albumin:globulin ratio was higher ($P < 0.05$), while change from baseline triglyceride concentrations were lower ($P < 0.05$) over time in dogs fed the SCFP diet. Change from baseline globulin concentrations tended to be lower ($P < 0.10$), while change from baseline total bilirubin and creatine phosphokinase concentrations tended to be higher ($P < 0.10$) over time in dogs fed the SCFP diet. The other serum chemistry measures were not affected by diet or time.

Change from baseline of unstimulated lymphocytes (% of PBMC) and stimulated IFN-γ secreting T cells (% of lymphocytes) were lower ($P < 0.05$), while stimulated T cells (% of lymphocytes) were higher ($P < 0.05$) in dogs fed the SCFP diet than those fed with the control diet (Table 2). Change from baseline stimulated cytotoxic T cells (% of lymphocytes) tended to be lower ($P < 0.10$) in dogs fed with the SCFP diet than those fed with the control diet. All other T cell populations were not different between groups. Change from baseline NK cell and APC (B cells and monocytes) populations were not altered by treatment (Table 3). Change from baseline TNF-α concentrations of control cell culture supernatants tended to be lower ($P < 0.10$) in dogs fed with the SCFP diet than those fed with the control diet, but cells stimulated with TLR agonists were not affected by diet (Table 4).

Change from baseline serum SOD concentrations were higher ($P < 0.05$) in dogs fed with the SCFP diet than those fed with the control diet (Table 5). None of the other serum immune or oxidative stress markers were affected by treatment. Change from baseline mRNA expression of catalase was lower ($P < 0.05$) in dogs fed the SCFP diet than those fed the control diet (Table 6), but the expression of other genes was not affected by diet.

Change from baseline measures of skin and coat health were variable and region-specific. Change from baseline TEWL in the back region was lower ($P < 0.05$) in dogs fed with the control diet than those fed with the SCFP diet (Table 7). In contrast, change from baseline TEWL in the ear region tended to be lower ($P < 0.10$) in dogs fed the SCFP diet than those fed the control diet. Change from baseline sebum concentrations tended to be higher ($P < 0.10$) in the inguinal and ear regions of dogs fed with the SCFP diet than those fed with the control diet. Other change from baseline skin and coat measures were not affected by diet.

DTH response was not measured at baseline but was affected by diet and time at the end of the treatment phases (Table 8). As expected, wheal diameter increased over time for all injections. The response to PHA was higher ($P < 0.05$) and the response to ConA tended to be higher ($P < 0.10$) in dogs fed with the SCFP diet than those fed with the control diet.

Discussion

In recent decades, pet humanization has transformed the ways in which pet owners choose to feed their pets. Dogs are now considered a part of the family, driving owners to seek out premium, high-quality formulas often made with functional ingredients. Functional ingredients claim to provide health benefits beyond basic nutrition, such as immune support

Table 2. Week 10 change from baseline T cell populations of dogs consuming a SCFP-supplemented or control diet

Item	Δ Control	Δ SCFP	SEM	*P*-value
Unstimulated				
Lymphocyte, % of PBMC[1]	8.30[a]	−3.89[b]	4.19	0.0440
T cell, % of lymphocyte	−8.17	−3.20	3.12	0.2721
Helper T cell, % of lymphocyte	3.93	3.17	2.02	0.7911
Cytotoxic T cell, % of lymphocyte	−15.61	−20.40	5.42	0.2858
Helper: cytotoxic T cell ratio	10.48	13.96	9.54	0.7993
IFN-γ secreting T cell, % of lymphocyte	3.75	0.88	1.50	0.3568
IFN-γ secreting helper T cell, % of lymphocyte	0.00	0.01	0.01	0.2220
IFN-γ secreting cytotoxic T cell, % of lymphocyte	12.82	14.32	4.56	0.8174
Stimulated[2]				
Lymphocyte, % of PBMC[1]	−1.75	−6.28	2.05	0.1301
T cell, % of lymphocyte	−12.29[b]	−4.01[a]	3.23	0.0350
Helper T cell, % of lymphocyte	4.88	0.56	2.28	0.1388
Cytotoxic T cell, % of lymphocyte	-6.32	-21.13	5.68	0.0759
Helper: cytotoxic T cell ratio	2.15	13.32	4.30	0.1989
IFN-γ secreting T cell, % of lymphocyte	2.18[a]	−10.94[b]	4.09	0.0309
IFN-γ secreting helper T cell, % of lymphocyte	−1.53	−3.76	2.27	0.4916
IFN-γ secreting cytotoxic T cell, % of lymphocyte	8.31	10.87	4.46	0.6876

[1]PBMC = peripheral blood mononuclear cells.
[2]Cells were stimulated with cell stimulation cocktail (phorbol 12-myristate 13-acetate, ionomycin, brefeldin A, and monensin) for 4 h.

Table 3. Week 10 change from baseline natural killer cell and antigen-presenting cell populations of dogs consuming a SCFP-supplemented or control diet

Item	Δ Control	Δ SCFP	SEM	*P*-value
Natural killer cell, % of lymphocyte	-0.53	3.74	1.78	0.1006
Antigen Presenting Cells				
B cell, % of lymphocyte	2.10	1.57	0.92	0.6526
B cell, MHC II+, % of B cell[1]	0.06	-0.41	0.62	0.5892
Monocyte, % of white blood cell	6.90	1.60	2.56	0.1541
Monocyte, MHC II+, % of monocyte[1]	2.11	-3.43	4.94	0.4339

[1]B cells or monocytes that present major histocompatibility complex class II (MHC II).

or anti-inflammatory properties. Yeast-derived products are commonly added for their functional properties. Improvements to feed efficiency, growth performance and quality of animal products have been observed in several livestock species due to SCFP supplementation (Hristov et al., 2010; Kidd et al., 2013; Zaworski et al., 2014; Chen et al., 2020) and has stimulated interest in its use as a functional ingredient in companion animal diets. Previous research on yeast-based products in dogs has demonstrated that supplementation of yeast cell walls and their components (i.e., MOS and β-glucans) may modulate host microbiota (Swanson et al., 2002a; Grieshop et al., 2004) and alter fecal fermentative-end products (Swanson et al., 2002a; Theodoro et al., 2019), but the effects on immune function are not well defined. Early studies investigating the effects of yeast-based product supplementation on immune cell numbers and function were limited (Grieshop et al., 2004; Middelbos et al., 2007), possibly due to the lack of canine-specific research techniques at the time. However, supplementation of MOS in dogs has recently been shown to increase circulating CD4+ lymphocytes (43.7% vs. 45.6%) and the CD4+:CD8+ lymphocyte ratio (2.5 vs. 2.9; Pawar et al., 2017) and yeast cell wall supplementation has resulted in a higher (37% increase) phagocytic index in peripheral monocytes (Theodoro et al., 2019).

There is currently limited information on the functional properties of SCFP in companion animal diets. Lin et al. (2019) supplemented SCFP in dogs via gelatin capsules (0, 125, 250, and 500 mg/d) and reported positive effects on GI health outcomes, immune function outcomes, and inflammatory markers. However, our study investigated a slightly different dosage and delivery method of SCFP than the dogs evaluated by Lin et al. (2019). When SCFP intake was calculated based on the average BW (10.3 kg) reported by Lin et al. (2019), supplementation was equal to approximately 12.1, 24.3, and 48.5 mg SCFP/kg BW in the 125, 250, and 500 mg/d treatment groups, respectively. In the present study, the active SCFP ingredient was included as 27% of the SCFP product (i.e., active ingredient + carrier). Because the SCFP product was formulated into an extruded kibble diet and dogs were fed to maintain BW, this resulted in dogs consuming slightly different doses of SCFP depending on their energy requirements. When calculating the dose of SCFP based on daily food intake, dogs in the present study consumed an average of 30.1 ± 3.6 mg SCFP/kg BW per day throughout the treatment period. Furthermore, effects on oxidative stress and skin and coat health have not been thoroughly investigated, and to our knowledge, the functionality of SCFP has not been tested when included as an ingredient in an extruded kibble diet. In the present study,

Table 4. Week 10 change from baseline TNF- α concentrations (pg/mL) in cell culture supernatant of dogs consuming a SCFP-supplemented or control diet

Agonist[1]	Δ Control	Δ SCFP	SEM	*P*-value
Control, pg/mL	−116.72	−342.11	92.02	0.0957
LPS (TLR4), pg/mL	525.98	276.48	167.47	0.3011
Zymosan (TLR2), pg/mL	25.20	301.50	666.41	0.7716
Poly I:C (TLR3), pg/mL	545.55	132.56	172.15	0.1007
Resiquimod (TLR 7/8), pg/mL	2515.40	1505.74	1078.27	0.5169

[1]LPS mimics a bacterial challenge, zymosan mimics a yeast challenge, and poly I:C and resiquimod mimics a viral challenge.

Table 5. Week 10 change from baseline oxidative stress and immune function marker concentrations of dogs consuming a SCFP-supplemented or control diet

Measure	Δ Control	Δ SCFP	SEM	*P*-value
Lipopolysaccharide-binding protein, μmol/L	−4.38	2.37	4.72	0.3197
Lysozyme, ng/mL	−0.24	−0.21	0.08	0.7826
Malondialdehyde, nmol/mL	−1.32	−0.64	0.8	0.5521
Superoxide dismutase, ng/mL	−1.21[b]	2.84[a]	1.39	0.0304
8-isoprostane, pg/mL	20.18	20.85	8.57	0.9560
α-tocopherol, μg/mL	−0.76	−2.42	0.92	0.2160
Trolox equivalent antioxidant capacity (TEAC), μM Trolox	−67.42	26.16	40.37	0.1120
CAP-e fasted[1]	712.1	329.1	415.6	0.5204
CAP-e 2 h. postprandial[1]	507.1	223.3	450.1	0.6602

[1]Cell-based antioxidant protection in erythrocytes assay. Results provided as mean fluorescence intensity, where low fluorescence indicates less oxidative stress to RBC, and higher fluorescence indicates a higher level of stress to RBC.
[ab]Mean values within a row with unlike superscript letters differ ($P < 0.05$).

Table 6. Week 10 change from baseline whole blood gene expression fold change of dogs consuming a SCFP-supplemented or control diet

Measure	Δ Control	Δ SCFP	SEM	p-value
Cyclooxygenase-2	0.08	-0.06	0.09	0.2039
Glutathione peroxidase	0.20	0.22	0.08	0.1995
Myeloperoxidase	0.25	0.08	0.16	0.3532
Superoxide dismutase	0.27	0.50	0.11	0.5570
Catalase	0.20[a]	0.17[b]	0.10	0.0156
Glutathione reductase	-0.42	-0.27	0.22	0.5444

[ab]Mean values within a row with unlike superscript letters differ ($P < 0.05$).

we investigated measures of skin and coat health and further measured circulating immune cell numbers and activity, antioxidant status, and oxidative stress marker concentrations of adult dogs fed an SCFP-supplemented extruded diet. All dogs remained healthy throughout the study. CBC were within reference ranges for healthy dogs. Despite some serum chemistry markers (corticosteroid-induced alkaline phosphatase; alanine transaminase) being slightly above the reference ranges, no adverse clinical signs were noted during the study.

Table 7. Week 10 change from baseline hair and skin scores, sebum concentrations[1], hydration status[2], and transepidermal water loss (TEWL)[3] of dogs consuming a SCFP-supplemented or control diet

Measure	Δ Control	Δ SCFP	SEM	*P*-value
Skin score[4]	-0.03	-0.02	0.09	0.6877
Hair score[5]	-0.11	-0.03	0.09	0.5562
Sebum concentration (back)[6], arbitrary unit	3.00	1.44	0.93	0.3276
Sebum concentration (inguinal)[7], arbitrary unit	-0.04	1.48	0.61	0.0893
Sebum concentration (ear)[8], arbitrary unit	22.97	38.82	7.22	0.0572
Hydration (back)[6], arbitrary unit	0.05	-0.88	0.43	0.1468
Hydration (inguinal)[7], arbitrary unit	0.44	2.30	2.65	0.4583
Hydration (ear)[8], arbitrary unit	-1.58	-4.49	3.29	0.5076
TEWL (back)[6], g/h/m²	-6.47[b]	-3.82[a]	0.99	0.0410
TEWL (inguinal)[7], g/h/m²	-0.25	0.84	1.01	0.3465
TEWL (ear)[8], g/h/m²	0.85	-1.18	0.77	0.0545

[1]Measured using a sebumeter, μg/cm².
[2]Measured using a corneometer, arbitrary units.
[3]Measured using a tewameter, g/h/m².
[4]Skin scores: 1 = dry; 2 = slightly dry; 3 = normal; 4 = slightly greasy; 5 = greasy.
[5]Hair scores: 1 = dull, coarse, dry; 2 = poorly reflective, nonsoft; 3 = medium reflective, medium soft; 4 = highly reflective, very soft; 5 = greasy.
[6]Back measurements were taken on the right side along the spine between the rib and hip bone.
[7]Inguinal measurements were taken on the left side.
[8]Ear measurements were taken on the left side towards the inside of the ear.
[ab]Mean values within a row with unlike superscript letters differ ($P < 0.05$).

To our knowledge, the effects of SCFP supplementation on skin and hair coat scores or skin biophysical parameters have not been previously investigated in dogs. Supplementation of SCFP did not affect subjective skin and coat scores or hydration status in the current study. The tendency for higher change from baseline in sebum concentrations in dogs fed SCFP, however, may provide benefits. Sebum is thought to protect the epidermis by lubricating the stratum corneum and hair follicle (Dunstan et al., 2000). It also gives the hair coat a glossy appearance, which is desired by pet owners. Although sebum concentrations increased in SCFP-fed dogs, the change did not result in an excessively greasy hair coat, as mean hair coat scores were "medium reflective/medium soft" for both groups.

TEWL measurements provide valuable information for assessing the integrity of the skin barrier and estimating the skin's moisture retention properties (Pinnagoda et al., 1990, Watson et al., 2002; Fluhr et al., 2006). TEWL outcomes varied by body site in our study, where TEWL values were higher in the back region but lower on the ears of SCFP-fed dogs. Higher TEWL values indicate greater water loss and are consistent with increased damage to epidermal barrier function. TEWL measures must be interpreted carefully, however, as they can be altered by sampling methods (e.g., open chamber probe, closed chamber probe, hair clipping, and the

Table 8. Week 10 delayed-type hypersensitivity measurements[1] of dogs consuming a SCFP-supplemented or control diet

Measure					p-value	
	Control	SCFP	SEM	Diet	Time	Diet*Time
Saline			0.24	0.6751	<0.0001	0.9452
15 min	1.92	2.38				
30 min	1.38	1.74				
45 min	0.86	0.69				
60 min	0.09	0.18				
24 h	0.01	0.00				
48 h	0.01	0.00				
72 h	0.01	0.00				
PHA[2]			0.65	<0.0001	<0.0001	0.0200
15 min	3.40	5.94				
30 min	2.60	5.59				
45 min	1.53	4.69				
60 min	0.65[b]	3.37[a]				
24 h	0.90	1.69				
48 h	0.07	0.00				
72 h	0.07	0.00				
ConA[2]			1.27	0.0653	<0.0001	0.8623
15 min	4.74	5.99				
30 min	3.17	5.09				
45 min	1.83	3.60				
60 min	1.13	1.57				
24 h	7.32	9.55				
48 h	5.47	7.36				
72 h	2.31	2.47				
Vaccine[2]			0.73	0.7794	<0.0001	0.9395
15 min	4.26	4.95				
30 min	2.96	4.30				
45 min	1.77	3.53				
60 min	1.10	2.15				
24 h	0.42	0.20				
48 h	1.03	1.08				
72 h	0.68	0.48				

[1]Average wheal diameter, mm.
[2]PHA, phytohaemagglutinin; ConA, concanavalin A; vaccine, Duramune Max5 Dog Vaccine (Elanco Animal Health, Greenfield, IN).

use of anesthesia; Watson et al., 2002; Oh and Oh, 2010). It has also been observed that TEWL measurements are subject to significant site-to-site, day-to-day, and dog-to-dog variation (Lau-Gillard et al., 2010), which may account for the discrepancy in TEWL data between body sites and treatment groups in the present study. Throughout our TEWL pilot studies, however, the ear region produced the most consistent values among dogs in our cohort, which we suspect was due to a lack of interference from the hair coat. Despite clipping the hair coat prior to measurement, it was difficult to ensure the TEWL probe maintained direct contact with the skin on the back region. Thus, TEWL values from the ear region may be more reliable for interpretation, indicating that reduced TEWL on the ears of SCFP-fed dogs may be due to enhanced skin integrity.

The DTH response is an intradermal test that indicates the in vivo cell-mediated immune response (Kim et al., 2000). This response is characterized by the activation of phagocytes and antigen-specific cytotoxic T cells and the production of proinflammatory cytokines. It has been shown that mannoproteins, a component of yeast cell walls, can induce hypersensitivity reactions and release cytokines, such as TNF-α and IFN-γ (Chaka et al., 1997; Pietrella et al., 2001). In our study, DTH response was not measured at baseline but was affected by diet and time at week 10. As expected, wheal diameter increased over time for all injections. In response to PHA and ConA, a greater increase in wheal diameter was observed in dogs fed the SCFP-supplemented diet than those fed the control diet. Diet and time interactions were observed for DTH response to the nonspecific lymphocyte mitogen PHA in SCFP-fed dogs. In contrast, the diet tended to influence DTH response in SCFP-fed dogs after stimulation with ConA, another nonspecific lymphocyte mitogen. Similar DTH results were reported in dogs supplemented with MOS (Pawar et al., 2017) and mannoproteins (Kroll et al., 2020) following intradermal inoculation with PHA. Pawar et al. (2017) reported that DTH response (i.e., skin thickness) to PHA inoculation was significantly higher in the MOS group when compared with controls, and Kroll et al. (2020) reported diet and time effects as well as time and age interactions after PHA injection. Increased DTH response to nonspecific antigens PHA and ConA may suggest an enhanced cell-mediated immune response due to SCFP supplementation.

Dietary supplementation with yeast-based products has been previously shown to modulate circulating immune cell populations and immune function in dogs (Swanson et al., 2002a; Grieshop et al., 2004; Middelbos et al., 2007; Pawar et al., 2017; Theodoro et al., 2019; Lin et al., 2019; Kroll et al., 2020). The lower change from baseline unstimulated lymphocytes observed in SCFP-supplemented dogs in the current study were similar to that reported in previous dog studies investigating yeast supplementation. Supplementation with MOS increased (15.55% vs. 20.40%; Swanson et al., 2002a) or tended to decrease (2.2 10^3/μL vs. 1.7 10^3/μL; Grieshop et al., 2004) lymphocyte populations. Lin et al. (2019) did not report changes in lymphocyte populations. However, Lin and colleagues reported dogs having lower total WBC counts, showing a linear effect with increasing SCFP dose (0 vs. 125, 250, and 500 mg/d), potentially due to an enhanced immune capacity requiring fewer immune cells. This was supported by immune cell population data demonstrating increases in MHC-II+ B cells, effector T cells (IFN-γ secreting helper and cytotoxic T cells), and monocytes (Lin et al., 2019) in SCFP-supplemented dogs, which is not consistent with our data. In the present study, change from baseline populations of stimulated IFN-γ secreting T cells and cytotoxic T cells decreased in SCFP-fed dogs. Change from baseline stimulated T cells decreased in both treatment groups, although populations were significantly lower in the control group. It is difficult to interpret the changes observed in immune cell populations among SCFP-supplemented and control dogs in the present study. However, the decrease in circulating lymphocytes among SCFP-fed dogs may suggest a more balanced immune response. In addition to functional TLR stimulation, utilizing additional assays to investigate immune function (i.e., lymphocyte proliferation and/or phagocytic activity of leukocytes) may aid future studies in interpreting the changes observed in immune cell numbers.

An additional immune response observed in the study by Lin et al. (2019) was a significant reduction in TNF-α concentrations in the cell supernatant of SCFP-supplemented dogs. In cells stimulated with TLR2, TLR3, TLR4, and TLR7/8 agonists, an overall effect of controls (0 mg/d SCFP) vs. all SCFP treatments (125, 250, and 500 mg/d) was observed, with cells obtained from dogs supplemented with SCFP producing less TNF-α than cells collected from control dogs (Lin et al., 2019). These agonists represent yeast (zymosan, TLR2), bacterial (lipopolysaccharide, TLR4), and viral (polyinosinic:polycytidylic acid, TLR3; resiquimod, TLR7/8) challenges to the immune system. A reduction in the pro-inflammatory cytokine TNF-α coupled with reduced lymphocyte populations may have suggested a more moderated immune response due to SCFP supplementation. However, despite some numeric differences in TNF-α production in our study, this hypothesis cannot be confirmed due to the lack of significant differences in TNF-α concentrations between treatment groups and high biological variation in TNF-α concentrations between dogs.

Mannans isolated from *S. cerevisiae* have been shown to have antioxidant properties in vitro (Krizková et al., 2001), and zymosan derived from glucans in the yeast cell wall have been shown to increase antioxidant function in tumor-bearing mice (Liu et al., 2011). In the present study, SCFP supplementation increased serum SOD concentrations and decreased catalase mRNA expression, suggesting that antioxidant defenses may function more efficiently in SCFP-fed dogs. To maintain a homeostatic level of oxidative stress, powerful enzymatic systems such as SOD and catalase directly counteract the formation of reactive oxygen species. Superoxide is a major oxidant produced during oxidative stress and is scavenged by the enzyme SOD. The reaction creates hydrogen peroxide, which is subsequently detoxified by glutathione or catalase (Eaton, 2006). Previous data on dogs supplemented with MOS (15 g/kg of the diet) indicated no influence of MOS on erythrocytic antioxidants (Pawar et al., 2017), and oxidative stress markers (i.e., SOD and MDA) were not different in dogs supplemented with SCFP (125, 250, and 500 mg/d) or the placebo (Lin et al., 2019). However, SCFP-supplemented dogs (500 mg/d) challenged with exercise and transport stress exhibited increased concentrations of serum TAC and reduced concentrations of serum TBARS, suggesting that SCFP may provide some protection from oxidative damage under conditions of stress (Varney et al., 2021). Using an induced environmental stress model, such as exercise or transport stress, may aid future studies in detecting changes to the antioxidant status or oxidative stress in healthy dogs. Further research on the antioxidant status and oxidative stress markers of dogs fed SCFP is warranted, as our data suggest SCFP-supplementation increases the activity of antioxidant enzymes SOD and catalase and may reduce oxidative damage.

The treatment duration and experimental design of the present study allowed for a thorough, longitudinal assessment of the effects of SCFP supplementation in which each dog could serve as their own control. However, the experimental design can also be considered a limitation of the study, as it may have increased variability due to seasonal changes. Seasonal changes (e.g., relative humidity and room temperature) may have impacted skin and hair coat measures, as dogs in the present study had daily outdoor access across multiple seasons. However, giving dogs in the study outdoor access provided a more accurate comparison to pet dogs. Variability in skin and hair coat outcomes may also be attributed to the use of anesthesia (Oh and Oh, 2010) or interference from the hair coat (Momota et al., 2013). Another potential limitation of the study was the measurement of 8-isoprostane in serum rather than urinary samples. Measurement of urinary rather than plasma or serum 8-isoprostane has been proposed as a better indicator of oxidative stress in humans because arachidonic acid, the metabolic precursor of isoprostanes, is widely distributed in cell membranes throughout tissues, providing a comprehensive reflection of oxidative stress activation in the entire body (Montero et al., 2000; Monnier et al., 2006). Moreover, 8-isoprostane concentrations fluctuate throughout the day, hence urinary determinations are likely to provide a more reliable estimation of the 24 h fluctuations in isoprostane production than blood sampling at fixed time points (Monnier et al., 2006; Ito et al., 2019). Logistically, metabolic cages would likely be required to carry out urine sampling over a 24-h period in dogs. Although such methodology is more difficult to use, it would allow for the accurate measurement of urinary 8-isoprostane and a more accurate measurement of oxidative stress.

Conclusions

Our data suggest SCFP may act as a functional ingredient in dog foods to beneficially impact skin and coat health, immunity, and antioxidant status when included in an extruded diet. SCFP was shown to improve skin and coat health by improving skin barrier function and increasing sebum concentrations, which act to lubricate and protect skin while giving the hair coat a glossy appearance. SCFP had effects on some parameters of cell-mediated and humoral immunity by enhancing the DTH response to nonspecific lymphocyte mitogens (PHA and ConA) and modulating circulating lymphocyte populations. Finally, SCFP supplementation was shown to support key antioxidant defense systems through modulation of enzymes SOD and catalase. Our data suggest that SCFP can be included in extruded dog foods as a functional ingredient to support antioxidant status and skin integrity. SCFP supplementation may be most beneficial to geriatric and/or working dogs and dogs with skin sensitivities.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of Interest Statement

M.R.P. is employed by Blue Buffalo Co. Ltd and S.A.N. is employed by Cargill, Inc. All other authors have no conflicts of interest.

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