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Effects of a *Saccharomyces cerevisiae* fermentation product-supplemented diet on fecal characteristics, oxidative stress, and blood gene expression of adult dogs undergoing transport stress

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Abstract

Previously, a *Saccharomyces cerevisiae* fermentation product (SCFP) was shown to positively alter fecal microbiota, fecal metabolites, oxidative stress, and circulating immune cell function of adult dogs. The objective of this study was to measure the effects of SCFP on fecal characteristics, serum oxidative stress biomarkers, and whole blood gene expression of dogs undergoing transport stress. Sixteen adult pointer dogs [8M, 8F; mean age = 6.7 ± 2.1 yr; mean body weight (BW) = 25.5 ± 3.9 kg] were used in a randomized crossover design study. All dogs were fed a control diet for 4 wk, then randomly assigned to a control or SCFP-supplemented diet (formulated to include approximately 0.13% of the active SCFP ingredient) and fed to maintain BW for 11 wk. A 6-wk washout preceded the second 11-wk experimental period with dogs receiving opposite treatments. After 11 wk, fresh fecal and blood samples were collected before and after transport in a van for 45 min. Change from baseline data (i.e., before and after transport) were analyzed using the Mixed Models procedure of SAS 9.4, with $P < 0.05$ being significant and $P < 0.10$ being trends. Change in serum malondialdehyde concentrations increased ($P < 0.05$) and serum 8-isoprostane concentrations tended to increase ($P < 0.10$) in dogs fed SCFP, but decreased ($P < 0.05$) in control dogs after transport. Other serum markers were unaffected by diet during transport stress. Fecal dry matter percentage tended to be affected ($P < 0.10$) by diet during transport stress, being reduced in control dogs, but stable in dogs fed SCFP. Other fecal characteristics were unaffected by diet during transport stress. Genes associated with activation of innate immunity were impacted by diet in response to transport stress, with blood cyclooxygenase-2 and malondialdehyde mRNA expression being increased ($P < 0.05$) in control dogs, but stable or decreased in dogs fed SCFP. Expression of other genes was unaffected by diet during transport stress. These data suggest that the benefits of feeding a SCFP during transport stress may be mediated through suppression of innate immune cell activation.

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 fecal characteristics and oxidative stress of dogs undergoing transport stress. Using a randomized crossover study design, 16 adult pointer dogs were used to compare changes in fecal characteristics, oxidative stress marker concentrations, and gene expression when fed a SCFP-supplemented diet or control diet. After transport, change in serum malondialdehyde concentrations increased and serum 8-isoprostane concentrations tended to increase in dogs fed SCFP, but decreased in control dogs. Fecal moisture percentage tended to be affected by diet during transport stress, being reduced in control dogs, but stable in dogs fed SCFP. Blood cyclooxygenase-2 and myeloperoxidase mRNA gene expression was affected by diet during transport stress, being increased in control dogs, but stable or decreased in dogs fed SCFP. In conclusion, these data suggest that the benefits of feeding a SCFP during transport stress may be mitigated through suppression of innate immune cell activation rather than through suppressing oxidative damage to lipids.

Key words: canine nutrition, postbiotic, yeast product

Abbreviations: AAPH, 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride; BW, body weight; CAP-e, cell-based anti-oxidant protection in erythrocytes; CNS, central nervous system; COX-2, cyclooxygenase-2; DM, dry matter; GI, gastrointestinal; IgA, immunoglobulin A; MDA, malondialdehyde; MPO, myeloperoxidase; SCFP, *Saccharomyces cerevisiae* fermentation product; SOD, superoxide dismutase; TEAC: Trolox equivalent anti-oxidant capacity

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Introduction

Stress refers to any external or internal stimulus that evokes a biological response, and this response to a perceived stressor is known as a stress response (Yaribeygi et al., 2017). The stress response, which is largely dependent on the type, duration, and severity of the stressor, can lead to a variety of events within the body, from disruptions in homeostasis to the development of life-threatening diseases. Acute stress is characterized as adaptive because it quickly responds to defend the organism from danger (Selye, 1956). Acute stress elicits hormone release by the sympathetic nervous system and hypothalamic-pituitary-adrenocortical axis to make energy stores available for the body's immediate use, diverting energy to the tissues that become more active during stress (e.g., skeletal muscles and the brain). Acute stress also activates the innate immune system, causing cells to migrate to the tissues most vulnerable to assault, such as the skin, to aid in pathogen clearance and wound healing (Dhabhar and McEwen, 1997). Cyclooxygenase-2 (COX-2) and myeloperoxidase (MPO) are key genes involved in innate immunity and are involved in this response (Feng et al., 1995; Arnhold, 2020).

Although the body can adapt to short-term stress, the acute stress response can become chronic and maladaptive when activated repeatedly (Selye, 1956; Ketchesin et al., 2017). Chronic stress has been shown to suppress the immune system (Khansari et al., 1990) and adversely affect the gastrointestinal (GI) tract's absorptive capabilities, permeability, mucus secretion, and inflammation (Collins, 2001). Previous research investigating the impacts of stress in canines has demonstrated that transportation- and/or exercise-induced stress modulates oxidative stress responses, GI inflammation, hematological parameters, immune cell trafficking and effector function, and enzyme and hormone activities (Ochi et al., 2013; Ferreira et al., 2014; Fazio et al., 2015; Dhabhar, 2018; DeClue et al., 2020; Zannoni et al., 2020). Transportation, exercise, and other naturally occurring stressors (e.g., loud noises, new places, changes in routine) are common in today's world and inevitable events for most dogs, which can lead to both acute and chronic stress.

While there is little research specifically investigating dietary interventions to reduce transportation stress in dogs at the molecular level (e.g., biomarkers in feces and blood), diet has been previously shown to impact behavioral signs of stress and anxiety in dogs (DeNapoli et al., 2000; Beata et al., 2007; Cracknell and Mills, 2008; Landsberg et al., 2015; Pike et al., 2015; Titeux et al., 2021). Moreover, it has been shown that dietary supplementation with functional ingredients, such as plant-based compounds and dietary fibers, can support GI health (Swanson et al., 2002), promote changes in biochemical parameters (Spears et al., 2004; Pasquini et al., 2013; Cortese et al., 2015), or improve brain function that may increase longevity and reduce behavioral disorders (Sechi et al. 2015, 2017) in dogs.

Saccharomyces cerevisiae fermentation product (SCFP) is a functional ingredient that may have the potential to ameliorate oxidative stress and inflammation during periods of acute stress, as it has been previously shown to positively impact GI health, immune function, and oxidative stress in adult dogs (Lin et al., 2019; Varney et al., 2021; Wilson et al., 2022). SCFP is a dry product produced via *S. cerevisiae* fermentation and includes residual yeast cells, yeast cell wall fragments, bioactive fermentation metabolites, and media used during fermentation. The exact composition of SCFP is proprietary; however, yeast products are commonly known to include components of the yeast cell wall, such as mannanoligosaccharides and β-glucans, which have been shown to improve intestinal health and barrier function by increasing fecal *Bifidobacterium* and *Lactobacillus* populations, elevating ileal IgA concentrations, inhibiting the expression of inflammatory mediators, and enhancing the expression of tight junction proteins associated with intestinal permeability (Swanson et al., 2002; Grieshop et al., 2004; Han et al., 2017). Furthermore, mannan oligosaccharides and β-glucans within the cell wall and metabolites from fermentation have been implicated in yeast's immunomodulatory properties. Through digestion, these components encounter the gut-associated lymphoid tissue, influencing its immune function and systemic immunity (Field et al., 1999). Finally, mannans isolated from *S. cerevisiae* have been shown to possess anti-oxidant properties in vitro (Krizková et al., 2001), and zymosan derived from glucans in the yeast cell wall have been shown to increase anti-oxidant function in tumor-bearing mice (Liu et al., 2011). Thus, the composition of *S. cerevisiae* and bioactive yeast fermentation metabolites have the potential to support GI health, immunity, and oxidative stress parameters. Further investigation is warranted to determine if SCFP may mitigate cellular damage or disruptions to homeostasis during periods of acute stress.

The objectives of this study were to determine the effects of an SCFP-supplemented diet on fecal characteristics, fecal immunoglobulin A (IgA) and calprotectin concentrations, serum anti-oxidant status and oxidative stress biomarkers, and whole blood gene expression of dogs undergoing transport stress. We hypothesized that, without negatively impacting fecal characteristics, dogs fed the SCFP-supplemented diet would have enhanced anti-oxidant status and reduced concentrations of serum oxidative stress markers after transport stress compared with dogs fed the 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 BW = 25.5 ± 3.9 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

Before the study, blood samples were collected for serum chemistry measures and complete blood count to confirm health. A crossover study began with a 4-wk adaptation phase followed by two 11-wk experimental periods. After 11 wk, fasted (12 h overnight) blood samples were collected immediately before and after canines were transported in carriers (0.9 m × 0.6 m × 0.7 m) with 3 to 4 dogs per van for 45 min. Fresh fecal samples were collected from each

dog before and within 1 d after transport. There was a 6-wk washout phase between experimental periods to decrease the possibility of treatment carryover effects. Given recent data from our laboratory (Lin et al., 2022), this period of time should have provided plenty of time for gut adaptations and microbiota to stabilize prior to the start of the next experimental period. Dogs were fed the control diet during the adaptation and washout phases, then randomly allotted to a SCFP-supplemented or control diet. Both dietary treatments tested were extruded kibble diets formulated to meet all Association of American Feed Control Officials (AAFCO, 2020) nutrient profiles 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 ± 3.5 mg SCFP/kg BW per day (range: 21.7 to 37.9 mg SCFP/kg BW). Dogs were weighed and body condition scores were assessed using a 9-point scale (Laflamme, 1997) once per week prior to the morning feeding.

Table 1. Ingredient and analyzed 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
Nitrogen-free extract[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 × % nitrogen-free extract)].

Fecal scoring, sample collection, and analysis

Fresh fecal samples were collected for scoring, pH, dry matter (DM), IgA, and calprotectin at week 11 (before and within 1 d after transport stress) of each experimental period. All fecal samples collected were scored according to the following scale: 1) hard, dry pellets, small hard mass; 2) hard, formed, dry stool, remains firm and soft; 3) soft, formed, and moist stool, retains shape; 4) soft, unformed stool, assumes shape of container; 5) watery, liquid that can be poured. Fecal pH was measured immediately using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman Instruments Inc., Fullerton, CA), and then aliquots were collected. One aliquot of fresh feces was collected for IgA and calprotectin analysis. These samples were immediately transferred to sterile cryogenic vials (Nalgene, Rochester, NY), quickly frozen in dry ice, and stored at –80 °C for later analysis. Another aliquot was used for fresh fecal DM determination and was measured according to AOAC (2006) using a 105 °C oven.

Fecal protein extraction

Fecal proteins were extracted according to Vilson et al. (2016). Fecal samples (500 mg) were vortexed with 1.5-mL extraction buffer containing 50 mM-EDTA (ThermoFisher, Waltham, MA) and 100 µg/L soybean trypsin inhibitor (Sigma, St. Louis, MO) in 1X PBS/L percent bovine serum albumin (Tocris Bioscience, Bristol, UK). Phenylmethanesulphonyl fluoride (12.5 µL, 350 mg/L; Sigma, St. Louis, MO) was added into each tube, followed by centrifugation at 10,000 × *g* at 25 °C for 10 min. The supernatants were collected for measurements of IgA and calprotectin using commercial ELISA kits (IgA: #MBS018650; calprotectin: #MBS030023, MyBioSource, San Diego, CA).

Blood sample collection

Fasted (12 h overnight) blood samples were collected for anti-oxidant status and oxidative stress measures after week 11 of each experimental period (before and after transport in a van for 45 min). Blood samples were collected via jugular or cephalic puncture. Samples were immediately transferred to appropriate vacutainer tubes, with some blood going into BD Vacutainer Plus plastic whole blood tubes (#363706; Lavender with K_2EDTA additive; Becton Dickinson, Franklin Lakes, NJ), some blood going into BD Vacutainer SST tubes (#367988 and #367983; Becton Dickinson) for serum separation, and some blood going into PAXgene Blood Tubes (#762165; Qiagen, Valencia, CA). Serum was isolated by centrifugation 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 aliquoted to cryovials and stored at –80 °C until analysis.

Serum oxidative stress markers

Circulating oxidative stress markers [8-isoprostane, Trolox equivalent anti-oxidant capacity (TEAC); malondialdehyde (MDA); and superoxide dismutase (SOD)] concentrations were measured using commercial ELISA kits (8-isoprostane: #MBS2611970, TEAC: # MBS169313, MDA: #MBS2605193,

SOD: #MBS2104718, MyBioSource, San Diego, CA) according to the manufacturer's instructions. 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 cell-based anti-oxidant protection in erythrocytes (CAP-e) assay.

CAP-e assay

To test for anti-oxidants in serum that were likely to be bio-available at the cellular level in vivo, 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 anti-oxidant protection assay, each canine serum sample was tested in quadruplicate, using human erythrocytes to detect anti-oxidant compounds present in 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 10x 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, anti-oxidant compounds are 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′-dichlorofluorescein 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 anti-oxidants 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 were 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). cDNA were 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), MPO (UniqueAssayID: qCfaCID0034597), and 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).

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 DM and ash according to AOAC (2006; methods 934.01 and 942.05), with organic matter 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).

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 dog was considered a random effect. Change from baseline (i.e., before and after transport) differences between treatments (16 replicates per treatment) were 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 $P < 0.10$ being trends. Reported pooled standard errors of the mean were 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. Clinical signs (e.g., vomiting) were not observed during transport. Dogs maintained their BW, food intake, and health throughout the study, but 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 allotted to the SCFP group.

Fecal characteristics and fecal protein concentrations

Change from baseline values (i.e., before and after transport stress) were used to evaluate the impacts of transport stress on outcomes in the present study. Most of the fecal characteristics measured, including fecal pH, fecal score, and concentrations of fecal IgA and calprotectin were not different between

treatments when challenged with transport stress (Table 2). Change in fecal DM %, however, tended to be lower ($P < 0.10$) in dogs fed the control diet than dogs fed the SCFP diet. The LS means of the variables analyzed are presented in Supplementary Table S1.

Serum oxidative stress markers

Change from baseline serum MDA concentrations were higher ($P < 0.05$) and change from baseline serum 8-iso-prostane concentrations tended to be higher ($P < 0.10$) in dogs fed the SCFP-supplemented diet than those fed the control diet (Table 3). The other serum markers of anti-oxidant status (i.e., α-tocopherol, TEAC, CAP-e) and oxidative stress marker (i.e., SOD) responses to transport stress were not affected by supplementation with SCFP. The LS means of the variables analyzed are presented in Supplementary Table S2.

Whole blood gene expression

Change from baseline mRNA expression of COX-2 and MPO were affected by diet (Table 4). Change from baseline mRNA expression of MPO decreased ($P < 0.05$) in dogs fed the SCFP-supplemented diet, but increased in those fed the control diet. Change from baseline mRNA expression of COX-2 remained stable after transport in SCFP-fed dogs, but increased ($P < 0.05$) in the control group. The mRNA expression of glutathione peroxidase, SOD, catalase, and glutathione reductase was not affected by diet in response to transport stress. The LS means of the variables analyzed are presented in Supplementary Table S3.

Table 2. Change from baseline fecal characteristics of dogs consuming a SCFP-supplemented or control diet before and after transport stress

Measure	Δ Control	Δ SCFP	SEM	*P*-value[1]
Fecal pH	−0.47	−0.15	0.17	0.2063
Fecal score[1]	−0.33	−0.22	0.19	0.6692
Fecal dry matter, %	−1.89	0.06	0.72	0.0653
Fecal IgA, mg/g	1.32	1.02	0.67	0.7561
Fecal calprotectin, µg/g	0.09	0.07	0.05	0.7936

[1]Fecal scores: 1 = hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape;4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.

Discussion

Our previous study reported changes in circulating immune cell percentages and effector function, anti-oxidant status and oxidative stress marker concentrations, and measures of skin and coat health of adult dogs fed an SCFP-supplemented extruded diet under normal conditions (no stressor; Wilson et al., 2022). This study was done to test whether long-term SCFP consumption could also provide benefits to healthy adult dogs during an acute stress challenge in the form of transport stress, with fecal characteristics, fecal protein concentrations, markers of antioxidant status, and oxidative stress markers being the primary outcomes. Change in fecal DM percentage tended to decrease in control dogs, but remained stable in dogs fed SCFP after transport, suggesting that SCFP may be able to modulate stress-induced diarrhea. Change in serum MDA concentrations increased and serum 8-isoprostane concentrations tended to increase in SCFP-supplemented dogs compared with controls, indicating a higher degree of lipid peroxidation in SCFP-treated dogs due to acute stress. Finally, whole blood mRNA gene expression of COX-2 and MPO increased in control dogs yet remained stable or decreased, respectively, in dogs fed SCFP, suggesting that SCFP supplementation may suppress activation of the innate immune response during acute stress.

The central nervous system (CNS) communicates with the GI tract through the gut-brain axis, which refers to the bidirectional communication and transfer of information from the CNS to the enteric nervous system. In response to environmental stress, including transportation, the CNS regulates enteric immunity through the activation of leukocytes, expression of inflammatory mediators, and synthesis of secretory IgA to protect the intestinal lumen from bacteria, viruses, and tissue trauma (Campos-Rodríguez et al., 2013; de Jonge, 2013; Jukic et al., 2021). Although the stress coming from transportation may often lead to loose stools or diarrhea in dogs and domesticated livestock, most of the fecal characteristics in the current study were not affected. However, change in fecal DM% tended to be lower in dogs fed the control diet than dogs fed the SCFP diet post-transport, suggesting that SCFP may be able to limit negative changes to stool quality. Because the change in fecal DM% was very small and fecal

Table 3. Change from baseline oxidative stress marker concentrations of dogs consuming a SCFP-supplemented or control diet before and after transport stress

Measure	Δ Control	Δ SCFP	SEM	*P*-value
Malondialdehyde, nmol/mL	−0.52[b]	2.52[a]	0.96	0.0337
Superoxide dismutase, ng/mL	1.10	0.93	1.24	0.9254
8-Isoprostane, pg/mL	−13.11	26.54	16.15	0.0935
α-Tocopherol, µg/mL	−1.81	−1.63	0.49	0.7883
Trolox equivalent antioxidant capacity (TEAC), µM Trolox	−18.51	−3.50	16.83	0.5113
CAP-e[1]	−173.0	402.8	357.70	0.2646

[1] CAP-e: cell-based anti-oxidant protection in erythrocytes assay. Results provided as mean fluorescence intensity, where low fluorescence indicates less oxidative stress to red blood cells, and higher fluorescence indicates a higher level of stress to red blood cells.
[a,b]Mean values within a row with unlike superscript letters differ ($P < 0.05$).

Table 4. Change from baseline whole blood gene expression fold change of dogs consuming a SCFP-supplemented or control diet before and after transport stress

Measure	Δ Control	Δ SCFP	SEM	*P*-value[1]
Cyclooxygenase-2	0.20[a]	0.02[b]	0.05	0.0461
Glutathione peroxidase	−0.15	0.01	0.08	0.5272
Myeloperoxidase	0.70[a]	−0.24[b]	0.20	0.0383
Superoxide dismutase	0.51	0.32	0.09	0.2372
Catalase	0.34	0.27	0.07	0.7146
Glutathione reductase	0.33	2.33	1.10	0.7629

[1]Statistics were conducted using ΔΔCt values to generate *P*-values; data are reported as fold change (2^-ΔΔCt) in relation to a housekeeping gene (RPS5).
[a,b]Mean values within a row with unlike superscript letters differ ($P < 0.05$).

scores and the inflammatory marker calprotectin were not altered with transport stress or due to diet, further investigation is necessary.

Oxidative stress refers to an imbalance between the generation of oxidants and their elimination systems (i.e., antioxidants) in favor of oxidants, leading to a disruption of redox signaling and control and/or molecular damage (Sies et al., 2017). Recent findings suggest that exposure to acute and chronic stressors can increase levels of oxidative stress (Lin et al., 2004; Haussmann et al., 2012; Treidel et al., 2013; Marasco et al., 2017). In the current study, changes in serum MDA and 8-isoprostane concentrations depended on the diet fed, with increases in dogs fed SCFP and decreases in dogs fed the control diet. This response suggests an increased level of lipid peroxidation in SCFP-treated dogs following transport stress. MDA and F_2-isoprostanes, including 8-isoprostane, have been extensively investigated as markers of lipid peroxidation and are considered reliable biomarkers of oxidative stress in vivo (Kadiiska et al., 2005; Niki, 2014). Generation of MDA and 8-isoprostane occurs through the peroxidation of arachidonic acid, and these harmful end-products are often implicated in the pathogenesis of diseases (e.g., cancer, diabetes, asthma, atherosclerosis, Alzheimer's disease, and Parkinson's diseases; Dalle-Donne et al., 2006; Giustarini et al., 2009; Tsikas, 2017).

Our data does not reveal a clear explanation as to why supplementation of SCFP resulted in changes to serum MDA and 8-isoprostane concentrations after transport stress, requiring further investigation. However, measurement of urinary rather than plasma or serum markers of 8-isoprostane has been proposed as a better indicator of oxidative stress in humans because arachidonic acid, their metabolic precursor, 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, pre-analytical factors must also be considered when assessing the degree of lipid peroxidation in lipid-rich samples such as plasma or serum, as MDA is particularly sensitive to storage conditions, analytical processes, and hemolysis that could lead to artificially high concentrations (Dreissigacker et al., 2010; Yoshida et al., 2013; Tsikas et al. 2016, 2017). This is a limitation of our study, as serum was the sole biological fluid harvested for the measurement of MDA and 8-isoprostane, and samples were stored at -80 °C as opposed to immediate analysis following blood sampling.

Similar to oxidative stress marker response, changes in whole blood gene expression depended on dietary treatment. While mRNA expression of COX-2 remained stable and MPO expression decreased in dogs fed SCFP, the expression of both genes increased in dogs fed the control diet following transport stress. MPO catalyzes the formation of reactive oxygen intermediates that aid in microbial killing by neutrophils (Klebanoff et al., 2013), and COX-2 catalyzes the formation of prostaglandins and thromboxanes that aid in inducing swelling and inflammation at the site of injury. In dogs, transport stress is often perceived as an acute stressor, which can lead to frequent activation of the innate immune system in the absence of pathogens or tissue damage with repeated car rides. This can ultimately lead to chronic inflammation, wherein MPO and COX-2 have been shown to act as mediators in the development of numerous inflammatory diseases and contribute to tissue damage through excessive generation of reactive oxygen species (Iadecola, 2004; Aratani, 2018). Although circulating immune cell populations and effector function were not measured in this study, the observed changes in whole blood mRNA gene expression after transport stress provide some insight into the modulation of innate immune activation due to SCFP supplementation. In future studies, performing flow cytometry or hematology may be used to evaluate circulating immune cells before and after transport stress to aid in the interpretation of these results.

This study evaluated the impacts of long-term SCFP supplementation to healthy adult dogs during an acute stress challenge. We used transport stress as a noninvasive model to induce acute stress in dogs, which is a limitation of this study due to the physiological and behavioral differences in how dogs respond to unfamiliar stimuli. Although the dogs used in our study had not previously experienced car rides, transportation in a vehicle may have been too mild a stressor to detect changes in innate immune activity that may have presented under a more severe stress model. Alternative noninvasive methods, such as exercise or repeated exercise stress, may induce stress more effectively in future studies investigating the acute and chronic stress responses in dogs, respectively (Pastore et al., 2011; Zannoni et al., 2020; Elias et al., 2021; Varney et al., 2021). As discussed above, using serum as the primary biological fluid to measure oxidative stress and antioxidant status in dogs may have also limited our assessment of the impacts of SCFP supplementation during transport stress, primarily due to the distribution of circulating biomarkers within the body and pre-analytical factors. Additional outcome measures, such as changes in fecal metabolites and fecal microbiota, may aid future studies in determining the ways in which SCFP may modulate GI health during acute stress. Moreover, cortisol measurements (blood or salivary) may provide additional insight into the impacts of dietary SCFP on the stress response, as elevated cortisol levels reflect activation of the hypothalamic-pituitary-adrenocortical axis (Vincent and Michell, 1992).

Conclusions

In conclusion, our data suggest SCFP may act as a functional ingredient in dog foods to beneficially modulate stress-induced diarrhea and suppress innate immune activation after a transport stress challenge. SCFP is suggested to modulate stress-induced diarrhea through the increase of fecal DM%, although further investigation is needed as the change in fecal DM%

was minor, and fecal scores and inflammatory fecal markers were not altered due to transport stress or diet. Furthermore, investigation is necessary to determine how SCFP modulates oxidative stress under stressful conditions, as SCFP-fed dogs exhibited increased MDA and 8-isoprostane levels, which is indicative of lipid peroxidation due to transport stress or diet. Use of a different stress model, such as exercise stress, to induce acute stress as well as alterations to blood and fecal sample analysis may aid in investigation. Finally, SCFP was shown to modulate the expression of two key inflammatory mediators during acute stress (COX-2 and MPO), suggesting SCFP may suppress activation of the innate immune response. Our data suggest that SCFP can be included in as a functional ingredient in extruded dog foods aimed at reducing stress. SCFP may be most beneficial to less adaptable senior and/or geriatric dogs, or dogs predisposed to travel anxiety.

Supplementary Data

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

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Conflicts of Interest Statement

M.R.P. was employed by Blue Buffalo Co. Ltd during conceptualization, execution, analysis, and interpretation of this study, and is now employed by Mars Pet Nutrition. S.A.N. is employed by Cargill, Inc. All other authors have no conflicts of interest.

Literature Cited

American Association of Cereal Chemists (AACC). 1983. *Approved methods*. 8th ed. St Paul, MN: American Association of Cereal Chemists.

Aratani, Y. 2018. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Arch. Biochem. Biophys*. 640:47–52. doi:10.1016/j.abb.2018.01.004

Arnhold, J. 2020. The dual role of myeloperoxidase in immune response. *Int. J. Mol. Sci.* 21:8057. doi:10.3390/ijms21218057

Association of American Feed Control Officials (AAFCO). 2020. *Official publication*. Champaign, IL: AAFCO.

Association of Official Analytical Chemists (AOAC). 2006. *Official methods of analysis*. 17th ed. Gaithersburg, MD: Association of Official Analytical Chemists.

Beata, C., E. Beaumont-Graff, V. Coll, J. Cordel, M. Marion, N. Massal, N. Marlois, and J. Tauzin. 2007. Effect of alpha-casozepine (Zylkene) on anxiety in cats. *J. Vet. Behav* 2:40–46. doi:10.1016/j.jveb.2007.02.002

Budde, E. F. 1952. The determination of fat in baked biscuit type dog foods. *J. Assoc. Off. Agric. Chem*. 35:799–805.

Campos-Rodríguez, R., M. Godínez-Victoria, E. Abarca-Rojano, J. Pacheco-Yépez, H. Reyna-Garfias, R. E. Barbosa-Cabrera, and M. E. Drago-Serrano. 2013. Stress modulates intestinal secretory immunoglobulin A. *Front. Integr. Neurosci*. 7:86. doi:10.3389/fnint.2013.00086

Collins, S. M. 2001. Stress and the gastrointestinal tract IV. Modulation of intestinal inflammation by stress: Basic mechanisms and clinical relevance. *Am. J. Physiol. Gastrointest. Liver Physiol*. 280:G315–G318. doi:10.1152/ajpgi.2001.280.3.G315

Cortese, L., M. Annunziatella, A. T. Palatucci, S. Lanzilli, V. Rubino, A. Di Cerbo, S. Centenaro, G. Guidetti, S. Canello, and G. Terrazzano. 2015. An immune-modulating diet increases the regulatory T cells and reduces T helper 1 inflammatory response in Leishmaniosis affected dogs treated with standard therapy. *BMC Vet. Res*. 11:295. doi:10.1186/s12917-015-0610-7

Cracknell, N. R., and D. S. Mills. 2008. A double-blind placebo-controlled study into the efficacy of a homeopathic remedy for fear of firework noises in the dog (*Canis familiaris*). *Vet. J*. 177:80–88. doi:10.1016/j.tvjl.2007.04.007

Dalle-Donne, I., R. Rossi, R. Colombo, D. Giustarini, and A. Milzani. 2006. Biomarkers of oxidative damage in human disease. *Clin. Chem*. 52:601–623. doi:10.1373/clinchem.2005.061408

DeClue, A. E., S. Axiak-Bechtel, C. F. Cowan, Y. Zhang, J. Amorim, R. Halpin, G. N. Melillo, and C. Hagan. 2020. Transportation and routine veterinary interventions alter immune function in the dog. *Top. Comp. Anim. Med* 39:100408. doi:10.1016/j.tcam.2020.100408

DeNapoli, J. S., N. H. Dodman, L. Shuster, W. M. Rand, and K. L. Gross. 2000. Effect of dietary protein content and tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in dogs. *J. Am. Vet. Med. Assoc*. 217:504–508. doi:10.2460/javma.2000.217.504

Dhabhar, F. S. 2018. The short-term stress response - mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Front Neuroendocrinol*. 49:175–192. doi:10.1016/j.yfrne.2018.03.004

Dhabhar, F. S., and B. S. McEwen. 1997. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. *Brain Behav. Immun*. 11:286–306. doi:10.1006/brbi.1997.0508

Dreissigacker, U., M. T. Suchy, N. Maassen, and D. Tsikas. 2010. Human plasma concentrations of malondialdehyde (MDA) and the F2-isoprostane 15(S)-8-iso-PGF(2alpha) may be markedly compromised by hemolysis: evidence by GC-MS/MS and potential analytical and biological ramifications. *Clin. Biochem*. 43:159–167. doi:10.1016/j.clinbiochem.2009.10.002

Elias, B., M. Starling, B. Wilson, and P. McGreevy. 2021. Influences on infrared thermography of the canine eye in relation to the stress and arousal of racing greyhounds. *Animals* 11:103. doi:10.3390/ani11010103

Fazio, F., S. Casella, C. Giannetto, E. Giudice, and G. Piccione. 2015. Characterization of acute phase proteins and oxidative stress response to road transportation in the dog. *Exp. Anim*. 64:19–24. doi:10.1538/expanim.14-0032

Feng, L., Y. Xia, G. E. Garcia, D. Hwang, and C. B. Wilson. 1995. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J. Clin. Invest*. 95:1669–1675. doi:10.1172/JCI117842

Ferreira, C. S., R. S. Vasconcellos, R. S. Pedreira, F. L. Silva, F. C. Sá, F. S. Kroll, A. P. Maria, K. S. Venturini, and A. C. Carciofi. 2014. Alterations to oxidative stress markers in dogs after a short-term stress during transport. *J. Nutr. Sci* 3:e27. doi:10.1017/jns.2014.47

Field, C. J., M. I. McBurney, S. Massimino, M. G. Hayek, and G. D. Sunvold. 1999. The fermentable fiber content of the diet alters the function and composition of canine gut associated lymphoid tissue. *Vet. Immunol. Immunopathol*. 72:325–341. doi:10.1016/s0165-2427(99)00148-8

Giustarini, D., I. Dalle-Donne, D. Tsikas, and R. Rossi. 2009. Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers. *Crit. Rev. Clin. Lab. Sci*. 46:241–281. doi:10.3109/10408360903142326

Grieshop, C. M., E. A. Flickinger, K. J. Bruce, A. R. Patil, G. L. Czarnecki-Maulden, and G. C. Fahey. 2004. Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. *Arch. Anim. Nutr*. 58:483–493. doi:10.1080/00039420400019977

Han, F., H. Fan, M. Yao, S. Yang, and J. Han. 2017. Oral administration of yeast β-glucan ameliorates inflammation and intestinal barrier

in dextran sodium sulfate-induced acute colitis. *J. Funct. Foods* 35:115–126. doi:10.1016/j.jff.2017.05.036

Haussmann, M. F., A. S. Longenecker, N. M. Marchetto, S. A. Juliano, and R. M. Bowden. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proc. Biol. Sci.* 279:1447–1456.doi:10.1098/rspb.2011.1913

Iadecola, C. 2004. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat. Rev. Neurosci.* 5:347–360. doi:10.1038/nrn1387

Jensen, G. S., X. Wu, K. M. Patterson, J. Barnes, S. G. Carter, L. Scherwitz, R. Beaman, J. R. Endres, and A. G. Schauss. 2008. In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo-controlled, crossover study. *J. Agric. Food Chem.* 56:8326–8333. doi:10.1021/jf8016157

de Jonge, W. J. 2013. The gut's little brain in control of intestinal immunity. *ISRN Gastroenterol* 2013:630159. doi:10.1155/2013/630159

Jukic, A., L. Bakiri, E. F. Wagner, H. Tilg, and T. E. Adolph. 2021. Calprotectin: From biomarker to biological function. *Gut* 70:1978–1988. doi:10.1136/gutjnl-2021-324855

Kadiiska, M. B., B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, et al. 2005. Biomarkers of oxidative stress study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radic. Biol. Med.* 38:698–710. doi:10.1016/j.freeradbiomed.2004.09.017

Ketchesin, K. D., G. S. Stinnett, and A. F. Seasholtz. 2017. Corticotropin-releasing hormone-binding protein and stress: from invertebrates to humans. *Stress* 20:449–464. doi:10.1080/10253890.2017.1322575

Khansari, D. N., A. J. Murgo, and R. E. Faith. 1990. Effects of stress on the immune system. *Immunol. Today* 11:170–175. doi:10.1016/0167-5699(90)90069-l

Klebanoff, S. J., A. J. Kettle, H. Rosen, C. C. Winterbourn, and W. M. Nauseef. 2013. Myeloperoxidase: A front-line defender against phagocytosed microorganisms. *J. Leukoc. Biol.* 93:185–198. doi:10.1189/jlb.0712349

Krizková, L., Z. Duracková, J. Sandula, V. Sasinková, and J. Krajcovic. 2001. Antioxidative and antimutagenic activity of yeast cell wall mannans in vitro. *Mutat. Res.* 497:213–222. doi:10.1016/s1383-5718(01)00257-1

Laflamme, D. P. 1997. Development and validation of a body condition score system for dogs: a clinical tool. *Canine Pract* 25:10–15.

Landsberg, G. M., I. Mougeot, S. Kelly, and N. W. Milgram. 2015. Assessment of noise-induced fear and anxiety in dogs: Modification by a novel fish hydrolysate supplemented diet. *J. Vet. Behav* 10:391–398. doi:10.1016/j.jveb.2015.05.007

Lin, H., E. Decuypere, and J. Buyse. 2004. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 1. Chronic exposure. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* 139:737–744. doi:10.1016/j.cbpc.2004.09.013

Lin, C. Y., C. Alexander, A. J. Steelman, C. M. Warzecha, M. R. C. de Godoy, and K. S. Swanson. 2019. Effects of a *Saccharomyces cerevisiae* fermentation product on fecal characteristics, nutrient digestibility, fecal fermentative end-products, fecal microbial populations, immune function, and diet palatability in adult dogs. *J. Anim. Sci.* 97:1586–1599. doi:10.1093/jas/skz064

Lin, C. -Y., A. R. Jha, P. M. Oba, S. M. Yotis, J. Shmalberg, R. W. Honaker, and K. S. Swanson. 2022. Longitudinal fecal microbiome and metabolite data demonstrate rapid shifts and subsequent stabilization after an abrupt dietary change in healthy adult dogs. *Anim. Microbiome* 4:46. doi:10.1186/s42523-022-00194-9

Liu, X. L., N. Lin, D. Zan, J. J. Yuan, and D. L. Cai. 2011. Effect of zymosan on antioxidant and immune function of S180 tumor-bearing mice. *Cell Biochem. Biophys.* 60:225–229. doi:10.1007/s12013-010-9143-7

Marasco, V., A. Stier, W. Boner, K. Griffiths, B. Heidinger, and P. Monaghan. 2017. Environmental conditions can modulate the links among oxidative stress, age, and longevity. *Mech. Ageing Dev.* 164:100–107. doi:10.1016/j.mad.2017.04.012

Monnier, L., E. Mas, C. Ginet, F. Michel, L. Villon, J. P. Cristol, and C. Colett. 2006. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 295:1681–1687. doi:10.1001/jama.295.14.1681

Montero, A., K. A. Munger, R. Z. Khan, J. M. Valdivielso, J. D. Morrow, A. Guasch, F. N. Ziyadeh, and K. F. Badr. 2000. F(2)-isoprostanes mediate high glucose-induced TGF-beta synthesis and glomerular proteinuria in experimental type I diabetes. *Kidney Int.* 58:1963–1972. doi:10.1111/j.1523-1755.2000.00368.x

Niki, E. 2014. Biomarkers of lipid peroxidation in clinical material. *Biochim. Biophys. Acta* 1840:809–817. doi:10.1016/j.bbagen.2013.03.020

Ochi, T., I. Nishiura, M. Tatsumi, Y. Hirano, K. Yahagi, Y. Sakurai, Y. Sudo, H. Koyama, Y. Hagita, Y. Fujimoto, et al. 2013. Effects of transport stress on serum alkaline phosphatase activity in beagle dogs. *Exp. Anim.* 62:329–332. doi:10.1538/expanim.62.329

Pasquini, A., S. Roberti, V. Meucci, E. Luchetti, S. Canello, G. Guidetti, and G. Biagi. 2013. Association between body condition and oxidative status in dogs. *Food Nutr* 4:191–196. doi:10.4236/fns.2013.48A023

Pastore, C., F. Pirrone, F. Balzarotti, M. Faustini, L. Pierantoni, and M. Albertini. 2011. Evaluation of physiological and behavioral stress-dependent parameters in agility dogs. *J. Vet. Behav* 6:188–194. doi:10.1016/j.jveb.2011.01.001

Phillips, F. C., G. S. Jensen, L. Showman, R. Tonda, G. Horst, and R. Levine. 2019. Particulate and solubilized β-glucan and non-β-glucan fractions of *Euglena gracilis* induce pro-and anti-inflammatory innate immune cell responses and exhibit antioxidant properties. *J. Inflamm. Res* 12:49–64. doi:10.2147/JIR.S191824

Pike, A. L., D. F. Horwitz, and H. Lobprise. 2015. An open-label prospective study of the use of l-theanine (Anxitane) in storm-sensitive client-owned dogs. *J. Vet. Behav* 10:324–331. doi:10.1016/j.jveb.2015.04.001

Prosky, L., N. G. Asp, T. F. Schweizer, J. W. DeVries, and I. Furda. 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *J. Assoc. Off. Anal. Chem.* 71:1017–1023.

Sechi, S., F. Chiavolelli, N. Spissu, A. Di Cerbo, S. Canello, G. Guidetti, F. Fiore, and R. Cocco. 2015. An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: Preliminary results. *J. Vet. Med* 2015:412501. doi:10.1155/2015/412501

Sechi, S., A. Di Cerbo, S. Canello, G. Guidetti, F. Chiavolelli, F. Fiore, and R. Cocco. 2017. Effects in dogs with behavioural disorders of a commercial nutraceutical diet on stress and neuroendocrine parameters. *Vet. Rec.* 180:18. doi:10.1136/vr.103865

Selye H. 1956. *The Stress of Life*. New York, NY: McGraw-Hill Book Company.

Sies, H., C. Berndt, and D. P. Jones. 2017. Oxidative Stress. *Annu. Rev. Biochem.* 86:715–748. doi:10.1146/annurev-biochem-061516-045037

Spears, J. K., C. M. Grieshop, and G. C. Fahey, Jr. 2004. Evaluation of stabilized rice bran as an ingredient in dry extruded dog diets. *J. Anim. Sci.* 82:1122–1135. doi:10.2527/2004.8241122x

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. -P. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey. 2002. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. *J. Nutr.* 132:980–989. doi:10.1093/jn/132.5.980

Titeux, E., S. Padilla, B. M. Paragon, and C. Gilbert. 2021. Effects of a new dietary supplement on behavioural responses of dogs exposed to mild stressors. *Vet. Med. Sci* 7:1469–1482. doi:10.1002/vms3.560

Treidel, L. A., B. N. Whitley, Z. M. Benowitz-Fredericks, and M. F. Haussmann. 2013. Prenatal exposure to testosterone impairs

oxidative damage repair efficiency in the domestic chicken (*Gallus gallus*). *Biol. Lett*. 9:20130684. doi:10.1098/rsbl.2013.0684

Tsikas, D. 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal. Biochem*. 524:13–30. doi:10.1016/j.ab.2016.10.021

Tsikas, D., S. Rothmann, J. Y. Schneider, M. T. Suchy, A. Trettin, D. Modun, N. Stuke, N. Maassen, and J. C. Frölich. 2016. Development, validation and biomedical applications of stable-isotope dilution GC-MS and GC-MS/MS techniques for circulating malondialdehyde (MDA) after pentafluorobenzyl bromide derivatization: MDA as a biomarker of oxidative stress and its relation to 15(S)-8-iso-prostaglandin F2α and nitric oxide (NO). *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. 1019:95–111. doi:10.1016/j.jchromb.2015.10.009

Tsikas, D., S. Rothmann, J. Y. Schneider, F. M. Gutzki, B. Beckmann, and J. C. Frölich. 2017. Simultaneous GC-MS/MS measurement of malondialdehyde and 4-hydroxy-2-nonenal in human plasma: effects of long-term L-arginine administration. *Anal. Biochem*. 524:31–44. doi:10.1016/j.ab.2016.08.009

Varney, J. L., C. N. Coon, and S. A. Norton. 2021. PSV-B-20 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) postbiotic in Labrador retrievers during exercise and transport stress. *J. Anim. Sci*. 99(Suppl.3):332–333.doi:10.1093/jas/skab235.611

Vilson, A., A. Hedhammar, A. Reynolds, J. Spears, E. Satyaraj, R. Pelker, C. Rottman, B. Björkstén, H. Hansson-Hamlin. 2016. Immunoglobulins in dogs: Correspondence and maturation in 15 litters of German shepherd dogs and their dams. *Vet. Rec. Open* 3:e000173. doi:10.1136/vetreco-2016-000173

Vincent, I. C., and A. R. Michell. 1992. Comparison of cortisol concentrations in saliva and plasma of dogs. *Res. Vet. Sci*. 53:342–345. doi:10.1016/0034-5288(92)90137-q

Wilson, S. M., P. M. Oba, S. A. Koziol, C. C. Applegate, K. Soto-Diaz, A. J. Steelman, M. R. Panasevich, S. A. Norton, and K. S. Swanson. 2022. Effects of a *Saccharomyces cerevisiae* fermentation product-supplemented diet on circulating immune cells and oxidative stress markers of dogs. *J. Anim. Sci*. 100:1–11. doi:10.1093/jas/skac245

Yaribeygi, H., Y. Panahi, H. Sahraei, T. P. Johnston, and A. Sahebkar. 2017. The impact of stress on body function: a review. *EXCLI J* 16:1057–1072. doi:10.17179/excli2017-480

Yoshida, Y., A. Umeno, and M. Shichiri. 2013. Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity in vivo. *J. Clin. Biochem. Nutr*. 52:9–16. doi:10.3164/jcbn.12-112

Zannoni, A., M. Pietra, A. Gaspardo, P. A. Accorsi, M. Barone, S. Turroni, L. Laghi, C. Zhu, P. Brigidi, and M. Forni. 2020. Non-invasive assessment of fecal stress biomarkers in hunting dogs during exercise and at rest. *Front. Vet. Sci* 7:126. doi:10.3389/fvets.2020.00126