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A proof of principle study investigating the effects of supplemental concentrated brewer's yeast on markers of gut permeability, inflammation, and fecal metabolites in healthy non-challenged adult sled dogs

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Abstract

Yeast-derived β-glucans impact immunity, though their effects on gut permeability and inflammation are less understood. Most research has investigated other components of the yeast cell wall, such as the prebiotic mannan- and fructo-oligosaccharides. The objective of this study was to assess the effects of feeding a concentrated yeast product on markers of inflammation (serum amyloid A [**SAA**] and haptoglobin [**Hp**]) and oxidative status (malondialdehyde [**MDA**]), fecal products of fermentation, and gut permeability. Nineteen privately owned domestic Siberian huskies, and one Alaskan husky (9 females: 5 intact, 4 spayed; 11 males: 3 intact, 8 neutered), with an average age of 4.8 ± 2.6 yr and body weight (**BW**) of 25.6 ± 4.1 kg, were used in this study. Dogs were blocked and randomly allocated to one of two diet groups. Ten dogs received a dry extruded diet. The other 10 received the same diet top dressed with yeast for a daily β–glucan dose of 7 mg/kg BW for 10 wk. Fecal collection, for evaluation of fecal metabolites, and scoring occurred weekly. Gut permeability was assessed using the chromium-labeled ethylenediamine tetra-acetic acid (**Cr-EDTA**) and iohexol markers prior to the initiation of dietary treatment and after 10 wk of treatment. Blood samples were collected premarker administration and 0.5, 1, 2, 3, 4, 5, and 6 h postadministration. Fasting concentrations of SAA, Hp, and MDA were measured on weeks −1, 2, 4, and 8. Incremental area under the curve (**I-AUC**) was calculated for serum iohexol and Cr-EDTA concentrations. All data were analyzed using PROC GLIMMIX of SAS with dog as random effect, and week as fixed effect and repeated measure. Dogs receiving treatment tended to have decreased I-AUC of Iohexol ($P = 0.10$) and Cr-EDTA ($P = 0.06$) between baseline and cessation of treatment compared to the change over time in I-AUC for control (**Ctl**) dogs. Treatment dogs had lower Hp concentrations ($P \le 0.05$) than Ctl. There were no differences between treatments for SAA and MDA concentrations ($P > 0.05$). Fecal arabinose concentrations were greater in treatment (**Trt**) dogs ($P \le 0.05$) compared to Ctl, though no other fecal metabolites were affected by treatment. There was no difference in the relative frequency of defecations scored at any fecal score between Trt and Ctl dogs, and mean score did not differ between groups ($P > 0.10$). These data suggest that concentrated brewer's yeast may have the potential to reduce gut permeability without impacting inflammatory status and markers of health in adult dogs.

Lay Summary

This study evaluated the effects of concentrated brewer's yeast on gut health in dogs. Nineteen Siberian Huskies and one Alaskan husky were blocked and randomly allocated to one of two groups. Treatment dogs received a yeast supplement for 10 wk, while control dogs received no supplement. Dogs were administered two markers to assess intestinal permeability prior to start of treatment and following 10 wk of treatment. Blood samples were collected and analyzed for markers of inflammatory status (serum amyloid A [**SAA**] and Haptoglobin [**Hp**]) and oxidative status (serum malondialdehyde [**MDA**]). Fecal samples were collected weekly to assess fecal score as well as fecal metabolite concentrations. Intestinal permeability was reduced in treatment dogs following treatment, and no change was observed in the control group. Treatment dogs had lower Hp concentrations than control (**Ctl**), but there were no differences between treatments for SAA and MDA. Fecal arabinose concentrations were significantly greater in the treatment group when compared to control. There were no differences in the relative frequency of defecations scored at any fecal score between treatment and Ctl dogs, nor did mean score differ between the groups. This study suggests that concentrated brewer's yeast may reduce gut permeability and inflammation without detrimentally impacting markers of health in adult dogs.

Key words: antioxidant status, beta-glucan, canine, inflammatory status, intestinal permeability, *Saccharomyces cerevisiae*

Abbreviations: AAFCO, Association of American Feed Control Officials; APP, acute phase protein; BCFA, branch chain fatty acid; BW, body weight; CBC, complete blood count; Cr-EDTA, chromium-labeled ethylenediamine tetra-acetic acid; Ctl, control; ELISA, enzyme-linked immunosorbent assay; FI, feed intake; Hp, haptoglobin; HPLC, high-performance liquid chromatography; I-AUC, incremental area under the curve; IL, interleukin; MDA, malondialdehyde; ROS, reactive oxygen species; SAA, serum amyloid a; SCFA, short-chain fatty acid; TLR2, toll-like receptor 2; TNF-α, tumor necrosis factor alpha; Trt, treatment

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Introduction

The use of yeast components as functional ingredients in canine diets has garnered interest due to potential immune modulatory and gut health enhancing effects (Pillemer and Ecker, 1941; Novak and Vetvicka, 2009). Yeast is a fermentable source of carbohydrates and protein that, when supplemented in the diet, may increase nutrient digestibility in dogs (Beloshapka et al., 2013). Beyond its role in digestion, the gut acts as a physical barrier against invading pathogens, helping to maintain host health (McKay and Perdue, 1993). Supplemental yeast has a protective effect against factors negatively impacting gut permeability, such as maintaining the integrity of the intestinal barrier and stimulating the immune system, both critical to the prevention of luminal bacteria and endotoxins from translocating into portal and systemic circulation (Generoso et al., 2011). These outcomes are believed to be the result of B-vitamins, antioxidants, and the cell-membrane-derived mannan-, fructo-oligosaccharides, and β-glucans found in yeast products, the last of which has been reported to reduce gut inflammation, while improving gut and overall health in animal and human models (Martins et al., 2013).

β-Glucans are naturally occurring polysaccharides found in the cell walls of yeast as well as other fungi, cereal grains, algae, lichens, and some bacteria (Novak and Vetvicka, 2009). Yeast-derived β-glucans have been reported to directly augment innate antimicrobial defense mechanisms and act as immunostimulants in mice and dogs (Lee et al., 2001; Stuyven et al., 2010), stimulating the release of nitric oxide, arachidonic acid metabolites, and cytokines (Jung et al., 2003; Suram et al., 2006; Chen and Seviour, 2007). Yeast-derived β-glucans function by binding to specific receptors on macrophages, neutrophils, monocytes, dendritic cells, and natural killer cells to exert their immune-modulatory effects (Stuyven et al., 2010). Further, yeast β-glucans have protective effects against immune challenges caused by bacteria, viruses, and parasites in humans (Willment et al., 2001; Gantner et al., 2003; Brown and Gordon, 2005) and mice (Brown et al., 2003; Generoso et al., 2011). Dectin-1 has been identified as the major β-glucan receptor, and is found in large quantities on macrophages and neutrophils (Brown and Gordon, 2001).

Serum amyloid A (**SAA**) and haptoglobin (**Hp**) are positive acute phase proteins (**APPs**) produced following activation of the innate immune system that act as nonspecific markers of inflammation in dogs (Cerón et al., 2005). Dectin-1 acts collaboratively with toll-like receptor 2 (**TLR2**) to produce the major proinflammatory cytokines, triggering the production of APPs (Gantner et al., 2003; Cerón et al., 2005). Dogs supplemented with a yeast-derived β-glucan had reduced serum concentrations of proinflammatory interleukin (**IL**)-6, and increased concentrations of anti-inflammatory IL-10, further substantiating the immune-modulatory effects of β-glucan in dogs (Rychlik et al., 2013). While yeast and β-glucans have a well-established role in supporting immune function, their effects on the gut in dogs are largely unknown.

Intestinal barrier function refers to the ability of the gut epithelium to separate harmful bacteria and endotoxins from the body (MacFie et al., 2005; Generoso et al., 2011). When the gut is injured or suffers a barrier failure, caused by changes in the diet, stressors, or medications (Soeters, 2008), this facilitates the transport of harmful substances from the lumen into portal and systemic circulation (Generoso et al., 2011). This increase in pathogenic bacteria in systemic circulation activates the immune system, triggering the production of various immune cells, and can lead to systemic inflammation (Generoso et al., 2011). While yeast contains many active components, β-glucans are thought to exert numerous beneficial effects on intestinal permeability (Generoso et al., 2011; Che et al., 2017; Han et al., 2017). Intestinal permeability has historically been evaluated in dogs and other species using ^{51}Chromium-labeled ethylenediamine tetra-acetic acid (^{51}**Cr-EDTA**) and a combination of two sugars (Klenner et al., 2009; Frias et al., 2012). Iohexol, a water-soluble nonionic contrast medium, has also been evaluated as a comparable marker of intestinal permeability to ^{51}Cr-EDTA in dogs (Frias et al., 2012). Both iohexol and ^{51}Cr-EDTA permeate the intestinal membrane via paracellular transport and have been used to detect early stages of intestinal diseases in humans and dogs (Klenner et al., 2009; Frias et al., 2012). When iohexol and ^{51}Cr-EDTA were administered concurrently to dogs, the percent recovery of both markers in serum was linearly associated, though the lower molecular weight of ^{51}Cr-EDTA resulted in greater recovery (Frias et al., 2012). The use of a radiolabeled marker increases health risks and requires additional training and caution to work with, particularly when using these radioactive compounds in the field. A non-radiolabeled form of Cr-EDTA has been successfully utilized in human (Ten Bruggencate et al., 2006) and calf (Amado et al., 2019) subjects to evaluate intestinal permeability, eliminating the complexities associated with the radioactive form. To the authors' knowledge, Cr-EDTA has not been utilized in dogs.

Though SAA and Hp are well-established markers of inflammatory status, no studies to the authors' knowledge have evaluated the effects of a concentrated yeast product on SAA and Hp concentration as markers of inflammatory status in dogs. In addition, there is a dearth of research investigating the role played by yeast products on gut permeability in dogs. As such, the objectives of this study were to assess the effects of feeding a concentrated yeast product on gut permeability as measured with Cr-EDTA and iohexol, serum markers of gut inflammation (SAA, Hp), fecal products of fermentation (short-chain fatty acids [**SCFA**], branched-chain fatty acids [**BCFA**]), and antioxidant status measured via serum malondialdehyde (**MDA**) concentrations in adult dogs. We hypothesized that dogs receiving the concentrated brewer's yeast would have lower concentrations of circulating inflammatory markers, lower gut permeability, and lower serum concentrations of MDA than dogs receiving the control diet only. In addition, we hypothesized that fecal score would be improved in the dogs receiving the concentrated brewer's yeast compared to dogs receiving only the control diet.

Materials and Methods

Animals and housing

This study was approved by the University of Guelph's Animal Care Committee (Animal Use Protocol #4412). Nineteen privately owned domestic Siberian huskies, and one Alaskan husky (9 females: 5 intact, 4 spayed; 11 males: 3 intact, 8 neutered), with a mean (± SD) age of 4.8 ± 2.6 yr and body weight (**BW**; ± SD) of 25.6 ± 4.1 kg, were used in this study from August 2020 to November 2020. Dogs were housed at an off-site facility (Rajenn Siberian Huskies, Ayr, ON, Canada) that was previously visited and approved by the University of Guelph's Animal Care Services. Throughout the study,

dogs were housed in free-range, outdoor kennels ranging in size from 3.5 to 80 m^2. Each kennel contained between 2 and 9 dogs. Dogs had ad libitum access to fresh water, constant access to shelter, received daily socialization, and the same amount of weekly exercise.

Diet and study design

Dogs were blocked by age, sex, and BW prior to being randomly allocated to one of two diet groups: control (**Ctl**) (*n* = 10; 5 males, 4 neutered, 1 intact; 5 females, 3 spayed, 2 intact; average age (± SD) = 4.80 ± 2.82 yr, average BW = 25.43 ± 3.65 kg) or treatment (**Trt**; *n* = 10; 6 males, 4 neutered, 2 intact; 4 females, 1 spayed, 3 intact; average age = 4.80 ± 2.62 yr, average BW = 25.40 ± 4.47 kg).

All dogs were acclimated to a Ctl diet (Acana Adult Large Breed, Champion Petfoods LT, Morinville, AB, Canada; Table 1) for 4 wk prior to the study start. The Ctl diet met or exceeded all National Research Council (NRC, 2006) and Association of American Feed Control Officials (AAFCO, 2016) nutrient recommendations for adult dogs. Following the 4-wk diet acclimation period, Ctl dogs continued to receive the Ctl diet for the remainder of the study, while dogs on Trt were fed the Ctl diet top-dressed with a concentrated yeast product (F.L. Emmert Company, Cincinnati, OH, USA; Table 2). The yeast product was supplemented to provide a β-glucan dose of 7 mg/kg BW per day (Rychlik et al., 2013). Throughout both the acclimation and study period, dogs were fed once daily at 1700 h to maintain their typical feeding regimen. All dogs were fed individually, which allowed for monitoring of food consumption and proper allocation of dietary treatments. Any orts (offered, refused, and treatment) were weighed and recorded daily. Dogs had access to ad libitum fresh water throughout the entire study period, except for 12 h prior to each gut permeability assessment to encourage voluntary consumption of the markers. Feed intake was initially determined based on historical feeding records and reassessed throughout the study to ensure all dogs maintained their initial BW throughout the study.

Table 1. Diet nutrient content[1] on an as-fed basis and ingredient composition[2] of the control diet as reported on the commercial product

Nutrient contents	Analyzed content
Metabolizable energy, kcal/kg (calculated)[3]	3651.85
Moisture, %	9.02
Protein, %	33.37
Fat, %	14.44
Ash, %	7.29

[1]Typical analysis of nutrient content of the kibble. All kibble utilized in this study was sourced from the same lot.
[2]Ingredient composition: Fresh chicken meat, chicken meal, turkey meal, red lentils, whole green peas, field beans, fresh chicken giblets (liver, heart, and kidney), herring meal, fresh cage-free eggs, fresh whole flounder, herring oil, chicken fat, sun-cured alfalfa, green lentils, whole yellow peas, pea fiber, fresh chicken cartilage, dried brown kelp, fresh whole pumpkin, fresh whole butternut squash, fresh whole parsnips, fresh kale, fresh spinach, fresh mustard greens, fresh turnip greens, fresh whole carrots, fresh apples, fresh pears, freeze-dried chicken liver, freeze-dried turkey liver, fresh whole cranberries, fresh whole blueberries, chicory root, turmeric, milk thistle, burdock root, lavender, marshmallow root, and rosehips.
[3]Calculated metabolizable energy based on modified Atwater values and presented on an as-fed basis.

Table 2. Nutrient content[1] on an as-fed basis of the yeast supplement

Nutrient contents	Analyzed content
Metabolizable energy, kcal/kg (calculated)[1]	2820.00
Moisture, %	7.00
Crude protein, %	45.00
Crude fat, %	0.75
Crude fiber, %	6.00
Ash, %	7.50
Carbohydrates, %	33.75
NDF	9.40
Beta glucans, %	3.20
Mannan oligosaccharides, %	≥1.00

[1]Typical analysis of nutrient content of the yeast supplement. All yeast supplement utilized in this study was sourced from the same lot.
[2]Calculated metabolizable energy based on modified Atwater values and presented on an as-fed basis.

Blood sample collection and analysis

Fasting blood samples were collected on weeks −3, 0, 5, and 7 to assess whole blood hematology (complete blood count; **CBC**; 1 mL) and on weeks −3 and 7 to assess serum biochemistry parameters as markers of health and nutritional status (4 mL). Blood samples were collected via cephalic venipuncture with a Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) after an overnight, 12 h fast. Whole blood samples were kept on ice before being submitted to Animal Health Laboratories (University of Guelph, Guelph, ON, Canada) for hematology analysis using an Advia 2120 hematology analyzer (Siemens Global, Munich, Germany). Blood samples were centrifuged at 2,000 × *g* for 20 min at 4 °C using a Beckman J6-MI centrifuge (Beckman Coulter, Indianapolis, IN, USA), and serum was aliquoted and submitted to Animal Health Laboratories (University of Guelph) for serum biochemistry analysis using a cobas 6000 c501 analyzer (Roche Diagnostics International AG, Rotkreuz, Switzerland). Hematological samples were analyzed for blood leukocyte count, erythrocyte count (**RBC**), hemoglobin (**Hb**), hematocrit (**Hct**) (RBC × MCV), mean cell volume (**MCV**), mean cell hemoglobin (Hb/RBC), mean corpuscular hemoglobin concentration (Hb/Hct), red cell distribution width, platelet count, mean platelet volume, plateletecrit, and total solid protein as well as segmented neutrophil, lymphocyte, monocyte, and eosinophil counts. Serum samples were analyzed for calcium, phosphorus, magnesium, sodium, potassium, chloride, carbon dioxide, anion gap, sodium:potassium (**Na:K**) ratio, total protein, albumin, globulin, albumin:globulin ratio, urea, creatine, glucose, cholesterol, total bilirubin, conjugated bilirubin, free bilirubin, alkaline phosphatase (**ALP**), steroid-induced ALP, gamma-glutamyl transferase, alanine aminotransferase, creatine kinase, amylase, lipase, and calculated osmolarity using a cobas 6000 c501 analyzer (Roche Diagnostics International AG).

Concentrations of SAA, Hp, and MDA were evaluated on weeks −1, 2, 4, and 8. A 5 mL fasted blood sample was collected via cephalic venipuncture from each dog into a serum Vacutainer (Becton, Dickinson and Company) using a winged infusion set (Terumo Surflo Winged Infusion Set, 21 G × 0.75, Terumo Medical Corporation, Vaughn, ON, Canada). Samples were centrifuged and serum aliquoted as described above. SAA was analyzed using a multispecies sandwich

enzyme-linked immunosorbent assay (**ELISA**) (Phase SAA, Tridelta Development Ltd, Maynooth, County Kildare, Ireland), Hp with a Phase Haptoglobin colorimetric Assay (Tridelta Development Ltd), and MDA via a Canine MDA ELISA (MyBioSource Inc., San Diego, CA, USA). All kits were used following the manufacturer's instructions, and had previously been validated for use in dogs (Eckersall et al., 1999, Hp; Martinez-Subiela et al., 2003, SAA; Phungviwatnikul et al., 2020, MDA).

Gut permeability was assessed on weeks −2 and 9 using Cr-EDTA and Iohexol markers. The Cr-EDTA solution was prepared similarly to the methods of Binnerts et al. (1968). Briefly, the solution was prepared by dissolving 95.4 g of chromium chloride in 600 mL of double-distilled water (DDH_2O). Next, 133.4 g of disodium ethylenediamine tetra-acetate (EDTA) was dissolved in 1,000 mL of DDH_2O. The two solutions were combined and boiled at 60 °C for 1 h. Following boiling, 3.874 g of calcium chloride was added to neutralize excess EDTA, pH was adjusted to 6.0, and the volume was adjusted to 2 liter. Iohexol was dosed at 2.0 mL iohexol/kg BW (Omnipaque-240, Bayer Schering Pharma, Berlin, Germany; Klenner et al., 2009) and Cr-EDTA at 0.1 g Cr/kg BW (Amado et al., 2019).

Prior to assessing intestinal permeability, water was withheld for 12 h overnight to encourage voluntary consumption of the Cr-EDTA and iohexol markers. Dogs were catheterized for the sampling period. Briefly, dogs' forearms were shaved and a topical anesthetic (EMLA cream [2.5% lidocaine and 2.5% prilocaine], Astra Pharmaceuticals, L.P. Wayne, PA, USA) was applied. 20 minutes were allocated to allow activation of the anesthetic, at which point the front legs were cleaned with 4% chlorhexidine, 70% alcohol, and 0.5% chlorhexidine in that order. A 20 G cephalic catheter (Insyte-W 20 G × 1.1, Becton Dickinson Canada Inc., Mississauga, ON, Canada) was placed and a 5-mL blood sample was taken immediately after placement. A three-way stopcock (Cardinal Health Canada, Vaughan, ON, Canada) was affixed to each catheter and flushed with 0.5 mL of 125 United States Pharmacopeia (USP) heparinized saline and locked with 0.5 mL of 495 USP heparinized saline (Sandoz Canada Inc., Boucherville, QC, Canada). Following placement of the cephalic catheters, the Cr-EDTA and Iohexol markers were administered. Dogs were provided the opportunity to consume the markers independently, and any remaining marker was administered orally via syringe. Samples were collected 30 min, 1, 2, 3, 4, 5, and 6 h post-marker administration. For all samples, 5 mL of blood was collected into a 10 mL serum Vacutainer (Becton, Dickinson and Company). After every sample collection, catheters were flushed with 0.5 mL of 125 USP heparinized saline and locked with 0.5 mL of 495 USP heparinized saline (Sandoz Canada Inc.). Heparin was administered and blood was drawn through separate ports of the three-way stopcock (Cardinal Health Canada). Sampling ports were kept consistent for each stopcock to prevent heparin contamination. Samples were kept on ice until they were centrifuged, and serum was aliquoted as described above. Serum iohexol concentrations were analyzed using a multispecies FIT-GFR ELISA (BioPAL Inc., Worcester, MA, USA), following the manufacturer's instructions previously validated for use in dogs (Ortín-Piqueras et al., 2018). Serum samples were diluted 10-fold with a diluent consisting of 4% (w/v) 1-butanol, 0.01% (w/v) EDTA, 0.01% (w/v) Triton X-100, and 1% (w/v) tetramethylammonium hydroxide, before analysis of Cr-EDTA concentration via Inductively Coupled Plasma Mass Spectrometry (Trent University Water Quality Centre, Peterborough, ON, Canada).

Fecal sample collection and analysis

Fecal collection and scoring were conducted over 2 d weekly. Fecal scores were assessed using a 5-point visual scoring system where 1 = hard/dry and 5 = watery/diarrhea (Moxham, 2001); a score of 2.5 was considered ideal. Defecations were identified, scored, photographed, and collected within 15 min of being voided. For fecal scoring, a secondary evaluator re-scored all defecations using photographs. The average of the two scores was used for all analyses. The primary (on-site) and secondary evaluators remained consistent throughout the study.

For all fecal samples, whole samples were collected, and all visible contaminants were removed. Samples were then transferred to a Whirl-pak bag (Thermo Fisher Scientific, Waltham, MA, USA) to be manually homogenized. Following homogenization, samples were stored in sterile 50 mL centrifuge tubes (Thermo Fisher Scientific) and kept on ice before being stored at −20 °C until further analysis. Samples were analyzed for fecal metabolites, including SCFA (acetic acid, propionic acid, and butyric acid), BCFA (isobutyric acid and isovaleric acid), lactic acid, lactose, glucose, xylose, and arabinose using an Agilent HP1000 series high-performance liquid chromatography system (**HPLC**; Agilent Technologies, Santa Clara, CA, USA). Samples were prepared and analyzed using the methods described in Neijat et al. (2019) and Templeman et al. (2020).

Statistical analysis

Data were analyzed using statistical software SAS (v. 9.4; SAS Institute Inc., Cary, NC, USA). Complete blood count and biochemistry data, as well as mean daily feed intake, mean BW, SAA, Hp, MDA, and fecal metabolite data were analyzed using a mixed model via PROC GLIMMIX of SAS where dog was treated as a random effect, and week as a fixed effect and repeated measure. A Tukey's HSD test was used to separate means. Incremental area under the curve (**I-AUC**) was first calculated from serum iohexol and Cr-EDTA concentrations in Microsoft excel (Excel 2018, Microsoft Corp., Redmond, WA, USA) and the resulting values for each week, as well as the difference between the weeks, were analyzed using a mixed model via PROC GLIMMIX of SAS, where dog was treated as a random effect, and week as a fixed effect and repeated measure. Assumptions of residuals for all parameters were assessed using the Shapiro–Wilk to test normality. A Tukey's HSD test was used to separate means. Interobserver reliability on fecal scoring was conducted by calculating Kappa statistics using the PROC FREQ procedure in SAS. Fecal score data were analyzed using PROC FREQ of SAS with a Chi-square test to compare fecal scores and groups. Significance was declared when $P \le 0.05$ and a trend when $0.05 < P \le 0.10$.

Results

Mean daily feed intake and bodyweight

Mean daily food intake (**FI**) did not differ between Trt and Ctl ($P > 0.10$) but differed by week ($P \le 0.05$, data not shown). Feed intake at week 6 was greater than weeks 0 and 1 ($P \le 0.05$) for the pooled data (data not shown). Mean weekly BW

did not differ between Trt or Ctl ($P > 0.10$) but differed by week with a group by week interaction effect ($P \le 0.05$, data not shown). BW at week 1 was greater than at weeks 2 to 6, at week 0 was greater than weeks 4 to 6, and at week 7 was greater than week 6 ($P \le 0.05$, data not shown). The mean weekly BW of Ctl dogs was significantly lower at weeks 4, 5, and 6 when compared to week 0 ($P \le 0.05$, data not shown). No differences were observed in the Trt group ($P > 0.10$, data not shown).

CBC and biochemistry

Data related to the standard serum veterinary diagnostic measurements and markers of nutritional and health status are presented in Supplementary Tables S1 and S2. All dogs remained healthy throughout the study period, and all mean CBC and serum biochemistry values were within the standard reference range (Animal Health Laboratory, University of Guelph) aside from eosinophil counts which were above reference values, and Na:K ratio and cholesterol, which were below reference values. When compared to Ctl, Trt dogs had lower mean blood cell volume ($P \le 0.05$; Supplementary Table S1) and phosphorus levels ($P \le 0.05$) and tended to have lower cholesterol and lipase ($P \le 0.10$; Supplementary Table S2).

Intestinal permeability

At week −2, I-AUC was greater in Trt dogs for Iohexol ($P \le 0.05$) and tended to be greater for Cr-EDTA ($P \le 0.10$) when compared to Ctl dogs (Table 3). There were no differences in I-AUC between Trt and Ctl dogs for either Iohexol or Cr-EDTA markers at week 9 ($P > 0.10$, Table 3). Treatment dogs tended to have reduced I-AUC in both Iohexol and Cr-EDTA between weeks −2 and 9 when compared to the change in I-AUC for the Ctl dogs ($P \le 0.10$, Table 3).

Fecal scores and fecal metabolites

There was no difference in the relative frequency of defecations scored at any fecal score (1 to 5) between Trt and Ctl dogs ($\chi^2 = 6.04$, $P > 0.10$; data not shown). When pooled across all dogs, the mean fecal scores at weeks 0 and 3 were greater than week 7 ($P \le 0.05$; data not shown), but no other weeks differed from each other. The mean fecal score over the entire study period for Trt (2.8 ± 0.10) and Ctl (2.7 ± 0.10) were not different ($P > 0.10$; data not shown) and there was no group by week interaction effect ($P > 0.10$; data not shown).

Fecal metabolite data is reported in Table 4. No differences were observed between Trt and Ctl dogs for any fecal SCFA (acetic acid, propionic acid, and butyric acid), BCFA (isobutyric acid and isovaleric acid), or for lactose, glucose, and xylose ($P > 0.10$). Treatment dogs had greater arabinose concentrations than Ctl dogs ($P \le 0.05$). There were no group by week interaction effects ($P > 0.10$). When data for the two diet groups was pooled, week had no effect on propionic or lactic acids ($P > 0.10$); however, all other metabolites differed by week ($P \le 0.05$). Fecal lactose concentrations were greater at week 0 than week 7, but no other weeks differed from each other ($P \le 0.05$). Fecal glucose concentration at weeks 0, 1, 3, and 4 were greater than week 8 ($P \le 0.05$). Fecal xylose concentration at week 4 was greater than week 8 ($P \le 0.05$). Fecal arabinose concentration at week 3 was greater than weeks 6, 7, and 8. Fecal acetic acid concentration at weeks 0 to 5 were greater than week 8 ($P \le 0.05$). Fecal propionic acid concentration did not differ between week ($P > 0.05$). Fecal butyric acid concentration was greater at week 1 than weeks 0, 7, and 8 ($P \le 0.05$). Fecal isobutyric acid concentration was greater at weeks 0 and 1 than week 8 ($P \le 0.05$). Fecal isovaleric acid concentration was greater at week 1 than week 8 ($P \le 0.05$). Total SCFA concentrations were lower at week 8 than weeks 1 to 4 ($P \le 0.05$). There were no differences in total BCFA concentrations over time ($P > 0.10$).

Inflammatory markers and marker of antioxidant status

The results for the inflammatory markers SAA and Hp, as well as MDA, the marker of antioxidant status, are reported in Table 5. Serum Hp concentrations were greater for Trt than Ctl over the duration of the study ($P \le 0.05$). Serum Hp concentrations were lower in Trt at week 8 compared to week −1

Table 3. Treatment least square means for Iohexol and Chromium-EDTA I-AUC at week −2 and 9 for control and yeast-supplemented treatment dogs.

Measurement	Treatment[1]			*P*-value[2]
	Trt	Ctl	SEM	Treatment
Week −2				
I-AUC[3]				
Iohexol, µg/mL*h	32.82[a]	12.17[b]	7.29	0.05
Cr-EDTA, ppb/h	45.84	23.83	9.40	0.10
Week 9				
I-AUC				
Iohexol, µg/mL*h	16.23	16.33	2.47	0.98
Cr-EDTA, ppb/h	18.60	22.94	2.10	0.15
Difference week −2 and 9[3]				
Iohexol, µg/mL*h	−14.55	1.48	6.92	0.10
Cr-EDTA, ppb/h	−23.68	−0.55	8.60	0.06

[1]Trt, treatment dogs; Ctl, control dogs; SEM $n = 10$ for treatment and $n = 8$ for control.
[2]Significantly different when $P \le 0.05$ and a tendency declared when $0.05 < P \le 0.10$.
[3]Difference = Week 9 – Week −2.

Table 4. Mean fecal lactic acid, SCFA, and BCFA data for weeks 0 to 8 for control and yeast-supplemented dogs

	Week										Trt vs. Ctl[1]			*P*-value		
Parameter[3]	0	1	2	3	4	5	6	7	8	SEM	Trt	Ctl	SEM	Treatment	Week	Trt × Wk[2]
Glucose	1.28[ab]	1.38[a]	0.73[bc]	1.10[abc]	1.13[abc]	0.92[abc]	0.73[bc]	0.82[abc]	0.60[c]	0.15	1.01	0.93	0.07	0.40	≤0.01	0.63
Xylose	1.13[ab]	1.25[ab]	1.17[ab]	1.20[ab]	1.34[a]	1.17[ab]	1.05[ab]	1.04[ab]	1.02[b]	0.07	1.18	1.13	0.03	0.27	0.02	0.67
Arabinose	2.19[ab]	2.12[ab]	1.67[ab]	2.97[a]	1.51[b]	1.42[b]	1.05[b]	0.95[b]	0.89[b]	0.34	1.99*	1.29	0.15	≤0.01	≤0.01	0.16
Lactic acid	5.98	4.62	5.38	4.10	5.16	2.74	4.84	2.84	5.41	1.06	4.36	4.77	0.64	0.66	0.24	0.84
Acetic acid	27.11[a]	28.70[a]	29.63[a]	28.12[a]	26.64[a]	26.35[a]	25.26[ab]	24.30[ab]	20.73[b]	1.32	26.56	26.07	0.60	0.57	≤0.01	0.79
Propionic acid	14.95	14.85	16.83	15.57	16.17	15.16	15.58	14.22	12.94	0.99	15.20	15.09	0.64	0.90	0.15	0.74
Butyric acid	5.31[bc]	6.72[a]	6.24[abc]	5.59[abc]	6.46[ab]	5.68[abc]	5.83[abc]	5.24[bc]	4.97[c]	0.33	5.85	5.72	0.16	0.57	≤0.01	0.43
Isobutyric acid	4.19[a]	4.14[a]	3.80[ab]	4.00[ab]	3.87[ab]	3.78[ab]	3.83[ab]	3.80[ab]	3.49[b]	0.15	3.85	3.90	0.08	0.63	0.05	0.21
Isovaleric acid	1.18[ab]	1.31[a]	1.30[a]	1.23[a]	1.11[ab]	1.25[a]	1.04[ab]	1.04[ab]	0.88[b]	0.29	1.16	1.14	0.05	0.66	≤0.01	0.73
Total SCFA[4]	47.37[ab]	50.27[a]	52.69[a]	49.35[a]	49.27[a]	47.18[ab]	46.67[ab]	43.76[ab]	38.65[b]	2.14	47.62	46.87	1.06	0.62	≤0.01	0.77
Total BCFA[5]	4.26	5.06	4.76	4.93	4.70	4.70	4.51	4.74	4.20	0.21	4.61	4.69	0.10	0.61	0.04	0.22

[1]Trt or Ctl.
[2]Interaction effect between treatment and control and week.
[3]All data presented in nanomoles per gram (nmol/g).
[4]Total SCFA; acetic acid, propionic acid, butyric acid.
[5]Total BCFA; isobutyric acid and isovaleric acid.
[a,d]Values in a row (within week) with different superscript are different ($P \leq 0.05$).
[†]Indicates that the treatment group mean significantly differed ($P \leq 0.05$) from the equivalent control group mean.
[*]Indicates that the treatment group mean tended to differ ($P \leq 0.10$) when compared with the equivalent control group mean.

Table 5. Hp; mg/mL, SAA; ng/mL, and MDA; nmol/mL concentrations in control and treatment dogs across week

	Group*Week					Week					Trt vs. Ctl[2]			*P*-value		
Parameter	−1	2	4	8	SEM[1]	−1	2	4	8	SEM[1]	Trt	Ctl	SEM	Trt × Wk[3]	Wk	Treatment
Hp, mg/mL																
Trt	1.42	1.49	0.87	0.76	0.18	1.26[a]	1.12[ab]	0.78[b]	0.78[b]	0.12	1.13[†]	0.84	0.09	0.14	<0.01	0.01
Ctl	1.11	0.75	0.7	0.8	0.16											
SAA, ng/mL																
Trt	ND	10.04	43.97	45.77	41.24	ND	10.45	52.95	30.89	29.16	33.26	29.6	18.71	0.63	0.41	0.89
Ctl	ND	10.86	61.94	16.01	41.24											
MDA, nmol/mL																
Trt	27.95	27.67	34.78	31.22	2.47	27.19[ab]	25.53[b]	32.64[a]	31.40[a,b]	1.73	30.41	27.98	1.41	0.78	<0.01	0.22
Ctl	26.44	23.39	30.49	31.58	2.64											

[1]n = 18, 16, 17, and 15 for week −1, 2, 4, and 8, respectively.
[2]Trt or Ctl.
[3]Interaction effect between treatment and week.
[a,b]Values in a row (within group) with different superscript are significantly different ($P \leq 0.05$).
[†]Pooled treatment values in a row with different symbols are significantly different ($P \leq 0.05$).

and 2 ($P \leq 0.05$) but did not differ from week 4. There were no differences in Hp concentrations in the Ctl group over the duration of the study ($P > 0.05$).

Concentrations of SAA were not detectable in any dog, regardless of diet group at week −1. There were no differences within diet group or between Trt and Ctl at any week ($P > 0.05$).

Serum MDA concentration increased in both Trt and Ctl groups over the course of the study. Concentrations of MDA in the Trt group tended to be greater at week 2 than week 4 ($P < 0.10$, Table 5). There were no significant differences in serum MDA concentrations in the Ctl group. When data were pooled for both diet groups at each week, MDA concentrations at week 4 were greater than week 2 ($P \leq 0.05$, Table 5).

Discussion

Sled dogs that received the concentrated yeast product tended to reduce gut permeability, had marginally affected fecal metabolites, had no effect on SAA and MDA status, and may have resulted in decreased concentrations of serum Hp after 8 wk of feeding. In addition, the concentrated yeast product had no effect on mean daily FI or weekly BW. Average daily feed intake was significantly different between week, though these changes can be attributed to seasonal variations impacting daily caloric requirements of dogs housed outdoors. While there was a significant diet group by week interaction effect, there were no differences between Trt and Ctl BW at any week. This suggests a general acceptability of the supplement at the given dose of 7 mg β-glucan/kg BW and a potential to reduce intestinal permeability.

The results of the present study generally agree with those reported in literature. Both live yeast products, as well as isolated yeast components, have been used to reduce intestinal permeability and improve overall gut health in pigs (Che et al., 2017), mice (Generoso et al., 2011; Han et al., 2017), and chickens (Brümmer et al., 2010). Piglets and mice exposed to a gastrointestinal challenge that were supplemented with live yeast maintained intestinal permeability comparable to that of unchallenged control animals (Generoso et al., 2011; Che et al., 2017). Moreover, day-old chicks who were fed a yeast supplement had improved gastrointestinal health, seen in greater production of mucus secreting goblet cells, when compared to un-supplemented control chicks (Brümmer et al., 2010). In a vaccination challenged group of dogs, apparent total tract digestibility of dry matter and crude protein, as well as energy digestibility, were greater in a yeast β-glucan supplemented group when compared to control (Traughber et al., 2020). The data presented herein suggest that 10 wk of concentrated yeast supplementation improved gut permeability; therefore, yeast supplementation may improve the gastrointestinal health of healthy, adult dogs. As this study utilized only healthy dogs, future studies evaluating the effects of concentrated yeast at the given dose in dogs exercising, growing, or experiencing chronic inflammation would further solidify the positive effects of yeast on gut health. A further limitation of the current study is that dogs were not blocked by gut permeability, largely due to the difficulty in rapidly analyzing gut permeability markers. Future studies should block groups based on gut permeability measures when gut permeability is the primary outcome investigated.

There are several markers that can be utilized for the evaluation of intestinal permeability in dogs, each with their

advantages and disadvantages (Klenner et al., 2009; Frias et al., 2012). The two-sugar test utilizes saccharides—such as lactulose, rhamnose, sucrose, and mannitol—and quantifies their subsequent recovery in plasma or urine as a ratio of the administered mono- and disaccharide (Frias et al., 2004; Klenner et al., 2009). The sugars are difficult to quantify in plasma, requiring extensive and complex sample preparation, and to quantify their recovery in urine, urinary catheterization and metabolic chambers are required for total urine collection over 6 or 24 h (Frias et al., 2004; Allenspach et al., 2006; Klenner et al., 2009). In addition, these sugars may be degraded by intestinal bacteria, leading to inaccurate assessments of intestinal permeability (Frias et al., 2004; Allenspach et al., 2006; Klenner et al., 2009). Radiolabeled markers, such as the gold standard ^{51}Cr-EDTA, are effective for both urine and blood quantification, and pass through the intestines undegraded (Frias et al., 2004; Frias et al., 2012). However, working with a radioactive marker restricts accessibility as they require additional training prior to use (Frias et al., 2012). While the soluble Cr indicator has been used in intestinal permeability studies in other species, such as rats (Ten Bruggencate et al., 2005), to the knowledge of the authors, this was the first study assessing the use of Cr-EDTA in dogs. Since the use of iohexol is well-validated in dogs (Frias et al., 2012), it was administered concurrently to evaluate the validity of Cr-EDTA as an intestinal marker. Previous evaluation of iohexol and ^{51}Cr-EDTA as markers of intestinal permeability reported that both were equally sensitive to changes in intestinal permeability in dogs, agreeing with the results of this study (Frias et al., 2012). As both iohexol and Cr-EDTA were able to quantify a difference in gut permeability between the Trt and Ctl groups at week −2, and a tendency for a difference at week 9, they can be considered comparable markers of intestinal permeability and either could be considered for future permeability studies, though more than one marker should be utilized to ensure accuracy.

Dogs in the Trt group had significantly greater fecal arabinose concentrations compared to Ctl; however, no differences were observed between diet groups for any other fecal metabolite, or for relative frequency of fecal score. The increased production of arabinose in the present study suggests an increase in saccharolytic fermentation. Saccharolytic metabolism is the breakdown of sugars to produce energy, and is preferred to proteolytic metabolism, the breakdown of proteins in the colon which results in the production of BCFAs and putrefactive compounds (Jackson and Jewell, 2019; Jewell et al., 2020). However, as only arabinose was increased, further study is warranted, such as evaluation of colonic microbiota, to determine if there were indeed any positive effects on the colonic microbiome. Fecal score was not affected by yeast supplementation, as all dogs, regardless of treatment, maintained an ideal fecal score throughout the study period. Strompfová et al. (2021) found no changes in fecal score in dogs treated with hydrolyzed brewer's yeast. Traughber et al. (2020) supplemented dogs with a yeast-derived β-glucan and found no differences in fecal SCFA, BCFA, or other products of fermentation following a vaccination challenge. It is important to note that as there are a variety of forms of yeast supplements, including isolated yeast cell wall extracts, live and heat-killed yeast, as well as several different preparations, there may be different components in these supplements that elicit beneficial outcomes. The lack of differences seen in all other fecal metabolites, as well as in fecal score and frequency, suggest no detrimental effect of the yeast supplement on overall dog health. A lack of unfavorable effects on a healthy population of adult dogs, especially when supplemented at a greater level than previously reported, is an encouraging result to continue investigating canine populations that could benefit from yeast inclusion, such as dogs experiencing chronic inflammatory conditions like irritable bowel syndrome, cancer, or obesity to determine if yeast would have a notable impact on inflammation in a chronically inflamed population.

In the present study, serum Hp concentrations in the Trt dogs decreased throughout the study period, suggesting that dogs receiving the concentrated yeast experienced a decreased acute phase response, which could be due to reduced systemic inflammation. It is unclear why the Trt group had greater inflammatory status at the beginning of the study. All dogs were screened prior to study onset, as well as throughout the study, and displayed no indicators of ill health, including no changes in FI, BW, or fecal parameters. Hp concentrations in dogs in other studies range from 0 to 3 g/L (Eckersall et al., 1999; Martínez-Subiela et al., 2004), which agrees with the findings of the present study. However, the Hp data were not supported by the other inflammatory marker, SAA. Concentrations of SAA were nondetectable in any dog, regardless of diet group, at week −1, and no differences were observed between Trt and Ctl dogs at any week following. Inflammatory stimuli trigger the acute phase response, a nonspecific reaction that occurs shortly after tissue injury with the goal of returning the animal to homeostasis (Ebersole and Cappelli, 2000). It is a complex reaction that results in a variety of systemic effects, including affecting the concentrations of APPs (Kushner, 1988; Eckersall, 1995). Both SAA and Hp are positive APPs, proteins that increase in response to inflammation, that are produced by the proinflammatory cytokine IL-6, with SAA additionally requiring IL-1 (Kushner, 1988). During the acute phase response, concentrations of SAA and Hp increase (Cerón et al., 2005). Other studies in dogs have reported SAA concentrations ranging from nondetectable to 69.6 mg/L (Yamamoto et al., 1994; Martínez-Subiela et al., 2004), which is in alignment with the concentrations in the present study. While serum Hp concentrations were decreased in the Trt group, indicating reduced inflammatory status in these dogs at the end of study, SAA concentrations were only detected in 20% of the samples evaluated. It is unclear why SAA concentrations were not reliably detected in these dogs as previous studies have validated its efficacy as a consistent and sensitive marker of inflammation in both healthy and diseased dogs (Martínez-Subiela et al., 2004; Christensen et al., 2014; Jitpean et al., 2014). Jitpean et al. (2014) evaluated SAA concentrations in healthy dogs, using the same assay kit as in the present study, and found that healthy dogs SAA concentrations were <10 mg/L, which agrees with the results of the present study. The increased concentrations in some dogs after week 2 could be attributed to exercise or seasonal impacts on SAA concentrations (Ducharme et al., 2009), though does not explain why most dogs had concentrations below the detectable limit of the ELISA. No studies have evaluated if seasonal changes impact APP concentrations, a variable which could have had an impact on SAA concentrations in these dogs. Owing to the lack of agreement between these two inflammatory markers, further evaluation of inflammatory status in dogs supplemented with concentrated brewer's yeast is required. Analysis of additional markers of inflammation, such as TNF-α, IL-1, IL-6, or IL-10, would further

elucidate the effects of the concentrated yeast on inflammatory status in healthy dogs.

Serum MDA concentrations, as a marker of lipid peroxidation, increased in both Trt and Ctl groups over the course of the study, and overall were greater in all dogs at week 4 than week 2, regardless of treatment. Oxidative stress is caused by an excess of reactive oxygen species (**ROS**), and can be due to overproduction of ROS, a reduction in antioxidants responsible for the degradation of ROS, or a combination of both factors (McMichael, 2007; Macotpet et al., 2013). This overproduction of ROS results in lipid peroxidation, which causes potentially fatal damage to cells and tissues by changing the structure of proteins, lipids, and DNA (Khanna et al., 2012). Serum MDA concentrations are commonly used as a marker of oxidative stress in humans (Moore and Roberts, 1998), pigs (Nilzén et al., 2001), cats, (McMichael, 2007) and dogs (McMichael, 2007; Crnogaj et al., 2010). Crnogaj et al., (2010) reported that dogs experiencing infection had a mean MDA concentration of 36.90 ± 13.95 μmol/L, while healthy dogs had a mean concentration of 8.13 ± 1.78 μmol/L. These elevated concentrations of MDA are in line with those in the present study at all weeks, suggesting that the dogs in the present study were likely experiencing higher levels of oxidative stress than would be considered normal for clinically healthy dogs. This could be attributed to their outdoor housing, as pigs who were reared outdoors had greater serum concentrations of MDA compared to those raised indoors (Nilzén et al., 2001), though additional research is needed to substantiate this effect. The increase in serum MDA concentration at week 4 could be attributed to changes in seasonal temperature and coincided with a moderate increase in FI, suggesting that this increase was a result of increasing oxidative processes associated with increased production of heat from metabolism (LeBlanc and Diamond, 1986).

In conclusion, supplementation of a concentrated brewer's yeast product to achieve a β-glucan dose of 7 mg/kg BW was well accepted by the dogs, and had no effect on daily FI, weekly BW, or relative frequency and mean fecal score. Although SAA and MDA concentrations were not affected by treatment, Hp decreased in the Trt group throughout the study period, suggesting that inflammatory status may be moderately reduced in this healthy population of dogs. In agreement, Trt dogs tended to have reduced gut permeability. The use of both markers in the present study allowed for the evaluation of non-radiolabeled Cr-EDTA in contrast to the validated marker iohexol, and the results presented herein suggest that both performed in a similar manner, although iohexol may be more accurate. As gut permeability tended to be lower in Trt dogs' post-supplementation, future research in dogs experiencing either an acute or chronic inflammatory condition where the intestinal barrier may be compromised would further elucidate the effects of brewer's yeast on gut health in dogs.

Supplementary Data

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

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Author's contributions

J.R.T., A.K.S., and M.A.S. designed the experiment. L.M.R., J.G.L., and P.S. conducted the research, L.M.R. analyzed the data, N.A. assisted with laboratory analysis, and all authors contributed to the writing of the manuscript. A.K.S. and M.A.S. had primary responsibility for the final content. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors, L.M.R., J.R.T., T.T.Y., N.A., W.P., M.A.S., J.G.L., and P.S. have no conflicts of interest. T.A. is an employee of the F.L. Emmert Company. A.K.S. is the Champion Petfoods Chair in Canine and Feline Nutrition, Physiology and Metabolism and additionally consults for Champion Petfoods. A.V. is the Royal Canin Veterinary Diets Endowed Chair in Canine and Feline Clinical Nutrition and declares that they serve on the Health and Nutrition Advisory Board for Vetdiet. A.V., M.A.S., and A.K.S. have received honoraria and research funding from various pet food manufacturers and ingredient suppliers.

Literature Cited

Allenspach, K., J. M. Steiner, B. N. Shah, N. Berghoff, C. Ruaux, D. A. Williams, J. W. Blum, and F. Gaschen. 2006. Evaluation of gastrointestinal permeability and mucosal absorptive capacity in dogs with chronic enteropathy. *Am. J. Vet. Res*. 67:479–483. doi:10.2460/ajvr.67.3.479

Amado, L., H. Berends, L. N. Leal, J. Wilms, H. Van Laar, W. J. J. Gerrits, and J. Martín-Tereso. 2019. Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. *J. Dairy Sci*. 102:3994–4001. doi:10.3168/jds.2018-15847

Association of American Feed Control Officials. AAFCO. 2016. *AAFCO manual*. West Lafayette (IN): AAFCO Inc.

Beloshapka, A. N., S. E. Dowd, J. S. Suchodolski, J. M. Steiner, L. Duclos, and K. S. Swanson. 2013. Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. *FEMS Microbiol. Ecol*. 84:532–541. doi:10.1111/1574-6941.12081

Binnerts, W. T., A. T. Van't Klooster, and A. M. Frens. 1968. Soluble chromium indicator measured by atomic absorption in digestion experiments. *Vet. Rec*. 82:470.

Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for beta-glucans. *Nature* 413:36–37. doi:10.1038/35092620

Brown, G. D., and S. Gordon. 2005. Immune recognition of fungal β-glucans: immune recognition of fungal β-glucans. *Cell. Microbiol*. 7:471–479. doi:10.1111/j.1462-5822.2005.00505.x

Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β-glucans. *J. Exp. Med*. 197:1119–1124. doi:10.1084/jem.20021890

Brümmer, M., C. Jansen van Rensburg, and C. Moran. 2010. *Saccharomyces cerevisiae* cell wall products: the effects on gut morphology and performance of broiler chickens. *S. Afr. J. Anim. Sci*. 40:14–21. doi:10.4314/sajas.v40i1.54125

Cerón, J. J., P. D. Eckersall, and S. Martínez-Subiela. 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet. Clin. Pathol*. 34:85–99. doi:10.1111/j.1939-165X.2005.tb00019.x

Che, L., Q. Xu, C. Wu, Y. Luo, X. Huang, B. Zhang, E. Auclair, T. Kiros, Z. Fang, Y. Lin, et al. 2017. Effects of dietary live yeast supplementation on growth performance, diarrhoea severity, intestinal permeability and immunological parameters of weaned piglets challenged with enterotoxigenic *Escherichia coli* K88. *Br. J. Nutr*. 118:949–958. doi:10.1017/S0007114517003051

Chen, J., and R. Seviour. 2007. Medicinal importance of fungal β-(1→3), (1→6)-glucans. *Mycol. Res*. 111:635–652. doi:10.1016/j.mycres.2007.02.011

Christensen, M. B., R. Langhorn, A. Goddard, E. B. Andreasen, E. Moldal, A. Tvarijonaviciute, J. Kirpensteijn, S. Jakobsen, F. Persson, and M. Kjelgaard-Hansen. 2014. Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs. *Can. Vet. J*. 55:161–168

Crnogaj, M., R. Petlevski, V. Mrljak, I. Kis, M. Torti, N. Kucer, V. Matijatko, I. Sacer, and I. Stokovic. 2010. Malondialdehyde levels in serum of dogs infected with Babesia canis. *Vet. Med*. 55:163–171. doi:10.17221/77/2010-vetmed

Ducharme, N. G., L. A. Fortier, M. S. Kraus, S. Hobo, H. O. Mohammed, M. P. McHugh, R. P. Hackett, L. V. Soderholm, and L. M. Mitchell. 2009. Effect of a tart cherry juice blend on exercise-induced muscle damage in horses. *Am. J. Vet. Res*. 70:758–763. doi:10.2460/ajvr.70.6.758

Ebersole, J. L., and D. Cappelli. 2000. Acute-phase reactants in infections and inflammatory diseases: acute-phase reactants in infections and inflammatory diseases. *Periodontology 2000* 23:19–49. doi:10.1034/j.1600-0757.2000.2230103.x

Eckersall, P. D. 1995. Acute phase proteins as markers of inflammatory lesions. *Comp. Haematol. Int*. 5:93–97. doi:10.1007/bf00638925

Eckersall, P. D., S. Duthie, S. Safi, D. Moffatt, N. U. Horadagoda, S. Doyle, R. Parton, D. Bennett, and J. L. Fitzpatrick. 1999. An automated biochemical assay for haptoglobin: prevention of interference from albumin. *Comp. Haematol. Int*. 9:117–124. doi:10.1007/BF02600369

Frias, R., S. Sankari, and E. Westermarck. 2004. 51Cr-EDTA absorption blood test: an easy method for assessing small intestinal permeability in dogs. *J. Vet. Intern. Med*. 18:156–159. doi:10.1892/0891-6640(2004)18<156:cabtae>2.0.co;2

Frias, R., K. Strube, W. Ternes, M. C. Collado, T. Spillmann, S. Sankari, and E. Westermarck. 2012. Comparison of 51Chromium-labeled ethylenediamine tetra-acetic acid and iohexol as blood markers for intestinal permeability testing in Beagle dogs. *Vet. J*. 192:123–125. doi:10.1016/j.tvjl.2011.04.024

Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by Dectin-1 and toll-like receptor 2. *J. Exp. Med*. 197:1107–1117. doi:10.1084/jem.20021787

Generoso, S. V., M. L. Viana, R. G. Santos, R. M. E. Arantes, F. S. Martins, J. R. Nicoli, J. A. N. Machado, M. I. T. D. Correia, and V. N. Cardoso. 2011. Protection against increased intestinal permeability and bacterial translocation induced by intestinal obstruction in mice treated with viable and heat-killed *Saccharomyces boulardii*. *Eur. J. Nutr*. 50:261–269. doi:10.1007/s00394-010-0134-7

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

Jackson, M. I., and D. E. Jewell. 2019. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. *Gut Microbes* 10:298–320. doi:10.1080/19490976.2018.1526580

Jewell, D., M. Jackson, J. Hall, and D. Badri. 2020. Feeding microbiome-targeting ingredients increases fecal butyrate, plant-origin antioxidants, and anti-inflammatory compounds in dogs. *Curr. Dev. Nutr*. 4:689. doi:10.1093/cdn/nzaa050_012

Jitpean, S., B. S. Holst, O. V. Höglund, A. Pettersson, U. Olsson, E. Strage, F. Södersten, and R. Hagman. 2014. Serum insulin-like growth factor-I, iron, C-reactive protein, and serum amyloid A for prediction of outcome in dogs with pyometra. *Theriogenology* 82:43–48. doi:10.1016/j.theriogenology.2014.02.014

Jung, K., Y. Ha, S. Ha, D. Han, D.-W. Kim, W. K. Moon, and C. Chae. 2003. Antiviral effect of Saccharomyces cerevisiae b-glucan to swine influenza virus by increased production of interferon-c and nitric oxide. *J. Vet. Med. B*. 51:72–76. doi:10.1111/j.1439-0450.2004.00732.x

Khanna, S., D. Pande, R. Negi, K. Karki, R. S. Khanna, and H. D. Khanna. 2012. Oxidative stress induced damage in benign and malignant breast diseases: histopathological and biochemical aspects. *J. Stress Physiol. Biochem*. 8:209–214.

Klenner, S., M. Coenen, K. Failing, M. Hewicker-Trautwein, W. Ternes, J. Verspohl, and T. Spillmann. 2009. Estimation of intestinal permeability in healthy dogs using the contrast medium iohexol. *Vet. Clin. Pathol*. 38:353–360. doi:10.1111/j.1939-165X.2009.00136.x

Kushner, I. 1988. The acute phase response: an overview. *Methods Enzymol*. 163:373–383. doi:10.1016/0076-6879(88)63037-0

LeBlanc, J., and P. Diamond. 1986. Effect of meal size and frequency on postprandial thermogenesis in dogs. *Am. J. Physiol. Endocrinol. Metab*. 250:E144–E147. doi:10.1152/ajpendo.1986.250.2.e144

Lee, J. -N., D. -Y. Lee, I. -H. Ji, G. -E. Kim, H. N. Kim, J. Sohn, S. Kim, and C. -W. Kim. 2001. Purification of soluble β-glucan with immune-enhancing activity from the cell wall of yeast. *Biosci. Biotechnol. Biochem*. 64:837–841. doi:10.1271/bbb.65.837

MacFie, J., B. S. Reddy, M. Gatt, P. K. Jain, R. Sowdi, and C. J. Mitchell. 2005. Bacterial translocation studied in 927 patients over 13 years. *Br. J. Surg*. 93:87–93. doi:10.1002/bjs.5184

Macotpet, A., F. Suksawat, P. Sukon, K. Pimpakdee, E. Pattarapanwichien, R. Tangrassameeprasert, and P. Boonsiri. 2013. Oxidative stress in cancer-bearing dogs assessed by measuring serum malondialdehyde. *BMC Vet. Res*. 9:101. doi:10.1186/1746-6148-9-101

Martinez-Subiela, S., L. J. Bernal, and J. J. Ceron. 2003. Serum concentrations of acute-phase proteins in dogs with leishmaniosis during short-term treatment. *Am. J. Vet. Res*. 64:1021–1026. doi:10.2460/ajvr.2003.64.1021

Martínez-Subiela, S., J. J. Cerón, and P. J. Ginel. 2004. Effects of different glucocorticoid treatments on serum acute phase proteins in dogs. *Vet. Rec*. 154:814–817. doi:10.1136/vr.154.26.814

Martins, M. S., N. K. Sakomura, D. F. Souza, F. O. R. Filho, M. O. S. Gomes, R. S. Vasconcellos, and A. C. Carciofi. 2013. Brewer's yeast and sugarcane yeast as protein sources for dogs. *J. Anim. Physiol. Anim. Nutr. (Berl)* 98:948–957. doi:10.1111/jpn.12145

McKay, D. M., and M. H. Perdue. 1993. Intestinal epithelial function: the case for immunophysiological regulation. *Dig. Dis. Sci*. 38:11. doi:10.1007/BF01308592

McMichael, M. A. 2007. Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats. *J. Am. Vet. Med. Assoc*. 231:714–720. doi:10.2460/javma.231.5.714

Moore, K., and L. J. Roberts. 1998. Measurement of lipid peroxidation. *Free Radic. Res*. 28:659–671. doi:10.3109/10715769809065821

Moxham, G. 2001. Waltham feces scoring system—a tool for veterinarians and pet owners: how does your pet rate? *Vet. Focus* 11:4–25.

National Research Council. NRC. 2006. *Nutrient requirements of dogs and cats*. 2nd rev. ed. Washington (DC): The National Academies Press.

Neijat, M., J. Habtewold, R. B. Shirley, A. Welsher, J. Barton, P. Thiery, and E. Kiarie. 2019. Bacillus subtilis Strain DSM 29784 modulates the cecal microbiome, concentration of short-chain fatty acids, and apparent retention of dietary components in shaver white chickens during grower, developer, and laying phases. *Appl. Environ. Microbiol*. 85:e00402–e00419. doi:10.1128/AEM.00402-19

Nilzén, V., J. Babol, P. C. Dutta, N. Lundenheim, A. -C. Enflält, and K. Lundström. 2001. Free range rearing of pigs with access to pasture grazing-effect on fatty acid composition and lipid oxidation products. *Meat Sci*. 58:267–275. doi:10.1016/s0309-1740(00)00164-9

Novak, M., and V. Vetvicka. 2009. Glucans as biological reponse modifiers. *Endocr. Metab. Immune Disord. Drug Targets* 9:67–75. doi:10.2174/187153009787582423

Ortín-Piqueras, V., T. Spillmann, M. Pöytäkangas, D. E. Vaccaro, S. Sankari, and R. Frías. 2018. Determination of iohexol in canine plasma—strong correlation between enzyme-linked immunosorbent assay, high-performance liquid chromatography, and neutron activation analysis. *Scand. J. Lab. Anim. Sci*. 44:7. doi:10.23675/sjlas.v44i0.535

Phungviwatnikul, T., H. Valentine, M. R. C. de Godoy, and K. S. Swanson. 2020. Effects of diet on body weight, body composition, metabolic status, and physical activity levels of adult female dogs after spay surgery. *J. Anim. Sci*. 98:skaa057. doi:10.1093/jas/skaa057

Pillemer, L., and E. E. Ecker. 1941. Anticomplementary factor in fresh yeast. *J. Biol. Chem*. 137:139–142. doi:10.1016/S0021-9258(18)72984-0

Rychlik, A., R. Nieradka, M. Kander, M. Nowicki, M. Wdowiak, and A. Kołodziejska-Sawerska. 2013. The effectiveness of natural and synthetic immunomodulators in the treatment of inflammatory bowel disease in dogs. *Acta Vet. Hung*. 61:297–308. doi:10.1556/AVet.2013.015

Soeters, P. B. 2008. Probiotics: did we go wrong, and if so, where? *Clin. Nutr*. 27:173–178. doi:10.1016/j.clnu.2008.02.003

Strompfová, V., I. Kubašová, D. Mudroňová, L. Štempelová, M. Takáčová, B. Gąsowski, K. Čobanová, and A. Maďari. 2021. Effect of hydrolyzed yeast administration on faecal microbiota, haematology, serum biochemistry and cellular immunity in healthy dogs. *Probiotics Antimicrob. Proteins* 13:1267–1276. doi:10.1007/s12602-021-09765-9

Stuyven, E., F. Verdonck, I. Van Hoek, S. Daminet, L. Duchateau, J. P. Remon, B. M. Goddeeris, and E. Cox. 2010. Oral administration of β-1,3/1,6-glucan to dogs temporally changes total and antigen-specific IgA and IgM. *Clin. Vaccine Immunol*. 17:281–285. doi:10.1128/cvi.00344-09

Suram, S., G. D. Brown, M. Ghosh, S. Gordon, R. Loper, P. R. Taylor, S. Akira, S. Uematsu, D. L. Williams, and C. C. Leslie. 2006. Regulation of cytosolic phospholipase A2 activation and cyclooxygenase 2 expression in macrophages by the β-glucan receptor. *J. Biol. Chem*. 281:5506–5514. doi:10.1074/jbc.M509824200

Templeman, J. R., E. Thornton, C. Cargo-Froom, E. J. Squires, K. S. Swanson, and A. K. Shoveller. 2020. Effects of incremental exercise and dietary tryptophan supplementation on the amino acid metabolism, serotonin status, stool quality, fecal metabolites, and body composition of mid-distance training sled dogs. *J. Anim. Sci*. 98:1–12. doi:10.1093/jas/skaa128

Ten Bruggencate, S. J. M., I. M. J. Bovee-Oudenhoven, M. L. G. Lettink-Wissink, M. B. Katan, and R. van der Meer. 2006. Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J. Nutr*. 136:70–74. doi:10.1093/jn/136.1.70

Ten Bruggencate, S. J. M., I. M. J. Bovee-Oudenhoven, M. L. G. Lettink-Wissink, and R. Van der Meer. 2005. Dietary fructooligo saccharides increase intestinal permeability in rats. *J. Nutr*. 135:837–842. doi:10.1093/jn/135.4.837

Traughber, Z., F. He, and M. R. C. de Godoy. 2020. PSVI-24 immunological effects of a purified 1,3/1,6 β-glucan supplementation in retorted canine diets. *J. Anim. Sci*. 98:313. doi:10.1093/jas/skaa278.558

Willment, J. A., S. Gordon, and G. D. Brown. 2001. Characterization of the human β-glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem*. 276:43818–43823. doi:10.1074/jbc.M107715200

Yamamoto, S., S. Miyaji, Y. Ashida, K. Otabe, E. Momotani, and Y. Rikihisa. 1994. Preparation of anti-canine serum amyloid A (SAA) serum and purification of SAA from canine high-density lipoprotein. *Vet. Immunol. Immunopathol*. 41:41–53. doi:10.1016/0165-2427(94)90056-6