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# *Saccharomyces cerevisiae* Dehydrated Culture Modulates Fecal Microbiota and Improves Innate Immunity of Adult Dogs

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**Abstract:** *Saccharomyces cerevisiae* yeast culture can be dehydrated, and it has a potential prebiotic effect. This study evaluated the effects of supplementing increasing levels of dehydrated yeast culture (DYC) of *Saccharomyces cerevisiae* (Original XPC™, Diamond V, Cedar Rapids, IA, USA) on fecal microbiota, nutrient digestibility, and fermentative and immunological parameters of healthy adult dogs. Eighteen adult male and female dogs with a mean body weight of 15.8 ± 7.37 kg were randomly assigned to three experimental treatments: CD (control diet), DYC 0.3 (control diet with 0.3% DYC) and DYC 0.6 (control diet with 0.6% DYC). After 21 days of acclimation, fecal samples were collected for analysis of nutrient digestibility, microbiota and fecal fermentation products. On the last day, the blood samples were collected for the analysis of immunological parameters. The microbiome profile was assessed by the Illumina sequencing method, which allowed identifying the population of each bacterial phylum and genus. The statistical analyses were performed using the SAS software and the Tukey test for multiple comparison ($p < 0.05$). Our results suggest that the addition of DYC increased the percentage of the phyla Actinobacteria and Firmicutes ($p = 0.0048$ and $p < 0.0001$, respectively) and reduced that of the phylum Fusobacteria ($p = 0.0008$). Regardless of the inclusion level, the yeast addition promoted reduction of the genera *Allobaculum* and *Fusobacterium* ($p = 0.0265$ and $p = 0.0006$, respectively) and increased ($p = 0.0059$) that of the genus *Clostridium*. At the highest prebiotic inclusion level (DYC 0.6), an increase ($p = 0.0052$) in the genus *Collinsella* and decrease ($p = 0.0003$) in *Prevotella* were observed. Besides that, the inclusion of the additive improved the apparent digestibility of the crude fiber and decreased the digestibility of crude protein, nitrogen-free extract and metabolizable energy ($p < 0.05$). There were no significant changes in the production of volatile organic compounds. However, an increase in propionate production was observed ($p = 0.05$). In addition, the inclusion of yeast resulted in an increased phagocytosis index in both treatments ($p = 0.01$). The addition of 0.3 and 0.6% DYC to the diet of dogs wase able to modulate the proportions of some phyla and genera in healthy dogs, in addition to yielding changes in nutrient digestibility, fermentative products and immunity in healthy adult dogs, indicating that this additive can modulate fecal microbiota and be included in dog nutrition.

**Keywords:** bacteria; beta-glucan; fermentation; illumina; mannan oligosaccharides; prebiotic

## 1. Introduction

Prebiotics are substrates that are selectively used by host microorganisms and promote health benefits [1]. By modulating the intestinal microbiota, prebiotics can alter the host physiology and improve the fight against innumerable metabolic and immune infections and diseases, such as obesity, diabetes, and inflammatory bowel disease [2]. Yeasts are an example of prebiotics used in nutrition, such as *Saccharomyces cerevisiae*.

Structurally, the yeast cell wall of *S. cerevisiae* is composed of two fractions: one formed by beta-1,3/1,6-glucans and chitin, and another comprising mannoproteins partially formed by mannan oligosaccharides (MOS) [3]. *S. cerevisiae* can be dehydrated and is used as the commercial product Original XPC™ (Diamond V, Cedar Rapids, IA, USA). Its final composition includes MOS, beta-glucans, nucleotides, organic acids, polyphenols, amino acids, vitamins and minerals.

The MOS present in the *Saccharomyces cerevisae* cell wall is able to increase *lactobacilli* populations [4] and fecal *bifidobacterial* populations [5], which are considered beneficial bacteria to the host, and it appears to preserve the integrity of the gut absorption surface [6]. Another component, the beta-glucan, can selectively stimulate the growth of *lactobacilli* populations in a rat model [7], which also suggests a prebiotic activity [8].

Some studies have evaluated the supplementation of prebiotics with similar compositions of *S. cerevisiae* for modulation of fermentative products in dogs and obtained lower concentrations of phenols and indoles [9], reduced fecal ammonia excretion [10], and an increase in short chain fatty acids (SCFA) [11].

Regarding immunological parameters, supplementation with MOS present in the yeast cell wall can induce an increase in white cell blood concentrations, stimulating the immune response against pathogens [12], in addition to reducing inflammatory activity and improving innate immunity [11,13].

Considering these potential benefits, the aim of this experiment was to evaluate the effect of a food enriched with increasing levels of a commercial product called Original XPC™ (Diamond V, Cedar Rapids, IA, USA) (OXPC) composed of dehydrated yeast culture on fecal microbiota, nutrient digestibility, and fermentative and immunological parameters in healthy adult dogs.

## 2. Materials and Methods

### 2.1. Ethics

All experimental procedures were approved by the Ethics Research Committee for Animal Welfare of the School of Veterinary Medicine and Animal Science, University of São Paulo (protocol number 9148270415).

### 2.2. Animals

The experiment was carried out at the Nutritional Development Center and Bromatology Laboratory of the Premier Pet company, located in Dourado, São Paulo, Brazil. Eighteen adult male and female dogs, with a mean weight of 15.8 $\pm$ 7.37 kg and mean age of 3.3 $\pm$ 1.58 years, were included in this investigation (Table 1). The health status was confirmed prior to the beginning of the experiment. During the experiment, the dogs were housed individually in kennels with dimensions of 2.0 $\times$ 5.60 m and solarium of 2.0 $\times$ 4.90 m.

### 2.3. Experimental Design

The animals were randomly assigned to one of three experimental treatments, resulting in 6 replicates per treatment. The whole experiment last 25 days, which included an acclimation period of 14 days for diet adaptation; after this period, total fecal samples were collected for analysis of nutrient digestibility every day for a week (7 days). On the next 3 days, feces were collected immediately after defecation with sterile gloves for fecal pH, fecal fermentation products, and microbiota analysis. On the last day, the blood samples were collected for analysis of immunological parameters.

**Table 1.** Descriptive information of the animals included in the study.

| Animal | Diet | Sex | Breed | Weight (Kg) | Age (Years) |
|---|---|---|---|---|---|
| Animal 1 | DC | Male | Whippet | 11.6 | 1.5 |
| Animal 2 | DC | Female | English Setter | 24.4 | 1.7 |
| Animal 3 | DC | Female | Cocker Spaniel | 10.5 | 6.0 |
| Animal 4 | DC | Male | Beagle | 12.0 | 5.0 |
| Animal 5 | DC | Female | Beagle | 12.8 | 5.0 |
| Animal 6 | DC | Male | Golden Retriever | 29.0 | 3.3 |
| Animal 7 | OXPC 0.3 | Male | Beagle | 12.2 | 4.0 |
| Animal 8 | OXPC 0.3 | Female | Beagle | 10.4 | 4.0 |
| Animal 9 | OXPC 0.3 | Female | Whippet | 9.1 | 1.2 |
| Animal 10 | OXPC 0.3 | Female | English Bulldog | 12.6 | 2.6 |
| Animal 11 | OXPC 0.3 | Male | West Highland White Terrier | 8.4 | 1.1 |
| Animal 12 | OXPC 0.3 | Male | Labrador Retriever | 30.4 | 4.2 |
| Animal 13 | OXPC 0.6 | Male | Beagle | 14.5 | 5.7 |
| Animal 14 | OXPC 0.6 | Female | Beagle | 7.8 | 1.5 |
| Animal 15 | OXPC 0.6 | Male | French Bulldog | 15.2 | 2.4 |
| Animal 16 | OXPC 0.6 | Male | English Setter | 26.9 | 1.6 |
| Animal 17 | OXPC 0.6 | Male | Beagle | 11.4 | 4.0 |
| Animal 18 | OXPC 0.6 | Female | Golden Retriever | 25.0 | 4.5 |

### *2.4. Diets*

A control diet was formulated to meet the requirements of AAFCO [14] for adult dogs under maintenance, and the prebiotic was included in different concentrations. The additive used in this study was Original XPC™ (Diamond V, Cedar Rapids, Iowa, USA) (OXPC), composed of dehydrated yeast culture of *S. cerevisiae*, with approximately 11.50% moisture, 14.90% crude protein, 1.30% fat, 25.20% crude fiber, and 8.50% ash. The OXPC is produced through the fermentation of selected liquids and cereal grains and raw ingredients with *S. cerevisiae*. After this process, the entire culture medium is dried without destroying the yeast factors, B-vitamins, and other nutritional fermentation products to form the final product.

Experimental treatments included increasing levels of the additive, as follows: DC (control diet without OXPC), OXPC 0.3 (control diet with 0.3% OXPC), and OXPC 0.6 (control diet with 0.6% OXPC). All diets were isonutritive and formulated with the same ingredients, differing only by the addition and concentration of OXPC, which was proportionally compensated by starch between diets (Table 2). Diets were extruded at the Pet Unit of Premier Pet, Dourado—SP (Brazil), and all ingredients were obtained from a single batch in order to avoid variability among treatments.

All animals were fed sufficient amounts of calories according to the National Research Council's energy requirement [15] for the maintenance of adult dogs, calculated as 95 kcal $\times$ (BW) $^{0.75}$ per day, with water offered ad libitum. The daily total amount of the food was divided into two equal portions, offered at 07:00 a.m. and 03:30 p.m. The feeders were removed 30 min after offering the diets. Consumption and food leftovers were measured and recorded throughout the experiment.

**Table 2.** Ingredient and chemical composition of the experimental diets with and without the additive Original XPC™ (OXPC).

| Item | Diets [1] | | |
|---|---|---|---|
| | DC | OXPC 0.3 | OXPC 0.6 |
| Ingredients (%) | | | |
| Starch | 1.00 | 0.70 | 0.40 |
| Dehydrated yeast culture | – | 0.30 | 0.60 |
| Corn grain | 20.91 | 20.91 | 20.91 |
| Poultry viscera meal | 36.00 | 36.00 | 36.00 |
| Broken rice | 30.00 | 30.00 | 30.00 |
| Poultry fat | 8.20 | 8.20 | 8.20 |
| Liquid palatability enhancers | 2.00 | 2.00 | 2.00 |
| Powdered palatability enhancers | 0.50 | 0.50 | 0.50 |
| Potassium chloride | 0.43 | 0.43 | 0.43 |
| Common salt | 0.30 | 0.30 | 0.30 |
| Premix mineral/vitamin [2] | 0.52 | 0.52 | 0.52 |
| Antifungal | 0.10 | 0.10 | 0.10 |
| Antioxidant | 0.04 | 0.04 | 0.04 |
| Chemical composition (% of dry matter) | | | |
| Ash | 6.65 | 5.92 | 6.09 |
| Crude protein | 35.66 | 31.25 | 33.37 |
| Fat | 15.63 | 16.38 | 14.36 |
| Nitrogen-free extract [3] | 35.35 | 39.09 | 39.58 |
| Crude fiber | 6.71 | 7.36 | 6.59 |
| Gross energy (kcal/g) | 5.23 | 5.22 | 5.14 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC) and OXPC 0.6 (control diet with 0.6% OXPC); [2] Addition per kilogram of product: Iron 100 mg, Copper 10 mg, Manganese 10 mg, Zinc 150 mg, Iodine 2 mg, Selenium 0.3 mg, Vitamin A 18000IU, Vitamin D 1200IU, Vitamin E 200IU, Thiamine 6 mg, Riboflavin 10 mg, Pantothenic Acid 40 mg, Niacin 60 mg, Pyridoxine 6 mg, Folic Acid 0.30 mg, Vitamin B12 0.1 mg, and Choline 2000 mg; [3] Nitrogen-free extract was calculated by the formula NFE = 100 − (Moisture + crude protein + fat + crude fiber + ash).

### *2.5. Determination of the Coefficients of Apparent Digestibility of Nutrients*

The apparent digestibility coefficients (ADC) of the nutrients were determined by the total fecal collection method, according to AAFCO [14] recommendations. Individual food consumption was recorded daily, as well as the quantities offered and rejected. Feces were collected within a 24 h period for 7 days, subsequently weighed and conditioned in individual plastic bags, previously identified, closed, and stored in a freezer (−15 °C) for further analysis. At the end of the collection period, they were thawed and homogenized, forming a single sample per animal (feces pool). The content of dry matter (DM), crude protein (CP), ethereal extract in acid hydrolysis, a.k.a. fat (EEAH), ashes, and crude fiber (CF) from food and feces were analyzed according to the methodology described by AOAC [16]. All analyses were conducted in duplicate and repeated when the coefficient of variation was greater than 5%.

Nitrogen-free extract (NFE) was calculated by the difference between DM and the sum of CP, EEAH, CF, and ashes. The gross energy (GE) of food and feces was determined on a calorimetric pump (1281, Parr Instrument Company, Moline, IL, USA). Based on the results obtained in the laboratory, ADC of DM, organic matter (OM), CP, EEAH, CF, and NFE of the diets were calculated. These calculations were performed with the following formula: CDC of the nutrient (%) = [ingested nutrient (g) − excreted nutrient (g)]/(ingested nutrient (g))] × 100.

### *2.6. Determination of Fecal Score, Fecal pH, and Ammoniacal Nitrogen*

The fecal score was evaluated according to grading scores from 0 to 5, of which 0 = liquid stools; 1 = pasty and shapeless stools; 2 = soft, malformed stools that assume the shape of the collection container; 3 = soft, formed, and moist stools that mark the floor;

4 = well-formed and consistent stools that do not mark the floor; 5 = well-formed, hard, and dry stools. Values between 3 and 4 were considered as ideal fecal score [17].

For determination of fecal pH, a 2 g sample of feces was separated and diluted in 18 mL of distilled water within 30 min after defecation. Determination was carried out with a digital benchtop pH meter (Digimed, DM-20, São Paulo, SP, Brazil), according to the methodology adapted from Walter et al. [18]. For quantification of fecal ammoniacal nitrogen, stool samples were collected within 30 min after defecation. The sampling process, as well as the distillation, were performed according to Sá et al. [19].

### *2.7. Evaluation of Volatile Organic Compounds in Feces*

Fresh feces were collected from the animals within 30 min after defecation and were quickly homogenized; 0.5 g of sample was placed in a sealed 20 mL glass vial with a leak-proof metal cap and double-sided silicone/Teflon. Samples were stored and maintained at −20 °C. The samples were evaluated by gas chromatography coupled to mass spectrometry (GC-MSD) (Agilent Technologies, Santa Clara, CA, USA) using an Agilent 7890 A gas chromatograph (CG) and an Agilent 5975C mass sorting detector (MSD), according to an adapted methodology [20,21]. The NIST mass spectra library of 2008 was used to identify the compounds detected.

### *2.8. Determination of Short-Chain (SCFA) and Short Branched Chain Fatty Acids (SBCFA) and Lactic Acid in Feces*

Stool samples were collected within 30 min after defecation. Subsequently, they were homogenized and weighed for the quantification of SCFA and SBCFA. Three grams of feces were acidified with 9 mL of 16% formic acid in a 15 mL falcon tube. The determination of the short and short branched chain fatty acids was performed by gas chromatography (Shimadzu Corporation, Kyoto, Japan) according to Erwin et al. [22].

Lactic acid was measured according to the methodology described by Pryce et al. [23], by the spectrophotometry method at 565 nm (500 to 570 nm). After collection, the feces were homogenized and mixed with 9 mL of distilled water (1: 3 $v/v$).

### *2.9. Determination of Biogenic Amines*

Five grams of fresh stool were collected in duplicate within 30 min after defecation and stored in 7 mL of 5% trichloroacetic acid and refrigerated. Subsequently, the samples were centrifuged at 10,000× $g$ for 20 min at 4 °C, and the supernatant was filtered on qualitative filter paper. The residue was extracted two more times using 7 mL and 6 mL of 5% trichloroacetic acid. The supernatants were combined for further determination of the biogenic amines. The determination and separation of the biogenic amines were performed by high-performance liquid chromatography by reverse phase column ion pairing and subsequently quantified by fluorimetry after post-column derivation with second ophthalaldehyde [24]. The amines were identified by comparing the retention time of the peaks found in the samples with those of the amines of the standard solution, according to the methodology described by Gomes et al. [25].

### *2.10. Determination of Fecal Bacteria Concentration by Illumina Sequencing Technology*

After a 7-day digestibility period, fecal samples were immediately and aseptically collected for 3 days for microbiota determination. The samples did not have contact with any other surface besides sterile gloves. The DNA extraction was performed using the Mobio Power Soil kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the methodology described by McInnes et al. [26]. After extraction, the DNA concentration was quantified using a Qubit® 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA).

Amplifications of the 16S rRNA gene were generated using a Fluidigm Access matrix (Fluidigm Corporation, South San Francisco, CA, USA) in combination with a Roche High Fidelity Fast Start Kit (Roche, Indianapolis, IA, USA). For this step, primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)

291 bp-fragment of the V4 region were used [27]. After this, Fluidigm primer specific forward (CS1 tag) and inverse (CS2 tag) were added according to the Fluidigm protocol. To confirm the quality of the regions and the sizes of the amplicons, the fragment analyzer (Advanced Analytics, Ames, IA, USA) was used.

A pool of DNA was generated by combining equimolar amounts of the amplified fragments from each sample. The pooled samples were selected by gel size from 2% E-gel agarose (Life Technologies, Grand Island, New York, NY, USA) and extracted using Qiagen gel purification kit (Qiagen, Valencia, CA, USA). To confirm the appropriate profile and average size, the sorted and cleaned clustered products were run on an Agilent Bioanalyzer.

The characterization of the microbial community through the Illumina sequencing was performed on a MiSeq using V3 reagents (Illumina Inc., San Diego, CA, USA) at the W.M. Keck Center for Biotechnology at the University of Illinois. Fluidigm tags were removed using FASTX-Toolkit (version 0.0.13); to process the resulting sequence data, QIIME 1.8.1 was used according to Caporaso et al. [28]. The sequence data were imported from demultiplexed fastq files, and we filtered out low-quality sequencing reads considering a quality score threshold of 25. After that, the sequences were grouped into operational taxonomic units (OTUs) using OTU open reference and were compared against the reference database OTU Greengenes 13_8 using a 97% similarity threshold.

An even sampling depth of sequences per sample was used for assessing alpha and beta diversity measures. A total of 917,433 reads were obtained, with an average of 50,436 reads (range = 17,666–93,602) per sample. Rarefaction curves based on observed species, Chao1, and phylogenetic distance (PD) whole tree measures plateaued, suggesting enough sequencing depth. The dataset was rarified to 16,300 reads for analysis of diversity and species richness. Principal coordinate analysis (PCoA) was performed, using both weighted and unweighted unique fraction metric (UniFrac) distances, to measure the phylogenetic distance between sets of taxa in a phylogenetic tree as the fraction of the branch length of the tree, on the 97% OTU composition and abundance matrix [29]. The unweighted distance checks for the presence or absence of different taxa of microbial communities between/among samples, whereas the weighted distance investigates proportional changes in the microbial community.

### *2.11. Phagocytosis and Oxidative Burst Test*

In order to perform this assay, blood leukocytes (lymphocytes, neutrophils, and monocytes) were incubated with a fluorescent reagent that indicated the production of reactive oxygen species in the basal state, and after carrying out phagocytosis of *Staphylococcus aureus* bacteria, which indicated the percentage and intensity of phagocytosis. Cells were incubated with the DCFH-DA reagent in phosphate buffered saline (PBS) and DCFH and fluorochrome-labeled (propidium iodide) labeled *S. aureus* and maintained at 37 °C for 20 min. After this period, the red cells were disrupted with a lysis solution and washed with PBS until a clear-looking sample was obtained. This sample was then read on a FACS Calibur (Becton & Dickinson, Franklin Lakes, NJ, USA) flow cytometer.

### *2.12. Lymphoproliferation Test*

The assay was performed in 96-well microtiter plates with U-shaped bottom. Blood lymphocytes were obtained by separation into iron particles and, after purification and washes in RPMI-1640 medium, were added to the wells in a concentration equivalent to $1 \times 10^5$ cells/well in 200 µL/well. The mitogens used were Concanavalin A and Phytohemagglutinin. The plates were incubated for 72 h at 37 °C in 5% $CO_2$ atmosphere. After 72 h of incubation, cells were collected and evaluation of proliferation was performed on a FACS Calibur (Becton & Dickinson, Franklin Lakes, NJ, USA) flow cytometer. For the analysis of fluorescence data, the values of the percentage of cell divisions and the index of cell proliferation were considered. In order to obtain and analyze the results, we used the software Cell Queste Flow Jo (TreeStar).

### *2.13. Immunophenotyping of Lymphocytes*

The number of CD4/CD8+ CD4+ CD8+ lymphocytes (CD3/CD4+/CD45R−) and CD4/CD8+ lymphocytes (CD3/CD8+/CD45R−) was assessed. Mononuclear cells ($2 \times 10^5$ cells/mL) were incubated in microtubes (1.5 mL) with CD3 (1:100), CD4 (1:10), CD8 (1:20), CD21 (1:100), and CD45R (1:100) (Serotec Antibody, Biolegend and eBioscience), diluted in 100 µL of cytometry buffer (PBS containing 0.5% bovine serum albumin and 0.02% sodium azide). The isotypic antibodies for background definition were included in the assay. Cells were incubated for 20 min at 4 °C, protected from light. At the end of the incubation period, the samples were washed twice with buffer for cytometry in a volume of 1000 µL/microtube. Finally, the cells were resuspended in 500 µL of phosphate buffer. The population of cells with low size (FSC) and low complexity (SSC) according to the delimited gate was selected as the lymphocyte population. From this selection, the different populations of lymphocytes were determined. The acquisition and analysis of 10,000 cells were performed using the flow cytometry technique.

### *2.14. Statistical Analysis*

The results were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA, 2004). The normality of the residues was verified by the Shapiro–Wilk test (PROC UNIVARIATE) and the variances compared by the F test. The statistical assumptions underwent logarithmic or square root transformation, and then analysis of variance was performed by PROC GLM of the SAS with the means compared by the Tukey test at 5% of significance, as well as by simple polynomial regression, considering 2 degrees of freedom (linear and deviation).

The abundances observed for phyla and genera of each animal were evaluated by means of a generalized linear model, considering binomial distribution and a logit link function. The model included the fixed effect of the treatments (OXPC levels: 0.0, 0.3% or 0.6%) in addition to the random effect of the residue. The Tukey multiple comparison test was performed to identify which specific means differed at 5% significance level. All analyses were performed using the PROC GENMOD of the SAS procedure from the Statistical Analysis System, version 9.4 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

There was no difference between the treatments in relation to the average daily consumption of DM, NM, OM, CP, EEAH, CF, ash, and GE ($p > 0.05$; Table 3). The inclusion levels assessed for OXPC did not influence the ADC of DM, MO, ash, EEAH, and GE ($p > 0.05$; Table 3). The presence of additive decreased the digestibility of NFE ($p = 0.04$), CP ($p = 0.01$), and the metabolizable energy of the diets ($p < 0.01$), but increased the digestibility of CF ($p < 0.001$).

There were no differences for fecal production in organic matter, dry matter, and fecal score ($p > 0.05$; Table 4).

The presence of OXPC increased the propionic acid amount compared to that in the control group ($p = 0.05$). The other variables of intestinal fermentation did not differ between the treatments in this study ($p > 0.05$; Table 4). There was no difference among treatments regarding VOCs ($p > 0.05$; Table 5).

Regarding fecal microbiota, the alpha diversity was measured to determine the number of OTUs and then to give a basic measure of the bacterial diversity within each sample. All samples showed similar rarefaction curves regardless of treatment, indicating that these samples had similar diversity and no treatment effect (Figure 1). The principal coordinate analysis (PCoA) measures the overall bacterial genera relatedness, where the samples with similar bacterial communities are localized in similar positions in the diagram. Figures 2 and 3 suggest that OXPC supplementation at both levels (0.3% and 0.6%) did not have a beta-diversity effect on fecal microbiota.

**Table 3.** Intake of nutrients, apparent digestibility coefficients of nutrients, and metabolizable energy from experimental diets with different doses of the additive Original XPC™ (OXPC) given to the adult dogs.

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | CO | OXPC 0.3 | OXPC 0.6 | | |
| | | Consumption (g/day) | | | |
| Natural matter | 197.33 | 170.67 | 198.00 | 15.07 | 0.75 |
| Dry matter | 187.95 | 156.28 | 180.54 | 14.46 | 0.69 |
| Organic matter | 184.21 | 160.56 | 185.94 | 14.54 | 0.76 |
| Crude protein | 70.37 | 53.34 | 66.08 | 5.37 | 0.45 |
| Ethereal extract in acid hydrolysis | 30.83 | 27.95 | 28.44 | 2.38 | 0.89 |
| Crude fiber | 13.25 | 12.55 | 13.05 | 1.05 | 0.97 |
| Ash | 13.12 | 10.11 | 12.06 | 0.99 | 0.50 |
| Nitrogen-free extract | 69.76 | 66.72 | 78.37 | 5.91 | 0.74 |
| Gross energy (Kcal/day) | 1031.29 | 891.74 | 1018.82 | 80.56 | 0.77 |
| | | Apparent digestibility coefficient (%) | | | |
| Dry matter | 85.84 | 85.09 | 85.43 | 0.72 | 0.76 |
| Organic matter | 89.10 | 88.44 | 88.35 | 0.58 | 0.62 |
| Ash | 40.08 | 31.82 | 40.41 | 3.13 | 0.12 |
| Crude protein | 90.56 $^{a}$ | 86.90 $^{c}$ | 88.62 $^{b}$ | 0.74 | 0.01 |
| Crude fiber | 60.95 $^{b}$ | 74.41 $^{a}$ | 72.13 $^{a}$ | 2.03 | <0.001 |
| Ethereal extract in acid hydrolysis | 94.44 | 95.78 | 95.72 | 0.79 | 0.42 |
| Nitrogen-free extract | 91.02 $^{a}$ | 89.24 $^{b}$ | 88.15 $^{b}$ | 0.72 | 0.04 |
| Gross energy | 89.69 | 88.78 | 88.60 | 0.56 | 0.36 |
| Metabolizable energy (Kcal/kg of food consumed) | 4100 $^{a}$ | 4003 $^{b}$ | 3902 $^{c}$ | 33.38 | <0.01 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC) and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean. $^{a,b,c}$ mean in the lines followed by the same letters do not differ by Tukey test ($p > 0.05$).

The predominant fecal phyla present in all dogs included Firmicutes, Fusobacteria, and Bacteriodetes (Table 6). Together, Firmicutes and Fusobacteria constituted about 85–88% of the bacterial sequences, and Bacteroidetes contributed 8–12% of the sequences. An increase ($p = 0.0048$) in the abundance of fecal Actinobacteria was observed as the dose of the OXPC diet increased (Table 6). Besides that, the concentration of Firmicutes increased ($p < 0.0001$) while Fusobacteria decreased with the additive inclusion ($p = 0.0008$).

**Table 4.** Fecal quality and production, concentration of lactic acid, short and branched chain fatty acids, and biogenic amines of feces from dogs fed with different doses of the additive Original XPC™ (OXPC).

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | CD | OXPC 0.3 | OXPC 0.6 | | |
| Fecal production, fecal quality and lactic acid | | | | | |
| Fecal production g MN/dog/day | 85.34 | 73.54 | 88.54 | 14.46 | 0.75 |
| Fecal production g MS/dog/day | 26.16 | 23.11 | 26.37 | 3.86 | 0.80 |
| Fecal score | 3.97 | 3.88 | 3.97 | 0.05 | 0.42 |
| Fecal pH | 6.66 | 6.56 | 6.44 | 0.10 | 0.34 |
| N ammoniacal | 130.53 | 150.72 | 165.67 | 24.33 | 0.60 |
| Lactic acid | 13.22 | 16.30 | 11.69 | 3.56 | 0.62 |
| Short chain fatty acids. mmol/Kg of dry matter | | | | | |
| Acetic acid | 55.02 | 86.47 | 84.17 | 10.00 | 0.07 |
| Propionic acid | 25.77 [b] | 42.98 [a] | 40.80 [a] | 4.94 | 0.05 |
| Butyric acid | 9.41 | 12.04 | 13.00 | 2.16 | 0.49 |
| SCFA [3] total | 90.21 | 141.5 | 137.98 | 10.05 | 0.07 |
| Branched chain fatty acids. mmol/Kg of dry matter | | | | | |
| Valeric acid | – | – | – | – | – |
| Iso-valeric acid | 2.20 | 2.58 | 3.40 | 0.59 | 0.36 |
| Iso-butyric acid | 1.95 | 2.00 | 2.55 | 0.39 | 0.50 |
| SBCFA [4] total | 4.16 | 4.58 | 5.96 | 0.55 | 0.42 |
| Total fatty acids | 94.36 | 146.08 | 143.94 | 16.46 | 0.07 |
| Biogenic amines. mg/Kg of feces in the of natural matter | | | | | |
| Tyramine | 80.38 | 12.47 | 65.91 | 43.88 | 0.53 |
| Putrescin | 130.54 | 92.14 | 106.35 | 26.33 | 0.59 |
| Cadaverine | 54.42 | 18.31 | 37.29 | 24.57 | 0.56 |
| Spermidine | 41.22 | 34.80 | 41.25 | 5.67 | 0.65 |
| Phenylethylamine | – | 4.25 | 3.04 | – | – |
| Tryptamine | 1.81 | 3.18 | 2.44 | – | - |
| Total amines | 271.32 | 155.35 | 223.95 | 41.25 | 0.56 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC) and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean; [3] SCFA. short chain fatty acids; [4] SBCFA. short branched chain fatty acids. [a,b] mean in the lines followed by the same letters do not differ by Tukey test ($p > 0.05$).

**Table 5.** Mean percentage of the peak area of the most abundant volatile organic compounds present in feces from dogs fed with different doses of the additive Original XPC™ (OXPC).

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | DC | OXPC 0.3 | OXPC 0.6 | | |
| Acetic acid | 12.88 | 13.78 | 12.56 | 3.19 | 0.95 |
| Butanoic acid | 4.52 | 6.26 | 8.33 | 1.47 | 0.28 |
| Ethanol | 1.49 | 3.66 | 7.08 | 1.48 | 0.08 |
| Indol | 6.80 | 14.10 | 7.82 | 2.45 | 0.09 |
| Phenol | 1.59 | 4.18 | 4.26 | 1.10 | 0.24 |
| Propanoic | 11.73 | 15.01 | 14.76 | 2.29 | 0.54 |
| 2-piperidinone | 2.35 | 2.87 | 1.86 | 0.53 | 0.43 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC) and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean.

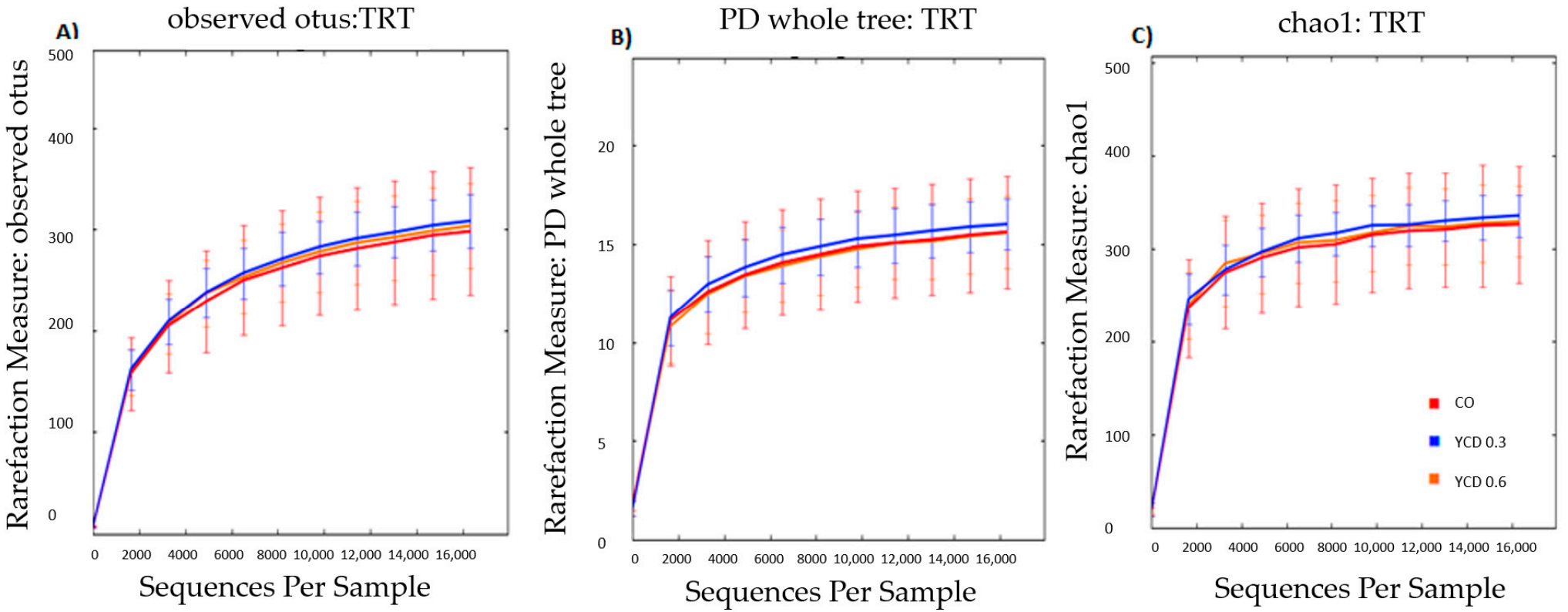


**Figure 1.** Alpha diversity: Rarefaction curves based on observed operational units (observed OTU) (**A**), phylogenetic distances (PD whole tree) (**B**) and metrics (Chao1) (**C**) according to the diet consumed by the animals. X axis represents the sequence depth (16,300 readings/sample), lines represent the mean of each group (red = control diet, blue = control diet with 0.3% OXPC, and orange = control diet with 0.6% OXPC).

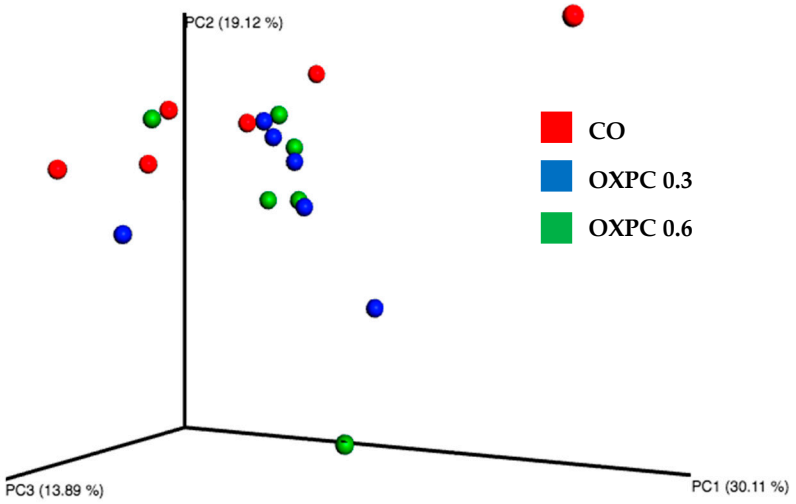


**Figure 2.** Beta diversity: Principal coordinate analysis (PCoA) of the unweighted portion of the unique metric fraction (Unifrac), according to the diet consumed by the animals. The plot showing clustering of microbial communities from feces of dogs fed with 0% (red), 0.3% (blue), and 0.6% (green) of OXPC. The closer the items, the more similar the microbial communities in the samples.

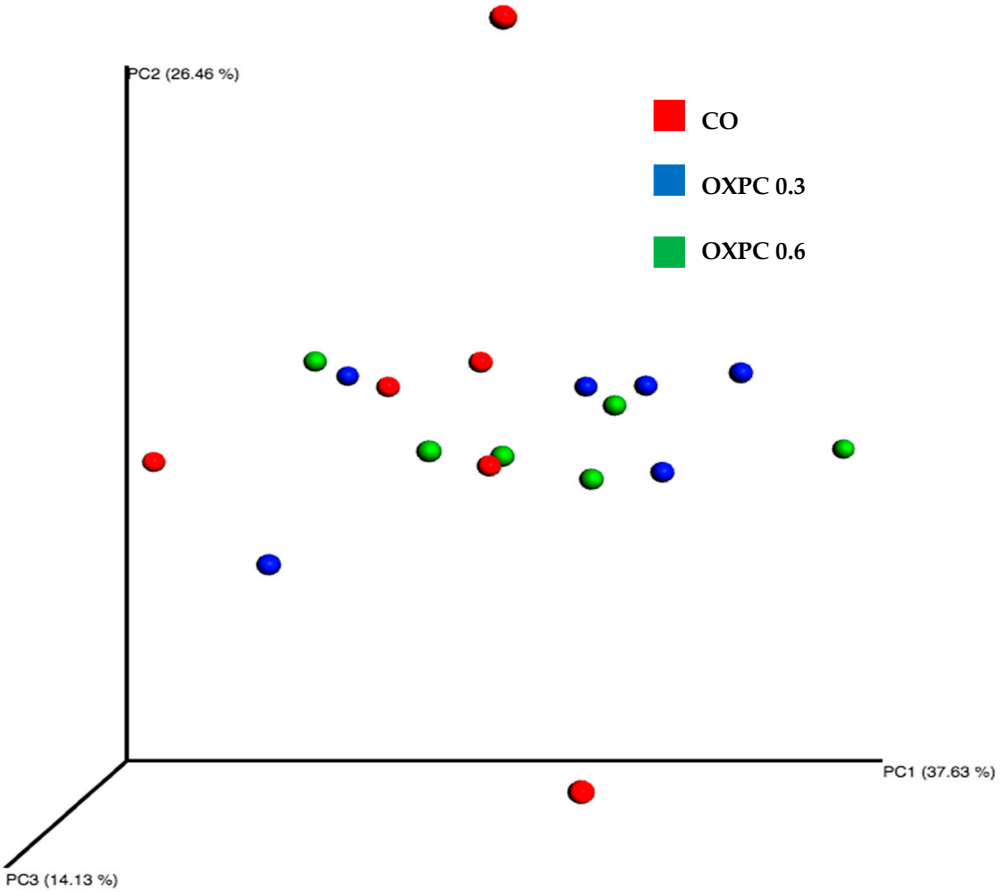


**Figure 3.** Beta diversity: Principal coordinate analysis (PCoA) of the weighted portion of the unique metric fraction (Unifrac), according to the diet consumed by the animals. The plot showing clustering of microbial communities from feces of dogs fed with 0% (red), 0.3%(blue), and 0.6% (green) OXPC. The closer the items, the more similar the microbial communities in the samples.

**Table 6.** Prominent bacterial phyla (expressed as percentage of total sequences) in feces of dogs fed with different doses of the additive Original XPC™ (OXPC).

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | CO | OXPC 0.3 | OXPC 0.6 | | |
| Unassigned bacteria | 0.011 ± 0.0004 | 0.010 ± 0.0004 | 0.019 ± 0.0006 | 0.0078 | 0.9886 |
| Actinobacteria | 0.160 $^{b}$ ± 0.0016 | 0.703 $^{a,b}$ ± 0.0034 | 1.869 $^{a}$ ± 0.0055 | 0.3311 | 0.0048 |
| Bacteroidetes | 11.990 ± 0.0133 | 9.370 ± 0.0119 | 8.150 ± 0.0112 | 30.550 | 0.0768 |
| Deferribacteres | 0.006 ± 0.0003 | 0.019 ± 0.0006 | 0.0101 ± 0.0004 | 0.0088 | 0.9754 |
| Firmicutes | 60.050 $^{b}$ ± 0.0200 | 70.380 $^{a}$ ± 0.0186 | 70.760 $^{a}$ ± 0.0186 | 5.937 | <0.0001 |
| Fusobacteria | 25.500 $^{a}$ ± 0.0178 | 17.960 $^{b}$ ± 0.0157 | 17.620 $^{b}$ ± 0.0156 | 41.330 | 0.0008 |
| Proteobacteria | 2.280 ± 0.0061 | 1.560 ± 0.0051 | 1.570 ± 0.0051 | 0.5619 | 0.5709 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC), and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean. $^{a,b}$ mean in the lines followed by the same letters do not differ by Tukey test ($p > 0.05$).

The predominant fecal bacterial genera were *Clostridium* (18–25%), *Fusobacterium* (16–25%), and *Blautia* (7–11%) (Table 7). The fecal concentrations of *Prevotella*, *Allobaculum*, and *Fusobacterium* were lower after including OXPC ($p$ = 0.0003, $p$ = 0.0265, and $p$ = 0.0006 respectively; Table 7). The *Clostridium* proportion increased with OXPC inclusion ($p$ = 0.0059; Table 7) and the *Collinsella* proportion was also greater when the highest prebiotic level was supplemented ($p$ = 0.0052; Table 7).

**Table 7.** Prominent bacterial genera (expressed as percentage of total sequences) in feces of dogs fed different doses of the additive Original XPC™ (OXPC).

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | DC | OXPC 0.3 | OXPC 0.6 | | |
| Unassigned bacteria | 0.012 ± 0.0004 | 0.010 ± 0.0004 | 0.020 ± 0.0006 | 0.0078 | 0.9886 |
| Actinobateria | | | | | |
| *Bifidobacterium* | 0.056 ± 0.0010 | 0.090 ± 0.0012 | 0.016 ± 0.0005 | 0.062 | 0.8422 |
| *Collinsella* | 0.050 $^{b}$ ± 0.009 | 0.427 $^{b}$ ± 0.0027 | 1.441 $^{a}$ ± 0.0049 | 0.256 | 0.0052 |
| *Slackia* | 0.054 ± 0.0009 | 0.186 ± 0.0018 | 0.412 ± 0.0026 | 0.093 | 0.3835 |
| Bacteroidetes | | | | | |
| *Bacteroides* | 6.946 ± 0.0104 | 7.392 ± 0.0107 | 6.465 ± 0.0100 | 2.356 | 0.8188 |
| *Parabacteroides* | 0.126 ± 0.0014 | 0.070 ± 0.0011 | 0.079 ± 0.0011 | 0.044 | 0.9446 |
| *Prevotella* | 3.044 $^{a}$ ± 0.0070 | 0.584 $^{b}$ ± 0.0031 | 0.561 $^{b}$ ± 0.0031 | 0.355 | 0.0003 |
| S24-7 | 0.102 ± 0.0013 | 0.009 ± 0.0004 | 0.007 ± 0.0003 | 0.036 | 0.6529 |
| Other [Paraprevotellaceae] | 0.441 ± 0.0027 | 0.419 ± 0.0026 | 0.234 ± 0.0020 | 0.162 | 0.7954 |
| [Paraprevotellaceae] | 0.045 ± 0.0009 | 0.035 ± 0.0008 | 0.017 ± 0.0005 | 0.019 | 0.9581 |
| [Paraprevotellaceae] [Prevotella] | 1.287 ± 0.0070 | 0.863 ± 0.0031 | 0.789 ± 0.0031 | 0.355 | 0.6505 |
| Deferribacteres | | | | | |
| *Mucispirillum* | 0.006 ± 0.0003 | 0.019 ± 0.0006 | 0.011 ± 0.0004 | 0.009 | 0.9754 |
| Firmicutes | | | | | |
| *Lactobacillus* | 0.003 ± 0.0002 | 0.402 ± 0.0026 | 0.003 ± 0.0002 | 0.230 | 0.0841 |
| *Streptococcus* | 0.015 ± 0.0005 | 0.061 ± 0.0010 | 0.007 ± 0.0003 | 0.033 | 0.8391 |
| *Turicibacter* | 0.108 ± 0.0013 | 0.017 ± 0.0005 | 0.010 ± 0.0004 | 0.047 | 0.6865 |
| Other Clostridiales | 0.290 ± 0.0022 | 0.417 ± 0.0026 | 0.183 ± 0.0017 | 0.137 | 0.7529 |
| Clostridiales | 0.433 ± 0.0027 | 0.368 ± 0.0025 | 0.457 ± 0.0028 | 0.092 | 0.9696 |

**Table 7.** *Cont.*

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | DC | OXPC 0.3 | OXPC 0.6 | | |
| Other Clostridiaceae | 0.175 ± 0.0017 | 0.209 ± 0.0019 | 0.305 ± 0.0023 | 0.043 | 0.8914 |
| Clostridiaceae | 2.641 ± 0.0065 | 2.730 ± 0.0067 | 2.530 ± 0.0064 | 0.348 | 0.9768 |
| *Clostridium* | 18.185 [b] ± 0.0158 | 24.513 [a] ± 0.018 | 25.043 [a] ± 0.0177 | 3.931 | 0.0059 |
| *Pseudoramibacter Eubacterium* | 0.041 ± 0.0008 | 0.020 ± 0.0006 | 0.004 ± 0.0003 | 0.024 | 0.9024 |
| Other Lachnospiraceae | 0.547 ± 0.0030 | 0.840 ± 0.0037 | 0.801 ± 0.0036 | 0.191 | 0.8033 |
| Lachnospiraceae | 3.769 ± 0.0078 | 3.402 ± 0.0074 | 4.975 ± 0.0089 | 0.754 | 0.3603 |
| *Blautia* | 7.122 ± 0.0105 | 10.870 ± 0.013 | 9.698 ± 0.0121 | 1.573 | 0.0667 |
| *Coprococcus* | 0.073 ± 0.0011 | 0.134 ± 0.0015 | 0.198 ± 0.0018 | 0.039 | 0.8358 |
| *Dorea* | 2.404 ± 0.0063 | 3.790.0078 | 3.899 ± 0.0079 | 0.620 | 0.2579 |
| *Roseburia* | 0.031 ± 0.0007 | 0.007 ± 0.0004 | 0.017 ± 0.0005 | 0.009 | 0.9543 |
| [Ruminococcus] | 2.930 ± 0.0069 | 4.378 ± 0.0084 | 4.314 ± 0.0083 | 0.127 | 0.3235 |
| *Peptococcus* | 0.956 ± 0.0040 | 1.299 ± 0.0046 | 0.700 ± 0.0034 | 0.622 | 0.5734 |
| Peptostreptococcaceae | 0.538 ± 0.0030 | 0.237 ± 0.0020 | 0.365 ± 0.0025 | 0.192 | 0.6946 |
| Ruminococcaceae | 1.639 ± 0.0052 | 1.488 ± 0.0049 | 1.526 ± 0.0050 | 0.277 | 0.9762 |
| *Fecalibacterium* | 5.828 ± 0.0096 | 4.650 ± 0.0086 | 5.355 ± 0.0092 | 1.162 | 0.6535 |
| *Ruminococcus* | 0.300 ± 0.0022 | 0.190 ± 0.0018 | 0.098 ± 0.0013 | 0.127 | 0.7235 |
| *Megamonas* | 0.665 ± 0.0033 | 0.980 ± 0.0040 | 1.970 ± 0.0057 | 0.685 | 0.1052 |
| *Phascolarctobacterium* | 0.276 ± 0.0021 | 0.220 ± 0.0019 | 0.151± 0.0016 | 0.103 | 0.8935 |
| [Mogibacteriaceae] | 0.038 ± 0.0008 | 0.001 ± 0.0001 | 0.0003 ± 0.0001 | 0.011 | 0.8000 |
| Erysipelotrichaceae | 2.825 ± 0.0068 | 2.383 ± 0.0062 | 2.199 ± 0.0060 | 0.525 | 0.7750 |
| *Allobaculum* | 6.905 [a] ± 0.0104 | 3.850 [b] ± 0.079 | 3.996 [b] ± 0.0080 | 3.318 | 0.0265 |
| *Catenibacterium* | 0.455 ± 0.0027 | 1.014 ± 0.0041 | 1.016 ± 0.0041 | 0.349 | 0.4300 |
| *Clostridium* | 0.024 ± 0.0006 | 0.018 ± 0.0006 | 0.020 ± 0.0006 | 0.011 | 0.9975 |
| *Coprobacillus* | 0.056 ± 0.0010 | 0.183 ± 0.0017 | 0.016 ± 0.0005 | 0.041 | 0.5815 |
| [Eubacterium] | 0.781 ± 0.0036 | 1.697 ± 0.0053 | 0.906 ± 0.0039 | 0.390 | 0.2840 |
| Fusobacteria | | | | | |
| Other Fusobacteriaceae | 0.611 ± 0.0032 | 0.643 ± 0.0033 | 0.692 ± 0.0034 | 0.070 | 0.9849 |
| *Fusobacterium* | 24.888 [a] ± 0.0177 | 17.319 [b] ± 0.015 | 16.928 [b] ± 0.0153 | 4.106 | 0.0006 |
| Proteobacteria | | | | | |
| *Sutterella* | 0.894 ± 0.0038 | 0.487 ± 0.0028 | 0.300 ± 0.0022 | 0.277 | 0.3770 |
| *Campylobacter* | 0.000 ± 0.0000 | 0.051 ± 0.0009 | 0.016 ± 0.0005 | 0.028 | 0.8022 |
| Succinivibrionaceae | 0.031 ± 0.0007 | 0.004 ± 0.0003 | 0.278 ± 0.0022 | 0.103 | 0.2653 |
| *Anaerobiospirillum* | 1.328 ± 0.0047 | 0.865 ± 0.0038 | 0.956 ± 0.0040 | 0.371 | 0.7132 |
| Enterobacteriaceae | 0.024 ± 0.0006 | 0.148 ± 0.0016 | 0.015 ± 0.0005 | 0.084 | 0.6069 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC), and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean. [a,b] mean in the lines followed by the same letters do not differ by Tukey test ($p > 0.05$).

There was no difference for CD4+ and CD8+ lymphocytes, CD4+/CD8+ ratio, oxidative burst (baseline and SAPI and PMA induced), and lymphocyte proliferative response ($p > 0.05$; Table 8). However, the phagocytosis index was higher with inclusion of OXPC compared to that of control ($p = 0.01$).

**Table 8.** Results of lymphocyte immunophenotyping, phagocytosis test, proliferation, and oxidative burst of dogs fed different doses of the additive Original XPC™ (OXPC).

| Item | Diets [1] | | | SEM [2] | *p* Value |
|---|---|---|---|---|---|
| | DC | OXPC 0.3 | OXPC 0.6 | | |
| CD4+ % (T helper cells) | 28.66 | 28.33 | 28.83 | 1.28 | 0.96 |
| CD8+ % (cytotoxic T cells) | 15.16 | 14.33 | 16.83 | 0.87 | 0.15 |
| CD4+/CD8+ | 6.26 | 2.16 | 1.56 | 1.77 | 0.16 |
| Basal oxidative burst | 216.17 | 206.50 | 196.83 | 8.00 | 0.26 |
| Oxidative burst SAPI [3] | 497.83 | 575.83 | 507.00 | 29.45 | 0.15 |
| Oxidative burst PMA [4] | 764.33 | 778.00 | 774.67 | 60.51 | 0.98 |
| Phagocytosis index | 264.67 [b] | 295.17 [a] | 297.17 [a] | 7.38 | 0.01 |
| Proliferation index | 310.33 | 355.50 | 356.17 | 16.14 | 0.10 |

[1] DC (control diet), OXPC 0.3 (control diet with 0.3% OXPC), and OXPC 0.6 (control diet with 0.6% OXPC); [2] SEM, standard error of the mean; [3] SAPI, *Staphylococcus aureus* conjugated with propidium iodide; [4] PMA, phorbol 12-myristate 13-acetate. [a,b] mean in the lines followed by the same letters do not differ by Tukey test ($p > 0.05$).

## 4. Discussion

Few studies have evaluated nutrient digestibility in dogs fed with diets supplemented with additives similar to OXPC, such as MOS mixed with basal diet [10], spray-dried yeast cell wall [30], *S. cerevisiae* live yeast [31], or *S. cerevisiae* fermentation product [6], among others. Different results were found with regard to nutrient digestibility that mentioned increases, decreases, and even non-alteration. Therefore, there is no consensus on the influence of the addition of this additive on digestibility parameters. Nonetheless, in this study, there was a decrease in ADC of CP and NFE and an increase in the ADC of the CF. The increase in intestinal microbial biomass caused by the inclusion of prebiotics in the diet may reduce ADC of CP [30]. Fecal bacterial mass enhances fecal protein content, which implicates in less ADC compared to the control group. Ideally, the true digestibility coefficient of the protein should be evaluated, in order to avoid considering fecal microbial protein content [32]. In regard to NFE digestibility, the OXPC consisted of dehydrated yeast culture, which has soluble fiber in its composition. That may have increased the viscosity of the bolus and impaired the interaction of pancreatic enzymes with the substrate, thereby decreasing the rate of carbohydrate digestion by pancreatic amylase [33].

Although the ADC of CF increased, this result must be evaluated with caution. The methodology used to determine CF was not entirely satisfactory. The laboratory technique is deficient because it yields low estimates of the fiber fraction present in the samples, destroying all of the soluble fraction and part of the insoluble fraction [34]. The main limitation is related to the fact that it does not separate cellulose from hemicellulose and causes loss of lignin (which is not considered carbohydrate) and hemicellulose. This method provides values that may change due to very drastic digestion, which leads to the loss of some components, and therefore, the values and differences obtained in our study may not be accurate [35]. The differences found in the metabolizable energy content of the foods may be actually a reflection of the small variations in the crude energy of the diets and levels and types of fibers.

Regarding fecal pH, no differences were observed among treatments, as well as in the study developed by Swanson et al. [4], who supplemented dogs with 2 g of FOS plus 1 g MOS. It is known that lactate produced by lactobacillus can lower fecal pH [36], and in this study, the authors attributed the non-detection of some bacterial species that consume lactate to this absence of differences in the results of fecal pH. Besides that, the SCFAs are absorbed quickly in the intestine, and may not be possible to identify in large amounts in feces that could have masked minor effects on pH [4].

In our study, the addition of OXPC at the concentrations of 0.3 and 0.6% were not capable of altering this genus population, which may have been implicated in the lack of

lactic acid alteration. In addition, in a study by Vickers et al. [37], the authors evaluated the fermentation characteristics of different substrates found in canine diets and could observe an increase in lactate with the use of FOS; however, when MOS was used, this product had its concentration decreased. *Saccharomyces cerevisae* processing or concentration also may explain the differences in these results.

Volatile organic compounds (VOCs) reflect differences in diet, intestinal microbiota, and exposure to chemical contaminants, as they are usually generated by the metabolism of intestinal microorganisms [38]. In this study, the main fecal VOCs did not change among treatments, which can be considered a positive effect, once it is related to lower stool odor [39]. In dogs, the presence of fecal VOCs was identified with the inclusion of prebiotics [40].

Total SCFAs did not change with OXPC addition, which can be explained by their rapid absorption by colonocytes, reducing fecal detection by presenting smaller quantities in feces [4]. These findings corroborate other studies [9,12,31]. SCFAs are associated with cell proliferation due to their role in the energy metabolism of colonocytes and are among the products generated in prebiotic fermentation [41]. Among them, propionic acid showed a higher fecal concentration in dogs treated with OXPC. The MOS present in OXPC reduces colonization by pathogenic bacteria in the intestine by competitive exclusion [30], which may result in an increase in propionic acid.

Biogenic amines are putrefactive compounds that can cause damage to intestinal health [42]. The extra source of energy promoted by fermentable carbohydrates, undigested protein, and their metabolites are used by bacteria for protein synthesis, decreasing the fecal concentration of protein-derived fermentation compounds [43]. No difference between treatments was observed for biogenic amines, which also corroborates the results of Swanson et al. [4].

According to Slavin [44], the level of inclusion of the prebiotic, as well as its source and time of use, can influence its effect. In a recent study, Perini et al. [45] compared the efficacy of prebiotics over 30 and 60 days of supplementation, and observed some changes in fermentation products over time. Therefore, the period of 21 days may not have been long enough to observe the effects of these prebiotics on the fermentative products evaluated in this study.

The microbial balance in the gastrointestinal tract is mostly determined by diet, and prebiotics can influence the gastrointestinal microbiota [46]. Likewise, evaluating the effect of adding a fermented *S. cerevisae* dry product in vitro, Possemiers et al. [47] did not find strong changes in the microbial community composition of the mucosal associated microbiota.

The predominant microbial phyla in the canine and feline gut, reported by previous investigations are *Firmicutes, Bacteroidetes, Proteobacteria, Fusobateria*, and *Actinobacteria*. *Fusobacteria* is one of the predominant phyla in the intestinal microbiome (intestine or feces). It frequently represents 10% or more of the genera sequences that inhabit the intestine [32]. This characteristic was also observed in this study, where the predominant fecal phyla present in all dogs included *Firmicutes, Fusobacteria, Bacteriodetes, Proteobacteria, Actinobacteria*, and *Deferribacteres*.

Middelbos et al. [48] phylogenetically characterized the fecal microbiota of healthy dogs with 454 pyrosequencing for the first time. The dominant phyla were Fusobacteria (23–40% of the readings), followed by *Firmicutes* (14–28% of the readings), *Bacteroidetes* (31–34% of the readings), *Actinobacteria* (0.8–1.4% of the readings), and *Proteobacteria* (5–7% of readings). Although *Fusobacteria* was not the most dominant, it was among the phyla with the highest proportion. It was also observed in the study of Beloshapka et al. [46], in which *Fusobacteria* and *Firmicutes* constituted about 75–80% of the bacterial sequences, with *Bacteroidetes, Proteobacteria*, and *Actinobacteria* contributing only about 10–15%, 5%, and 2–3% of the sequences, respectively.

An increase in *lactobacilli, bifidobacterium*, and aerobic bacteria was reported when supplementing the diet of healthy dogs with a combination of 2 g FOS and 1 g MOS [4], as FOS is preferentially fermented by lactic acid-producing bacteria [49]. This effect may have

been evidenced by the type of additive used and its processing, and for this reason, this result was not observed in our study.

The abundance of fecal *Actinobacteria* increased with the highest dose of OXPC in the diet and has also been observed in cats fed a diet containing a prebiotic [50]. This increase was correlated to microbiota adaptation of the prebiotic, which led to the increase in a genus belonging to the phylum *Actinobacteria*. The same may have occurred in this study, where the presence of OXPC stimulated the increase in the genus *Collinsella,* which belongs to the phylum *Actinobacteria* that was responsible for this increase. In the same way, an increase in *Clostridium* was observed, which may be correlated with the increase in the *Firmicutes* phylum, to which it belongs.

The members of the phylum *Firmicutes*, especially those of the genus *Clostridium*, can provide some benefits to the animals and are positively correlated with the oxidation of carbohydrates [51]. Although clostridial species are not all considered negative [52], an increase in the population of a potentially pathogenic bacterial genus could be considered a disadvantage of adding OXPC to dog's diet. Despite this, the animals did not shown clinical signs of infection, so it is reasonable to associate the increase in *Clostridium* with non-pathogenic strains.

*Allobaculum* also belongs to the *Firmicutes* phylum and is associated with weight regulation and regulation of hormones known to influence energy homeostasis (e.g., leptin) [53]. *Fusobacterium* reduction was observed with the inclusion of OXPC in the diet of the animals. This effect is considered beneficial since this genus is associated with gastrointestinal diseases [54,55].

Previous studies have shown an association of the genus *Prevotella* with diets containing high concentrations of carbohydrates [56,57]. However, the addition of OXPC reduced the concentration of this genus to the detriment of the others, which highlights the importance of conducting investigations on the interactions between bacterial populations and dietary substrates. We hypothesized that a greater inclusion of OXPC would allow the capture of evident differences among the bacterial groups.

Among the immunological tests performed in the study, the phagocytosis index presented greater activity in the animals that were supplemented with OXPC (0.3 and 0.6) compared to control. Few studies have assessed the effect of including this prebiotic on the immunity of pets; the pioneers were Middelbos et al. [3], who did not find differences in the immunity of dogs with the use of blends containing beet pulp, cellulose or blends of cellulose, fructooligosaccharides, and yeast cell wall at 2.5% in the diet. However, another study that evaluated MOS supplementation observed an increase in the total percentage of white blood cells [4]. A more recent study demonstrated that the inclusion of 1.0% of a commercial blend containing MOS, FOS, GOS, and beta-glucan in healthy dogs increased the polymorphonuclear cell count, phagocytosis index, and oxidative burst in supplemented animals compared to the control group [58]. Finally, Lin et al. [59] observed that supplementation of 0.2% yeast cell wall fractions to dogs tended to increase fecal IgA concentrations. All of the aforementioned suppliers concluded that this finding is related to positive modulation of the immune system.

Studies in other species show that the beta-glucans and MOS contained in *S. cerevisiae* have been identified as agents capable of triggering strong antigenic stimuli and immune responses. Beta-glucan is designated as an immunological response modifier, for when recognized by the organism, it has the ability to trigger a series of events in the immune response. Kubala et al. [60] reported that modulation of cellular activity by beta-glucan begins with the activation of macrophages, endothelial and dendritic cells, and B and T cells. In addition, they involve the specific immune response by inducing the expression of various cytokines such as TNF, IL-6, IL-8, and IL-12 [61]. Therefore, the composition of the OXPC treatment (0.3 and 0.6) explains the improvement in the index of phagocytosis in supplemented dogs.

## 5. Conclusions

According to the results, the addition of 0.3 and 0.6% OXPC in the diet of dogs was able to alter some phyla and genera abundances to increase propionic acid production and the phagocytosis index in healthy adult dogs with minor alteration in digestibility. Other studies should evaluate higher doses of OXPC supplementation and its effects on the intestine of healthy dogs and those suffering from gastrointestinal disorders.

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