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Companion Animal Nutrition

Food supplemented with a novel fiber blend containing soluble and insoluble fiber supported growth and fecal parameters indicative of gastrointestinal health in kittens

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Abstract

Prebiotic fiber plays a major role in the gastrointestinal health of animals. While research investigating the effects of prebiotic fiber in adult companion animals has expanded, studies in growing animals are limited. This study evaluated the effects of a patented fiber blend (with soluble and insoluble fiber and fiber-bound polyphenols) on kitten gastrointestinal health. Twenty kittens 4–9 mo of age were randomized to a control or test food after a 14-d prefeed period. The test food had a similar nutrient composition to the control food with the exception of a higher fiber content, due to the inclusion of the novel fiber blend, containing ground pecan shells, flaxseed, dried beet pulp, dried citrus pulp, and pressed cranberries. Kittens were fed for 150 d, with food intake monitored daily and body weight measured weekly. Fecal and blood samples were collected during the last week of the prefeed period (baseline), and for fecal samples on d 15, 29, 43, 57, 85, 114, and 142, and for blood samples on d 92 and 148 of the treatment period. Body weight and food intake changed as expected for cats of this age. The test food beneficially affected fecal score, which was higher in kittens fed the test food ($P < 0.001$). A diet-by-day interaction was observed for fecal pH ($P = 0.002$), which was lower in kittens fed the test food from d 29 onward versus the control food, for which fecal pH remained similar to baseline throughout the study. A diet-by-day interaction was also seen for fecal moisture ($P = 0.015$), which was unchanged throughout the study in control-fed kittens, but was higher than baseline on all days except for d 29 in those fed the test food. Kittens fed the test food exhibited a higher concentration of total saccharolytic short-chain fatty acids (SCFAs) ($P = 0.002$) and a lower concentration of total proteolytic SCFAs ($P < 0.001$) in feces than kittens fed the control food. No effects of diet, day, or the interaction on serum immunoglobulin A or most inflammatory cytokines were seen. Overall, kittens fed a food formulated for growing kittens and fortified with this patented prebiotic fiber blend for 150 d grew and developed normally and had beneficial changes in stool characteristics, including fecal score, pH, and SCFA concentration. Thus, this prebiotic blend with soluble and insoluble fibers and fiber-bound polyphenols supports normal growth and promotes gastrointestinal health in kittens.

Lay Summary

Nutrition plays a major role in the gastrointestinal and overall health of mammals. Adequate nutrition is particularly important in the development of the gastrointestinal tract. A variety of nutritional strategies have been utilized to support gastrointestinal health in companion animals, including prebiotics. Prebiotics are substrates that are utilized by the microbes present in the gastrointestinal tract to provide a benefit to host health. While numerous studies have investigated the impacts of feeding prebiotic fibers to adult dogs and cats, research investigating their benefits in growing companion animals is more limited. In this study, kittens were fed either a food with a patented fiber blend containing soluble and insoluble fibers or a control food, and markers of digestive health were evaluated. Animals were fed for 150 d, with body weight monitored weekly and fecal and blood samples collected at multiple time points. Results showed that while both foods supported healthy growth, kittens fed the food containing the novel fiber blend had fecal characteristics indicative of more favorable gastrointestinal health compared to kittens fed the control food. These results were similar to those previously seen in adult cats and dogs and adds to our knowledge about prebiotics in pets.

Key words: cat, fiber, kitten, growth, nutrition, prebiotic

Abbreviations: AAFCO, Association of American Feed Control Officials; ALP, alkaline phosphatase; ALT, alanine transaminase; BUN, blood urea nitrogen; CBC, complete blood count; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; Flt3-L, FMS-related tyrosine kinase 3 ligand; GGTP, gamma-glutamyl transpeptidase; GI, gastrointestinal; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IgA, immunoglobulin A; IL, interleukin; IPF, immature platelet fraction; IRF, immature reticulocyte fraction; IU, international units; KC, keratinocyte chemoattractant; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCP-1, monocyte chemoattractant protein-1; MCV, mean corpuscular volume; ME, metabolizable energy; NFE, nitrogen-free extract; NM, neutered male; PDGF, platelet-derived growth factor; ppm, parts per million; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; RBC, red blood cells; RDW, red cell distribution width; SCF, stem cell factor; SCFA, short-chain fatty acids; SDF-1, stromal cell-derived factor-1; SF, spayed female; TNF, tumor necrosis factor; WBC, white blood cell

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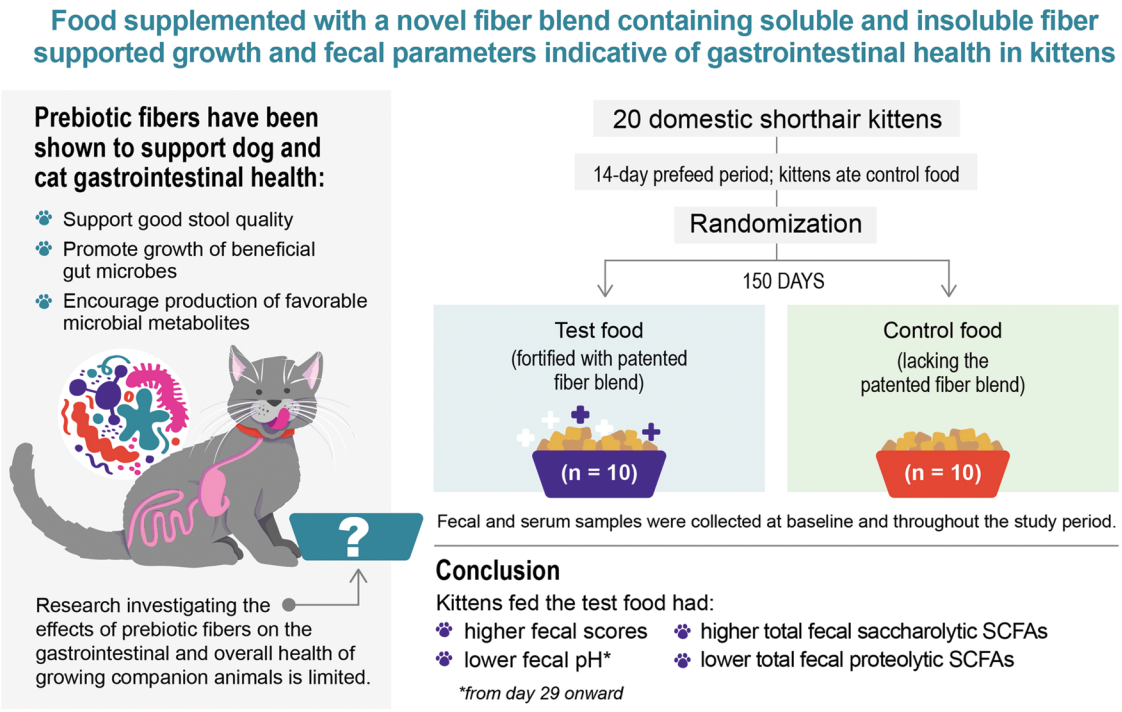
Graphical abstract



Introduction

The gastrointestinal (GI) tract, and the microbes that reside in the gut (the GI microbiome) are fundamental for supporting the digestive process and overall wellbeing of mammals. The GI tract works to break down and utilize nutrients that an animal needs to survive, with the GI microbiome providing further support for these processes. Across species, previous work has shown that a healthy GI microbiome is essential for proper nutrient absorption and metabolism, maintenance of intestinal epithelial integrity and osmolarity, and gut immunomodulation (reviewed in Lyu et al., 2020) (Minamoto et al., 2012; Lyu et al., 2020).

Proper nutrition is particularly crucial in the development of the GI tract and, specifically, the GI microbiome. According to the National Research Council, cats reach maturity at about 12 mo of age, but studies suggest that growth may extend beyond this point (National Research Council, 2006; Gross et al., 2010; Merenda et al., 2021; Salt et al., 2022). Adequate nutrition during growth is essential to support proper maturation and development and sets a foundation for health in adulthood (Hemmings, 2016; Gaillard et al., 2022). Imbalance in the GI microbiome can result in decreased nutrient absorption, poor stool quality, and an impaired immune response, all of which can have negative health impacts for growing animals (Minamoto et al., 2012; Lyu et al., 2020). Therefore, supporting GI health is of paramount importance for growing cats.

Prebiotic fiber supplementation has been identified as a strategy to support GI health in humans and companion animals (Wernimont et al., 2020b; Pilla and Suchodolski, 2021; Lee et al., 2022a). Prebiotics are substrates that are selectively utilized by the host microbiome to confer a health benefit and are often supplied as soluble fiber (Gibson et al., 2017). Studies in adult cats and dogs have shown that prebiotic fibers can positively impact GI health by promoting good stool quality, supporting growth of beneficial gut microorganisms, and encouraging production of beneficial metabolites by the gut microbiota (Rochus et al., 2014; Panasevich et al., 2015; Garcia-Mazcorro et al., 2017; Jewell et al., 2022a, 2022b; Palmqvist et al., 2023; Belchik et al., 2024; Swanson et al., 2025).

The patented prebiotic fiber blend used in this study includes both soluble and insoluble fibers with fiber-bound polyphenols, supplied by ground pecan shells, flaxseed, dried beet pulp, dried citrus pulp, and pressed cranberries (Jackson and Jewell, 2017). This combination of ingredients, which provides soluble and insoluble fiber, was chosen in order to provide a combination of benefits to the animal: soluble fiber has a high water-holding capacity and is readily fermented by gut microbes, while insoluble fiber aids in stool-bulking and promotes regular stool frequency. Pecan shells are predominantly composed of insoluble fiber, including cellulose and lignin (Dolan et al., 2016), while flaxseed provides moderately fermentable fiber, including mucilage (Puligundla and Lim 2022). Pressed cranberries, citrus pulp, and beet pulp provide moderately fermentable hemicellulose and pectin (Biagi et al., 2010; Varnaite et al., 2022). The ingredients in the blend also contain fiber-bound plant compounds, including flavanones, flavonols, and lignans, which can be liberated from the fiber matrices through the process of microbial fermentation and made accessible to the animal (Jackson and Jewell, 2019; Fritsch et al., 2022b). Previous work has shown that a therapeutic food containing this patented fiber blend at a higher inclusion level than used in this study rapidly and effectively resolved clinical outcomes in adult cats with constipation or diarrhea (Wernimont et al., 2020a). Furthermore, a later study found that healthy cats fed a food containing the same inclusion level of the fiber blend used in this study had favorable stool quality, decreases in proteolytic short chain fatty acids (SCFAs), often referred to as branched chain fatty acids, and an increase in the production of beneficial postbiotics (Jewell et al., 2022a, 2022b). Similarly,

studies in healthy dogs and those with chronic gastroenteritis/enteritis demonstrated that dietary interventions consisting of this prebiotic blend positively affected GI microbiome signatures and improved clinical signs of GI disease (Fritsch et al., 2022a, 2022b). While past studies have investigated the effects of feeding prebiotics, including the fiber blend used in this study, to adult dogs and cats, similar work in growing companion animals is more limited. A study was previously conducted to assess the impact of this patented prebiotic fiber blend in growing puppies and showed that, after consuming a food with this fiber blend for at least 90 d, the animals grew and developed normally and exhibited markers of improved GI health (McGrath et al., 2024). The primary objective of the present study was to assess the effects of a kitten food fortified with this novel fiber blend containing ground pecan shells, flaxseed, dried beet pulp, dried citrus pulp, and pressed cranberries on fecal parameters indicative of digestive health in healthy kittens, including fecal score, pH, and SCFA concentration. The underlying hypothesis was that kittens fed this food would experience fecal characteristics indicative of favorable GI health, similar to what has been shown in adult dogs and cats, and that they would continue to grow and develop as expected for kittens of this age.

Methods

This study was approved by the Hill's Pet Nutrition Institutional Animal Care and Use Committee (CP985) and was conducted in accordance with Hill's Global Animal Welfare Policy.

Animals

Male and female domestic shorthair kittens between 4 and 9 mo of age that were in good health and weighed at least 2 kg were eligible for participation in the study. Kittens were excluded if they had a history of food allergy or poor eating behavior, a history of certain prior conditions, including chronic GI disease, or received an antibiotic intervention within the last month.

Kittens enrolled in the study could be removed based on veterinary discretion if they: 1) experienced excessive weight loss (>15% of their initial body weight); 2) did not eat for 2–3 d; 3) were diagnosed with a medical condition for which they would not benefit to remain on the study; or 4) received any antibiotics, anti-inflammatory medications, anti-allergic medications, or medications related to gut transit time.

Animals were group-housed for a majority of the testing period, with the exception of feeding times and fecal collections. Housing and care were provided by Ontario Nutri-Lab Inc. (Fergus, Ontario, Canada). All study animals were allowed normal socialization and enrichment activities with other animals, and cats had daily opportunities for enrichment through access to care personnel. Toys and climbing structures were provided to encourage play and exercise. Windows within the animal rooms provided a natural light cycle, while the rooms were also equipped with full-spectrum LED lights. Fresh, clean drinking water was provided ad libitum, and at no time were animals subjected to any procedures expected to cause pain or distress.

Foods

Key nutrient levels of the control food and test food are summarized in Table 1, with ingredients of each food provided in Table 2. Both foods were formulated using Concept5 (CFC Tech Services, Inc., Pierz, Minnesota, USA), and nutritional analysis of finished foods was conducted by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA) using official methods published by AOAC International (AOAC International, 2019). Both the control and test foods were formulated to be complete and balanced for growing kittens, according to the Association of American Feed Control Officials (AAFCO) Official Publication (Association of American Feed Control Officials, 2024). The control food was similar in terms of nutrient composition compared with the test food, though the test food had higher soluble, insoluble, dietary, and crude fiber due to the inclusion of the fiber blend. The patented fiber blend added to the test food consisted of ground pecan shells, flaxseed, dried beet pulp, dried citrus pulp, and pressed cranberries. In place of the fiber blend, the control food included added whole grain oats and cracked pearled barley.

Table 1. Analyzed[1] nutrient composition of the control food and test food on a dry matter basis.

Nutrient	Control food	Test food
Moisture, % as fed	6.31	6.54
ME[2] (calculated), kcal/kg	4336	4285
Ash, %	7.2	6.2
Crude protein, %	39.1	39.1
Crude fat, %	22.3	21.8
NFE[3] (calculated), %	30.6	30.5
Crude fiber, %	0.7	2.5
Total dietary fiber, %	5.2	8.7
Insoluble fiber, %	4.1	7.1
Soluble fiber, %	1.2	1.6
C20:5n3 EPA, %	0.19	0.20
C22:6n3 DHA, %	0.13	0.14
Calcium, %	1.25	0.91
Phosphorus, %	0.94	0.81
Potassium, %	0.84	0.86
Sodium, %	0.46	0.45
Taurine, ppm	4910	4708

[1]Nutritional analysis was conducted by Eurofins Scientific, Inc. (Des Moines, IA, USA) using official methods of analysis published by Association of Official Analytical Collaboration International (AOAC International, 2019).
[2]Metabolizable energy. Calculated ME based on modified Atwater values.
[3]Calculated NFE as follows: % NFE = 100% – (% ether extract + % crude protein + % ash + % crude fiber).
DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IU, international units; ME, metabolizable energy; NFE, nitrogen-free extract; ppm, parts per million.

Study design

Kittens completed a 14-d prefeed period, in which they were fed the control food. Once the prefeed period was complete, kittens were stratified by sex and body weight, then randomly assigned to one of two groups, where they either continued eating the same food consumed during the prefeed period (control group) or were transitioned to the test food containing the patented fiber blend (test group). Animals were fed their respective foods for the entire 150-d testing phase; the amount of food offered to each kitten was sufficient to allow for ad libitum access, with the exception of the day preceding blood collection. If kittens consumed close to the total amount of food provided, their food offering was increased the following week. Kittens were weighed weekly to evaluate growth and to adjust feeding amounts. Physical exams were conducted by the

Table 2. Select ingredients in the control and test food as a percentage of the total recipe.

Ingredient, %[1]	Control food	Test food
Chicken	30.9	35.0
Brown rice	13.2	12.5
Wheat gluten	12.0	10.4
Chicken fat	10.0	9.8
Fiber blend[2]	–	6.6
Egg product	5.6	3.0
Whole grain oats	5.5	–
Whole grain wheat	5.0	10.3
Cracked pearled barley	5.0	–
Chicken liver flavor	3.0	3.0
Flaxseed	1.5	2.0
Lactic Acid	1.2	1.2
Calcium sulfate	1.2	0.50
L-Lysine	1.1	1.2
Fish oil	1.1	1.1
DL-Methionine	0.40	0.40
Taurine	0.35	0.35
Vitamin premixes[3]	0.23	0.22
Mineral premixes[4]	0.10	0.08

[1]Values are shown as percent of the total recipe. All study foods were formulated to meet AAFCO requirements for growing kittens (AAFCO, 2024). Ingredients included at < 1.0% in the test and control recipes are not shown, but included potassium chloride, calcium carbonate, choline chloride, sodium tripolyphosphate (control only), iodized salt, dicalcium phosphate, oat fiber, mixed tocopherols for freshness, natural flavors, magnesium oxide, and beta-carotene.
[2]Fiber blend included dried beet pulp, ground pecan shells, dried citrus pulp, and pressed cranberries.
[3]Vitamin premixes included the following individual vitamin compounds: vitamin E, L-ascorbyl-2-polyphosphate, niacin, thiamine mononitrate, calcium pantothenate, pyridoxine hydrochloride, vitamin A, riboflavin, biotin, vitamin B12, folic acid, and vitamin D3.
[4]Mineral premixes included the following individual mineral compounds: ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, and sodium selenite.
AAFCO, Association of American Feed Control Officials.

attending veterinarian at the start of the prefeed period and at the end of the testing phase (d 150).

Assessments

Fecal samples

Fecal samples were collected during the last week of the prefeed period (baseline), every other week for the first 2 mo of the study, and monthly after the first 2 mo until study completion. Whole feces were collected within 30 min of stool production and were scored on a 1–5 scale (grade 1 indicated stool with no solid form and > 75% liquid and grade 5: meant stool was well-formed, cylindrically shaped and > 80% firm) (Hall et al., 2013; Jewell et al., 2022a, 2022b). If it was nearing the end of the week and a fresh sample had not yet been obtained, an overnight sample was collected instead. Out of 160 stool samples, 25 (15.6%) were collected overnight. Once all litter was removed, the stool sample was placed in a Thinky container (Thinky U.S.A., Inc. Laguna Hills, CA) and homogenized until visually uniform. If any litter could not be removed, the sample was not used for analysis. After homogenization, pH measurements were performed via electrode (Hanna Instruments, Woonsocket, RI). The sample was then aliquoted into labelled tubes for further analysis. For the fecal moisture analysis, the sample was kept at room temperature until moisture assessment and drying was performed, which occurred within 5 h of collection to avoid dehydration. The remaining samples were snap frozen and stored at −70°C. Additional analyses of fecal matter included ammonia, calprotectin, immunoglobulin A (IgA), and SCFA composition. Ammonia in feces was analyzed using the indophenol blue method (Utomo et al., 2022). Fecal calprotectin and IgA were analyzed by MLM Medical Labs, Inc. (Oakdale, MN, United States). Fecal IgA was assessed using the feline IgA enzyme-linked immunosorbent assay quantitation set (Product #ab190547, AbCam; Cambridge, MA, USA) according to the manufacturer's instructions. Fecal calprotectin was assessed using the feline calprotectin enzyme-linked immunosorbent assay kit (Product #MBS094297, MyBioSource; San Diego, CA, USA) according to the manufacturer's instructions. and fecal SCFA composition was analyzed by Metabolon, Inc. (Morrisville, NC, United States) via liquid chromatography with tandem mass spectrometry according to Metabolon method TAM135.

Blood and serum samples

Blood samples were collected on the last day of the prefeed period (baseline) and on d 92 and 148 of the treatment period. Kittens were fasted for at least 12 h prior to collection. Approximately 7 mL was collected at each time point (2 mL into an ethylenediaminetetraacetic acid [EDTA] tube and 5 mL into a serum-separating tube). EDTA tubes were inverted several times and placed in the refrigerator. Samples were shipped on ice to Hill's Pet Nutrition Center (Clinical Lab) for complete blood count (CBC) and serum chemistry analysis upon arrival. Serum separator tubes were allowed to clot for 20 to 30 min before being centrifuged at 3000 rpm for 10 min. Serum was aliquoted into labelled tubes for further analysis. Whole blood was analyzed for CBC immediately after collection (Sysmex XN 1000-V, Sysmex America, Inc., Lincolnshire, IL, United States). Serum chemistry was analyzed within 24 h of collection (Cobas c501, Roche Diagnostics, Indianapolis, IN, United States) and the remainder of the serum sample was then frozen at −70°C until needed for further analysis. Inflammatory cytokines were analyzed using a 19-plex feline cytokine/chemokine immunology multiplex assay according to manufacturer's instructions (MILLIPLEX® Feline Cytokine/Chemokine Magnetic Bead Panel, MilliporeSigma, Burlington, MA, United States). Serum IgA was analyzed by MLM Medical Labs, Inc. (Oakdale, MN, United States) using the feline IgA enzyme-linked immunosorbent assay quantitation set (Product #ab190547, AbCam; Cambridge, MA, USA) according to the manufacturer's instructions.

Statistical analyses

The fecal variables of fecal score, moisture, pH, ammonia, calprotectin, IgA, and SCFAs, and the serum variables of IgA and inflammatory cytokines, were analyzed using linear mixed-model analysis (PROC GLIMMIX in SAS®, version 9.4 [SAS Institute Inc., Cary, NC]), with diet, day, and the interaction as fixed effects in the model. The best variance-covariance to model correlation between the repeated measures was selected using the corrected Akaike Information Criterion fit statistic. The Kenward-Roger adjustment (DDFM=KR) was used to adjust the error degrees of freedom in the F-test and standard error (SE) for the means for the presence of multiple random effects in the model (Kenward and Roger, 1997). The post day 0 timepoints were compared to d 0 within each diet

using the SLICEDIFF = Diet ADJUST = DUNNETT option to control the Type I error rate. The two foods were compared at each time point using SLICEDIFF = Day ADJUST = SIMULATE to control the Type I error rate. Effects were considered significant at $P \leq 0.050$.

Body weight and food intake data were analyzed using a random coefficient model that included both fixed and random intercept and slope estimates. Linear, quadratic, and higher order models were evaluated. For the serum inflammatory cytokines data, any measurements that were not detected, and thus less than the lowest level of detection, were replaced with the value of one half of the lowest level of detection. The data were ln-transformed prior to statistical analysis and analyzed as described above. Data were back-transformed for reporting purposes. Effects were considered significant at $P < 0.05$.

Hypothesis testing was not performed on the safety laboratory measures (serum chemistry and CBC). Rather, summary statistics (number, standard deviation, minimum, median, and maximum) were calculated for each lab parameter at each time point, using PROC MEANS in SAS®, version 9.4 (SAS Institute Inc., Cary, NC).

Results

Animals

A total of 20 kittens were enrolled ($n = 10$ kittens per group) and none were removed prior to study completion. There were no differences between groups with respect to sex, weight, or age ($P > 0.05$). Each group had five neutered males and five females. The mean ages in the control and test groups were 0.75 ± 0.04 yr and 0.73 ± 0.07 yr, respectively ($P = 0.294$). Physical examinations were unremarkable, with no abnormal findings seen in either group, except for one case of stomatitis identified during the end-of-study physical exam.

Food intake and body weight

Caloric intake (kcal/d) increased linearly for both the control group ($P = 0.042$) and test group ($P = 0.003$) (Figure 1). There was no impact of diet or the diet-by-day interaction on caloric intake ($P = 0.544$ and $P = 0.868$, respectively).

Mean body weight at baseline was 3.75 ± 1.01 kg for the control group and 3.77 ± 0.99 kg for the test group ($P = 0.958$). Analysis of body weight showed that both the control and test groups grew throughout study duration, but the linear trend was not significant in either group ($P > 0.05$). There was no impact of diet or the diet-by-day interaction on body weight ($P = 0.995$ and $P = 1.00$, respectively; results not shown).

Fecal variables

Data from fecal endpoints are presented in Table 3. An effect of diet was observed for fecal score, in which kittens fed the test food with the patented fiber blend had a higher fecal score than those fed the control food ($P < 0.001$, Supplemental Fig. 1A). Both diet and day effects were observed for fecal IgA ($P = 0.014$ and $P = 0.020$, respectively), with fecal IgA concentration higher in kittens fed the test food than those fed the control food (Supplemental Fig. 1B). A diet-by-day interaction was observed for fecal pH ($P = 0.002$). While similar between groups at baseline and d 15, fecal pH was lower in kittens fed the test food at all subsequent timepoints compared with those

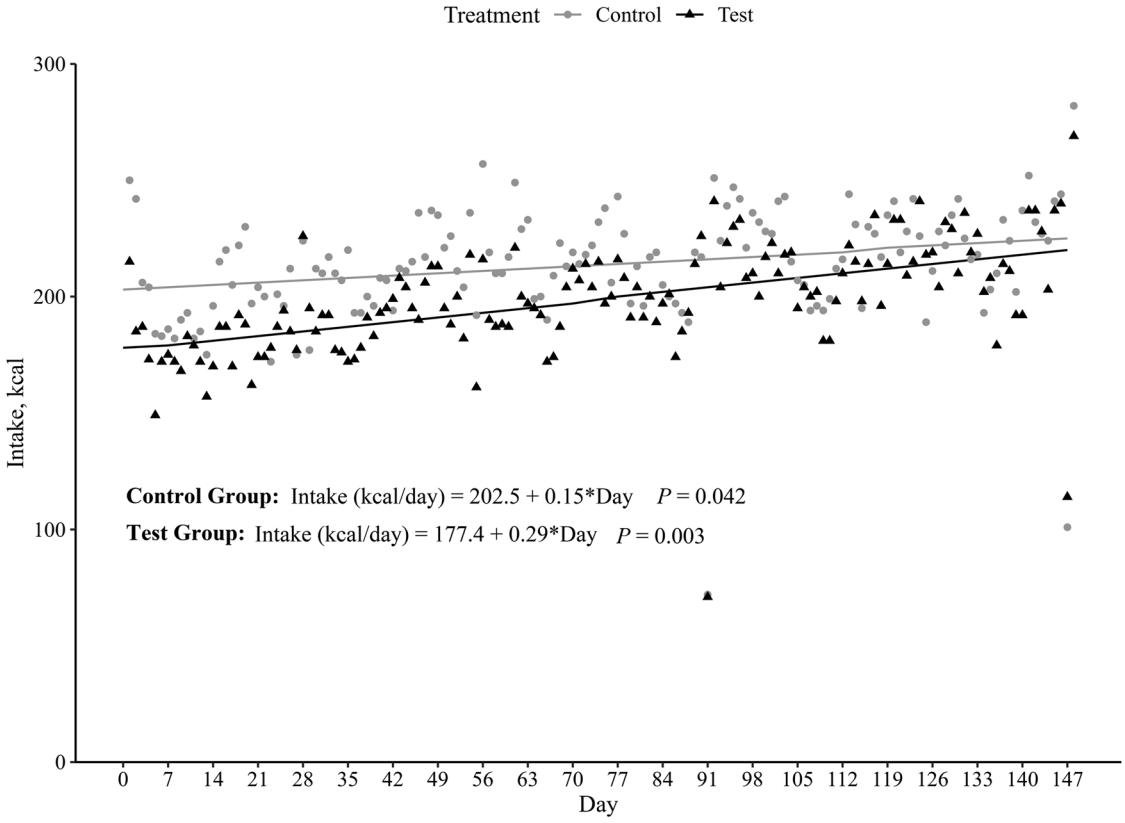


Figure 1. Daily mean food intake in kilocalories throughout the study duration for kittens fed the test food containing the novel fiber blend or control food lacking the novel fiber blend.

Table 3. Effect of diet, day, and the interaction on fecal variables (score, moisture, pH, ammonium, calprotectin, and IgA) in healthy kittens fed the test food containing the novel fiber blend or control food lacking the novel fiber blend.

				P value		
Variable	Control group ($n=10$)	Test group ($n=10$)	SE	Diet	Day	Diet × Day
Score	4.475	4.938	0.073	**<0.001**[a]	0.159	0.282
pH	5.882	5.609	0.045	**<0.001**[a]	**<0.001**[a]	**0.002**[a]
Moisture, %	59.02	62.50	0.91	**0.015**[a]	**0.002**[a]	**0.015**[a]
Ammonium, mmol/g	0.0490	0.0394	0.0042	0.125	0.089	0.073
Calprotectin, ng/g	305	262	14	0.053	0.517	0.794
IgA, mg/g	2.39	3.56	0.30	**0.014**[a]	**0.020**[a]	0.234

Fecal scores were measured on a scale of 1–5, with 1 being extremely loose, watery stool and 5 being optimal stool quality.
[a]Bolded *P* values are significant at $P<0.050$.
IgA, immunoglobulin A; SE, standard error.

Table 4. Effect of diet, day, and the interaction on fecal short chain fatty acids (measured in µg/g) in healthy kittens fed the test food containing the novel fiber blend or control food lacking the novel fiber blend.

		Mean		*P* value		
SCFA	Control group ($n=10$)	Test group ($n=10$)	SE	Diet	Day	Diet × Day
2-Methylbutyric acid	150.7	97.1	5.5	**<0.001**[a]	0.316	0.349
Acetic acid	3478	4267	113	**<0.001**[a]	**0.004**[a]	0.121
Butyric acid	2717	1892	108	**<0.001**[a]	0.895	0.402
Hexanoic acid	408	130	28	**<0.001**[a]	0.984	0.767
Isobutyric acid	192.9	134.7	6.3	**<0.001**[a]	0.224	0.128
Isovaleric acid	218.0	148.4	7.0	**<0.001**[a]	0.368	0.139
Propionic acid	1668	2705	92	**<0.001**[a]	**0.001**[a]	**0.025**[a]
Valeric acid	1624	1366	57	**0.002**[a]	0.900	0.924
Saccharolytic SCFAs (acetic + butyric + propionic acid)	7863	8864	223	**0.002**[a]	**0.012**[a]	0.429
Proteolytic SCFAs (2-methylbutyric + isobutyric + isovaleric acid)	562	380	18	**<0.001**[a]	0.358	0.172

[a]Bolded *P* values are significant at $P<0.050$.
SCFA, short-chain fatty acid; SE, standard error.

fed the control food, for which fecal pH remained similar to baseline throughout the study period (Supplemental Fig. 1C). A diet-by-day interaction was also observed for fecal moisture ($P=0.015$), where moisture was unchanged throughout the study in kittens fed the control food, but was higher than baseline on all days except for d 29 in those fed the test food (Supplemental Fig. 1D).

Fecal SCFA data are presented in Table 4. Both diet and day effects were observed for acetic acid ($P<0.001$ and $P=0.004$, respectively), where the acetic acid concentration was higher for kittens fed the test food than those fed the control food. A diet by day interaction was observed for propionic acid ($P=0.025$), where propionic acid was higher than baseline for each subsequent fecal collection day in the test group, but remained unchanged from baseline in the control group. Dietary treatment affected 2-methylbutyric acid, butyric acid, hexanoic acid, isobutyric acid, isovaleric acid ($P<0.001$ for each), and valeric acid ($P=0.002$), with each of these SCFAs present at a higher concentration in the feces of kittens fed the control food compared with the feces of kittens fed the test food.

There was an effect of diet and day on total saccharolytic SCFA concentration, which included the sum of acetic acid, propionic acid, and butyric acid ($P=0.002$ and $P=0.012$, respectively), where total saccharolytic SCFA concentration was higher for kittens fed the test food compared with those fed the control food (Table 4; Supplemental Fig. 2). There was an effect of diet on total proteolytic SCFA concentration (Supplemental Fig. 3), which included 2-methylbutyric acid, isobutyric acid, and isovaleric acid ($P<0.001$), where lower concentrations of total proteolytic SCFAs were seen in kittens fed the test food compared with those fed the control food.

Blood and serum variables

There was an effect of dietary treatment on stromal cell-derived factor-1 (SDF-1) ($P=0.013$), with the test group having a higher concentration of serum SDF-1 than the control group (Table 5). A day effect was observed for the cytokines Fas, FMS-related tyrosine kinase 3 ligand, platelet-derived growth factor-BB, and stem cell factor ($P<0.05$). In addition, an effect of day was observed for serum IgA concentration ($P<0.001$). All CBC and chemistry parameters were reviewed and deemed clinically normal by the attending veterinarian (Supplemental Tables 1 and 2).

Discussion

Research investigating the effects of prebiotic fiber on the GI health and overall wellbeing of growing animals is limited. As such, the objective of the present study was to evaluate the

Table 5. Effect of diet, day, and the interaction on inflammatory cytokines in serum (pg/mL) in healthy kittens fed the test food containing the novel fiber blend or control food lacking the novel fiber blend.

		Mean ± SE		*P* values		
Cytokine	Test day	Control group ($n=10$)	Test group ($n=10$)	Diet	Day	Diet × Day
Fas	0	49.7 ± 8.1	54.2 ± 8.8	0.812	**0.004**[a]	0.494
	92	38.6 ± 6.3	32.8 ± 5.3			
	148	44.5 ± 7.2	41.7 ± 6.8			
Flt-3L	0	126 ± 15	133 ± 16	0.897	**<0.001**[a]	0.669
	92	140 ± 21	144 ± 22			
	148	154 ± 17	152 ± 16			
GM-CSF	0	45.1 ± 6.3	38.7 ± 6.3	0.886	0.230	0.494
	92	36.5 ± 12.7	50.0 ± 12.7			
	148	38.0 ± 6.4	39.3 ± 6.4			
IFN-γ	0	500 ± 98	492 ± 96	0.900	0.994	0.761
	92	490 ± 96	496 ± 97			
	148	517 ± 101	469 ± 92			
IL-1β	0	152 ± 31	153 ± 31	0.951	0.684	0.665
	92	144 ± 29	152 ± 31			
	148	148 ± 30	132 ± 27			
IL-2	0	72 ± 17	79 ± 19	0.823	0.119	0.670
	92	66 ± 16	75 ± 18			
	148	65 ± 15	64 ± 15			
IL-4	0	829 ± 177	792 ± 169	0.735	0.939	0.449
	92	833 ± 178	822 ± 176			
	148	900 ± 192	714 ± 152			
IL-6	0	476 ± 97	481 ± 98	0.936	0.921	0.776
	92	464 ± 95	508 ± 104			
	148	476 ± 97	459 ± 94			
IL-8	0	67.5 ± 8.3	66.5 ± 8.2	0.810	0.669	0.698
	92	70.5 ± 8.6	70.2 ± 8.6			
	148	73.7 ± 9.0	66.8 ± 8.2			
IL-12(p40)	0	612 ± 121	552 ± 109	0.799	0.109	0.865
	92	652 ± 129	613 ± 121			
	148	665 ± 132	635 ± 126			
IL-13	0	118 ± 13	143 ± 16	0.209	0.721	0.621
	92	111 ± 15	145 ± 20			
	148	113 ± 15	141 ± 19			
IL-18	0	575 ± 134	602 ± 140	0.465	0.794	0.432
	92	523 ± 160	820 ± 250			
	148	519 ± 138	691 ± 183			
KC	0	1.80 ± 0.52	1.78 ± 0.54	0.690	0.653	0.823
	92	1.74 ± 0.53	2.30 ± 0.67			
	148	2.07 ± 0.60	2.35 ± 0.66			
MCP-1	0	6986 ± 639	6983 ± 639	0.838	0.965	0.397
	92	6954 ± 636	6958 ± 636			
	148	7293 ± 667	6751 ± 617			
PDGF-BB	0	7784 ± 979	6054 ± 761	0.378	**0.015**[a]	0.464
	92	5949 ± 748	5313 ± 668			
	148	7469 ± 939	7306 ± 919			
RANTES	0	86 ± 8	103 ± 9	0.391	0.872	0.643
	92	87 ± 13	101 ± 15			
	148	94 ± 11	101 ± 11			
SCF	0	407 ± 47	381 ± 44	0.586	**0.008**[a]	0.484
	92	458 ± 66	435 ± 63			
	148	508 ± 55	433 ± 47			
SDF-1	0	2254 ± 475	3340 ± 703	**0.013**[a]	0.907	0.277
	92	2310 ± 486	3244 ± 683			
	148	1614 ± 340	4011 ± 844			
TNF-α	0	163 ± 52	123 ± 39	0.751	0.805	0.435
	92	121 ± 38	136 ± 44			
	148	159 ± 50	126 ± 40			

[a]Bolded *P*-values are significant at $P<0.050$.
Flt3-L, FMS-related tyrosine kinase 3 ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; KC, keratinocyte chemoattractant; MCP-1, monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; SCF, stem cell factor; SDF-1, stromal cell-derived factor-1; SE, standard error; TNF, tumor necrosis factor.

effects of a patented prebiotic fiber blend, consisting of ground pecan shells, flaxseed, dried beet pulp, dried citrus pulp, and pressed cranberries, on the growth and GI health of kittens.

Kittens fed the test food with the novel fiber blend had great stool quality, and the novel fiber blend resulted in mean fecal scores of 4.9 or above at all timepoints. This is consistent with previous work in adult cats, in which stool scores were favorable when both healthy cats and those with mild GI distress were fed a food containing this patented fiber blend (Wernimont et al., 2019a, 2019b; Fritsch and Gross, 2021; Jewell et al., 2022a). Stool quality is a general marker of GI health; therefore, consistently high stool scores indicate that kittens fed the test food maintained favorable GI health throughout the duration of the study. The interaction of diet and day had an effect on fecal pH, where fecal pH was lower in the test group from d 29 onward. This was consistent with previous findings which showed that adult dogs and cats had a reduced fecal pH after consuming a test food with this fiber blend (Jackson and Jewell, 2019; Wernimont et al., 2019a, 2019b; Fritsch and Gross, 2021). A lowered fecal pH helps to inhibit the growth of pathogenic bacteria and the production of harmful metabolites (Slavin, 2013; Moreno et al., 2022), and is indicative of SCFA production via saccharolytic fermentation by the gut microbiota (Yamamura et al., 2023). Fecal moisture was higher in the test group than the control group for most fecal collection days, though this higher moisture content did not reduce stool quality, as described above. An increase in fecal moisture is consistent with results seen in healthy adult cats fed the same fiber blend (Wernimont et al., 2019a).

The elevated saccharolytic SCFA concentration in the test group compared with the control group throughout the study suggests that kittens fed the test food containing the patented fiber blend experienced more saccharolytic fermentation versus kittens fed the control food. The SCFAs produced by gut microbes have been shown to provide a variety of beneficial effects on host health by supporting intestinal homeostasis, providing fuel for intestinal epithelial cells, and strengthening gut barrier function (Parada Venegas et al., 2019; Lavelle and Sokol, 2020; Gasaly et al., 2021). Moreover, dogs with chronic enteropathy have been shown to have lower fecal SCFA concentrations than healthy dogs (Minamoto et al., 2019), though, interestingly, the opposite was seen in cats with chronic enteropathy, driven largely by a high concentration of butyrate in these diseased cats (Miller et al., 2023). While total fecal SCFAs were higher in the test group compared with the control group in this study, a lower butyric acid concentration was seen in the feces of kittens fed the test food. Butyrate is a major energy source for the cells in the colon (Roediger, 1980), and has been shown to increase with prebiotic fiber consumption in adult cats and dogs (as reviewed in Pilla and Suchodolski 2021). The lower butyric acid concentration seen in the test group compared to the control could be explained by the control food having whole grain oats in the recipe, which were not present in the test food. Oats are rich in beta-glucans and have been linked to elevated fecal butyrate levels in humans and animal models (Hallert et al., 2003; Fabiano et al., 2023), so their inclusion in the control food may have elevated butyrate production by the gut microbiota. To better understand the impact of the patented fiber blend on butyrate production, future studies should use a control food that avoids whole grain oats. Another potential explanation for the lower butyrate seen in the test group is that, as obligate carnivores, the feline gut microbiome may produce and utilize SCFAs differently in response to fiber consumption than the gut microbiome of non-obligate carnivores. The lower concentration of fecal butyrate observed in kittens fed the food containing the fiber blend compared to the control group is consistent with previous findings in adult cats. Specifically, in one study investigating the effects of different inclusion levels of the patented fiber blend, the percent change in fecal butyric acid compared to baseline was lower in adult cats fed the test food that contained a similar inclusion level of the blend, compared to those with a lower percentage, or none, present in the food (Jewell et al. 2022a). Additionally, one study found that cats with chronic enteropathy had a higher proportion of SCFAs in the feces present as butyrate than healthy control cats (Miller et al., 2023). Work investigating the impact of this patented fiber blend on feline gut microbiome composition and microbial metabolism is in progress, and results will be presented in a future publication. To better understand if this result is due to the ingredient profiles of the study foods, or related to a unique response of the feline gut microbiome to fiber supplementation, future studies utilizing different study foods are warranted. Regardless, the elevated fecal concentrations of acetic acid and propionic acid in kittens fed the test food demonstrate that this novel fiber blend encouraged saccharolytic fermentation.

Kittens fed the test food exhibited lower fecal proteolytic SCFA concentrations compared with kittens fed the control food, which is in accordance with findings from studies in adult cats fed this fiber blend (Fritsch and Gross, 2021; Jewell et al., 2022a). Proteolytic SCFAs are products of protein fermentation by gut microbes, and an increase in proteolytic SCFA production is associated with higher levels of harmful compounds, including inflammatory uremic toxins and polyamines, as well as detrimental effects on GI health (Diether and Willing, 2019; Jackson and Jewell, 2019). As a whole, the reduction in total fecal proteolytic SCFAs and the increase in total fecal saccharolytic SCFAs in the test group compared with the control group indicates that the kitten gut microbiota shifted away from proteolytic fermentation towards the more beneficial process of saccharolysis (Jackson and Jewell, 2019).

In serum, IgA is present in a monomeric form, and can be transformed into secretory IgA in the mucosal lumen (Patel and Jialal, 2024). Secretory IgA is abundant in mucosal tissue, particularly the GI tract, and is important for maintaining a healthy GI microbiome composition, supporting the intestinal barrier, and providing anti-inflammatory effects (Yang and Palm, 2020; Patel and Jialal, 2024). Fecal IgA is also a marker of intestinal immune function (Stokes and Waly, 2006). A higher concentration of IgA in the feces, as seen in kittens fed the test food, may indicate that the patented fiber blend supported intestinal barrier function, potentially through microbiota-mediated mechanisms (Wells et al., 2017). Our results are consistent with recent studies in dogs that showed that prebiotics can elevate serum and fecal IgA levels; however, an earlier meta-analysis did not find any effects of prebiotics on serum IgA in dogs. (Patra, 2011; Lee et al., 2022b; Wilson et al., 2024; Zhang et al., 2025b). In cats, previous work has demonstrated that cats fed a food enriched with resistant starch had higher fecal IgA concentrations compared to when they were fed foods containing a blend of prebiotic and probiotic ingredients (Lee et al., 2022c). However, studies investigating the impact of fiber on fecal and serum IgA concentrations in cats are lacking, and more research is needed.

Of the serum inflammatory cytokines analyzed, only SDF-1 was different between the test group and the control group. However, one of the kittens in the test group was reported to have stomatitis during the final physical exam, which is characterized by inflammation of the mucous membranes of the mouth. This event may have resulted in the mean elevated SDF-1 levels reported in the test group since increased SDF-1 has been associated with periodontal disease in humans (Havens et al., 2008). Because SDF-1 was the only cytokine that was elevated in the test group and there have been no other reports about the effects of prebiotics on SDF-1, this finding is unlikely to be clinically relevant.

As described previously, the ingredients contained in the patented fiber blend are known to contain fiber-pound polyphenols, including flavanones, flavonols, and lignans (Jewell et al., 2022a, 2022b). Previous work has demonstrated that healthy cats fed a food containing a similar inclusion level of this patented fiber blend had elevated concentrations of hesperidin, hesperetin, ponciretin, secoisolariciresinol diglucoside, secoisolariciresinol, and enterodiol in the feces after 10 d (Jewell et al., 2022a). Similarly, healthy dogs fed this patented fiber blend had elevated fecal concentrations of hesperetin, hesperidin, ponciretin, secoisolariciresinol diglucoside, secoisolariciresinol, and enterodiol after 10 d (Jewell et al., 2022b). Evaluation of the impact of this prebiotic fiber blend on kitten fecal polyphenol concentrations is in progress, and results will be presented in a future publication.

One limitation of this study is that the kittens were nearing the end of their growth phase, and newly weaned kittens were not included in the study population. An older kitten population was utilized due to the consideration that a more stable gut microbiome composition would be present compared with younger kittens, and therefore it would be easier to detect the effect of the patented fiber blend on GI health. Kittens are typically weaned when they are 8–10 wk old, and the period immediately post-weaning is characterized by major changes to the gut microbiota (Zhang et al., 2025a). Using older kittens ensured that animals would be well past this period of adjustment by the gut microbiome. However, future work investigating the potential impact of this patented fiber blend in young, freshly weaned kittens is warranted. Additionally, some kittens in the study were littermates, and were not stratified across treatment groups. Because of this, the study did not control for a potential genetic influence on kittens' responses to our test food. Another limitation of this study is that, in order to keep the macronutrient composition between the control and test foods as similar as possible, there were several differences between the ingredient profiles of the study foods. For example, whole grain oats and cracked pearled barley, which have been shown to work with the gut microbiota and exhibit beneficial effects on GI and overall health in humans, were included in the control food but not the test food (de Godoy et al., 2013; Tosh and Bordenave, 2020; Fabiano et al., 2023). This may have contributed to the elevated butyrate seen in the control group, as described previously. Further examination of this fiber blend in foods with different ingredient and nutrient compositions, including studies with fewer ingredient differences between study foods, are warranted to gain a more comprehensive understanding of how this fiber blend impacts growing cats.

Conclusions

Study findings reveal that kittens fed a food formulated for growing kittens and fortified with a patented prebiotic fiber blend continued to grow and develop normally and exhibited stool characteristics indicative of good GI health, including favorable stool quality, lower fecal pH from d 29 onward, elevated production of saccharolytic SCFAs, reduction in proteolytic SCFAs, and higher fecal IgA, compared with kittens fed the control food. The beneficial impacts of the patented fiber blend were similar to those previously seen in growing puppies and adult cats and dogs. Thus, this patented fiber blend containing soluble and insoluble fibers and fiber-bound polyphenols supports normal growth and favorable GI health, and a gut environment supportive of fiber fermentation in kittens, and contributes to our overall understanding of prebiotics in companion animals.

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Author Contributions

Allison McGrath provided data curation, project supervision, and interpretation of the data. Elizabeth Morris provided project supervision and interpretation of the data. Michael Faurot provided project supervision and interpretation of the data. Cheryl Stiers assisted in the study design and execution. John Brejda provided statistical analysis of the data. All authors reviewed and provided revisions and approval of the published version of the manuscript.

Supplementary Data

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

Conflict of interest statement. Allison McGrath, Michael Faurot, Cheryl Stiers, and Elizabeth Morris, and are employees of Hill's Pet Nutrition, Inc.; John Brejda is a subcontractor of Hill's Pet Nutrition, Inc.

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Data Availability Statement

The datasets analyzed for this study can be made available upon request.

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