MISCANTHUS GRASS AS A NOVEL FUNCTIONAL FIBER SOURCE IN EXTRUDED FELINE DIETS

BY

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THESIS

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ABSTRACT

Over the past few decades, there has been a growing interest in dietary fiber as an important nutrient in both human and animal nutrition. The driving force behind this growing interest has largely been the population's desire to consume "functional foods" to support health and aid in disease prevention. With the increasing humanization of pets, consumers have focused on pet health and wellness, as affected by nutrition. Consumers often have a desire to provide their animals with pet food products that are not only complete and balanced, but also reflect their personal nutritional interests and philosophy. A wider range of products and ingredients is needed in order to supply these nutritional benefits and meet the growing expectations of pet parents. The objective of this research was to evaluate the novel dietary fiber source, miscanthus grass, in comparison to traditional dietary fiber sources, and their effects on parameters related to gastrointestinal health, nutrient apparent total tract digestibility (**ATTD**) and fecal characteristics of adult cats. Four dietary treatments were evaluated (n = 7 cats/treatment), differing only in dietary fiber source. The diets were formulated to meet or exceed the AAFCO (2018) nutrient profiles of adult cats and contained either 7% cellulose (**CO**), 9% miscanthus grass fiber (**MF**), a blend of 7% miscanthus grass fiber plus 2% tomato pomace (**MF+TP**), or 11% beet pulp (**BP**) to achieve a target total dietary fiber (**TDF**) content of 15% in each treatment. The study was a completely randomized design using twenty-eight neutered adult, domesticated shorthair cats (19 females and 9 males, mean age 2.21 ± 0.03 yr; mean BW 4.58 ± 0.7 kg, mean body condition score 5.6 ± 0.6). Cats were randomly assigned to one of the four dietary treatments and were fed twice a day to maintain body weight for an experimental period of 21 d. On the last 4 d of the experimental period, a fresh fecal and total fecal collection were performed. A fresh fecal sample was collected for each cat within 15 min of defecation and was used to evaluate fecal dry matter

(**DM**) content, fecal score, pH, and fermentative end-product concentrations. A fasted blood sample was collected at baseline and at the end of the 21-d period. Serum chemistry and complete blood count were analyzed to verify the health status of all animals. Data were analyzed using SAS version 9.4 with the mixed model procedure. The treatments were well accepted by the cats, and daily food intake (DM basis) was similar across all groups ($P > 0.05$). Additionally, treatment did not have an effect on fecal output (as-is or DM basis), fecal score, or fecal pH ($P > 0.05$). All diets had nutrient digestibility coefficients close to or above 80%, indicating that they were well digested by the animals. The ATTD of DM (78.3-82.7%), organic matter (**OM**) (81.8-86.3%), and crude protein (**CP**) (83.1-84.6%), were similar for all treatments ($P > 0.05$). However, ATTD of acid hydrolyzed fat was higher for the CO group (94.5%) when compared with the MF (91.7%) and MF+TP (91.2%) groups ($P < 0.05$), with BP (92.6%) being intermediate. Additionally, the BP treatment had significantly higher TDF digestibility (54.2%) in contrast with all other treatments (MF=19.1%, MF+TP=25.5%, CO=21.8%) ($P < 0.05$). Digestible energy (**DE**) of the CO diet (3.9 kcal/g) was higher than for the MF+TP diet (3.7 kcal/g) ($P < 0.05$), while MF and BP diets were similar to all treatments. While there was no difference ($P > 0.05$) in fecal ammonia and phenol concentrations among groups, fecal indole and total phenol and indole concentrations were highest for the MF and MF+TP groups compared with CO and BP ($P < 0.05$). Cats fed BP had the highest fecal concentrations of total short-chain fatty acids (**SCFA**), acetate, and propionate ($P < 0.05$), while butyrate concentrations were similar among all treatments ($P > 0.05$). Total branched-chain fatty acids (**BCFA**), isobutyrate, and isovalerate concentrations were higher for the MF+TP group than for the CO and BP groups ($P < 0.05$), with the MF group being intermediate. A similar trend for valerate concentration was observed with the MF+TP treatment being higher than the BP treatment ($P <$

0.05), with CO and MF treatments being intermediate. Cats fed BP differed in β-diversity compared with cats fed CO, MF, and MF+TP. However, α-diversity was greater for cats fed MF and MF+TP in contrast with cats fed BP. As no adverse effects on health, fecal score, or macronutrient ATTD were observed with the inclusion of 9% miscanthus grass fiber, or a miscanthus grass blend, the data suggest that it is a viable alternative to the traditional sources of dietary fiber, being most comparable to cellulose.

Key words**:** cats, dietary fiber, fecal microbiota, miscanthus grass, nutrient digestibility, postbiotics

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CHAPTER 1: INTRODUCTION

The number of pet owning households has increased steadily in the United States over the past years. In the 2019-2020 National Pet Owners Survey conducted by the American Pet Products Association, it was reported that 67% of households own a pet, corresponding to almost 85 million homes (APPA, 2020). This number has increased over 10% since the first survey was conducted in 1988. Of these homes, 42.7 million own at least one cat. With an increase in pet ownership comes an increase in spending on corresponding products and services within the pet industry. The total industry expenditure for 2020 is estimated to be around $99 billion. Veterinary services and related products sales were a large portion of this expenditure in 2019 ($23.9 billion). However, pet food and treats comprise the majority of the market share with $36.9 billion spent on these products in 2019 (APPA, 2020).

As consumers increase their knowledge and understanding of how nutrition plays an integral role in preventing disease and maintaining their own health, they begin to make the same associations for their pets and the nutritional products that they consume (Schleicher et al., 2019). Following this trend, recent interest in the mechanisms of dietary fiber action as they pertain to human nutrition and their role in helping to mitigate gastrointestinal diseases, diabetes, and other health problems has led to an increased interest in companion animal nutrition as well (Anderson et al., 2009). The most commonly used sources of dietary fiber for companion animals are cellulose and beet pulp (de Godoy et al., 2013). However, fiber sources can vary greatly in their composition and consistency, as well as the physiological benefits that they provide. Multiple fiber sources with diverse physico-chemical and physiological properties are likely necessary to obtain the desired optimal health benefits; novel fiber ingredients may assist in achieving this goal.

Limited data are available evaluating the use of miscanthus grass as a novel dietary fiber ingredient in companion animal diets, especially for felines. The aim of this research was to evaluate the effects of miscanthus grass and novel fiber blends on the gastrointestinal health, macronutrient digestibility, and fecal characteristics of adult cats in comparison to traditionally used dietary fiber sources, cellulose and beet pulp.

LITERATURE CITED

American Pet Products Association. 2020. APPA National Pet Owners Survey: Pet Industry Market Size & Ownership Statistics. 2019-2020 Survey. http://www.americanpetproducts.org/press_industrytrends.asp. Accessed 5 October 2020.

Anderson, J. W., P. Baird, R. H. Davis, S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205. doi:10.1111/j.1753-4887.2009.00189.x.

de Godoy, M. R. C., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients. 5:3099-3117. doi:10.3390/nu5083099.

Schleicher, M., S. B. Cash, and L. M. Freeman. 2019. Determinants of pet food purchasing decisions. Can. Vet. J. 60:644-650.

CHAPTER 2: LITERATURE REVIEW

DEFINITION AND CLASSIFICATION OF DIETARY FIBER

The United States Food and Drug Administration (**FDA**) has defined "dietary fiber" as "non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates determined by the FDA to have physiological effects that are beneficial to human health", as defined in the Nutrition and Supplemental Facts label final rule in 2016 (FDA, 2020). Fibers that are naturally occurring in fruits, grains, and other foods are considered "intrinsic and intact" if they have not been removed from their natural source. Isolated and synthetic non-digestible carbohydrates that have been identified and approved by the FDA in the definition of dietary fiber include beta-glucan soluble fiber, psyllium husk, cellulose, guar gum, pectin, locust bean gum, hydroxypropylmethylcellulose, mixed plant cell wall fibers, arabinoxylan, alginate, inulin/inulin-type fructans, high amylose starch, galactooligosaccharide, polydextrose, resistant maltodextrin/dextrin, cross linked phosphorylated resistant starch 4, and glucomannan (FDA, 2020). A benefit to human health must be demonstrated for consideration as a dietary fiber. According to the FDA, this can include lowering blood glucose and (or) lowering cholesterol concentrations, lowering blood pressure, increasing frequency of bowel movements, increasing mineral absorption in the intestinal tract, and reducing energy intake.

Several strategies exist for the classification of dietary fiber. These include classification based on chemical properties (i.e., monosaccharide composition, bonding, polymerization), nutritional properties (i.e., fermentability, solubility, viscosity), physiological functions (i.e, prebiotic fibers), and plant functions (i.e., structural, cell wall component) (Tungland and Meyer, 2002; Dai and Chau, 2016). Chemical analysis can be used to quantify dietary fiber in a given

ingredient or feed using the properties of solubility to isolate different fiber fractions. Components of the soluble fiber fractions include pectins, gums, beta-glucans, mucilages and oligosaccharides, while insoluble components include cellulose, hemicelluloses, and lignin (Fahey et al., 2019). The three most common methods of fiber analyses used in the United States are crude fiber, detergent fiber, and total dietary fiber (**TDF**) (Fahey et al., 2019). Crude fiber analysis results in the quantification of cellulose in addition to incomplete parts of the lignin and hemicelluloses. Detergent fiber analyses result in the quantification of cellulose, lignin, and insoluble hemicelluloses. This classification can be further subdivided into acid detergent fiber that quantifies cellulose and lignin, and neutral detergent fiber that quantifies cellulose, lignin, and hemicelluloses. Analysis of TDF includes lignin and all non-starch polysaccharides, including cellulose, soluble hemicelluloses, and insoluble hemicelluloses. The current method for measuring and reporting dietary fiber for animal feeds is crude fiber. However, the validity of this measurement has been questioned as many of the hemicelluloses, phenolic compounds, and lignin are lost in this method of analysis, leading to a value that underestimates true fiber content by 30-50% (Fahey et al., 2019).

HEALTH BENEFITS AND PHYSIOLOGICAL EFFECTS

Although dietary fiber is not a nutritional requirement for cats, a growing interest has been observed in the addition of fiber to feline diets (National Research Council, 2006). This mirrors the growing interest that also has been observed in human nutrition, as many health benefits have been associated with fiber intake. In humans, dietary fiber has been shown to improve metabolic imbalances by lowering blood cholesterol concentration, improving glycemic response, and reducing the risk of type-2 diabetes mellitus and cardiovascular disease (Anderson

et al., 2000; Ziai et al., 2005; Sierra et al., 2002; Bazzano et al., 2003). It also has been shown to support gastrointestinal health by promoting laxation, reducing the risk of colon cancer, and mitigating diseases such as irritable bowel syndrome (Anderson et al., 2009; Chen and Vitetta, 2018; El-Sahey et al., 2017). With the increase in the humanization of household pets, consumers have developed an interest in providing their animals with diets that also provide these health benefits. In pets, dietary fiber was shown to help control glycemia and maintain glucose homeostasis, promote laxation, support gastrointestinal health, and potentially improve immune function (Nelson et al., 2000; Massimino et al., 1998; Bueno et al., 2000b; Zoran, 2008; Swanson et al., 2002). In cats, dietary fiber also has been shown to decrease the unwanted symptoms of hairballs (i.e., retching, vomiting, coughing), reduce trichobezoar formation, and facilitate excretion of hair in the feces (Benyen et al., 2011; Loureiro et al., 2014; Weber et al., 2015). Additionally, formulations including higher amounts of dietary fiber have been used as a tool to help combat the growing problem of pet obesity. Dietary fiber helps to achieve this by diluting caloric density of pet foods, and enhancing satiety (German, 2006).

Many of these health benefits can be attributed to the mechanisms of dietary fiber in the gastrointestinal tract. Insoluble dietary fiber and soluble dietary fiber help to increase fecal bulk, which helps to decrease intestinal transit time and maintain regular bowel movements (Dai and Chau, 2016). Because most insoluble dietary fiber is not fermented, these fibers are present in the feces. The fibers pull water into the fiber matrix leading to an increase in stool mass. Although soluble dietary fiber may be fermented and not present in the feces itself, the byproducts that are produced as a result of fermentation (i.e., short-chain fatty acids (**SCFA**) and gas) and increased numbers of fecal microbiota can add to fecal bulk (Dai and Chau, 2016). In addition to bulk, the viscosity of the fiber can increase the thickness of digesta passing through the small intestine,

causing certain nutrients to have reduced rates of absorption. This can help to blunt postprandial glycemic response by slowing glucose absorption. Cholesterol concentrations also may be reduced by the same mechanism (Dai and Chau, 2016). Additionally, fermentable fibers promote overall gastrointestinal health by acting as a fuel source for beneficial bacteria in the gut. These bacteria use the process of fiber fermentation to obtain energy from a substrate that would otherwise go undigested and unused by the host. Through this process, microbiota produce SCFA, which can be absorbed and used to maintain the health and function of intestinal cells (Dai and Chau, 2016).

It is important to acknowledge that there is a large variety of dietary fibers, and their physiological functions depend on many factors such as composition and physicochemical properties. Blending a variety of dietary fiber types and sources can help achieve specific physiological functions and health benefits when incorporated in pet food products.

TRADITIONAL FIBER SOURCES IN FELINE DIETS

Historically, cellulose and beet pulp have been the most commonly utilized and researched dietary fiber sources in companion animal diets. Cellulose is mainly comprised of insoluble, non-viscous fiber. The composition of microcrystalline cellulose varies little. In the literature, reported ranges of TDF have been recorded from 91.6- 99.9%, with 92.0- 97.0% being insoluble dietary fiber (Godoy et al., 2013). Commercial sources of cellulose such as wood cellulose are also common, but may contain an amorphous structure and include other substances, potentially leading to more compositional variability. Beet pulp is comprised of both soluble and insoluble fibers, as well as viscous and non-viscous portions. The composition of beet pulp is typically less consistent than cellulose, including varying quantities of pectin,

cellulose, and hemicelluloses. Reported TDF values vary from 57.0- 82.6%, and the insoluble fiber to soluble fiber ratio varies from 1.9:1 to 5.3:1 (Godoy et al., 2013).

Sunvold et al. (1995a) used an in vitro assay to evaluate the fermentability of various fiber sources using feline fecal inoculum. Citrus pectin, guar gum, locust bean gum, Solka Floc (cellulose), beet pulp, rice bran, xanthan gum, gum karaya, gum talha, carob bean gum, and gum arabic were tested as common or potential sources of dietary fiber in feline diets. These fibers were fermented for 24 h, and organic matter disappearance as well as total SCFA production were greatest ($P < 0.05$) for citrus pectin, guar gum, and locust bean gum, indicating the highest fermentability. As expected, Solka Floc, a purified cellulose, had the lowest organic matter disappearance as well as total SCFA production ($P < 0.05$), indicating low fermentability. Sunvold et al. (1995a) then tested how the addition of fibers varying in fermentability affected stool quality and digestibility of macronutrients when added to feline diets. The control diet with no supplemental fiber source (1.7% TDF) was reported to have the highest dry matter **(DM**) and organic matter (**OM**) apparent total tract digestibility (**ATTD**) (88.0% and 91.6%, respectively), while the diet supplemented with the highly fermentable fibers (3.91% citrus pectin, 3.34% locust bean gum, 2.22% carob bean gum, 1.66% guar gum) had the lowest DM and OM ATTD (61.3% and 63.7%, respectively) in addition to the lowest protein and lipid ATTD (59.0% and 39.6%, respectively). Cats eating the diet supplemented with highly-fermentable fibers had high fecal scores that indicated soft and poor-quality stools. Sunvold et al. (1995a) concluded that moderately fermentable fibers may be a more ideal choice in feline diets to provide beneficial fermentative end-products without compromising nutrient digestibility or fecal characteristics.

Bueno et al. (2000a,b) also evaluated various fiber sources in feline diets. Four extruded test diets were used in two studies including cellulose as a low-fermentable fiber source (8.8%

TDF), beet pulp as a moderately-fermentable fiber source (8.4% TDF), pectin/gum arabic as a highly-fermentable fiber source (8.6% TDF), and a control diet with no additional fiber source (3.0% TDF). Twenty-eight female cats received one of the four treatment diets for a minimum of 15 d for diet adaptation. Following this period, a test solution was perfused through the large intestine of each cat, followed by the removal of the gastrointestinal tract. The colon was then separated and tissue samples were collected for analysis. It was observed that cats fed the diet including cellulose had a significantly higher colonic weight/kg of body weight when compared with the no-fiber control diet ($P < 0.05$). It was hypothesized that the increased colonic weight was associated with the tactile response and the abrasive action of the non-fermentable fibers present in the gut (Bueno et al., 2000b). The energetics of the colon mucosa were measured by tissue oxygen consumption when placed in a glucose media substrate. Tissue energetics per gram of colonic tissue were highest in the cats fed the highly fermentable fiber diet and lowest for the no-fiber control group ($P < 0.05$), indicating that increasing fermentable fiber can promote gut mucosa metabolism and induce proliferation. However, cats fed the highly fermentable pectin/gum arabic diet experienced significant weight loss over the 15 d diet adaptation period and decreased food and water intake compared with the other treatment groups ($P < 0.05$) (Bueno et al., 2000b). Additionally, the pectin/gum arabic group had significantly lower colonic fluid recovery ($P < 0.05$) resulting in poor stool quality and odor (Bueno et al., 2000a). When evaluating short-chain fatty acid flux in the colon, it was observed that acetate and butyrate absorption was highest for the cats fed the beet pulp diet, while cellulose had the lowest, and pectin/gum arabic and the no-fiber control group were intermediate. The absorption of propionate did not differ significantly among treatments ($P > 0.05$) (Bueno et al., 2000a). These findings by Bueno et al. (2000a,b) also indicated that moderately fermentable fibers, such as beet

pulp, may be the most beneficial in feline diets as they can provide the tactile advantages of non-fermentable fiber as well as the advantages of fermentable fibers with minimal drawbacks.

Another study done by Barry et al. (2010) evaluated three fiber sources, cellulose, fructooligosaccharide (**FOS**), and pectin, and their effect on macronutrient digestibility, fecal characteristics, fermentative end products, and fecal microbiota. The fiber sources were added to the base diet at an inclusion level of 4%. Analysis of the diets indicated that the nutrient composition was similar among treatments with the exception of TDF content. The cellulose diet had a TDF concentration of 7.9%, whereas TDF concentration of pectin and FOS diets were 6.7% and 3.6%, respectively. However, the lower TDF content of the FOS diet was due to the lack of quantification of fructans in the TDF method. A TDF concentration of 7.6% in the FOS diet was calculated. This value was derived by accounting for the 3.6% TDF content intrinsically present in the base formula in addition to the 4% FOS supplementation. In terms of ATTD, no difference was reported in DM or OM digestibility across treatments ($P > 0.05$). Crude protein (**CP**) ATTD was highest for the cellulose group (90.5%) with pectin having the lowest (87.4%), and FOS being intermediate (88.0%). Acid hydrolyzed fat (**AHF**) digestibility was significantly higher for cats fed cellulose (95.8%) and FOS (95.3%) than pectin (92.5%) ($P < 0.001$). Cats fed different treatments did not differ in terms of fecal pH, fecal DM, or fecal output on an as-is or DM basis. A significant difference was reported in fecal score with FOS (2.8) and pectin (2.7) resulting in higher scores than cellulose (2.0) on a 5-point scale with 1 being hard, dry pellets, and 5 being watery liquid that can be poured ($P < 0.001$). However, the fecal scores of all treatments fell within an ideal range of 2.0 to 3.0. Several fecal protein catabolites were evaluated, and were generally observed in higher concentrations in the pectin and FOS treatment groups. Fecal ammonia, 4-methyl phenol, and branched-chain fatty acid (**BCFA**) concentrations

were reported to be significantly higher in the cats fed the FOS and pectin diets ($P < 0.05$). Fecal indole concentrations were higher in the FOS group (2.4 μmol/g of fecal DM) when compared with the cellulose group (1.4 μmol/g of fecal DM) with the pectin group being intermediate (2.1 μmol/g of fecal DM). The cats fed the pectin treatment also had the highest total fecal biogenic amine concentrations (115.6 μmol/g of fecal DM) followed by FOS (76.1 μmol/g of fecal DM) and cellulose treatments (20.7 μmol/g of fecal DM) ($P < 0.001$). While increases in some of these protein catabolites are not desirable due to odor, or association with negative health outcomes, the pectin treatment group, and to a lesser extent the FOS treatment group also were associated with an increase in carbohydrate-associated fermentative end-products that promote gut health. The cats fed pectin and FOS had higher butyrate concentrations than cats fed cellulose (94.3 μmol, 97.3 μmol, and 39.2 μmol/g fecal DM, respectively) ($P < 0.05$), and pectin had the highest propionate concentrations (109.0 μmol/g fecal DM). Concentrations of acetate and total SCFA also were reported to be highest in pectin followed by FOS and then cellulose. This study indicates that lower inclusion levels (4%) of readily fermentable dietary fibers (i.e., pectin, FOS) can be well utilized in commercial diets. Lower levels of fermentable fibers evaluated by Barry et al. (2010) did not produce the negative effects (i.e., poor stool quality and nutrient digestibility) observed with higher inclusion levels in the studies Sunvold et al. (1995a) and Bueno et al. (2000a,b), while still providing benefits to gut health by increasing SCFA production. Reducing the inclusion of highly fermentable fiber to 1-2% may be recommended in order to reduce the effects of undesirable putrefactive compounds.

Butowski et al. (2019) reported similar results in cats when comparing a high protein (74.4% DM basis) and high fat (19.0%) raw meat diet (1.3% TDF) to a raw meat diet with a 4% fiber inclusion (2% cellulose and 2% inulin (66.6% CP, 15.4% crude fat, 12.9% TDF) and

commercially available kibble diet (41.5% CP, and 16.1% crude fat). Macronutrient ATTD was higher for the raw diet when compared with the raw + fiber diet. However, all digestibility values for these diets were above 90%, indicating that both diets were well digested. Fecal output (g/d) on an as-is basis was similar for the cats fed the raw diet and the raw plus fiber diet. However, the addition of fiber led to a higher fecal DM output for the cats fed the raw plus fiber diet (8.1 g/d) than the raw diet (4.4 g/d) ($P < 0.05$). Fecal score was evaluated on a scale of 1 to 5, with 1 being hard, dry feces and 5 being watery diarrhea, and was observed to be significantly higher for cats fed the raw plus fiber diet (3.5) compared with cats fed the raw diet (1.8) ($P < 0.05$). Fecal pH was numerically lower for the cats fed the raw plus fiber diet (7.0) than the raw diet (7.6); however, the difference was not significant between these treatments ($P > 0.05$). Similarly, fecal total SCFA concentration was numerically higher for the raw plus fiber diet group (364.1 μmol/g of fecal DM) than the raw diet group (296.2 μmol/g), but the difference was not significant ($P > 0.05$). In this study, low levels of fiber inclusion (4%) were sufficient to impact fecal qualities such as DM and fecal score. However, the differences in SCFA concentration and fecal pH were not significantly affected.

In addition to cellulose and beet pulp, fruit fibers also have been often used in companion animal nutrition. These ingredients are generally well accepted by pet owners and have a positive tag appeal in pet food products. Fruit fibers are broadly characterized by higher concentrations of pectin and hemicelluloses, and good water-binding properties in contrast with cellulose (Fischer, 2009). Despite increased interest and use of fruits and fruit fibers in pet food products, literature on this topic is sparse. Only a few studies have evaluated the chemical composition and fermentative characteristics of these ingredients (Sunvold et al., 1995 a-d; Swanson et al., 2001).

Most of these studies evaluated fruit pomaces (i.e., apple, grape, tomato), which are byproducts of juice and puree manufacturing for human consumption.

Total dietary fiber concentration among different fruit pomaces varies from 79% for apple to as low as 57 and 55% for tomato and grape, respectively. Similarly, the ratios of insoluble:soluble fiber also differ among them, being higher for tomato and grape (13:1 and 11:1) and lower for apple pomace (6:1). Because of the greater concentration of soluble fiber, apple pomace has been reported to result in greater SCFA production after 24 h of in vitro fermentation using canine inoculum in contrast with grape pomace (2.1 and 0.83 mmol/g, respectively) (Swanson et al., 2001). In vivo studies evaluating the effects of these dietary fiber ingredients on nutrient digestibility, fecal quality, metabolites and microbiota are lacking, especially in felines. Fekete et al. (2001) evaluated graded levels of apple pomace (0, 10, 20, and 40%) in meat-based diets for adult cats. Inclusion up to 20% of this ingredient did not affect diet palatability, but reduced nutrient digestibility.

Among the fruit pomaces, tomato pomace is, arguably, the most common ingredient used in pet food formulations. This ingredient is mostly used as a fiber source, but it also contains lycopene and beta-carotene, which are antioxidants, making it an attractive ingredient for the pet food industry. In complete and balanced diets, tomato pomace often is used in combination with other fiber containing-ingredients including pulse seeds and fractions (e.g., pea, pea fiber, lentils), cereal grains (e.g., barley and oats), and insoluble fiber sources (e.g., cellulose and miscanthus grass). However, evaluation of tomato pomace and fiber blends containing this ingredient are lacking in companion animal nutrition literature.

MISCANTHUS GRASS AS A NOVEL DIETARY FIBER IN FELINE DIETS

Miscanthus x giganteus is a C4 perennial grass that grows well in temperate climates and produces large quantities of biomass, reaching heights of up to 4 m in a single growing season and producing up to 40 tons of DM per hectare (Babich et al., 2019). Originally cultivated to provide cellulosic biomass for biofuel production, the interest in *Miscanthus x giganteus* has since spread to other industries for uses such as bedding material for production animals, paper products, and phytomanagement of polluted soils (Van Weyenberg et al., 2015; Danielewicz and Suma-Slusarska, 2019; Pidlisnyuk et al., 2019). This grass is a hybrid of *Miscanthus sacchariflorus* and *Miscanthus sinensis* that has triploid chromosomes and, therefore, produces sterile seeds. Because of this, the risk of invasive spread of this grass is very low, but propagation must be done by separating and cultivating the rhizomes of established plants (Babich et al., 2019). Besides its high productivity, environmental and ecological benefits are large incentives for industries and producers interested in this crop. Possibly one of the most advantageous characteristics of this grass is its ability to grow on marginal land that may not be suitable for other crop growth. Miscanthus grass makes efficient use of nitrogen, making the use of fertilizer unnecessary after the rhizomes are matured, as well as efficient water use, requiring minimal agricultural inputs while yielding high outputs. Additionally, no tilling is needed after initial planting, reducing the greenhouse gases associated with production (Shepherd et al., 2020). Further ecological advantages include improving soil structure, increasing water-holding capacity, and promoting earthworm species diversity and abundance while reducing field run-off, erosion, and chemical leaching (McCalmont et al., 2015).

Several studies have evaluated the lignocellulosic composition of this grass in the interest of using it in the production of biofuel. Bauer and Ibanez (2014) used two different methods of

analysis to determine the cellulose content of miscanthus grass and reported the extracted biomass to be composed of 41.9-44.7% cellulose on a dry weight basis. Babich et al. (2019) reported biomass composition as 50.34- 52.13% cellulose, 24.83- 25.76% hemicelluloses, and 12.02- 12.58% lignin. Low ash composition also was noted (2.7% DM basis), and mineral composition included varying concentrations of potassium, chloride, nitrogen, and sulfur depending on soil composition and plant maturity at harvest. Naturally occurring xylooligosaccharides (**XOS**) can also be found in miscanthus grass. A study completed by Chen et al. (2014) evaluated methods of extracting XOS from the hemicellulose portion of the miscanthus grass biomass using autohydrolysis, and researchers were able to obtain XOS yields of up to 13.5% of the initial biomass. This finding is especially significant for the nutritional use of *Miscanthus x giganteus* as XOS may provide a prebiotic-like effect, but more research is needed in this area to confirm (Li et at., 2015).

The interest in utilizing *Miscanthus x giganteus* for nutritional purposes is relatively new, and the current research is limited, especially in feline nutrition. The use of this ingredient in companion animal diets would be to provide a source of novel dietary fiber, primarily comprised of insoluble fibers. This ingredient can be used in formulations of complete and balanced diets, treats and snacks, but also as a carrier in mineral premixes and for diets making natural, non-GMO, and grain-free claims. Donadelli and Aldrich (2019, 2020) evaluated miscanthus grass as a fiber source in canine and feline diets and compared it to traditionally used fiber sources, beet pulp and cellulose. The fiber fractions of the dietary fiber sources were evaluated in both trials and the miscanthus grass was reported to consist of 95% DM, 47.6% crude fiber, 56.5% acid detergent fiber, 77.7% neutral detergent fiber, and 13.7% acid detergent lignin on a DM basis. Total dietary fiber composition (DM basis) of the miscanthus grass (90.0%) was reported to be

lower than that of cellulose (102.6%), and higher than beet pulp (62.4%). The same trend was noted for insoluble fiber with miscanthus grass being intermediate (82.7%), cellulose being the highest (100.0%), and beet pulp being the lowest (36.0%). As expected, soluble fiber followed an inverse relationship with miscanthus grass having intermediate concentration (7.3%), cellulose the lowest (2.6%), and beet pulp the highest (26.4%). In a 14 d feeding trial completed by Donadelli and Aldrich (2019) using 12 beagles, no adverse health effects were reported in dogs eating a complete and balanced dry extruded diet with a 10% inclusion rate of the miscanthus grass (20.0% TDF diet). When compared with the other treatments with 10% inclusion rates of cellulose (20.5% TDF diet) and beet pulp (17.6% TDF diet), it was reported that the effects of miscanthus grass on nutrient digestibility and fecal quality were most similar to the effects of the cellulose treatment group. Dogs in the cellulose and miscanthus grass treatment groups had higher CP and crude fat ATTD when compared with the beet pulp group in addition to firmer feces. In terms of TDF ATTD, beet pulp was the highest (63.0%), miscanthus grass was intermediate (46.1%), and cellulose had the lowest (37.5%) ($P < 0.0001$). These findings indicate that miscanthus grass could be comparable to cellulose as a novel dietary fiber ingredient in dry extruded canine diets.

A feline feeding trial was completed by Donadelli and Aldrich (2020) using the same treatments as in the canine trial. The fiber sources were included in a complete and balanced extruded feline diet at a rate of 10%, resulting in a miscanthus grass treatment diet with 13.8% TDF, cellulose treatment diet with 14.5% TDF, and a beet pulp treatment diet with 10.9% TDF. Twelve American shorthair cats participated in the feeding trial in a replicated 3x3 Latin Square design. Each period consisted of 9 d of diet adaptation, followed by 5 d of total fecal collection. Apparent total tract digestibility of macronutrients and fecal quality were evaluated. No adverse

health effects were noted for cats receiving any treatment. Dietary treatments also did not have an effect on food intake, defecation frequency, or fecal output on an as-is basis ($P > 0.05$). Fecal score was evaluated on a scale from 1 to 5 with 1 being liquid diarrhea, and 5 being hard dry pellets, and a score of 3.5 was considered ideal. The miscanthus grass treatment resulted in a significantly higher fecal score that was closer to ideal (3.3) than the beet pulp treatment (2.8), with cellulose being intermediate (3.2) ($P < 0.05$). Fecal output on a DM basis was higher for miscanthus grass and cellulose compared with beet pulp (34.2%, 33.6%, and 27.0%, respectively). In terms of ATTD, DM, OM, and gross energy digestibility were higher for cats fed the beet pulp diet than the miscanthus grass and cellulose diets ($P < 0.0001$). Crude protein ATTD was highest for cellulose (86.1%), followed by miscanthus grass (85.8%), and then beet pulp (84.2%). The miscanthus grass group had lower crude fat ATTD than cellulose and beet pulp (85.0, 89.6, and 89.2%, respectively) ($P < 0.0001$). The ATTD of TDF was highest for cats fed the beet pulp diet (39.7%), followed by miscanthus grass (20.8%), then cellulose (12.2%) groups. With the exception of TDF, ATTD coefficients were all close to or greater than 80%, indicating that all treatments were well digested by the cats. These results demonstrated that the inclusion of miscanthus grass in extruded feline diets caused no severe adverse effects in a short-term feeding trial measuring animal health indices, nutrient digestibility, and fecal quality. Similar to the canine trial, it was concluded that miscanthus grass may be considered as an alternative fiber source to cellulose in feline diets.

Further testing done by Donadelli and Aldrich (2020) evaluated the effect of an extruded diet including miscanthus grass on hairball management in cats in a replicated crossover design. In each period, twelve American shorthair cats were fed either a control diet or a diet containing 10% inclusion of miscanthus grass at the expense of rice flour. This formulation resulted in

treatments with similar nutrient profiles with the exception of lower TDF content in the control diet (5.4%) and higher TDF content in the miscanthus grass treatment (14.1%). Food intake, defecation frequency, and fecal DM content were not affected by treatment ($P > 0.05$). The miscanthus grass treatment resulted in a higher fecal output on an as-is basis compared with the control (47.0 g/d/cat, and 28.8 g/d/cat, respectively) ($P < 0.05$). Fecal score also was evaluated on a scale from 1 to 5, with 1 being liquid diarrhea and 5 being hard dry pellets, and a score of 3.5 was considered ideal. The miscanthus grass treatment resulted in a significantly higher fecal score than the control (4.0 and 3.4, respectively), with the control group having scores closer to the ideal range. Inclusion of miscanthus grass in extruded feline diet had no significant effect on fecal total hair clump count, hair clump count per cat per day, hair clump size, or total hair clump weight ($P > 0.05$) in contrast with cats fed the control diet. However, hair retained in the strainer and total fecal hair weight tended to be higher for the miscanthus grass group, possibly indicating that inclusion of miscanthus grass in extruded diets prevented the formation of hair clumps in the gastrointestinal tract of cats. Total hair weight (mg) per dry feces weight (g) and number of hair clumps per dry feces weight (g) were significantly higher for the control group than the miscanthus grass treatment ($P < 0.05$). The researchers hypothesize that this could indicate that the passage of larger quantities of digesta could promote the movement of hair clumps more regularly through the gastrointestinal tract. The animals used in this trial were shorthaired cats with no history of hairball occurrences, and these factors could have influenced the results of the study. More research is necessary to determine the effectiveness of miscanthus grass in the nutritional management of hairballs in felines.

Knowledge of dietary fiber's health benefits is known throughout the companion animal industry, and interest ingredients that provide novel sources of dietary fiber has increased.

Widening the pool of dietary fiber sources can help to expand formulation options and potential functional benefits to support the health and wellness of companion animals. *Miscanthus x giganteus* is a readily available, economical, and ecologically friendly fiber source that has attracted industry attention. As a dietary fiber source, it has many positive marketing attributes including natural, non-GMO, non-wood, non-byproduct, and the potential to be grown as a certified organic ingredient. Research exploring the use of *Miscanthus x giganteus* in feline diets is limited and focuses on macronutrient digestibility, fecal quality, and hairball management. The effect of miscanthus grass on gastrointestinal health and as a main constituent of fiber blends has yet to be evaluated. These factors should be investigated to further enhance the knowledge of this novel fiber as a functional ingredient in feline diets.

THESIS OBJECTIVES

The objective of this research was to evaluate the effect of *Miscanthus x giganteus* and a novel fiber blend of miscanthus grass and tomato pomace on gastrointestinal tolerance, apparent total tract macronutrient digestibility, and fecal fermentative end-products and microbiota in comparison with traditionally utilized dietary fiber sources in extruded diets for adult felines.

LITERATURE CITED

Anderson, J. W., L. D. Allgood, A. Lawrence, L. A. Altringer, G. R. Jerdack, D. A. Hengehold, and J. G. Morel. 2000. Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: Meta-analysis of 8 controlled trials. Am. J. Clin. Nutr. 71:472-479.

Anderson, J. W., P. Baird, R. H. Davis, S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205.

Babich, O. O., O. V. Krieger, E. G. Chupakhin, and O. V. Kozlova. 2019. Miscanthus plants processing in fuel, energy, chemical and microbiological industries. Foods Raw Mater. 7:403-411. doi:10.21603/2308-4057-2019-2-403-411.

Barry, K. A., B. J. Wojcicki, I. S. Middelbos, B. M. Vester, K. S. Swanson, and G. C. Fahey, Jr.. 2010. Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. J. Anim. Sci. 88:2978-2987.

Bauer, S. and A. B. Ibanez. 2014. Rapid determination of cellulose. Biotechnol. Bioeng. doi:10.1002/bit.25276.

Bazzano, L. A., J. He, L. G. Ogden, C. M. Loria, and P. K. Whelton. 2003. Dietary fiber intake and reduced risk of coronary heart disease in US men and women: The National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. Arch. Intern. Med. 163:1897-1904.

Benyen, A. C., D. H. J. Saris, and J. Middelkoop. 2011. Clinical signs of hairballs in cats fed a diet enriched with cellulose. Am. J. Anim. Vet. Sci. 6:69-72. doi:10.3844/ajavsp.2011.69.72.

Bueno, A. R., T. G. Cappel, G. D. Sunvold, R. A. Moxley, G. A. Reinhart, and E. T. Clemens. 2000a. Feline colonic microbes and fatty acid transport: Effects of feeding cellulose, beet pulp, and pectin/gum arabic fibers. Nutr. Res. 20:1319-1328.

Bueno, A. R., T. G. Cappel, G. D. Sunvold, G. A. Reinhart, and E. T. Clemens. 2000b. Feline colonic morphology and mucosal tissue energetics as influenced via the source of dietary fiber. Nutr. Res. 20:985-993.

Butowski, C. F., D. G. Thomas, W. Young, N. J. Cave, C. M. McKenzie, D. I. Rosendale, and E. N. Bermingham. 2019. Addition of plant dietary fibre to a raw red meat high protein, high fat diet, alters the faecal bacteriome and organic acid profiles of the domestic cat (*Felis catus*). PLoS One. 14:1-19. doi:10.1371/journal.pone.0216072.

Chen, J. and L. Vitetta. 2018. Inflammation-modulating effect of butyrate in the prevention of colon cancer by dietary fiber. Clin. Colorectal Cancer. 17:541-544. doi:10.1016/j.clcc.2018.05.001.

Chen, M. H., K. D. Rausch, M. E. Tumbleson, V. Singh, M. J. Bowman, and B. S. Dien. 2014. Autohydrolysis of Miscanthus x giganteus for the production of xylooligosaccharides (XOS): Kinetics, characterization and recovery. Bioresour. Technol. 155:359-365. doi:10.1016/j.biortech.2013.12.050.

Dai, F. J. and C. F. Chau. 2016. Classification and regulatory perspectives of dietary fiber. J. Food Drug Anal. 25:37-42.

Danielewicz, D. and B. Surma-Slusarska. 2019. *Miscanthus* × *giganteus* stalks as a potential non-wood raw material for the pulp and paper industry. Influence of pulping and beating conditions on the fibre and paper properties. Ind. Crops Prod. 141:1-11. doi:10.1016/j.indcrop.2019.111744.

Donadelli, R. A., and C. G. Aldrich. 2019. The effects on nutrient utilization and stool quality of Beagle dogs fed diets with beet pulp, cellulose, and Miscanthus grass. J. Anim. Sci. 97:4134-4139. doi:10.1093/jas/skz265.

Donadelli, R. A., and C. G. Aldrich. 2020. The effects of diets varying in fibre sources on nutrient utilization, stool quality and hairball management in cats. J. Anim. Physiol. Anim. Nutr. 104:715-724. doi:10.1111/jpn.13289.

El-Salhy, M., S. O. Ystad, T. Mazzawi, and D. Gundersen. 2017. Dietary fiber in irritable bowel syndrome. Int. J. Mol. Med. 40:607-613. doi:10.3892/ijmm.2017.3072.

Fahey, G. C. Jr., L. Novotny, B. Layton, and D. R. Mertens. 2019. Critical factors in determining fiber content of feeds and foods and their ingredients. J. AOAC Int'l. 102:52-62.

Fekete, S., I. Hullar, E. Andrasofszky, Z. Rigo, and T. Berkenyi. 2001. Reduction of the energy density of cat foods by increasing their fibre content with a view to nutrients' digestibility. J. Anim. Physiol. Anim. Nutr. 85:200–204. doi: 10.1046/j.1439-0396.2001.00332.x.

Fischer, J. Fruit fibers. In Fiber Ingredients: Food Applications and Health Benefits; Cho, S.S., Samuel, P., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2009; pp. 427-438.

Food and Drug Administration. 2016. Food labeling: Revision of the nutrition and supplement facts labels. Fed. Regist. 81:33741-33999.

Food and Drug Administration. 2020. Questions and answers on dietary fiber. https://www.fda.gov/food/food-labeling-nutrition/questions-and-answers-dietary-fiber#define_dietary_fiber. (Accessed 6 November 2020).

German, A. J. 2006. The growing problem of obesity in dogs and cats. J. Nutr. 136:1940S-1946S.

de Godoy, M. R. C., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients. 5:3099-3117.

Li, Z., S. M. Finegold, P. H. Summanen, and T. Komoriya. 2015. In vitro study of the prebiotic xylooligosaccharide (XOS) on the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. Int. J. Food Sci. Nutr. 66:919-922. doi:10.3109/09637486.2015.1064869.

Loureiro, B. A., G. Sembenelli, A. P. J. Maria, R. S. Vasconcellos, F. C. Sa, N. K. Sakomura, and A. C. Carciofi. 2014. Sugarcane fibre may prevents hairball formation in cats. J. Nutr. Sci. 3:1-5.

Massimino, S. P., M. I. McBurney, C. J. Field, A. B. R. Thomson, M. Keelan, M. G. Hayek, and G. D. Sunvold. 1998. Fermentable dietary fiber increases GLP-1 secretion and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. J. Nutr. 128:1786-1793.

McCalmont, J. P., A. Hastings, N. P. McNamara, G. M. Richter, P. Robson, I. S. Donnison, and J. Clifton-Brown. 2017. Environmental costs and benefits of growing Miscanthus for bioenergy in the UK. GCB Bioenergy. 9:489-507. doi:10.1111/gcbb.12294.

National Research Council. 2006. Nutrient requirements of dogs and cats, 2nd . National Academy Press, Washington, D. C.

Nelson, R. W., E. C. Feldman, L. A. Neal, P. H. Kass, S. E. DeVries-Concannon, C. T. Kiernan, J. C. Scott-Moncrieff, and D. J. Davenport. 2000. Effect of dietary insoluble fiber on control of glycemia in cats with naturally acquired diabetes mellitus. J. Am. Vet. Med. Assoc. 216:1082-1088. doi:10.2460/javma.2000.216.1082.

Pidlisnyuk, V., J. Trögl, L. Erickson, T. Stefanovska, J. Popelka, G. Hettiarachchi, and L. Davis. 2019. Potential phytomanagement of military polluted sites and biomass production using biofuel crop *Miscanthus x giganteus*. Environ. Pollut. (Oxford, U. K.). 249:330-337. doi:10.1016/j.envpol.2019.03.018.

Shepherd, A., E. Littleton, J. Clifton-Brown, M. Martin, and A. Hastings. 2020. Projections of global and UK bioenergy potential from *Miscanthus* × *giganteus*—Feedstock yield, carbon cycling and electricity generation in the 21st century. GCB Bioenergy. 12:287-305. doi:10.1111/gcbb.12671.

Sierra, M., J. J. Garcia, N. Fernandez, M. J. Diez, and A. P. Calle. 2002. Therapeutic effects of psyllium in type 2 diabetic patients. Eur. J. Clin. Nutr. 56:830-842.

Sunvold, G. D., G. C. Fahey, Jr., N. R. Merchen, L. D. Bourquin, E. C. Titgemeyer, L. L. Bauer, and G. A. Reinhart. 1995a. Dietary fiber for cats: *In vitro* fermentation of selected fiber sources by cat fecal inoculum and *in vivo* utilization of diets containing selected fiber sources and their blends. J. Anim. Sci. 73:2329-2339.

Sunvold, G. D.. G. C.Fahey, N. R. Merchen, E. C. Titgemeyer, L. D. Bourquin, L. L. Bauer, and G. A. Reinhart. 1995b. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. J. Anim. Sci. 73:1099–1109.

Sunvold, G. D., G. C.Fahey, N. R. Merchen, and G. A. Reinhart. 1995c. In vitro fermentation of selected fibrous substrates by dog and cat fecal inoculum: Influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. J. Anim. Sci. 73:1110–1122.

Sunvold, G. D.; Hussein, H. S.; Fahey, G. C., Jr.; Merchen, N. R.; Reinhart, G. A. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. J. Anim. Sci. 1995d, 73, 3639–3648.

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

Swanson, K. S., C. M. Grieshop, G. M.Clapper, R. G. Shields, T. Belay, N. R. Merchen, G. C. Fahey. 2001. Fruit and vegetable fiber fermentation by gut microflora from canines. J. Anim. Sci. 79:919–926

Tungland, B. C., and D. Meyer. 2002. Nondigestible oligo and polysaccharides (dietary fibre): Their physiology and role in human health and food. Compr. Rev. Food. Sci. Food Safety. 1:73-92. doi:10.1111/j.1541-4337.2002.tb00009.x.

Van Weyenburg, S., T. Ulens, K. De Reu, I. Zwertvaegher, P. Demeyer, and L. Pluym. 2015. Feasibility of Miscanthus as alternative bedding for dairy cows. Vet. Med. (Prague, Czech Repub.). 60:121-132.

Weber, M., L. Sams, A. Feugier, S. Michel, and V. Biourge. 2015. Influence of the dietary fibre levels on faecal hair excretion after 14 days in short and long-haired domestic cats. Vet. Med. Sci. 1:30-37. doi:10.1002/vms3.6.

Ziai, S. A., S. Akhoondzadeh, A. Dastpak, A. Rezai, B. Larijani, H. Fakhrzadeh, F. Bandarian, H. N. Badi, and T. Emami. 2005. Psyllium decreased serum glucose and glycosylated

hemoglobin significantly in diabetic outpatients. J. Ethnopharmacol. 102:202-207. doi:10.1016/j.jep.2005.06.042.

Zoran, D. L. 2008. Nutritional management of feline gastrointestinal diseases. Top. Companion Anim. Med. 23:200-206. doi:10.1053/j.tcam.2008.08.003.

CHAPTER 3:
MISCANTHUS GRASS AS A NOVEL FUNCTIONAL FIBER SOURCE IN EXTRUDED FELINE DIETS

ABSTRACT

Although dietary fiber is not considered an essential nutrient in a complete and balanced diet for felines, it provides a substrate for fermentation by the gut microbiota, thus promoting gastrointestinal health through the production of fermentative metabolites, as well as improving laxation. Dietary fiber has also commonly been utilized in companion animal diets to dilute caloric density. This is an especially useful strategy in the formulation of "lite" or weight-loss promoting diets. The aim of this research was to evaluate the novel dietary fiber source, miscanthus grass, in comparison to traditional dietary fiber sources, and their effects on fecal quality, apparent total tract digestibility (**ATTD**), and fecal fermentative end-products and microbiota of healthy adult cats. Four dietary treatments were evaluated, differing only in dietary fiber source. The diets were formulated to meet or exceed the AAFCO (2018) nutritional requirements for adult cats and contained either 7% cellulose (**CO**), 9% miscanthus grass fiber (**MF**), a blend of 7% miscanthus grass fiber plus 2% tomato pomace (**MF+TP**), or 11% beet pulp (**BP**). Diets were formulated to have similar nutrient composition and to achieve a target total dietary fiber (**TDF**) content of 15%. The animal study was conducted using a completely randomized design with twenty-eight neutered adult, domesticated shorthair cats (19 females and 9 males, mean age 2.2 ± 0.03 yr; mean body weight 4.6 ± 0.7 kg, mean body condition score 5.6 ± 0.6). Cats were randomly assigned to one of four dietary treatments and were fed twice daily to maintain body weight. The experimental period comprised 21 d. A fresh fecal and total fecal collection period were performed during the last 4 d of the 21 d trial period. During the collection period, food intake and total fecal output were evaluated to determine macronutrient

ATTD. A fresh fecal sample was collected from each cat within 15 min of defecation and was used to evaluate fecal dry matter (**DM**) content, fecal score, pH, fecal ammonia concentration, short-chain fatty acid (**SCFA**) and branched-chain fatty acid (**BCFA**) concentrations, as well as fecal phenol and indole concentrations. A fasted blood sample was collected at baseline and at the end of the 21-d period. Serum chemistry and complete blood count were analyzed to ensure that good health status was maintained for all animals. All dietary treatments were well accepted by the cats, and daily food intake (DM basis) was similar across all groups ($P > 0.05$). Additionally, dietary treatment did not affect fecal output (as-is or DM basis), fecal score, or fecal pH ($P > 0.05$). All diets had digestibility coefficients close to or above 80%, indicating that they were well digested by the animals. The ATTD of DM (78.3-82.7%), organic matter (**OM**) (81.8-86.3%), and crude protein (**CP**) (83.1-84.6%) were similar among treatment groups ($P > 0.05$). However, ATTD of acid hydrolyzed fat (**AHF**) was highest for cats fed the CO treatment (94.5%) when compared with cats fed the MF (91.7%) and MF+TP (91.2%) treatments ($P < 0.05$), with cats fed the BP diet (92.6%) having intermediate AHF digestibility. Additionally, BP had significantly higher TDF ATTD (54.2%) than all other treatments (MF=19.1%; MF+TP=25.5%; CO=21.8%) ($P < 0.05$). Digestible energy (**DE**) of the CO diet (3.9 kcal/g) was higher than for the MF+TP diet (3.7 kcal/g) ($P < 0.05$), while MF and BP diets were similar in DE to all treatments. While there was no difference ($P > 0.05$) in fecal ammonia and phenol concentrations among groups, fecal indole and total phenol and indole concentrations were highest for the MF and MF+TP groups compared with the CO and BP groups ($P < 0.05$). Cats fed the BP diet had the highest concentrations of total SCFA, acetate, and propionate ($P < 0.05$), while butyrate concentrations were similar for all treatments ($P > 0.05$). Total branched-chain fatty acids, isobutyrate, and isovalerate concentrations were higher for cats fed the MF+TP diet

in contrast with cats fed CO and BP ($P < 0.05$), with cats fed MF being intermediate. A similar trend for fecal valerate concentration was observed, with cats fed the MF+TP diet having greater concentrations than cats fed the BP diet ($P < 0.05$); intermediate concentrations of fecal valerate were observed in cats fed CO and MF diets. As no adverse effects on health, fecal quality, or ATTD of macronutrients were observed with the inclusion of 9% miscanthus grass fiber, or a miscanthus grass fiber blend with tomato pomace, the data suggest that miscanthus grass fiber and the blend are viable alternatives to the traditional dietary fiber sources used in commercial extruded feline diets, being most comparable to cellulose.

***Key words*:** cats, dietary fiber, fecal microbiota, miscanthus grass, nutrient digestibility, postbiotics

INTRODUCTION

Since the 1950's, when the term "dietary fiber" was first introduced, several attempts have been made by the scientific community and regulatory bodies to provide a clearer and more encompassing definition of this term (Dai and Chau, 2016). This is because a diverse group of substances and ingredients fall under this umbrella term, which differ in many aspects including origin, physico-chemical properties and physiological effects. In 2016, the Food and Drug Administration issued a final ruling on the definition of "dietary fiber". They defined it as: "non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates determined by the Food and Drug Administration to have physiological effects that are beneficial to human health" (FDA, 2016). Many studies have shown the broad range of health benefits associated

with dietary fiber intake by humans, with a major focus on gut health (Anderson et al., 2009). This interest has spread to companion animal nutrition as well.

The most traditionally used sources of dietary fiber in companion animal diets are cellulose and beet pulp (de Godoy et al., 2013). These ingredients, like most fiber sources, have distinct fiber profiles that allow them to provide different physiological effects. Fiber characteristics such as viscosity, solubility, and fermentability determine the functional effects of various dietary fiber sources. Cellulose is made up almost entirely of insoluble, non-viscous fiber. Purified sources of cellulose are highly uniform in composition, while other commercially available sources may be byproducts of other industries resulting in more variable compositions. Beet pulp has a mixed composition, consisting of viscous, non-viscous, soluble, and insoluble fibers. The ratios of these fiber portions can be inconsistent, leading to more variability in this product (de Godoy et al., 2013).

Miscanthus grass is an ingredient that has the potential to act as a novel dietary fiber source in companion animal diets. This grass is largely composed of insoluble fibers, making it compositionally similar to cellulose. It also contains naturally occurring xylooligosaccharides that may provide a prebiotic-like effect; however, more research is required to determine this. As an ingredient, miscanthus grass offers many positive marketing attributes such as “natural”, non-GMO, non-byproduct, and potentially organic. Limited data are available on the use of miscanthus grass in monogastric diets, especially as regards its effects on gastrointestinal health. Therefore, the goal of this research was to compare the effects of miscanthus grass fiber to traditional dietary fiber sources and their effects on gastrointestinal tolerance, apparent total tract macronutrient digestibility, and fecal fermentative end-products in adult cats fed extruded diets.

MATERIALS AND METHODS

Animals and Experimental Design

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Twenty-eight neutered, adult domesticated shorthair cats (19 females and 9 males, mean age 2.2 ± 0.03 yr; mean BW 4.6 ± 0.7 kg, mean body condition score 5.6 ± 0.6) were used in a completely randomized design. At the start of the experiment, all cats were adapted to the CO diet for 7 d. After this control adaptation period, all cats were randomly assigned to one of four treatment diets and were fed for 21 d to maintain body weight. During the last 4 d of this period, a total fecal and fresh fecal collection was performed.

Cats were group housed for 20 h of the day, and individually housed in stainless steel cages for 4 h per day for feeding. Feeding occurred twice a day from 0800-1000 and 1500-1700. Cats had free access to water at all times. Food refusals were weighed and recorded after each feeding. Body weights and body condition scores were measured and recorded weekly. Cats were housed in the Edward R. Madigan Laboratory in a climate-controlled room with a 14 h light and 10 h dark cycle. Human socialization periods took place at a minimum of two times per week, and cats had access to behavioral enrichments such as scratching posts.

Diets

Four diets were formulated to meet or exceed the AAFCO (2018) nutrient profile for adult cats (n = 7 cats/treatment). They were formulated with similar ingredient composition, except for the dietary fiber sources being tested, and to have similar nutrient composition and a targeted TDF content of 15%. To achieve this target, the diets were formulated to contain 7%

cellulose (CO), 9% miscanthus grass fiber (MF), 7% miscanthus grass fiber plus 2% tomato pomace **(**MF+TP), or 11% beet pulp (BP) (**Table 3.1**).

Sample Collection

For the duration of the 4 d fecal collection period, cats were housed individually. All feces were collected during this time and composited by cat to determine total fecal output. Each sample also was evaluated for fecal score on a 5-point scale (1 = hard, dry pellets; 2 = hard formed, remains firm and soft, 3 = soft, formed and moist stool; 4 = soft, unformed stool; or 5 = watery, liquid that can be poured), and then samples were stored at -20°C for later analysis to determine ATTD of macronutrients.

A fresh fecal sample was collected from each cat within 15 min of defecation during the 4 d fecal collection period. These samples were also evaluated for fecal pH and score, and dry matter. Then, they were aliquoted to determine ammonia, SCFA, BCFA, phenol and indole concentrations. To determine DM content, duplicates of approximately 2 g of feces were dried in a forced-air oven at 105°C. For determination of fecal ammonia, SCFA and BCFA concentrations, 3 g of each fresh sample was placed in a Nalgene bottle and mixed with 3 mL of 2N hydrochloric acid, and stored at -20°C for later analysis. Duplicates of 2 g of each fresh sample were placed into plastic test tubes, covered with parafilm, and stored at -20°C for later analysis of phenols and indoles. Fecal samples allocated for microbiota analysis were stored in 2 ml cryovials and stored at -80^{o}C until analysis.

On 0 and 21 d of the experimental period, cats were fasted overnight, and a blood sample was collected to evaluate blood metabolites and health status. Cats were sedated before collecting 5 mL of blood via jugular venipuncture. For complete blood count analysis, 1 mL of

blood from each cat was placed in EDTA vacutainer tubes and 4 mL was placed in serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood analyses were completed by the Clinical Pathology Laboratory at the University of Illinois College of Veterinary Medicine (Urbana, IL).

Sample Preparation

Experimental diets were subsampled and ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a size 10 mesh screen resulting in 2 mm average particle size used for proximate laboratory analysis. Total fecal samples were composited for each animal and partially dried in a forced air oven at 57 °C. After drying, they were also ground in a Wiley mill to a 2 mm particle size.

Chemical analyses

After the diet and feces samples were prepared, DM and ash content were determined following the AOAC procedures (2006; methods 934.01 and 942.05). Crude protein concentration was evaluated using the Official Method of AOAC International (2002) by measuring total nitrogen with a LECO TruMac (model 630-300-300, Leco Corporation, St. Joseph, MI). Fat content of diets and fecal samples were determined using acid-hydrolysis and ether extraction following the methods of the Budde (1952) and the American Association of Cereal Chemists (1983). Bomb calorimetry was utilized to determine gross energy of the samples using a Parr 6200 calorimeter (Parr Instruments Co., Moline, IL). Further analysis of the fecal samples was completed to determine TDF content according to Prosky et al. (1992) and the Official Method of AOAC International (2006; methods 985.29 and 991.43). Diet samples were

analyzed using the same methods to determine TDF as well as soluble dietary fiber (**SDF**) and insoluble dietary fiber (**IDF**) contents.

Gas chromatography was used to measure SCFA and BCFA concentrations in the fresh fecal samples using a modified method of Sunvold et al. (1995). These analyses were completed using a Hewlett-Packard gas chromatograph **(**Model 5890A HewlettPackard, Avondale, PA) equipped with a flame ionization detector on a column (1.8 m x 4 mm i.d.) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 chromosorb W AW (Supelco, Bellefonte, PA). Nitrogen was used as the carrier gas at a flow rate of 45 mL/min. Oven temperature was set at 125ºC, injection port at 175ºC, and detector port at 180ºC. Fecal phenol and indole concentrations were measured using a Thermo Scientific TRACE 1300 Gas Chromatograph coupled with a FID in duplicate according to the modified procedure of Flickinger et al. (2003). The internal standard used was 5-methylinodle. Following this method, 1 µl sample was injected at 220 ºC at splitless mode. A Nukol Supelcol column (60 m length, 0.32 mm diameter) with a film thickness of 0.25 µm was used to separate the phenolic compounds. Oven temperature was held at 150 ºC for 1 min, and then increased at 25 ºC per min until reaching 200 ºC and held at this temperature for 35 min. Ammonia concentration was measured according to the procedures of Chaney and Marbach (1962).

Statistical Analysis

Data were analyzed using the MIXED Model procedures of SAS version® 9.4 (SAS Institute Inc., Cary, NC). Animal was used as the random effect, and treatment diet was used as the fixed effect in the statistical model. Data normality was checked using the UNIVARIATE procedure, comparing all treatment least-squares means. Experiment-wise error was controlled

for using Tukey adjustment. The significance level was set at a probability of $P < 0.05$. Pooled standard errors of the mean also were obtained using the MIXED model procedure.

DNA Extraction, Amplification, Sequencing, and Bioinformatics

Total DNA extraction from fresh fecal samples was completed using a Mo-Bio PowerSoil kit (MO BIO Laboratories, Inc., Carlsbad, CA). A Qubit® 3.0 Fluorometer (Life technologies, Grand Island, NY) was used to quantify DNA concentration prior to amplification and sequencing. A Fluidigm Access Array (Fluidigm Corporation, South San Francisco, CA), in combination with Roche High Fidelity Fast Start Kit (Roche, Indianapolis, IN), were used for amplification of the 16S rRNA gene. The primers 515F (5′- GTGYCAGCMGCCGCGGTAA -3′) and 806R (5′- GGACTACNVGGGTWTCTAAT -3′), targeting a 292 bp-fragment of V4 region, were used for amplification (primers synthesized by IDT Corp., Coralville, IA; Caporaso et al., 2012). Fluidigm specific primer, forward and reverse tags, were added in accordance with the Fluidigm protocol. A Fragment Analyzer (Advanced Analytics, Ames, IA) was used to verify the quality of amplicon region and size. A DNA pool was generated through the combination of equimolar amounts of the amplicons from each sample. The pooled samples were selected by size on a 2% agarose E-gel (Life Technologies, Grand Island, NY) and extracted using a Qiagen gel purification kit (Qiagen, Valencia, CA). The pooled, size-selected, and cleaned products were then run on an Agilent Bioanalyzer in order to confirm appropriate profile and mean size. The Roy J. Carver Biotechnology Center at the University of Illinois performed Illumina sequencing on a MiSeq using v3 reagents (Illumina Inc., San Diego, CA). A FASTX-Toolkit (version 0.0.14) removed the Fluidigm tags. Analysis of sequences was completed using QIIME 2.0 (Caporaso et al., 2010) and DADA2 (version 1.14; Callahan et al., 2016). The high quality

(quality value ≥ 20) sequence data, derived from the sequencing process, were de-multiplexed. An opened-reference OTU clustered the sequences into operational taxonomic units (**OTU**), choosing against the SILVA 138 reference OTU database with a 97% similarity threshold (Quast et al., 2013). The OTUs observed fewer than two times (singletons), as well as OTUs with less than 0.01% of the total observations, were discarded. An average of 47,315 reads were obtained, with a total of 1,324,844 reads. The number of reads ranged from 39,416 to 56,474 per sample. To analyze for diversity and species richness, the dataset was rarified to 39,415 reads. Weighted and unweighted unique fraction metric (UniFrac) distances were performed by principal coordinate analysis (PCoA) (Lozupone et al., 2005).

RESULTS

The four treatment diets were formulated to contain similar nutrient composition (**Table 3.1**). This was confirmed through the chemical analysis of the diets (**Table 3.2**). Dry matter content ranged from 91.9 to 94.2%. Acid-hydrolyzed fat content was very similar for all treatments, with CO being approximately one percentage unit higher. CP content ranged from 29.6 to 31.3%. All diets were close to the targeted TDF content of 15%, with CO containing 15.1% TDF, MF with 15.0%, MF+TP with 15.7%, and BP with 15.7%. The CO, MF, and MF+TP diets had similar SDF contents (3.4, 3.8, and 3.2%, respectively), while BP had a higher SDF content of 6.1%. The CO, MF, and MF+TP diets also had similar IDF content (11.7, 11.2, and 12.5%, respectively) while BP had a lower IDF content of 9.6%.

Treatment did not have a significant effect on daily food intake (DM), wet fecal output (g/d), fecal DM output (g/d), fecal score, or fecal pH ($P > 0.05$) (**Table 3.3**). Additionally, ATTD of DM, OM, and CP were similar across treatment ($P > 0.05$). Dry matter digestibility ranged

from 78.3 to 82.7%. Acid hydrolyzed fat digestibility of CO (94.5%) was significantly higher compared with MF and MF+TP (91.7 and 91.2%, respectively) ($P < 0.05$) with BP being intermediate (92.6%). The BP diet had the highest TDF digestibility (54.2%) when compared with all other treatments (average 22.1%) ($P < 0.05$). Digestible energy (kcal/g), which was calculated by subtracting fecal gross energy from diet gross energy, was higher for CO (3.94 kcal/g) than MF+TP (3.72 kcal/g) ($P < 0.05$), with MF and BP being intermediate.

Cats fed the MF+TP and MF diets had significantly higher total fecal phenol and indole concentrations than CO and BP fed cats ($P < 0.05$) (**Table 3.4**). Fecal indole concentration followed the same pattern ($P < 0.05$), while fecal phenol concentration was not significantly affected ($P > 0.05$). Fecal ammonia concentration was also similar across all treatments ($P > 0.05$). BP resulted in the highest concentrations of total SCFA, acetate, and propionate ($P < 0.05$), while butyrate concentrations were similar for all treatments ($P > 0.05$). Total BCFA, isobutyrate, and isovalerate concentrations were higher in the MF+TP group than the CO and BP groups ($P < 0.05$), with MF being intermediate. A similar trend for valerate concentration was observed with the MF+TP group being higher than the BP group ($P < 0.05$), with CO and MF groups being intermediate.

The fecal microbiota composition for cats fed different dietary fibers was comprised of 7 phyla (**Fig. 3.1**) with Firmicutes, Bacteriodota, Proteobacteria, and Actinobacteria corresponding to more than 90% of the total sequences. Cats fed MF and MF+TP treatments had greater ($P < 0.05$) relative abundances of Firmicutes in relation to the BP treatment. Bacteriodota phylum was increased ($P < 0.05$) in cats fed BP in contrast with MF. Proteobacteria was increased ($P < 0.05$) in cats fed BP in contrast with all other dietary groups. A total of 38 families, 102 genera, and 66 species were identified; within those, over 10 and 20 taxonomic groups for family (**Table 3.5**)

and genera (**Table 3.6**) differed ($P < 0.05$) among treatments, respectively. The relative abundance of Bacteroidaceae was greater ($P < 0.05$) for cats fed BP in comparison with those fed CO, but did not differ for cats fed MF and MF+TP treatments. Cats fed BP also had greater ($P < 0.05$) relative abundance of Prevotellaceae in contrast with cats fed all other dietary treatments. Relative abundance of Oscillospiraceae, Butyricicoccaceae, and Anaerovoracaceae were consistently higher ($P < 0.05$) in cats fed CO, MF, and MF+TP treatments in contrast with the BP treatment, whereas Succinivibrionaceae was consistently lower ($P < 0.05$) in cats fed those dietary treatments when compared with the BP treatment (**Table 3.5**).

The relative abundance of *Collinsella,* a genus within the family Coriobacteriaceae, and phylum, Actinobacteria, was greater ($P < 0.05$) in cats fed the CO treatment (4.0%) in contrast with BP (2.4%), and intermediate in cats fed the MF or MF+TP treatments (3.5 and 3.0%, respectively). In contrast, relative abundance of *Prevotella* was greater in cats fed BP when compared with cats fed CO (9.2%), MF (5.4%), and MF+TP (6.0%). In addition, the relative abundance of several genera were consistently higher in cats fed CO, MF, and MF+TP in contrast with BP including *Clostridia UCG-014*, Butyricicoccaceae *UCG-009*, *Colidextrobacter*, *Oscillibacter*, and *Megasphaera.* Cats fed BP (4.3%) had greater relative abundance of *Succinivibrio* than cats fed CO (1.8%) and MF+TP (1.0%), with MF (0.8%) being lowest (**Table 3.6**).

Beta-diversity based on weighted (**Fig. 3.2a**) and unweighted (**Fig. 3.2b**) UniFrac analysis showed that fecal microbial community composition of cats fed the BP treatment differed (P and q-value < 0.05) in comparison with cats fed CO, MF, and MF+TP treatments. Alpha-diversity was measured as Pielou evenness, Faith's phylogenetic diversity, and Shannon entropy (**Fig. 3.3**). Fecal microbial diversity and richness based on the Pielou evenness index

(**Fig. 3.3a**) revealed that cats fed the BP treatment had lower α-diversity than cats fed the MF+TP treatment ($P < 0.05$ and q-value < 0.1). Similarly, α-diversity of cats fed BP was lower than cats fed other dietary treatments based on Faith's phylogenetic diversity (P- and q-value < 0.05; Fig. 3.3b), and lower (P- and q-value < 0.05) than cats fed MF and MF+TP based on Shannon entropy index (**Fig. 3.3c**).

Blood analysis was performed to determine health status of the cats during the experimental period. Serum metabolites (**Table 3.7**) were within normal ranges observed in healthy adult cats and no treatment× time interaction or main treatment effect were observed. At baseline, however, creatinine levels of cats assigned to the MF (1.7 mg/dL) and BP (1.6 mg/dL) treatments were slightly above the reference values. Similarly, cats fed CO (1.6 mg/dL), MF (1.8 mg/dL), and BP (1.6 mg/dL) treatments, on 21 d of the experimental period, were slightly higher than the reference range (0.5 to 1.5 mg/dL). However, this outcome was independent of dietary treatment. Additionally, glucose concentrations were above the normal reference range for all treatments. However, this temporary increase in plasma glucose has been attributed as a side effect of the sedation used during blood sample collection. Based on complete blood cell count (data not provided) and serum chemistry results, cats were considered healthy during the experimental period.

DISCUSSION

Diet, Food Intake, and Fecal Characteristics

The experimental diets all were formulated to maintain similar ingredient and nutrient composition, differing only in dietary fiber source (cellulose, beet pulp, miscanthus grass fiber, miscanthus grass fiber and tomato pomace blend). Minimal variations in ingredient inclusion

rates were necessary to obtain the targeted TDF content of 15%. Chemical composition of the diets fell within a relatively narrow range, with the CO treatment having a slightly higher AHF and CP concentration, as well as a gross energy value. This could be due to the slightly higher inclusion level of poultry by-product meal in this diet. Overall, diets were very close to their target TDF content of 15%. Small variations are expected as TDF content can be affected by plant's growing conditions, time of harvest, and plant maturity, among other things. The BP diet had higher levels of SDF and lower IDF as was expected based on the typical fiber profile of this ingredient (de Godoy et al., 2013). The CO, MF, and MF+TP diets were similar in SDF and IDF content, which was as expected due to the similar fiber profile of cellulose and miscanthus grass (Donadelli and Aldrich, 2019). It was predicted that the MF+TP blend might have slightly lower TDF, SDF, and IDF content than MF as 2% tomato pomace was added at the expense of 2% of the miscanthus grass fiber. The composition of tomato pomace was reported by Swanson et al. (2001) as 56.9% TDF, 4.2% SDF, and 52.7% IDF (DM basis) in comparison to miscanthus grass composition reported by Donadelli and Aldrich (2019) as 90% TDF, 7.3% SDF, and 82.7% IDF (DM basis). However, only lower SDF values were observed for the MF+TP blend compared with MF alone (3.2 and 3.8%, respectively), possibly due to the expected TDF variability of plant byproducts that was previously mentioned, or the low inclusion level of the tomato pomace.

Food intake (g/d) on an as-fed and DM basis did not differ among treatment groups. Similarly, Donadelli and Aldrich (2019) saw no effect on food intake when cellulose, beet pulp, and miscanthus grass were added to the formula at a 10% inclusion rate. Detweiler et al. (2019) evaluated diets including either 15.5% beet pulp (17.1% TDF), 9.6% cellulose (15.1% TDF), or 14% soybean hulls (16.6% TDF) and observed that cats fed the diet containing beet pulp had

lower intake than the diet containing soybean hulls due to feed refusals. This indicates that although no effect on palatability was observed in this study with an inclusion level up to 9% miscanthus grass (15.0% TDF), or up to 10% inclusion (13.8% TDF; Donadelli and Aldrich, 2019), higher inclusion levels and, subsequently, higher TDF contents may impact palatability and must be considered in practical utilization of this ingredient.

Similar fecal scores were observed for all treatments ranging from 1.81 to 2.18 on a 5-point scale. Previous research reported similar fecal scores with 8% inclusion of cellulose (11.2% TDF) and 12.5% inclusion of beet pulp (10.6% TDF), 1.8 and 2.3, respectively (Sunvold et al., 1995). Fecal output (g/d) on an as-is basis, as well as on a DM basis, were not significantly different among treatments. However, numerically, cats fed BP had the highest fecal output on an as-is basis and the lowest on a DM basis. This is due to the higher soluble fiber content of beet pulp that has a higher water-holding capacity, therefore increasing fecal water content and overall fecal mass. A similar effect was reported in felines by Detweiler et al. (2019) and in other studies across multiple species including canines and swine (Burkhalter et al., 2001; Serena et al., 2008).

Apparent Total Tract Macronutrient and Energy Digestibilities

Many studies have reported that dietary fiber sources can impact the digestibility of other macronutrients depending on their level of inclusion and fiber profile. Kienzle et al. (1991) reported that addition of dietary fiber significantly decreased OM digestibility by cats, and Sunvold et al. (1995) reported decreased OM and DM digestibility by cats when compared with a diet with no added fiber source. The DM and OM digestibility coefficients reported by Sunvold et al. (1995) for the diet containing 12.5% beet pulp (DM: 80.4%; OM: 83.8%) and 8.1% cellulose (DM: 81.0%; OM: 83.5%) were similar to values obtained for the diets in this study

containing 11% beet pulp (DM: 82.7%; OM: 86.3%) and 7% cellulose (DM: 79.1%; OM: 82.5%). When comparing diets with 10% inclusions of cellulose, beet pulp, and miscanthus grass, Donadelli and Aldrich (2019) reported that cats fed beet pulp (DM: 81.1%; OM: 85.9%) had significantly higher DM and OM coefficients than cellulose (DM: 75.5%; OM: 79.4%) and miscanthus grass (DM: 76.2%; OM: 80.5%). While the DM and OM digestibility coefficients reported in the current research were reported to be just a few percentage units higher than similar treatments evaluated by Donadelli and Aldrich (2019), DM and OM digestibility did not vary among dietary treatments herein. All diets were well digested by adult cats.

No difference in CP digestibility was detected among treatments in this study with a range of 83.1 to 84.6%. However, Donadelli and Aldrich (2019) observed the cellulose treatment to have a significantly higher CP digestibility than the beet pulp treatment with miscanthus grass being intermediate (86.1, 85.8, and 84.2%, respectively). In contrast, in this study, while not significant, the CP digestibility of the BP treatment was reported to be numerically lower than for all other treatments. Many similar effects have been reported due to beet pulp's moderate level of fermentability compared with cellulose and other fiber sources with greater concentrations of insoluble fiber. Greater fermentation may result in increased microbial proliferation causing more microbial protein to be present in the feces. The quantification of this microbial nitrogen during analysis can lead to underestimations of actual crude protein digestibility (Detweiler et al., 2019; Sunvold et al., 1995; Rossoni Serão and Fahey, 2013).

The CO treatment resulted in higher AHF digestibility than the treatments containing miscanthus grass fiber (MF and MF+TP). A similar effect was reported by Donadelli and Aldrich (2019). The lipid content of the cellulose diet was slightly higher compared with the other treatments in both of these studies, which could have contributed to the higher digestibility.

Another possible factor could be the higher lignin content of miscanthus grass compared with cellulose or beet pulp, measured by Donadelli and Aldrich (2019) to be 13.68%, 0.73%, and 6.38%, respectively. Lignin has been reported to bind bile acids, inhibiting their action during lipid digestion, and potentially lowering fat digestibility (Rodriguez-Gutierrez et al., 2019). Digestible energy (kcal/g) followed the same pattern as AHF digestibility. The lower fat digestibility and DE can be beneficial tools in the development of diets for overweight and obese cats, which is a serious clinical condition in the pet population. According to these data, miscanthus grass fiber may avoid further reductions in dietary fat content, which may assist maintaining palatability of weight management diets. This is important since weight management or loss diets tend to be formulated with higher concentrations of dietary fiber and lower fat content, resulting in poor acceptance, especially by cats. However, further studies should evaluate the impact of utilization of miscanthus grass fiber on fecal bile acid concentrations of cats. Since lignin can bind with bile acids in the gastrointestinal tract, it is possible that greater amounts of bile acids will be excreted in the feces, lowering their ability to recycle via enterohepatic circulation. This could lead to increased requirements of dietary taurine for cats.

Total dietary fiber ATTD was highest for the cats fed BP than for all other treatments. This was expected as beet pulp has been shown to be moderately fermented in the feline intestinal tract in comparison to cellulose, which has a low fermentative potential (Sunvold et al., 1995). Both of the treatments including miscanthus grass fiber (MF, 19.1 and MF+TP, 25.5%) were similar to cellulose (21.8%) in this regard, as they had greater IDF content, which is poorly fermented and, therefore, excreted in higher quantities in the feces. Donadelli and Aldrich reported a similar TDF digestibility coefficient (20.8%) with the inclusion of 10% miscanthus

grass. While not significantly different, the MF+TP treatment had a numerically higher TDF digestibility than did MF and CO treatments. This could be due to the inclusion of tomato pomace in the fiber blend that was reported by Swanson et al. (2001) to have a higher fermentation potential than cellulose using an in vitro model with canine fecal inoculum.

Fecal Fermentative End-Products and Microbiota, and Serum Chemistry

Short-chain fatty acids are the major organic end products of saccharolytic fermentation, with increased concentration indicating increased fermentative processes. While not an entirely accurate representation of complete SCFA production in the large intestine, fecal SCFA concentration has been utilized by researchers as a non-invasive method of estimating the production of these fermentative end-products by the gut microbiota. Total fecal SCFA concentration was highest in the BP group (583.7 µmole/g, DM basis) compared with all other treatments. Detweiler et al. (2019) evaluated higher levels of beet pulp (15.5% inclusion; 17.1% TDF) that resulted in higher levels of total SCFA (699.7 µmole/g, DM basis), and also observed the beet pulp treatment to produce the highest total SCFA compared with cellulose and soybean hulls. Fischer et al. (2012) reported similar results when evaluating a diet including 15.5% beet pulp (26% TDF) in overweight cats. This increased production of SCFA indicates that beet pulp has higher fermentability compared with the other fiber substrates evaluated, which is supported by the findings of Sunvold et al. (1995) who observed that beet pulp had a higher OM disappearance and total SCFA production than did cellulose in an in vitro assay using feline fecal inoculum. A decrease in gut lumen and fecal pH also is associated with higher fermentative activity as the buildup of these metabolites start to acidify the environment. However, no difference in fecal pH was observed, with values ranging from 7.1 (BP) to 7.7 (CO).

When evaluating the fecal SCFA on an individual basis, the same trend was observed for fecal acetate and propionate concentrations, being highest for the BP group. Our findings also are supported by Detweiler et al. (2019) who reported that cats fed beet pulp had significantly higher fecal concentrations of acetate (459.2 µmole/g, DM basis) and propionate (139.0 µmole/g) compared with cats fed diets with no additional fiber, cellulose, or soybean hulls (average acetate 219.6 µmole/g; average propionate 62.0 µmole/g) ($P < 0.05$). Fischer et al. (2012) also observed that when compared with diets containing wheat bran, sugarcane fiber, and a diet with no added fiber source (average acetate 217 mM/kg DM; average propionate 95.7 mM/kg), cats fed beet pulp had significantly higher fecal concentrations of acetate (427 mM/kg) and propionate (214 mM/kg) ($P < 0.05$). No statistical differences were observed in fecal butyrate concentration across treatments. However, CO and MF treatments had numerical values (24.4 µmole/g and 25.5 µmole/g, respectively) that grouped closer together, while MF+TP and BP treatments also were more closely grouped (34.2 µmole/g and 32.0 µmole/g). It is well established that SCFA play a significant role in maintaining gastrointestinal health as they provide energy to colonocytes, reduce inflammation, and have been implicated in the inhibition of cancer (Zhang and Davies, 2016). While available substrate is an important factor affecting SCFA production, complex factors such as removal of fermentative wastes and microbial population composition also play a critical role and are important to consider when evaluating the relationships between dietary components and fermentative metabolites (Zhang and Davies, 2016).

The fermentation of protein by microbiota in the large intestine results in end-products such as ammonia, phenols, indoles, and BCFA. Increases in these compounds often are seen as a negative outcome as they are considered putrefactive compounds that can lead to unwanted fecal malodor (O'Neill and Phillips, 1992). No difference was observed in fecal ammonia (1.4 to 1.7

mg/g DM) or phenol concentration (0.037 to 0.053 µmole/g, DM) among treatments. Detweiler et al. (2019) also reported no significant difference in these concentrations among treatments including beet pulp, cellulose, and soybean hulls as fiber sources, and a treatment with no added fiber source. Total phenol and indole and individual indole concentrations followed similar patterns of being higher in the treatment groups containing miscanthus grass fiber (MF and MF+TP). While the indole compound can help to maintain gut homeostasis by promoting barrier functions, regulating inflammation, and possibly aid in satiety, it can also be metabolized into indoxyl sulfate, which is a uremic toxin that has been associated with negative health outcomes in humans such as cardiovascular disease and chronic kidney disease (Zhang and Davies, 2016). Barry et al. (2010) reported higher indole concentrations (1.4 µmole/g, DM) with lower inclusion levels of cellulose (4% inclusion; 7.9% TDF), while Detweiler et al. (2019) reported lower indole concentrations (0.7 µmole/g, DM) with higher inclusion levels of cellulose (9.6%; 15.1% TDF). The inclusion level of cellulose and indole concentration in the current study (7% inclusion; 0.97 µmole/g, DM) were intermediate to values reported in the previous studies. In contrast, Detweiler et al. (2019) observed a higher level of indole (1.4 µmole/g, DM) with a higher inclusion rate of beet pulp (15.5% inclusion; 17.1% TDF).

Increased BCFA concentration indicates that higher levels of peptides and amino acids are present in the large intestine, and are available for fermentation. Cats fed the MF+TP diet had greater total BCFA concentrations than cats fed CO and BP treatments. Previous research by Barry et al. (2010) indicated that the addition of rapidly fermentable fibers (fructooligosaccharides and pectin) increased total BCFA concentrations compared with cellulose. This could explain the effect reported with addition of tomato pomace in the MF+TP fiber blend, as higher levels of rapidly fermented pectin are generally observed in fruit

byproducts (de Godoy et al., 2013). However, the total BCFA concentrations reported by Barry et al. (2010) were much higher (44.0 to 63.9 µmole/g, DM) with low inclusions (4%) of cellulose, FOS, and pectin than were observed in the current study (12.06 to 22.68 µmole/g, DM).

The use of dietary fiber has been an effective strategy in the modulation of gut microbiota to support gastrointestinal and systemic host health. Metabolites produced by gut microbiota (e.g., SCFA) are involved in the beneficial health effects on the host. These metabolites have been described as postbiotics (Tsilingiri and Rescigno, 2013), a term that is ill-defined by the scientific community (Żółkiewicz et al., 2020). The domestic cat, despite being a strict carnivore and having a short and unsacculated colon, has considerable capacity for hindgut microbial fermentation and production of fermentative end-products (Brosey et al., 2000). In companion animal nutrition, a few studies have evaluated the effects of dietary fiber in the modulation of gut microbiota in cats (Terada et al., 1993; Sparkes et al., 1998; Kanakupt et al., 2011; Barry et al., 2012; Barry et al., 2014; Garcia-Mazcorro et al., 2017; Lyu et al., 2020); however, most of those studies evaluated the effects of soluble and (or) prebiotic sources (e.g., FOS, lactosucrose, pectin, XOS) in contrast to cellulose or a no-added fiber diet. Thus, the effect of miscanthus grass fiber and tomato pomace on fecal microbiota has not been evaluated previously. Characterization of the feline gut microbiota have shown that Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria are dominant phyla in adult healthy cats (Desai et al., 2008; Barry et al., 2012; Garcia-Mazcorro et al., 2011; Barry et al., 2014). Our findings agree with current literature, even though the relative abundance of each phylum may differ among individuals and based on experimental methods used.

The phyla, Firmicutes, Bacteroidetes (Bacteroidota), and Actinobacteria, are considered important producers of metabolites that have direct beneficial effect on gut and host health (Suchodolski, 2016). A recent study evaluating the effects of dietary XOS supplementation on the fecal microbiota of healthy cats reported that diets containing either 0.04% or 0.4% of XOS, at the expense of cellulose, resulted in increased relative abundance of *Collinsellla* (2.6-4.4%) and decreased abundance of *Megasphaera* (0.80-0.82%) in contrast with cats fed a control diet containing 1% cellulose (1.7 and 1.3%, respectively) (Lyu et al., 2020). Similar relative abundance of *Megasphaer*a was observed in cats fed CO, MF, and MF+TP diets (range: 0.8-1.3%) in this study. Lyu et al. (2020) also reported increased relative abundance of Ruminococcaceae, Erysipelotrichaceae, and Lachnospiraceae in contrast to cats fed the control diet. In the current study, cats fed CO, MF and MF+TP treatments had increased relative abundance of *Allobaculum*, a genus within the Erysipleotrichaceae family, and a few genera within the family Ruminococcaceae (i.e., *Candidatus Soleaferrea*, *Incertae Sedis, and Phocea*). Garcia-Mazcorro et al. (2017) reported increased relative abundance of Veilonellaceae and decreased relative abundance of Gammaproteobacteria in fecal samples of healthy cats during FOS and inulin supplementation. In the current study, a greater relative abundance of *Megasphaera,* a genus within the family Veilonellaceae, and a lower relative abundance of *Succinivibrio*, a genus within the family Succinivibrionaceae and class Gammaproteobacteria, were observed in fecal samples of cats fed CO, MF, or MF+TP treatments in contrast with cats fed the BP treatment. More recently, Butowski et al. (2019) evaluated the fecal microbial communities of cats fed kibble, raw, and raw+fiber diets. The fiber sources included in the kibble diet were beet pulp and inulin (% inclusion not provided), and in the raw+fiber diet 2% of inulin and 2% of cellulose were included (as-is basis). In general, lower relative abundance of

Collinsella (0.03%), *Bacteroides* (0.2%), and greater relative abundance of *Prevotella* were reported for cats fed the kibble diet in comparison with our findings. The relative abundance of *Succinivibrio* of cats fed BP (4.3%) were greater than the relative abundance of cats fed the kibble diet (1.2%) reported by Butowski et al. (2019). Differences in relative abundance of microbial taxa among studies can be affected by many variables including animal variation, and differences in methods including DNA extraction, variable region and primers used for sequencing, bioinformatic procedures, and reference databased utilized.

From this study, it is clear that different dietary fibers exert distinctive effects on the modulation of the feline gut microbiota. Cats fed CO, MF, and MF+TP treatments had greater microbial taxa similarities among them in contrast with cats fed the BP treatment. This effect was evident based on the presence and absence of particular taxa (unweighted UniFrac), as well as their relative abundance (weighted UniFrac). Microbial diversity has been used as an indicator of gut health, as lower microbial diversity has been associated with clinical conditions including irritable bowel disease and small cell lymphoma in cats (Marsilio et al., 2019). Therefore, increased α-diversity observed in cats fed diets containing miscanthus grass fiber may support gut health by maintaining microbial richness and evenness in adult cats. However, healthy cats supplemented with either FOS or apple pomace had decreased α-diversity when compared with baseline values (Hall et al., 2020). Overall, there were apparent microbial benefits across all dietary treatments. In addition, all cats in this study were healthy and, therefore, microbial shifts should be evaluated on different dietary fiber sources and amounts may be used to modulate gut microbiota and their metabolites in the hindgut of felines.

Serum chemistry and complete blood count analysis were within reference ranges for healthy adult cats. Creatinine was an exception, and observed to be slightly higher than the

reference range for MF and BP treatments at baseline, and CO, MF, and BP treatments at the end of the trial period. These deviations from the reference range were small, and could be due to individual variation among cats. No effect of treatment was observed. Additionally, glucose concentrations were above the normal range. However, as previously mentioned, this has been observed as a side effect of the sedation used during the blood collection. The results of the blood analysis and lack of clinical symptoms indicates that the treatments did not result in any negative health outcomes.

IMPLICATIONS

The findings from this research indicate that miscanthus grass fiber is an advisable dietary fiber for adult felines. The addition of miscanthus grass fiber and the MF+TP blend had no detrimental effects on animal health, fecal quality, or macronutrient digestibility. Diet inclusion of miscanthus grass fiber up to 9% (15% TDF) had no negative effect on voluntary food intake, indicating that it had acceptable palatability to the cats. The resulting concentrations of fecal fermentative-end products were more similar to those observed in the CO group than in the BP group, as expected from the similarities in the fiber profile of these ingredients. In conclusion, miscanthus grass can be utilized by the pet food industry as an economical and environmentally conscious ingredient that can provide flexibility in the formulation of diets that aim to maximize the health benefits of dietary fiber. Miscanthus grass fiber can be effectively used as a base ingredient to develop fiber blends in combination witj more soluble and fermentable dietary fiber, including prebiotic sources, which might be beneficial to improve SCFA production and modulate gut microbiota. In this study, inclusion of 2% TP in combination with MF resulted in small numerical increases in fecal SCFA concentrations, suggesting that

fiber blends can be used to support gut and host health. Inclusion of dietary fibers was an effective strategy to modulate feline gut microbiota. Cats fed diets containing miscanthus grass fiber had greater α-diversity than cats fed BP. Future studies should further evaluate nutraceutical uses, additional fiber blends, and diet formats, as miscanthus grass fiber can be a functional ingredient in multiple dietary platforms, including weight management, gut health, and hairball control.

TABLES AND FIGURES

Table 3.1: Ingredient composition of treatments containing traditional and novel fiber sources for adult felines

	Treatment[1]			
Ingredient, % as-is	**CO**	**MF**	**MF+TP**	**BP**
Poultry by-product meal	40.31	40.00	38.31	37.81
Brewers rice	32.00	30.00	32.00	30.00
Poultry fat	8.50	8.81	8.50	9.00
Yellow corn	5.00	5.00	5.00	5.00
Corn gluten meal 60%	5.00	5.00	5.00	5.00
AFB palatant	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Choline chloride	0.13	0.13	0.13	0.13
Potassium chloride	0.10	0.10	0.10	0.10
BHT antioxidant	0.10	0.10	0.10	0.10
Mineral premix[2]	0.18	0.18	0.18	0.18
Vitamin premix[3]	0.18	0.18	0.18	0.18
Cellulose	**7.00**	0.00	0.00	0.00
Miscanthus grass	0.00	**9.00**	**7.00**	0.00
Beet pulp	0.00	0.00	0.00	**11.00**
Tomato pomace	0.00	0.00	**2.00**	0.00

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.
[2]Provided per kg diet: 17.4 mg manganese (MnSO4), 284.3 mg iron (FeSO4), 17.2 mg copper (CuSO4), 2.2 mg cobalt (CoSO4), 166.3 mg zinc (ZnSO4), 7.5 mg iodine (KI), and 0.2 mg selenium (Na2SeO3).
[3]Provided per kg diet: 11,000 IU vitamin A, 900 IU vitamin D3, 57.5 IU vitamin E, 0.6 mg vitamin K, 7.6 mg thiamin, 11.9 mg riboflavin, 18.5 mg pantothenic acid, 93.2 mg niacin, 6.6 mg pyridoxine, 12.4 mg biotin, 1,142.1 μg folic acid, and 164.9 μg vitamin B12

Table 3.2: Chemical composition of treatments containing traditional and novel fiber sources for adult felines

	Treatment[1]			
Item	**CO**	**MF**	**MF+TP**	**BP**
Dry matter, %	94.2	93.1	92.4	91.9
	----- % DM basis -----			
Organic matter	93.9	93.5	93.8	93.1
Ash	6.1	6.5	6.2	6.9
Acid hydrolyzed fat	17.6	16.7	16.2	16.2
Crude protein	31.3	30.9	29.6	30.3
Total dietary fiber	15.1	15.0	15.7	15.7
Soluble dietary fiber	3.4	3.8	3.2	6.1
Insoluble dietary fiber	11.7	11.2	12.5	9.6
Gross energy, kcal/g	4.7	4.6	4.6	4.5

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.

Table 3.3: Food intake, fecal characteristics, and total tract apparent macronutrient digestibility of adult felines fed dietary treatments containing traditional and novel fiber sources

	Treatment[1]				
Item	**CO**	**MF**	**MF+TP**	**BP**	**SEM[2]**
Food intake, as-is	57.3	57.1	62.9	64.9	4.83
Dry matter, g/d	54.0	53.1	58.1	59.7	4.49
Fecal output, g/d (as is)	27.5	24.6	29.5	37.5	3.97
Fecal output, g/d (DM basis)	11.6	11.3	12.4	10.4	1.21
Fecal score	2.0	1.8	2.0	2.2	0.10
Fecal pH	7.7	7.5	7.3	7.1	0.21
Digestibility, %					
Dry matter	79.1	78.3	78.7	82.7	1.40
		-----% DM basis -----			
Organic matter	82.5	81.8	82.1	86.3	1.19
Acid hydrolyzed fat	94.5[a]	91.7[b]	91.2[b]	92.6[ab]	0.57
Crude protein	84.1	84.6	83.7	83.1	1.29
Total dietary fiber	21.8[b]	19.1[b]	25.5[b]	54.2[a]	5.44
Digestible energy, kcal/g	3.94[a]	3.74[ab]	3.72[b]	3.85[ab]	0.05

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.
[2] Standard error of the mean.
[a-b] Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3.4: Fecal fermentative-end products for adult felines fed treatments containing traditional and novel fiber sources

	Treatment[1]				
Item (µmole/g DM basis)	**CO**	**MF**	**MF + TP**	**BP**	**SEM[2]**
Total Phenols/Indoles	1.0[b]	1.8[a]	1.8[a]	0.6[b]	0.18
Phenols	0.0	0.1	0.1	0.1	0.01
Indoles	1.0[b]	1.7[a]	1.7[a]	0.5[b]	0.17
Total short-chain fatty acids	168.0[b]	189.2[b]	256.5[b]	583.7[a]	42.87
Acetate	99.1[c]	121.3[bc]	161.4[b]	390.2[a]	27.13
Propionate	44.5[b]	42.4[b]	61.0[b]	161.5[a]	13.66
Butyrate	24.4	25.5	34.2	32.0	3.54
Total branched-chain fatty acids	13.1[b]	17.2[ab]	22.7[a]	12.1[b]	2.08
Isobutyrate	2.9[b]	3.6[ab]	4.6[a]	3.1[b]	0.35
Isovalerate	4.5[b]	5.3[ab]	7.4[a]	4.2[b]	0.65
Valerate	5.7[ab]	8.3[ab]	10.2[a]	4.8[b]	1.29
Ammonia, mg/g DM	1.2	1.4	1.7	1.4	0.15

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.
[2] Standard error of the mean.
[a-b] Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3.5 Fecal microbial composition (%) at the family level for cats fed treatments containing traditional and novel fiber sources.

		Treatment[1]				
Phylum	Family	CO	MF	MF+TP	BP	SEM[2]
Actinobacteria	Bifidobacteriaceae	0.75[a]	0.02[b]	0.26[ab]	0.53[ab]	0.178
	Coriobacteriaceae	3.97[a]	3.53[ab]	2.98[ab]	2.38[b]	0.431
Bacteroidota	Bacteroidaceae	9.40[b]	10.39[ab]	12.26[ab]	13.80[a]	1.280
	Prevotellaceae	12.33[b]	7.99[b]	9.50[b]	17.19[a]	1.536
	Rikenellaceae	0.40	0.62	0.60	0.02	0.207
	Tannerellaceae	1.54[ab]	1.88[ab]	2.10[a]	1.29[b]	0.232
Firmicutes	Erysipelotrichaceae	5.58[ab]	7.86[a]	7.14[ab]	4.97[b]	0.929
	RF39	0.03[b]	0.30[a]	0.00[b]	0.00[b]	0.084
	Clostridiaceae	1.13[ab]	0.41[b]	0.72[ab]	1.63[a]	0.355
	Butyricicoccaceae	1.53[a]	1.48[a]	1.38[a]	0.45[b]	0.241
	Oscillospiraceae	3.13[a]	3.21[a]	3.06[a]	0.93[b]	0.373
	Eubacterium coprostanoligenes group	0.68[a]	0.52[a]	0.51[a]	0.01[b]	0.171
	Anaerovoracaceae	2.13[a]	2.80[a]	2.44[a]	0.49[b]	0.469
Proteobacteria	Succinivibrionaceae	1.76[b]	0.84[c]	1.02[bc]	4.25[a]	0.282

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.
[2] Standard error of the mean.
[a-b] Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3.6 Fecal microbial composition at the genus level for cats fed treatments containing traditional and novel fiber sources.

		Treatment[1]				
Phylum	Genus	CO	MF	MF+TP	BP	SEM[2]
Actinobacteria	*Bifidobacterium*	0.75^{a}	0.02^{b}	0.26^{ab}	0.53^{ab}	0.178
	Collinsella	3.97^{a}	3.53^{ab}	2.95^{ab}	2.38^{b}	0.431
Bacteroidota	*Bacteroides*	9.40^{b}	10.39^{ab}	12.26^{ab}	13.80^{a}	1.280
	Paraprevotella	0.01^{b}	0.12^{ab}	0.20^{a}	0.00^{b}	0.043
	Prevotella	9.22^{b}	5.42^{b}	6.03^{b}	14.80^{a}	1.460
	Alistipes	0.40^{ab}	0.47^{ab}	0.54^{a}	0.02^{b}	0.173
	Parabacteroides	1.54^{ab}	1.88^{ab}	2.10^{a}	1.29^{b}	0.232
	Allobaculum	0.46^{ab}	0.75^{a}	0.31^{ab}	0.08^{b}	0.193
Firmicutes	*RF39*	0.03^{b}	0.30^{a}	0.00^{b}	0.00^{b}	0.084
	Clostridia UCG-014	2.07^{ab}	2.26^{ab}	2.55^{a}	1.42^{b}	0.363
	Butyricicoccus	0.73^{a}	0.56^{ab}	0.45^{ab}	0.19^{b}	0.152
	Butyricicoccaceae UCG-009	0.74^{a}	0.88^{a}	0.82^{a}	0.22^{b}	0.177
	Colidextribacter	1.23^{a}	1.16^{a}	1.22^{a}	0.58^{b}	0.156
	Oscillibacter	0.61^{a}	0.50^{a}	0.44^{a}	0.13^{b}	0.103
	Candidatus soleaferrea	0.26^{ab}	0.33^{a}	0.30^{a}	0.11^{b}	0.065
	Incertae sedis	0.18^{ab}	0.52^{a}	0.29^{a}	0.00^{b}	0.087
	Phocea	0.22^{a}	0.16^{ab}	0.09^{ab}	0.03^{b}	0.056
	Eubacterium coprostanoligenes group	0.68^{a}	0.52^{a}	0.51^{a}	0.01^{b}	0.171
	Mogibacterium	0.24^{ab}	0.29^{ab}	0.43^{a}	0.00^{b}	0.138
	Eubacterium brachy group	0.39^{a}	0.36^{a}	0.22^{ab}	0.08^{b}	0.066
	Eubacterium nodatum group	0.78^{ab}	0.90^{a}	0.89^{a}	0.32^{b}	0.182
	Megasphaera	1.15^{a}	0.77^{ab}	1.26^{a}	0.28^{b}	0.290
Proteobacteria	*Succinivibrio*	1.76^{b}	0.84^{c}	1.02^{bc}	4.25^{a}	0.282

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp.

[2] Standard error of the mean.

[a-c] Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3.7 Serum metabolites of adult felines fed treatments containing traditional and novel fiber sources.

Item	Reference Range[2]	*Day 0*				SEM[3]	*P* - Value		
		CO[1]	MF	MF+TP	BP		Trt	Day	Trt*Day
Creatinine (mg/dL)	0.5-1.5	1.5	1.7	1.4	1.6	0.08	0.07	0.03	0.82
BUN[3] (mg/dL)	6.0-30.0	21.9	21.9	20.3	21.1	0.79	0.30	0.72	0.72
Total protein (g/dL)	5.1-7.0	6.4	6.3	6.5	6.3	0.12	0.83	0.20	2.30
Albumin (g/dL)	2.5-3.8	3.3	3.4	3.2	3.3	0.06	0.37	0.01	0.02
Globulin (g/dL)	2.7-4.4	3.1	2.9	3.3	3.0	0.11	0.14	0.32	0.27
Ca (mg/dL)	7.6-11.4	9.1	9.0	9.1	9.0	0.10	0.83	<.01	0.22
P (mg/dL)	2.7-5.2	4.8	4.9	5.1	4.9	0.21	0.64	0.64	0.98
Na (mmol/L)	141-152	147.1	147.0	146.6	145.9	0.60	0.79	<.01	0.07
K (mmol/L)	3.9-5.5	4.1	4.1	4.2	4.2	0.18	0.45	<.01	0.11
ALT (SGPT)	8.0-65.0	49.7	41.4	46.3	51.7	6.67	0.69	0.01	0.78
Cl (mmol/L)	107-118	118.3	118.3	117.7	117.7	0.62	0.66	<0.01	0.13
Glucose (mg/dL)	68-126	146.6	152.9	151.1	167.0	14.85	0.54	0.21	0.10
Total Bilirubin (mg/dL)	0.1-0.3	0.1	0.1	0.1	0.1	0.01	0.25	0.67	0.57
Total Cholesterol (mg/dL)	66-160	140.6	158.0	146.9	157.0	13.09	0.91	0.14	0.10
Triglycerides (mg/dL)	32-154	34.6	40.6	44.7	39.0	4.45	0.54	0.05	0.67
Bicarbonate (mmol/L)	16-24	19.1	18.7	19.3	18.6	0.47	0.16	<0.01	0.23

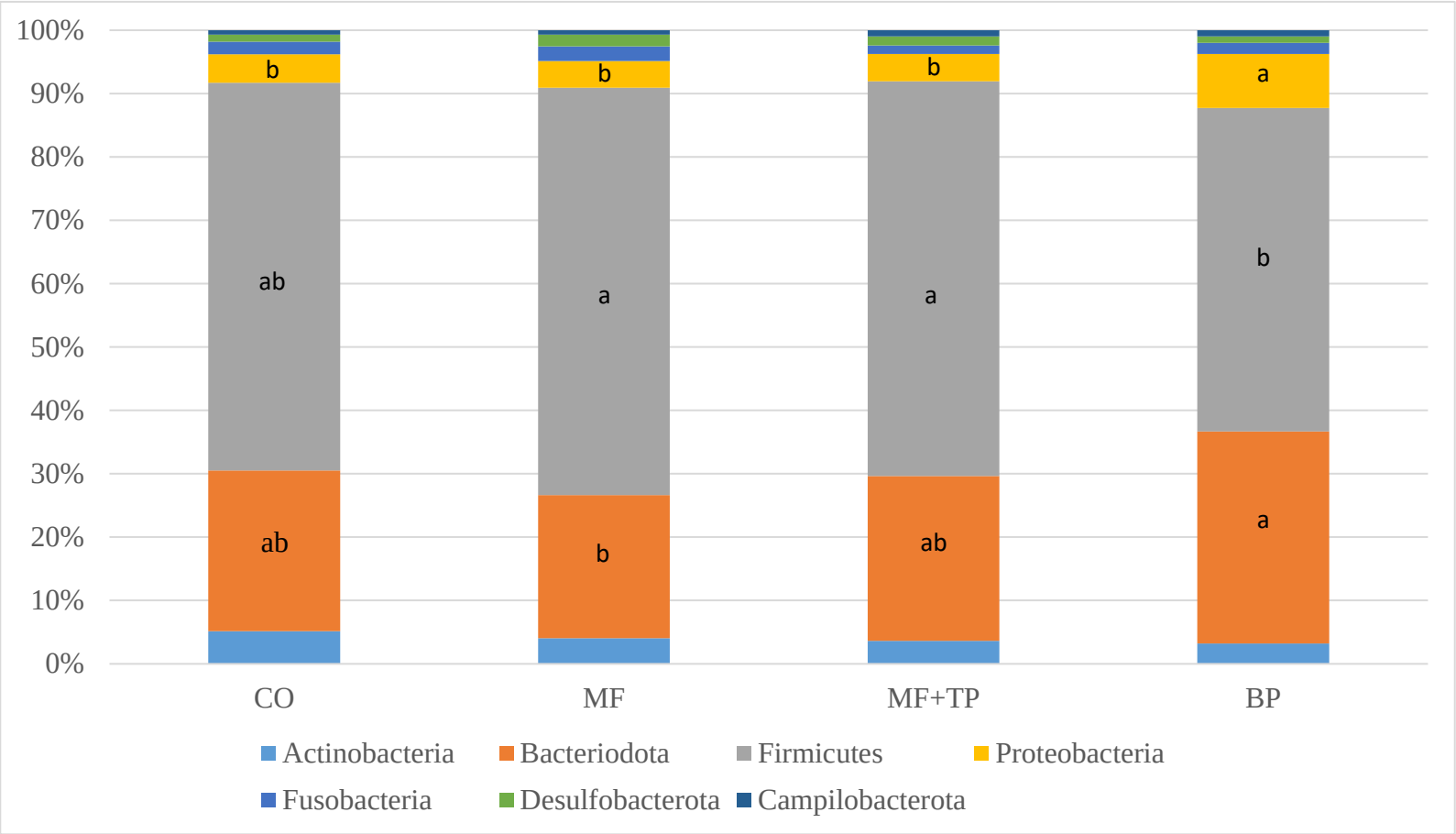
[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp
[2] References ranges were provided by the University of Illinois Veterinary Diagnostic Laboratory
[3] SEM = Standard error of the mean; BUN = Blood urea nitrogen

Table 3.7 (cont.) Serum metabolites of adult felines fed treatments containing traditional and novel fiber sources (**cont'd**)

Item	Reference Range[2]	*Day 21*				SEM[3]	***P* - Value**		
		CO[1]	MF	MF+TP	BP		Trt	Day	Trt*Day
Creatinine (mg/dL)	0.5-1.5	1.6	1.8	1.5	1.6	0.08	0.07	0.03	0.82
BUN[3] (mg/dL)	6.0-30.0	22.1	21.7	20.0	21.7	0.79	0.30	0.72	0.72
Total protein (g/dL)	5.1-7.0	6.3	6.3	6.5	6.5	0.12	0.83	0.20	2.30
Albumin (g/dL)	2.5-3.8	3.2	3.4	3.3	3.4	0.06	0.37	0.01	0.02
Globulin (g/dL)	2.7-4.4	3.0	2.9	3.2	3.1	0.11	0.14	0.32	0.27
Ca (mg/dL)	7.6-11.4	9.3	9.4	9.4	9.2	0.10	0.83	<.01	0.22
P (mg/dL)	2.7-5.2	4.7	4.9	5.1	5.0	0.21	0.64	0.64	0.98
Na (mmol/L)	141-152	148.0	149.0	148.0	149.3	0.60	0.79	<.01	0.07
K (mmol/L)	3.9-5.5	4.0	3.5	4.0	3.7	0.18	0.45	<.01	0.11
ALT (SGPT)	8.0-65.0	61.1	54.4	51.0	58.9	6.67	0.69	0.01	0.78
Cl (mmol/L)	107-118	116.7	116.7	116.1	118.0	0.62	0.66	<0.01	0.13
Glucose (mg/dL)	68-126	175.3	139.4	130.3	118.7	14.85	0.54	0.21	0.10
Total Bilirubin (mg/dL)	0.1-0.3	0.1	0.1	0.1	0.1	0.01	0.25	0.67	0.57
Total Cholesterol (mg/dL)	66-160	159.9	156.1	147.3	158.7	13.09	0.91	0.14	0.10
Triglycerides (mg/dL)	32-154	31.7	39.3	37.6	35.9	4.45	0.54	0.05	0.67
Bicarbonate (mmol/L)	16-24	18.3	18.6	18.6	16.9	0.47	0.16	<0.01	0.23

[1] CO = Cellulose; MF = M-Fiber; MF+TP = M-Fiber + Tomato Pomace; BP = Beet Pulp
[2] References ranges were provided by the University of Illinois Veterinary Diagnostic Laboratory
[3] SEM = Standard error of the mean; BUN = Blood urea nitrogen

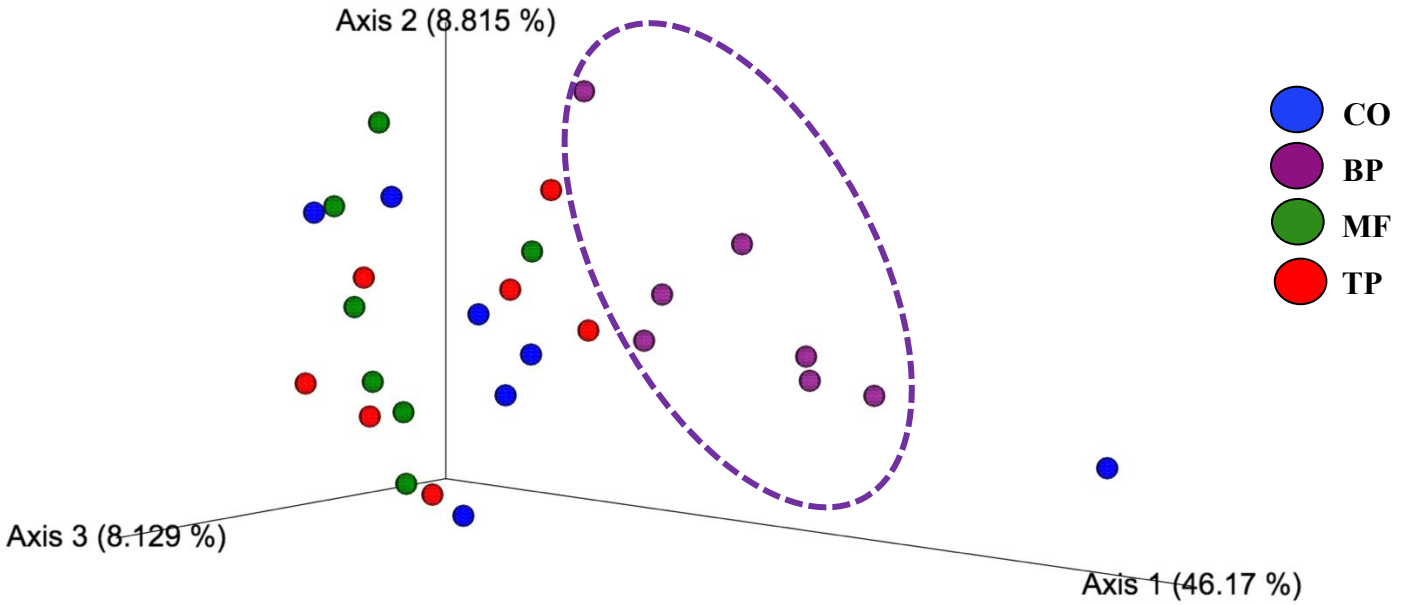
Figure 3.1 Fecal microbial composition at the phyla level for cats fed diets containing traditional and novel fiber sources.



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace
[a-b] Means in the same row with different superscript letters are different ($P < 0.05$).

Figure 3.2 Principal coordinated plots of weighted (A), and unweighted (B) UNIFRAC distances of fecal microbial communities of cats fed diets containing traditional and novel fiber sources.

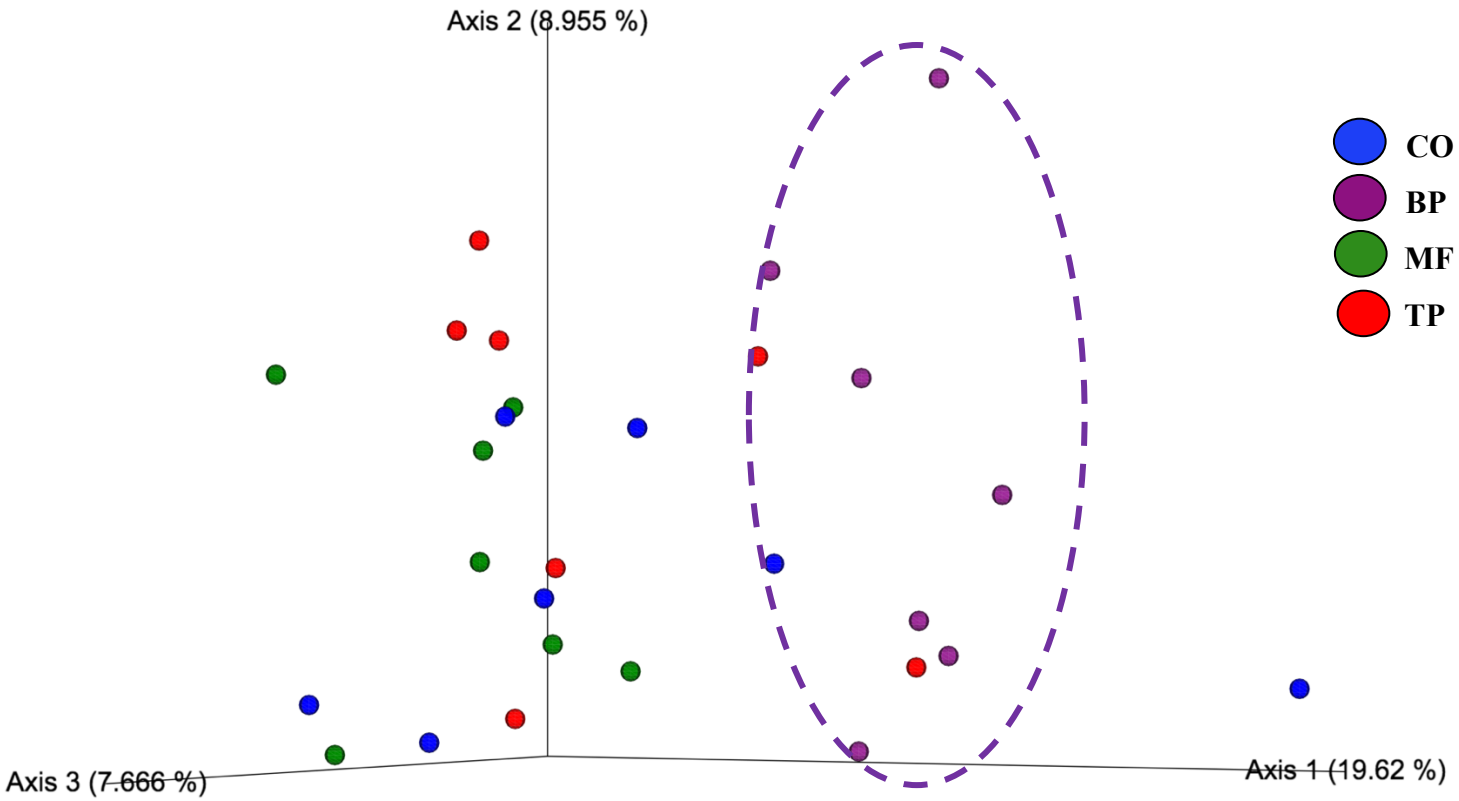
(A)



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace

Figure 3.2 (cont,)

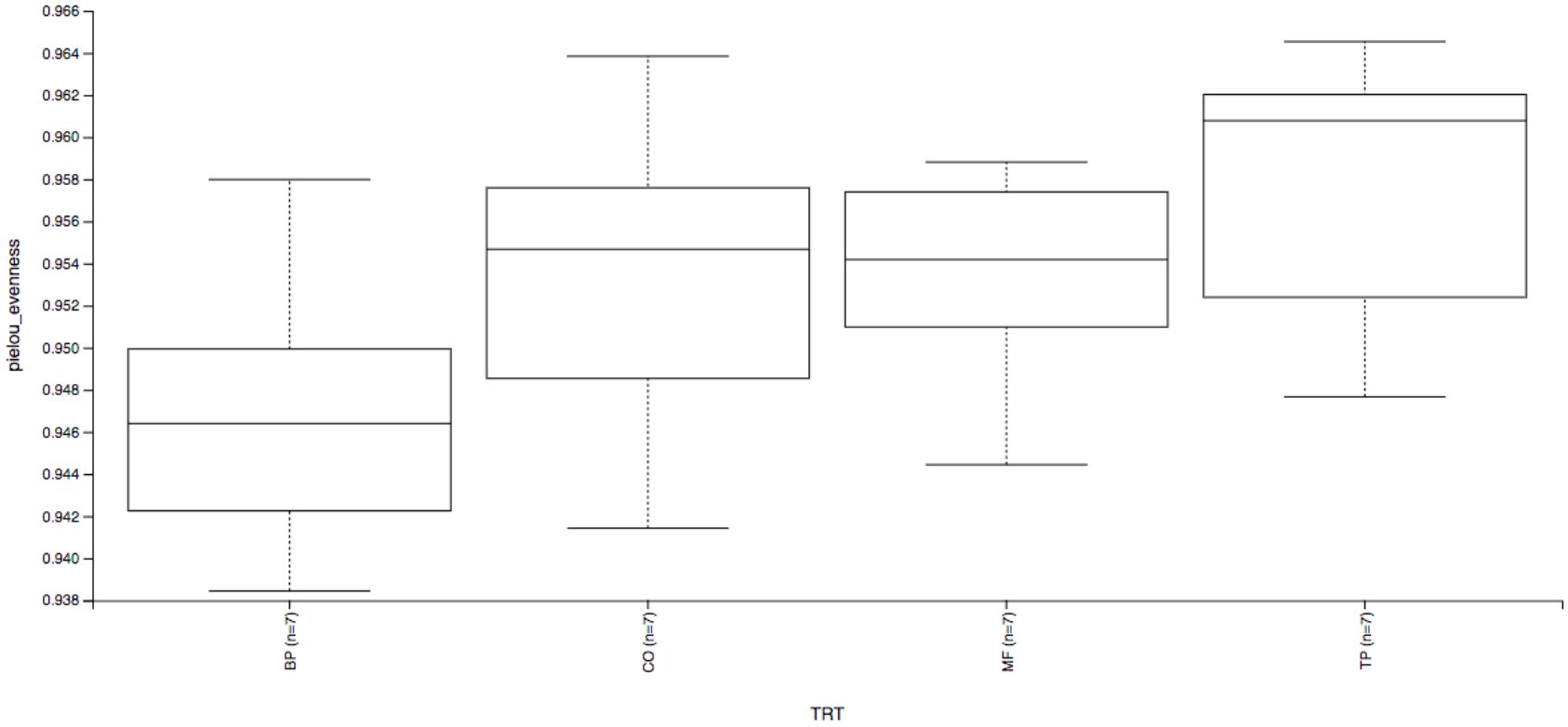
(B)



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace

Figure 3.3 Alpha-diversity analysis of fecal microbial communities, measured by Pielou evenness (A), Faith's phylogenetic diversity (B), and Shannon entropy (C) of cats fed diets containing traditional and novel fiber sources.

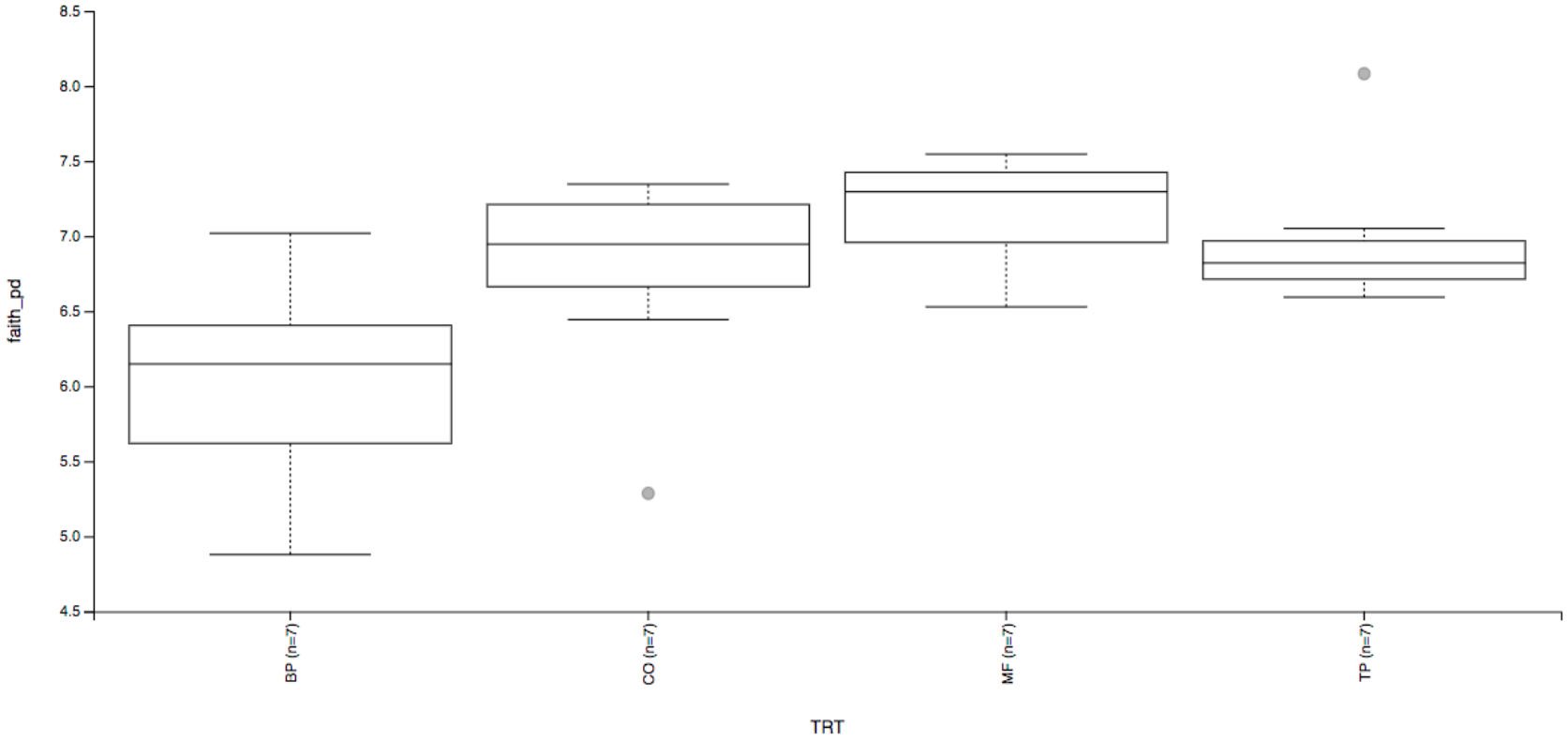
(A)



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace

Figure 3.3 (cont.)

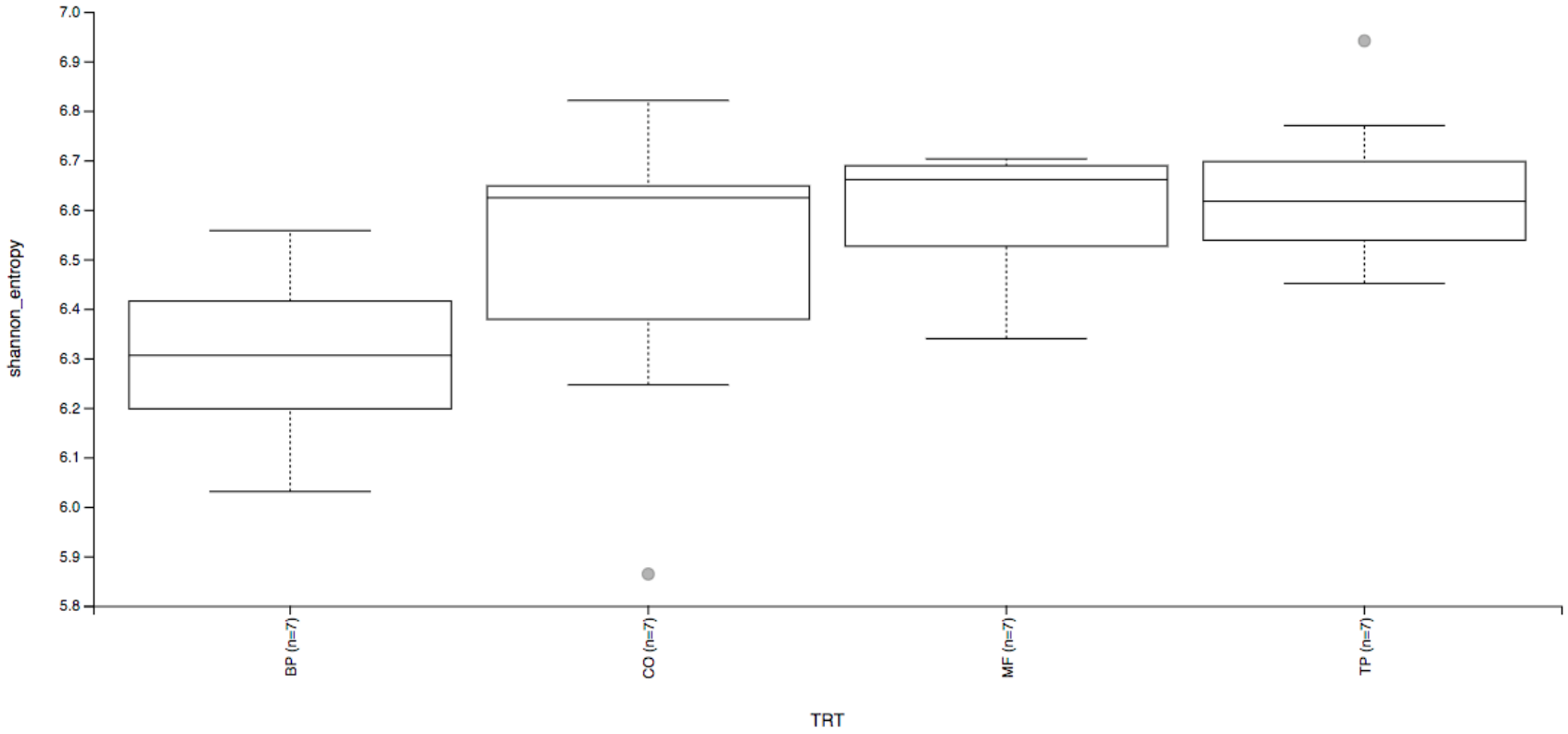
(B)



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace

Figure 3.3 (cont.)

(C)



ABBR: CO = Cellulose, BP = Beep pulp, MF = Miscanthus grass, TP = Blend of miscanthus grass plus tomato pomace

LITERATURE CITED

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

Anderson, J. W., P. Baird, R. H. Davis, S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205.

AOAC. 2006. Official methods of analysis. 17th ed. AOAC, Arlington, VA.

Association of American Feed Control Officials (AAFCO). 2018. Official Publication. Champaign, IL.

Barry, K. A., B. J. Wojcicki, I. S. Middelbos, B. M. Vester, K. S. Swanson, and G. C. Fahey Jr. 2010. Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. J. Anim. Sci. 88:2978-2987. doi:10.2527/jas.2009-2464.

Barry, K. A., I. S. Middelbos, B. M. Vester Boler, K. S. Swanson, and G. C. Fahey. 2012. Effects of dietary fiber on the feline gastrointestinal metagenome. J. Proteome Res. 11:5924-5933. doi:10.1021/pr3006809.

Barry, K. A., D. C. Hernot, J. Van Loo, G. C. Fahey, and M. R. C. de Godoy. 2014. Fructan supplementation of senior cats affects stool metabolite concentrations and faecal microbiota concentrations, but not nitrogen partitioning in excreta. J. Anim. Sci. 92:4964-4971. doi:10.2527/jas.2013-7510.

Brosey, B. P., R. C. Hill, and K. C. Scott. 2000. Gastrointestinal volatile fatty acid concentrations and pH in cats. Am. J. Vet. Res. 61:359–361. doi:10.2460/ajvr.2000.61.359.

Budde, E. F. 1952. The determination of fat in baked biscuit type of dog foods. J. AOAC 35:799-805.

Burkhalter, T. M., N. R. Merchen, L. L. Bauer, S. M. Murray, A. R. Patil, J. L. Brent, and G. C. Fahey, Jr. 2001. The ratio of insoluble to soluble fiber components in soybean hulls affects ileal and total-tract nutrient digestibilities and fecal characteristics of dogs. J. Nutr. 131:1978-1985.

Butowski, C. F., D. G. Thomas, W. Young, N. J. Cave, C. M. McKenzie, D. I. Rosendale, and E. N. Bermingham. 2019. Addition of plant dietary fibre to a raw red meat high protein, high fat diet, alters the faecal bacteriome and organic acid profiles of the domestic cat (*Felis catus*). PLoS One. 14: e0216072. doi:10.1371/journal. pone.0216072.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature. Methods. 13:581-583. doi:10.1038/nmeth.3869.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 7: 335-336. doi:10.1038/nmeth.f.303.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, and R. Knight. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6:1621-1624. doi:10.1038/ismej.2012.8.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Dai, F. J. and C. F. Chau. 2016. Classification and regulatory perspectives of dietary fiber. J. Food Drug Anal. 25:37-42.

de Godoy, M. R. C., K. R. Kerr, and G. C. Fahey, Jr. 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients. 5:3099-3117. doi:10.3390/nu5083099.

Desai, A. R., K. M. Musil, A. P. Carr, and J. E. Hill. 2008. Characterization and quantification of feline faecal microbiota using cpn60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. Vet. Microbiol. 137:120–128. doi:10.1016/j.vetmic.2008.12.019.

Detweiler, K. B., F. He, H. F. Mangian, G. M. Davenport, and M. R. C. de Godoy. 2019. Extruded feline diets formulated with high inclusion of soybean hulls: effects on apparent total tract macronutrient digestibility, and fecal quality and metabolites. J. Anim. Sci. 97:1042-1051. doi:10.1093/jas/skz014.

Donadelli, R. A. and C. G. Aldrich. 2019. The effects on nutrient utilization and stool quality of Beagle dogs fed diets with beet pulp, cellulose, and Miscanthus grass. J. Anim. Sci. 97:4134-4139. doi:10.1093/jas/skz265.

Fischer, M. M., A. M. Kessler, L. R. M. de Sá, R. S. Vasconcellos, F. O. Roberti Filho, S. P. Nogueria, M. C. C. Oliveira, and A. C. Carciofi. 2012. Fiber fermentability effects on energy and macronutrient digestibility, fecal traits, postprandial metabolite responses, and colon histology of overweight cats. J. Anim. Sci. 90:2233-2245. doi:10.2527/jas.2011-4334

Flickinger, E. A., E. M. Schreijen, A. R. Patil, H. S. Hussein, C. M. Grieshop, N. R. Merchen, and G. C. Fahey, Jr. 2003. Nutrient digestibilities, microbial populations, and protein

catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci. 81:2008-2018. doi:10.2527/2003.8182008x.

Food and Drug Administration. 2016. Food labeling: Revision of the nutrition and supplement facts labels. Fed. Regist. 81:33741-33999.

Garcia-Mazcorro, J. F., D. J. Lanerie, S. E. Dowd, C. G. Paddock, N. Grutzner, J. M. Steiner, R. Ivanek, and J. S. Suchodolski. 2011. Effect of a multi-species synbiotic formulation on faecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. FEMS Microbiol. Ecol. 78:542–554. doi:10.1111/j.1574-6941. 2011.01185.x.

Garcia-Mazcorro, J. F., J. R. Barcenas-Walls, J. S. Suchodolski, and J. M. Steiner. 2017. Molecular assessment of the fecal microbiota in healthy cats and dogs before and during supplementation with fructo-oligosaccharides (FOS) and inulin using high-throughput 454-pyrosequencing. Peer J. 8:5:e3184. doi:10.7717/peerj.3184.

Hall, J. A., M. I. Jackson, and D. E. Jewell. 2020. Chronic kidney disease in cats alters response of the plasma metabolome and fecal microbiome to dietary fiber. PLosONE. 5: e0235480. doi:10.1371/journal. pone.0235480.

Kanakupt K., B. M. Vester Boler, B. R. Dunsford, and G. C. Fahey, Jr. 2011. Effects of short-chain fructooligosaccharides and galactooli- gosaccharides, individually and in combination, on nutrient digestibility, faecal fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats. J. Anim. Sci. 89:1376 -1384. doi:10.2527/jas.2010-3201.

Kienzle, E., H. Meyer, and R. Schneider. 1991. Investigations on palatability, digestibility and tolerance of low digestible food components in cats. J. Nutr. 121:S56-S57. doi:10.1093/jn/121.suppl_11.S56

Lozupone, C., and R. Knight. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71:8228-8235. doi:10.1128/AEM.71.12.8228-8235.2005.

Lyu, Y., S. Debevere, H. Bourgeois, M. Ran, B. J. G. Broeckx, L. Vanhaecke, T. Van de Wiele, and M. Hesta. 2020. Dose-dependent effects of dietary xylooligosaccharides supplementation on microbiota, fermentation and metabolism in healthy adult cats. Molecules. 25:50030. doi:10.3390/molecules25215030.

Marsilio, S., R. Pilla, B. Sarawichitr, B. Chow, S. L. Hill, M. R. Ackermann, J. S. Estep, J. A. Lidbury, J. M. Steiner, and J. S. Suchodolski. 2019. Characterization of the fecal microbiome in cats with inflammatory bowel disease or alimentary small cell lymphoma. Sci. Rep. 9:1–11. doi:10.1038/s41598-019-55691-w.

O'Neill, D. H., and V. R. Phillips. 1992. A review of the control of odour nuisance from livestock buildings: Part 3. Properties of the odorous substances which have been identified in livestock wastes or in the air around them. J. Agric. Eng. Res. 53:23-50. doi:10.1016/0021-8634(92)80072-Z.

Prosky, L., N. G. Asp, T. F. Schwizer, J. W. De Vries, and I. Furda. 1992. Determination of insoluble and soluble dietary fiber in foods and food products: Collaborative study. J. Assoc. Off. Anal. Chem. 75:360-367.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glockner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41(D1):D590-D596. doi:10.1093/nar/gks1219.

Rodriguez-Gutierrez, G., F. Rubio-Senent, A. Lama-Munoz, A. Garcia, and J. Fernandez-Bolanos. 2014. Properties of lignin, cellulose, and hemicelluloses isolated from olive

cake and olive stones: Binding of water, oil, bile acids, and glucose. J. Agric. Food Chem. 62:8973-8981. doi:10.1021/jf502062b.

Rossoni Serão, M. C. and G. C. Fahey Jr. 2013. Companion animal nutrition as affected by dietary fibre inclusion. In: J. A. Delcour and K. Poutanen, editors, Fibre-Rich and Wholegrain Foods. Woodhead Publishing, Swaston, Cambridge, UK. p. 407-420.

Serena, A., H. Jørgensen, and K. E. Bach Knudsen. 2008. Digestion of carbohydrates and utilization of energy in sows fed diets with contrasting levels and physicochemical properties of dietary fiber. J. Anim. Sci. 86:2208-2216. doi:10.2527/jas.2006-060.

Sparkes, A. H., K. Papasouliotis, G. Sunvold, G. Werrett, G. Gruydd-Jones, and E. A. Egan. 1998. Effect of dietary supplementation with fructooligosaccharides on faecal flora of healthy cats. Am. J. Vet. Res. 59:436–440.

Suchodolski, J. S. 2016. Diagnosis and interpretation of intestinal dysbiosis in dogs and cats. Vet. J. 215:30-37. doi:10.1016/j.tvjl.2016.04.011.

Sunvold, G. D., G. C. Fahey Jr, N. R. Merchen, L. D. Bourquin, E. C. Titgemeyer, L. L. Bauer, and G. A. Reinhart. 1995. Dietary fiber for cats: In vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. J. Anim. Sci. 73:2329-2339. doi:10.2527/1995.7382329x.

Swanson, K. S., C. M. Grieshop, G. M. Clapper, R. G. Shields, Jr., T. Belay, N. R. Merchen, and G. C. Fahey, Jr. 2001. Fruit and vegetable fiber fermentation by gut microflora from canines. J. Anim. Sci. 79:919-926. doi:10.2527/2001.794919x.

Terada, A., H. Hara, S. Kato, T. Kimura, I. Fujimori, K. Hara, T. Maruyama, and T. Mitsuoka. 1993. Effect of lactosucrose (4G-B-d-galactosylsucrose) on faecal flora and faecal putrefactive products of cats. J. Vet. Med. Sci. 55:291-295. doi:10.1292/jvms.55.291.

Tsilingiri K. and M. Rescigno. 2013. Postbiotics: What else? Benef. Microbes. 4:101-107. doi:10.3920/BM2012.0046.

Zhang, L. S. and S. S. Davies. 2016. Microbial metabolism of dietary components to bioactive metabolites: Opportunities for new therapeutic interventions. Genome. Med. 8:1-18. doi:10.1186/s13073-016-0296-x.

Żółkiewicz, J., A. Marzec, M. Ruszczyński, and W. Feleszko. 2020. Postbiotics—A step beyond pre- and probiotics. Nutrients. 12:2189. doi:10.3390/nu12082189.